## OCCASION

This publication has been made available to the public on the occasion of the 50th anniversary of the United Nations Industrial Development Organisation.

## DISCLAIMER



## FAIR USE POLICY



## CONTACT

Please contact publications@unido.org for further information concerning UNIDO publications.

For more information about UNIDO, please visit us at www.unido.org

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION
Vienna International Centre, P.O. Box 300, 1400 Vienna, Austria
Tel: (+43-1) 26026-0 · www.unido.org · unido@unido.org

RESTRICTED

DP/ID/SER.B/630
21 September 1988
ORIGINAL: ENGLISH

17076

# PROCESSING OF STEVIA

## SI/DRK/87/802

## DEMOCRATIC PEOPLE'S REPUBLIC OF KOREA

Terminal report*

Prepared for the Government of
the Democratic People's Republic of Korea
by the United Nations Industrial Development Organization,
acting as executing agency for the United Nations Development Programme

Based on the work of Peter J. Hylands, UNIDO consultant

Backstopping officer: R.O.B. Wijesekera
Chemical Industries Branch

United Nations Industrial Development Organization

Vienna

11

* This document has not been edited.

## CONTENTS

## A. SUMMARY

The Consultant has investigated the current state of Stevia processing in DPRK. A crude extract is already in production, used principally in the soft-drink industry. The Consultant was able to make specific recommendations regarding improvements to this process.

The research activity of the Stevia research institute was also evaluated and the Consultant was able to make proposals about the current research (mainly directed towards the isolation of purified compounds and their biotransformations) as well as suggestions for future activity.

The main problem with the programmes may be summarised as lack of investment. Production equipment should be upgraded by the provision of stainless-steel equipment, research requires research appartus and training. The production of Stevia in DPRK is worth continuing, and should be encouraged, but considerable investment is required. Utilisation of Stevia has rapidly reached a stage of which considerable development is necessary but it is technically feasible and would be of considerable benefit to the country. For example, the entire soft-drinks industry in wholly dependent on this material.

If the Government wishes to initiate a Development Project from this study, the report and findings below would form the basis of such a Project Document.

## B. FINDINGS AND OBSERVATIONS

### 1. Background

With its lack of arable land, and in any case its climate not permitting the successful cultivation of beet or cane, the provision of sugar to the foodstuff industry for a growing market is a severe problem for the Government of DPRK. The Government has therefore encouraged the investigation of alternative sources of sweetening material.

A major initiative was the establishment, in 1979, of a Stevia research programme in the Foodstuff Research Institute of the Light Industry Academy of Science in Pyongyang, currently in association with the Korea Rakwon Jesam Trading Corporation.

The Government of DPRK therefore requested help in development of Stevia processing by a letter of 17th July, 1987 from the Deputy Director-General, Fifth Department, Ministry of Foreign Trade. The subsequently-prepared project description is attached as Annexe 1.

Stevia rebaudiana (Bertoni) Bertoni, the source plant for this study, is a shrubby perennial plant, originally introduced into DPRK from Paraguay via Japan. It produces a range of diterpene glycosides which have the property of being exceptionally sweet in taste. These compounds may be extracted from the leaves and, when purified, used in low concentrations in foodstuffs to replace sugar. For example, it is used at a level of 0.1% stevioside in fruit-flavoured fruit drinks.

One tonne of Stevia leaves contains about 50kg of stevioside, the main principle (which, being 200 times as sweet as sucrose, is equivalent with about 10 tonnes of sugar), and ca 20kg rebaudioside A (which is 300 times as sweet as sucrose and has a more pleasant taste than has stevioside) which is so equivalent with ca 6 tonnes of sugar. Other minor sweet components are also present. Since about 2 tonnes of Stevia leaves may be harvested per hectacre, the total sweetening potential of Stevia is about 40 tonnes sugar equivalents/hectacre, compared with 4-5 tonnes sucrose/hectacre from beet or cane. Structures of some Stevia glycosides are shown in Annexe 2.

Internationally, Stevia sweeteners are used extensively in South America (Paraguay and Brazil) and Japan. Recent estimates put the production of stevioside in Japan at about 1000 tonnes/year. Furthermore, no adverse effects have been reported from the use of Stevia products in human foodstuffs.

It is thus highly desirable and economically sound to cultivate Stevia leaves and extract the sweetening agents for use in the foodstuff industry in DPRK and as a material for export.

### 2. Scientific background

#### 2.1 The products under study in DPRK

Four different Stevia products are currently under investigation in DPRK:

2.1.1 Stevia leaves are extracted with water, partially purified (either by a lime-process or electrochemically and by ion-exchange chromatography) to give a yellowish-green solution containing about 2% dry matter of which ca 60% is sweetener compounds. One L of this dilute solution has a sweetening power equivalent with ca 2 kg sucrose. It is either used as such or, more frequently, concentrated under vacuum (see 3.2) to give a more concentrated extract with a total sweetening power of ca 17kg sucrose equivalent/L. The solution in certain food industry applications, particularly soft drinks. It is subsequently referred to in this report as PARTIALLY PURIFIED STEVIA EXTRACT.

2.1.2 Alternatively, this latter material may be purified by adsorption chromatography and dried to give a yellowish solid containing ca 74% of sweeteners. It is further purified by crystallisation from 95% methanol to give a colourless solid, said to contain ca 90% stevioside. The impurities are mainly rebaudioside A and other minor sweet compounds together with other polar organic molecules. This is the material known in SPRK as, and subsequently referred to in this report as, STEVIOSIDE.

2.1.3 The mother liquors from this crystallisation are recrystallised to give another colourless solid said to contain ca 90% rebaudioside A. The impurities are mainly stevioside and other, unknown, materials. This is the substance known in DPRK as, and subsequently referred to as, MONOSIDE.

2.1.4 The mother liquors from the second crystallisation may then be subjected to an enzymatic process (see below) to yield a yellowish solid containing a complex mixture of mainly mono- and diglucosylated stevioside derivatives (about 50% in total), the remainder being polyglucosylated products and some unreacted sweet glycosides with other non-sweet polar organic substances. This is the material known in DPRK as, and subsequenlty referred to as, ALPHASIDE.

2.2 Comments on the production of ALPHASIDE

Since stevioside has a somewhat bitter taste whereas rebaudioside A has not (its sensory behaviour very closely approximating to that of sucrose) and is in any case 1.5 times as sweet as stevioside, a programme was initiated to attempt the synthesis of rebaudioside A from stevioside, so increasing the total sweetening power available from the leaf extract and at the same time improving the quality of the taste.

Straightforward chemical synthesis is not possible since there is no means of exclusively directing the glucose moiety required to the specific site at the $\alpha$-sugar (position 3) on the existing disaccharide chain in stevioside. An enzymatic transformation was thus proposed.

The first task of the Stevia research programme was thus to locate a source of the enzyme capable of performing this transformation. To do this, many soil samples from locations all over DPRK were obtained. These were tested for their ability to undertake this $\alpha$-glucosylation reaction. Bacteria contained in the promising samples were isolated and from these one was selected for further study and a Bacillus strain was isolated.

The product of the reaction was however found to be not the desired rebaudioside A but a range of new substances, having... extra glucose residues in the $\beta$-sugar of the disaccharide (attached at C-13) and also the single glucose attached to C-19 in stevioside. Organoleptic assessment of this mixture showed it to have a desirably sweet taste (not bitter) but less intense than that of stetioside.

Nonetheless it is the wish of the Government to develop this process to the pilot scale in order to use the residue from the production of STEVIOSIDE and MONOSIDE.

2.3 The degree of industrialisation

PARTIALLY PURIFIED STEVIA EXTRACT is produced on an industrial scale in various food processing factories all over DPRK and is used in local food applications. STEVIOSIDE, MONOSIDE and ALPHASIDE are produced only in the Stevia research project of the Foodstuff Research Institute. Batch-wise production of STEVIOSIDE and MONOSIDE has been successful on non-continuous processes starting from ca 20kg batches of Stevia leaves. ALPHASIDE has been produced in very small, experimental quantities only.

3. Current Status in DPRK

3.1 STEVIA RESEARCH PROJECT OF THE FOODSTUFF RESEARCH INSTITUTE

3.1.1 Buildings and laboratories.

The Foodstuff Research Institute comprises a two storey building with 3 laboratories of an area of ca 50m$^2$ on the ground floor and offices on the first floor. The laboratories are equipped with tiled benches and concrete floors and are serviced with water, drainage and electricity.

More details of the equipment in place is provided below.

The pilot-scale facility is a separate building of total area 480m$^2$ comprising mainly one room about 4.8m in height. This is serviced by water, drainage and electricity. A small boiler provides steam.

A small room to the side serves as a microbiology laboratory and a large cupboard provides incubating space for 2 oscillating shakers, each capable of holding 24 250ml flasks. Heating is by means of an infra-red lamp but no fine control of conditions is possible.

3.1.2 Personnel and activities

The Foodstuff Research Institute employs in total 207 staff (113 scientifically trained). Of these, the Stevia research programme includes about 2( staff including 3 chemists, 3 food science technologists, 2 acteriologists and 3 chemical engineers.

In addition to the Stevia work, other activities of the Food Research Institute include studies on millet, tobacco and fruit and vegetable processing.

3.1.3 Stevia research plan and activities

The study began with a plant breeding programme, carried out by staff of the Central Botanic Garden, Pyongyang, who have achieved the effective selection of a high-yielding strain: total sweetening componenets 11.5% including 6.7% stevioside and 3.9% rebaudioside A, at the same time minimising the level of the bitter material rebaudioside C (or dulcoside B, 0.86%).

This strain is now being cultivated in every country of DPRK and the Government has undertaken a programme of cultivation and the production of 10000 tonnes Stevia leaves. Current production said to be about 2000 tonnes per year from the cultivation of 1000 hectacres around the country.

Laboratory scale research has been undertaken to optimise conditions for the extraction and purification of Stevia extract and purified components, using very small scale equipment (sufficient for the processing of ca 1 kg leaves). This has been successfully (in part) transferred to larger scale: now, batches of 20kg leaves can be dealt with using a batch-wise process described below, but transfer between unit operations is poor. Considerable difficulties with the transglucosylation reaction, principally due to problems in the scaling up of the bacterial growth chambers. Pilot scale equipment has been constructed for the growth of bacteria and the isolation of the derived enzyme (capacity of initial growth chamber 200 L and that of final growth chamber 1000 L) but this has failed to produce acceptable results.

Details of the optimised processes are explained below, together with details of the successful industrial process for the production of PARTIALLY PURIFIED STEVIA EXTRACT.

### 3.1.4 Analysis

Analytical procedures in the laboratories devoted to the Stevia research programme use standard techniques. Assessment of the purity of Stevia extracts and isolated products is difficult because of their highly polar nature and the close similarities between the derivatives. Currently this is attempted bu the use of thin-layer chromatographic methods (silica gel Chromorods SII; $CHCl_3$:MeOH:$H_2O$ (30:20:4) as solvent) which can only give partial resolution of the individual substances. Quantification is achieved by analysis using an IATROSCAN TH-10.

### 3.1.5 Extraction

Dried (13.5% moisture) Stevia leaves arrive by truck from the fields where they have been dried in the sun for about 7 days in thin layers spread on mats laid on the ground. The usual harvest time is October. They are extracted in batches of ca 20 kg into hot water (50 - 60°) for 1.5 - 2 hr in open vessels (ca 1 m in diameter). The dark brown-green liquid is transferred in aliquots using stainless steel buckets to be filtered through cloth and the filtrate is similarly transferred for further purification.

### 3.1.6 Purification

The filtered extract is acidified with concd HCl to pH 3 and transferred to open electrolytic chambers (ca 50 x 50 x 50 cm) to be purified by the passage of 5v (3 - 4 amp/L for 20 - 30 min) between a series of 19 parallel Al electrodes (ca 40 x 40 x 40 cm) placed 2.5 cm apart at 40 - 50°, during which the pH rises to ca 6. The material is then transferred as before to a stainless steel filtration vessel and filtered through cloth. The filtrate (light yellow in colour) is passed, under gravity, through a column ca 1 m high (i.d. 15 cm) of Amberlite IR 120 ($H^+$) and then through a similar column of Amberlite 45 ($OH^-$). The pale yellow solution (of the PARTIALLY PURIFIED STEVIA EXTRACT) is then selectively absorbed onto Diaion HP 20 resin in a similar way and the eluate is rejected. When the resin is saturated (assessed by taste of the eluate), passage is stopped and the crude Stevia glycosides are desorbed by elution with 50% EtOH (3 x the volume of the bed). The alcohol is removed by vacuum distillation (60°) to give a slightly yellowish solid.

### 3.1.7 Crystallisation

This material is dissolved in 95% MeOH (using weight of solvent 6 x that of solid) at 60°, filtered, cooled to ca 10° for about 1 - 2 days, whereupon colourless crystals of STEVIOSIDE form. These are separated from the supernatant liquid by batch-wise centrifugal separation and dried at 80 - 100°. The purity is said to be ca 90%, the main impurities being rebaudioside A and other sweet and non-sweet organic substances.

A second, identical crystallisation of the mother liquors from the above process (without addition of further solvent) is said to produce another crop of crystals, this time of MONOSIDE. Again, the purity is claimed to be 90%, the main impurities being stevioside and other sweet and non-sweet organic molecules.

The mother liquors from the second crystallisation are used as a substrate for the transglucosylation reaction.

3.1.8 Transglucosylation

The process for this reation is a very complex procedure to be undertaken in DPRK.

Below are details of the laboratory scale process.

The pure strain of bacteria is grown up (media and growth conditions in a 250 ml flask having been optimised): maize starch 2%, corn steep liquor 0.6%, $K_2HPO_4$ 0.1%, $MgSO_4.7H_2O$ 0.02%, $Na_2CO_3$ 0.07% at 35 - 40 for 32 hr. An additional 0.03% $Na_2CO_3$ is then added to maintain the pH near to 10 and the culture maintained for 72 hr, after which time no more enzyme is liberated to the medium and the titre of the medium is about 40 u/ml (1 unit being defined as that quantity of enzyme capable of effecting the transglucosylation of 1 micromole stevioside/min). The liquid is then filtered with the aid of kieseguhr to yield the enzyme-containing liquid.

At the same time, starch is digested to provide glucose which is then reacted with the enzyme-containing fluid and by centrifugal separation to remove unreacted starch, a solution containing a transglucosylation enzyme/glucose complex is obtained. It is stored at 4°, the actitity again being monitored by the complex's ability to perform the transglucosylation reaction. Laboratory conditions for the above preparatory processes have been optimised.

The complex is then added to another vessel containing more hydrolysed starch solution and malt extract and to this, the dried mother liquors from the crystallisation of MONOSIDE (prepared by evaporation of the methanol and water under reduced pressure) is added. The reaction is allowed to proceed for 12 - 13 hr. With a 6 fold excess of transglucosylation enzyme complex, the degree of reaction achieved is about 70 - 75%. The solution is boiled for 10 - 20 min to stop the reaction, filtered and then the liquid is selectively absorbed on a column of HP 20 resin. As before, all sweet glycosides are initially absorbed but, by careful adjustment of the flow rate, the transglucosylated products (which have a lower affinity for the resin than do the unreacted (more polar) compounds) are desorbed in favour of the latter.

The early eluate from the column is thus a solution containing principally the transglucosylated products. When unreacted material is detected in the eluate, pre-colation is stopped and the eluate is concentrated under reduced pressure to give a yellowish solid known as ALPHASIDE. Elution of the column with 50% EtOH provides a solution of unreacted starting material which may be recycled.

The ALPHASIDE is hygroscopic and typical analytical results are shown in the Table below:

Composition of transglucosylated product

| transglucosylated products | % |
|---|---|
| polyglucosylstevioosides | 6.0 |
| triglucosylstevioside | 16.0 |
| diglucosylstevioside | 25.0 |
| monoglucosylstevioside | 22.0 |
| unreacted products | |
| rebaudioside A | 11.5 |
| rebaudioside C | 6.0 |
| stevioside | 7.0 |
| rebaudioside B | 2.4 |
| dulcoside A | 1.8 |
| steviolbioside | 2.1 |
| hydrolysis products | 0.2 |

It can be seen that transglucosylated products account for about 70% of the total.

It must be emphasised that the conditions described above have been optimised only on a laboratory scale (initial culture vessel capacity 250ml).

A schematic of the existing process at the Foodstuff Research Institute is attached as Annexe 6.

## 3.2 STATE OF INDUSTRIALISATION

Of the 2000 tonnes Stevia leaves currently produced in DPRK, 50% is processed in small units (ca 10 - 20 tonnes/yr) throughout the country. The bulk (1000 tonnes/yr) is handled at the purpose-built factory at Sam Suk District, about 15km east of Pyongyang, in a region of considerable Stevia cultuvation. It has a staff of 21, working 24 hr/day in 3 shifts of 7.

Extraction here uses a continuous, screw-type extractor with a jacketed extraction pipe of 60 cm diameter which raised dried leaves against a current of descending water at 60°.

Contact time is about 90 min at a rate of 150 kg/hr. The extract so produced (500 - 600 L/hr) is stored in 2000 L vessels before being digested with lime at 40 (pH > 10). After vacuum filtration through cloth, the extract is passed through towers of cation then anion exchange resin at 50 L/hr. The total sweetener content is about 1% which is raised by vacuum concentration at 80 to 10%. This solution is the commercial product - PARTIALLY PURIFIED STEVIA EXTRACT. It is about 17 times as sweet as solid sucrose and the solution is packed in 50 L plastic drums for shipment to soft drink processing factories.

The most significant factors about this factory are (a), since the equipment is of mild steel, the quality of the product is poor - very dark in colour due to dissolved iron and (b) a lime purification is used. Because of the lability of the sweeteners at high pH values, this is kept as low as possible ( 10) and this leads only to partial purification.

Quality control at the factory was achieved by a simple method of hydrolysis, extraction in chloroform and gravimetric determination. In-process controls were non-existent.

The factory was not operating during the visit of the Consultant.

4. Consultant's activities

The Consultant spent his time in DPRK in detailed and intensive discussion with personnel of the Stevia research programme, Foodstuff Research Institute, visited the Institute's laboratories and pilot scale facilities on a number of occasions and made one visit to the Pyongyang Sam Suk Factory (see Job Description, output 1). He studied in depth the laboratory and discussed problems with staff and made a number of practical suggestions (see 5 and 6 below; output 2). He was able to obtain a thorough understanding of the work of the Stevia research programme and its proposed pilot scale project and so establish the required scale of the bench/pilot extraction and purification facility and thus produce the required process flow sheet (output 3). He was also able to make very detailed and specific recommendations with regard to the pilot scale transglucosylation facility itself (output 4).

5. Consultants's comments on section 3

5.1 STEVIA RESEARCH PROJECT OF THE FOODSTUFF RESEARCH INSTITUTE

5.1.1 Buildings and laboratories

The analytical laboratory is an adequate facility, sufficiently well serviced with water, drainage and electricity and had the air of being well-run. Analytical equipment was however inadequate for work on the polar Stevia glycosides, the reliance on thin-layer chromatographic separation being inappropriate. However, the existing equipment did give workable results and was operated as efficiently as possible given the prevailing shortages of consumable items.

The pilot laboratory was a sound building but servies were inadequate. No steam line was available, nor was a piped cooling system. Pumps were few and unsatisfactory. Equipment for continuous pilot scale extraction work (such as jacketed vessels for extraction and evaporation), condensers, pumps, resin towers of appropriate size, etc. was not present. All development was extemporaneous, the transfers by hand between unit operations, using stainless steel buckets, being partially unsatisfactory.

The microbiology laboratory was also entirely unsatisfactory. This is not surprising since at no time in the early stages of the research programme did the need for on seem likley and so its later provision was an afterthought when the need for a microbiological stage was seen. Thus no provision for clean air had been made, incubation facilities were primitive and growth conditions were therefore not controllable. Contamination was very likely to occur under these conditions.

5.1.2 Personnel and activities

The senior staff of the Stevia research programme were hard working and extremely capable. Much effort had obviously been put into the success of the project by the seniot scientific officer within the limitations of his facilities. He was also cogniscent of recent developments in Stevia processing by careful study of the foreign scientific literature. The junior staff were also capable and the laboratory was well-run. Every assistance was given to the Consultant.

5.1.3 Stevia research plan and activities

The activities of the Stevia research programme have been carried out in a logical manner. Laboratory scale studies were carried out meticulously and gave consistent results. The method for the production of PARTIALLY PURIFIED STEVIA EXTRACT has been successfully transferred to the food processing industry (see 5.2) and those for the preparation of STEVIOSIDE and MONOSIDE to the batch-wise pilot (ca 20 kg) in the former process. The staff are hampered by lack of equipment on all levels.

With regard to the enzymatic process, small scale work had been partially successful (but not in the original aim of converting stevioside to rebaudioside A) and on this basis, some pilot scale equipment had been constructed with intermediate and final growth chamber capacities of 200 L and 1000 L respectively (sufficient to process the output of 50 kg batches of Stevia leaves at a total level of 15 tonnes/yr). None of this equipment was of use however because of two reasons: (a) the lack of intermediate scale fermentation equipment in order to optimise growth conditions in deep culture (abortive attempts had been made to transfer directly from the 250 ml scale to 200 L and then to 1000 L but this was without success), and (b) the total lack of control over the growth conditions (pH, temperature, aeration, agitation, etc.).

5.1.4 Analysis

Within the existing limitation of equipment, analytical procedures are adequate. Not enough attention has however been paid to quality of the final products and in-process controls.

5.1.5 Extraction

The process for extraction on the pilot scale is satisfactory but wasteful. No real possibility of exhaustive extraction is possible without a continuous extractor. Again, adequate temperature control is not possible without jacketed vessels. The transferance to purification steps is unsatisfactory and a pumping system is urgently required.

5.1.6 Purification

Stevia glycosides are unstable under strongly alkaline conditions so traditional lime-based methods of initial purification are not entirely satisfactory. The electrichemical method currently adopted at the Institute is efficient and in line with international practice (Japanese patents exist for the process) but is hampered by lack of suitably sized chambers. Filtration is acceptable but should be facilitated by the provision of suitable pumping equipment. Subsequent ion exchange chromatography is also appropriate but again lack of equipment causes difficulties and losses.

5.1.7 Crystallisation

No suitable crystallising vessels were available, nor were means of heating, cooling or filtering of the solutions. A centrifugal separator was in use and this is adequate. Suitable scale equipment for the drying of the products and for recovery of solvent was not present.

The Consultant was not convinced by the analytical results presented that the two stage crystallisation process is as selective as described. Lack of suitable analytical equipment prevented further on-the-spot investigation but in any case totally inadequate quality control to ensure batch-to-batch consistency had been attempted. Neither had attempts been made to improve the quality of the final crystalline products by recrystallisation. The claimed 90% (with no declaration concerning the nature of the impurities) is wholly unacceptable internationally.

5.1.8 Transglucosylation

This is an ambitious project and the Consultant has severe doubts about its economic viability. The table below shows that the extra yield to be obtained by the extremely complex procedure is of the order of only 20% of the total.

Potential (theoretical) yields of Stevia sweeteners using existing process

| 1 tonne of dry Stevia leaves could yield | degree of sweetness compared with sucrose | sucrose equivalent tonnes (%) |
|---|---|---|
| 54 kg STEVIOSIDE | 200 | 10.8 (50) |
| 20 kg MONOSIDE | 300 | 6.0 (29) |
| 37 kg ALPHASIDE | 120 | 4.4 (21) |

The product is a complex mixture (see Table in 3.1.8) and would need to be spray dried (but one is not currently available) and, using the bacterium currently employed, produced a material less sweet than stevioside.

In any case, transfer of conditions to a large scale from the small laboratory scale investigated is impossible as is industrialisation of this process.

If, on the other hand, an organism capable of producing and enzyme for the conversion STEVIOSIDE → MONSIDE were to be found, it would certainly be worthwile investing in intermediate scale growth chambers in order to optimise growth conditions for deep culture, prior to the design of a true and complete pilot scale process. MONOSIDE is the desirable product internationally, having a considerably sweeter and more acceptable taste than STEVIOSIDE.

Assuming that all the stevioside were able to be converted to monoside, the total theoretical yield of the desirable MONOSIDE from 1 tonne Stevia leaves would be ca 74 kg, equivalent with 22.2 tonnes sucrose. Since there is an international market for this latter product and not for the others, this suggested course of action seems the most effective.

5.2 STATE OF INDUSTRIALISATION

The equipment of the factory at Sam Suk was barely adequate.

Since there are considerable problems in the use of the electrochemical purification method (but see below) on an industrial scale, this factory was operating using a more traditional lime-based process. This is satisfactory for base-stable compounds and can produce very effective purifications. With unstable compounds like the Stevia glycosides however, in order not to cause excessive alkali-induced decomposition, the lime concentration was kept low and the temperature of the digestion was also low. This had the effect of producing only a partial purification causing the impurities to be carried through to the end product and also considerable denaturing of the ion-exchange resins (the subsequent process) with dramatic loss in efficiency.

Also, the use of mild steel vessels caused much discolouration of the product and rusting of the vessels themselves.

Lack of in-process controls (particularly with regard to reagent specifications) and end-product quality controls was very evident. The analytical procedure used was inadequate since it was not specific. No control over final impurities was carried out. The product would be wholly unacceptable internationally.

A food processing factory at Wonsan has been equipped with two small electrochemical purification chambers as a trial. Quality of the product is, as expected, considerably better than that produced at Sam Suk bu quantities produced are very small.

C. Consultant's recommendations

1 Activities of the Stevia research programme

1.1 Laboratory scale

The urgent provision of more appropriate analytical equipment. There is an obvious need for high performance liquid chromatography, the only reliable method directly applicable to both the routine and experimental analysis of Stevia glycosides.

This is needed for experimental and development work as well as being applicable to the routine quality control of samples submitted from the factories of purified componenets.

The staff of the Stevia research project could operate this equipment without further training.

1.2 Pilot Scale

1.2.1 The provision of a continuous pilot/bench scale (50 kg dried Stevia leaves/batch) facility to replace the existing small, batch-wise apparatus for the study of all the processes involved in the prepartion PARTIALLY PURIFIED STEVIA EXTRACT, STEVIOSIDE and MONOSIDE. This would require all equipment from the jacketed extraction vessel to drying equipment for the final products and solvent recovery.

Only when this is provided can real development work be carried out to enable the more efficient processing of Stevia leaves to be conducted throughout the country.

1.2.2 More thorough attention must be paid to in-process controls, particularly reagent specifications, including water.

1.2.3 More thorough attention must be paid to the precise conditions for ion-exchange chromatogrphay, esecially flow rates and hence optimal times for pre-colation, though this is dependent on the provision of equipment in 1.2.1.

1.2.4 The quality of material presented to the international market must be excellent and claimed purity must be substantiated by detailed analysis of impurities. Quality control of the final products is lacking at the moment and it is strongly recommended that much more attention be paid to the factor, though it is recognised that this is to a large extent dependant on the provision of analytical equipment detailed in 1.1.1.

1.2.5 Attempts should be made to increase quality by the investigation of the introduction of a re-crystallisation step as a final stage in the purification of STEVIOSIDE and MONOSIDE.

1.3 Transglucosylation studies

1.3.1 The Consultant recommends strongly that the development work to increase the scale of the enzymatic transglucosylation reaction be terminated, pending the successful outcome of more laboratory scale studies in order to discover a convenient source of an enzyme capable of carrying out the transformation STEVIOSIDE → MONOSIDE.

1.3.2 In the Consultant's opinion, pilot scale development for the production of the ALPHASIDE mixture is not warranted on economic grounds.

Hence provision of a pilot scale plant for the production of ALPHASIDE should be suspended, pending results of 6.1.3.1 Once the source of an appropriate enzyme has been found, then the recommendation 1.3.3 should be followed.

1.3.3 That intermediate scale (13 and 30 L, of the type illustrated in Annexe 8) fermentation vessels be provided, which will enable fine control of growth conditions in deep culture to be made. This is essential for the successful transfer at a later stage of the enzymatic reaction STEVIOSIDE → MONOSIDE.

1.3.4 The activities outlined in 1.3.1, 1.3.2 and 1.3.3 will be greatly facilitated by an injection of appropriate expertise from scientist technologists well versed in microbiological fermentation and industrial scale enzymatic processes. These visitors could come from either DPRK itself (from industrial plants using deep culture such as for amylase production) or abroad.

2 Industrial process

If the lime-based process is to be continued in the factory, considerable development work at the Foodstuff Research Institute is required using this as a first step. Research workers were of the opinion that their studies (using a more efficient electrochemical process) would be directly applicable to the large scale; this is not the opinion of the Consultant since the nature and level of impurities differ between the two processes. Thus,

2.1 More development work with a view to optimising the conditions for the lime-process is required.

2.2 More development work to establish true optimal conditions for the ion exchange process is required, paying particular attention to optimising concentration of solutions to be purified, flow rates and time of use before regeneration of the resin.

2.3 In particular, an improved system of in-process controls and final product quality control should be established. In the opinion of the Consultant, the Stevia research project laboratory could act as an appropriate analytical facility if it were provided with apporpriate analytical equipment.

If it is the aim to introduce the electrochemical purification step to the smaller scale factories, then more development work is necessary, particularly with regard to improving the scale of the electrolytic chambers with the eventual aim of arriving at a continuous process.

In the Consultant's opinion, this is at present a very long term aim, and development work on the electrochemical purification should be concerned only with the subsequent production of pure stevioside and monoside (the latter perhaps by enzymatic conversion in the future).

In any case, it should be the aim of the Government to provide stainless steel equipment in the factory, a step which will of itself go a long way to improving the quality of the products.

A process flow-sheet is attached as Annexe 7.

3 Summary of recommendations

Clearly the production of Stevia sweeteners in DPRK is currently on two levels.

First, there is the industrial production of the crude, partially purified extract already in use in the food industry. The preparation of this material is a relatively simple process which could be improved by the provision of better equipment and a system of in-process and quality controls. Some development work at the Research Institute could help overcome some of the current diffculties. With improvements along these lines, the production should continue and will be able to fulfill a need in the domestic market.

Secondly, there is the aim of the Stevia research project to industrialise the production of stevioside and monoside in a pure form. This is a laudable aim and has every possibility of producing material for the international marker as well. However, the resources for this study are inadequate. The Consultant has suggested some specific analytical equipment. He has also made a number of specific recommendations about the organisation of this study.

At the level of the enzymatic transformation, while the Consultant agrees that in theory the enzymatic synthesis of monoside is a valid aim, he cannot recommend the design of a pilot scale plant at the moment for the production of this alphaside mixture, which has no international market, as far as the Consultant is aware, and is likely to prove highly uneconomic in production.

On the other hand, there is every need for the continuation of laboratory scale studies to find a source of the desired enzyme. To this end, considerable improvements are required in the design of the microbiology laboratory. Eventually, at the moment of transfer to larger scale, bench-scale fermentation vessels for deep culture are an absolute requirement, otherwise, as the staff of the Stevia research programme have already found in trying to scale up the existing transglucostylation process, insuperable difficulties will be encountered.

The staff of the Stevia research programme, Foodstuff Research Institute are to be congratulated on their achievements, particularly bearing in mind that it was only about 10 years ago that the potential of Stevia sweeteners became internationally recognised. If they are to succeed, however, in their aim of industrialisation of the purified materials, much more investment is required.

ANNEXE 1

# UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION

## PROJECT PROPOSAL

### PART A - BASIC DATA

| | |
|---|---|
| COUNTRY | : Democratic People's Republic of Korea |
| PROJECT NUMBER | : SI/DRK/87/802 |
| PROJECT TITLE | : Processing of Stevio |
| SCHEDULED START | : October 1987 |
| SCEHDULED COMPLETION | : December 1987 |
| ORIGIN AND DATE OF OFFICIAL REQUEST | : Letter dated 13 July 1987 from Deputy Director-General, Fifth Department, Ministry of Foreign Trade to Resident Representative transmitted vide Resident Representative's letter dated 17 July 1987. |
| GOVERN COUNTERPART AGENCY | : Food Stuff Institute of Light Industry Science, Pyongyang |
| UNIDO CONTRIBUTION | : US$ 17,100 |
| GOVERNMENT CONTRIBUTION | : In kind |
| CURRENCY REQUIRED | |
| FOR UNIDO INPUTS | : US$ 17,100 |
| CONVERTIBLE | : US$ 17,100 |
| OTHER | : - |
| UNIDO SUBSTANTIVE SECTION | : IO/T/CHEM |
| PROGRAMME COMPONENT CODE | : J13400 |

oOo

**PART B - NARRATIVE**

## 1. Objectives

### a) Development Objective

- Development of manufacturing of sweetening material from stevio leaves replacing sugar, thus encouraging the basic food processing chemical sector based on local raw material.

### b) Immediate Objective

- Development of production of stevioside and monoside by testing and verifying the laboratory process established by the Light Industry Scientific Institute, and designing specifications of pilot plants to facilitate large-scale production in the long-run.

## 2. Special Considerations

None

## 3. Background and Justification

DPR Korea produces only about 300,000 tons of sugar per annum. About 90 per cent of this production is consumed by the foodstuff industry.

Arable land being limited, the country could not undertake programme for development of sugarcane and beat. Besides, soil and climatic conditions of the country are not favourable for production of these raw materials for sugar. With the development of foodstuff industry, the demand for sugar is increasing every day. The government is therefore encouraging use of alternative sweetening materials particularly stevio plant juice.

The country is growing stevio plants from which annually about 2000 tons of dry leaves are produced. The dry stevio leaves contains 6 to 7 per cent of stevio fluid, and a total sweetening component of 10 to 11 per cent. In other words, one ton of processed stevio leaves gives 50 kgs of stevio juice and 25 kgs of rebandioside 'A' which are respectively 200 and 300 times sweeter than ordinary beat sugar. This means 17.5 tons of stevio sugar would have the sweetening effect of about 44 tons of ordinary sugar.

The Light Industry Scientific Institute has successfully developed at laboratory scale a process of extraction of stevioside and monoside sweetener by processing of stevio plants. The process includes transfer of alpha-glucosylstevioside from stevio, separation of stevioside and monoside, crystallization of monoside, its successive extraction, the electrical and chemical purification of stevio extraction and quantitative determination of stevio sweetener. (Please see attached technical data on processing of stevio)

.../

Based on the success of the laboratory scale processing, the government has undertaken programme for production of 10,000 tons of dry stevio leaves/year and established small demonstration units to encourage production of stevio sugar in every district. This is consistent with the established priority of the government to develop basic food processing chemical sector industries based on the utilization of local raw materials.

Light Industry Scientific Institute (LISI) has good technical personnel to design the pilot plant once the process design is verified and established. It is also informed that in the chemical laboratory of LISI the stevio sweetener now being processed is quite extensively used and in the villages stevio leaves are boiled and used as a sweetener. LISI confirmed that there is no health hazard for use of stevio and the government therefore undertook a programme of popularising its use by setting up a few pilot plants in the rural/semi-urban locations.

Before launching the programme, however, the government urgently needs assistance to further test and improve the electrical and chemical purification process to ensure quality of the products and design specifications of pilot plants to be installed under this priority programme. The government therefore requested for urgent UNIDO assistance in this regard.

## 4. Outputs

1) Tested and confirmed the process of electrical and chemical purification of stevio extraction with recommendations for improvement of the process.

2) Designed specifications of pilot plant for production of stevioside and monoside sweetener based on the process developed and verified.

## 5. Activities

| Activities for Output 1 | To be completed by |
|---|---|
| 1.1 Tested and verified of the laboratory processing of stevio sweetener particularly the electrical and chemical purification of extraction | Month 1 |
| 1.2 Confirmation of the laboratory process and recommendations for improvements, if any | Month 1 |
| 1.3 Submission of the findings | Month 1 |
| **Activities for Output 2** | |
| 2.1 Designed a Pilot Plant specifications based on established laboratory process | Month 2 |
| 2.2 Submission of specifications and consultations | Month 2 |

## 6. Inputs

### a) Government Inputs

#### National Staff

At present 20 specialists of the Light Industry Scientific Institute are working in the project including 3 senior chemical researchers, 4 food chemists, 3 bacteriologists and 3 mechanical engineers.

This team would continue to work with the international consultants for the proposed UNIDO project.

Other National inputs

In addition, the government, among others, would provide office accommodation, transport, secretarial help and interpretation facilities to the international consultant.

b) UNDP/UNIDO Inputs

International staff

| | | | |
|---|---|---|---|
| BL 11-51 | Analytical chemist with experience in design of pilot plant specifications | 2 m/m | US$ 17,000 |
| BL 51-00 | Sundries | | US$ 100 |
| BL 99-99 | Project Total | 2 m/m | US$ 17,100 |

7. Evaluation Plans

The project will be subject to evaluation, in accordance with the policy and procedures established for this purpose by UNIDO.

8. Proposed Work Plan

The following Work Plan has been tentatively established which may be readjusted by the consultant in consultation with the government on arrival at the project site:

| | |
|---|---|
| - Approval of the project | October 1987 |
| - Fielding of expert | October/November 1987 |
| - Testing and verification of laboratory process | November 1987 |
| - Designing of pilot plant specifications | December 1987 |
| - Final discussion and recommendations | December 1987 |

**PART C - CLEARANCE AND APPROVAL**

| | | |
|---|---|---|
| **PROPOSAL SUBMITTED BY:** | M.A. Mansur, IDO, PPD/AREA/AP | Date: 7.3.87 |
| **IN CO-OPERATION WITH:** | Mrs. Tcheknavorian-Asenbauer, Head, IO/T/CHEM | Date: 7 August 1987 |
| **CLEARED BY:** | Mr. B. Jamilla, Acting Head, AREA/AP | Date: 10/8/87 |
| | Mr. A. Vassiliev, D-D-G/ DIO | Date: 10/8/87 |
| **APPROVED BY:** | Mr. A. Hacini, Director, PPT/PRA | Date: |

UNIDO

# PROJECT BUDGET/REVISION



| 3. COUNTRY | 4. PROJECT NUMBER AND AMENDMENT | 5. SPECIFIC ACTIVITY |
|---|---|---|
| DPR KOREA | ST/DRK/87/ | |

10. PROJECT TITLE

Processing of Stevia

| 16. INTERNATIONAL EXPERTS (Functional titles required except for line 11-99) | 16. TOTAL m/m | 16. TOTAL $ | 17. 1987 m/m | 17. 1987 $ | 18. m/m | 18. $ | 19. m/m | 19. $ | 20. m/m | 20. $ |
|---|---|---|---|---|---|---|---|---|---|---|
| 11-01 | | | | | | | | | | |
| 02 | | | | | | | | | | |
| 03 | | | | | | | | | | |
| 04 | | | | | | | | | | |
| 05 | | | | | | | | | | |
| 06 | | | | | | | | | | |
| 07 | | | | | | | | | | |
| 08 | | | | | | | | | | |
| 09 | | | | | | | | | | |
| 10 | | | | | | | | | | |
| 11 | | | | | | | | | | |
| 12 | | | | | | | | | | |
| 13 | | | | | | | | | | |
| 14 | | | | | | | | | | |
| 15 | | | | | | | | | | |
| 16 | | | | | | | | | | |
| 11-99 Short term consultants | 2.0 | 17,000 | 2.0 | 17,000 | | | | | | |
| 11-99 Sub-total—International experts* | 2.0 | 17,000 | 2.0 | 17,000 | | | | | | |

21. REMARKS

* If more than 16 experts are required check here ☐ and attach continuation sheet 1A. This sub-total must include all experts.

UNIDO

# PROJECT BUDGET/REVISION



| 4. PROJECT NUMBER SI/DRK/87/ | 16. TOTAL m/m | 16. TOTAL $ | 17. 1987 m/m | 17. 1987 $ | 18. m/m | 18. $ | 19. m/m | 19. $ | 20. m/m | 20. $ |
|---|---|---|---|---|---|---|---|---|---|---|
| GPAS EXPERTS (functional titles required) | | | | | | | | | | |
| 12-01 | | | | | | | | | | |
| 12-02 | | | | | | | | | | |
| 12-03 | | | | | | | | | | |
| 12-09 Sub-total—GPAS experts* | | | | | | | | | | |
| ADMINISTRATIVE SUPPORT PERSONNEL | | | | | | | | | | |
| 13-03 Clerks, secretaries, drivers | | | | | | | | | | |
| 13-05 Freelance interpreters (non-UNDP projects) | | | | | | | | | | |
| 13-09 Sub-total—Administrative support personnel | | | | | | | | | | |
| UN VOLUNTEERS (functional titles required) | | | | | | | | | | |
| 14-01 | | | | | | | | | | |
| 14-02 | | | | | | | | | | |
| 14-03 | | | | | | | | | | |
| 14-04 | | | | | | | | | | |
| 14-09 Sub-total—UN Volunteers* | | | | | | | | | | |
| 15-03 Project travel | | | | | | | | | | |
| 15-05 UNDP personnel costs (excluding UNIDO staff mission costs) | | | | | | | | | | |
| NATIONAL EXPERTS (functional titles required) | | | | | | | | | | |
| 17-01 | | | | | | | | | | |
| 17-02 | | | | | | | | | | |
| 17-03 | | | | | | | | | | |
| 17-04 | | | | | | | | | | |
| 17-05 | | | | | | | | | | |
| 17-09 Sub-total—National experts* | | | | | | | | | | |
| 19-09 TOTAL—PERSONNEL COMPONENT | | | | | | | | | | |

* If additional individual budget lines are required, check here ☐ and attach continuation sheet 1A. These sub-totals must include budget lines listed on page 1A

| 4. PROJECT NUMBER SI/DRK/87/ | 16. TOTAL m/m | 16. TOTAL $ | 17. 1987 m/m | 17. 1987 $ | 18 m/m | 18 $ | 19. m/m | 19. $ | 20. m/m | 20. $ |
|---|---|---|---|---|---|---|---|---|---|---|
| SUBCONTRACTS | | | | | | | | | | |
| 21-00 Subcontracts | | | | | | | | | | |
| TRAINING | | | | | | | | | | |
| 31-00 Individual fellowships | | | | | | | | | | |
| 32-00 Study tours; UNDP group training | | | | | | | | | | |
| 33-00 In-service training | | | | | | | | | | |
| 34-00 Non-UNDP group training | | | | | | | | | | |
| 35-00 Non-UNDP training | | | | | | | | | | |
| 30-00 TOTAL – TRAINING COMPONENT | | | | | | | | | | |
| EQUIPMENT | | | | | | | | | | |
| 41-00 Expendable equipment | | | | | | | | | | |
| 42-00 Non-expendable equipment | | | | | | | | | | |
| 43-00 Premises | | | | | | | | | | |
| 40-00 TOTAL – EQUIPMENT COMPONENT | | | | | | | | | | |
| MISCELLANEOUS | | | | | | | | | | |
| 51-00 Sundries | | 100 | | 100 | | | | | | |
| 52-00 Hospitality (non-UNDP projects) | | | | | | | | | | |
| 53-00 Support costs (IDC and DC projects only) | | | | | | | | | | |
| 50-00 TOTAL – MISCELLANEOUS COMPONENT | | 100 | | 100 | | | | | | |
| SURPLUS/DEFICIT | | | | | | | | | | |
| 81-00 Surplus/Deficit (ADM/FS use only) | | | | | | | | | | |
| 99-00 PROJECT TOTAL | 2.0 | 17,100 | 2.0 | 17,100 | | | | | | |
| * COST SHARING (UNDP/IPF projects only) | | | | | | | | | | |
| * NET UNDP CONTRIBUTION | | | | | | | | | | |

*For information only – not for PAD input

MINISTRY OF FOREIGN TRADE
DEMOCRATIC PEOPLE'S
REPUBLIC OF KOREA

P. O. BOX 504, PYONGYANG
CABLE: MICOM, PYONGYANG
TELEX: 8349 KP

Dear Mr.Wiberg

13 July,1987.

Subject; Government Priorities for IDF and SIS Projects
For UNIDO financing

Refering to your letter of 25 June1987 on the above Subjects,government priorities on the IDF and SIS projects for UNIDO financing are as follows;



IDF Projects

1. Improving Pulp and Paper Mill Operations of Hyesan Plant
2. Assis tance in Establishment of an Experimental Pilot Plant for Milk Processing.
3. Salt Production from Sea Water.
4. Gold ore Processing.
5. Techno-economic study on the Estblishment of a Plant for Production of Cryolite
6. Metrology
7. Improvment of Metallurgical Products Quality through Surface Finishing and Treatment ( Study Tour connecting with SI/DRK/85/803 )
8. Sewage Disposal
9. Dry Quenching Technology for Coke Production.
10. Feltspat

SIS Projects

1. Processing of Stevia
2. Cyclodextrine Process
3. Antibiotics
4. Liquified Petrolleum Gas
5. Pre-engineering Study on the Hyesan Pulp and Paper Mill.
6. Assistance in Removing Rust from Vessels

Requested information By UNIDO on the above IDF and SIS projects will be provided to you as soon as possible.

Yours Sencerely

Deputy Director General
5th Department of MFT
Pyongyang.

Mr. Carl-Erik Wiberg
Resident Representative
of UNDP

UNITED NATIONS DEVELOPMENT PROGRAMME

OFFICE OF THE RESIDENT REPRESENTATIVE

PYONGYANG, DPR KOREA



PRO 303 UNIDO

17 July 1987

Dear Mr. Tandon,

Many thanks for your letter of 23 June, with which was enclosed copy of a letter Mr. Alexandrenne had written on 19 June to H.E. Mr. Choe Jong Gun, Minister of Foreign Trade.

In a meeting with Mr. Han Tae Hyok, Deputy Director-General of the Fifth Department, Ministry of Foreign Trade, on 14 July, to which I was accompanied by Mr. Melder, Deputy Resident Representative, and Mr. Farooque, UNIDO/JPO, we discussed, inter alia, DPRK Government priorities for IDF and SIS assistance. In this regard, I am enclosing copy of a letter from Mr. Han, dated 13 July, which contains Government priorities for IDF and SIS assistance. As Mr. Han explained to me, priority and urgency should in this particular context be regarded as more or less synonymous concepts, i.e. the more urgent activities are listed first. Incidentally, on the list of proposed IDF projects numbering as many as 10, the Pilot Fruit Processing project (US/DRK/87/050) is not included. Since this project has apparently already been approved for Italian special purpose funding, Mr. Han did not think it necessary to include it. However, we are keenly awaiting your reply to our latest query as to the status of this IDF project.

Mr. Han has promised to provide me with technical data and background information for each of the newly proposed projects for IDF and SIS assistance, respectively.

On the question of Government cost-sharing with UNDP, which was discussed in the meeting Mr. Alexandrenne had with the Minister of Foreign Trade last month, I was assured by Mr. Han that this is being actively considered by the

... ... ...



Mr. N.N. Tandon
Director
Area Programmes Division
UNIDO
Vienna, Austria

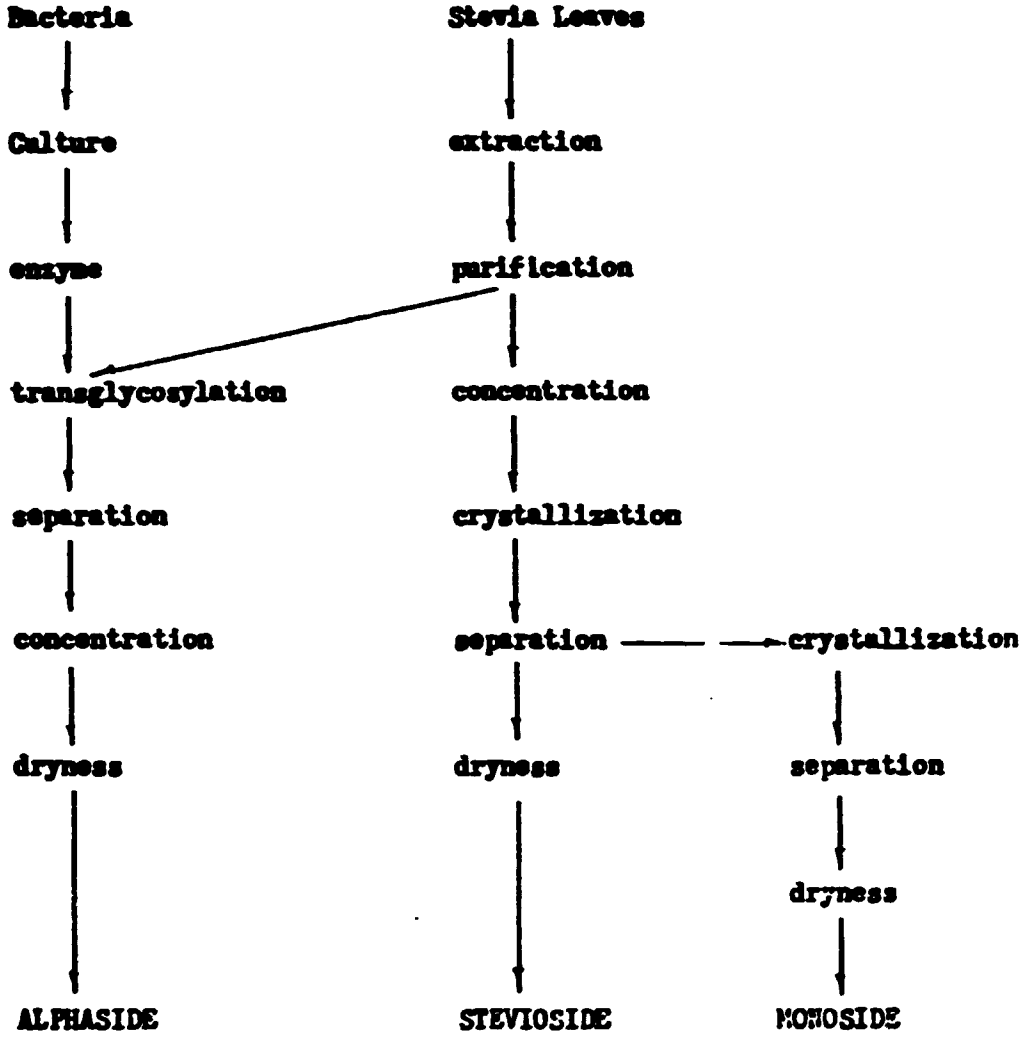
Government and he will presumably revert to me on this subject in due time. Cost-sharing would be required for the more "capital-intensive" projects among those identified by the recent UNIDO mission, i.e. liquefication of coal, and energy conservation and diversification with special reference to chemical industries. There is also the non-metallic beneficiation technology which would necessitate a suitable mix of IPF and Government cost-sharing, should it be found necessary to embark on this type of technology within the compass of the current IPF period. However, you will please appreciate that remaining programmable IPF resources are indeed very limited.

It was a pleasure seeing you here in Pyongyang. Please convey my sincere regards also to Mr. Alexandrenne.

Yours sincerely,

Carl-Erik Wiberg
Resident Representative

- Flowsheet of the Stevia sweetener



- Technical condition

1. Extraction

| | |
|---|---|
| solvent | $H_2O$ |
| temperature | 50 - 60° C |

2. Purification

I) Electrochemical Purification

| | |
|---|---|
| Electrode | Al |
| voltage | 5v |
| temperature | 40 -50°C |

2) Purification by ion-exchange resin

kind of resin

| | |
|---|---|
| cation | Amberlite IR-120 |
| anion | Amberlite IR-45 |

3. Concentration

| | |
|---|---|
| temperature | 60°C or below |

4. Crystallization

| | |
|---|---|
| solvent | $CH_3OH$ |
| temperature | 10°C or below |
| time (Stevioside) | 1 - 2 d |
| (Monoside ) | 3 - 4 d |

5. Dryness

I) Crystall (Stevioside, Monoside)

temperature 80 - 100' C

2) Alphaside

- Spray dryness

temperature

input of tower I50 - I60 'C

output of tower 90 - I00 'C

6. Culture of bacteria

kind of bacteria Bacillus sp. No. 825

temperature 35 - 40 'c

time 2 - 3 d

7. Transglycosylation

Amount of enzyme (per I g stevioside) 40 unit

Amount of starch (per I g stevioside) I-2g

temperature 50 - 60 'C

8. Separation of Alphaside

kind of resin

Dia ion HP - 20

- Chemical nature and property of the Stevia sweetener

| Product | Stevioside | Ronoside | Alphaside |
|---|---|---|---|
| chemical name | stevioside | Rebaudioside A | α-Glucosylstevioside |
| Formula of molecular | $C_{38}H_{60}O_{18}$ | $C_{44}H_{70}O_{23}$ | $C_{44-56}H_{70-90}O_{23-33}$ |
| Weight of molecular | 805 | 967 | 967-1291 |
| Purity, % or more | 90 | 90 | - |
| Nonglycosylated stevioside, % or below | - | - | 10 |
| Moisture, % | 6 - 8 | 6 - 8 | 6 - 8 |
| Taste | sweet | good sweet | good sweet |
| Degree of sweetness to Sugar | 200 | 300 | 120 |

ANNEXE 2

R.O.B. Wijesekera/la
11 November 1987

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION

## JOB DESCRIPTION

SI/DRK/87/802/11-51

**Post title** Consultant in Analytical Chemistry and the Chemical Technology of Natural Products

**Duration** 2 man months (including upto 2 weeks home-base work as needed)

**Date required** ASAP

**Duty station** Pyongyang (Foodstuffs Institute of Light Industry Science)

**Purpose of project**

i) to develop a scale up process for the production of stevioside derivatives based on the laboratory scale processes already established;
ii) establish the appropriate design and specifications for a pilot plant to facilitate large scale production of stevioside and its derivates.

**Duties :** The consultant, among others, is expected to:

1. Evaluate in detail the information and results available at the Light Industry Science Institute (LISI) Pyongyang on processing of Stevio and compare it with international experience and standards, with a view to commercialising the process.
2. Participate in evaluating and improving the laboratory scale extraction of the plant material from Stevio spp. and the separation and purification of stevioside products.
3. Prepare the process design including process flow sheet for a bench-scale unit for stevio extraction.
4. Prepare a drawing of the process design thus established for pilot plant operation for processing of stevioside and related products and recommend the most appropriate design and specifications for a pilot plant.

The consultant will also be expected to prepare a report covering the avobe activites with appropriate recommendations for further action by the Government.

..../..

| | |
|---|---|
| Qualifications | Chemist or Chemical Technologist with extensive experience in processing of natural raw materials by extraction techniques with a specific knowledge of analytical requirements and preparation of specification of pilot plants. |
| Language | English |

Background information DPR Korea produces only about 300,000 tons of sugar per annum. About 90 per cent of this production is consumed by the foodstuff industry.

Arable land being limited, the country could not undertake programme for development of sugarcane and beat. Besides, soil and climatic conditions of the country are not favourable for production of these raw materials for sugar. With the development of foodstuff industry, the demand for sugar is increasing every day. The Government is therefore encouraging use of alternative sweetening materials particularly stevio plant juice.

The country is growing Stevia rebaudiana from which annually about 2000 tons of dry leaves are produced. The dry stevio leaves generate 6 to 7 per cent of stevio extract, and a total of 10 - 11 per cent sweetening principles. In other words, one ton of processed stevio leaves gives 50 kgs of stevio extract and 25 kgs. of rebaudioside 'A' which are respectively 200 and 300 times sweeter than ordinary beat suger. This means 17.5 tons of stevio sugar would have the sweetening effect of about 44 tons of ordinary sugar. Stevioside and extracts have been widely used in Japan since the mid 1970's as sweetening agents, tast modifiers and sugar substitutes, and there have been no reported adverse effects.

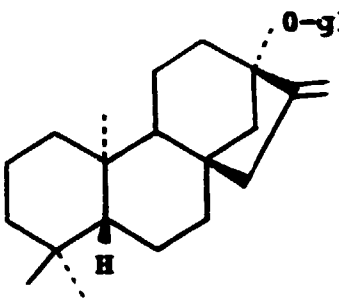
The Light Industry Scientific Institute has successfullydeveloped at laboratory scale a process of extraction of stevioside and monoside sweetener by processing of stevio plants. the process includes transfer of alpha glucosylstevioside from stevio, separation of stevioside and monoside, crystallization of monoside, its successive extraction, the purification of the stevioside and the quantitative determination of the sweetening effect.

Based on the success of the laboratory scale processing, the government has undertaken a programme for production of 10,000 tons of dry stevio leaves/year and established small demonstration units to encourage production of stevio sugar in every district. This is consistent with the established priority of the government to develop basic food processing chemical sector industries based on the utilization of local raw materials.

Before launching the programme, however, the government urgently needs assistance to further test and improve the purification process to ensure quality of the products, and design specifications of pilot plants to be installed under this priority programme. The Government therefore requested for urgent UNIDO assistance in this regard.

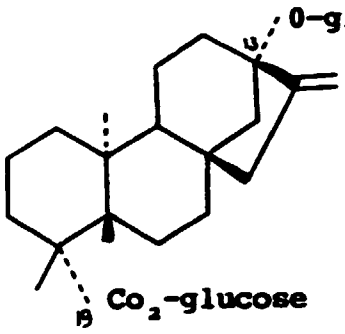
ANNEXE 3

STRUCTURE OF SOME STEVIA GLYCOSIDES




$Co_2$-glucose

STEVIOSIDE




$Co_2$-glucose

REBAUDIOSIDE A

## ANNEXE 4

## LIST OF PERSONS CONTACTED AND THEIR AFFILIATIONS

1. United Nations Development Programme, Pyongyang,

   WIBERG Carl-Erik, Resident Representative
   FAROOQUE Imran, Junior Professional Officer

2. Ministry of Foreign Trade

   HAN Tae Hok, Deputy Director General, Fifth Department

3. Korean Rakwon Jesam Trading Corporation

   LI Chung Ui**, Manager, Foreign Trade Department
   KIM Gui Hyon, Vice-Director
   KIM Quong Ha*, Manager

4. Foodstuff Research Industrial INstitute

   KIM Tong Su, Director
   KIM Te Qu, Chief, Stevia study project

5. Central Botanic Garden

   IM Rok Je, Director
   RO Che Min, Research Scientist

6. Sam Suk Stevia Factory

   KIM Te Ha, Manager

7. KIM Quong Ha*, guide
   LI Chung Ui**, interpreter

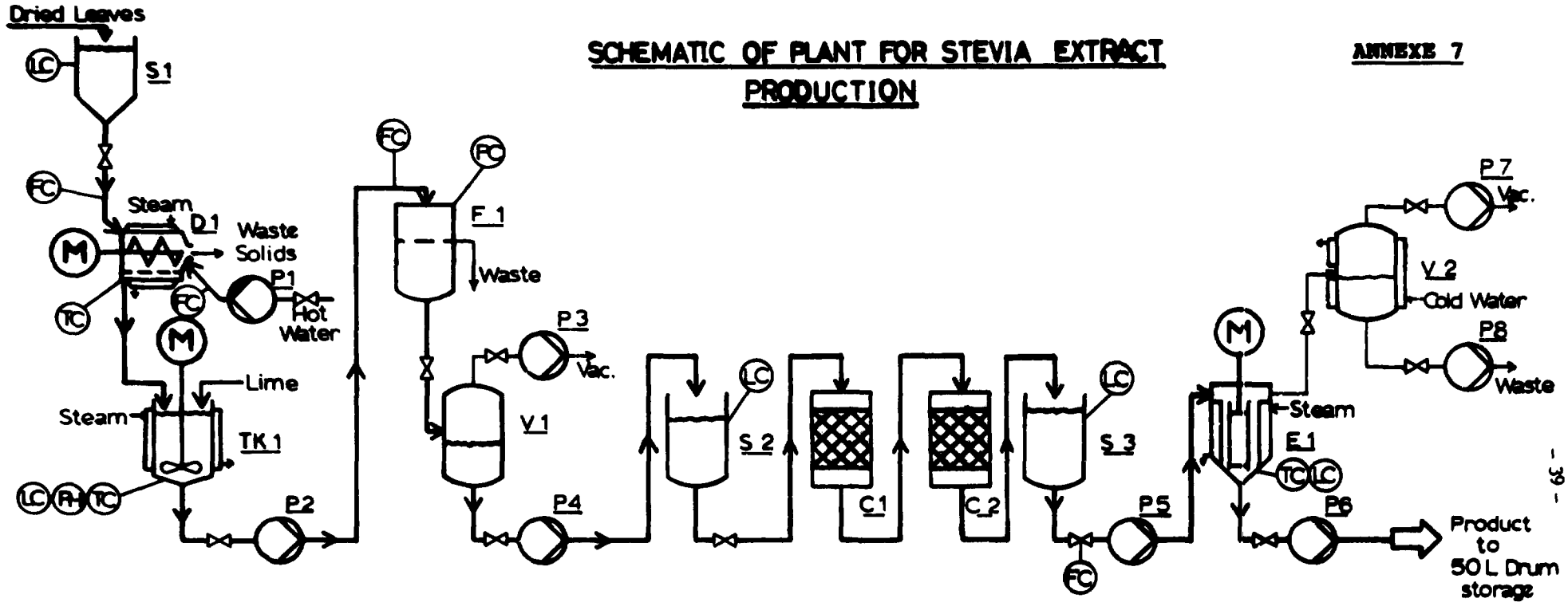
## ANNEXE 5

## REFERENCES

Current structure of stevioside as a sweetening agent for human use, Kinghorn, A.D. and Soejarto, D.D., 1985, in Economic and Medicinal Plant Research, I, Academic Press, New York.

ANNEXE 7

# SCHEMATIC OF PLANT FOR STEVIA EXTRACT PRODUCTION




| UNIT NUMBER | S 1- 3 | D 1 | TK 1 | F 1 | V1-2 | C 1- 2 | E 1 | P1- 8 |
|---|---|---|---|---|---|---|---|---|
| NAME | Storage | Screw-type Extractor | Tank | Filter | Vac. Receivers | Columns | Evaporator | Pumps |
| TECHNICAL DATA | S1-Feedrate to process 150 kg/h<br>S2-3-sized for continuous operation | Q = 5-600 L/h<br>T = 60 °C<br>I.D. = 60cm<br>V = 1000 L | V = 2000 L<br>T = 40 °C<br>pH = 10 | Cloth type<br>Q = 5-600 L/h<br>Batch | P < 1 atm. | Q = 50 L/h | Feed = 1% pure<br>Prod. = 10% ....<br>T = 80 °C<br>P < 1 atm. | Various |
| CONTENTS | S1 - Leaves<br>S2-3 - Extract | Leaf-Water mix | Lime digestion of Extract | Solid waste & Extract filtrate | V1-Extract. filt.<br>V2-Evap. condate | C-1 Cation IEX<br>C-2 Anion IEX | Extract Concentrate | Various |
| NOTES | 1) Not to scale. 2) No recycles or stream heat exchange shown. 3) Duplicate tankage (eg TK 1) not shown. 4) D 1 is operated at 45° to horizontal. 5) Column regeneration not shown. 6) Column pumping may be needed. | | | | | | | |

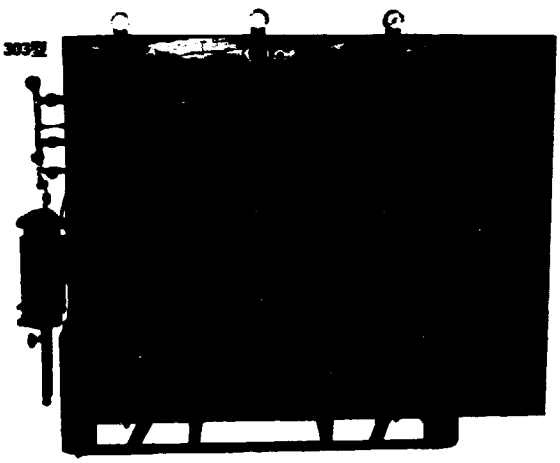
ANNEXE 8

TYPICAL JAR FERMENTER

MJB

# JAR-FERMENTER

MSJ 303型



ＭＢ型（卓上用）2，5，10l

ＭＳＪ型（一般用）10 ～ 30l

ＭＰＦ型（工場用）50～1000l

自動消泡装置各種

其の他特殊醗酵槽

カタログ進呈

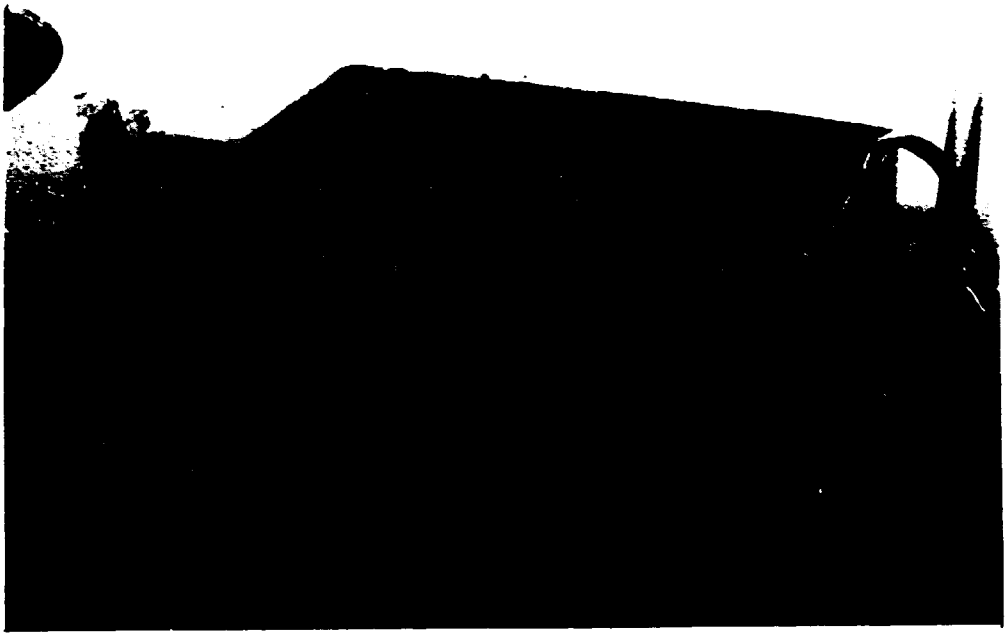
株式会社 丸菱理化装置研究所

本社　東京都千代田区神田錦町43　Tel (252) 7236－7・7297

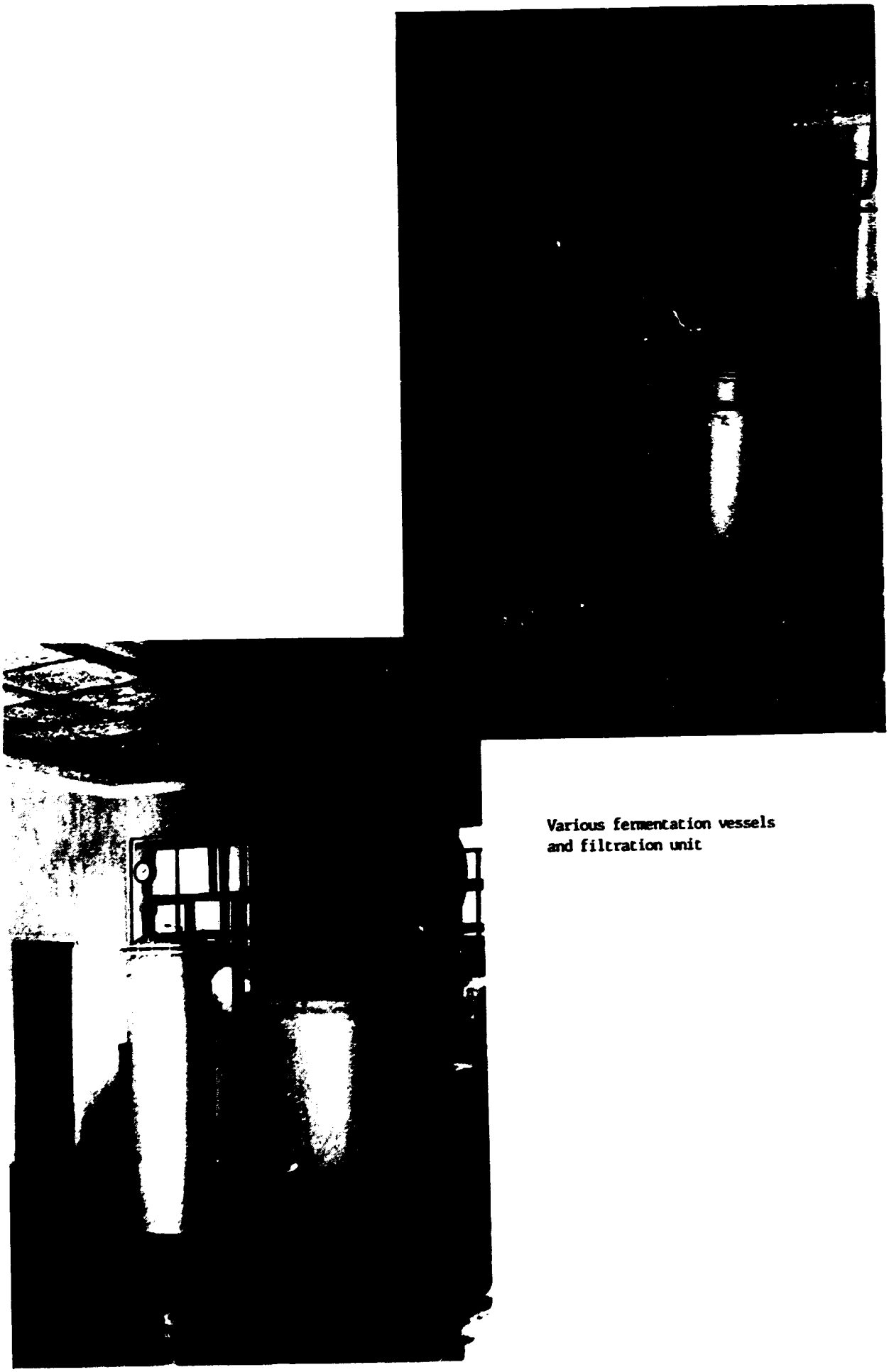
工場　東京都葛飾区奥戸新町４３２　Tel (697) 6 7 4 6

ANNEX 9

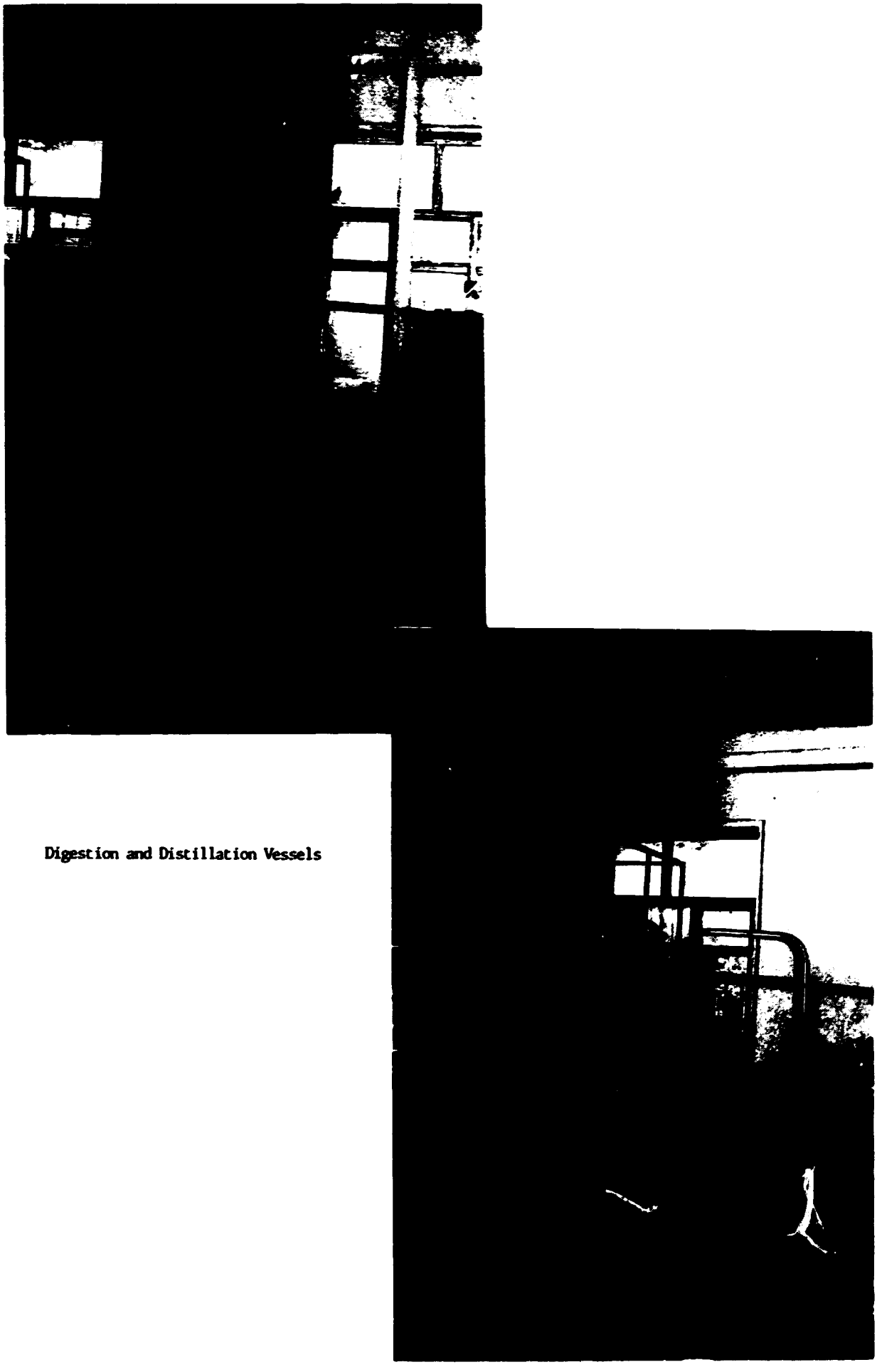
PHOTOGRAPHS TAKEN AT SILVIA RESEARCH INSTITUTE



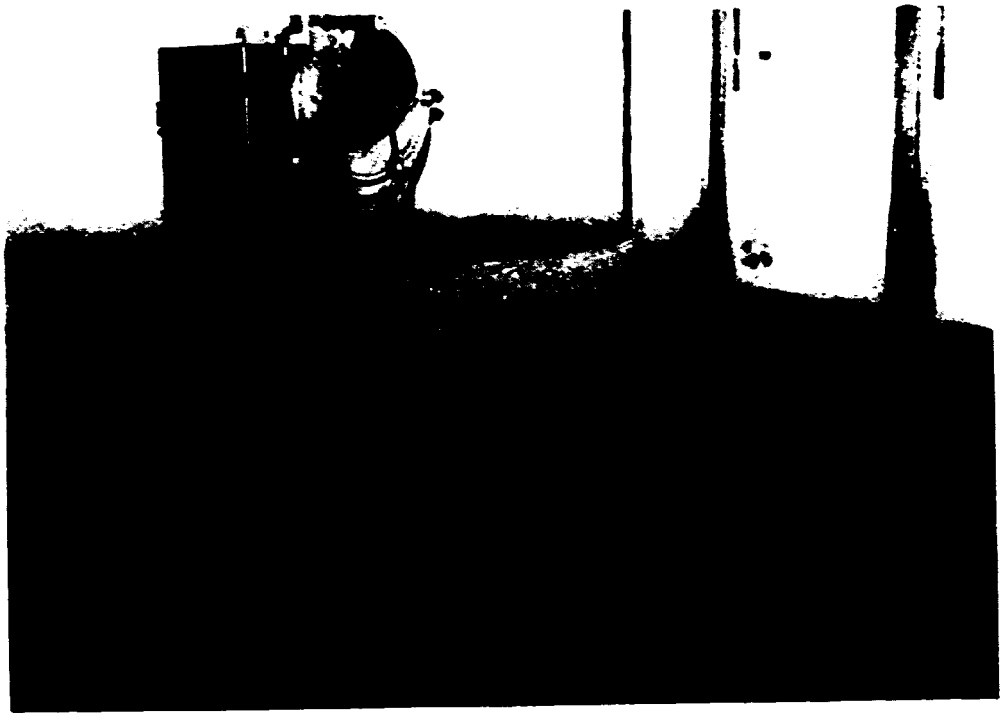
Development Electrolytic Chamber



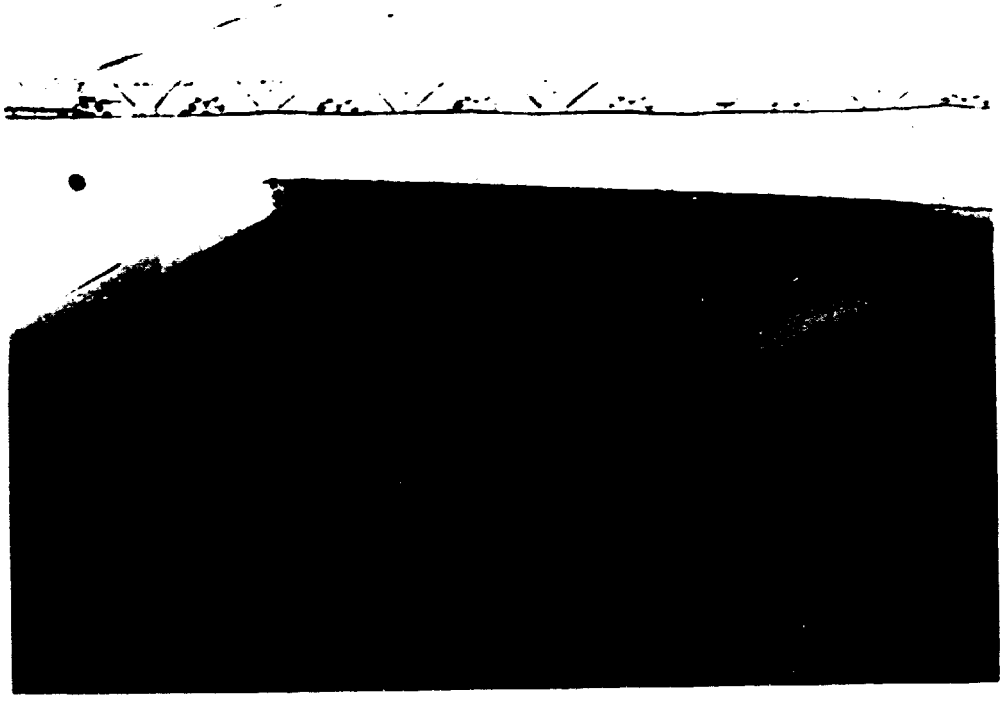
Various fermentation vessels
and filtration unit



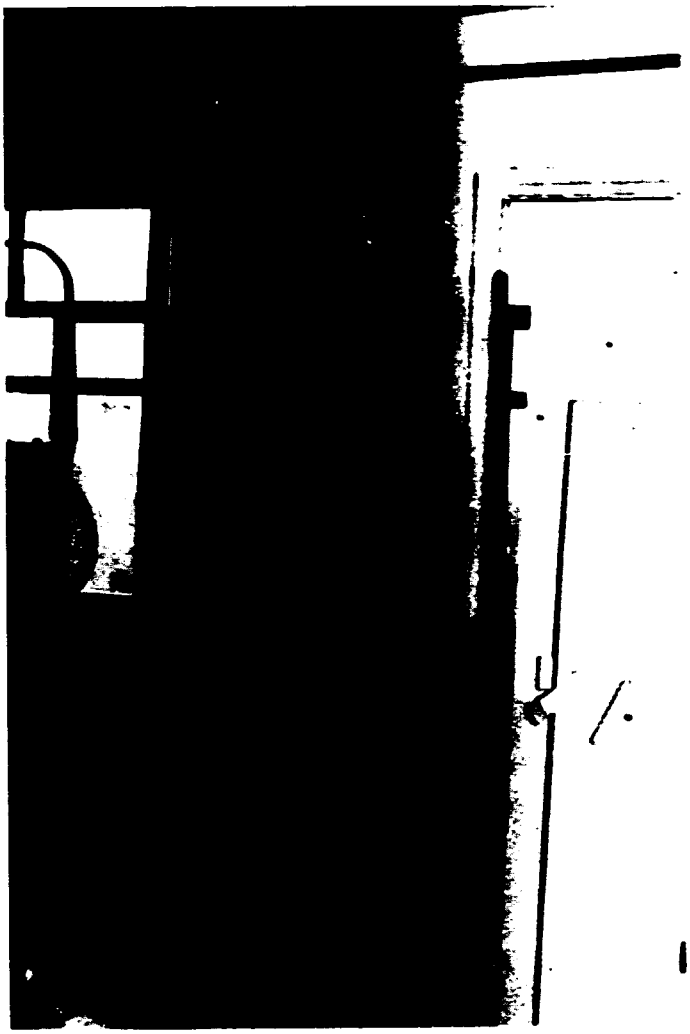
Digestion and Distillation Vessels



Microbiology Laboratory



Incubator



Centrifugal separator

## ANNEXE 10

## PHOTOGRAPHS TAKEN AT SAM SUK STEVIA FACTORY

Sacks of dried Stevia

Hopper

Screw-type extractor

Digestion and filtration towers

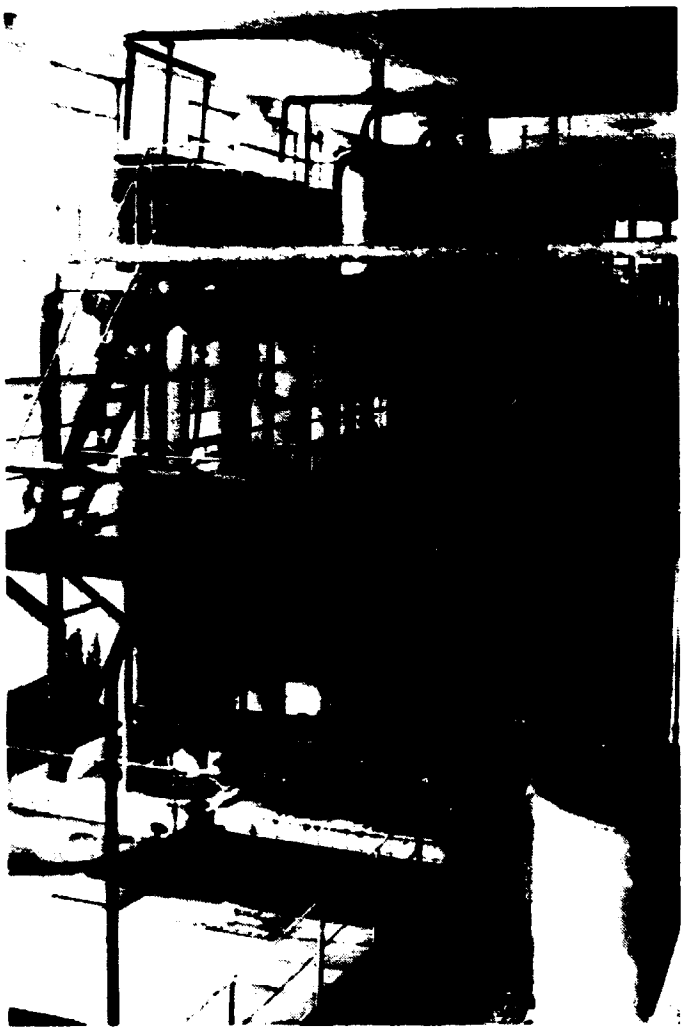
Ion-exchange towers and storage vessels

Evaporation Unit

General view showing
hopper (back LHS) , inclined extractor
(visuing to RHS), storage veseels
(front) and digestion vessel(Front RHS)

General view

Showing filtration towers and
storage vessels



General View of ion-exchange towers
and storage units

Drums of finished Stevia extract

RESTRICTED

DP/ID/SER.B/630/Add.1
6 November 1988
ORIGINAL: ENGLISH

17076
(2 of 2)

# PROCESSING OF STEVIA

## SI/DRK/87/802

## DEMOCRATIC PEOPLE'S REPUBLIC OF KOREA

Terminal Report (Supplement)*

Prepared for the Government of the
Democratic People's Republic of Korea
by the United Nations Industrial Development Organization,
acting as executing agency for the United Nations Development Programme

Based on the work of Peter J. Hylands, UNIDO Consultant

Backstopping officer: R.O.B. Wijesekera
Chemical Industries Branch

United Nations Industrial Development Organization
Vienna

1/74

* This document has not been edited.

V.88 30647

SUPPLEMENT TO PROCESSING OF STEVIA
SI/DRK/87/802

109005432 CA: 109(1)5432w JOURNAL
Improved isolation and purification of stevioside
AUTHOR(S): Adduci, John; Buddhasukh, Duang; Ternai, Bela
LOCATION: Dep. Chem., La Trobe Univ., Bundoora, 3083, Australia
JOURNAL: J. Sci. Soc. Thailand DATE: 1987 VOLUME: 13
NUMBER: 3 PAGES: 179-83 CODEN: VKSTDB ISSN: 0303-8122
LANGUAGE: English

108221992 CA: 108(25)221992a JOURNAL
An easy method for the preparation of sophorose from stevioside
AUTHOR(S): Kusakabe, Isao; Kusama, Satoru; Murakami, Kazuo
LOCATION: Inst. Appl. Biochem., Univ. Tsukuba, Sakura, Japan, 305
JOURNAL: Agric. Biol. Chem. DATE: 1987 VOLUME: 51
NUMBER: 8 PAGES: 2255-6 CODEN: ABCHA6 ISSN: 0002-1369
LANGUAGE: English

108203438 CA: 108(23)203438v JOURNAL
Potential sweetening agents of plant origin. Part XII. Mass spectral analysis of some derivatives and in vitro metabolites of steviol, the aglycone of the natural sweeteners, stevioside, rebaudioside A, and rubusoside
AUTHOR(S): Compadre, C. M.; Hussain, R. A.; Nanayakkara, N. P. D.; Pezzuto, J. M.; Kinghorn, A. D.
LOCATION: Coll. Pharm., Univ. Illinois, Chicago, IL, 60612, USA
JOURNAL: Biomed. Environ. Mass Spectrom. DATE: 1988
VOLUME: 15 NUMBER: 4 PAGES: 211-22 CODEN: BEMSEN ISSN: 0887-6134 LANGUAGE: English

108186353 CA: 108(19)186353r PATENT
Purification of Stevioside from Stevia rebaudiana leaves
INVENTOR(AUTHOR): Arashi, Hideyo
LOCATION: Brazil
ASSIGNEE: Arashi Agro Desenvolvimento Ltda.
PATENT: Brazil Pedido ; BR 8700543 A DATE: 870616
APPLICATION: BR 87543 (870206)
PAGES: 6 pp. CODEN: BPXXDX LANGUAGE: Portuguese CLASS: C13D-001/14A

108106288 CA: 108(13)106288p JOURNAL
Absence of mutagenesis induced by the stevioside from Stevia rebaudiana (Bert.) Bertoni
AUTHOR(S): Flores, Renato Zamora; Cechin, Sonia Terezinha Zanini; Rodrigues da Silva, Andrea Cristina
LOCATION: Cent. Cienc. Nat. Exatas, UFSM, 97111, Sant Maria, Brazil
JOURNAL: Cienc. Cult. (Sao Paulo) DATE: 1987 VOLUME: 39
NUMBER: 4 PAGES: 417-18 CODEN: CCUPAD ISSN: 0009-6725
LANGUAGE: Portuguese

108093198 CA: 108(11)93198e JOURNAL
Quantitative determination of stevioside by HPLC in serum, urine, and excrement
AUTHOR(S): Yuan, Yisheng; Qian, Jianqiu
LOCATION: Nanjing Mil. Gen. Hosp., Nanjing, Peop. Rep. China,
JOURNAL: Zhongcaoyao DATE: 1987 VOLUME: 18 NUMBER: 11
PAGES: 491-2 CODEN: CTYAD8 ISSN: 0253-2670 LANGUAGE: Chinese

107235121 CA: 107(25)235121g JOURNAL
Use of steviosides in pickles prepared with soy sauce
AUTHOR(S): Gu, Linang
LOCATION: Nantong Soy Prod. Fact., Nantong, Peop. Rep. China,
JOURNAL: Zhongguo Tiaoweipin DATE: 1987 NUMBER: 6 PAGES: 12-14 CODEN: ZHTIE7 LANGUAGE: Chinese

107218002 CA: 107(23)218002b PATENT
Method for obtaining sweetening sugars from leaves of Stevia
INVENTOR(AUTHOR): Takahashi, Tsutomu
LOCATION: Brazil
ASSIGNEE: Arashi Agro-Desenvolvimento Ltd.
PATENT: Brazil Pedido ; BR 8605325 A DATE: 870317
APPLICATION: BR 865325 (861024)
PAGES: 12 pp. CODEN: BPXXDX LANGUAGE: Portuguese CLASS: C07H-015/24A; A23L-001/236B

107216227 CA: 107(23)216227e JOURNAL
Stevioside
AUTHOR(S): Bakal, Abraham I.; Nabors, Lyn O'Brien
LOCATION: ABIC Int. Consult., Inc., Pine Brook, NJ, USA
JOURNAL: Food Sci. Technol. DATE: 1986 VOLUME: 17
NUMBER: Altern. Sweeteners PAGES: 295-307 CODEN: FSTEEM
LANGUAGE: English

107198610 CA: 107(21)198610u JOURNAL
Quantitative analysis of glycosidic sweeteners from Stevia rebaudiana and their hydrolysis products by high performance liquid chromatography (HPLC)
AUTHOR(S): Alvarez, Mauro; Kusumoto, Ines Tomoco
LOCATION: Dep. Farm.-Bioquim., Univ. Estadual Maringa, 87020, Maringa, Brazil
JOURNAL: Arq. Biol. Tecnol. DATE: 1987 VOLUME: 30
NUMBER: 2 PAGES: 337-48 CODEN: ABTTAP ISSN: 0365-0979
LANGUAGE: Portuguese

107196584 CA: 107(21)196584g JOURNAL
**Chemical characteristics and biological safty of a glycosidic sweetener, stevioside**
AUTHOR(S): Yoshihira, Kunitoshi; Matsui, Michiko; Ishidate, Motoi
LOCATION: Kokuritsu Eisei Shikenjo, Japan,
JOURNAL: Tokishikoroji Foramu DATE: 1987 VOLUME: 10 NUMBER: 3 PAGES: 281-9 CODEN: TOFOD5 ISSN: 0287-8712 LANGUAGE: Japanese

107174429 CA: 107(19,174429q PATENT
**Enzymic manufacture of rebaudioside A**
INVENTOR(AUTHOR): Nishibashi, Hideji; Katabami, Tadashi; Matsubayashi, Tadao
LOCATION: Japan,
ASSIGNEE: Dainippon Ink and Chemicals, Inc.
PATENT: Japan Kokai Tokkyo Koho ; JP 87146599 A2 ; JP 62146599 DATE: 870630
APPLICATION: JP 85287197 (851220)
PAGES: 10 pp. CODEN: JKXXAF LANGUAGE: Japanese CLASS: C12P-019/44A; C07H-015/24B; C12P-019/44J; C12R-001/465J

107149170 CA: 107(17)149170r JOURNAL
**Potential sweetening agents of plant origin. Part X. Characterization and feeding deterrent effects on the aphid, Schizaphis graminum, of some derivatives of the sweet compounds, stevioside and rebaudioside A**
AUTHOR(S): Nanayakkara, N. P. D.; Klocke, J. A.; Compadre, C. M.; Hussain, R. A.; Pezzuto, J. M.; Kinghorn, A. D.
LOCATION: Coll. Pharm., Univ. Illinois, Chicago, IL, 60612, USA
JOURNAL: J. Nat. Prod. DATE: 1987 VOLUME: 50 NUMBER: 3 PAGES: 434-41 CODEN: JNPRDF ISSN: 0163-3864 LANGUAGE: English

107133019 CA: 107(15)133019n PATENT
**Rebaudioside A-high Stevia rebaudiana strain**
INVENTOR(AUTHOR): Morita, Toyoshige
LOCATION: Japan,
ASSIGNEE: Nakasato, Takanori
PATENT: Japan Kokai Tokkyo Koho ; JP 8796025 A2 ; JP 6296025 DATE: 870502
APPLICATION: JP 85229384 (851014)
PAGES: 6 pp. CODEN: JKXXAF LANGUAGE: Japanese CLASS: A01H-005/00A; A23L-001/22B; A01H-001/00

107078872 CA: 107(9)78872j JOURNAL
**Effects of boron on the growth, yield, and contents of stevioside and rebaudioside A of kaa he-e (Stevia rebaudiana)**
AUTHOR(S): Sheu, Bor Wen; Tamai, Fujio; Motoda, Yoshiharu
LOCATION: Natl. Pingtung Inst. Agric., Pingtung, Taiwan,
JOURNAL: Nogaku Shuho (Tokyo Nogyo Daigaku) DATE: 1987 VOLUME: 31 NUMBER: 4 PAGES: 265-72 CODEN: TNDNAG ISSN: 0375-9202 LANGUAGE: Japanese

107078301 CA: 107(9)78301x JOURNAL
**Inhibition of monosaccharide transport in the intact rat liver by stevioside**
AUTHOR(S): Ishii, Emy L.; Schwab, Andreas J.; Bracht, Adelar
LOCATION: Dep. Farm. Bioquim., Univ. Maringa, 87100, Maringa, Brazil
JOURNAL: Biochem. Pharmacol. DATE: 1987 VOLUME: 36 NUMBER: 9 PAGES: 1417-33 CODEN: BCPCA6 ISSN: 0006-2952 LANGUAGE: English

107078200 CA: 107(9)78200p JOURNAL
**Extraction and utilization of steviosides**
AUTHOR(S): Wu, Yinpi
LOCATION: Guangzhou No. 10 Pharm. Fact., Guangzhou, Peop. Rep. China,
JOURNAL: Yiyao Gongye DATE: 1987 VOLUME: 18 NUMBER: 5 PAGES: 228-31 CODEN: YIGODN LANGUAGE: Chinese

106100883 CA: 106(13)100883r JOURNAL
**Refining of Stevia sweeteners and their application in health food**
AUTHOR(S): Liu, Qixun
LOCATION: Hunan Inst. Anal. Test, Hunan, Peop. Rep. China,
JOURNAL: Zhongguo Tiaoweipin DATE: 1986 NUMBER: 8 PAGES: 10-13 CODEN: ZHTIE7 LANGUAGE: Chinese

106065992 CA: 106(9)65992b JOURNAL
**Chemistry, metabolism and biological activity of steviol (ent-13-hydroxykaur-16-en-19-oic acid), the aglycone of stevioside**
AUTHOR(S): Pezzuto, John M.
LOCATION: Coll. Pharm., Univ. Illinois, Chicago, IL, 60612, USA
JOURNAL: Stud. Org. Chem. (Amsterdam) DATE: 1986 VOLUME: 26 NUMBER: New Trends Nat. Prod. Chem. 1986 PAGES: 371-86 CODEN: SOCHDQ ISSN: 0165-3253 LANGUAGE: English

106048798 CA: 106(7)48798a JOURNAL
**Utilization of subsidiary materials in food processing. II. Properties of commercial natural sweeteners**
AUTHOR(S): Miyashiro, Ryuji
LOCATION: Kagawa Prefect. Ferment. Food Exp. Stn., Japan, 761-44
JOURNAL: Kagawa-ken Hakko Shokuhin Shikenjo Hokoku DATE: 1986 NUMBER: 78 PAGES: 72-4 CODEN: KHSSAX ISSN: 0368-5640 LANGUAGE: Japanese

106048794 CA: 106(7)48794w JOURNAL
Effects of two concentrations of stevioside on renal function and mean arterial pressure in rats
AUTHOR(S): Melis, Marcia Salomao; Sainati, Ana Rita; Maciel, Rui Errerias
LOCATION: Inst. Biol., State Univ. Campinas, Sao Paulo, Braz il
JOURNAL: IRCS Med. Sci. DATE: 1986 VOLUME: 14 NUMBER: 10
PAGES: 973 CODEN: IMSCE2 ISSN: 0268-8220 LANGUAGE: Englis h

106048274 CA: 106(7)48274c JOURNAL
Studies on the .beta.-1,3-glucanase system of Streptomyces. Part V. Transglucosylation into stevioside by the enzyme system from Streptomyces sp
AUTHOR(S): Kusama, Satoru; Kusakabe, Isao; Nakamura, Yasuyuki; Eda, Shigeru; Murakami, Kazuo
LOCATION: Inst. Appl. Biochem., Univ. Tsukuba, Sakura, Japan , 305
JOURNAL: Agric. Biol. Chem. DATE: 1986 VOLUME: 50
NUMBER: 10 PAGES: 2445-51 CODEN: ABCHA6 ISSN: 0002-1369
LANGUAGE: English

106015861 CA: 106(3)15861n JOURNAL
Production of sweet substances in Stevia rebaudiana. I. Simple determination of sweet glucosides in Stevia plant with a thin layer chromato-scanner and their accumulation patterns with plant growth
AUTHOR(S): Yoshida, Shigekata
LOCATION: Fac. Agric., Nagoya Univ., Aichi, Japan, 470-01
JOURNAL: Nippon Sakumotsu Gakkai Kiji DATE: 1986 VOLUME: 5 5 NUMBER: 2 PAGES: 189-95 CODEN: NISAAJ ISSN: 0011-1848
LANGUAGE: Japanese

106015289 CA: 106(3)15289g JOURNAL
Analytical standards and quantitative analysis of steviosides
AUTHOR(S): Pagliosa, Frida Maciel; Mezzomo da Silva, Maria Cladis; Vinade, Maria Elisabeth do Canto; Frizzo, Sonia Maria Bittencourt
LOCATION: Cent. Cienc. Nat. Exatas, UFSM, Santa Maria, Brazi l
JOURNAL: Cienc. Nat. (St. Maria, Braz.) DATE: 1985
VOLUME: 7, PAGES: 49-53 CODEN: CNATD5 ISSN: 0100-8307
LANGUAGE: Portuguese

106004036 CA: 106(1)4036w PATENT
Sweetener from new Stevia species
INVENTOR(AUTHOR): Morita, Toyoshige
LOCATION: Japan,
ASSIGNEE: Morita Kagaku Kogyo Co., Ltd.
PATENT: Japan Kokai Tokkyo Koho ; JP 86202667 A2 ; JP 61202667 DATE: 860908
APPLICATION: JP 8543217 (850304)
PAGES: 6 pp. CODEN: JKXXAF LANGUAGE: Japanese CLASS: A23L -001/22A

105224678 CA: 105(25)224678b JOURNAL
Sweet glycosides from the Stevia plant
AUTHOR(S): Crammer, Bernard; Ikan, Raphael
LOCATION: Minist. Justice, 94342, Jerusalem, Israel
JOURNAL: Chem. Br. DATE: 1986 VOLUME: 22 NUMBER: 10
PAGES: 915-16, 918 CODEN: CHMBAY ISSN: 0009-3106
LANGUAGE: English

105207668 CA: 105(23)207668f JOURNAL
Thin layer chromatographic detection and liquid chromatographic determination of stevioside and rebaudioside A in beverages and foods following reverse phase column chromatography
AUTHOR(S): Fujinuma, Kenji; Saito, Kazuo; Nakazato, Mitsuo; Kikuchi, Yoko; Ibe, Akihiro; Nishima, Taichiro
LOCATION: Div. Food Hyg., Tokyo Metrop. Res. Lab. Public Health, Tokyo, Japan, 160
JOURNAL: J. - Assoc. Off. Anal. Chem. DATE: 1986 VOLUME: 6 9 NUMBER: 5 PAGES: 799-802 CODEN: JANCA2 ISSN: 0004-5756
LANGUAGE: English

105207621 CA: 105(23)207621k JOURNAL
Studies on toxicity of stevioside
AUTHOR(S): Chen, Yongde; Lu, Guangzhong; Li, Xinlan
LOCATION: Hubei Epidemic Prevent. Stn., Hubei, Peop. Rep. China,
JOURNAL: Shipin Kexue (Beijing) DATE: 1986 VOLUME: 80,
PAGES: 42-5 CODEN: SPKHD5 ISSN: 0253-8997 LANGUAGE: Chine se

105170813 CA: 105(19)170813w JOURNAL
Stevioside, the sweet glycoside of Stevia rebaudiana, inhibits the action of atractyloside in the isolated perfused rat liver
AUTHOR(S): Ishii, Emy L.; Bracht, Adelar
LOCATION: Lab. Liver Metab., Univ. Maringa, 87100, Maringa, Brazil
JOURNAL: Res. Commun. Chem. Pathol. Pharmacol. DATE: 1986
VOLUME: 53 NUMBER: 1 PAGES: 79-91 CODEN: RCCCB8 ISSN: 00 34-5164 LANGUAGE: English

105150024 CA: 105(17)150024m PATENT
Processing of stevia rebaudiana leaves
INVENTOR(AUTHOR): Alvarez, Mauro; Couto, Amaury Cezar Cruz
LOCATION: Brazil
ASSIGNEE: Fundacao Universidade Estadual de Maringa; Banco
(cont. next page)

do Brasil S. A.-FIPEC
PATENT: Brazil Pedido ; BR 8402752 A DATE: 860114
APPLICATION: BR 842752 (840607)
PAGES: 15 pp. CODEN: BPXXDX LANGUAGE: Portuguese CLASS: C 07H-001/08A; C07F-003/02B; A23L-001/236B

105149752 CA: 105(17)149752r DISSERTATION
Studies on the sweet principle of Lippia dulcis and on steviol, the aglycon of stevioside
AUTHOR(S): Compadre, Cesar Manuel
LOCATION: Health Sci. Cent., Univ. Illinois, Chicago, IL, USA
DATE: 1985 PAGES: 230 pp. CODEN: DABBBA LANGUAGE: English
CITATION: Diss. Abstr. Int. B 1986, 47(1), 204 AVAIL: Univ. Microfilms Int., Order No. DA8605094

105132359 CA: 105(15)132359v JOURNAL
Extraction of total steviosides by macro-reticular resins
AUTHOR(S): Bian, Shouzheng; Sun, Hongfeng; Liu, Lili; Zhou, Husheng
LOCATION: Lianyungang Tradit. Chin. Med. Fact., Lianyungang, Peop. Rep. China,
JOURNAL: Zhongcaoyao DATE: 1986 VOLUME: 17 NUMBER: 6
PAGES: 12-13 CODEN: CTYAD8 ISSN: 0253-2670 LANGUAGE: Chinese

105132174 CA: 105(15)132174f PATENT
Manufacture of rebaudioside A from stevioside by microbial .beta.-1,3-glucosyltransferase
INVENTOR(AUTHOR): Nishibashi, Hideji; Katabami, Tadashi; Oyama, Mikio; Matsubayashi, Tadao
LOCATION: Japan,
ASSIGNEE: Dainippon Ink and Chemicals, Inc.
PATENT: Japan Kokai Tokkyo Koho ; JP 8696995 A2 ; JP 6196995 DATE: 860515
APPLICATION: JP 84217913 (841017)
PAGES: 6 pp. CODEN: JKXXAF LANGUAGE: Japanese CLASS: C12P-019/56A; C12P-019/56J; C12R-001/465J

105116910 CA: 105(14)116910n PATENT
Recovery of stevioside
INVENTOR(AUTHOR): Kumar, Sampath
LOCATION: USA
ASSIGNEE: Levy, Harold; Sorkin, Harry W.; Rogers, Edna Y.; Bruno, Charles F.
PATENT: United States ; US 4599403 A DATE: 860708
APPLICATION: US 785200 (851007)
PAGES: 5 pp. CODEN: USXXAM LANGUAGE: English CLASS: 536018100; C07H-001/08A

105098251 CA: 105(11)98251t PATENT
Sweetener compositions.
INVENTOR(AUTHOR): Ueda, Kazuo
LOCATION: Japan,
ASSIGNEE: Asahi Chemical Industry Co., Ltd.
PATENT: Japan Kokai Tokkyo Koho ; JP 8685164 A2 ; JP 6185164 DATE: 860430
APPLICATION: JP 84206155 (841003)
PAGES: 3 pp. CODEN: JKXXAF LANGUAGE: Japanese CLASS: A23L-001/22A

105041420 CA: 105(5)41420s JOURNAL
Studies on isolation and purification of steviosides from Stevia by ion-exchange method
AUTHOR(S): Zi, Xueli; Li, Renbiao; Zhou, Ruiming
LOCATION: Dep. Chem., Yunnan Normal Univ., Yunnan, Peop. Rep. China,
JOURNAL: Zhongguo Tiaoweipin DATE: 1986 NUMBER: 1 PAGES: 12-13 CODEN: ZHTIE7 LANGUAGE: Chinese

105041419 CA: 105(5)41419y JOURNAL
Extraction of steviosides from Stevia by modified water extraction-alcohol precipitation methods
AUTHOR(S): Zi, Xueli; Li, Renbiao; Zhou, Ruiming
LOCATION: Dep. Chem., Yunnan Normal Univ., Yunnan, Peop. Rep. China,
JOURNAL: Zhongguo Tiaoweipin DATE: 1986 NUMBER: 2 PAGES: 17-18 CODEN: ZHTIE7 LANGUAGE: Chinese

105021171 CA: 105(3)21171h JOURNAL
Determination of stevioside in Stevia rebaudiana by reversed-phase high-performance liquid chromatography
AUTHOR(S): Gagliardi, Luigi; Amato, Andrea; Basili, Arnaldo; Cavazzutti, Gilberto; Galeffi, Corrado; Bolasco, Adriana; Chimenti, Franco; Gattavecchia, Enrico; Tonelli, Domenica
LOCATION: Ist. Super. Sanita, Rome, Italy
JOURNAL: Ann. Chim. (Rome) DATE: 1986 VOLUME: 76 NUMBER: 1-2 PAGES: 39-43 CODEN: ANCRAI ISSN: 0003-4592 LANGUAGE: English

104223658 CA: 104(25)223658n TECHNICAL REPORT
Stevioside: an updating of its chemical and biological properties
AUTHOR(S): Salvatore, G.; Paganuzzi, A. Stammati; Silano, V.; Aureli, A. Dracos
LOCATION: Lab. Tossicol. Comp. Ecotossicol., Ist. Super. Sanita, Italy
JOURNAL: Rapp. ISTISAN DATE: 1984 NUMBER: Istisan 84/13
PAGES: 34 pp. CODEN: RAISEF LANGUAGE: English

104205717 CA: 104(23)205717b JOURNAL
Absorption, distribution, metabolism and excretion of stevioside in rats
AUTHOR(S): Nakayama, Kunio; Kasahara, Daigo; Yamamoto, Fumihiro
LOCATION: Omiya Res. Lab., Nikken Chem. Co. Ltd., Omiya, Japan, 330
JOURNAL: Shokuhin Eiseigaku Zasshi DATE: 1986 VOLUME: 27 NUMBER: 1 PAGES: 1-8 CODEN: SKEZAP ISSN: 0015-6426
LANGUAGE: English

104184958 CA: 104(21)184958p JOURNAL
Determination of the stevioside content in leaves of Stevia rebaudiana Bertoni
AUTHOR(S): Pryluka, M.; De Cernadas, R. R.
LOCATION: Dep. Tecnol. Aliment., INTI, 1001, Buenos Aires, Argent.
JOURNAL: Rev. Agroquim. Tecnol. Aliment. DATE: 1985 VOLUME: 25 NUMBER: 4 PAGES: 611-14 CODEN: RATLAB ISSN: 0034-7698 LANGUAGE: Spanish

104128831 CA: 104(15)128831q PATENT
Stable artificial, low-caloria sweetener composition in acidic media
INVENTOR(AUTHOR): Takizawa, Koichi; Kamata, Mitsuo
LOCATION: Japan,
ASSIGNEE: Ajinomoto Co., Inc.
PATENT: Japan Kokai Tokkyo Koho ; JP 85221056 A2 ; JP 60221056 DATE: 851105
APPLICATION: JP 8477181 (840417)
PAGES: 7 pp. CODEN: JKXXAF LANGUAGE: Japanese CLASS: A23L-001/236A; A23L-001/008; A23L-002/008

104122808 CA: 104(15)122808f JOURNAL
Effects of indomethacin on the action of stevioside on mean arterial pressure and on renal function in rats
AUTHOR(S): Melis, Marcia Salomao; Maciel, Rui Errerias; Saint, Ana Rita
LOCATION: Inst. Biol., State Univ. Campinas, Sao Paulo, Brazil
JOURNAL: IRCS Med. Sci. DATE: 1985 VOLUME: 13 NUMBER: 12 PAGES: 1230-1 CODEN: IMSCE2 LANGUAGE: English

104106307 CA: 104(13)106307m JOURNAL
Method of extraction and purification of stevioside from Stevia rebaudiana
AUTHOR(S): Zan, Fengsheng
LOCATION: No. 1 Food Fact., Mudanjiang, Peop. Rep. China,
JOURNAL: Huaxue Shijie DATE: 1986 VOLUME: 27 NUMBER: 1 PAGES: 31-3 CODEN: HUAKAB ISSN: 0367-6358 LANGUAGE: Chinese

104087289 CA: 104(11)87289p JOURNAL
Isolation of sweet compounds from Stevia rebaudiana
AUTHOR(S): Aquino, R. P.; Behar, I.; Biondi, A.; De Simone, F.; Scalesse, G.
LOCATION: Dip. Chim. Sostanze Nat., Univ. Naples, Naples, Italy
JOURNAL: Boll. - Soc. Ital. Biol. Sper. DATE: 1985 VOLUME: 61 NUMBER: 9 PAGES: 1247-52 CODEN: BSIBAC ISSN: 0037-8771 LANGUAGE: Italian

104050085 CA: 104(7)50085t PATENT
Improvement of stevia sweetener flavors
INVENTOR(AUTHOR): Kasori, Akiyo; Kawasaki, Mitsuyasu
LOCATION: Japan,
ASSIGNEE: Riken Vitamin Co., Ltd.
PATENT: Japan Kokai Tokkyo Koho ; JP 85188035 A2 ; JP 60188035 DATE: 850925
APPLICATION: JP 8444537 (840308)
PAGES: 4 pp. CODEN: JKXXAF LANGUAGE: Japanese CLASS: A23L-001/22A

104018778 CA: 104(3)18778k JOURNAL
Studies of purification of crude stevioside preparations
AUTHOR(S): Zhang, Jiaji; Xian, Ruishan
LOCATION: Changsha Inst. Agric. Mod., Changsha, Peop. Rep. China,
JOURNAL: Zhongguo Tiaoweipin DATE: 1985 NUMBER: 5 PAGES: 1-3 CODEN: ZHTIE7 LANGUAGE: Chinese

104004838 CA: 104(1)4838t PATENT
Stevioside-.gamma.-cyclodextrin inclusion compounds as sweeteners
LOCATION: Japan,
ASSIGNEE: Sanraku-Ocean Co., Ltd.; Dainippon Amami K. K.; Tama Biochemical Co., Ltd.
PATENT: Japan Kokai Tokkyo Koho ; JP 8598957 A2 ; JP 6098957 DATE: 850601
APPLICATION: JP 83204891 (831102)
PAGES: 9 pp. CODEN: JKXXAF LANGUAGE: Japanese CLASS: A23L-001/22A

104002933 CA: 104(1)2933w JOURNAL
Hydrolysis of Stevia rebaudiana glycosides with the gastric juice of Megalobulimus paranaguensis
AUTHOR(S): Ferraresi, Maria de L.; Bracht, Ana M. Kelmer; Bracht, Adelar
LOCATION: Dep. Farm. Bioquim., Univ. Maringa, 87100, Maringa, Brazil
JOURNAL: Arq. Biol. Tecnol. DATE: 1985 VOLUME: 28 NUMBER: 3 PAGES: 399-412 CODEN: ABTTAP ISSN: 0365-0979
LANGUAGE: English

103211164 CA: 103(25)211164q JOURNAL
Determination of steviosides by high performance liquid chromatography
AUTHOR(S): Nie, Hongyong
LOCATION: Hunan Bur. Control Import Export Commod., Changsha , Peop. Rep. China.
JOURNAL: Shengwu Huaxue Yu Shengwu Wuli Jinzhan DATE: 1985 VOLUME: 64, PAGES: 63-5 CODEN: SHYCD4 ISSN: 0253-9918 LANGUAGE: Chinese

103195095 CA: 103(23)195095k JOURNAL
Effects of stevioside on the frequencies of chromosomal aberrations and sister chromatid exchanges in the D-6 cell of Chinese hamster
AUTHOR(S): Nadamitsu, Shinsaku; Segawa, Michiharu; Sato, Yojuro; Kondo, Katsuhiko
LOCATION: Fac. Home Econ., Hiroshima Women's Univ., Hiroshima, Japan, 734
JOURNAL: Hiroshima Daigaku Sogo Kagakubu Kiyo, 4 DATE: 1985 VOLUME: 10, PAGES: 57-62 CODEN: HDSKEK LANGUAGE: English

103177144 CA: 103(21)177144w JOURNAL
Study on the purification of steviosides with the use of ion-exchange resins
AUTHOR(S): Cheng, T. F.; Chang, C. Y.; Chang, W. H.
LOCATION: Grad. Inst. Food Sci. Technol., Natl. Taiwan Univ., Taipei, Taiwan.
JOURNAL: Chung-kuo Nung Yeh Hua Hsueh Hui Chih DATE: 1985 VOLUME: 23 NUMBER: 1-2 PAGES: 178-90 CODEN: CKNHAA ISSN: 0578-1736 LANGUAGE: Chinese

103177127 CA: 103(21)177127t JOURNAL
A method for the isolation of stevioside from leaves of Stevia rebaudiana Bert
AUTHOR(S): De Cernadas, R. R.; Pryluka, M.
LOCATION: Dep. Tecnol. Aliment., INTI, 1001, Buenos Aires, Argent.
JOURNAL: Rev. Agroquim. Tecnol. Aliment. DATE: 1985 VOLUME: 25 NUMBER: 2 PAGES: 268-72 CODEN: RATLAB ISSN: 0034-7698 LANGUAGE: Spanish

103177011 CA: 103(21)177011a JOURNAL
Worldwide research on steviosides and their applications
AUTHOR(S): Tang, Xuezhong; Wu, Xiukun
LOCATION: Shenyang Fourth Pharm. Fact., Shenyang, Peop. Rep. China.
JOURNAL: Zhongguo Tiaoweipin DATE: 1985 NUMBER: 4 PAGES: 1-9, 13 CODEN: ZHTIE7 LANGUAGE: Chinese

103177003 CA: 103(21)177003z JOURNAL
A new sweetener containing glycosyl stevioside.
AUTHOR(S): Kikuchi, Yoshiaki
LOCATION: Sanyo Kokusaku Pulp Co. Ltd., Japan.
JOURNAL: Shoku no Kagaku DATE: 1985 VOLUME: 85, PAGES: 52-6 CODEN: SNKAD4 LANGUAGE: Japanese

103159410 CA: 103(19)159410a PATENT
Low-calorie sweetener composition
LOCATION: Japan.
ASSIGNEE: Sekisui Chemical Co., Ltd.
PATENT: Japan Kokai Tokkyo Koho ; JP 8575252 A2 ; JP 6075252 DATE: 850427
APPLICATION: JP 83183269 (830930)
PAGES: 4 pp. CODEN: JKXXAF LANGUAGE: Japanese CLASS: A23L-001/236A

103159172 CA: 103(19)159172z JOURNAL
Separation and quantitative determination of stevioside by reversed-phase HPLC
AUTHOR(S): Peng, Qihua; Fan, Shaowen; Wang, Wenlong
LOCATION: Wenzhou Ind. Sci. Inst., Wenzhou, Peop. Rep. China.
JOURNAL: Sepu DATE: 1985 VOLUME: 2 NUMBER: 1 PAGES: 6-10 CODEN: SEPUER LANGUAGE: Chinese

103156767 CA: 103(19)156767m JOURNAL
A study on the conditions of extraction of steviosides from Stevia leaves
AUTHOR(S): Chang, C. Y.; Chang, W. H.
LOCATION: Grad. Inst. Food Sci. Technol., Natl. Taiwan Univ., Taipei, Taiwan.
JOURNAL: Chung-kuo Nung Yeh Hua Hsueh Hui Chih DATE: 1985 VOLUME: 23 NUMBER: 1-2 PAGES: 168-77 CODEN: CKNHAA ISSN: 0578-1736 LANGUAGE: Chinese

103122029 CA: 103(15)122029g PATENT
Glucosylsteviobioside as a sweetener
LOCATION: Japan.
ASSIGNEE: Sanyo-Kokusaku Pulp Co., Ltd.
PATENT: Japan Kokai Tokkyo Koho ; JP 8537950 A2 ; JP 6037950 DATE: 850227
APPLICATION: JP 83146480 (830812)
PAGES: 7 pp. CODEN: JKXXAF LANGUAGE: Japanese CLASS: A23L-001/236A; A23L-001/22

103120310 CA: 103(15)120310t JOURNAL
Effect of stevioside and its related compounds on the induction of .alpha.-amylase biosynthesis
AUTHOR(S): Komai, Koichiro; Iwamura, Junichi; Morita,
(cont. next page)

Toyoshige; Hamada, Masayuki
LOCATION: Dep. Agric. Chem., Kinki Univ., Higashiosaka, Japan, 577
JOURNAL: Nippon Noyaku Gakkaishi DATE: 1985 VOLUME: 10 NUMBER: 1 PAGES: 113-17 CODEN: NNGADV ISSN: 0385-1559 LANGUAGE: English

103086668 CA: 103(11)86668d JOURNAL
Ion exchange methods in extraction and purification of steviosides from Stevia rebaudiana
AUTHOR(S): Zhou, Ren; Ran, Zhijun; Li, Qiang; Zi, Xueli; Rong, Yingxin; Li, Renbiao
LOCATION: Dep. Chem., Yunnan Normal Univ., Peop. Rep. China,
JOURNAL: Zhongguo Tiaoweipin DATE: 1984 NUMBER: 12 PAGES: 12-13 CODEN: ZHTIE7 LANGUAGE: Chinese

103052935 CA: 103(7)52935y PATENT
Removal of disagreeable after-taste of artificial sweeteners
LOCATION: Japan,
ASSIGNEE: Kondo, Kiyo; Kondo, Akiko
PATENT: Japan Kokai Tokkyo Koho ; JP 8527360 A2 ; JP 6027360 DATE: 850212
APPLICATION: JP 83136241 (830725)
PAGES: 4 pp. CODEN: JKXXAF LANGUAGE: Japanese CLASS: A23L-001/236A; A23L-001/228

103021249 CA: 103(3)21249y JOURNAL
Stevia rebaudiana Bertoni: an excellent natural sweetening agent
AUTHOR(S): De Levy, Ruth H. Galperin
LOCATION: Div. Quim., CHEMI S.R.L., 1012, Buenos Aires, Argent.
JOURNAL: Acta Farm. Bonaerense DATE: 1984 VOLUME: 3 NUMBER: 1 PAGES: 47-50 CODEN: AFBODJ ISSN: 0326-2383 LANGUAGE: Spanish

102219832 CA: 102(25)219832f PATENT
Modification of Stevia sweetener
LOCATION: Japan,
ASSIGNEE: Morita Kagaku Kogyo Co., Ltd.
PATENT: Japan Tokkyo Koho ; JP 8451257 B4 ; JP 5951257 DATE: 841213
APPLICATION: JP 7636798 (760401)
PAGES: 4 pp. CODEN: JAXXAD LANGUAGE: Japanese CLASS: A23L-001/22A

102202773 CA: 102(23)202773q JOURNAL
Metabolically activated steviol, the aglycone of stevioside, is mutagenic
AUTHOR(S): Pezzuto, John M.; Compadre, Cesar M.; Swanson, Steven M.; Nanayakkara, N. P. Dhammika; Kinghorn, A. Douglas
LOCATION: Coll. Pharm., Univ. Illinois, Chicago, IL, 60612, USA
JOURNAL: Proc. Natl. Acad. Sci. U. S. A. DATE: 1985 VOLUME: 82 NUMBER: 8 PAGES: 2478-82 CODEN: PNASA6 ISSN: 0027-8424 LANGUAGE: English

102147684 CA: 102(17)147684j JOURNAL
Preliminary studies on technological conditions for extraction of steviosides
AUTHOR(S): Li, Sishi
LOCATION: Xihu Stevioside Plant, Changde, Peop. Rep. China,
JOURNAL: Zhongguo Tiaoweipin DATE: 1984 NUMBER: 9 PAGES: 10-11 CODEN: ZHTIE7 LANGUAGE: Chinese

102060885 CA: 102(7)60885v JOURNAL
Role of column switching in semi-preparative liquid chromatography. Isolation of the sweetener stevioside
AUTHOR(S): Little, C. J.; Stahel, D.
LOCATION: Anachrom Ltd., Slough/Berkshire, UK, SL3 9YZ
JOURNAL: J. Chromatogr. DATE: 1984 VOLUME: 316, PAGES: 105-11 CODEN: JOCRAM ISSN: 0021-9673 LANGUAGE: English

102024947 CA: 102(3)24947q JOURNAL
Diterpenoid sweeteners. Synthesis and sensory evaluation of stevioside analogs with improved organoleptic properties
AUTHOR(S): DuBois, Grant E.; Stephenson, Rebecca A.
LOCATION: Chem. Synth. Lab., Palo Alto, CA, 94304, USA
JOURNAL: J. Med. Chem. DATE: 1985 VOLUME: 28 NUMBER: 1 PAGES: 93-8 CODEN: JMCMAR ISSN: 0022-2623 LANGUAGE: English

101228843 CA: 101(25)228843s PATENT
Glycosylsteviosides and glycosylrebaudiosides as sweetening agents
LOCATION: Japan,
ASSIGNEE: Ikeda Tohka Industry Co., Ltd.
PATENT: Japan Kokai Tokkyo Koho ; JP 84120073 A2 ; JP 59120073 DATE: 840711
APPLICATION: JP 82229417 (821228)
PAGES: 10 pp. CODEN: JKXXAF LANGUAGE: Japanese CLASS: A23L-001/236; C12P-019/56

101211584 CA: 101(23)211584k JOURNAL
Diterpenoid sweeteners. Synthesis and sensory evaluation of biologically stable analogs of stevioside
AUTHOR(S): DuBois, Grant E.; Bunes, Leonard A.; Dietrich, Paul S.; Stephenson, Rebecca A.
LOCATION: Chem. Synth. Lab., Palo Alto, CA, USA
JOURNAL: J. Agric. Food Chem. DATE: 1984 VOLUME: 32 NUMBER: 6 PAGES: 1321-5 CODEN: JAFCAU ISSN: 0021-8561

(cont. next page)

LANGUAGE: English

101209312 CA: 101(23)209312b JOURNAL
**Effects of three sweeteners on rat urinary bladder carcinogenesis initiated by N-butyl-N-(4-hydroxybutyl)nitrosamine**
AUTHOR(S): Hagiwara, Akihiro; Fukushima, Shoji; Fukushima, Sho; Kitaori, Masayoshi; Shibata, Michiko; Ito, Nobuyuki
LOCATION: Med. Sch., Nagoya City Univ., Nagoya, Japan, 467
JOURNAL: Gann DATE: 1984 VOLUME: 75 NUMBER: 9 PAGES: 763-8 CODEN: GANNA2 ISSN: 0016-450X LANGUAGE: English

101177332 CA: 101(20)177332q JOURNAL
**Extraction and isolation of stevioside from leaves of Stevia rebaudiana Bertoni indigenous to China**
AUTHOR(S): Huang, Jianping; Yang, Qiaoling; Hong, Weimin; Zheng, Yimin; Tang, Yongmao
LOCATION: Xiamen Tradit. Chin. Plant. Inst. Tradit. Chin. Propr. Drugs, Xiamen, Peop. Rep. China,
JOURNAL: Zhongcaoyao DATE: 1984 VOLUME: 15 NUMBER: 8 PAGES: 379 CODEN: CTYAD8 ISSN: 0253-2670 LANGUAGE: Chinese

101168527 CA: 101(19)168527f JOURNAL
**The role of column switching in analyzing complex samples**
AUTHOR(S): Little, C. J.; Stahel, O.; Hales, K.
LOCATION: Anachrom Ltd., Slough/Berks., UK,
JOURNAL: Int. J. Environ. Anal. Chem. DATE: 1984 VOLUME: 18 NUMBER: 1-2 PAGES: 11-23 CODEN: IJEAA3 ISSN: 0306-7319
LANGUAGE: English

101152264 CA: 101(17)152264f PATENT
**Stevioside analogs**
INVENTOR(AUTHOR): Dubois, Grant E.
LOCATION: USA
ASSIGNEE: Dynapol
PATENT: United States ; US 4454290 A DATE: 840612
APPLICATION: US 272798 (810611) *US 189243 (800922)
PAGES: 8 pp. Cont.-in-part of U.S. 4,332,830. CODEN: USXXAM
LANGUAGE: English CLASS: 536018100; C07H-015/24

101150202 CA: 101(17)150202k PATENT
**Flavor improvement of stevioside sweeteners**
LOCATION: Japan,
ASSIGNEE: Kondo, Kiyo; Kondo, Akiko
PATENT: Japan Kokai Tokkyo Koho ; JP 84102372 A2 ; JP 59102372 DATE: 840613
APPLICATION: JP 82211645 (821202)
PAGES: 3 pp. CODEN: JKXXAF LANGUAGE: Japanese CLASS: A23L-001/23; A23L-001/22

101150195 CA: 101(17)150195k PATENT
**Modified steviosides as sweeteners**
LOCATION: Japan,
ASSIGNEE: Dainippon Ink and Chemicals, Inc.
PATENT: Japan Kokai Tokkyo Koho ; JP 8448059 A2 ; JP 5948059 DATE: 840319
APPLICATION: JP 82159391 (820913)
PAGES: 9 pp. CODEN: JKXXAF LANGUAGE: Japanese CLASS: A23L-001/236; C08B-037/00

101127046 CA: 101(15)127046d JOURNAL
**Studies on the tissue culture of Stevia rebaudiana Bertoni and its components (I)**
AUTHOR(S): Miyagawa, Hideki; Fujita, Yoko; Fujioka, Naomi; Kohda, Hiroshi; Yamasaki, Kazuo
LOCATION: Sch. Med., Hiroshima Univ., Hiroshima, Japan, 734
JOURNAL: Shoyakugaku Zasshi DATE: 1984 VOLUME: 38 NUMBER: 1 PAGES: 12-18 CODEN: SHZAAY ISSN: 0037-4377
LANGUAGE: Japanese

101089251 CA: 101(11)89251g PATENT
**Glucopyranosylstevioside sweeteners from stevia**
LOCATION: Japan,
ASSIGNEE: Ikeda Toka Industry Co., Ltd.
PATENT: Japan Kokai Tokkyo Koho ; JP 8471662 A2 ; JP 5971662 DATE: 840423
APPLICATION: JP 82100271 (820610)
PAGES: 14 pp. CODEN: JKXXAF LANGUAGE: Japanese CLASS: A23L-001/236

101069379 CA: 101(9)69379b JOURNAL
**A phytochemical screening procedure for sweet ent-kaurene glycosides in the genus Stevia**
AUTHOR(S): Kinghorn, A. D.; Soejarto, D. D.; Nanayakkara, N. P. D.; Compadre, C. M.; Makapugay, H. C.; Hovanec-Brown, J. M.; Medon, P. J.; Kamath, S. K.
LOCATION: Coll. Pharm., Univ. Illinois, Chicago, IL, 60612, USA
JOURNAL: J. Nat. Prod. DATE: 1984 VOLUME: 47 NUMBER: 3
PAGES: 439-44 CODEN: JNPRDF ISSN: 0163-3864 LANGUAGE: English

101053330 CA: 101(7)53330t PATENT
**Microbial production of rebaudioside A**
LOCATION: Japan,
ASSIGNEE: Dainippon Ink and Chemicals, Inc.
PATENT: Japan Kokai Tokkyo Koho ; JP 8417996 A2 ; JP 5917996 DATE: 840130
APPLICATION: JP 82127007 (820721)
PAGES: 11 pp. CODEN: JKXXAF LANGUAGE: Japanese CLASS: C12P-019/56; A23L-001/22; C12P-019/56J; C12R-001/465J

101037391 CA: 101(5)37391h PATENT
Improvement of stevia sweetener flavor
LOCATION: Japan.
ASSIGNEE: Morita Kagaku Kogyo Co., Ltd.
PATENT: Japan Kokai Tokkyo Koho ; JP 8445848 A2 ; JP
5945848 DATE: 840314
APPLICATION: JP 82157902 (820909)
PAGES: 5 pp. CODEN: JKXXAF LANGUAGE: Japanese CLASS: A23L
-001/22