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DP/ID/SER.A/1358
15 June 1990
ORIGINAL: ENGLISH

18423

PROCESSING OF STEVIA

DP/DRK/88/008

DEMOCRATIC PEOPLE'S REPUBLIC OF KOREA

Technical report: Findings and recommendations*

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 Yaw-Awusu-Ansah and Janos Mikle

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* This document has not been edited.

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EXECUTIVE SUMMARY

The mission, defined by the terms of reference was executed between 4/9-4/30 1990.

PROJECT STATUS (as of 4/9/90)

The PSE process as defined by FRI and consisting of:

Electrolysis followed by filtration, trace metal/pigment removal using ion exchange resins, separation of stevioside (80-84% purity), is well documented and its execution is routinized.

Laboratory scale process/parameter optimization studies are severely handicapped due to the lack of minimal/adequate laboratory equipment.

Analytical back up for the project is nil, for all practical purpose. ALL available instruments are down, supplies are depleted.

WORK PERFORMED:

1. The lab, pilot, commercial processes were reviewed in detail while in execution on site.

2. Each unit operation (lab, pilot) was evaluated weighing advantages, disadvantages and their scaleability to larger pilot size.

3. Seventeen lab/pilot (combined) experiments were performed searching for process alternatives that would eliminate dependence on imported resins, and minimize capital investment.

4. The process related QA/QC methods were reviewed and a revised plan for an operational analytical service was defined.

5. The local fabrication facilities were visited and availability of materials checked.

6. The work plan (p. 16 of P.D.) was revised or updated in accordance with the prevailing situation and long term plans of FRI.

CONCLUSIONS

The budget's (p. 16 of P.D.) -UNIDO/UNDP component-elements are oddly proportioned due to perhaps too many incorrect assumptions.

Without basic lab/equipment, FRI cannot support the project with bench top optimization tests, or yield/purity, qualitative/quantitative data.

RECOMMENDATIONS

1. Provide in shortest possible time the FRI chemistry labs with the minimum but essential equipment that will permit them to become a working lab as opposed to a struggling one. (General chemistry lab inventory-to be provided by UNDP)

2. Expedite selection, acquisition of analytical equipment to be able to support lab/pilot scale R & D with:
   - Thin layer chromatography
   - Rod chromatography
   - High pressure liquid chromatography
   - A computer (PC) with printer for process optimization (UNDP)

3. Develop an R & D programme (supported by 1, 2) that will evaluate alternative process technology, before final commitment is made towards design/acquisition of a pilot plant. (ERI)

4. Consider the long term use of the pilot plant generated data: It has to be solid enough to be used in the design of full scale facilities for a diversity of end products.

1.0 BACKGROUND AND OBJECTIVES

Climatic incompatibility does not permit the cultivation of conventional sweetening crops such as sugar cane or sugar beet in the DPRK. The environment is, however, conducive for cultivation of *Stevia rebaudiana* (Bertoni) Bertoni. The non-caloric sweetener extracted from the plant is widely used in the soft drink industry in the DPRK, thus relieving the foreign exchange strain of the nation for the importation of sweeteners.

Currently the stevia sweetener is produced as crude extract and is used as such. Research efforts are underway to improve the processing technology and quality of the sweetener. These efforts are the mandates of the Foodstuff Research Institute of DPRK located in Pyongyang. In order to enhance the stevia research efforts of this Institute, assistance has been sought from UNIDO in terms of pilot plant equipment, design and fabrication of equipment, analytical equipment for both research and quality control and assurance, methodology development, training and experts assistance.

This joint mission of two professionals was more or less a familiarization mission with the following objectives:

a) Review, together with the staff of the FRI, the stevia project: lab, pilot scale, commercial scale processes, i.e. current status of PPSE, and PSE as applicable.

b) Present, implement QA/QC procedures as permitted by current limitations on hardware/reagents.

c) Present, demonstrate/implement alternative procedures for production of PPSE, PSE; confirm results on site.

d) Attempt to use industrially produced PPSE for PSE in lab scale.

e) If d above is sufficiently documented/confirmed, develop a principle, flow/process diagram for 50 kg/day size pilot unit.

f) Describe unit operation in e, specify key components. Identify components for which adequate local fabrication is available (Materials compatible with process/parameters, tools).

g) Present a revised/updated workplan using as input the most recent field data; (Assess validity of UNDP-UNIDO inputs 2.1, 11-01, 11-02, 11-51, 11-52, on page 16 of P.D.)

h) Analytical capability-upgrading/implementation. Prioritize the kinds of equipment and support urgently needed with personnel needed and availability at site.

i) Definition of a purpose-formulated training programme:-

- Define the scope of the programme, evaluate potential candidates for participation.
- Present training programme in detail: lectures, followed by lab/pilot plant training, duration/instructors, format, site, time frame.

2.0 REVIEW OF CURRENT PROCESSING METHOD

2.1 Laboratory

2.1.1. Extraction, Decolorization and Demineralization

Leaves containing both twigs and small stem parts are extracted by decoction with (8:1 H_2O:leaves) at 60-70° C for 90 min. The extract is filtered affording about 6.7

parts of extract of approximately 3.5% soluble solids. The residue is sequentially extracted twice in a similar manner using 5 parts of water instead of 8. The combined extracts serve as the first extracting solvent for subsequent batches of leaves. The spent leaves are said to contain 0.3% residual sweetener. Process control is limited; operators use refractometer to determine solids and a pH paper to assess pH of the extract. The extract is then placed in an electrochemical treatment vessel fitted with aluminum electrodes. The pH of the extract is adjusted to 3 with hydrochloric acid and the solution electrolysed for 30 min. at 5 V. constant voltage. After electrolysis the solution is gravity filtered. The filtrate is respectively passed through a cation and anion exchange resin columns for further decolorization and demineralization. The resulting effluent is almost colorless with a tint of green coloration. The solution is referred to as partially purified stevia extract (PPSE).

2.1.2. Purification of PPSE

PPSE is purified by loading the extract on an adsorption resin column (Diaion HP-20). The sweetener is then eluted with 50% ethanol. The ethanol is distilled off and the solution further concentrated and dried. The dried material ("EX") is dissolved in hot methanol (6 parts) and crystalized at 10° C; sometimes with seeding and stirring. The crystals, mainly stevioside are filtered and dried. The filtrate is allowed to stand at 10° C for a few days (~3) after which time more crystals, mainly rebaudioside A are formed. The crystals are filtered and vacuum dried. The filtrate referred to as the "mother liquor" is then dried and the solids are subjected to transglucosylation. It was reported by FRI staff that approximately 55 kg of stevioside, 15 kg of rebaudioside A and 35 kg of transglucosylated products can be obtained from one tonne of leaves.

2.1.3. Transglucosylation Process

According to the staff member in charge of this process, the enzyme being used, (an extracellular crude preparation from a *Bacillus sp.* #825) catalyses mainly α-glucosyl transfer reactions. The transfer products, therefore, contain some rebaudioside A.

The dried material from the mother liquor is said to be bitter, but when subjected to transglucosylation, a product of identical sweetness as stevioside, but cleaner in taste is said to be produced.

The bacteria culture said to be predominantly alkaliphilic *Bacillus sp.* #825, is grown in flasks containing 2% soluble starch, 0.5% peptone, 0.1% KH_2PO_4, 0.05% $MgSO_47H_20$, 0.5% yeast extract solids, and 1.0% Na_2CO_3. The pH of the media is maintained at between 10.5 and 11.0. In case of scarcity the peptone and yeast extract solids are replaced with corn steep liquor. The bacteria are cultured for 2-3 days after which time no more extracellular enzymes are secreted. The normal enzyme activity of the extracellular solution is about 40 units/mL (A unit activity defined as the amount of enzyme capable of catalysing the transglucosylation of 1 µmole of stevioside per min. at 40° C). The liquid is filtered on normal filter paper. The solution is used for transglucosylation.

The transglucosylation reaction is effected by adding to a solution of acid treated starch (prepared by treating potato starch with 7.5% HCl at room temperature for 7 days, washing with distilled water, neutralizing to pH 7.0 with 0.5% $NaHCO_3$, finally washing with distilled water and air drying into powder) the dried mother liquor solids. Transglucosylation enzyme and β-amylase are added to the solution. The mixture is incubated for 4-8 hours at 60° C with stirring. A degree of conversion of 70-75% is

said to be attained. The mixture is then boiled for 20 min. to inactivate the enzyme and the transfer products selectively adsorbed on to a column of HP-20 resin. The transfer products are selectively eluted with 50% ethanol. The ethanol is distilled off and the solids dried to yield the transfer products known as ALPHASIDE. The product from this process is said to be equally as sweet as stevioside, but without a bitter after taste. It is claimed that if stevioside is used as substrate, the resulting transfer product is mainly rebaudioside A. The laboratory process is schematically shown in Figure 1.

2.1.4. Remarks:

It was very clear that most of the unit operations being used have been adequately tested but not completely optimized, apparently due to the gross lack of analytical and proper laboratory hardware. Most of the parameters have either been arbitrarily chosen based on rationalized economics and limitations at the Institute or have been adapted from a publication available to the scientists. For example, the 1:8 leaves:water ratio has been chosen based on limitations on downstream water removal equipment and economics. It is said that the ratio leaves about 0.3% residual sweetener in a continuous counter-current extraction mode. It is, however, questionable whether this estimate is accurate taking into consideration the limited analytical capabilities available. It is also questionable whether the residual levels are consistent from batch to batch. Obviously there is the need to optimize every unit operation used in the process. In the case of extraction, key factors to optimize are; extraction temperature, time, and leaves:water ratio, with respect to yield of sweetener, and residual sweetness in leaves. The effect of front end size reduction of leaves on yield and facility of sweetener removal also warrants investigation.

Another operation which needs optimization is the transglucosylation reaction. For example the reaction is carried out at 60^{0} C; the optimal temperature of the enzyme.

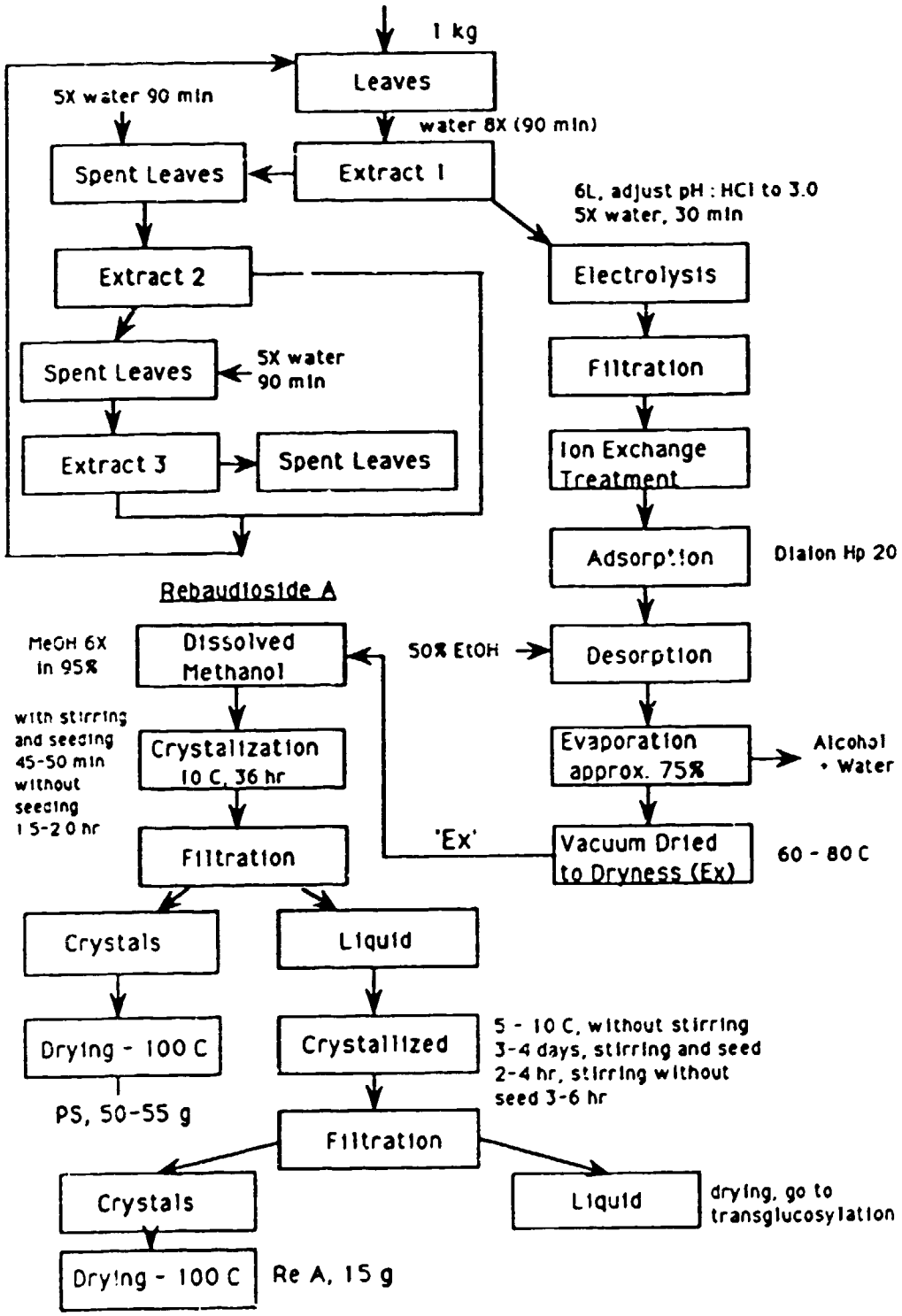


Fig 1. Lab Process for Stevioside and Rebaudioside A

However, enzyme stability data reviewed indicated that at this temperature the activity of the enzyme is reduced to approximately 50% in two hours. The choice of 60^{o} C is, therefore, not justifiable. Obviously the parameters for this operation need to be optimized.

It is clear that the process used by the FRI is workable. The combined electrolysis and ion exchange treatment which seems to be based on a Japanese patent is functional. It was, however, obvious that alternative processing routes have not been exhaustively tested. The facility has a chronic problem of not replenishing expendable items especially those requiring foreign exchange for importation. This problem makes it even more imperative that alternative process avenues less dependent on imported resins or reagents be explored.

The same problem has crippled the analytical capabilities of the Institute. The only quantitative analytical equipment supporting this project was an Iatroscan. Due to lack of chromarods the equipment is not operational. Chromarods obtained for this equipment were normally washed with chromic acid and reused sometimes for 50 times, till they are completely exhausted. It is not known whether such repeated cleaning and reuse allow reproduceable results to be obtained. It is questionable whether the provision of expendable items or additional equipment could provide lasting solution to this problem. Some minimum foreign exchange reserves should be provided to the Institute for purchase of expendable items, reagents and spare parts.

The personnel in the Institute are well trained, skillful and dedicated. They have obviously achieved an almost impossible feat considering the limitations on analytical reagents, equipment and labware.

2.2. Pilot Plant Facility - FRI

The floor plan, provided by FRI, is presented in Appendix 1. The facility of undetermined age does not meet the requirements of a food grade facility. Floor is of concrete that is chipped/cracked and ideal as microbial habitat. Doors, windows, traffic flow are such that infestation by flies, rodents is unavoidable.

The electrical installation/wiring is totally incompatible with the contemplated use of flammable/explosive solvents (for elution and crystallization) in pilot scale quantities.

2.2.1. Utilities

Steam: 1 MT/hr, pressure of 3 atm. upper limit of 5 atm.

Power: 380 V, 60 Hz, 230 A, 160 KW installed Power line with cross-sectional area of 75 mm^2.

Water: 5-6^0 C (winter), 18^0 C (summer). Emergency supplies of 50 m^3 above and 20 m^3 underground are kept to avoid dependence on city water which is delivered only for certain hours of the day (morning 2 hours, noon 3 hrs, etc.).

Vacuum: Not available.

Compressed air: Not available.

Refrigeration (plant): Not available. There is a 20 year old ammonia plant for which there are no parts available.

Distilled water: Not available.

2.2.2. Current Pilot Scale Process (FRI) for PSE (Purified Stevioside Extract)

2.2.2.1. Unit Operations Steps:

a) Aqueous extraction - One (1) part of leaves is immersed in eight (8) parts of water (city water from rusty steel storage tanks) of 45-55^{o} C, for 90 min., while occasionally stirring. After the 90 min. has elapsed the "brew" is strained, and the solids squeezed by hand. The extract is sieved by gravity, through cloth. (In one test conducted in our presence distilled water was used to generate extract for lab work). The moisture of the spent leaves was 72%, the dry substance (D.S.%) content of the extract was 3.0%.

b) Electrolysis - The pH or the extract is adjusted using hydrochloric acid to 3.0-3.5. This acidulated extract is loaded into the electrolyser where it is subjected to electrolysis applying 5V/DC for 30 min. The vessel has 15 (20 cm x 48 cm) plates of aluminum plates serving as electrodes. In this operation the leaf proteins (50-60 mg/100 mL as determined on site) are precipitated (pH - is near isoelectric point). Some pigments are either reduced or oxidized and precipitated. The aluminum electrodes are attacked by the HCl; $AlCl_3$ and $Al(OH)_3$ are generated. The latter acts as a floculating agent and the destabilized colloidal solute is precipitated. After filtration the extract is a clear greenish liquid. The staff of FRI perform this operation routinely and are satisfied with its effect.

Remark: The untreated "tank" water, the HCl-Al electrode interaction introduce

trace metals over and above those inherent (not determined) in the plant material. In other words, operations 1 & 2 introduce contaminants that must be removed in the subsequent operations.

c) Trace Metal Removal - Decolorization

Filtrate from the preceding operation is passed through two ion exchange columns connected in series. These are loaded with Amberlite IR 120 and IR 45 (5 litres each) respectively. The DS content of the feed is about 1%. Each column is capable of handling 30-40 times its volume of feed then the resin is regenerated.

- There is no routine measurement performed to confirm whether the undesirable trace components have been removed or not.
- Color is examined visually; water like to pale yellow is acceptable. It is not known what pigments are retained on the resins and still present in the eluant.

Remark: There are a variety of chelating agents/sequestrants available to scavenge trace metals. These may be a viable alternative to investigate before a process selection/decision is made.

- Likewise, removal of pigments can be effected using specific adsorbants, without having to rely on imported resins.

The SAM-SUK processing plant (to be described) is a good example to warn any process developer in the country against the use of ion exchange resins for stevia processing. There was a first supply for start up, and demonstration of an elegant approach. When that supply was exhausted, they were never replenished and the columns are fouled up, beyond salvage. The system cannot be effectively used any more, generating a product of questionable quality. In collaboration with the lab/pilot plant staff several experiments (to be discussed later) were performed on site to increase

awareness and focus attention on other workable alternatives, which are not dependent on ion-exchange resins or for that matter electrolysis.

d) Stevioside Separation

The liquid effluent from the ion exchange columns is reported to contain 1.4% sweetener (i.e. 2.3% D.S. total). This feedstock is passed through a column loaded with HP-20 (crosslinked, nonpolar polymer adsorbent). The retention capacity of the material is 7-8.0g/100 mL resin. The retained material is eluted using 50% or 75% ethanol. The eluant contains 3.0% stevioside (3.7% D.S. total). Upon desolventization, stevioside of 80-85% purity is obtained.

Remarks: This step is performed in the laboratory using one of the several columns with 500 mL resins. There is no sufficient resin to load a 5 liter size column in the pilot plant.

2.3 SAM SUK - Commercial production unit

The facility is situated twenty-five minutes away from Pyongyang. At the time of the visit the facility was operational. Information from Chief Engineer LI YONG SU and Engineer ZU IN HYOK,.was obtained through interpreters. According to these sources, the facility has been processing 1,000 MT dry leaves per year, over the past 10 years. The balance of the DPRK crop (1,000 MT) is processed by various smaller facilities. The end products are solutions of mixed STEVIA sweeteners, "standardized" to 8% and 20% concentrations respectively.

2.3.1. Current status

The process is essentially as described, in the diagram in Appendix IV of the project document (copy attached in Appendix II). The input material (referred to everywhere as LEAVES) is in fact the whole plant cut above ground, plus weeds. It is said no herbicides or pesticides are used in the growing of the crop. There is no provisions for segregation of leaf material from stems, weeds, and other contaminants.

The input is described invariably as having 12-13% moisture, and 6-7% total sweetener content, but the facility is not equipped to routinely perform these analyses on incoming materials. Proximate analysis would be very useful. Data regarding water (extracting solvent) quality, such as hardness, metals, microbial load, is not available. Prior to feeding the plant material to the extractor, it is sprayed with $AlCl_3$ solution (6 kg per 1 MT dry leaves) for dust suppression. Dust suppression is a valid concern but it does not require $AlCl_3$ which in the aqueous system guarantees substantial corrosion of the extractor (mild steel) and contamination of the extract with ferric derivatives. Due to lack of calibrated feeder, through-put was established based on 250 working days/year, 20 hour days. Best estimate is 200-220 kg/hr.

Water for extraction is added in 8:1 (water:solids) proportion without a flow-meter or any other measuring device. Given the fixed volume of the extractor and rpm of the screw conveyor, the residence time in the extractor is uncertain. In other words, the process is suboptimal and corrosive by definition. Process input is 9 parts (8 water, 1 leaves) from which two output streams are generated; 4-5 parts of aqueous extract and 5-4 parts of wet solids. If one assumes 100% extraction efficiency (not in this suboptimal process) the 14 kg sweetener from 200 kg leaves is in 1,600 kg of water (0.875% solution). At least 600 kg of this extract leaves with the wet solids due

to lack of a dewatering press. Thus in every hour, 5.25 kg or 37.5% of the available sweetener may be lost this way.

Further downstream the extract is treated with Calcium oxide, without any metering system. Solids, probably preweighed, are scooped into an overflow box. The final pH hovers around 10.5-11.5. In this step one expects that pigments are flocculated (by the $Al(OH)_3$ available from hydrolysis of $AlCl_3$) to enhance their subsequent removal in a plate and frame filter press. As input streams are undefined, the residence time in this step is uncertain. At pH values above 10 and depending on the temperature, as much as 40% of the available stevioside could be lost due to hydrolysis. That could substantially reduce the yield of sweetener in this process.

The filtered solution is passed through two ion exchange columns. (Cation and anion respectively) In these, trace metals are presumably removed and the color of the extract is enhanced. The resins are completely fouled as they are 10 years old. Regeneration procedures are said to be practised, but it is doubtful whether the columns are any good. Consequently the extract is either passed through the resin beds as a routine imposed by piping or by-passed, and fed to the evaporator. Evaporation takes place in a unit resembling a forced circulation evaporator, operated at 320 mmHg residual pressure. The finished product is said to be a 20% dry solids solution of sweeteners. Its appearance is less than desirable; blackish green, murky liquid. Obviously the quality of the sweetener will adversely affect the quality of finished food items in which they are used. Quality control is empirical. A solution of 8% sucrose is the reference and the concentrate is diluted to a sweetness level (sensory-subjective) equal to this reference. In summary it can be said that the facility is in dire need of rehabilitation. Process optimization, process and quality control, are essential for a better utilization of the input raw material. One cannot be but skeptical about records

showing 0.3-0.4% residual sweeteners in the spent solids, as there is no method or hardware available on site for their determination. It is reported that the degree of recovery is 80%, but this figure may be overly optimistic.

Remark: The SAM SUK plant cannot be called industrial without stretching ones imagination. It is simply a 200 kg/hr (solids) pilot facility, operated 20 hours per day.

3.0 ANALYTICAL CAPABILITIES-FRI

An inventory of existing analytical instruments, suitable for the stevia project was made. Results of prior experiments were well documented including purities, yields; that one could be convinced that some workable methods must be in place. The equipment available for the project were as follows:

a) Spectrophotometer - Hungarian made: MOM spectromom 195 D UV/Vis equipment with unknown model year is available. The unit is not operational. Nobody at FRI could indicate when it was last used.

b) Gas Chromatograph - A Checoslovakian-made, CHROM 4 - PRAHA (Not essential for stevia project) of unknown year of manufacture is available but the unit is not operational. There are no records to indicate when it was last in usable shape.

c) IATROSCAN Model TH-10 - (Locally known as synchrograph) In principle a ROD chromatograph with flame ionization detector (FID) assembly, connected to a chart recorder. This unit was most often referred to when qualitative/quantitative data from the past was discussed. Unit is out of commission, not faulty but lacking rods.

The total number of rods ever available to FRI for the Iatroscan was 40. Most of them were recycled (reused, reportedly 50 times after successive treatment with sulphochromic acid, and rinsing with distilled water), but they have all come to the end of their useful life. It was not divulged, since when the stevia research program has been completely without analytical backup.

Remark: The reliability of data obtained using the recycled rods is unknown and not documented.

During the mission, attempts were made to provide some minimal QA/QC programme. A method was introduced for TLC determination of stevioside and rebaudioside A. Attempts were made to obtain quantitative data based on the area of the TLC spots. As shown in Table 1 the correlation coefficients of the linear regression

TABLE 1

Linear Regression Analysis of TLC Spot Areas Against The Concentrations of Standards

Spotted Samples No.	Volume (μL)	Conc.(μg) ST	Conc.(μg) ReA	Spot Area (mm^2) ST	Spot Area (mm^2) ReA	Regression Equations y = area in TLC
	40	19.74	16.2	71.50	33.18	y = 0.761x + 57.1 (ST)
1	80	39.49	32.4	81.25	38.48	y = 0.339x + 27.6 (ReA)
	120	59.23	48.67	98.00	44.17	r(ST) = 0.988, r(ReA) =0.999
	40	19.74	16.22	49.50	28.27	y = 0.721x + 36.0(ST)
2	80	39.49	39.4	66.00	33.18	y = 0.295x + 23.1 (ReA)
	120	59.23	48.6	78.0	38.48	r(ST) =0.996, r(ReA) =0.965
	40	19.74	16.22	19.63	19.63	y = 0.343x + 13.5 (ST)
3	80	39.49	39.45	28.27	28.29	y = 0.540x + 10.0 (ReA)
	120	59.23	48.67	33.18	38.48	r(ST) =0.988, r(ReA)=0.958

analysis of spot areas versus concentration were very high. Thus it would be possible to use such approach for some form of QA tests. The major drawback of this approach is that due to unavoidable human error during spotting and also fluctuations in environmental conditions, the results from different runs were not very reproduceable (Table 1). The use of such analysis would only provide estimates rather than absolute values. It is expected that the method could be improved if a densitometer scan output is used rather than manual calculation of spot area which could significantly vary due to lateral diffusion or channelling. It was assessed that a TLC unit with manual applicator for preparing the plates and a densitometer for some form of quantitation of the spots could probably be the only reasonable analytical tool that could survive the chronic problem of not replenishing supplies in the Institute.

4.0 ALTERNATIVE/PROCESS/TECHNOLOGY - (SCREENING)

The experiments (lab/pilot) performed were designed to address both PPSE and PSE preparation. As mentioned earlier in this report, there is no analytical data available regarding leaf composition (Protein, fiber, ash-minerals, pigments petroleum ether solubles, water solubles, etc.)

Leaf proteins were determined on site using a protein strip meter normally used for clinical protein analysis. According to this method the protein content of the extract was between 50-60 mg/100 mL. Tested process alternatives are schematically represented in the Appendix III attached and some are briefly discussed below.

a) One kg of leaves were extracted with 8.0 kg of distilled water in the pilot plant, as per usual practice. (Temperature was maintained by injection of live steam into the "brew", at 55-60^{0} C.) From the extraction 2.4 kg of wet leaves and 7.0 kg of aqueous extract were obtained. The wet leaves were analyzed for moisture

content, which was reported to be 72%. Consequently, the 2.4 kg of wet leaves contain 1.728 kg of water and 0.672 kg of solids, including spent leaves. The original leaves were said to contain 120 g of water (12%) thus approximately 210 g of soluble solids were extracted (21%). The water soluble solids in the approximately 1.7 kg "moisture" can be called a loss. A portion of the extract (3 L) was evaporated (in open aluminum kettle) and reduced to 1 L, and a final dry solids content of ~ 9.0%.

- The sample (1L) was treated with acetic acid until the pH was 3.0-3.5 (Isoelectric range), mixed (tickled with a thermometer) for 30 min., while maintaining at 40-45^{o} C.
- EDTA (0.2%) was added (2 g/L) as a chelating agent, mixed for 1 hr. and filtered.
- Activated carbon (5.0%) was added, contacted for 30 min., then filtered to remove the absorbant (this operation took about 8 hours to complete due to poor equipment).

In the two unit operations, 460 mL of sample were lost due to inadequate filtration/glassware for quantitative routines. The experiment was abandoned as there was no point to pursue it for qualitative purposes.

Remark: Color of solution was dark reddish brown, with no visible green.

b) Samples of crude extract (I), (pH - 5.2) and CaO treated extract (II) (pH = 10) from SAM SUK were subjected to lab scale processing.

- The pH value of I was adjusted to 3.5 adding citric acid (50% solution) as chelating agent. The mixture was maintained at 50-55^{o} C for 30 min., and cooled to ambient temperature.

Acid activated clay (Euglehaudt's Filtrol Grade 160, chlorophyll specific) was added at 5% level. The slurry was agitated for 20 min. then the absorbant was removed by filtration. Only marginal color improvement was achieved.

- Extract II was slurried with Celite, then filtered. The pH of the filtrate was adjusted to about 7.0-7.1 with citric acid (10% solution). In order to partition stevioside (into the aqueous phase) pigment and the other sweeteners into the organic phase, an extraction with n-butanol was performed. The solvent was not selective enough for the pigment(s); accordingly the color improvement of the extract was marginal.

c) A pilot scale extraction (of reduced batch size) was performed, followed by electrolysis and filtration, to generate extract for further experiments. In parallel to the pilot work the following bench scale experiments were performed:

i) Samples of distilled water extracts from (a), (50 mL each at pHs of 7.0, 3.0, and 11.0 respectively) were each treated with 4.5 mL H_2O_2 (~ 30% solution) at 50° C, while stirring. No visible color improvement occurred. One mL of $AlCl_3$ (27% solution) was added, mixed well and allowed to stand for 30 min.. Subsequently the precipitate was filtered. The sample with pH = 7 showed a significant color improvement. There was no colorimeter or spectrophotometer to quantify improvement, so subjective (before and after) visual comparisons were made.

ii) The variant with the best response was repeated in 200 mL scale. The procedure from (c)i was expanded to include an absorbant treatment. The partially improved filtrate (H_2O_2, $AlCl_3$ treated) was warmed to 50-55° C, and 5% (by volume) activated carbon was added to it. The slurry was maintained for 30 min. at this temperature with occasional stirring. Following filtration a further improvement of the color was observed.

iii) Pilot plant produced extract that had been subjected to electrolysis (4V for 30 min., pH = 3) and filtered, was processed in the lab as follows:

- Citric acid (~ 2000 ppm wt basis) was added as chelating agent,
- Absorptive clay was added at 5% (wt) level
- Slurry was filtered

The resulting filtrate had a color equivalent to those samples that are usually obtained after passing through the cation exchange resin column. In other words, the belief in the necessity of ion exchange for color improvement started to be questioned.

d) Pilot plant produced filtered crude extract was treated with $NaAlO_2$ and filtered. Subsequently the filtrate was treated with 5% activated carbon for 5 min. and filtered. The resulting filtrate appeared to be equivalent, if not better, to the material produced using electrolysis, followed by ion exchange resin treatment. Unfortunately no absolute values on color or residual trace metal content could be assessed. Examples of treated solutions are pictorialy shown in the photograph shown in Fig. 2.

e) The experiment was performed in order to demonstrate that pure stevioside can be crystallized from relatively impure stevia extract. Dry stevia leaves (12% moisture) were ground to pass a 0.3 mm mesh size. The leaves were extracted with hot methanol (1:3 leaves:methanol) for 2 h. The extract was desolventised and then dissolved in water. The aqueous solution was extracted with n-butanol and the aqueous layer dried. The dried solids were dissolved in methanol (1:5, solids:mehtanol) and cooled to 0-5^0 C for 5 days. The crystals formed were filtered and dried. TLC examination of the crystals (dissolved in methanol) showed them to be pure stevioside (Fig. 3). This work demonstrated that, it was

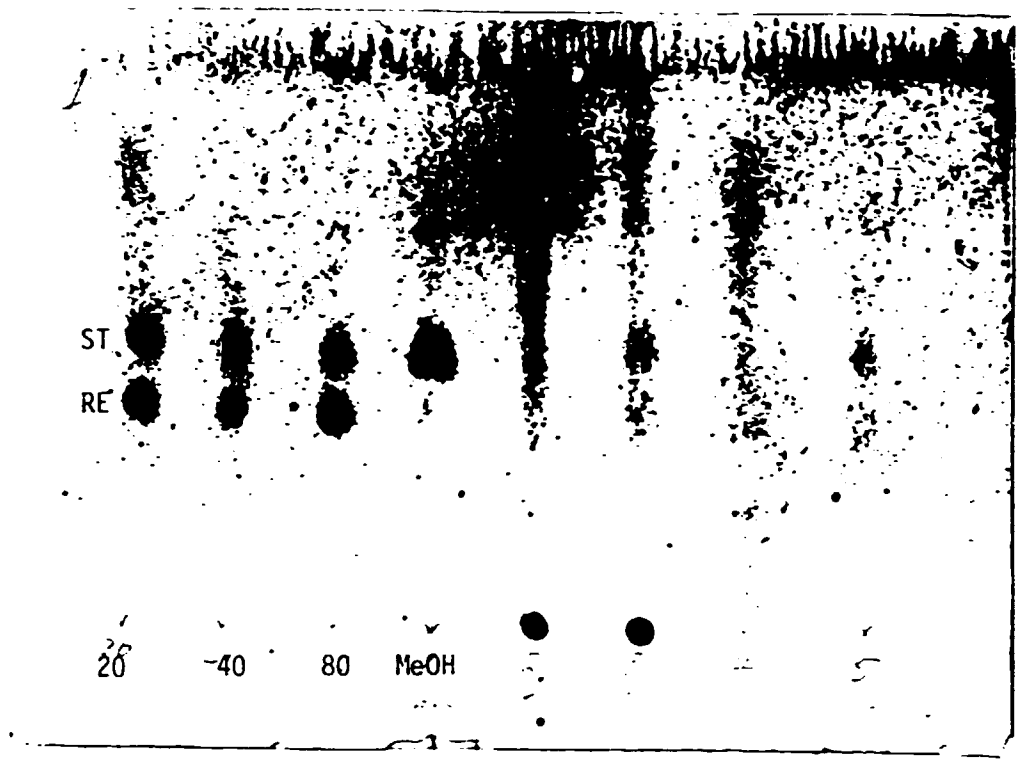
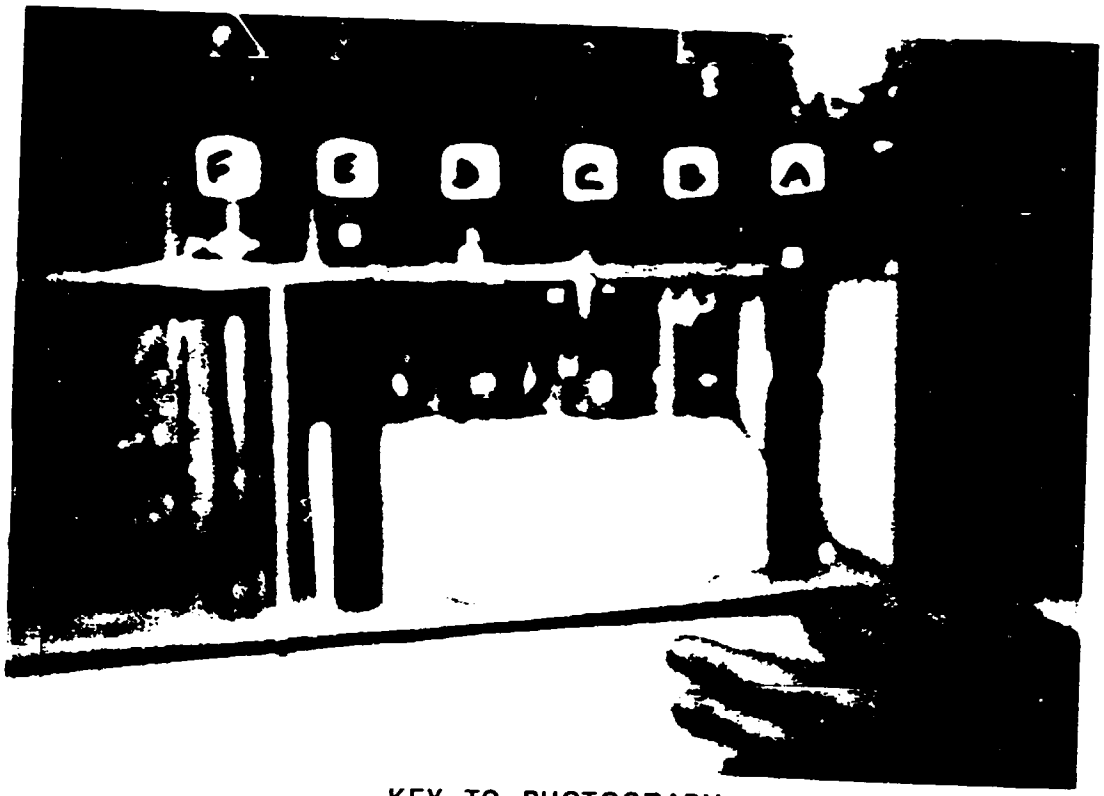


Fig. 3. A photocopy of TLC plate showing the purity of Stevioside Crystallized from relatively impure methanol extract.

ST, Stevioside, RE, Rebaudioside A.



KEY TO PHOTOGRAPH

A- Filtered Extract after Electrolysis.

B - Hydrogen peroxide Treated + Activated Carbon.

C - Na Aluminate Treated + Activated Carbon.

D - Lime Treated.

E - Lime Treated from Sam Suk.

F - Electrolysed and Ion- Exchange Treated.

Fig. 3. Photograph showing the color of samples treated by various methods.

not essential to pass the decolored, demineralized extract through HP-20 resins before crystallizing. Pure stevioside can be crystallized from the relatively impure extract. This would save the Institute the much needed foreign exchange which is needed for importing the resins.

Remarks:

- The descriptions given here are condensed for the sake of brevity. Detailed records (with volumes, proportions, pH values, DS contents, treatment sequences) of every experiment are in the possession of FRI with the proper annotations in Korean language (see Appendix III).
- The current FRI pilot scale process (aqueous extraction, electrolysis, trace metal/pigment removal) while workable, leaves a lot of questions unanswered. One can say for sure that solvent to solids ratio, temperatures, residence time in electrolyser, selection/efficiency of trace metal, pigment removal are not optimal. Optimization is needed for the individual unit operations.
- The screening tests presented in this report were performed with the intention of finding a cost effective alternative process, less dependent on imported ingredients. Without substantial optimization work, the likelihood of an efficient scaled-up version (pilot or industrial) of the process is remote.
- Prototype "products" have to be qualified as much as possible. Without target specification, any design choice may fall short of expectations when the finished products are ultimately defined!

  Example: The pilot plant should be able to produce as the result of 16-24 hours of operation:

  a, x kg of food grade (8%) solution of stevia sweetener

  b, y kg of food grade (20%) solution of stevia sweetener

  c, z kg of food grade powdered stevia sweetener

d. z' kg of food grade purified stevioside, of specific quality

e. q kg of alphaside if desired sweetness and quality

f. q kg of Rebaudioside A.

The tested process alternatives clearly demonstrated that there are other potential alternatives which are less dependent on imported resins. The most promising alternatives worth further studies before a process design principle can be formulated are the hydrogen peroxide, $AlCl_3$ treatment and the sodium aluminate treatment with subsequent activated carbon treatment.

5.0 PROCESS/DESIGN/CONSIDERATIONS

It is assumed here that a flexible, multipurpose unit, capable of producing a variety of intermediary products fits the mandate of FRI. It is further assumed that some components of the existing pilot plant can be used on and off for unit operations required in the overall process.

In order to avoid duplicating existing capability (aqueous extraction) 1000 L size batches (5, 45 gallon steel drums) of extract should be shipped form SAM SUK to FRI for pilot work. This material should be generated from leaves sprayed with water as dust supressant, should be free of solid impurities and have a DS content of about 5-6% or sweetener content of ~ 1.5%. (If the SAM-SUK facility is rehabilitated-(stainless steel extractor)-or the improved genetic variety of stevia containing 10-12% sweetener becomes commercially available, it is an added advantage.)

These are three process flow diagrams to be considered:

	A		B		C
	Filtered, crude extract, 1000 L (DS 5-6%, sweetener 1.5%)				
I	Electrolyses pH 3-6,4V,4A, 1/2 hour	I'	H_2O_2 treatment $AlCl_3$ "treatment" Reactor	I"	$NaAlO_2$ treatment pH = 7 Filter aid/Reactor
II	Filtration, 50°C	II'	Plate & Frame Filter	II"	Plate & Frame filter
	Filtrate, D.S~4.5%		Filtrate, DS~4.5%		Filtrate, DS~4.5%
III	IR 120 Resin	I'	Citric/Acetic Treatment, Activated Carbon Reactor	I"	Chelating Agent Activated Carbon Reactor
IV	IR 45 Resin				
		II'	Plate & Frame Filter	II"	Plate & Frame filter
	Sweetener Solution (DS~2.3, Sweetener 1.4%)		Sweetener solution (DS~2.3, Sweetener 1.4%)		Sweetener solution (DS~2.3, Sweetener 1.4%)
V	Evaporator	V'	- as V-	V"	- as V -
	Sweetener stocks: 10% 20% 50%				
VI	Spray Dryer	VI'	- as VI -	VI"	- as VI-
	Powdered sweetener(1) MeOH 6:1				
VII	Crystallyzer stevioside(2)	VII'	- as VII -	VII"	- as VII -
	Rebaudioside A"(3)				

The total output of the pilot plant is approximately 10.0 kg of powdered sweetener with the following contemplated composition:

Stevioside:	6.7 kg
Rebaudioside A:	1.1 kg
Steviolbioside:	0.44 kg
Dulcoside B:	1.10 kg
Dulcoside B:	0.66 kg

Following crystallization out of methanol/selective removal/retention on Diaion HP-20 resin about 6.0 kg purified stevioside and 1.0 kg of rebaudioside A can be produced. If after further testing alternative A is the preferred method, every unit operation with the exception of II (plate and frame filter available) will have to be provided for with adequately scaled up equipment. In other words, every step would require capital expenditure/import of materials.

Alternatives B, C need to be optimized (find the best response with the least treatment) yields need to be confirmed by reliable analytical methods. For both alternatives, there is equipment in place for steps I', II', I", II". (I', I" jacketed reactor, V = 1.4 m^3, anchor agitator at 120 rpm made of Cr/Ni/Ti (1, 18, 9%), provided with 4.5 Kw motor. Inside jacket pressure rated for 2 atm). II', II" Plate and frame filter, that has a surface of ~4.8 m^2 and can hold ~ 60 L solids.

If further laboratory studies substantiate the preliminary findings described in the previous chapter, the expenditures can be concentrated on items V, VI, VII. (Evaporator, spray dryer, crystallizer) Therefore, it is essential that FRI further investigates alternatives B & C and based on that data make their process option decision. At this point in time, we have unconfirmed trends, regarding the alternative procedures. It is FRI's internal decision whether they intent to pursue investigations along these

lines to arrive at the most appropriate process option mindful of the foreign exchange constraints in the country.

5.1. Unit operations - Equipment requirement

The pilot process should be defined in terms of powdered sweetener mix. An output of 10.0 kg per test was deemed adequate from the point of view of scaleability (to industrial level) and amount of refined fractions that can be derived from it for the purpose of test marketing, and tests in specific product formulations.

The pilot scale unit operations will be executed stagewise in a sequential manner, but these can be integrated into a fully continuous process when replicated in full scale. To avoid redundancy, no provision is made here for the extraction operation. Extract would be requested from the SAM-SUK facility, 1000 L for each planned test run.

5.1.1. I. Precipitation of proteins/Depigmentation

I.A. Electrolyser Tank of continuous design (flow rates in/out are equal) providing 30 min. residence time (optimized?). Volume - 100 L, output 200 L/h. Fitted with Aluminum electrodes, connected to an appropriate DC supply. This unit will process the 1000 L batch in five (5) hours. (To be designed/fabricated tested) Requires feed/discharge pumps to the plate and frame filter.

I.B. Hydrogen peroxide/Aluminum chloride treatment conditions yet to be optimized in FRI lab. Jacketed reactor vessel with agitator power drive, built of corrosive resistant material. Reactor (4 in Appendix I) of suitable design is available in pilot plant. V = 1.4 m^3, provided with anchor agitator, motor, reduction gear delivering 120 rpm. (Needs

thermometer, sighted glasses, and insulation) Need loading and discharge/feed pump piped to plate and frame filter.

I.C. $NaAlO_2$ treatment at pH = 7.

Adequate vessel is the same as described under I.B.

5.1.2. II FILTRATION of precipitated proteins/pigments

For all three versions (A,B,C) the existing plate and frame filter is adequate. Can hold up to 60 L sludge and has a surface area of 4.8 m^2.

Need filter cloth or paper, flexible hose connection to discharge pump of electrolyser or reactor.

5.1.3. III,IV Trace Metal Scavenging/Decolorization

In alternative A two separate steps are required. In both, the solution from II is passed through approximately 140 L of anion and cation exchange resins. For continuous operation in industrial scale two columns of each resin would be required: one onstream one regenerating.

Required: Stainless steel columns, flow meters (2) for measuring inflow/regenerating solution flow, pump with variable speed drive, flow totalizing counter.

In alternative B & C a chelating agent, then activated carbon is added to the filtrate and pumped from filter (8, Appendix I) into jacketed reactor (5,Appendix,I), identical to 4 described under IB,C. In the reactor chelating/pigment adsorption is completed. Filtration-operation IV B & C is effected on P & F filter (8, Appendix I). Eluate from III & IV A or filtrate from IV B & C is pumped to vessel (12, Appendix I), which can serve as feed tank.

5.1.4. V. Concentration by Evaporation

Required: Forced film evaporator complete with feed pump flow meter, instrumentation, vapour handling system (liquid ring vacuum

pump/barometric condenser rated for removal of 150-200 L/hour water.

In this unit sweetener stocks of 10, 20, 50% concentration can be generated. The 10% solution is suitable to be used as feed stock for the DIAION HP 20 column. The 50% solution could serve as feed to the spray dryer.

5.1.5. VI. Spray Dryer

The input material for this unit operation is nondescriptive regarding its properties. It is assumed that the forced film evaporator will be capable of producing a suitable input, say 40-60% D.S. that will be sufficiently qualified to allow for the selection of the atomizer system/design.

Since it is a small unit (approximately 15-25 L/h water removal) a one fan system is recommended. Heater choice is guided by (local) economics; the least cost version is electrically heated but it is more costly in operation than comparably sized gas fired units.

6.0 EVALUATION OF POTENTIAL FABRICATION FACILITIES/MATERIALS

A) MAN GYONG DAE in Pyongyang - Director Mr. KIM KWANG OB

According to Mr. KIM, the facility is capable to work from concept-through design-fabrication. The firm has prior experience in manufacturing pressure vessels, up to 150 atm, if needed.

A tour of the facility revealed that the activity is focused on gearbox manufacturing-finishing the cast iron/steel housing, machining gears, assembling, and painting. When asked where the vessel fabrication (cut, roll, form and weld) is taking place, the response was that such operations are subcontracted to small facilities. Thus during the visit we could not ascertain whether the firm has or has not the equipment needed for stainless steel welding.

B) DAEAN - Heavy Machinery Production Complex

Vice Managing Director - Mr. JONG HI CHANG

The facility is impressive, mainly geared towards fabrication of power plant components: steam turbines, generators, casings and shafts. We toured the fabrication shop where cylindical vessels with elliptical bottoms were in different phases of fabrication at the time of the visit. There was no indication of availability of stainless steel welding equipment. The main thrust seems to be components for the country's electrification program, in other words, mega projects. The equipment needed at FRI if contracted for fabrication by this firm, would have to compete (execution priority) with the mega project components.

Those materials required for the equipment sought by FRI, Stainless steel (SS) 302 (tanks, pipes, general chemical process equipment) SS 304 (heat exchanger tubing) SS 304 L (improved corrosion resistance), are not available in the country. The welding rods, welding apparatus are also lacking. The tools, expertise required to fabricate SS equipment with food grade/sanitary finish, that permits thorough cleaning, eliminates spots for microbial growth, are not available. The most likely solution to the needs of FRI is to import the key components to ensure that a model for standard is available, from which the R & D output could be transferred to industry for implementation.

7.0 GENERAL DISCUSSION

In relating our observations made on site to the output requirements, inputs, experts fielding and the budgetary breakdown made in the project directive, it becomes apparent that some erroneous assumptions were made during the preparation of the document. For example it seems, and understandably so, that the availability of basic standard glassware for a normal working laboratory at FRI was assumed. Similarly the

availability of a refrigeration plant, vacuum system, compressed air in the existing pilot plant at FRI was probably assumed. These, evidently important, fundamental equipment or service requirements are not mentioned in the project document. However, for a fruitful execution of the project it is imperative that these tools (basic laboratory glassware and equipment) and pilot plant service requirements be provided.

As previously discussed in this report, probably due to lack of proper laboratory glassware and analytical tools, the process parameters for stevia processing currently used at FRI are not properly optimized. In most cases processing factors have been studied one at a time and their effect pooled. In proper optimization work such an approach is not only unsatisfactory because of cost efficiency but it does not normally yield the desired results. Most process conditions assessed in this manner are later on found to be of suboptimal conditions when evaluated using alternative approaches such as factorial designs, EVOP, response surfaces, simplex or supersimplex optimization designs. To effectively optimize the process conditions in the various unit operations for the stevia processing consideration should be given to using some of these efficient and cost effective optimization approaches.

In most of these optimization designs, the use of micro-computers or personal computers become necessary. The pertinent process factors are normally considered simultaneously, thus, the statistical analysis of the resulting responses become complex. In the light of this an appropriate personal computer would be necessary for proper execution of this phase of the project.

Some specific input requirements seem not to be pertinently important for the project. For example laboratory equipment such as Gas chromotograph and IR spectrophotometer have been listed as requirements. One would expect these equipment

to be available in any functional Food Research Institute but their particular role as analytical or developmental tools in the stevia project is questionable. Also, need for training seems to have been overemphasized possibly based on an assumption that trained local expertise is seriously lacking. We have, on the other hand, observed that the local scientists are adequately trained. What is lacking is their exposure to modern equipment and some minimum training on these analytical equipment and control devices. In most cases we assess that a study tour or a few months (6) fellowship would suffice.

The requirement of an expert microbiologist seems to have been overemphasized. It was probably assumed that FRI was screening micro-organisms for their transglucosylation work. Our observation indicates that various micro-organisms (3) have already been isolated and one of them (Bacillus sp. #825) yields the required enzyme for the transglucosylation. What seems to be lacking is the enzymology aspect of the process; purifying the enzyme, stabilizing the enzyme, possibly immobilizing the enzyme for repeated applications and optimizing the process conditions. A microbiologist with industrial enzymology background can surely fulfill this task but it is not restrictive that a microbiologist should perform the task. A biochemist, food technologist or food scientist with experience in industrial enzymology and process optimization can expertly handle that task.

The budgetary requirements of the project as outlined in the project document seems to be severely strained. The specific area which seems to be grossly underbudgeted is the allocation for the pilot plant equipment. The possible reasons for this may include: (1) The assumption made about availability of necessary service facilities in the existing pilot plant. (2) The document was prepared about two years ago and since that period there have been significant changes in the dollar value as well

as inflational cost in pilot plant and laboratory equipment. For a successful execution of the project it seems that this budget would have to be reviewed with the aim of increasing it to reflect the current situation. To minimize the additional expenditure needed for the project, it would be desirable to consider obtaining used pilot plant equipment refurbished to their original specifications. Such equipment are normally available in the used equipment market for a fraction of the cost of a new unit. Also some reshuffling could be made to transfer funds from areas where the budget seems to be proportionately over estimated to those where they are under budgeted.

8.0 CONCLUSIONS

We conclude from the observations and work done during this mission that;

a) The initiated project is an important one that needs to be supported. The anticipated results are attainable if proper attention, support and management is provided.

b) The basic ingredients (raw materials and personnel) seem tc be available for proper execution of the project. What is lacking is the proper equipment (laboratory and pilot plant) to expedite the duties. Without basic lab equipment, FRI cannot support the project with bench top optimization work which is needed prior to pilot plant design.

c) The present FRI process is workable but dependent on key imported expendables which, due to foreign exchange constraints, cannot be nationally supported. Also process alternatives have not been fully explored. A workable process less dependent on imported expendables would be desirable.

d) The budget (UNIDO/UNDP) component-elements seem to be oddly proportioned due to perhaps too many incorrect assumptions. Also inflational effects necessitates revision of the budget.

9.0 RECOMMENDATIONS

9.1 Laboratory

a) Provide in shortest possible time the FRI chemistry labs with the minimum but essential equipment that will permit them to become a working lab as opposed to a struggling one. (General chemistry lab inventory-to be provided by UNDP) Details are provided in the Appendix IV.

b) Expedite selection, acquisition of analytical equipment to be able to support lab/ pilot scale R & D with:

- Thin layer chromatography
- Rod chromatography
- High pressure liquid chromatography
- A computer (PC) with printer for process optimization.

Details are provided in the Appendix IV (UNDP).

c) Develop an R & D programme (supported by a, b) that will evaluate alternative process technology, before final commitment is made toward design/acquisition of a pilot plant equipment. (FRI)

9.2. Pilot Plant Equipment

a) The improved capabilities resulting from the implementation of recommend-ations (a) and (b) will enable FRI to finalize the screening process alternatives initiated during this mission. additional information generated will support

the pilot process principle selected for implementation. At this time, this mission has concluded that the following can be recommended for the pilot plant.

- Forced film evaporator of stainless steel (food grade finish) construction, capable of evaporating 150-200 L/hr water, inclusive feed/ vacuum/condensor system. This unit operation will produce sweetener stock solution of 10, 20 and 50% concentrations. (UNDP)
- Spray dryer in s.s., food grade finish, capable of removing 20-30 L/hr water from incoming 40-50% sweetener stock solution, and producing a sweetener powder with 3-5% moisture. In order to properly select the atomizer, collector bags, etc., viscosity measurements are needed for the 10-60% range of solutions and particle size distribution analysis for the dried solids. (UNDP)
- Crystallizer (s.s.) of 100 L capacity, explosion proof design for the fractional crystallization of stevioside from spray dried sweetener : methanol (1:6) solution.

  Preliminary data indicated 90% recovery of input stevioside with a purity of 80%, when crystallizing at $10^{o}C$ for 36 hr. (This is an alternative procedure of the Diaion HP-20 retention/removal of stevioside) (UNDP)
- Pressure leaf filter/feed pump to separate PSE from mother liquor. (Mother liquor is recycled for crystallization of rebaudioside A). (UNDP)
- Vacuum tray dryer for desolventization of stevioside and rebaudioside A (methanol laden) crystals. (UNDP)

The front end of the pilot process is an open question and can be answered only upon completion of the screening experiments by FRI. Should it turn out that

alternative A (i.e. the current processing method) is the only viable solution the following equipment would be required:

- Electrolyzer of continuous flow design of 200 L/hr capacity, made of stainless steel, complete with feed and discharge pumps provided with variable speed drives, DC power source thermometer and electrodes. (UNDP)
- Two ion exchange resin columns of about 150 L capacity complete with resin, piped to regeneration system, consisting of feed tanks for 6% HCl, 2% NaOH, with pumps to deliver 1 L/100 g wet resin in case of the former and 1 L/130 g wet resin in the case of the latter. For a continuous operation, four columns will be required: 2 on stream, 2 in regeneration cycle/standby. (UNDP)

b) Consider the long term use of the pilot plant generated data. It has to be solid enough to be used in the design of full scale facilities for diverse end products.

9.3 Budgetary Recommendations

Due to budget constraints the following trade offs need to be considered:

- The pilot plant is going to operate using crude extract of an agreed upon acceptable quality from SAM SUK. This would permit deleting the cost of a pilot scale extractor. But in order to have extract, FRI will need a truck to haul 5-10 drums (200 L fresh extract each) for testing, when required. (DPRK)
- The pilot facility must be given a face lift consisting of:
  - a) Proper finish of the floors
  - b) Pest/insect control
  - c) Upgrading the electrical works to explosion proof standards. (DPRK)
- The pilot plant must be provided with a refrigeration unit (for crystallization)

and a source of compressed air (to blow P & F filter and pressure leaf filter). (DPRK)

- Budget provisions 11-01, 11-02 (currently available 8 m/m) should be pooled and allocated to process engineer specialized in organic technology. This could lead to saving of 2 m/m. Of the remaining 6 m/m one is to be allocated for project engineering, offsite.

Approximately US $20,000 can be transferred to 41-00.

Budget provisions 11-51, 11-52, should be pooled and covered by industrial microbiologist, biochemist or food scientist, specialized in enzymatic processing and with strong analytical background. This could save 1 m/m and allow the transfer of US $10,000 to 41-00.

Budget item 31-00 should be reduced proportionately with the project size. Pilot design does not include self tuning adaptive PID loops, digital or supervisory process control, just indestructible "military specs". Therefore the process control 8 m/m is rated as "overkill". Microbiology training is an inflated item, considering the project goals (8 m/m). These two items could be replaced by a 6 months fellowship at CFTRI, MYSORE INDIA, which is a reputable facility for graduate studies, in ENGLISH.

Estimated cost: US $21-26,000.

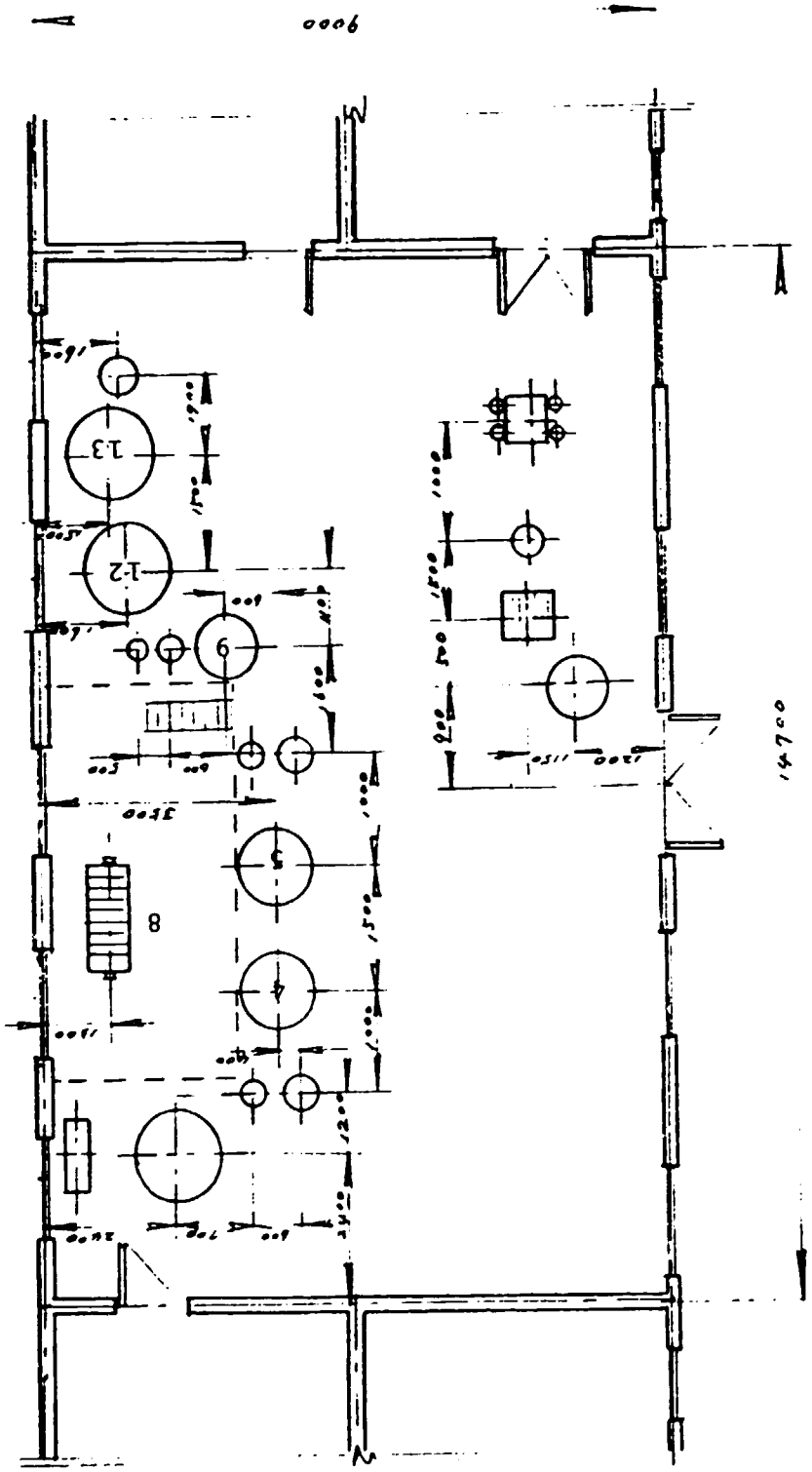
The US $30,000/saved should be transferred to item 41-00.

The rehashed budget would total US $120,000 for item 41-00, which though still relatively low, may be adequate for the purpose especially if used equipment are considered.

Budget item 32-00 should be scheduled as soon as possible, the obvious destination being Peoples Republic of China. This may also result in some cost savings as the original budget seems to have been tailored for Japan. Any such saving should be transferred to 41-00 since this is grossly under budgeted in the Project Document.

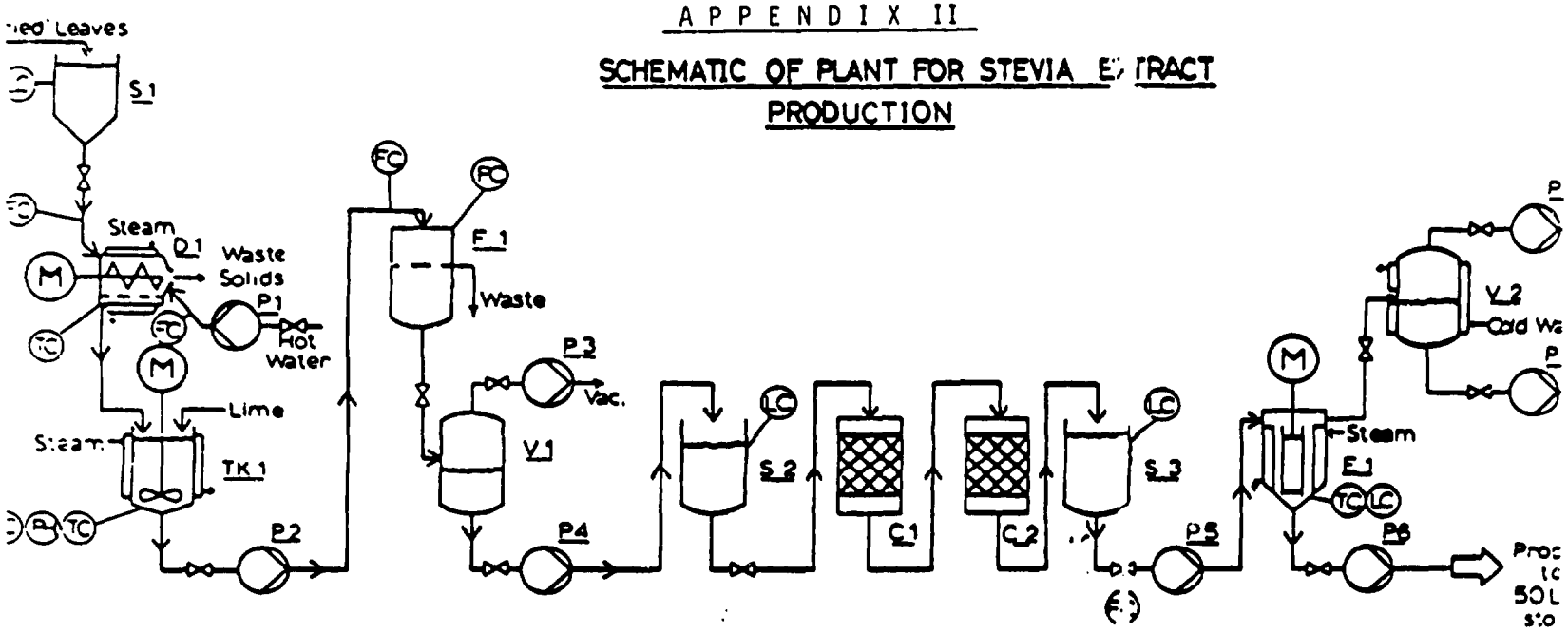
APPENDIX I

FRI Lay Out



APPENDIX II

SCHEMATIC OF PLANT FOR STEVIA EXTRACT PRODUCTION



UNIT NUMBER	S 1-3	D 1	TK 1	F 1	V1-2	C 2	E 1	P1-6
NAME	Storage	Screw-type Extractor	Tank	Filter	Vac. Receivers	Columns	Evaporator	Pumps
TECHNICAL DATA	S1-Feedrate to process-150 kg/h S2-3-sized for continuous operation	Q = 5-600 L/h T = 60°C ID = 60cm V = 1000 L	V = 2000 L T = 40°C pH = 10	Cloth type Q = 5-600L/h Batch	P < 1 atm.	Q = 50 L/h	Feed = 1% pure Prod = 10% --- T = 80°C P < 1 atm.	Various
CONTENTS	S1 - Leaves S2-3 - Extract	Leaf-Water mix	Lime digestion of Extract	Solid waste & Extract filtrate	V1-Extract, filt. V2-Evap.condat	C-1 C ion IEX C-2 A ion IEX	Extract Concentrate	Various
NOTES	1) Not to scale. 2) No recycles or stream heat exchange shown. 3) Duplicate tanks (eg TK1) not shown. 4) D1 is operated at 45° to horizontal. 5) Column regeneration not shown. 6) Column pumping may be needed.							

APPENDIX III

Alternate Approaches for Stevia Processing Evaluated During the Mission

(Flow chart - Written in Korean by National Counterpart)

FRI 방법-1

밀 5 kg W 13.4%

+물 40 l

-휴산칼슘 5 g

우리기 (60)°c (55-65), 90 min, 교반, 1차

DM 3.4%, pH 6.7 — W 76.2%

우림액 29.5 l — 우림찌꺼 13.44 kg

27% + HCl 110 ml

pH 3.0 조절

전기화학정제 50°c (44), 30 min, 5 V (4)

여과

전정 여액 27 l, DM 2.5%, pH 4.9 — 정제찌꺼 4.7 kg, W 91%

양수지정제 SK-111, V_R 4 l, SV 1

양수정 당액 l, DM %, pH

음수지정제 AD-41, V_R 4 l, SV 1

음수정 당액 28 l, DM 1.2%, pH 9

당흡착 HP-20, V_R 4 l, SV 1

흡착당여량 l

+ 75% (50%) EtOH 1/3 배용-R

당탈착 SV 0.5

탈착당액 l

증류

농축건고 — 회수알콜 % l

엑스 g, W %

+ 95% MeOH 1 : 6

용해 60~65°c

결정화 10°c, 2 h (교반) - 시결정

분리

+ 냉MeOH l

씻기

+물 25 l

2차우리기 60°c, 90 min, 교반

찌꺼 W % kg — 2차우림액 DM pH

+물 25 l

3차우리기

찌꺼 W % kg — 3차우림액 DM pH

+물 l

씻기

정제찌꺼 씻은물 l, DM %

씻기 +물 12 l — 양수지씻은물 l, DM %

씻기 +물 12 l — 음수지씻은물 l, DM %

씻기 +물 4 l — 흡착수지씻은물 l, DM %

씻기 +물 8 l — 흡착수지씻은물 l, DM %

ST결정모액 l

결정화 5°c, 6 h (교반) - 시결정

분리 — 결정모액 l

+ 냉MeOH l

씻기 — 증류

건조 100°c — 농축건고 g

MO결정 g

JARLOS

No. 1

식물종
일(가루 0.3mm) 250 g
| + MeOH 750 g = 960 ml
추출(환류) 2 h
여과 ——— 추출액 1.050 g
290 ml
농축 잔액 잔사 70 g
| + 물 140 ml
현탁
| + 디에틸에테르 70 g = 95 ml
잇기
분별 ———
| |
물층 195 ml 에테르층 ml
| + n-BuOH 200 g = 255 ml
추출 (실온, 10 min, 1회)
분별 ———
| |
BuOH층 375 ml 물층 83 ml
| |
농축건고 DM 50 g 농축건고
| + MeOH 1:5 에 250 g (310 ml) |
용해 잔사 g
|
결정화 0~5 °C
하루밤(16 h) 5.5 d
|
분리 ——— 결정모액 324 ml, DM
|
씻기 냉 MeOH 100 ml
|
건조 100 °C
|
ST
결정 4.9 g 3.23 g. mp 0.4 °C
무색 침상

yaw

No. 2

Sam Suk.

석회정제액(공장) 200 ml pH 10.8 DM

↓ + 폐황산

pH조절 pH 7.1 → pH 3.0 (10% 산 4 ml, 15% 산 30 ")

↓ + FILTROL 160 10 g

전제 고반 5 min, 정치 5 ", 고반 5 "

↓

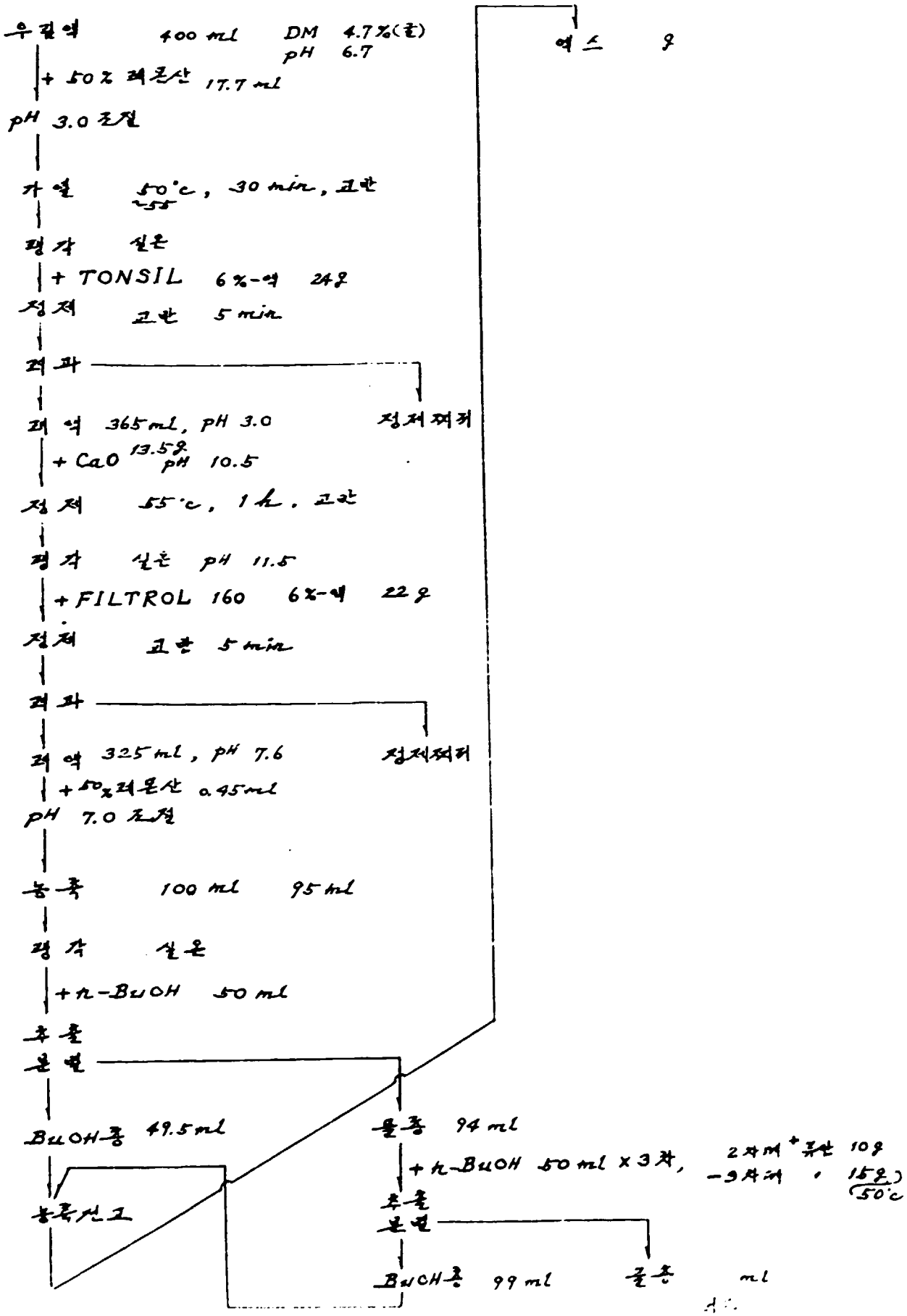
여과 ——— 정제찌꺼기

↓

여액 170 ml

yaw

No. 3


우질액 400 ml DM 4.7%(중)
pH 6.7
+ 50% 레몬산 17.7 ml
pH 3.0 조절
가열 50°c, 30 min, 교반
~55
냉각 실온
+ TONSIL 6%-액 24g
정제 교반 5 min
여과
여액 365 ml, pH 3.0
+ CaO 13.5g pH 10.5
정제 55°c, 1 h, 교반
냉각 실온 pH 11.5
+ FILTROL 160 6%-액 22g
정제 교반 5 min
여과
여액 325 ml, pH 7.6
+ 50% 레몬산 0.45 ml
pH 7.0 조절
농축 100 ml 95 ml
냉각 실온
+ n-BuOH 50 ml
추출
분별
BuOH층 49.5 ml
물층 94 ml
+ n-BuOH 50 ml x 3차,
농축건고
추출
분별
BuOH층 99 ml
물층 ml
여스 g
정제제거
정제제거
2차에 + 규산 10g
3차에 " 15g
50°c

yaw

No. 4

우질액 (일 500 g) 400 mL, DM 4.7 %, pH 6.7

↓

진공농축 (1/4 V) 100 mL, DM 21.2 %

| + 50% 황산 11.3 mL

pH 3.0 조절

↓

가열 50°C, 30 min, 교반

↓

냉각 20 ~ 25°C

| + 규조토 5 ~ 6 % 5 g

↓

여과 ——— 정제찌꺼

↓

여액 ≒ 100 mL, pH 3.2

↓

가열 50°C

| + CaO pH 10.5 7.9 g

↓

가열 70 ~ 80°C, 15 min, 교반

↓

냉각 20 ~ 25°C

| + 규조토 5 ~ 6 % 5 g

↓

여과 ——— 정제찌꺼

↓

여액 33 mL, pH

| + n-BuOH 50 mL

↓

추출

분별 ———

↓

BuOH층 ___ mL

물층 mL

↓

농축건고

g

| + MeOH 1 : 2

↓

용해

↓

전정화

↓

분리 ——— 전정오액 mL

↓

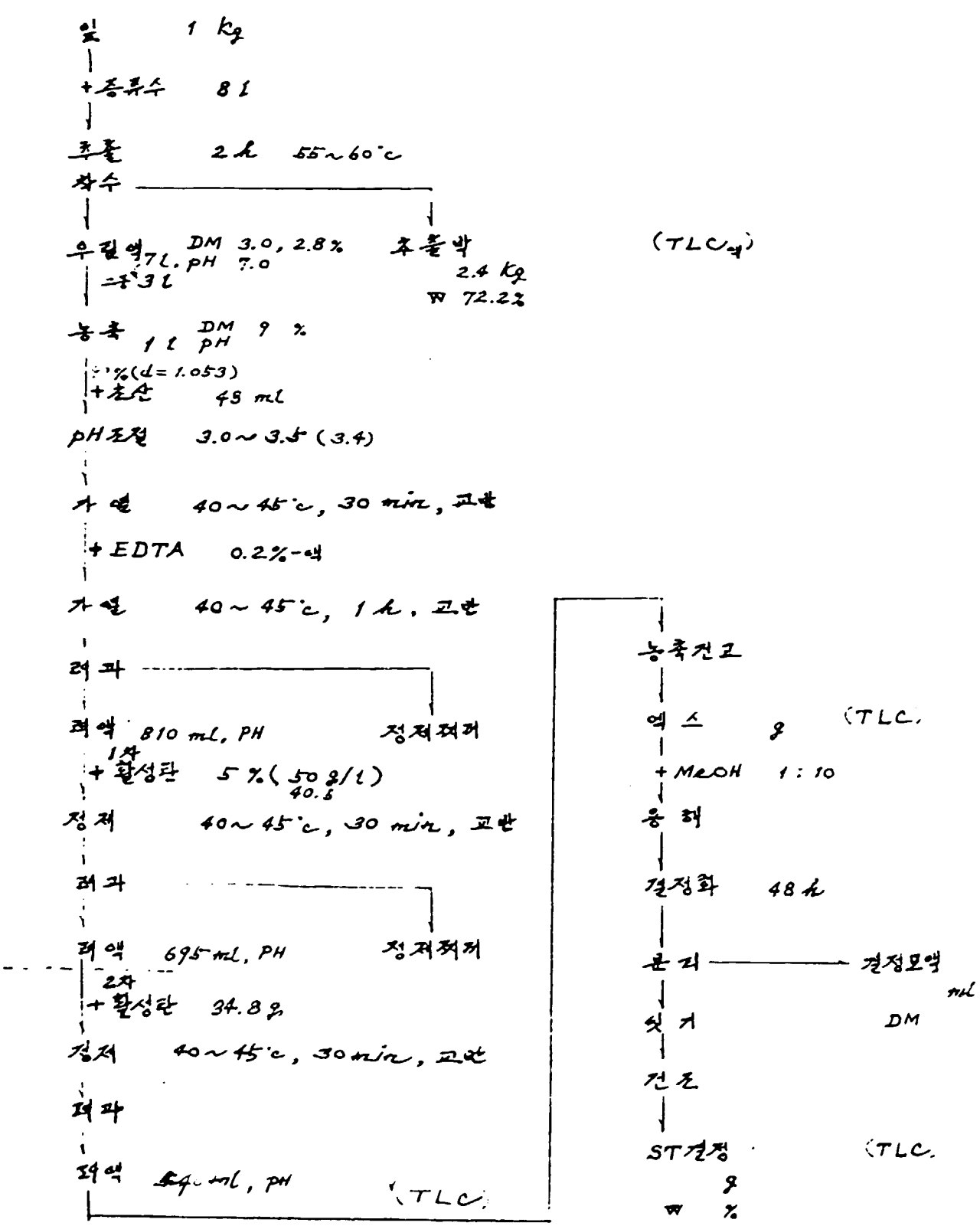
실기

↓

전조

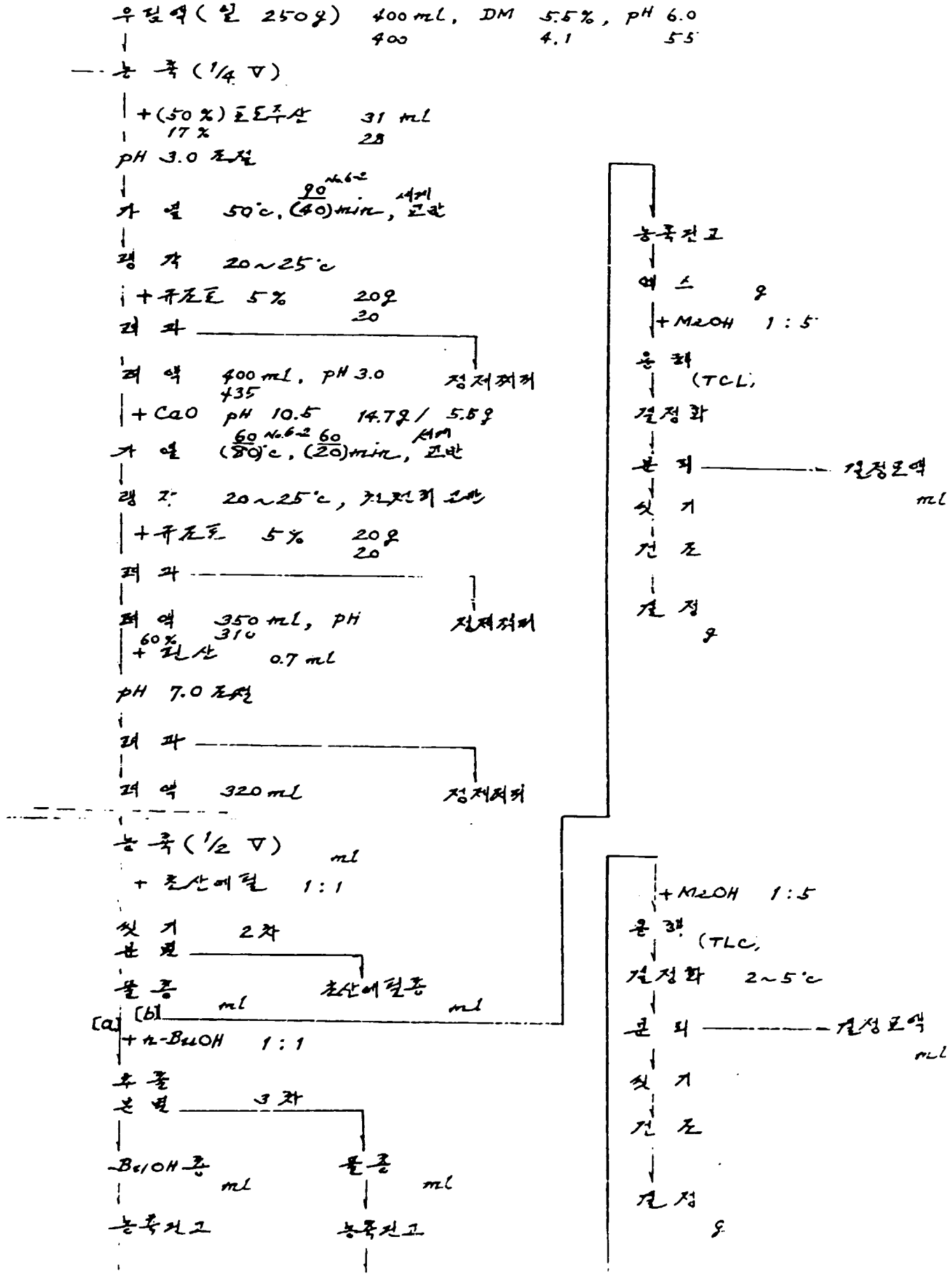
Janos

No. 5


잎 1 kg
+증류수 8 l
추출 2 h 55~60°c
착수
우림액 7 l. DM 3.0, 2.8%
pH 7.0
농축 3 l
추출박 2.4 kg ₩ 72.2%
(TLC액)
농축 1 l DM 9 %
pH
%(d=1.053)
+초산 48 ml
pH조절 3.0~3.5 (3.4)
가열 40~45°c, 30 min, 교반
+EDTA 0.2%-액
가열 40~45°c, 1 h, 교반
여과
여액 810 ml, PH
정제처리
+활성탄 1차 5 %(50 g/l) 40.5
정제 40~45°c, 30 min, 교반
여과
여액 695 ml, PH
정제처리
+활성탄 2차 34.8 g
정제 40~45°c, 30 min, 교반
여과
여액 ml, pH
(TLC)
농축건고
엑스 g
(TLC)
+MeOH 1 : 10
용해
결정화 48 h
분리
결정모액 ml
씻기 DM
건조
ST결정
(TLC)
g
₩ %

yaw

No. 6-1, 2


우질액 (일 250g) 400 ml, DM 5.5%, pH 6.0
400 4.1 5.5
농축 (1/4 V)
+ (50%) 포도주산 31 ml
17% 28
pH 3.0 조절
가열 50°C, (40) min, 서서히 교반
No.6-2
냉각 20~25°C
+ 규조토 5% 20g
20
여과
여액 400 ml, pH 3.0
435
정제처리
+ CaO pH 10.5 14.7g / 5.5g
가열 (80)°C, (20) min, 서서히 교반
No.6-2 60 60
냉각 20~25°C, 서서히 교반
+ 규조토 5% 20g
20
여과
여액 350 ml, pH
310
정제처리
+ 60% 인산 0.7 ml
pH 7.0 조절
여과
여액 320 ml
정제처리
농축 (1/2 V)
ml
+ 초산에틸 1:1
씻기 2차
분별
물층
초산에틸층
ml
ml
[a]
[b]
+ n-BuOH 1:1
유출
분별 3차
BuOH층
ml
물층
ml
농축건고
농축건고
농축건고
예스 g
+ MeOH 1:5
용해
(TLC)
결정화
분리
결정모액
ml
씻기
건조
건정 g
+ MeOH 1:5
용해
(TLC)
결정화 2~5°C
분리
결정모액
ml
씻기
건조
건정 g

yaw

No. 7

우림액 (물 250g) 400 ml, DM 6 %, pH 5.8

농축 (1/4 V) ; ml

+ % 푸마르산 ml
(17 % 포도주산 32 ml)

pH 3.0 조절

가열 55°c, (90 40) min, 교반

냉각 20~25°c

+ 규조토 5% 20g

여과 ——————— 제거

여액 326 ml, pH 3.1

+ 30% NaOH 6 ml

pH 10.0 조절

가열 50°c, 30 min, 정치

냉각 20~25°c

+ 규조토 20g

여과 ——————— 제거

여액 378 ml, pH 9.6

+ 4% 황산카리AL 16 ml

pH 8.5 조절

가열 50°c, 30 min, 정치

냉각

여과 ——————— 제거

여액 382 ml, pH 8.2

+ % 레몬산 ml

pH 7.0 조절

농축 (1/2 V) ml

Jamos

No. 8 (정성)

우림액 DM 2.8%
pH 7.0
50 ml

↓ + 30% H_2O_2 4.5 ml, 적정, 교반
정제 50~55°C

↓ + HCl
pH 3.0 조절

↓ + 27% $AlCl_3$ 0.2% 1 ml
실온, 15 min, 교반
적과

↓
적액

↓ + NaOH
pH 6.0 조절
(7.0)

↓ + 활성탄 5% 2g
자연 50°C, ~~45~~ 30 min, 교반

↓
적과

↓
적액

yaw

No. 9

전처정제액 DM 2.5%, pH 4.9 200 ml

↓ + 생석회 CaO pH 10.5 0.15 g

가 열 80°c, 15 min, 상온

↓

냉 각 20~25°c

↓ + 규조토 5% 10 g

여 과

↓

여 액 158 ml

↓ + 피론산 20% 0.13 ml

pH 7.0 조정

↓

가 열 60°c, 30 min, 상온

↓ + 활성탄 10% 15 g

가 열 40°c, 5 min, 온만

↓

냉 각 20~25°c

↓ + 규조토 2% 3 g

여 과

↓

여 액 123 ml

yaw

No. 10

200 ml

0.13 g

10 g

150 ml

+ 주석산 17% 0.15 ml

tartaric acid

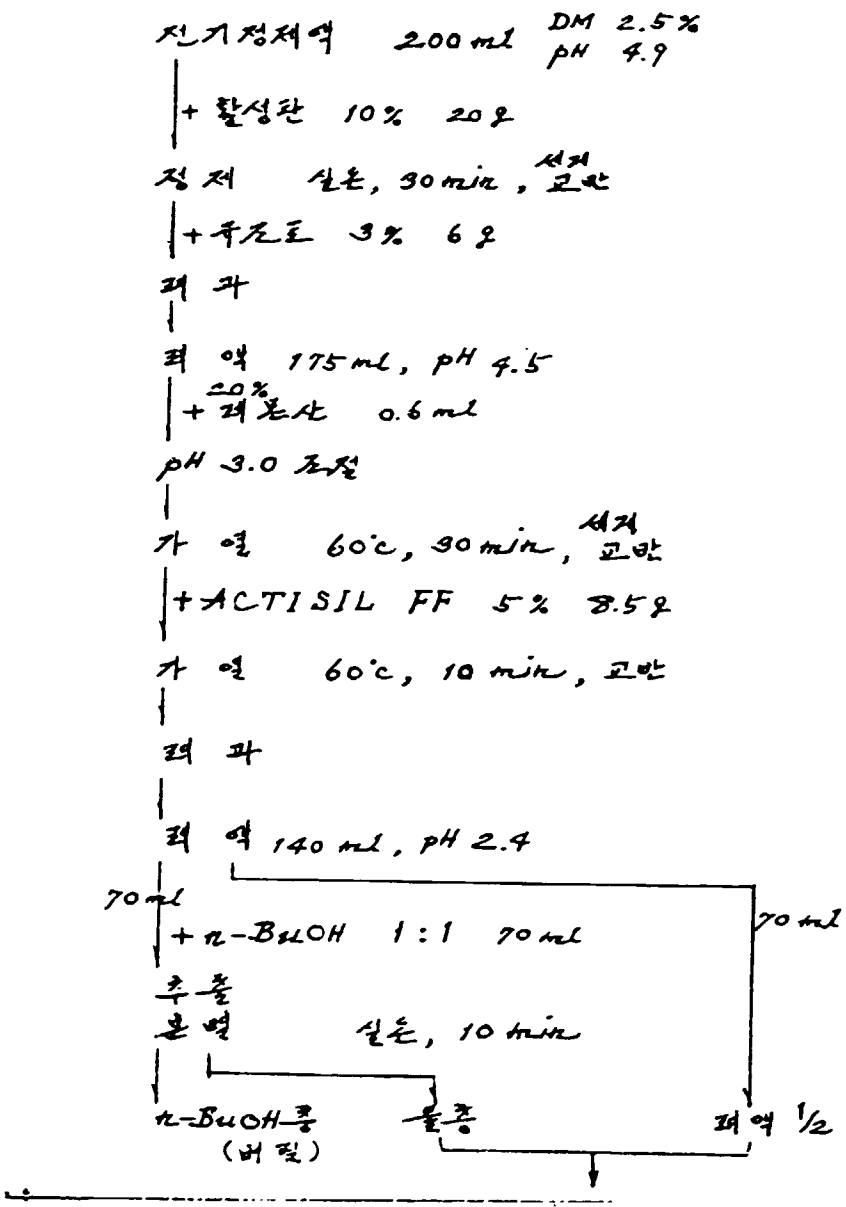
14.5 g

3 g

115 ml

yaw

No. 11


전기정제액 200 ml DM 2.5% pH 4.9
+ 활성탄 10% 20 g
정제 실온, 30 min, 서서 고반
+ 규조토 3% 6 g
여 과
여 액 175 ml, pH 4.5
+ 20% 레몬산 0.6 ml
pH 3.0 조절
가 열 60°C, 30 min, 서서 고반
+ ACTISIL FF 5% 8.5 g
가 열 60°C, 10 min, 고반
여 과
여 액 140 ml, pH 2.4
70 ml
+ n-BuOH 1:1 70 ml
70 ml
추출
분별 실온, 10 min
n-BuOH층 (버림)
물층
여액 1/2

Janos

No. 12

우장액 DM 2.8%, pH 7.0 (동유수유일액)
200 ml

↓

가열 45~50°c

↓ + 30% H_2O_2 18 ml

정제 10 min, 교반

↓ + 27% $AlCl_3$ 4 ml, 혼합후 정치 30 min

여과

↓

여액 150 ml, pH 약 3

↓ + 활성탄 5% 7.5g

가열 50°c, 30 min, 교반

↓

여과

↓

여액 135 ml, pH 2.9

↓ + 키토산 2.000 ppm (2.0g/l) 0.27g (가루), pH 2.2

가열 50°c, 10 min, 교반

↓

냉각 20~25°c

↓ + ACTISIL FF 5% 6.75g
실온, 5 min, 교반

여과

↓

여액 115 ml, pH

yaw

No. 13

- 우절액 200 ml DM 3.5% pH 5.4
- 가열 50°c
- + 20% 포도주산 17 ml
- pH 3.0 조절
- + 30% H_2O_2 20 ml
- 가열 50°c, 30 min, 서서 교반
- + 27% $AlCl_3$ 3.0 ml
- 가열 50°c, 30 min. 서서 교반
- 냉각 20~25°c
- + 규조토 3% 6 g
- 여과
- 여액 ml, pH 2.1
- + 퍼론산
- pH 3.0 조절
- 가열 50°c, 20 min, 약 교반
- + 활성탄 10% g
- 가열 50°c, 10 min, 교반
- + $Ca(OH)_2$ g
- pH 7.0 조절
- + 규조토 2% g
- 여과
- 여액 ml.

yaw

No. 14

- 우절액 200 ml DM 3.5% pH 5.4
- 가열 50°c
- + 30% H_2O_2 20 ml
- 가열 50°c, 10 min, 교반
- + 27% $AlCl_3$ 4 ml
- 가열 50°c, 30 min, 교반
- 냉각
- + 규조토 2% 4 g
- 여과
- 여액 175 ml, pH 2.8
- + 활성탄 5% 8.75 g
- 가열 50°c, 30 min, 교반
- + 규조토 2% 3.5 g
- 여과
- 여액 150 ml, pH 2.9
- + EDTA 2.000 ppm 0.3 g
- 가열 50°c,
- + 30% NaOH 2.5 ml
- pH 7.0 조절
- 10 min, 교반, 50°c
- 냉각 + ACTISIL FF 5% 7.5 g
- 5 min, 교반
- 여과
- 여액 140 ml, DM 3.5%

No. 14 (탈대)

2.50 ℓ
우질액 DM 3.5%, pH 6.3

+ H_2O_2 30% 250 mℓ (10%)
50°c, 10 min, 교반

+ $AlCl_3$ 27% 50 mℓ (0.5%)

전처 50°c, 30 min, 교반
냉각

+ 규조토 50 g (2%)

여과

여액 2.20 ℓ
DM 3.5%
pH 2.5

저거 0.45 kg
w 84.1%

+ 활성탄 110 g (5%)
50°c, 30 min, 교반

+ 규조토 44 g (2%)

전처
여과

여액 1.99 ℓ
DM 2.6%
pH

저거

+ EDTA (2000 ppm) 4.0 g
2.0 g/ℓ
50°c, 교반

+ NaOH 30%, 45 mℓ
pH 7.0
10 min, 교반, 50°c

+ ACTISIL FF 100 g (5%)
5 min, 교반, 실온(냉각)

여과

저거

여액 1.78 ℓ
DM 3.3%
pH 5.4

FRI-2 (탈대)

2.50 ℓ
우질액 DM 3.5%, pH 6.3

+ $NaAlO_2$ 25%, 50 mℓ → pH 11.4

+ HCl 30%, 30 mℓ → pH 7.0

정저 실온, 30 min, 흡합정치

여과

여액 1.61 ℓ
DM 2.0%

저거 0.820 kg
w 91.9%

+ 활성탄 80 g (5%)
50°c, 30 min, 교반

전처
여과

여액 1.45 ℓ
DM 1.9%
pH 5.0

저거

yaw

No. 15

우절액 200 mL DM 3.95% pH 5.4

↓ + NaOH 30% 0.3 mL pH 6.5~7.0

가열 50°C

↓ + H_2O_2 30% 20 mL

교반 15 min, 50°C

↓ + $AlCl_3$ 27% 4 mL

교반 30 min, 50°C

↓ + 규조토 2% 4 g

냉각 20-25°C

↓

여과

↓

여액 150 mL, pH 3.3

↓ + 활성탄 5% 7.5 g

가열 50°C, 5 min, 교반

↓ + 규조토 2% 3 g

여과

↓

여액 134 mL. pH 3.5

↓ + 과황산 2.000 ppm 0.27 g (2.0 g/L)

교반 10 min, 실온

↓ + ACTISIL FF 2% 2.7 g

교반 5 min, 실온

↓

여과

↓

여액 127 mL

FRI-2	FRI-3
우유액 100 mL DM 3.5% pH 5.4	100 mL, DM 3.5%, pH 5.4
+ $NaAlO_2$ 25% 2 mL → pH 11.5	+ $AlCl_3$ 25% 2 mL → pH 3.4
+ HCl 27%(30%) 1.3 mL (1.2 ") → pH 7.0	+ CaO(소) 0.0g → pH 7.0
정제 고압정치 30 min, 실온	
침과	
침액 50 mL, DM 2.0%	63 mL, DM 3.5%
+ 활성탄 5%	
가열 50°C, 30 min, 교반	
침과	
침액 35 mL, DM 2.0%	44 mL, DM 2.9%

APPENDIX IV

Essential lab equipment

- Beakers (10, 50. 250 mL, 1 L, 2 L, 4 L, 6 each)
- Erlenmeyer suction flasks (0.5, 1.0, 2.0, 4.0 L, 2 each)
- Buchner filters (5, 10, 20 cm, 1 each)
- Volumetric flasks (50, 100, 500 mL, 1 L, 2 L, 2 each)
- Volumetric pipette (1, 5, 10, 25 mL, 2 each)
- Delivering pipettes (1, 5, 10, 25 mL, 2 each)
- Burette (50 mL, 4)
- Pipette bulbs (4)
- Round bottom flasks with ground joints (0.5, 1, 2, 3 L, 2 each)
- Reflux condensers, cooler-condensers to fit above
- Electric heating mantle (to fit each of above)
- Rheostats for control of mantles
- Erlenmeyer flasks (100, 200, 500 mL, 2 each)
- Hot plates with magnetic stirrer (2)
- Teflon coated stirring bars
- S.S. beakers (0.5, 1,0, 4.0 L, 1 each)
- Overhead stirrers (2)
- Stands/clamps (4 each)
- Constant temp bath with heating/refrigeration unit, built in circulation pump
- pH meter (with temperature compensation), pH standard solutions or tablets, spare electrode
- Top loading (four decimal accuracy) balance
- Digital analytical balance
- Moisture (DS%) IR meter (spare bulb)

- Filter papers (5, 10, 20 cm diameter)
- Vacuum rotary evaporator (with waterbath, 1), hoses and clamps
- Vacuum pumps (lab) 2
- Lab size distilled water plant
- Shaker incubator

Equipment for Analytical (QA/QC) and R & D Support Services

a) TLC capability (qualitative/semiquantitative)

- Developing chambers (2)
- Applicator (manual) (1)
- Plates (20)
- Binder
- Silicagel "G" (10 kg)
- Color reagents:-p-anisaldehyde, ferric chloride and/or cinol
- Optical densitometer

b) Iatroscan TH-10 upgrade

- External electronic integrator with print out
- Chromorod SII (100)

c) Variable wavelength UV/Vis scanning spectrophotomter with UV and Vis set (3 each) of cuvettes, printing outlet, papers and pens.

d) HPLC unit with dual pump for gradient elution, manual injector system, variable wavelength detector and integrator with print out device.

Accessories needed for the HPLC unit would be;

- Degassing system

- Solvent filters
- Sample filters
- Injection syringes compatible with injector port
- Print-out papers for integrator
- Spare parts for pump
- Stainless steel tubing and tubing cutting device
- Columns: LiChrosorb NH_2 (5 um) or any Silica NH_2 bonded column

  Guard columns
- Fitting to columns
- Standards for quantitation
- Solvents, HPLC Grade, Acetonitrile, and methanol
- Sep-Pak cartridges
- Water treatment and filtration system to prepare HPLC grade water

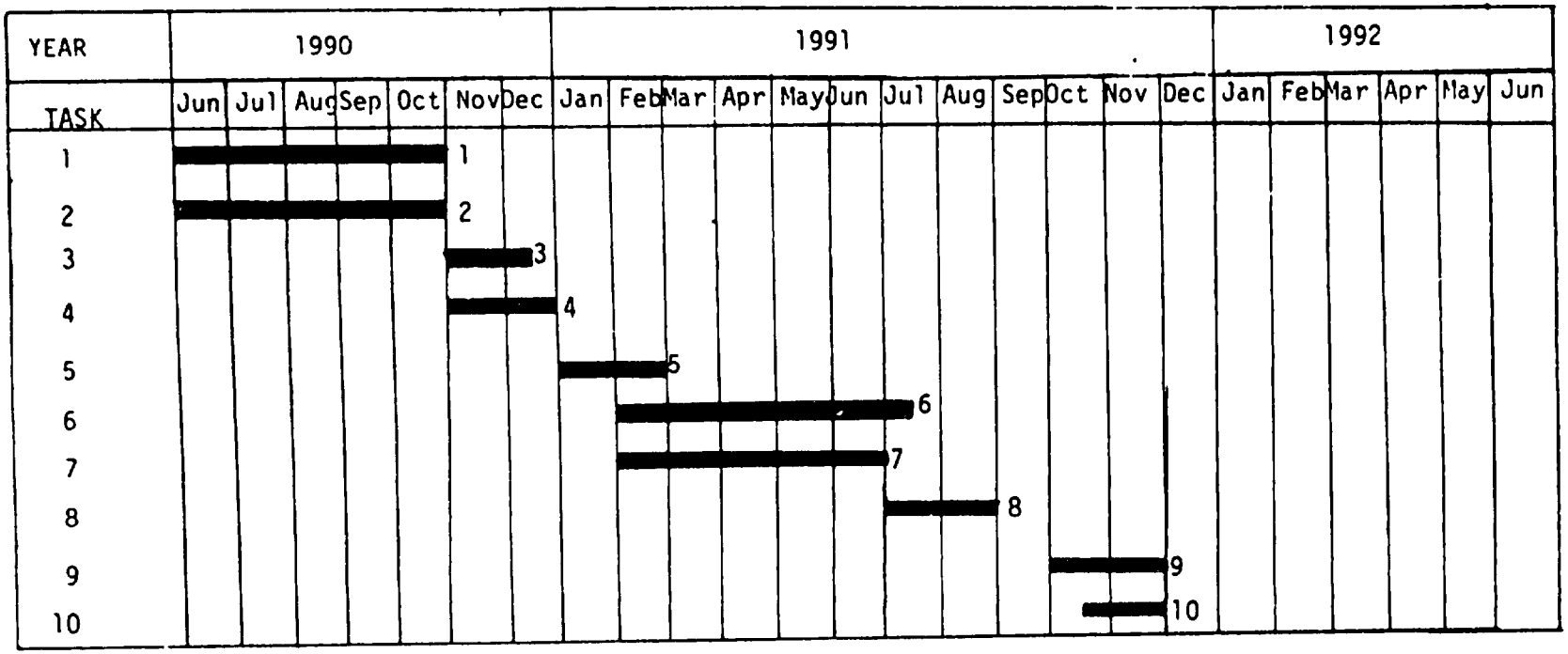
e) Lab scale (5 L) fermenter complete unit with controls, agitator constant temperature fluid system

f) Lab microscope

g) MS-DOS PC with 60-80 MB hard disk and two floppy disks to outlet (5 1/2" and 3 1/2"). A printer and monitor. Printing papers and cartidges. Diskettes, cables, etc. SAS PC option statistical software package with instructions. Simplex and supersimplex optimization software packages with instructions. WordPerfect software package with instructions.

APPENDIX V

A REVISED PROJECT SCHEDULE FOR THE STEVIA PROCESSING



KEY TO TASKS

1. Order/deliver essential lab equipment.
2. Replicate experiments(B, C paths) and generate samples(FRI).
3. Analytical methods/equipment testing and optimization (Food Scientist, UNDP).
4. Process alternative selection (FRI).
5. Layout,installation drawings, final specs of materials(off site,UNDP).
6. Order/deliver equioment, components (UNDP).
7. Pilot plant upgrade; Electricals,refrigeration etc. (FRI).
8. Installation,supervision, commissioning (UNDP).
9. Pilot scale test production runs.
10. Quality assurance and control, transglucosylation process testing.