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PROCESSING OF STEVIO

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DEMOCRATIC PEOPLE'S REPUBLIC OF KOREA

Technical report: Work performed on quality control 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 Sukhdev S. Handa
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Vienna

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TABLE OF CONTENTS

		Page
Abstract		i
I.	Introduction	1
II.	Activities	1
III.	Conclusions	13
IV.	Recommendations	13
Acknowledgements		14

Annexures

1.	Job Description		15
2.	List of persons contacted		16
3.	Protocols for standardization		17
4.	Quality Control Instruments procured through UNDP Support		18
5.	(a)	References - Review articles on Stevioside	19
	(b)	References on extraction methods of Steviosides	20
	(c)	References on Assay methods for steviosides	21
	(d)	References on estimation of Pesticide/insecticide residue	23

ABSTRACT

The consultant was briefed by the Backstopping Officer (BSO) in Vienna on 7th July, 1996. He carried out the mission as given in his job description from 10-28.7.96 (Annex.1).

For the production of high quality finished product Partially Purified Stevia Extract (PPSE/Stevio), it is absolutely essential to use good uniform quality of raw material (leaves of *Stevia rebaudiana*). Out of the six parameters laid down for the raw material (Annex 3) data was generated on five parameters viz. foreign material, moisture content, ash content (both total ash and acid insoluble ash), water and alcohol extractives and stevioside/rebaudioside content. However, the sixth parameter for the determination of pesticide/insecticide residue was not done as the information provided by the NPD was that no insecticide/pesticide is used on the crop of *S. rebaudiana*. However, it was suggested that as and when any insecticide/pesticide is used, the estimation of its residue must be done and a number of references on the methods of estimation of insecticide/pesticide residue has been provided by the expert (Annex 5d). Exhaustive referenced information on review articles (Annex 5a), extraction methods (Annex 5b) and assay methods for steviosides (Annex 5c) was also provided by the expert to the NPD/counterpart staff.

Since water is the only solvent used for the extraction of leaves of *Stevia rebaudiana* for the production of partially purified stevia extract (PPSE)/stevio, it was considered essential to get the analysis of water done. The analysis was done at the Institute of City Maintenance (Table 6).

Nine parameters were designed for the standardization of the finished product (PPSE/Stevio) under the protocols for standardization (Annex 3). Five batches of the PPSE prepared during June/July, 1996 were subjected to analysis under the present study and the data so generated are given in tables 7-16. The analysis indicates that the finished product (PPSE/Stevio) is 197-226 times sweeter than sugar (2% aqueous solution) and contains stevioside 37.4 to 45.2% and rebaudioside A 25.6 to 27.8% as analyzed through HPLC after demonstrating Calibration Curve for stevioside and rebaudioside.

The finished product (PPSE/Stevio) is levorotatory exhibiting optical rotation between -35.3 to -36.0 and an extinction coefficient 0.023-0.070 ($E^{1\%}_{1cm}$ OD at 420nm) in water. The product is highly soluble in water but in alcohol (96%) the solubility is 2.28 - 2.74 g/100 ml. Total ash is not more than 0.316%, acid insoluble not more than 0.071% and moisture content not more than 10.5%. Heavy metal estimation done at Analytical Centre, Academy of Sciences, Pyongyang indicated presence of lead (1.7 mg/kg) and Mercury 8.5×10^{-4} mg/kg. The heavy metal content are far less than the prescribed limit by WHO.

I. INTRODUCTION

With the financial and technical support of UNDP/UNIDO and constant encouragement provided by the Government of Democratic Republic of Korea, the Food Research Institute of the Light Industry Academy of Science has developed prototype extraction procedure and at a national factory a commercial scale operation to produce Partially Purified Stevia Extract (PPSE) is ongoing. The DPRK expects to augment its supplies of ordinary sugar (ca. 3000,000 t/y) now primarily utilized by the Food and Beverages industry, with the use of sugar substitutes as sweetening agents. Extracts of the plant *Stevia rebaudiana* have been widely utilized in Japan since the mid 70's as sweetening agents taste modifiers and sugar substitutes. They are generally accepted as safe food additives. At present the DPRK cultivates Stevia plants which generate ca. 2000 tons of dry leaves/year, and programme to increase this is already underway.

The expert on current mission with instructions from the Project Manager at UNIDO, Head Quarters is to primarily lay down quality control parameters for the raw material and the finished product PPSE. The persons contacted by the expert during his mission are given in Annex 2.

II. ACTIVITIES

The expert on mission with the active cooperation of NPD and counterpart staff devised protocols to develop standards for *Stevia rebaudiana* leaves (raw material) and for Stevio (PPSE, the finished product) (Annex 3). Exhaustive literature information on review articles (Annex 5a), extraction methods (Annex 5b) and assay methods for steviosides (Annex 5c) in addition to literature information on estimation of pesticide/insecticide residue (Annex 5d) was provided by the expert to the NPD/counter-part staff. Through the financial support of UNDP, the institute has all the requisite instruments (Annex.4) necessary for standardization and quality control except Atomic Absorption Spectrophotometer for estimation of heavy metals. The counterpart staff was familiarized with the details of the methods of quality control of both the raw material and of the finished product. Five batches of Stevia produced on 7th, 14th, 21st, 28th June and 5th July were selected for quality control parameters. Details of the methods used for quality control are given below.

1. Determination of Foreign matter

Stevia rebaudiana leaves should be entirely free from pathogenic organisms, and as free as possible from microorganisms, insects, and other animal contamination, including animal excreta. No abnormal odour, discoloration, slime or signs of deterioration of the leaves should be detected. Whenever the leaves of Stevia rebaudiana are required to be stored prior to processing for the production of PPSE, these should be kept in a clean and hygienic place, so that no contamination occurs. Special care should be taken to avoid formation of molds, since they may produce aflatoxins. Thorough macroscopic examination of the raw material (leaves) has been advised to be carried out for the entire lot of leaves before processing and all pieces of stones, sand, soil and foreign matter must be removed. Foreign matter is the material consisting of any or all of the following:

(a) Parts of the plant or material other than the leaves of *Stevia* rebaudiana with specified limits.
(b) Any organism, part or product of an organism other than the leaves of *Stevia rebaudiana*.
(c) Mineral admixtures not adhering to the leaves of *Stevia rebaudiana*, such as soil, stones, sand and dust.

The method used for determining foreign matter is as follows:

Weighed about 250 g of the sample out of the whole batch of the leaves of *Stevia rebaudiana* and spread it in a thin layer to sort out any foreign matter (flowers, pieces of stem, petiole or any other matter specified above under a, b & c) either by visual inspection or using magnifying lens (6X or 10X). Calculated the content of foreign matter in g per 100 g of the leaves of *Stevia rebaudiana* on dry weight basis (Table 1). The range of foreign matter varies from 6.3 to 10.0 per 100 g of the leaves.

TABLE 1

Foreign material in the leaves of *Stevia rebaudiana* (raw material)

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Flowers (a) %	3.1	3.5	3.7	3.3	3.1
Stem pieces etc.(b) %	5.1	3.0	2.6	3.8	6.9
Total foreign matter (a+b) %	8.2	6.5	6.3	7.1	10.0

Each reading under a&b is mean of 5

2. Moisture Content

Moisture content in the leaves of *Stevia rebaudiana* and in the PPSE were determined through loss on drying method by heating to 100 - 105°C. Weighed accurately about 2 - 5 g of the material (leaves of *Stevia rebaudiana* in case of the raw material and the PPSE in case of the finished product) in a previously dried and tared flat weighing bottle. Dried the sample by drying in an oven at 100 - 105°C for 5 hours. Dried until two consecutive weighing do not differ by more than 5 mg. Calculated the loss of weight which indicates amount of moisture present. The leaves contained moisture from 11.00 - 13.5% (Table 2) and the PPSE contained moisture between 8.34 - 10.5% (Table 3).

TABLE 2

Moisture content in the leaves of *S.rebaudiana*

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Moisture (%)	11.8	12.5	12.0	11.0	13.5

Mean of three readings

TABLE 3

Moisture content in Stevio (PPSE)

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Moisture (%)	10.1	10.5	8.34	10.3	9.41

Mean of three readings

3. Determination of ash

The presence of ash in *Stevia rebaudiana* leaves has been estimated as total ash and as acid insoluble ash.

Total ash: This test is designed to measure the amount of material remaining after ignition. Physiological ash is derived from the plant tissue itself and 'non physiological ash ' is the residue after ignition of the extraneous matter (e.g. sand and soil) adhering to the surface. The procedure adopted in this study determines both kinds of ashes and is referred to as the "total ash" test. The procedure used for determining total ash is as follows:

Weighed accurately into a previously ignited , cooled and tared silica crucible, 2-3 g of powdered *Stevia rebaudiana* leaves. Spread the powdered material in an even layer in the crucible, ignited the material by gradually increasing the heat to 500-600°C until free from carbon. Cooled in a desiccator and weighed. Calculated the content of total ash in mg/g on moisture free basis (Table 4 for leaves and Table 5 for PPSE).

TABLE 4

Ash content in the leaves of *S. rebaudiana* (% on moisture free basis)

Item	Ash %	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Stevia leaves	Total ash	8.50	8.54	8.38	8.60	8.41
	Acid insoluble ash	2.29	2.30	2.24	2.32	2.25

Mean of three readings

TABLE 5

Ash content in Stevio (PPSE)

% Ash	Total ash	0.257	0.302	0.100	0.316	0.211
	Acid insoluble ash	0.0568	0.0674	0.0215	0.0712	0.0458

Mean of three readings

Acid insoluble ash: Acid insoluble ash is the residue obtained after boiling the total ash with diluted hydrochloric acid, and igniting the washed insoluble matter left on the filter. This determination measures the presence of silica, especially sand and siliceous earth. The procedure adopted for determining Acid insoluble ash is as follows:

To the crucible containing the total ash, added 25 ml of hydrochloric acid (70 g/L) TS, covered with watch glass and boiled gently for 5 minutes. Rinsed the watch glass with 5 ml of hot water and added this liquid to the crucible. Collected the insoluble matter on an ashless filter paper and washed with hot water until the filtrate is neutral. Transferred the filter-paper containing the insoluble matter to the original crucible, dried on a hot plate and ignited to constant weight. Calculated the content of acid insoluble ash in mg/g of *Stevia* leaves on dry weight basis (Table 4 for leaves and Table 5 for PPSE).

4. Determination of Extractable matter in Water and Alcohol

Weighed accurately, about 4.0 g of coarsely powdered dried leaves of *Stevia rebaudiana* in a glass-stoppered conical flask. Added 100 ml solvent (water in case of determination of water soluble extractive and 96% alcohol in case of determination of alcohol soluble matter) and weighed. Shook well and allowed to stand for one hour. Attached a reflux condenser to the flask and boiled gently for one hour, cooled and weighed. Readjusted to the original weight with the specified solvent for the stevia leaves. Shook well and filtered rapidly through dry filter. Transferred 25 ml of the filtrate to a tared flat-bottomed dish and evaporated to dryness on a water-bath. Dried at 105°C for 6 hours, cooled in a desiccator for 30 minutes and weighed without delay. Calculated the content of extractable matter on dry weight basis (Table 6).

TABLE 6

Water and Alcohol extractives of leaves of *S. rebaudiana* (% on dry wt. basis)

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
H_2O Extractive %	41.9	41.6	42.1	42.0	41.3
96 % Alcohol Extractive %	26.8	26.6	27.0	27.0	26.5

Mean of three readings

5. Determination of Heavy metals in PPSE

Estimation of lead and mercury in PPSE done at Analytical Centre, Academy of Science, Pyongyang using Atomic absorption spectrophotometry indicated that the presence of these heavy metals is far below the limits prescribed by WHO (Table 7).

TABLE 7

Heavy metal estimation* in Stevio (PPSE)

Pb	1.7 mg/kg
Hg	8.5×10^{-4} mg/kg

* Analytical centre, Academy of Science, Pyongyang

6. Water Analysis

Hot water at 55-65°C is the solvent at atmospheric pressure used for extracting milled (15-20 mesh) *Stevia rebaudiana* leaves keeping water to leaves ratio 1:9/1:11 with mild agitation for 60 minutes. The slurry is filtered and the sediment recycled for an additional extraction. The filtered or drained extract from the second extraction is used as part of the feed water for next extraction. Water being the sole solvent used for extraction, its quality has to be maintained. Therefore, periodical analysis of water used for extraction has been strongly recommended. The NPD helped to get the analysis of water (currently being used) done from the Institute of City Maintenance, Pyongyang and the report given in Table 8. It indicates pH 7.2 - 8.0 besides the range of concentration of Ca^{++}, Mg^{++}, Na^{+}, HCO_3^- SO_4^{2-} and Cl^-. It is very essential to monitor quality of water so that the pH does not exceed this range since there is likelihood of hydrolysis of Steviosides at and above pH 9.

TABLE 8

Analysis* of water used for extraction of stevio

	Ca^{2+}	Mg^{2+}	$Na^+ + K^+$	HCO_3^-	SO_4^{2-}	Cl^-
mg/l	22~ 41	6~ 13	1~ 13	91~ 166	7~ 20	4~ 10

pH 7.2-8.0

*Institute of City maintenance, Pyongyang

7. Solubility of PPSE

The material is freely soluble in water, however, its solubility in alcohol (96%) is 2.28 to 2.74 g per 100 ml (Table 9).

TABLE 9

Stevio solubility in EtOH

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
g / 100 ml	2.43	2.74	2.28	2.57	2.40

24 ^{0}C ; 96 % EtOH Mean of three readings

8. Stability of PPSE

No stability study was conducted during the mission as it is well known to be stable even under extremes of temperatures (Progress in Sweeteners Ed. T.H. Grenby, Elsevier Applied Science 1989 pp. 190) and for practical industrial purposes has an unlimited shelflife and good stability in acid solutions.

9. Sweetness intensity of PPSE

2% aqueous sucrose solution was used as standard. An accurately weighed quantity of Stevio was dissolved in known volume of water. Aliquots of this standard solution were taken to make a series of dilutions. The organoleptic taste-testing of dilutions of PPSE aqueous solution was done by human volunteer and from the dilution giving comparable sweet taste to 2% aqueous Sucrose solution, intensity of sweetness was calculated. The volunteer rinsed mouth after tasting each dilution. The intensity of sweetness of PPSE thus calculated has been given in table 10. Thus, PPSE is 197-226 times more sweet than sucrose (when compared with 2% aqueous sucrose solution).

TABLE 10

Sweetener intensity of Stevio (PPSE)

Sucrose (%)	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
2.0	220	197	226	198	215

24 - 25 ^{0}C ; H_2O Mean of five Trials

10. Determination of Extinction Coefficient of aqueous solution of PPSE

One percent w/v PPSE aqueous solution with layer thickness of 1 cm the optical density indicated by E1 cm has been measured for each of five batches of PPSE on Shimadzu UV/vis-1202 Spectrophotometer and the values have been given in Table 11.

TABLE 11

Extinction Coefficient of Stevio (PPSE) in water

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
$E^{1\%}_{1\ cm}$, OD	0.048	0.065	0.023	0.070	0.032

420 nm — mean of three readings

11. Determination of Optical Rotation of PPSE

The optical rotation was measured at the wavelength of the D line of sodium (= 589.3 nm) at 25°, on a layer 1 dm thick.

PPSE was weighed accurately to obtain aqueous solution of known concentration and transferred to volumetric flask by means of water. Adjusted the contents of the flask to 25° by suspending the flask in a constant temperature bath. Made upto the volume with water at 25° and mixed well. Transferred the solution to the polarimeter tube within 30 minutes from the time the PPSE was dissolved and during this time interval maintained the solution at 25°. Determined the zero point of the polarimeter and then made five readings of the observed rotation of the PPSE solution at 25°. Took an equal number of readings in the same tube with water in place of the PPSE solution. The zero correction is the average of the blank readings, and is subtracted from the average observed rotation if the two figures are of the same sign or added if they are opposite in sign to obtain the corrected observed rotation. Calculated the specific optical rotation using the following formulae, dextrorotation and laevo-rotation being designated by (+) and (-) respectively.

$$[\alpha]\ D^{25} = 100\alpha/lc$$

where α = corrected observed rotation in degrees, at 25
D = *D line of sodium light* (λ = 589.3 nm)
l = length of the polarimeter tube in dm
c = concentration of stevio solution in o/o w/v

Mean of the five observations of each batch of PPSE manufactured was taken and optical rotation of five batches of PPSE prepared has been given in table 12.

TABLE 12

Optical rotation of Stevio (PPSE)

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
$[\alpha]^{25}_{D}$, ^{0}	-35.7	-35.7	-36.3	-35.3	-36.0

H_2O ; 25°C — Mean of 5 readings

12. HPLC analysis of *Stevia rebaudiana* leaves and PPSE for determination of Steviosides

Dried, powdered (30 mesh) Stevia rebaudiana leaves (0.5 g) were continuously extracted in a soxhlet apparatus with chloroform for 3 hours and the marc left behind was extracted with methanol for 5 hours. The methanolic extract was evaporated to dryness and the resulting residue was redissolved in 50 ml of propanol-water (49:1) for High Pressure Liquid Chromatographic analysis. Five such samples were subjected to HPLC analysis. (Table 13).

TABLE 13

HPLC Estimation of stevioside and rebaudioside in the leaves of Stevia rebaudiana

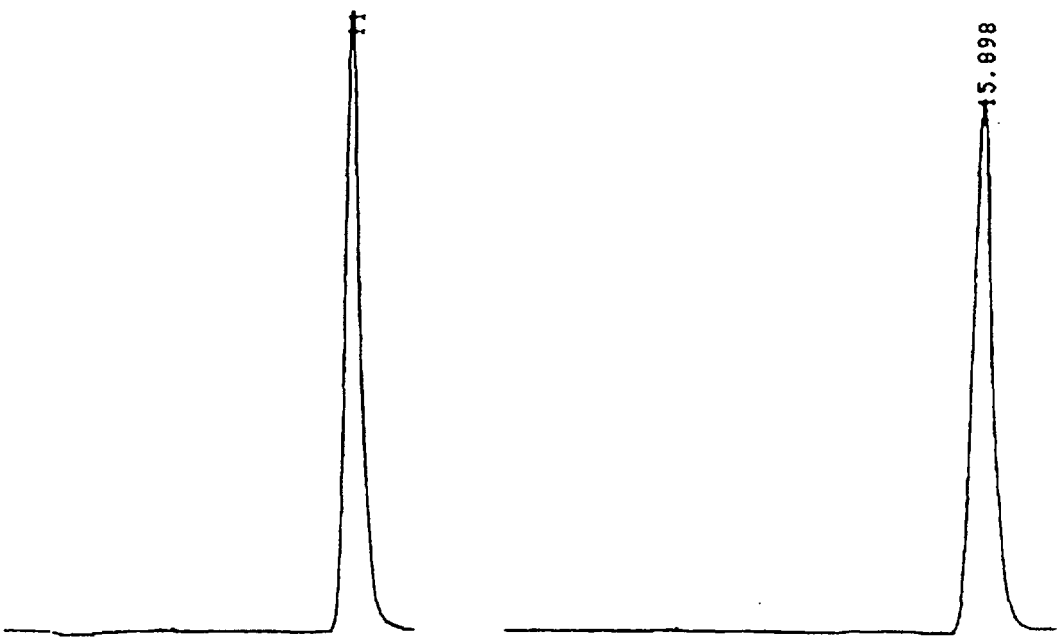
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Stevioside %	6.91	6.82	6.94	7.05	6.76
Rebaudioside A %	3.96	3.91	4.00	4.14	3.92

An accurately weighed quantity of the PPSE was dissolved in known volume of water and from this standard solution, appropriate dilutions were made with water for HPLC analysis.

Shimadzu Chromatograph comprising delivery pump LC-9A, autoinjector SIL-6B, UV-Vis detector SPD-6AV and C-R6A chromatopac recorder was employed. The operating conditions for HPLC were: ambient temperature, flow rate of eluting solvent 0.6 ml/min, wave length of UV detector, 210 nm, pressure of column 31-32 psi injection volume 2 µl.

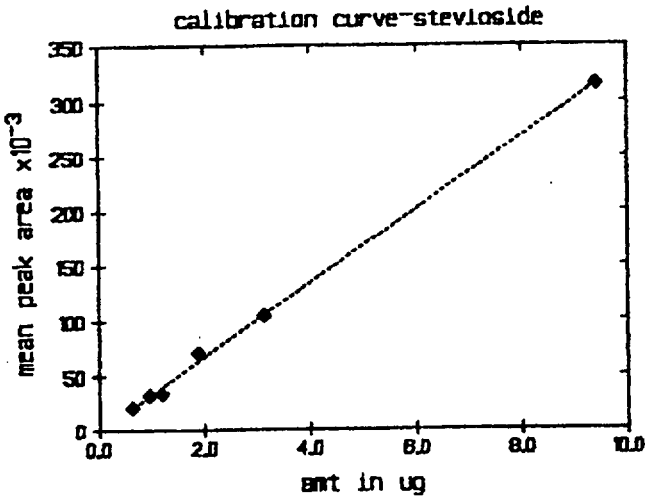
Standard solutions of stevioside and rebaudioside A were injected on to the column and their retention times determined. Fig. 1a, 1b shows peaks of steviode and rebaudioside.

Beer's law standard curves (Fig. 2a, 2b) were obtained by injecting different quantities of stevioside (0.628, 0.942, 1.178, 1.884, 3.14 and 9.42 µg) Table 14 and rebaudioside (0.536, 1.072, 1.34, 1.787, 2.68 and 5.36 µg) Table 15 on to the column in triplicate and measuring the resulting peak areas.

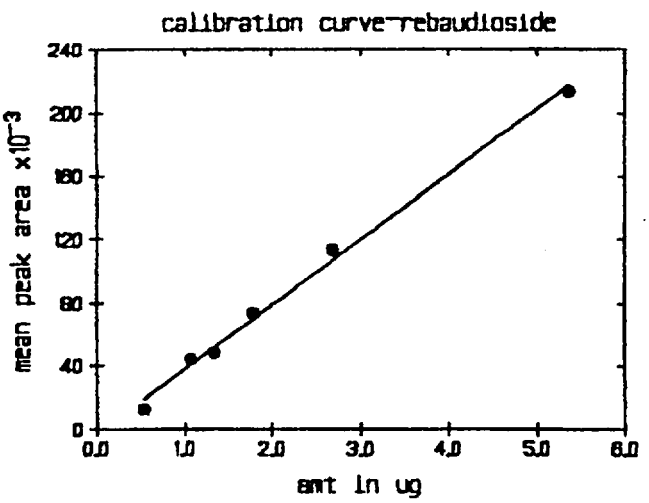


(a) (b)

Fig.1 HPLC chromotogram a) stevioside b) rebaudioside



(a)



(b)

Fig.2 Calibration curve for a) stevioside b)rebaudioside for HPLC analysis

TABLE 14

HPLC data of Stevioside Calibration Curve

S. No	Amount injected in µg (X Series)	Squares of Values of X (X^2)	Mean peak area (Y Series)	Multiple of corresponding x&y (XY)	Dilutions*
1	0.628	0.394	20893	13120.804	15
2	0.942	0.887	32121	30257.982	10
3	1.178	1.387	34175	40258.150	8
4	1.884	3.549	70771	133332.56	5
5	3.14	9.859	104824	329147.36	3
6	9.42	88.736	315396	2971030.3	1
Total (n=6)	17.192	104.81	578180	3517146.9	
Mean	2.865	17.47	96.363	586191.15	

$$b = \frac{\Sigma XY - (\Sigma X)(\Sigma Y)/n}{\Sigma X^2 - (\Sigma X)^2/n}$$

$$= 33492.98$$

$$a = \bar{y} - b\bar{x}$$

$$= 405.928$$

* Stock solution (4.71µg/µl)

Injection 2µl = 9.42µg

Regression equation for stevioside assay is

Y = 33492.98 X + 405.93 (r=0.999)

TABLE 15

HPLC data of Rebaudioside Calibration Curve

S. No	Amount injected in µg (X Series)	Squares of Values of X (X^2)	Mean peak area (Y Series)	Multiple of corresponding x&y (XY)	Dilutions*
1	0.536	0.287296	12495	6697.32	10
2	1.072	1.149184	44485	47687.92	5
3	1.34	1.7956	48540	65043.6	4
4	1.787	3.193369	73867	132000.3	3
5	2.68	7.1824	113572	304372.9	2
6	5.36	28.7296	213718	1145528.5	1
Total (n=6)	12.775	42.3374	506677	1701330.6	
Mean	2.1291	7.0562	84446.16	283555.1	

* Stock solution (2.68 µg/µl) Injection 2µl =5.36 µg

$$b = \frac{\Sigma XY - (\Sigma X)(\Sigma Y)/n}{\Sigma X^2 - (\Sigma X)^2/n}$$

$$= 41125.63$$

$$a = \bar{y} - b\bar{x}$$

$$= -3116.8$$

Regression equation for stevioside assay is

$Y = 41125.63\ X - 3116.8$ (r=0.997)

Under the HPLC conditions used in the present work stevioside and rebaudioside A gaveretentiontimes 11.0 min and 15.1 min respectively. Beer's law standard curve gave slopes of 33492.98 (Stevioside) and 41125.63 (rebaudioside); Y axis (peak area) intercepts of 405.9 (stevioside) and -3116.8 (rebaudioside) and the correlation coefficients of 0.999 (stevioside) and 0.997 (rebaudioside) by linear regression analysis. Table 16 shows the % of stevioside and rebaudioside in the PPSE.

TABLE 16

HPLC analysis of Stevio for stevioside and rebaudioside

Sweet component (%)	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Stevioside	45.2	37.4	43.7	37.9	42.0
Rebaudioside A	27.0	26.2	25.6	25.6	27.8

III. CONCLUSIONS

In recognition of the fact that the plant *Stevia rabaudiana* and the product prepared from it (PPSE/Stevio) hold promise to fill the great gap between the demand and supply of sweeteners in the country, UNDP/UNIDO financially supported very strongly and reinforced the efforts and technical expertise in strengthening the Food Research Institute by providing a pilot plant, modern quality control instruments and the services of international experts.

For the production of good and uniform quality of PPSE/Stevio, it is very essential to utilize standard quality of raw material. Therefore, protocols for the standardization of the raw material and for the finished product have been laid down. The detailed procedures for all the five parameters for raw material and 9 parameters for the finished product have been demonstrated and documented. Following the documented quality control parameters, the institute can now produce three grades of PPSE depending upon the content of total Steviosides estimated through HPLC. The estimation of heavy metals can be carried out at Analytical Centre of the Academy of Sciences where facilities of Atomic Absorption Spectroscopy exist. The F.R.I. may maintain records of quality control parameters as laid down both for the raw material and for the finished product. For obtaining raw material of uniform quality, it is essential to follow good Agrotechnological practices for the cultivation of *Stevia rebaudiana*. The Finished product (PPSE/Stevio) needs to be filled in packets/containers under dehumidified/hygienic conditions. Preparation of granules of PPSE has been suggested. The quality control laboratory is reasonably well equipped for carrying out quality control work but it needs to follow good laboratory practices (GLP).

The Food Research Institute is fully equipped with basic infrastructure, a modern pilot plant, good instrumentation facilities for carrying out quality control of both the raw material and of the finished product. The Institute has a good team of qualified and well trained scientific, engineering and quality control personnel who are now in a position to produce standardized products of Stevio.

IV. RECOMMENDATIONS

1. Quality control analytical profile as suggested (Annexure 3) for each batch of the raw material received for processing and for each batch of the PPSE produced should be prepared as regular routine. This will ensure quality production.

2. Appropriate arrangement may be made with the local institute having Atomic Absorption

Spectrophotometer to get heavy metal (Pb, Hg, AS) done for each batch of the PPSE produced.

3. Periodical analysis of water being used for extraction may be got done from the Institute of City maintenance.

4. Batchwise analysis of the stevio product will ensure not only the quality but also will help in making three grades of the product e.g. the batches containing 81% and above steviosides may be graded as Grade I and the batches containing 71-80% of steviosides pooled to be graded as Grade II and the batches containing 60-70% of steviosides may be combined and graded as Grade III.

5. Good manufacturing practices (GMP) need to be followed for quality assurance of the product.

6. Since the stevio project is of socio-economic importance to DPR Korea it needs further support both from the Government and UNDP/UNIDO especially with respect to adoption of GMP in hygienic environment conducive to follow GMP to ensure quality production. One instrumentation laboratory needs to follow Good Laboratory Practices (GLP) so that quality assurance of the product is practiced.

7. Variations in sweetener content in different batches produced from various lots of raw material are mainly due to excessive amount of extraneous material in the leaves. Therefore, it was strongly recommended that the raw material as soon as received at the Institute should be subjected to thorough garbling and sorting so that all extraneous material is removed before the leaves are powdered for extraction.

8. Although scientists have been duly trained in handling HPLC and UV spectrophotometer but need some training in handling Densitometer scanner.

9. Staff members may be exposed to Quality control Workshops and training programmes so that culture of quality production is inducted.

10. Packing of the finished product should be done under hygienically controlled environment room duly fitted with dehumidifier to avoid access of moisture in the finished product.

11. Discussed and explained the significance of granules of stevio powder and thus for the production of stevio granules, a granulator may have to be provided to the Institute.

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The expert also wishes to thank Mr. Ri Myong San, Mr. Jong San Hun, Mr. Cho Jong Nam, Officers, Directorate General Bureau for Cooperation with International Organizations (GIBCO) for discussion.

ANNEX 1

JOB DESCRIPTION
DP/DRK/88/008/11-52

Post Title:	Quality Control Expert
Duration:	0.5 m/m
Date required:	May 1996
Duty Station:	Pyongyang,Democratic Republic of Korea
Purpose of Project:	Enhancem Stevio Research Project to develop improved commercially viable processes for the production of sweetening agents from the plant *Stevia rebaudiana*, based on the laboratory research already carried out.
Duties:	The expert is required to carry out the following duties in collaboration with the National Project Director and the counter part staff. - Develop standards for the stevia powder - Train counterpart staff in techniques to be used in the quality control of stevia powder. - Assist in preparing a monograph for stevia powder containing purity, assay and limit tests. - Recommend methods for the further purification of stevia powder. The expert has to submit a final report embodying his findings and describing the work done and recommendations.
Qualifications:	A chemist with post graduate qualifications and with over 10 years experience in quality control of plant based products. Experience in working with stevia will be an advantage.

ANNEX 2

List of Persons contacted

Mr. G. F. Achikzad	Resident representative, UNDP, DPR Korea, Pyongyang.
Mr. Li Song Ho	Programme officer, UNDP, Pyongyang.
Dr. Kim Dong Su	Director National Project Director, Food Research Institute.
Dr. Kim Tae Gyu	Scientist, Food Research Institute, Pyongyang.
Mr. Ri Myong San	Directorate General Burea for Cooperation with with International Organizations (GIBCO).
Mr. Jong Sang Hun	GIBCO oficial
Mr. Cho Jong Nam	GIBCO official
Mr. Hong Li Suk	M.S. Analytical Chemist Food Research Institute.
Mr. Khim Yaung Cho	Food Processing Engineer Food Research Institute.

ANNEX 3

PROTOCOLS FOR STANDARDIZATION

RAW MATERIAL *(Stevia rebaudiana* leaves)

Foreign matter
Moisture Content
Total ash and acid insoluble ash
Water and alcohol soluble extractives
Pesticide/insecticide residue
Determination of steviosides

PRODUCT PPSE

Moisture content
Total ash and acid insoluble ash
Solubility
Stability
Sweetener intensity
Extinction Coefficient
Optical rotation
Heavy metal estimation
Determination of Stevioside and rebaudioside

ANNEX 4

QUALITY CONTROL INSTRUMENTS PROCURED THROUGH UNDP SUPPORT

1. UV-Vis Spectrophotometer (Shimadzu) with NEC Pinwriter P-20.

2. High Pressure Liquid Chromatograph (Shimadzu) with LC-9A pump, SIL-6B autoinjector SPD-6AV UN/Vis detector ASC-5 auto sample changer.

3. Dual Wave Length Flying Spot Scanner CS-9000 with FDV Floppy Disk Unit.

ANNEX 5(a)

REVIEW ARTICLES ON STEVIOSIDES

Compadre CM, Robbins EF and Kinghorn AD. The Intensely Sweet Herb, *Lippia Dulcis* Trev. Historical uses, field inquiries and constituents. *J. Ethnopharmacol.*, 15, 89-106, 1986.

Compadre CM, Pezzuto JM, Kinghorn AD and Kamath SK. Hernandulin: An Intensely Sweet Compound Discovered by Review of Ancient Literature. *Science*, 227, 417-419, 1985.

Gbile ZO and Adesina SK. Nigerian Flora and its Pharmaceutical Potential *J. Ethnopharmacol.*, 19, 1-16, 1987.

Handro W and Ferreira CM. XXVI *Stevia rebaudiana* (Bert.) Bertoni: Production of Natural Sweeteners., Biotechnol. in Agric. & Forestry Vol. 7. Med. & Aromat. Plants II (ed. YPS Bajaj) Springer Verlag, Berlin, Heiberg, 1989.

Hanson JR and Oliveira De BH. Stevioside and Related Sweet Diterpenoid Glycosides *Natr. Product Reports*, pp 301-309, 1993.

Kinghorn AD, Fillas F and Hussain RA. Structure- Activity Relationship of Highly sweet Natural Products., Studies in Natural Products Chemistry, Vol 15, Structure & Chem (Parte). (ed. Atta-ur-Rahman], Elsevier, PP 3-41, 1995

Kinghorn AD, and Soejarto DD. Stevioside, Alternative Sweeteners. Second Edition, Revised and Expanded (ed. Nabors Lyn O' Brien, Gelardi RC). Marcel Dekker Inc, PP 157-171, 1991.

Kinghorn AD, Biological active compounds from Plants reputed for Medicinal and Sweetening properties. J. Natr. Products, 50 (6), 1009-1024, 1987.

Kinghorn AD and Soejarto DD. Current Status of Stevioside as a Sweetening Agent for Human use. *Econ. & Med. Plant Res*, 1, 1-52, 1985.

Kinghrorn AD and Kennelly EJ. Discovery of Highly Sweet compounds from Natural Sources. *J. Chem. Educat* 72, 676-680,1995

Soejarto DD, Compadre CM, Medon PJ, Kamath SK and Kinghorn AD. Potential Sweetening Agents of Plant Origin II. Field Search for Sweet-Tasting *Stevia* Species. *Economic Botany* 37(1), 71-79, 1983.

ANNEX 5(b)

EXTRACTION METHODS FOR STEVIOSIDES

Banshu-Chomiryo Co. Ltd. Extraction of dried ground Stevia stalks and leaves using a methanol-chloroform mixture to yield a stevioside sweetener. Japanese examined Patent 5440 560 (1979) [Ja].

Cheng TF, Chang CY and Chang WH. A study on the purification of Stevioside with the use of ion-exchange resins. *J. Chinese Agric. Chem. Soc.* 23(1/2), 178-190, 1985.

Esakai S, Tanaka R and Kamiya S. Synthesis and taste of certain stevoil glycosides. *Agric. Biol. Chem.* 48 (7), 1831-1834, 1984.

Karniya S, Konishi F and Esaki S. Synthesis and taste of some analogs of stevioside. *Agric. & Biol. Chem.* 43 (9), 1863-1867 1979.

Kusama S, Kusakabe I, Nakamura Y, Eda S and Murakami K. Transglucosylation into stevioside by the enzyme system from streptomyces sp. *Agric & Biol Chem.* 50 (10), 2445-2451, 1986.

Maruzen Kasei Co. Ltd. A liquid extract of the sweet compound from the leaves of *Stevia rebaudiana* Bertoni is treated with an adsorbent consisting of an AL. Mg Complex oxide. Japanese Examined Patent 5512230 (1980) [Ja].

Mitsubishi Chemical Industry Co Ltd. Stevioside purified by a solution with an adsorbing resin followed by elution with a solvent and contact with an amphoteric ion exchange resin. Japanese Examined Patent 5523061 (1980) [Ja].

Nakayama K, Kasahara D and Yamamoto F. Absorption, distribution, metabolism and excretion of stevioside in rats. *J. Food Hyg. Soc. of Jpn* [Shokuhin Eiseigaku, Zasshi] 27(1), 1-8, 1986.

Nisshin Sugar Manufacturing Co. Ltd. Stevioside Sweetening compositions containing maltitol and fructose used for improving taste properties. Japanese Patent 5227225 (1977) [Ja].

Sanyo Kokusaku Pulp Co. Ltd. The flavour of synthetic sweetener composition is improved by the incorporation of the mother liquor obtained by the precipitation and separation of stevioside. Japanese Examined Patent 5414178 (1979([Ja].

Shinnakamura Kagaku Kogyo KK. Stevioside extract by treatment with barium hydroxide or acetate. Japanese Examined Patent JP 5742300B, (1982) [Ja].

Uchiyama T. Utilization of stevioside sweeteners in soft drinks and frozen desserts. *New Food Industry* 29(3), 26-29, 1987.

Wingard RE Jr, Brown JP, Enderlin FE, Dale JA, Hale RL and Seitr CT. Intestinal degradation and absorption of the glycosidic sweeteners stevioside and rebaudioside A. *Experientia* 36(5), 519-520, 1980.

Yokoyama Shiochiro and Hiroaki Sngiyama.Clarification of aqueous extract from *Stevia* leaves by using magnesia adsorbent. J. *Jpn. Soc. Food Sci. Tech.*, 37(11), 899-905, 1990.

Zaidan LBP, Dietrich SMC, Felippe GM. Effect of photoperiod on flowering and stevioside content in plants of *Stevia rebaudiana* Bertoni. *Jap. J. Crop Sciences*, 49(4), 569-574, 1980.

ANNEX 5(c)

ASSAY METHODS FOR STEVIOSIDES

Ahmed MS, Dobberstein RH and Farnsworth NR. *Stevia rebaudiana* I. Use of p-bromophenacyl bromide to enhance ultraviolet detection of water-soluble organic acids (steviolbioside and rebaudioside B) in high performance liquid-chromatographic analysis. *J. Chromatogr*, 192 (2), 387-393, 1980.

Ahmed MS, Dobberstein RH. Stevia rebaudiana II High-performance liquid chromatographic separation and quantitation of stevioside,rebaudioside A and rebaudioside C. *J. Chromatogr*, 236(2), 523-526, 1982.

Damayanova N, Boryana, Bankona V and Popov S. Separation and Quantitation of stevioside and rebaudioside A in plant extracts by normal phase HPLC and TLC. A comparison. *Phytochemical Analysis*, 5(2), 81-85, 1994.

Fullas F, Kim J, Compadre CM and Kinghorn AD. Sepearation of natural product sweetening agents using overpressured layer chromatogrphy. *J. Chromatogr.* 464, 213-219, 1989.

Ishima T, Katayama O and Yokoyama Y. Sensory evaluation of stevioside and newely isolated diterpene glycosides as sweeteners. *Int. Cong. Food Sci and Technol Abstracts*, pp 158, 1978.

Isima N and Kakayama O. Sensory evaluation of stevioside as a sweetner.Report of the National Food Res. Inst[Shokuryo Kenkyusho Knkyu Hokoku] No.31, 80-85, 1976.

Iwamura J, Kinoshita R, Ishima T, Katayama O, Morita T and Hirao N. Studies on the reaction and determination of steviosides II. Determination of stevioside by direct thin layer densitometry and of intensity of sweetners of steviosides. *J. Agr. Chem. Soc of Japan*, 56(2), 87-91, 1982.

Little CJ and Stahelo. Role of column switching in semi-preparative liquid chromatography: Isolation of the sweetener stevioside. *J. Chromatogr.* 316, 105-11, 1984.

Makapugay HC, Nanayakkara NPD and Kinghorn AD. Potential sweetening agents of plant origin. V. Improved high performance liquid chromatographic separation of *Stevia rebaudiana* sweet diterpene glycosides using linear gradient elution. *J. Chromatogr.*, 283, 390-395, 1984.

Paula N, Alavrez M and Vieira GE. Quantitative analysis of stevioside in the leaves of *Stevia rebaudiana* by near infrared reflectance sepectroscopy. *J. Sci. Food. Agric.* 59(3) 277-281, 1992.

Paula N, Alvarez M and Vieira GE. Determination of soluble stevioside and carbohydrates in leaves of *Stevia rebaudiana* by near infrared reflectance spectroscopy *Arg. Biol Technol* (Curitiba) 34(2), 361-374, 1991.

Schiffman SS, Barbara J and Wariwick ZS. Bitterness of sweteners as a function of concentration. *Brain Res.Bull.* 36(5), 505-573, 1995.

Sherma J and Norfolk. E. Quantitative TLC determination of Stevioside and Rebaudioside A in bererages. *J. Chromatogr.* 15(17), 2981-2988, 1992.

Soejarto DD, Kunghorn AD and Farnsworth NR. Potential sweetening agents of Plant Origin III. Organoleptic evaluation of *Stevia* leaf Herbarium samples for sweetners. *J. Nat. Prod.* 45(5), 590-599, 1982.

Striedner J, Gutjahr E, Czygan FC and Braneg G. Contribution to biotechnological production of sweeteners from *Stevia rebaudiana* Bertoni:Induction of stevioside accumulation in cell cultures by variation of the nutrient medium and the analysis of small amount of stevioside. *Acta Biotechnol* 11 (5), 501-504, 1991.

Yoshikawa S, Ishima T and Katayama O. Taste of components of stevioside. Abstracts of papers, American Chemical Society 177(1), AGFD 74, 1979.

ANNEX 5(d)

ESTIMATION OF PESTICIDE / INSECTICIDE RESIDUE

Aoki Y, Takeda M and Uchiyama M. Analysis of pesticide residues in foods. XVIII. Systematic analysis for organophosphorus pesticides by gas chromatography and influence of the flow rates of hydrogen and air on the response to flame-photometric detection. *Eisei Kagku [J.hyg. Chem]*,. 22(2), 81-90, 1976.

Appaiah KM, Nag UC, Kapur OP and Nagaraja KV. Colorimetric determination of carbaryl and its residue in vegetables. *J. Food. Sci. Technol.*, 20(5), 252-253, 1983.

Bache CA and Jisk DJ. Determination of organophosphorus insecticide residues by using the emission-spectrometer detector. *Analyt. chem.* 37(12), 1477-1480, 1965.

Bates JAR. General method for the determination of organophosphorus pesticide residues in food stuffs. *Analyst* Lond., 90, 453-466 , 1965.

Benecke R. Thieme H and Brotka J. Analysis of plant drugs for residues of organochlorine compounds I . Gas chromatographic determination of organochlorine insecticides. *Pharmazie* 41(2) 133-137, 1986.

Benson WR and Finocchiaro JM. Rapid procedure for carbaryl residues:modification of the official colorimetric method. *J. Ass. off. Agric. Chem.*, 48(3), 676-679, 1965.

Berg H and Sperlich W. Detection and determination of organo-arsenic compounds on fruits. *MittB1. GDCh-Fachgr. lebensmittel Chem. gerich H Chem*, 28(10-16) 298-304, 974 (German].

Beynon KI and Stoydin G. Application of an agar-diffusion procedure to pesticide-residue analysis and to the cholinestrase screening of candidate pesticide. *Nature*, Lond, 208, 748-750, 1965.

Gnusowki B, Zygmunt B and Majchrzykowa. Determination of biphenyl and 2-phnylphenol residues in herbal drugs and in condiments. Herba Pol. 27(4), 325-331, 1981.

Bolzoni L and Dagnino MR. Metoxuron residues in fresh tomatoes and tomato paste. *Ind. Conserve* 60 (1), 18-22, 1985.

Brauckhoff S, Thier HP. Residue analysis of carbamate insecticides by capillary gas chromatography with nitrogen-selective detection. *Lebensmittel Chem. Gerichtl. Chem.*, 40(5), 109-110, 1986.

Cano P, Dela Plaza JL and Munoz-Delgado L. Determination and persistence of several fungicides in post-harvest treated apples during their cold storage. *Agric. Food Chem.*, 35(1), 144-147, 1987.

Chioffi V. Detection of biphenyl and o-phenyl-phenol on lemons by thin-layer chromatography. *Boll. Laboratori Chim. Prov.*, 16(3), 366-376, 1965.

Copius-Peereboom JW. Food stuffs and their Additives.

Debska W and Domeracki, S. Determination of residual pesticides and their degradation products in vegetable drugs and crude drugs I. Content of organochlorine pesticides in Anthodium chamomillae. *Herba Pol.*, 19(1), 15-22, 1973.

Debska W and Gnusowski B. Analytical papers concerning determination of pesticide residues in medicinal plants. *Herba Pol.* 26(4), 257-266, 1980 [Polish].

Debska W, Gnusowski B and Zygmunt B. Determination of oxycarboxin residues in medicinal plants. *Analyst* (London), 104(245), 1191-1194, 1979.

Dickes GJ and Nicholas PV. Survey of fruits and vegetables [sampled in 1967] for organochlorine and organophosphorus insecticides. *J. Ass. Publ. Analyst*, 6(2), 60-66, 1968.

Farrow RP, Elkinsjun ER and Beacham LM. Vacuum-sublimation clean-up procedure for pesticide residue analysis. *J. Ass. Off. Agric. Chem.* 48(4), 738-744, 1965.

Fodor-Csorbe K, Komives T, Marton AF and Dutka F. Extension of the AOAC multi-residue methodology for the determination of thiocarbamate herbicide residues in food. *Nahrung* 24(10), 963-965, 1980.

Gabrio T and Ennet D. Gas-chromatographic determination of 1,3,5-triazineherbicide residues in drugs of vegetable origins. *Pharmazie*, 37(5), 375-377, 1982.

Gieger U. Determination of residues of fungicidal preservatives and antioxidants on fresh fruit by HPLC with automated column switching. *Lebensmittel Chem. Gerichlt Chem.*, 40(2), 25028, 1986.

Gnusowksi B. Determination of propyzamide residues in medicinal and spice herb raw materials and in soil. *Bromatol. Chem. Toksykor* 13(1), 23-26, 1980 [Polish].

Gorski T. Pesticide residues in teas consumed in Poland. *Rocz. Panstw. Zakl. Hig.*, 36(6), 433-437, 1985.

Hadorn H and Zuercher K. Isolation of crude extracts for the determination of insecticide residues in various food stuffs. *Mitt. Geb. Lebensmittelunters.u. Hyg*, 64(2), 266-274, 1973.

Hadorn H and Zuercher K. Determination of residues of chlorinated insecticides in cacao and chocolate. *Int. choc. rev.* 27(1), 2-11, 1972.

Henneberg M and Wasil-Kowska, J. Thin-layer chromatographic detection of residues of chlorinated hydrocarbon insecticides in crude drugs. *Herba pol.*, 15(4), 328-342, 1969.

Holland PT, McGhie TK. Multi-residue method for determination of pesticides in fruit, apples and berry fruits. *J. Assoc. Off Anal. Chem.* 66(4), 1003-1008, 1983.

Holmes DC and Wood NF. Removal of interfering substances from vegetable extracts before determination of organochlorine pesticide residues. *J. Chromat.*, 67(1) 173-174, 1972.

Hopper ML. Automated gel-permeation system for rapid separation of industrial chemicals and organophosphate and chorinated pesticides from fats. *J. Agric. Food Chem.*, 30(6), 1038-1041, 1982.

Hutzinger O and Jamieson WD. Identification of organochlorine pesticides in crude extracts by mass spectrometry. *Bull environ. contam. Toxicol.*, 5(6), 587-594, 1970.

Imanaka M, Shibata F, Matsunaga K and Ishida T. Hygiene-chemistry of harmful chemicals in foods V. Analysis of methyl carbamate pesticides in foods of animal origin. *Okayaina-ken kankyo Hoken Senta Nempo* 3, 195-200, 1979.

Ingrid FC, Erich R and Huber JFK. Determination of carbamate pesticides in fruits and vegetables by high pressure liquid chromatogrphy. *Z. Lebensm- Unters Forsch* 169(60), 438-443, 1979 [German].

Johnson LY. Collaborative study of a method for determining multiple chlorinated pesticide residue in fatty food. *J. Ass. Off. Agric. Chem.*, 48(3) 668-675, 1965.

Karanth NGK, Srimathi MS and Majumder SK. Chromogenic paper for ultra-rapid detection of organochlorine insecticide residues in vegetables. Bull. *Environ. Contain. Toxicol.*, 28(2), 221-224, 1982.

Kaufmar WM, Bills DD, and Hannan EJ. Identification of nanogram amounts of certain organochlorine ilsecticides. *J. Agric Fd Chem.* 20(3), 628-631, 1972.

Kitada Y, Sasaki M and Tanigawa K. Simultaneous liquid-chromatographic determination of thiabendazol, 2-phenylphenol and biphenyl residues in citrus fruits, without prior cleanup. *J. Assoc. Off. Anal Chem.*, 65 (6), 1302-134, 1982.

Laencina J, Alonso-Allende A, Gimenez JL and guzman G. Determination of organochlorine pesticide residues in paprika (Capsicum. annunm L.). Rev. Agroquim. Technol, Aliment. 18(3), 346-352, 1978.

Lafuente MT, Jadeo JL and Tuset JJ. Gas-chromatographic determination of fenpropimorph residues in citrus fruit. *J. Assoc. Off Anal. Chem.*, 69(5), 859-862, 1986.

Lakshminarayana V and Menon PK. Clean-up of cereal and milk for determination of residues of organochlorine insectides. *J. Fd. Sci. Technol*, 12(3), 144-146, 1975.

Lemperla E and Kemer E. Determination of fungicide residues on grapes and in must and wine after application of SCLEX (ORT 8840F, dichlozolin) [3-(3,5-dichlorophenyl)-5,5-dimethyloxazolidine-2,4-dione]. *Z. analyt. chem.*, 256(5), 353-356, 1971.

Leo Roberts. Separation of pesticide residues from lipids prior to gas-liquid chromatoraphic analysis. *J. Ass. Off Analyt. Chem.*, 50(1), 224-225, 1976.

Luckner R and Luckner M. Poposals for the DAB. VIII of the German Democratic Republic. Detection of DDT in official drugs. *Zentbl Pharm. Pharma Kother U. Lab-diagnostik*, 111(3), 267-280, 1972.

Ludwicki J, Kwast M, Zadrozinska J. Determination of residues of organo-phosphorus insectices in apples and pears by means of a technicon analyser. *Rocz. Panstw. Zakl,. Hig*, 34(1), 47-54, 1983 [Pol].

Ludwicki J. Determination of residues of organophosphorus insecticides in vegetables by means of an automatic analyser. *Rocz. Panstw. Zakl. Hig.*, 34(3), 289-293, 1983.

Mc. Caulley DF. An appraoch to the separation, identification and determination of atleast ten organophosphorus pesticide residues in raw agricultural products. *J. Ass. Off. agric. Chem.*, 48(3), 659-665, 1965.

Mestres R, Santapau C, Robin G and Portal E. Pesticide residues: DDT in milk of southern France. *Trav. Soc. Pharm. Montpellier*, 24(2), 148-160, 1965.

Mitchell LR. Collaborative study of the determination of endosulfan, endosulfan sulphate, tetrasul and tetradifon residence in fresh fruits and vegetables. *J. Ass. Off analyt Chem*, 59(1), 209-212, 1976.

Mueller B. Determination of pesticide residues in honey I. Semi-quantitative thin layer chromatographic determination of insecticide residues. *Nahrung*, 17(3), 381-386, 1972.

Mueller B. Semi-quantitative thin-layer chromatographic determination of herbicide residues. *Ibid*, 17(3), 387-392, 1973.

Newsome WH. Determination of daminozide residues on foods and its degradation to 1.1-dimethylhydrazine by cooking. *J. Agric. Food Chem.*, 28(2), 319-321, 1980.

Pestemer W and Mann W. Herbicide residues in some culinary herbs. *Z. Lebensm-Unters. Forsch.*, 171(4), 272-277, 1980.

Pluta J and Olszewski Z. Investigation on the extraction of chlorinated pesticides from several vegetable drugs. *Farm. Pol.*, 40 (4), 207-210, 1984.

Pluta J and Olszewski Z. Determination of chlorinated pesticides in tinctures and dried extracts. *Acta. Pol. Pharm.*, 37(6), 675-679, 1980 [Polish].

Porter ML and Burke JA. Isolation and clean-up procedure for low levels of organochlorine pesticide residues in fats and oils. *J. Ass. Off. analyt. chem* 56(3), 733-738, 1973.

Regula E and Wassermann L. Pesticide residues in poppy seed. *Z.Lebensm-Unters. Forsch*, 169(6), 444-446, 1979.

Roberts LA. Gas-chromatographic determination of di-chloromethane, 1,2-dichloroethane and trichloroethylene residues in spice oleoresins. *J. Ass. Off. Analyt. Chem.* 52(3), 477-481,1969.

Sobotka W, Janina. Determination of chlorinated hydrocarbon pesticide residues in fennel fruit. *Acta Pol. Pharm*, 31(3), 345-349, 1974.

Spittler TD, Arganer RJ, Lisk DJ, Mumma RO and Winnett G. Gas-liquid chromatographic determination of feavalerate insecticide residues in processed apple products and by-products. *J. Assoc. Off Anal. Chem.*, 65(5), 1106-111, 1982.

Stijve T. Determination of bromopropylate residues. *Dtsch. Lebensm. Rundsch.*, 76(4), 119-122, 1980.

Styve T and Cardinale E. Confirmation of the identity of heptachlor residues by hydroxy-derivative formulation on thin layers of aluminium oxide. *Mitt. Geb. Lebensmitt elunters. U. Hyg.* 63(2), 308-310, 1972.

Szokolay A, Uhnak J and Madaric A. Gas-chromatographic determination of residues of chlorinated insecticdes. *Chemicke Zvesti*, 25(6), 453-459, 1971.

Zadrozinska J. Application of fungi as a biological test for determining residues of several fungicides in plant material I Determination of benomyl, thiophanatemethyl, carbendazim and thiabendozole in lemons, oranges, grapefruit and banana. *Rocz. Panstco. Zakl. Hig.*, 30(1), 31-37, 1979.

Zarozinska J, Ludwicki JK and Klosinska J. Determination of triazine herbicide residues in plant material. *Roz. Panstw. Zakl. Hig.* 36(4), 313-321, 1985.