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THE DEVELOPMENT OF A RUBBERSEED PROCESSING TECHNOLOGY FOR THE PRODUCTION OF VEGETABLE OIL AND ANIMAL FEED*

US/GLO/81/103/21-00

Phase one: Literature review, field studies, laboratory tests and product and process development perspectives

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Abbreviations

Besides of the common abbreviations symbols and terms the following have been used in this study:

Technical abbreviations:

AV	=	Acid value
CN	=	Coconut
D	=	Dalton: molecular weight unit
FFA	=	Free fatty acids
GC	=	Gaschromatography
HPLC	=	High pressure liquid chromatography
MW	=	Molecular weight
MS	=	Mass spectroscopy
NMR	=	Nuclear magnetic resonance
PA	=	Phosphatidic acid
PC	=	Phosphatidyl choline
PE	=	Phosphatidyl ethanolamine
PI	=	Phosphatidyl inositol
RSM	=	Rubberseed meal
RSO	=	Rubberseed oil
SL Rps	=	Sri Lanka Rupies
THF	=	Tetrahydrofurane
TLC	=	Thin layer chromatography
UM	=	Unsaponifiable matter

Organisations:

CISIR	=	Ceylon Institute of Scientific and Industrial Research
RRIM	=	Rubber Research Institute of Malaysia
RRISL	=	Rubber Research Institute of Sri Lanka

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Abstract

In this study the composition of rubber seed (Hevea brasiliensis) and the frame work for the setting up of an industrial rubber seed processing technology is described. This report contains all information from a literature review, a field study in Sri Lanka and laboratory investigations carried out at the home laboratory of NATEC.

Until now in most of the rubber producing countries the rubber seeds are waste material. A rough estimate for the amount of seeds worldwide results in about 5 mio tons per year. Utilization of rubber seed is done in few places and very seldom on an economical scale. However, a lot of information about the utilization of rubber seed is already published in the literature.

The composition of the rubber seeds is investigated almost completely. The average total weight of the predried rubber seed is about 4,5 gram. The kernel of the rubber seed is roughly half the weight of the total seed. The kernel contains 40-50 % of a comparably highly unsaturated vegetable oil, 20 % protein and 40-50 % carbohydrates. Because of the fluffy structure of the kernels the best way for the separation of oil from the seeds is milling of the decorticated kernels in a chakku mill.

The unrefined oil is used for the production of alkyd resins for paints or in the production of soap together with coconut oil to obtain a somewhat softer soapbar. Refined oil could be used as an extender for lightly coloured drying oils (linseed oil) and because of its high nutritional value for human consumption. From the toxicological point of view neither the oil nor the meal will give insurmountable pro-

blems when used for human or animal nutrition, provided that several requirements have been met in the collection and storing of the seed and in the processing steps. The refining of rubber seed oil is somewhat difficult and can be done on an economical scale only if some requirements concerning the selection drying and storage of the seeds are fulfilled.

The rubber seed meal is presently used as fertilizer and also with a certain reluctance as feeding stuff for calves, pigs and broilers. Because of the hard and sharp shell of the rubber seed only dercorticated seeds should be used for animal feed to avoid damage of the intestines. The presence of hydrogen cyanide glycosides in rubber seed requires a special treatment to split the glycosides and sufficient storage of the kernels and the meal to obtain low HCN-levels. It could be shown in this study that a further problem in using rubber seed meal as feeding stuff is the aflatoxin residue caused by moulds. High levels of aflatoxins might cause strong deceases in animal breedings. The only way to avoid such high toxic substances in the meal would be a manual selection of the moulded kernels and a careful obeying of the storage conditions.

In the analytical investigations of this study it was shown that the crude rubber seed oil contains about 1 % of a polyisoprene material with a molecular weight of several hundred thousand Daltons. This material cannot be totally removed in the refining procedure and is responsible for the very low filtration rate of the oil after the bleaching step. It could also be shown by the analysis carried out on rubber seed from Sri Lanka that the oil contains a relatively high amount of phosphatidic acid. High contents of phosphatidic acids may cause emulsification problems in a neutralization step of the oil refining. To avoid this, only oils with a relatively low content of free fatty acids can be refined without problems. As a consequence only oil from fresh seeds quickly dried at a temperature of 80°C down to a water content of less than 5 % will be suitable raw material which may be processed on an economical scale.

In view of the above mentioned problems in processing of rubber seed on a pilot plant scale it is recommended to meet the following requirements:

- Seed collection quickly after seed fall,
- immediate drying at 80 °C and storing afterwards,
- decortication by small handrollers and separation of kernels from hulls and moulded ones manually.

Suitable places for carrying out these procedures on small scale with simple equipment are at rubber estates.

I Literature Survey

I 1. Introduction

This literature survey was carried out to compile the published present know how on rubber seed processing technology for the production of vegetable oil and feed. The survey is based on an online databank-recherche, where the following files have been used:

Chemical Abstracts (1967-1985)
Food Science and Technology Abstracts (1969 - 1985)
Biosis Prereviews (1977 - 1985)

The key words used for the oneline recherche were as follows:
rubber/seed or hevea, nutrition or food or feed or edible, not nutrition or food or feed or edible, process or technology or manufacturing or product. The printouts of the online-recherche are compiled in appendix 1.

To prepare a report of this literature-review, any online printout was examened with respect to the relevance for the different items of the project. Extended printouts were made from those titles, which seemed to give any contribution to the required information. Original documents were procured of those titles, which seemed to be of higher importance. Some further documents, which have not been found in the online review, but were mentioned in the quotations of the original literature are also included in this report.

In the following pages a compiled information from more than 300 titles of the documents of the online-literature-survey is given.

I 2. Information from literature

I 2.1. Early papers and reviews

Attempts, to utilize rubber seed, are not new at all. The Bibliography No. 10 of the Library of the Rubber Research Institute of Malaya, titled "Bibliography on Rubber seed Oil" covers the literature up to 1970. It started with chemical examinations of Para rubber seed, meal, oil and commercial evaluation done in the Imperial Institute (London) in 1903[1]. Lit.[2] quotes "Rutherford's Planters' Note Book" from 1913 to contain detailed estimates of the costs of collecting, drying and packing of seed, both for Malaya and Ceylon, as well as analyses of seeds and oil. In more recent time the Rubber Research Institute in Sri Lanka has contributed several papers [3,4,5]. Also in other areas, where Hevea is grown, this problem has been considered esp. in view of the oil [6,7,8], but also as feeding stuff [8a].

I 2.2. Composition of rubber seed

I 2.2.1. Composition of rubber seed (whole seed)

An example of a rough composition of fresh seed is given in lit. [3]:

kernel	:	41,2 %
shell	:	34,1 %
moisture	:	24,4 %

The moisture may go up as high as 35 %.
In four different crops from different plantations the ratio shell : kernel varied between 1:1 and 1:1,7 [7]. For most utilizations of rubber seed mainly the kernel is of importance, so its composition will be of more interest.

I 2.2.2. Composition of rubber seed kernel

Some compositions found in the literature are given in Tab.1.

Tab. 1 Composition of rubber seed kernels in %

ingr. \ ref.	[3]	[6]	[9]	[10]	[11]	[1]	[12]	[13]
oil	42,0	48,5	53,6/52,2	37,4	51,2	44,5	42,0	48,0
H_2O	5,0	8,5	3,3/ 3,2	6,8	4,3	14,7	5,0	0
cake	53,0							
protein		17,6	30,7/31,1	18,7	18,8		18,2	18,5
carbohydrates		22,9		30,7			27,2	
crude fiber			1,7/2,2	4,8	1,1		3,7	5,4
ash		2,5	5,3/5,5	1,4	3,4		3,2	3,4
Ca	0,12			0,25			0,11	
P	0,43			0,33			0,43	

These figures of course can give only indications about the composition, since starting materials, preparation and methods to determine the composition varied. In all, about 50% oil and 20% protein (moisture free basis) may be seen as relevant figures for the utilization of rubber seed. Similar oil contents of kernels were also reported in lit. [7] and [14].

I 2.2.3. Composition of Rubber seed oil

Rubber seed oil is a highly unsaturated oil. This is due to a relatively high content of linoleic acid and especially linolenic acid in the triglycerides. Some analyses from literature are given in Tab.2.

Tab. 2 Composition of rubber seed oil (rel. comp. in %)

fatty acids / ref.	[12]	[15]	[9]	[8]	[6]	[7]	[14]	[17]	[16]	[10]
14 : 0			0,5/ 0,9			0 - 0,2	0,2			
16 : 0	10,6	7,5-11,0	8,0/12,7		10,6/ 8,7	9,4-11,4	10,8	17-22	9,6	10,3/10,7
18 : 0	12,3	8,6-12,0	14,2/14,2	8,3	12,3/10,2	5,8- 9,4	12,6		13,4	23,8/23,2
20 : 0	1,0	0,3- 1,3	1,0/ 0,6		1,0/ 1,3				trace	
18 : 1	17,1	17 -30	24,6/24,7	21,9	17,1/20,2	21,4-27,5	28,2	19-22	24,2	47,2/49,2
18 : 2	35,5	30 -39	33,1/31,6	38,2	35,5/38,4	37.6-41,6	29,3	32-39	17,4	13,1/13,3
18 : 3	23,5	14 -26	19,9/14,4	24,3	23,5/21,2	14,6-20,1	19,1	21-26	24,9	5,3/ 4,0
free fatty acids %				5,2	5,4	10 -22				
unsaponifiable matter %			0,8/ 0,9	0,7		0,6- 1,5				

Most authors agree with the fatty acid composition of rubber seed oil given by Hilditch [15]. Variations are due to differences in crops [6,7,17]. Traces of 8:0, 9:0, 11:0, 10:1 and 14:1 fatty acids were found in addition [7].
Somewhat deviating results are given in Lit. [16] in so far, as additionally 9,6 % 7,13-Eicosadienoic acid and 1,0% 6,9,12,15-Octadecatetraenoic acid were found in oil from Malaysia. Very recently a rather uncommon composition of an Indian rubber seed oil was published [10] without discussing the deviations from the compositions found normally.
The individual glycerides of rubber seed oil were investigated by Gunstone and Padley [18] by argentation thin layer chromatography and lipolysis. As in most seed oils, the distribution of the fatty acids in the individual triglycerides are in a 1,3-random-2-random pattern. More than 50 % of all triglycerides contain at least one linolenic acid. Around 1 % unsaponifiable matter, probably consisting mainly of sterols [8], has been reported [7,8,9].
The content of free fatty acids is highly dependent on the storage conditions of the seed. In oil from fresh or preheated seed less than 0,5 ffa have been found [18a].
The phospholipid content in an oil,obtained by hexane extraction of whole rubber seed was around 1 % [8], it consisted mainly of phosphatidylethanolamine (0,58%), phosphatidylcholine (0,32%) and some phosphatidylserine (0,06%).
With respect to the objective of this study the most important fact from the reviewed data is the high content of linolenic acid of roughly 20%. This amount is in between that of linseed oil (30-60%) and considerably higher than in soybean oil (2 - 10%).

I 2.2.4. Composition of rubber seed meal

There are a lot of data about the composition of defatted (mostly expelled) rubber seed meal. Because of different materials and probably also partly because of different methods of analysis the fluctuations are rather broad. The mean values from 10 papers [3,6,10,12,13,19,20,21,22,23] are given in Tab. 3 with calculated standard deviations and extreme values.

Tab. 3

Avervage composition of defatted decorticated rubber seed meal

component	mean values (%) ± std. dev.	extreme values %
H_2O	8,2 ± 2,9	3,4 - 12,5
ether extract or oil	10,6 ± 2,7	4,4 - 14,7
crude protein	29,0 ± 4,0	22,9 - 34,3
crude fiber	6,6 ± 3,2	2,7 - 13,9
N-free extract or carbohydrates	39,7 ± 4,8	32,0 - 47,3
ash	5,1 ± 1,0	3,0 - 7,0

So roughly the rubber seed cake after pressing out of the oil consists of 30% protein, 10% oil and 40% carbohydrates. No investigations about the nature of the carbohydrates have been found in the literature.

About the contents of Ca and P several authors [10,19,20,22] give data, which result in mean values of 0,4% ± 0,2% for Ca and 0,6% ± 0,1% for P.

In lit. [22] a complete mineral composition is given (mean values of four different samples):

Ca: 0,88 %	P : 0,94 %	Fe: 147 mg/kg
Mg: 0,34 %	Cl: 0,18 %	Mn: 25 mg/kg
K : 1,54 %		Zn: 112 mg/kg
Na: 0,21 %		Cu: 32 mg/kg

The most important constituent of the meal of course is the protein. Its amino acid composition, as it has been found by several authors, is given in Tab. 4.

Tab. 4

<u>Amino acid composition of rubber seed rotein</u>

(% w.r.t. protein)

amino acid \ ref.	[10]	[12]	[13]	[21]	[22]	[23]	[24]
Isoleucine	7,10	3,1-4,2	2,71	3,16	3,38	3,1	3,8
Leucine	3,76	4,8-5,9	3,29	6,10	6,24	6,7	7,1
Lysine	2,35	2,8-4,2	2,60	2,98	3,34	5,4	3,6
Methionine	2,37	1,1-2,2		1,09	1,08	0,7	1,4
Cystine	0,91	1,4-2,0		1,33	1,38		2,9
Phenylalanine	3,04	2,8-3,8		[illegible],85	4,94	3,8	4,8
Threonine	3,26	2,8-3,1	1,79	3,13	3,24	2,8	3,8
Tyrosine	2,24	2,6-2,8		2,69	2,74	2,6	2,6
Tryptophan	2,05	1,2-1,4			1,38	1,3	
Valine	2,13	4,2-6,5	3,56	6,64	5,98	6,4	8,0
Histidine	3,34			1,80	2,03		2,3
Glycine	2,24			3,71	3,85		4,4
Arginine	7,60			9,40	10,24		9,4
Aspartic acid	15,80			10,32	11,25		11,2
Serine	1,78			4,25	4,86		4,8
Glutamic acid	17,70			14,95	15,87		15,7
Proline	6,15			4,39	4,28		4,4
Alanine	2,62			4,80	4,46		4,9

I 2.2.5. Minor components in rubber seed

These are mainly of interest as far as they may impair the utilisation of rubber seed in human nutrition or animal feed by the existance of hasardous components.

I 2.2.5.1. Hydrogen Cyanide

Para-rubber-seed contains the cyanogenic glycoside linamarin [25], the same as in manioc [21], which on hydrolysis yields hydrogen cyanide. The highest HCN-contents were found in fresh kernels (up to 0,223 % HCN calculated on a moisture free basis [18a].
Simply on storage the HCN is released by hydrolysis and thus reduced by evaporation (in fresh kernels 94 % of total HCN were bound [13]). This escaping HCN may be a certain toxic danger for the surroundings [4].

Tab. 5
Hydrogen cyanide content of rubber seed kernels [18a]

Period of storage (weeks)	% H_2O	HCN-content (mg/kg)
fresh	35,9	770
1	29,0	160
3	12,7	150
4	11,4	110
14	6,5	60
20	8,1	60

Similar results as shown in Tab. 5 were obtained by Narahari [26]. Fermented products already after one month had only around 2 mg/kg HCN [26,27]. So there are relatively simple methods to remove the HCN. In unfermented, solvent - or mechanically processed meal 55 resp. 60 mg/kg HCN were found [19].

I 2.2.5.2. Gossypol

Rubber seed meal (decorticated according to analysis) contained 40 - 410 mg/kg (solvent processed) and 100 - 570 mg/kg (mechanically processed) free gossypol. The ranges for total gossypol were 300 - 715 mg/kg and 300 - 800 mg/kg resp. [19]. These figures have to be compared with those of cotton seed meal containing normally 360 - 930 mg/kg free, 9000 - 15000 mg/kg total gossypol.

I 2.2.5.3. Saponine

1,8 % saponins were found in decorticated rubber seed kernels [28]. That is a similar level as in the meal of the oil containing leguminosea Pentaclethra macrophylla, Mucuma uriens and soybeans. After hydrolysis the sapogenins were compared by TLC. The three spots differed in Rf-values slightly from those in soybeans.

I 2.2.5.4. Tannins

0,4 to 0,5 % tannins were analysed in undecorticated rubber seeds [26]. They are located in the shell, since none was found in kernels.

I 2.2.5.5. Volatile inflammable substances

Thuch substances, probably carbohydrons, have been reported to be present in kernels [4]. They cause a certain risk of fire during drying of seeds in open fired driers as copra kiln. Nothing is reported about the amount of the volatile material present in the seeds.

I 2.3. Processing of rubber seed

For utilization of rubber seed the seeds have to be collected and processed beforehand. The fresh seeds optimally are collected from plantations every 3rd or 4th day [4,6]. Afterwards they are partially dried by sun [4,6a] or in ovens at 80 - 90°C [7]. This drying procedure to a moisture content of less than 5 % is essential to prevent enzymatic attacks (fat splitting) [3] during further storage of the material.

Starting with dried kernels [18a] (5-6% H_2O) the free fatty acids content began to rise after 12 weeks storage (also the H_2O-content increased somewhat). When the H_2O-content was kept low [18a] (3-4%) all the time (storage temp. 30-37°C), the ffa-content did not exceed 1% (in oil) during a storage period of at least 24 weeks. Sterilisation with hot steam improved these results somewhat. After 12 weeks however mould and insect attacks limited storability.
Microorganisms on rubber seed have been investigated in Brasilia [29].

Processing normally starts with dehulling. This has been done either manually after cracking the shell [3] or by means of a Kalyan type groundnut decorticator [11]. The latter technique resulted (after recycling) 95% kernels with 5% shells.
Seperation of kernels and shells by winnower and scoops was considered to be out of question [6].
In contrast to these more research oriented papers lit. [6a] reports, that 1976-1977 in South India (Tamil Nadu and Kerala) 97 commercial units have processed 12.070 tons of dry kernels. Dehulling here also was mostly done manually, only three mills used machinery and two a combination of machinery and hand labour.

Crude oil in a technical scale is obtained by expelling [4]. Adaption of an Anderson 1 expeller to rubber seed kernels has been described [4] (8-10% residual oil in meal). Addition of 10% molasses has facilitated removal of oil [10,6].
Expelling proofed to be very difficult due to jamming of the material inside the expeller chamber [11]. Cold hydrolyc pressing, successful in laboratory scale, gave high oil yield, but could not be adapted to cold crushing in the expeller [11]. According to lit. [5] the expeller cake may be either heat treated and pressed under high pressure to give some more oil, or more usually,it is solvent extracted afterwards.

In South India rubber seed kernels are processed in a rather large scale by groundnut oil millers with the same equipment as for groundnuts, that means rotary machines [6a]. Only a very few expellers were used. About 20 to 30 % molasses was added to the dried kernels resulting in an oil recovery of 30-40 % and cake recovery of 60-75 %.

Detoxification (HCN-removal), apart of the simple storage method, was achieved by fermentation (soaking the meal with tap water 1:3 for 24 hrs.) [26]. Soaking with ash solution or water or roasting (350°, 15 min.) were also applied for detoxification [21]. Without giving figures, roasting was estimated to be the best method [21], but already without special treatment there were no toxic effects to be seen in feeding experiments [21].

Refining of crude rubber seed oil has not been described much in the literature covered in this study. According to lit. [5] it can be refined in a similar manner as linseed oil.

I 2.4. Utilization of rubber seed

In this chapter the economical views for the utilization of rubber seed, given in several papers, will not be reviewed, because they may depend largely on the special situation of the local area at the time of publication. However, some figures about the availability of seeds will be given. For 1976 in Malaysia a production of 200-300 kg seeds/ha are reported [34]. The expectations for Kernataka, India, 1983 were only 95 kg/ha [7]. A similar figure can be calculated for Sri Lanka 1968 [4]. In still former times (1918, 1927, 1928) between 55 and 130 kg/ha have been found in Malaysia [29a]. The actual seed yield may vary very much [34] due to diseases and weather conditions [2,6a,29b].

Obviously already in 1929 serious attempts have been made to utilize rubber seed. An export of 7.905 tons from Sumatra intended for oil production and plans for a decortication plant in Malaya have been reported [29c]. The reasons why these activities have not been continued, are discussed in lit. [29b].

I 2.4.1. Rubber seed meal as animal feed

Judging from the literature, utilization of rubber seed as animal feed has aroused much interest. An Indian feed manufacturing company 1977 actually sold already feed containing 10 % RSM [6a]. Mostly the cake, obtained after pressing out much ot the oil, has been used. The worldwide annual potential of RSM has 1983 been estimated to be 1,68 x 10^6 t [10].
In none of the reviewed investigations toxic effects due to HCN or cyanogenic components have been found (the HCN-content in RSM mostly was in the order of 50 mg/kg.
Especially in pigs, given up to 30 % RSM (HCN-content

<60 mg/kg), no goitrous or anaemic symptoms were found. Neither daily gains nor carcass characteristics were affected by RSM [29d]. However a negative influence on fertility and hatchability of hen eggs was observed [30,31,12], even at a 10%-level [12]. This is probably not due to cyanogenic components. 10% RSM in the fodder of breeding cows also drastically affected the fertility of the herd (cited in lit. [5]).

Layerhens could be fed (without detrimental effect [31]) with up to 20% RSM. Probably due to an imbalance in amino acid composition (shortage in methionine and lysine) higher ratios led to negative influences on the eggs (size, thickness of shell). If additionally lysine and methionine were given, up to 30% RSM could be fed to layers (50 weeks) without negative effects [32]. Egg production decreased however with 40% or 50% RSM in the diet, whereas the quality of the eggs even increased [32]. In lit. [33] it is reported, that up to 20 % RSM substituting coconut meal even increased weight gains of broilers and that at a 25%-level egg production was not affected.

For broilers 20 % [33,12] to 25 % [34] RSM could be used in a basic diet of maize and soybeans meal. Giving additional lysine and methionine, up to 45% RSM were fed to broilers without negative influences [32]. If the basic diet was meat meal and maize, only 10 % RSM could be applied even in the presence of additional methionine [35]. Using fish meal and maize RSM could be rised to 20 % [35]. Pullets however could be fed with 40 % RSM in these diets, showing only slight signs of amino acid imbalance [35].

Chickens were also used to assess the appearent metabolizable energy; 2,80 kcal/g [10] and 2,86 kcal/g [32] have been reported but also 1,79 kcal/g [36]. The true metabolizable energy was even somewhat higher (3,00 kcal/g) [10].

The determined gross protein value was 47,0 [10] (chicken).

Poultry has been fed also whole kernels [21]. No toxic effects were seen up to 50 % kernels in the fodder, which had been pretreated by roasting or soaking with water or ash. Roasted RSM was accepted best by poultry or swine.

Also the rubber seed oil has been added to chicken fodder up to 8 % instead of coconut oil without any obvious drawbacks [37].

For growing swine only 10 % RSM could be included in the fodder. 20 % and especially 30 % resulted in poor weight gain and feed conversion efficiency [38]. Insufficient amino acid balance (lack of lysine and sulfur amino acids) is thought to be responsable. In lit. [5] however much better results are cited.

Growing calves seem to be better adapted for a RSM-containing diet. 30 % RSM replacing the same amount of cotton seed meal resulted in better results than 15 % or 0 % w.r.t. weight gain/kg feed or weight gain/cost [20]. Maintainance of health was good.

A rather similar investigation is described in lit. [39]. It is concluded that up to 30 % RSM can be incorporated in the fodder for growing calves.

Also in lit. [8a] up to 30 % RSM in fodder for calves and cows were used. The costs for fodder were thus reduced considerably without loss of performance. Similar positive results are reported in the papers [39a, 39b, 39c].

Feeding RSM instead of linseed meal to cows resulted even in 10 % increase of milk production [40].
Goats developed quite normally when fed with 35 % RSM in

their fodder for 3 months [41]. Very limited feeding experiments with sheep indicated a high digestability of RMS [41a].

I 2.4.2. Rubber seed meal in human nutrition

Only one article has been found, which exactly deals with this topic [42]. It reviews some older papers and gives some intentions for future work. Some other papers about feeding trials with rats, however, will be treated under the above head-line.
Lit. [23] quotes, that rubber seed is used already in the diet of people, living near the plantations. Detoxification may be done similar as with cassava (24 h soaking with water, boiling for half an hour).

Protein efficiency ratio of RSM (PER = gram weight gain/gram protein eaten) was studied in rats at 5, 10 an 20 % protein level. The HCN-content of the RSM was 34 mg/kg. At 5 % protein level the PER was only 0,9 as compared to casein (2,3). This is probably due to low methionine levels. At 20 % protein level (60% RSM in diet) PER was 1,4 (casein 1,6). No toxic effects were observed. The PER of RSM was in about the same order as that of other oil seeds. Former feeding trials with rats had been less satisfactory [43].

Also in lit. [22] RSM was found less good than soybean -, peanut- and casein-protein (10 % protein in basal diet fed to rats). PER for RSM here was even negative (but not for full fat kernels). Responses to amino acid supplementation suggests, that lysine and methionine are the most limiting amino acids in rubber seed protein. The conclusion is, that full fat rubber seed, although inferior to peanuts or soybeans, has some nutritional value, but defatting (by solvents) deteriorates the protein.

Lit. [13] on the contrary states, that the raw seed (with around 0,13 % to 0,23 % HCN!) has a low nutritional value and even caused death (rats, 10 % crude protein in the diet). Pretreatment by soaking with water and 1 h cooking reduced the HCN-content to less than 5 % of the original value. Even then the PER was only around 1/3 of that of casein, but close to that of traditional cereals like corn. Limiting amino acid was threonine.

At a lower level (7,4 - 12,4 % RSM in diet) RSM had no influence on weight gain, nitrogen digestibility and carcass composition of rats [24]. There was no difference when autoclaved RSM was used.

I 2.4.3. Technical utilization of rubber seed

Apart of occasionally remarks that RSM could be used as a fertilizer (which actually has been done in India [6a]), only the oil has been considered for technical utilization. Of course the high content of unsaturated fatty acids may make it useful for substitution of linseed oil in paints. These possibilities and the technical and economical situation in Sri Lanka are reviewed in lit. [5] (1973): Rubber seed oil can substitute 25 % linseed in oil paints. Its drying properties could be improved by reaction with maleic anhydride. Much more important however is its use in alkyd resins (reaction with glycerol and phthalic anhydride). These products were commercially produced in Sri Lanka in 1973. Heating rubber seed oil with sulfur results in the so called factice, a valuable aid for rubber and in mixing processes. Epoxidation leads to products useful to improve some properties of PVC.

In more recent time more papers have been published dealing with industrial use of rubber seed oil. So it has been reported, that the oil can replace 80 % of linseed oil in air-drying medium-oil alkyds [44] directly or after epoxidation [45]. Polymerisation of rubber seed oil with 20 - 45 % dicyclopentadiene resulted in a drying oil useful in low quality paints [46,47,48].

Rubber seed oil could be fractionated (solvents) in products with increased iodine value (useful in paints and resins) and products with lower iodine value (useful in soap manufacture) [49].

To utilise rubber seed oil in soaps (or as fatty acids), splitting studies have been performed [50,51]. The unsaturated fatty acids could be converted into stearic acid by electrochemical reduction [52].

Apart from these academic studies it is estimated, that 1976-77 1880 tons of rubber seed oil have been consumed in Tamil Nadu and Kerala (≈50 % of the total amount produced in India). This went mainly into the soap industry, only about 100 tons were used for paints [6a]. Sri Lanka exported 420 tons of oil in 1973 [5].

I 3. Summary

About utilization of rubber seed quite a lot of work has already been done in different countries. The oldest literature which was found in this study is dated 1903.

Many papers deal with the composition of the seeds. The kernel of rubber seed is roughly half the weight of the total seed. This kernel contains about 40 - 50 % oil, 20 % protein and 40 % carbohydrates.

The rubber seed oil contains around 20 % of linolenic acid, 35 % linoleic acid, ca. 25 % oleic acid and around 10 %

stearic- and palmitic acid each. Of course these figures fluctuate due to different investigations.

The amino acid composition of the protein has been analised several times. Main components are glutamic- and aspartic acid and arginine. The essential amino acids are all present in amounts ranging from ~ 1 % (methionine, tryptophan) till 6 % (leucine).

An important minor component in rubber seed is hydrogen cyanide. In fresh kernels an amount of up to 0.2 % has been reported. It is present as a glycoside. During storage of the kernels for several weeks the HCN-content drops below 100 mg/kg. Small amounts of gossypol (~ 0.1 %), saponins (1.8 %) and volatile inflammable substances have been found in the kernels, ~ 0.5 % tannins are located in the shells.

In processing of rubber seed it is important, to collect the seeds as soon as possible and dry them to moisture contents below 5 %. This limits liberation of free fatty acids and moulding.

The process mostly starts with cracking and manual decortication. In South India (1976 - 1977) about 12000 t kernels have been milled with ground nut equipments (rotary machines). Expellers have been used to press out the oil, but with unsatisfactory results.

Utilization of rubber seed is largely dependent on the availability. The figures about the seed yield vary between 55 kg/ha and 300 kg/ha. It depends largely on weather conditions, plant diseases and clone of the rubber tree. As a worldwide potential 1.7 mio tons (rubber seed meal) have been estimated 1983.

Rubber seed meal has been fed to pigs, hens and chickens, cows, calves and goats. It can replace up to 30 % of other feedstuff like linseed meal. Some fertility problems were observed with hens and cows. The HCN-content obviously is not a serious drawback in the utilization of rubber seed meal. Lysine and methionine are the limiting amino acids in rubber seed meal.

Rubber seed oil can replace up to 80 % linseed oil in paints of minor quality. Alkyd resins containing 25 % rubber seed oil instead of linseed oil have been produced on a commercial scale in Sri Lanka. In India 1880 tons of rubber seed oil have been consumed 1976 - 1977, mostly in soaps.

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II Field Study in Sri Lanka

II 1. Introduction

This part of the report deals with the revealed information during a field study in Sri Lanka which was carried out w.r.t. the technicological and economic situation of the utilization of rubber seed. From the information obtained by the literature study (see Part I) and the statements received from interviews and discussions with research scientists, estate managers, oil millers and production managers of paints and animal feed industries the frame work should be set up for a development of a technology for the production of vegetable oil and animal feed in the countries where rubber production is available. A list of these contacts and the people met for interviews in Sri Lanka is given in appendix 2.

II 2. Technical and technological aspects

II 2.1. Seed availability

This aspect of course is a very basic one, which has been hardly mentioned in the literature. It was learnt that, at least in Sri Lanka, it is a rather recent experience that the seed fall can fail completely in certain years. Taking into account the opinion of experts and estates managers as well as the poor information from the literature [1], the availability of rubber seed depends on a lot of various conditions and may hardly be influenced by agricultural techniques.

Amount and quality of the rubber seed growth depends on the following conditions:

1. Weather: The production of seeds from each type of clones varies from year to year according to the weathering conditions. In some years when it rains strongly during the flowering time from March to May the blooms are washed out and no seeds appear in summertime. In such years in these areas nearly no rubber seeds will be obtained. If there is high rainfall during the seedfall time from July to September a lot of seed may become moulded or will rot on the ground within a few days after the fall. In other years, when there is a drought from March to May, the blooms dry up and no seeds will appear in summertime.

2. Genetic: The different types of rubber seed trees produce different amounts of seeds. Until now only very few studies have been undertaken to determine the amount of rubber seed w.r.t. the different types of clones.

   New types of clones, developed in Malaysia and in the Rubber Research Institute of Sri Lanka, which give high yield in the production of Latex, produce rubber seed which amounts to less than 60 % of some of the older types.

3. Diseases: Phytophthora attacks the seed pods, causing them to rot. Oidium mildew destroys the flowers. Both diseases are largely under control now, but control is only done to protect the trees, not to save the seeds.

Depending on the type of clone and the weather conditions 50-150 seeds can be expected from one tree during falltime. Assuming that the stand of trees by hectare is about 275-350, depending on planting area, the production of seed/hectare in a year can vary from 12.500-52.500 . It has been estimated that 220 seeds will form about 1 kg. Therefore 50-250 kg (200 kg/ha in normal years) of rubber seed can be expected per hectare in one season.

A more detailed study about the seed yield/ha depending on site and clone in Sri Lanka is currently under way within the frame work of a doctoral thesis carried out at RRISL [2].

II 2.2. Collection of rubber seed

Seed collection is a tedious work because the seeds have an earthlike colour and the ground of the rubber plantations is mostly covered with leguminoses so that most of the nuts are hidden. Furthermore, some of the lands where rubber is growing is hilly and rocky - the latter is true especially in Sri Lanka and part of South India - and therefore seed collection cannot be done very effectively.

Thus, only 60 % of the total acres of rubber land in Sri Lanka are suitable for seed collection. In Sri Lanka currently about 250 000 ha are rubber land (increasing). From 150 000 ha (60 %) about 30 000 t seeds could be collected in normal years.

In Sri Lanka as well as in South India most of the seed collection is done by children from pendants of the rubber estates. At present in Sri Lanka the oil millers quote a price for the seed and traders then quote prices to shops situated in the villages. In some years when there was high demand of rubber seed, collection agents organized the seed collection also with adults, but always most of the seed collection was done by children. Using children for the collection is a cheap method but it should be mentioned that payment even for the children might go up with the growing marketing potentiality for the rubber seeds.

As collection centres factory houses of rubber estates or the factory houses of oil millers have been used in some cases. To avoid moulding, rotting or germinating of the seeds they have to be collected fairly fresh. Optimal collecting of the seeds on the same ground has to be repeated at least every 4th day if not more frequently.

II 2.3. Drying and storage of rubber seed

Before storage, the seed has to be dried (preferably to a moisture content, < 5 %). This is mainly to prevent moulding and rotting. But also lipase activity is reduced and shrinking of the kernel makes decortication easier. Drying has been normally done in the sun at the estates, but drying space and storage capacity sometimes may be limiting factors for the estates.

Oil millers reported about heap storage of undried seeds in their factories for 2-3 months without problems. The accidental moisture content, however, may have been low and also the oil was used for technical purposes only. Drying at 60 - 80°C resulted in more oil with less free fatty acids than drying in the sun (RRIM, Annual Report 1975, p. 160). Ovens should be preferred to smoke houses because phenols could diminish the oil yield (private communication Dr. Nadarajah). Storage of dried kernels in polythene bags slows down formation of free fatty acids and prevents mould growth.

II 2.4. Production of oil and cake

For the production of oil and cake from rubber seeds several methods have been used more or less successful. In Sri Lanka most of the seeds have been milled in copra mills. This however can be done only with undecorticated seeds because the kernels are too fluffy for the relatively wide screws of the copra mills. The throughput is much less than with copra and the wear of the machinery due to the shells is feared by the millers.

The oil produced is of medium or bad quality and the yield is about 60 % of the total content. The cake contains relatively large particles of the solid shell which might damage the throats and the intestines of cattles and pigs if it is used as animal feed. Therefore most of the rubber seed cakes produced in copra mills are used as manure.

Another technical drawback of milling rubber seeds on the copra expeller is that the seedfall season coincides with the coconut harvest. So the millers are unable to spare adequate capacity for the milling of rubber seeds as the mills are occupied then with the milling of copra. Therefore Messrs. Lever Brothers developed alternative milling processes. First trials in 1966 and 1967 were done with decorticated rubber seed kernels on a Anderson expeller as well as on a German EP expeller. The latter was an expeller in a pilot plant at CISIR in Colombo. The yield of processed oil in all these experiments was very low, so that more than 35 %, sometimes 50 % of the oil content of the kernels were left as a residue in the cake.

In 1972 an unsophisticated method of milling rubber seed used on the rubber estates by support of Messrs. Lever Brothers was introduced. This process involves crashing the decorticated seeds in a chakku mill, cooking the crashed kernels and finally espelling the oil by means of a manually operated press. With this arrangement an oil yield of ~50 % was obtained. The chakku mills were operated by bulls. It was reported that in South India (Kerala) chakku mills operated by electrical power exist and are being used for the same procedure of utilizing rubber seeds [3].

In many interviews disapproval was expressed mainly about two technical problems in rubber seed processing which seem to be of importance:

a) If the meal is to be used as feeding stuff, the shell should be either removed or ground very fine. The latter requires special mills and relatively high energy costs. Decortication even in the bigger scale experiments in Sri Lanka (Lever Brothers) has been done by hand so far. But also the rollers for rubber sheets, available at the estates, could be used to crack the shells, followed by a kind of winnowing/sieving. In India a special machinery is in use for this job.

b) The oil, at least that obtained from copra expellers, contains fines which makes the filtration step in the refining process very time consuming.

II 2.5. Consumption of rubber seed oil

It is reported in different statements that several trials were done in Sri Lanka to make use of rubber seed oil for the manufacture of laundry or washing soaps. The same is reported for Kerala and Tamil Nadu [4]. The intention to use RSO for the soap production was mainly, to replace parts of the coconut oil to obtain a somewhat softer soapstock. The commercial objective was to have available an oil of an appropriate quality which would not be more expensive than coconut oil.

From all statements it turns out that technically the darkening of the colour of the oil and the presence of unknown particles causes bleaching and filtration problems in the refining process. The filtration time in the bleaching process of RSO is ten-fold as much as that of coconut oil and thus the total process is rather ineffective. If not more than 10 % of refined RSO is used for the soapstock a fairly well coloured soap is obtained. If RSO is used, only 2 % is acceptable. Darkening during storage of the soap limits the use of RSO for soap manufacture and inquiries made by soap manufacturers show, that washing soap manufactured from RSO is of inferior quality and the consumers in Sri Lanka as well as in Kerala require a higher quality of soap. Nevertheless use is already made of rubber seed oil in a mixture together with coconut oil for the preparation of solid soap bars.

RSO has sometimes been used for paint manufacture. The oil contains about 35 % of linoleic and 25 % of linolenic acid, and therefore was used to replace part of linseed oil as a drying oil in paint industry. Because rubber seed oil does not contain sufficient amounts of linolenic acid it cannot replace linseed oil totally but is used as an extender of linseed oil. Whether unrefined or refined RSO is used in this application could not be established evidentially.

If RSO is used as an extender for linseed oil instead of soybean oil the drying oil may be used for all current paints but not for clear colourless lacquers, because the increasing darkening of the products containing RSO. With the current quality of the RSO no white coloured paints can be prepared. This, however, might be cured by using a better refined oil.

Most of the RSO in Sri Lanka has been used for the manufacture of alkyd resins. Inquiries with the manufacturers of alkyd resins assured, that the properties and the quality of the RSO obtained in the different milling processes is adequate for the production of alkyd resins and that there is lot of know-how at the different paint manufacturers to make use of the RSO. As linseed oil which has to be imported into Sri Lanka is replaced by RSO a saving of foreign currency is obtained. But this is true only if the cost for RSO do not exceed the cost and the import duty of linseed oil or other oils used as extender of drying oils (soybean oil). Therefore, the only limiting factors in this field are the availability of RSO and the costs of the oil in comparison to the costs of linseed or soybean oil.

In the years of large RSO production some of the RSO was exported to European countries (Netherlands, Germany) and was used for the production of oleo chemicals.

Not much emphasis is put on the possible use of RSO as an edible oil until now. The comparatively high amount of unsaturated fatty acids increases the nutritional value of the oil compared to coconut and palmoil. However, the relatively high content of linolenic acid in RSO and a slightly bitter and off-taste which might occur in the present quality of RSO has limited its use in human nutrition especially in a country where coconut oil and palmoil is available all over the year as a common product on a relatively cheap price level.

If - what has to be checked in laboratory trials - a better refined and highly stabilized product could be obtained from a crude RSO, a mixture of RSO and coconut or palmoil may be used as an edible fat with a high nutritional value.

The difficulties which occur in the refining of the oil, especially in the bleaching and filtration steps require further investigations.

II 2.6. Consumption of rubber seed cake

The rubber seed cake is used in Sri Lanka in some places as a fertilizer. This is done especially with those cakes coming from oil mills where copra expellers are used to produce the RSO. Large and hard pieces of the shells of the rubber seed give damage problems with cattles, pigs and poultry if this cake is used as animal feed.

In the inquiries with the estates managers and the production managers of the animal feed industry companies, which all showed general interest in making use of the rubber seed cake for animal feed at the cattle or pig farms, a certain reluctance was roticed. Growth inhibition and infertility as well as in some cases high dying rates were told to be found when rubber seed cakes have been used. In contrast to this predominantly negative statements, in many published papers the acceptability of rubber seed cake from decorticated seeds as a component up to 40 % in animal feed is reported. The Veterinary Research Institute of Sri Lanka in Kandy has recently carried out a feeding study for six months with undecorticated rubber seed meal using 16 cattles. Rubber seed cake was used up to 40 % in this feeding trial together with maize and fishmeal. No adverse effect is found in this feeding study so far. Growth of the cattle as well as fertility are reported to be in good order.

The inconsistancy of the statements about the suitability of rubber seed cake for animal feed may find its explanation in the lack of knowledge about the pretreatment and the dietary value of the rubber seed cakes applied. Too big particles of the rubber seed shell or the hydrogen cyanide content of fresh rubber seed kernels as well as the possibility of aflatoxin contamination are factors which might have had adverse effects to the animals. But these obstacles may be overcome from the technical point of view by using correct drying and storage conditions as well as by an adequate milling procedure.

A report from the RRIM [5] says, that rats have been fed with 40 % rubber seed meal in their diet without toxic effects. Broilers and chickens can tolerate up to 15 %. Mould formation can be eliminated by using 0,5 % Luprosil (Ca-propionate).

II 3. Economic aspects

In the consideration of economic aspects for the utilization of rubber seed in a developing country like Sri Lanka or India the figures for the profitability of a process will be influenced by several factors. The relatively short period of 3 or 4 months within a year the rubber seed is available, as well as the yearly fluctuating amount of seeds make any economic evaluation very difficult. In this chapter use is made of those cost figures which are based on fairly true facts at present which are revealed in the interviews but which may vary within a rather short period of time. Therefore, all calculated figures should only be taken as an indication of cost.

Currently about 250 000 ha are cultivated in Sri Lanka with Hevea brasiliensis. Because of the hilly structure of the land not more than 60 % are suitable for rubber seed collection. Assuming a yield of 200 kg seeds/ha about 30 000 t seeds (estimates as low as 9-12 000 t have also been given) should be available in a normal year in Sri Lanka. Since Sri Lanka produces about 4 % of the total world production of rubber, a rough estimate for the minimum amount of seeds available worldwide may be in the range of 500 000 to 1 000 000 t.

Milling whole seeds in a copra expeller yields a little more than 15 % oil. In view of the above mentioned 30 000 t seed the potential of RSO by this process in Sri Lanka will be

4 000-5 000 t/year. Milling decorticated kernels in small equipments (chakku-type, man-operated presses) yields 20-25 % oil w.r.t. kernels. This results in 3 000-3 500 t/year. Although the oil yield in this case is less than starting from whole seeds, the advantage of this process is, that a valuable feeding stuff is obtained (s. p.42).

Collection costs of seeds in Sri Lanka are hard to figure out. In the last year with a normal seed fall (1982) 0,3 - 0,4 SL Rps/kg have been paid. In 1985 it was expected to be 0,4 to 0,6 SL Rps/kg, but 1985 was a poor seed year, too. It can be assumed that in 1985 if it would have been a year with normal seed fall, about 0,4 SL Rps/kg should have to be paid for collection.

Once the seed has been collected, it has to be dried rather soon. No figures for drying costs were available. Drying costs depend on the local possibilities and weather conditions. In South India 1977 the prices for dried kernels were about 50 % to 100 % higher than for undried kernels. Probably the same relation would apply for drying of seeds in Sri Lanka.

If the seeds are to be milled in existing oil mills, they have to be transported. For 5 t-lots the transportation costs are 15-20 SL Rps/mile in Sri Lanka. Assuming an average distance of 50 miles between the places where most of the oil mills are situated (Colombo) and the growing areas, transportation costs will arise to about 0,2 SL Rps/kg which have to be added, thus the cost for undried seed at the mill will be 600 SL Rps/t.

Decortication, if done by machinery (groundnut decorticators) could not be calculated, because no distinct information about the equipment was available. If decortication is done in the growing areas, the hulls have to be cracked by an inexpensive roller system possibly a rubber sheet former. The cracked shells then have to be separated manually from the kernels. This separation should be less labour intensive than the collection of seed. So we assume the costs for manual decortication at the estates to be not more than 0,2 SL Rps/kg seed. In 1976-77 manual decortication costs amounted to 10-30 % of the costs of undried seeds in South India [6]. As compared to processing of seeds in the oil mills 0,1 SL Rps/kg seed are saved in this case because the shells (ca. 50 % of the whole seed) have not to be transported but could be used locally as fuel or fertiliser.

Milling expenses in small equipment (chakku, handpress) in the growing areas can be assumed roughly from investigations and calculations carried out by Lever Brothers in 1972. At that time processing costs were roughly 40 % of the costs of the kernels. If we calculate the price of 1 kg kernels at present to be 2 SL Rps (2 kg seeds ~ 1 kg kernels; 2 kg seeds take 0,8 SL Rps for collection, 0,8 SL Rps for drying and 0,4 SL Rps for decortication) then the milling expenses will be ~ 0,8 SL Rps/kg kernels.

From these figures the costs for crude oil and cake processed at the growing area can be estimated and is given in the following three examples:

Example 1:

Costs:

1 t kernels	2 000 SL Rps	
milling costs	800 " "	(in rel.to kernel costs 1972)
additional expenses: (manpower for organisation and supervising, transport costs in the growing areas)	600 "	
drums and bags for oil and cake	290 "	(in rel.to kernel costs 1972)
depreciation 20 %	230 "	(- " -)
transportation of 1 t to Colombo	200 "	
total costs for 220 kg oil + 700 kg cake (8 % milling loss)	4 120 SL Rps	

Selling Prices:

cake (3 SL Rps/kg ~ copra poonac)	2 100 SL Rps
oil (15 SL Rps/kg*))	3 300 " "
	5 400 " "
Profit:	1 300 SL Rps

*) According to a Sri Lanka paint producer the current rubberseed oil price is about 20 SL Rps/kg. At this price it starts to get too expensive for paint industry but at a price of 15 SL Rps/kg there would be a good demand.

Calculation for processing oil and cake at central milling places without decortication:

Example 2:

Costs:

1 t seed	400 SL Rps
transport	200 " "
milling costs + depreciation	2 250 " "
	2 850 SL Rps

Selling Prices:

150 kg oil (15 SL Rps/kg)	2 250 SL Rps
800 kg cake	250 " "
Profit:	- 350 SL Rps (loss)

Here the milling costs cannot be calculated. They must be considerably higher than for copra (~1 500 SL Rps/t CN oil), because the through-put for rubber seed is about only 1/10 of that of copra. Also the stronger wear of the machinery has to be considered.

It was reported by an oil miller that at the current price of rubber seed of 0,6 SL Rps/kg and the current price of RSO (20 SL Rps/kg) production of RSO for Sri Lanka might just be worthwhile, but production for export would be no business.

If the oil mills would start with decorticated material (done manually) too, the following result is calculated:

Example 3:

Costs:		
1 t kernels	2 200	SL Rps (transport included)
milling costs	2 200	" "
	4 400	" "
Selling Price:		
250 kg oil (15 SL Rps/kg)	3 750	" "
700 kg cake (3 SL Rps/kg)	2 100	" "
	5 850	" "
Profit:	1 450	" "

So also for oil mills decortication is of advantage. It is doubtful, however, whether this is done profitable by machinery, which could be employed for rubber seed only.

If one applies in these 3 examples the production costs only to the oil, a price between 18-19 SL Rps/kg oil can be calculated. So it is obvious, that the profit in case of decortication is mainly from the increased value of the cake.

Also without decortication the cake value probably could be increased by grinding. Thus in example 2 the 800 kg cake could sell for probably 1,50 SL Rps/kg = 1 200 SL Rps. It is not known, however, which costs will arise by this additional grinding step (investment in grinder, energy).

As can be seen from these examples, the profit rises if the endproducts are high priced products. The oil obtained in the examples 1-3 is only crude oil for industrial purposes. To raise the price two possibilities have to be considered seriously:

1. To refine the oil to get an edible oil (CN oil sells for ~ 30 SL Rps/kg in Sri Lanka).

2. To produce oleo chemicals out of the oil that means hydrolysis and processing of fatty acids and glycerol. The current prices of fatty acids on the world-market are given below (average figures Jan.-Oct. 1985):

palmitic acid (min. 93 %):	27 100 SL Rps/t
stearic acid (min. 92 %):	29 400 "
oleic acid (min. 71 %):	24 400 "
linoleic acid (min. 64 %):	30 400 "

Whether manufacture of fatty acids (or other oleochemicals like emulsifiers) is worthwhile, depends on the costs of RSO in relation to the costs of the now used starting material for these products. Certainly it is not favourable to base such a production on RSO alone, but due to demand and availability mixtures with other fats should be used.

II 4. Summary

From discussions with estate managers and oil millers it appeared that a problem for utilisation of rubber seed could be its fluctuating availability. Mainly due to weather conditions seed fall may fail completely in some years. In normal years ~ 200 kg seed/ha are expected.
In Sri Lanka occasional collection of seeds has been done by children. The oil millers quoted a price, agents organized the collection in the villages or at the estates.
The seeds have been dried in the sun or in smoke houses at the estates to prevent moulding during storage. Since the main seed fall coincides with the coconut season, the oil millers have to store the seeds for 2-3 months until they can be processed in the copra mills.
Using this equipment, only undecorticated seeds could be processed, since kernels are too fluffy for copra expellers. The throughput is low due to filtration problems of the oil. The hard shells increase the wear of the machinery.
In 1972 a simple equipment has been used directly at an estate for processing RSO from decorticated seeds. This obviously was similar to a process operating in South India in several places.

Use is already made of RSO in a mixture together with coconut oil for the preparation of solid soap bars. The advantage of this use is 1. the partial replacement of coconut oil which may be used as edible oil of hight quality and 2. a softer soap bar obtained because of the comparatively high amount of unsaturated fatty acids in the oil.

Disadvantages are the yellow colour of the oil and the darkening of the soap during storage if more than 5 % of the coconut oil is replaced by RSO.

From the economic point of view the use of RSO for soap preparation instead of coconut oil is only beneficial if the cost for the RSO is below that of the coconut oil.

Further uses are made of RSO as an extender of drying linseed oil in the paint industry. But because of the increasing darkening of products containing RSO it cannot be used for clear colourless lacquers. If imported linseed oil is replaced by RSO, a saving of foreign curency is obtained. But this is true only if the cost of RSO does not exceed the cost and the import duty of linseed oil or other oils (soybean oil) which are used as extender for drying oils.

Quality and properties of the already produced RSO is reported to be satisfactory for the production of alkyd resins. The only limiting factors in this field are the availability of RSO and the costs of the oil in comparison to the costs of linseed or soybean oil. With the current quality of the RSO no white coloured paints can be produced because of the above mentioned darkening of the products. This, however, might be cured by further refining.

Not much emphasis is put on the possible use of RSO as an edible oil for human consumption until now. The comparatively high amount of unsaturated fatty acids increases the nutritional value of the oil compared to coconut and palmoil. However, the content of linolenic acid in the RSO and a slightly bitter taste which occurs in the present quality of RSO has limited its use in human nutrition particularly in countries where coconut oil and palmoil is available all over the year as a common product for a relatively cheap price.

Therefore it has to be examined in laboratory trials whether a better refined and high qual[illegible] product could be obtained from crude RSO by a refining p[illegible]re which is more adapted to the properties of the RSO.

Rubber seed meal is mainly used as fertilizer. In the attempts to make use of RSM as animal feed unsteady results were obtained. Lack of knowledge of proper pretreatment of rubber seed meal is a possible reason for negative results in feeding trials. As the RSM is reported in the literature to be of good nutritional value, attention should be given in laboratory trials to reveal possible hazard substances in the minor components of the RSM.

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III Laboratory investigations

III 1. Introduction

A lot of knowledge about rubber seed, its composition and its utilization does exist already. Therefore within the laboratory investigations the work was concentrated on such areas, in which the knowledge was not sufficient for proper utilization. These areas were mainly the questions of refining and whether the oil can be used for human consumption. Another aspect of the laboratory trials was the problem of the minor components in rubber seed, which could hamper its utilization from a practical point of view.

III 2. Rubber seed

The starting material for the investigations were rubber seeds (≈ 80 kg) which had been collected under the guidance of the Rubber Research Institute at Sri Lanka at the plantations in August 1985. The seeds were collected within 3 or 4 days after seedfall and dried immediately in an air oven with air circulation at 70-80 °C to a water content of below 5 % (about 3 days). The seeds were shipped by air freight to our laboratory in Hamburg within 1 week. After arrival in the laboratory the seeds were stored at 0°C until use.

Because of the bad seed fall in 1985 in Sri Lanka, due to adverse weather conditions only 80 kg seeds could be sent to the Hamburg laboratory at that early date.

III 3. Rubber seed oil

III 3.1. Separation and Refining

To obtain the oil, the crushed kernels were extracted with hot petroleum ether (s. scheme 1). This process was chosen, to get as much oil as possible; it was not chosen as a direct simulation of a technical process. The way, the crude oil has been refined, follows more or less a standard laboratory method, which is designed to simulate the technical process, applied to fats used as edible oil. The method is outlined in scheme 2.

The fat content of the kernels, received from Sri Lanka, was 37 %. This is at the minimum of the range, previously reported in the papers (see part I, chapter 2.2.2., page 9). The reason may be the early harvest date.

The extraction process was carried out after one, two and four months storage of the seed at - 0°C. It is interesting to note, that even at this low storage temperature the acid value [1] of the crude oil had increased:

	Oct. 85	Nov. 85	Jan. 86
AV	6.4	13.9	17
oil yield (%)	32	24	25

The water content of the kernels at all three extraction trials was 7.6 %. So even at these low temperatures fat splitting enzymes were active. Lipase activity in these nuts could be demonstrated by a lipase screening test [2] (s. appendix 3, p. 1).

The increase in free fatty acids was also noticeable by the fact, that the crude oil from Nov. 85 emulsified much stronger in the desliming step than the oil from Oct. 85, so that no sludge could be separated. Thus the phospholipids were removed in the following neutralization/sodium carbonate-water glass-treatment together with the fatty acids. But also here the washing of the oil was difficult because of emulsion formation (yield only 64 % as compared with the yield of 78 % for these two steps with the first oil).

Parallel to the increase in free fatty acids during storage of the seeds, the oil yield under the rather simple extraction conditions decreased. Whether this will occur also under more practical conditions cannot be decided as yet.

Scheme 1

Crude RSO from rubber seed

15.10 kg seeds

↓ 1) cracking of the shell in a mechanic plate/press

2) manual separation of shells and moulded kernels → 7.10 kg shells (47.0 %), 0.82 kg kernels (moulded) (5.4 %)

7.19 kg good kernels (47.6 %)

↓ after mixing with 1 kg dry ice ground in a Stefan-Cutter
stirred with 35 l hexane and refluxed for 1 h, filtrated over a Seitz-filter (KO)
extraction repeated in the same manner with 20 l hexane each

combined hexane extracts

↓ evaporated at normal pressure, vacuum applied towards the end

2.27 kg crude RSO (32.0 %) (15 % w.r.t. seed)

Scheme 2

Refining of crude RSO

1200 g crude RSO

↓ desliming

addition of 1.2 g 50 % aqueous solution of citric acid at 70°C, stirring for 10 min. After cooling to 25°C addition of 18 g H_2O and stirring for 60 min. Then the oil was heated up to 60°C within 20 min while stirring and cooled down over night without stirring.

Centrifugation → 66 g sludge

66 g sludge ↓ drying → 56 g dry sludge

1082 g deslimed oil (90 %)

1023 g used for

↓ neutralization

in N_2-atmosphere heated up to 95°C, addition of 220 ml 0.8 N NaOH, stirred for 5 min, settled at 60°C. After drainage of the aqueous phase the oil was washed 3 times with 100 ml of hot saturated NaCl-solution, afterwards with 100 ml 0.1 N NaOH.

Soda water glass treatment

to the oil 3 % of 20 % Na_2CO_3-solution and 1.6 % of sodium silicate (water glass) were added and for 20 min life steam was injected. Afterwards the oil was washed with hot water 15 times until it was free of soap (pH neutral).

Drying was done at 105°C at 1 mbar for 30 min.

894 g neutralized oil (87 %)

↓

bleaching

stirred under N_2 with 18 g Tonsil ACCFF at 105°C/1 mbar for 20 min.

filtration*)

↓

284 g bleached oil (28 %)

deodorization

deodorized for 3 h at 230°C with 40 % H_2O (33 ml H_2O/h) in a laboratory deodorization equipment.

↓

241 g deodorized oil (85 %) overall yield: 19 %

A second refining trial was carried out. Starting from 1000 g crude oil a similar overall yield was obtained. Again the highest loss was in the bleaching step.

*) Filtration proved to be very difficult. Even the hot oil containing additional filter aid (Kristall Theorit, Seitz) was running only very slowly through a suction filter. The filter paper had to be changed very frequently and this explains the high losses of oil during this step.

III 3.2. Investigations of the rubber seed oil

III 3.2.1. Analytical characterisation of the triglycerides

III 3.2.1.1. Fatty acid composition

Glycerides: The composition of the fatty acids in the triglycerides of RSO is well known from the literature. A GLC analysis of the fatty acid composition (s. appendix 3, p. 1) of the oils used in this investigation is given below.

GLC-composition of RSO (fatty acid methylesters, area-%)

	RSO	
fatty acid	neutralized	fully refined
14:0	0.1	0.1
16:0	8.9	8.8
18:0	8.4	8.8
20:0	0.4	
18:1c	23.4	23.5
18:2cc	39.2	39.1
18:3ccc	18.8	16.8
18:3 isomers	0.9	1.4
	$\sum$ 99.2	$\sum$ 99.4

These compositions are well within the range, which is given in literature. In the fully refined oil obviously some isomerization of the 18:2ccc-fatty acid has taken place, either during the bleaching process (acid bleaching earth) or during the deodorization.

III 3.2.1.2. Gelpermeation chromatography of the oil

GPC (s. appendix 3, p. 2) of the intact oil separates the constituents according to their molecular weights. The GPC diagrams of crude RSO and fully refined RSO are shown in fig. 1 and 2. The area %, which is roughly weight %, is shown in the following table. The fractions are coordinated to the different types of lipids [3].

GPC-compositions of RSO (area %)			
	oil from extraction		
	October 1985		January 1986
fraction	crude RSO	refined RSO	crude RSO
free fatty acids + monoglycerides + unsaponifiable matter	3.7 %	0.3 %	7.1 %
diglycerides	1.8	1.9	1.7
triglycerides	93.1	95.9	90.1
dimeric triglycerides	0.1	0.6	./.
trimeric "	./.	./.	./.
MW > 10 000	1.3	1.2	1.1

The fraction with the lowest molecular weight contains fatty acids, monoglycerides and sterols. Mostly this fraction consists of fatty acids in case of crude RSO, what is evident in comparison to the refined RSO. In refined RSO hardly any free fatty acids should be present. The remaining 0.3 % thus is the upper limit for monoglycerides and sterols in crude RSO, since both components are hardly removed in the refining procedure. This assumption leaves 3.4 % free fatty acids in crude RSO which agrees well with the acid value of 6.4 (see p. 54, = 3.2 % free fatty acids).

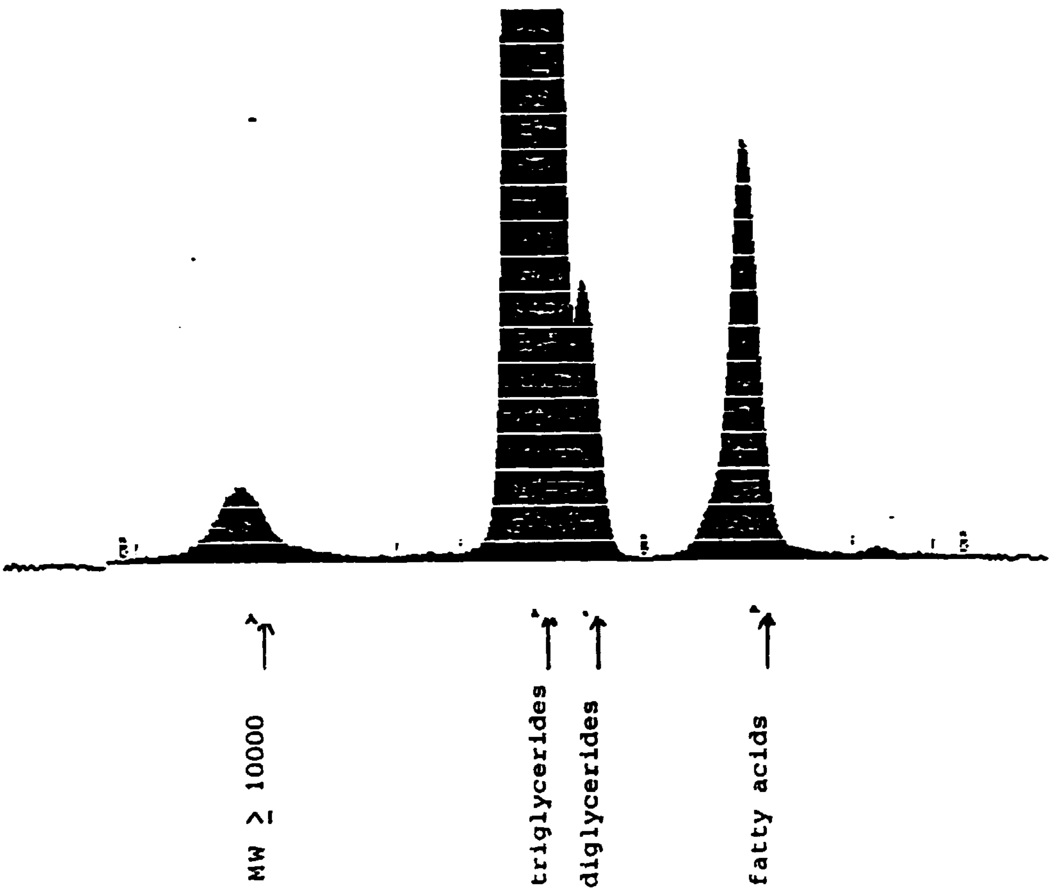


Fig. 1: Gelpermetaion chromatogram of crude RSO (Oct. 85)

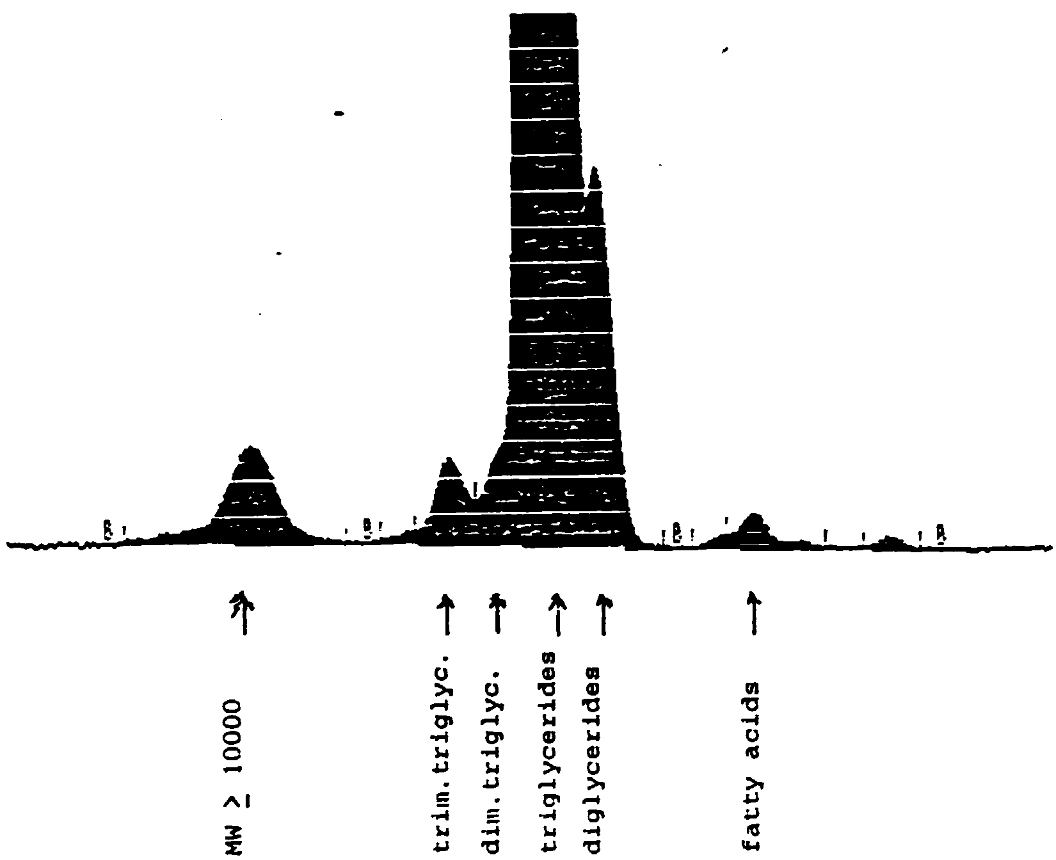


Fig. 2: Gelpermeation chromatogram of fully refined RSO

Diglyceride and triglyceride contents are a little bit higher in refined RSO than in crude RSO due to the fact, that free fatty acids have been removed.

The low content of dimeric triglycerides of 0.1 % or less in crude RSO is expected in the fresh extracted oil, where not much damage in the triglyceride molecules is caused by heat. A somewhat higher content after refining was found. That is due to the heat treatment in the deodorization step, where triglycerides containing hydroperoxy - or epoxy fatty acids are dimerised. Nevertheless the value of 0.6 % dimeric triglycerides is quite normal in refined oils.

The most interesting feature of the GPC-diagrams of the RSO is the substance with a rather high molecular weight of >10 000 D. The exact molecular weight of this fraction could not be determined by the column used in this experiment. Investigations concerning the nature of this substance are given in 3.2.2.2.

III 3.2.1.3. Oxidized fatty acids

From the GPC-results of monomeric triglycerides it is not possible to differentiate between those triglycerides containing oxidised or normal fatty acids. The composition of the normal fatty acids may be seen from GLC of the methylesters, but the methylesters of oxidised fatty acids normally are not assessed by GLC.

Applying a method to fatty acid methylesters, which is described for polar lipids (s. appendix 3, p. 3) 91.3 % unpolar esters (w.r.t. total ester content) [4], were found in the crude RSO extracted October 1985. In the fully refined RSO the proportion of these esters was 95.7 %. These figures show, that in RSO from fresh kernels most of the fatty acids in the triglycerides are not oxidized. The fact that in the fully refined oil the proportion of intact fatty acids is higher than in the crude oil, indicates, that the ox .ised fatty acids are enriched in constituents of the crude oil, which are removed during refining (phospholipids, free fatty acids). The figures are well in accordance with the results known for other vegetable oils.

III 3.2.1.4. Unsaponifiable matter

The amount of UM [5] in crude RSO was found between 1.1 % and 1.3 %. From the GPC analyses of this material about 57 % were determined as high molecular material, the remaining rest showed molecular weights around 300 D (see fig. 3). Thus the high molecular material is enriched in the UM.

In TLC two spots in the range of sterols were visible, which could not be identified satisfactorily, but by comparison with literature data are probably not one of the more common phytosterols like e. g. stigmasterol or sitosterol.

In refined RSO only between 0,3 - 0,4 % UM was found. In this UM only 20 % had a high molecular weight of > 10000 D. Since according to GPC the content of this polymer material shows a similarly high molecular weight in crude and refined oil, the considerable differences of the UM in both oils cannot be explained without further sophisticated investigations.

III 3.2.2. Minor constituents of rubber seed oil

In view of a possible use of RSO for human consumption, it was interesting to look for minor components in this oil, which could influence this utilization. For instance the content of a natural antioxidant is of high importance for

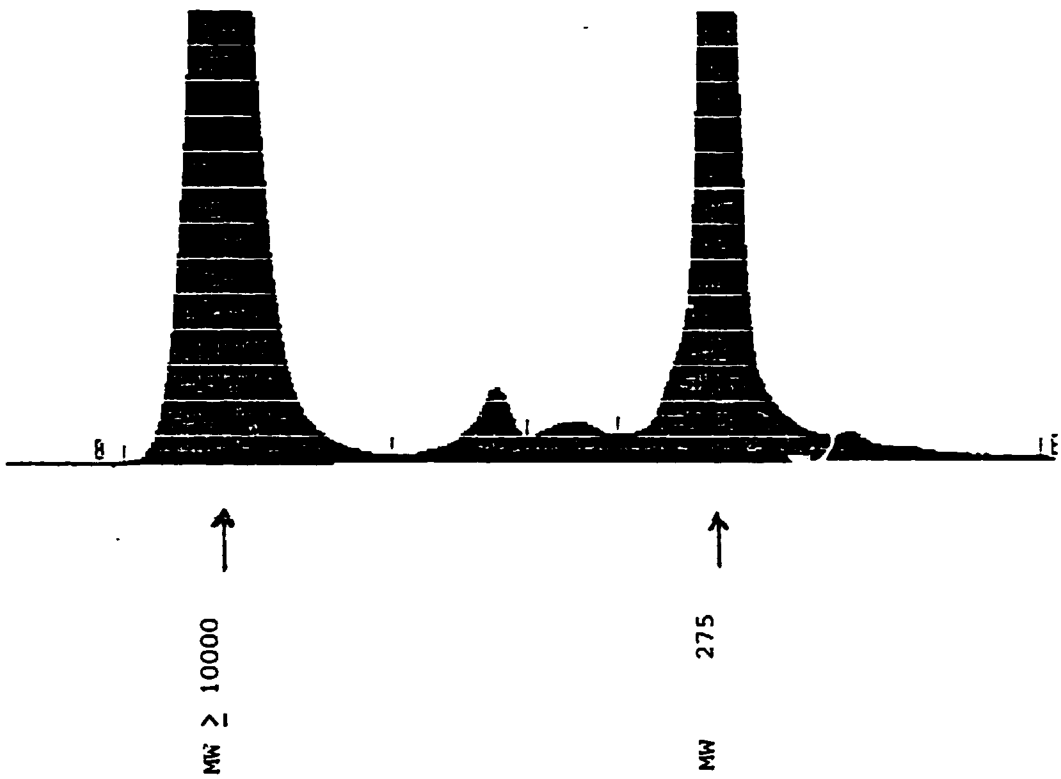


Fig. 3: Gelpermeation chromatogram of unsaponifiable matter of crude RSO

the taste keepability of such a highly unsaturated oil.

III 3.2.2.1. Tocopherols

A HPLC-diagram of crude rubber seed oil (method see appendix 3, p. 3) is shown in fig. 4. By cochromatography of a mixture of tocopherols (α - δ) and rubber seed oil it was demonstrated, that rubber seed oil does not contain tocopherols (fig. 5). Two main signals in RSO with a 10 % larger retention time than the neighbouring α- and γ-tocopherols were obtained. It is assumed, that these signals belong to tocotrienols. Tocotrienols and their esters are reported to be present in latex of Hevea brasiliensis [6,7,8].

III 3.2.2.2. Polyisoprene

One of the most interesting minor component which was found in RSO during this investigation is the high molecular weight material, which was detected in the GPC-diagram (s. III 3.2.1.2, p. 64). It was present in the oil in amounts of ~ 1 %. The assumption could be made, that this component does not contain oligomeric triglycerides or phospholipid micelles, since it is enriched in the unsaponifiable material of the oil.

To get some material for identification purposes the following procedure was applied:

1) Preparation of unsaponifiable material from crude RSO
2) Gelpermeation chromatography on preparative scale: 5 mg of the UM were separated on a polystyrene column, using tetrahydrofurane as eluent (s. appendix 3, p. 2). The first fraction was trapped and evaporated.

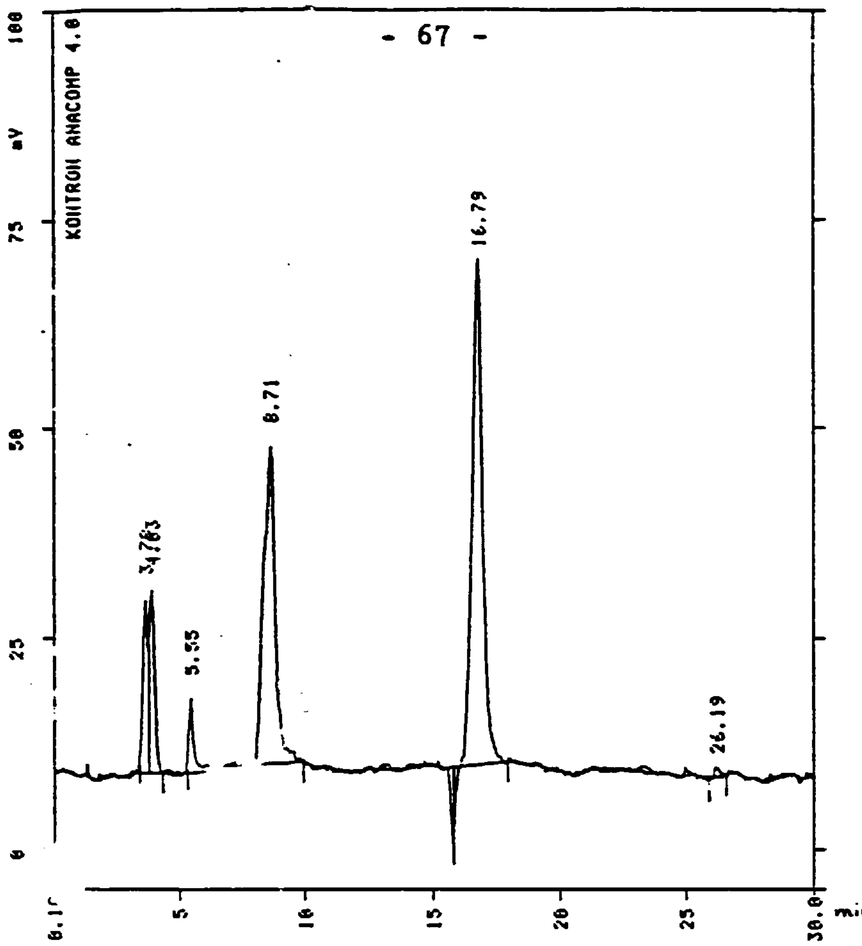


Fig. 4: HPLC-diagram of crude RSO. Spherisorb Si 60, iso-octane with 0,5 % iso-propanol, fluorescence-detection (ex 295 nm, em 325 nm)

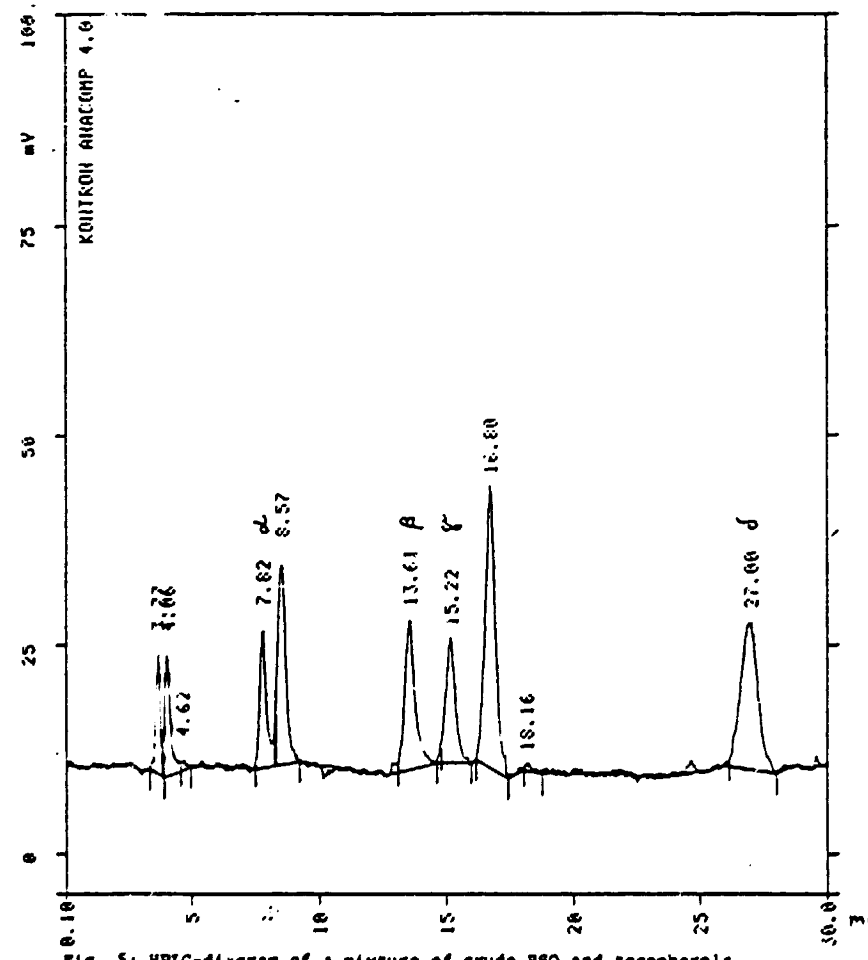


Fig. 5: HPLC-diagram of a mixture of crude RSO and tocopherols (isomeres indicated as $\alpha - \delta$); conditions as in fig. 4

The ^{1}H-NMR-spectrum (400 MHz) of this material in deuterated THF is shown in fig. 6. The signals indicated by x appear also in a spectrum of the blank material. They derive from impurities in the THF, used for fractionation and from the solvent used for the NMR-spectrum. The remaining three signals of the polymer have the characteristical chemical shifts for CH-protons, CH_2-protons and CH_3-protons with the relative intensities of 1 : 4 : 3.

This pattern is characteristic for isoprene increments

$$- CH_2 - CH = \overset{\overset{\displaystyle CH_3}{|}}{C} - CH_2 -$$

in trans- and cis-1,4-polyisoprene. Whether the configuration at the double bond is cis or trans could not be decided from the NMR-spectrum. Most probably it is cis, because Hevea brasiliensis produces cis-1,4-polyisoprene anyway in form of latex.

A further GPC-investigation was carried out, to get a more exact value for the molecular weight of this polyisoprene. Using a column set suitable for discriminating between much higher molecular weights than the set used for characterization of the RSO, molecular weights of several hundred thousand Daltons were found. The molecular weight distribtuion (volume-weight) is given in fig. 7. For polyisoprere in latex molecular weights of 200000 - 400000 D [9] or degrees of polymerisation of 8000 to 30000 [10] $\approx 5{,}4 \cdot 10^5$ to $2 \cdot 10^6$ D are reported . So the molecular weights of the polyisoprene molecules in RSO are about the same as those in latex.

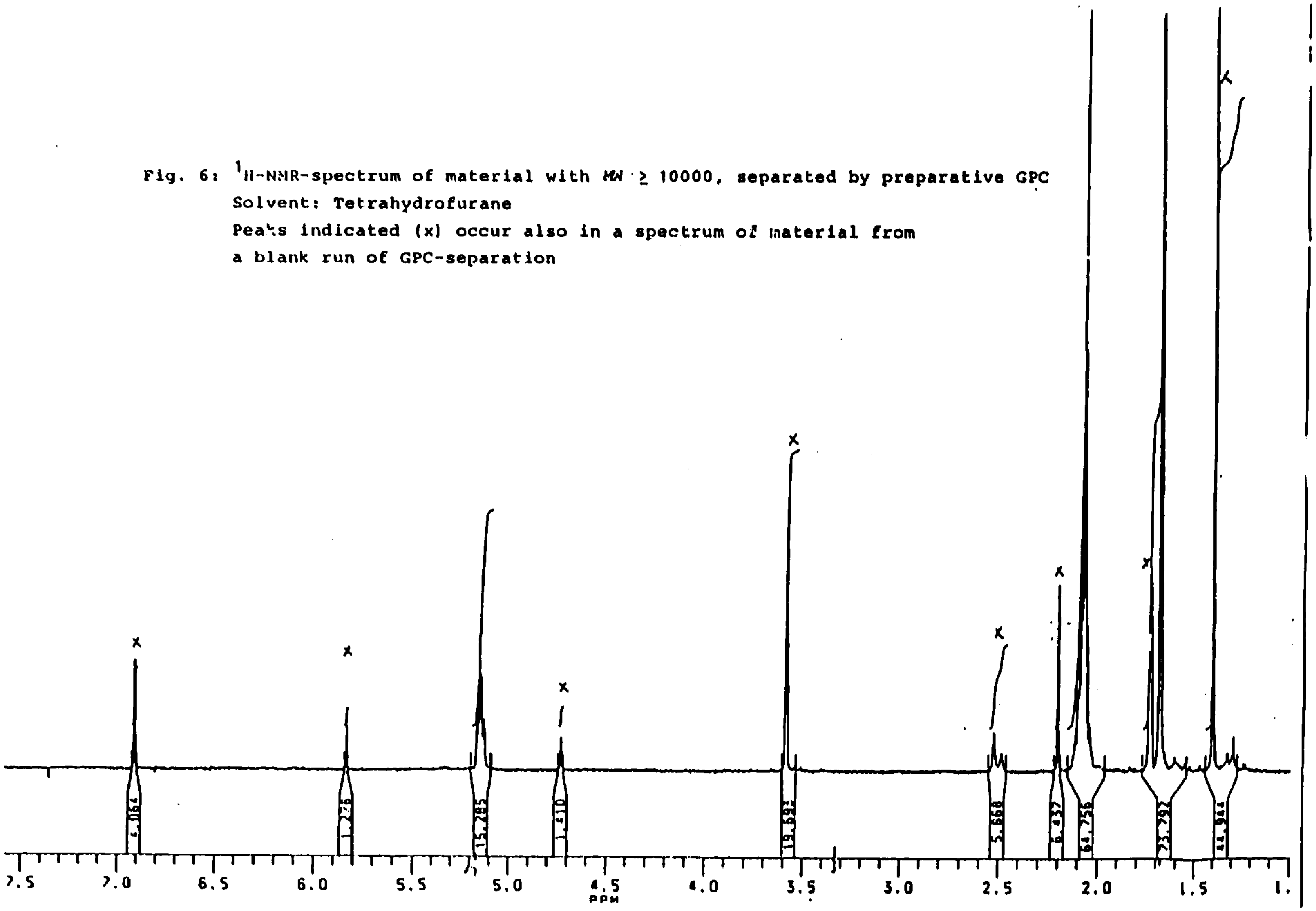
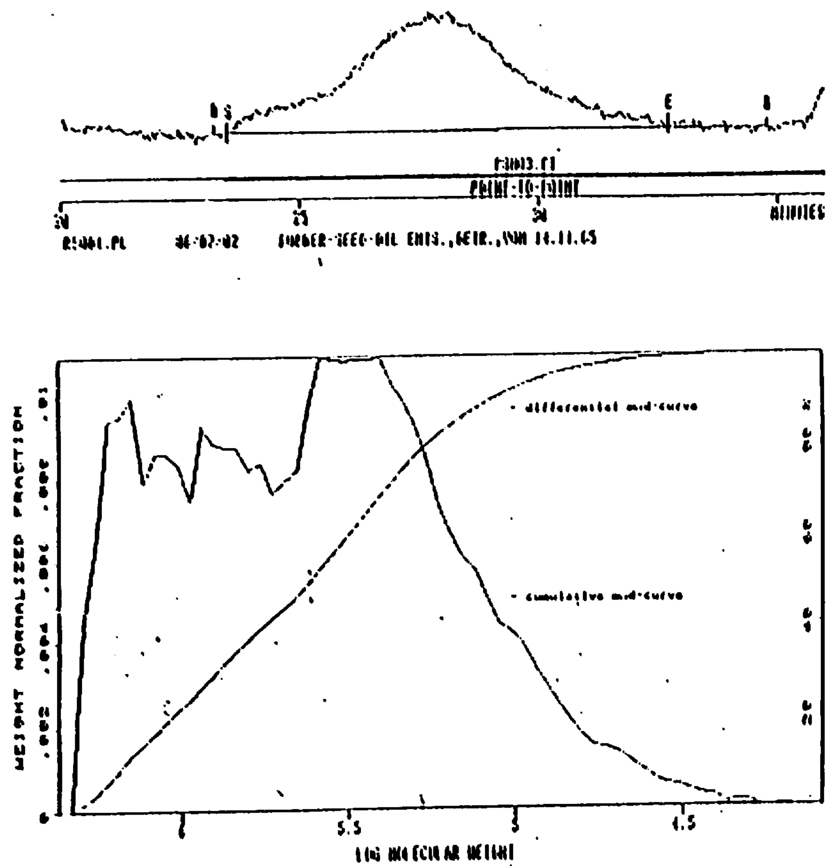


Fig. 6: ^{1}H-NMR-spectrum of material with $MW \geq 10000$, separated by preparative GPC
Solvent: Tetrahydrofurane
Peaks indicated (x) occur also in a spectrum of material from
a blank run of GPC-separation



GPCS DATA REPORT
CALCULATED AT: 14:46 ON 06/07/88

CHROMATOGRAM: RS001 INSTRUMENT: IA
RUBBER-SEED-OIL ENTS., GETR., VON 14.11.85
ACQUIRED: 06/07/88

CALIBRATION TABLE: SIL.1
WACKER AK SILICONE
BASE POINTS: 23.167 AND 34.730 MINUTES
SUMMATION FROM 23.482 TO 32.691 MINUTES
8.00 SECONDS PER DATA POINT

WEIGHT AVERAGE MW = 204,018

NUMBER AVERAGE MW = 77,699

POLYDISPERSITY = 2.626

Z AVERAGE MW = 548,626

DETAILED DATA TABLE

RT (MIN)	SLICE AREA	NORM AREA	NORM WT. (%)	NORM NO. (%)	MOLECULAR WEIGHT
23.48	0	0.00	0.00	0.00	2.065E+06
23.82	2752	0.45	4.25	0.02	1.805E+06
24.15	3143	1.09	14.26	0.10	1.523E+06
24.48	4521	2.50	22.61	0.22	9.575E+05
24.82	5343	5.41	30.02	0.41	7.006E+05
25.15	5627	7.62	36.27	0.67	5.406E+05
25.48	6307	10.17	42.70	1.10	4.475E+05
25.82	8021	13.17	48.56	1.60	3.604E+05
26.15	10647	17.20	55.16	2.62	3.017E+05
26.48	12623	22.46	61.59	4.15	2.474E+05
26.82	16243	28.71	66.72	6.25	2.027E+05
27.15	16415	35.60	72.64	9.37	1.661E+05
27.48	17607	43.37	80.22	13.35	1.361E+05
27.82	17649	51.41	83.24	18.47	1.115E+05
28.15	18451	59.50	89.28	24.74	9.164E+04
28.48	17102	67.03	92.44	31.66	7.530E+04
28.82	14495	73.00	94.00	39.04	6.590E+04
29.15	12353	79.60	96.51	46.53	5.557E+04
29.48	10331	84.29	97.67	53.70	4.604E+04
29.82	7725	88.13	98.47	60.71	3.916E+04
30.15	7063	91.25	99.02	67.43	3.325E+04
30.48	5048	93.75	99.39	75.06	2.770E+04
30.82	4300	95.09	99.62	80.51	2.352E+04
31.15	2894	97.55	99.82	86.76	1.931E+04
31.48	0	98.57	99.91	91.20	1.541E+04
31.82	2230	99.47	99.97	96.20	1.206E+04
32.15	2172	99.79	99.99	98.20	1.063E+04
32.48	0	100.00	100.00	100.00	8.769E+03
32.72	0	100.00	100.00	100.00	7.665E+03

Fig. 7: Molecular weight distribution of polyisoprene in RSO

3.2.2.3. Phosphorlipids

The crude oil, obtained Oct. 1985, had 260 ppm phosphorous. Applying a commonly used converting factor of 25, this results in 0.65 % phospholipds in this oil. In a TLC of this crude oil the main phospholipid to be seen is phosphatidic acid.

The relative phospholipid composition was assessed in the sludge, obtained from this oil (1-dimensional TLC and phosphorus determination in the spots of the phospholipids [11], see appendix 3, p. 3).

Phospholipid composition relative to total phospholipids assessed:

phosphatidic acid (PA):	46 %
phosphatidyl inositol (PI):	22 %
phosphatidyl choline (PC):	24 %
phosphatidyl ethanolamine (PE):	8 %

No other phospholipid of significance could be detected. These 4 phospholipids analysed in the sludge account only for 22 % of the total phospholipids, present in the crude oil. So the major part of the phospholipids remained in the "deslimed" oil and probably adds to the emulsion problems in the neutralization step. However, they are removed together with the soaps.

III 3.3. Quality assessment

III 3.3.1. Colour stability

The colour stability of RSO, exposed to air and daylight, was investigated (s. appendix 3, p. 4):

storage time (weeks)	iodine colour crude RSO	refined RSO
0	40+	3+
1	40-	3-
2 1/2	25	2
5	20	turbid
7	most material was polymerised, the remaining oils were only slightly yellow coloured	

During storage under these conditions, the colour was reduced in both oils. This is a normal behaviour with oils containing carotenoids as the main colouring matter. Carotenoids are oxidised by lipid-hydroperoxides, formed in the first autoxidation step of unsaturated fatty acids. This is in contrast to the statement that soap, containing 5 - 10 % RSO, gets a darker colour during storage (s. II 2.5., p. 40).

III 3.3.2. Taste and taste stability

Crude RSO as obtained after hexane extraction was a brownish clear somewhat sticky oil. A fresh sample with a relatively low acid value (6.4) had a mild pleasant nutty taste with only slightly soapy and bitter notes. The soapy and bitter taste was more pronounced in the sample with an acid value of 17.

The fully refined sample of RSO was almost colourless, the nutty flavour had disappeared, the taste was rather neutral.

III 3.3.2.1. Storage test with crude rubber seed oil

The acceptable taste of a fresh oil does not guarantee, that the taste will keep during longer storage. Especially the high proportion of linolenic acid in the triglycerides of RSO is vulnerable to the attack of oxygene, resulting in a bad taste. So it was of interest to see how long the taste of the oils will remain stable, and whether this can be influenced by an antioxidant.

Storage tests were done in half filled bottles under air and in darkness. To simulate the conditions in tropical countries storage temperature was 30°C. The taste was assessed by an experienced taste panel at intervals of 2 - 4 weeks. Main emphasis in the judgement was put on detecting taste deterioration. Taste intensity, as is often assessed, was not so important here, because the crude RSO started already at a relatively high level.

The results of the organoleptic judgements are compiled in Tab. 1. Crude RSO got a slightly seedy odor after 1 month which changed into a burnt note after 2 months. Taste deterioration became noticeable after 2 months. The soapy and bitter taste had increased, the oil now caused some irritation in the throat. A "burnt" taste was first noticed after 2 ½ months and became stronger and stronger to the end of the test period (5-6 months). At the end also a sour taste was perceived. Surprisingly no "varnish" taste was observed, as would have been expected because of the similarity of the triglycerides to linseed oil.

The crude RSO was rendered acceptable under these test conditions roughly up to 2 months. Addition of 1000 ppm synthetic α-tocopherol did not improve the taste stability of crude RSO, the kind and speed with which off-tastes developed were about the same as without tocopherol.

Tab. 1

Organoleptic judgement of rubber seed oil

all oils stored at 30 °C in total darkness

storage time [weeks]	crude RSO	refined RSO	Mixture of 80 % refined CN with 20 % crude RSO	Mixture of 80 % refined CN with 20 % refined RSO
initial	slightly soapy, bitter [a]	tasteless [a]	nutty, bitter [a]	tasteless [a]
2	slightly seedy [a]	slightly ranzid [a]	nutty, bitter [a]	tasteless [a]
4	slightly seedy [a]	slightly ranzid [a]	nutty, bitter [a]	tasteless [a]
8	slightly seedy [a]	slightly ranzid [a]	nutty, bitter [a]	tasteless [a]
10	slightly seedy little "burnt" [a]	slightly ranzid [a]	nutty, bitter [a]	tasteless [a]
15	" " stronger "burnt" [a]	faint taste of cod liver [a]	slightly soapy [a]	slightly ranzid [a]
20	throat irritating "burnt" taste [na]	faint taste of cod liver [a]	slightly throat irritating [a]	slightly ranzid [a]
25	strong sourish [na]	faint taste of cod liver [a]	slightly throat irritating [a]	slightly ranzid [a] + slightly sourish [a]

[a] = acceptable taste

[na] = non acceptable taste

III 3.3.2.2. Storage test with refined rubber seed oil

The refined RSO started with almost no taste. After 12 days a very slight rancid smell occurred, which probably derived only from the thin oil layer at the ground stopper. It did not increase on further storage and was not noticed in the taste. The latter became slightly seedy after 1 month and did not change much until after 3 1/2 months a faint taste of cod liver oil occurred. But up to the end of the test period (5 months) the taste of the refined RSO was still acceptable.

When 1000 ppm α-tocopherol was added to refined RSO, this oily and seedy taste was not observed. After almost 2 months a musty taste was firstly perceived, which only slightly increased until the end of the test period (5 months). So in this case the antioxidant had a positive effect; with crude RSO this effect probably is overuled by the more intensive basic taste of the oil itself

III 3.3.2.3. Storage testy of a mixture of rubber seed oil/ coconut oil

In a third test row the taste stability of a mixture of 20 % crude RSO and 80 % refined coconut oil was checked. Under the same test conditions as mentioned above, the taste of the mixture (which was nutty at the beginning) got slightly soapy and irritating to the throat after 3 1/2 months. After 5 months a slightly "burnt" taste was perceived in addition, but the mixture still was acceptable.

The mixture with 20 % refined RSO was quite neutral in taste at the beginning. A very slight rancid odour after 3 1/2 months did hardly increase until 5 months. At the end a slightly sourish note was perceived in addition.

III 4. Filtration of rubber seed oil

Some problems occured during refining of RSO, particularly in the bleaching step when bleaching earth has to be filtered off. It took more than 20 hours to filter off ≈ 1 kg RSO and severe losses of oil were observed, even at temperatures around 70°C and with a high rate of changing the filters.

To illustrate the problem, the filtration speed of RSO was compared with that of soybean oil:
2 ml oil of each kind were filtered through a Büchner funnel (diameter 1 cm) equipped with a paper filter ("Schwarzband", Schleicher u. Schüll). Then vacuum was applied and the time assessed, necessary for the oil to run through. Whereas the soybean oil passed the filter within 5 sec the RSO (neutralized, dried, clear oil) took 6-10 min.

III 4.1. Viscosity

In principle the slow filtration of RSO could be caused by a high viscosity. In comparison with soybean oil the RSO seems to be somewhat more sticky.

Measurement of the viscosities with a Rotovisko-apparatus resulted in the following viscosity figures:

	Viscosities (in mPa's) at	
	25°C	80°C
RSO	78.2	12.2
soybean oil	42.2	9.0

The viscosity differences would merely explain a factor 2-3 in filtration speed at ambient temperature. At higher temperatures hardly any difference in the viscosity was recognised. Therefore other reasons must be responsible for slow filtering rates in refining experiments, which are carried out at temperatures of 70 - 100°C

III 4.2. Filtration trials

In the following experiments the influence of RSO on the filter material was investigated. Using a glass filter (Glasfritte D 4, maximum of pore diameter 16 µm) soybean and RSO re-filtered in the sequence as given below:

1)	soybean oil	4 ml:	1	min filtration time
2)	"	4 ml:	1	" "
3)	"	2 ml:	½	" "
4)	RSO	2 ml:	1 ½	" "
5)	"	2 ml:	1 ½	" *) "
6)	"	4 ml:	9	" "
7)	soybean oil	2 ml:	5	" "
8)	filter washed with acetone			
9)	soybean oil	2 ml:	3	min "

This experiment shows the following:

1. The filtering rate of RSO is at least 3fold at the beginning of the process.

2. The filtering time increased also for soybean oil, after RSO had passed the filter.

3. If the RSO is prefiltered (No. 5), the filter properties obviously are not changed by this oil.

---

*) The oil in this experiment had been filtered through a glass filter before

4. The material that clogs the filter, cannot be washed out by acetone.

From these results it is concluded, that the filter picks up some material from the RSO, which partly blocks the pores and cannot be removed by polar solvents.

Another glassfilter D 4 (14 µm ∅) was completely blocked for soybean oil and even acetone, after filtration of 4 ml RSO.

Instead of paper also glass fiber sheets of different porosity were tested (samples delivered by Lehmann & Voss & Co., Hamburg)

1. AFS - 3 1/4", dustfilter, big pores
2. Lydair grade 251, medium density
3. " " 220, highest density

oil	filter times for 2 ml oil with filter type		
	1	2	3
soybean oil	3 sec	3 sec	11 sec
crude RSO	8 sec	9 sec	1 1/2 min

On addition of bleaching earth Tonsil ACCFF only filters 2 and 3 retained the bleaching earth. The filter times, however, increased to several minutes, because now the filter cake blocked the filtration.

III 4.3. Separation of polyisoprene

Because of the insufficient results of the filtration experiments with different filter types further trials were carried out to remove polyisoprene, using a technique which is based on an adsorption chromatography column [12].

Triglycerides and more polar constituents are adsorbed to silica gel from hexane solution. More unpolar molecules like carbohydrons (or very large molecules or micelles which do not fit into the pores of the SiO_2) are washed out.

Elution scheme:

500 g SiO_2 (Merck, activated at 160°C, 12h)
in hexane

d2 g crude RSO in 400 ml hexane →

1) 6 l hexane → 0.5 g liquid, MW < 380

82 g crude RSO in 400 ml hexane →

2) 1.5 l hexane → 1.5 g brownish wax, 50 % phospholipids

3) 4 l hexane → 19 g triglyceride

4) 8.5 l hexane : ether = 87 : 13 → 137 g triglycerides + fatty acids

5) 3.5 l ether → 7 g fatty acids, diglycerides

By this procedure the polyisoprene content of the RSO (fraction 4) has been reduced from 1.1 % to 0.4 %. The polyisoprene is concentrated in fraction 2 (although this contains also phospholipids, P = 2.0 % = 50 % phospholipids). This oil (fraction 4) was filtered over glass fiber filter (see below) in comparison to the starting oil:

oil	filtering time (2 ml oil) grade 251	grade 220
crude RSO	9 sec	1 1/2 min
SiO_2-treated RSO	6 sec	5 min

In case of the denser filter even longer filter times with the purified oil were obtained.

Some further experiments were carried out in order to reduce the filtration time of RSO:
By treatment of the oil with different adsorbents in order to adsorb the polyisoprene particles (-5 %, 10 min at 50°C). The following adsorbents were used:

Charcoal of different particle size
Kieselgel (Merck 7729, <0,08 mm)
Celite (filter aid)
Polyethylene (BASF, Lupolen 5270 Z)
Rubber (powdered tube)

None of them, when the treated RSO was filtered through paper (Schwarzband), gave a better filtration rate.

III 4.4. Dilution trials

III 4.4.1. Hexane as solvent

Small amounts of hexane reduce the viscosity very much and at the same time may dissolve some of the polyisoprene.

filtering times of crude RSO containing 0	5	10	20	30	50 % hexane
6-10 min	2-3 min	1 1/2-2 min	1 1/2 min	1/2 min	10 sec

(2 ml oil, Schwarzband)

So relatively small amounts of hexane seems to improve the filtration rate.

The same filtration times as with 30-50 % hexane could be obtained with crude RSO at 70-90°C (30-20 sec), but it was found that the high starting rate will soon be reduced because of blocking of the filter.

III 4.4.2. Oil as solvent

As a more appropriate solvent vegetable oils in mixtures of RSO were investigated. With mixtures of crude RSO and soybean oil = 1 : 1 no better filtration rates were obtained.

A mixture of 20 % crude RSO and 80 % coconut oil, however, showed the same filtratiion speed as coconut oil (at 50 - 55°C). Therefore, with 1 kg of a mixture of 80 % refined coconut oil and 20 % crude RSO a refining experiment was carried out on a somewhat larger scale.

The oil mixture was:

1) neutralized. Some emulsion problems were noticeable,

2) bleached with 1 % Tonsil ACCFF. After addition of filter aid Seitz Kristall Theorit the fat mixture filtered in quite a normal manner.

It remains to be checked on a pilot plan scale, whether is a practical way to overcome the filter problems wi RSO.

III 5. Composition of Rubber seed Meal

The RSMs of the three extractions (see III 3.1, page 54) were stored at -20°C prior to analysis. After drying at 120°C until constant weight, a water content of 8,8, 9,2 and 9.1 % was found for three extracted samples.

III 5.1. Residual oil content

The rubber seed meal of the first extraction was subjected to a further two hour extraction with diethylether. By this extraction an amount of 6,2 % soluble material was obtained.

A GPC-analysis of the ether extract showed that the composition of this residual oil is rather similar to the extracted crude RSO obtained as described on page 60.

constituent	area %
triglycerides	89.4
diglycerides	1.5
monoglycerides	0.3
fatty acids	4.9
dimeric triglycerides	1.3
trimeric "	1.0
MW > 10000	0.6

III 5.2. Water soluble materials

From the ether extracted meal the following results were obtained after further extraction with water:

Water soluble material:	33.5 %	(w.r.t. RSM)
Ash in " " :	6.5 %	"
Water soluble protein :	7.1 %	

calculated from nitrogen content (Kjeldahl) (F = 6.25)

water soluble carbohydrates are calculated from these figures

33.5 % - (6.5 % + 7.1 %) = 19.9 %

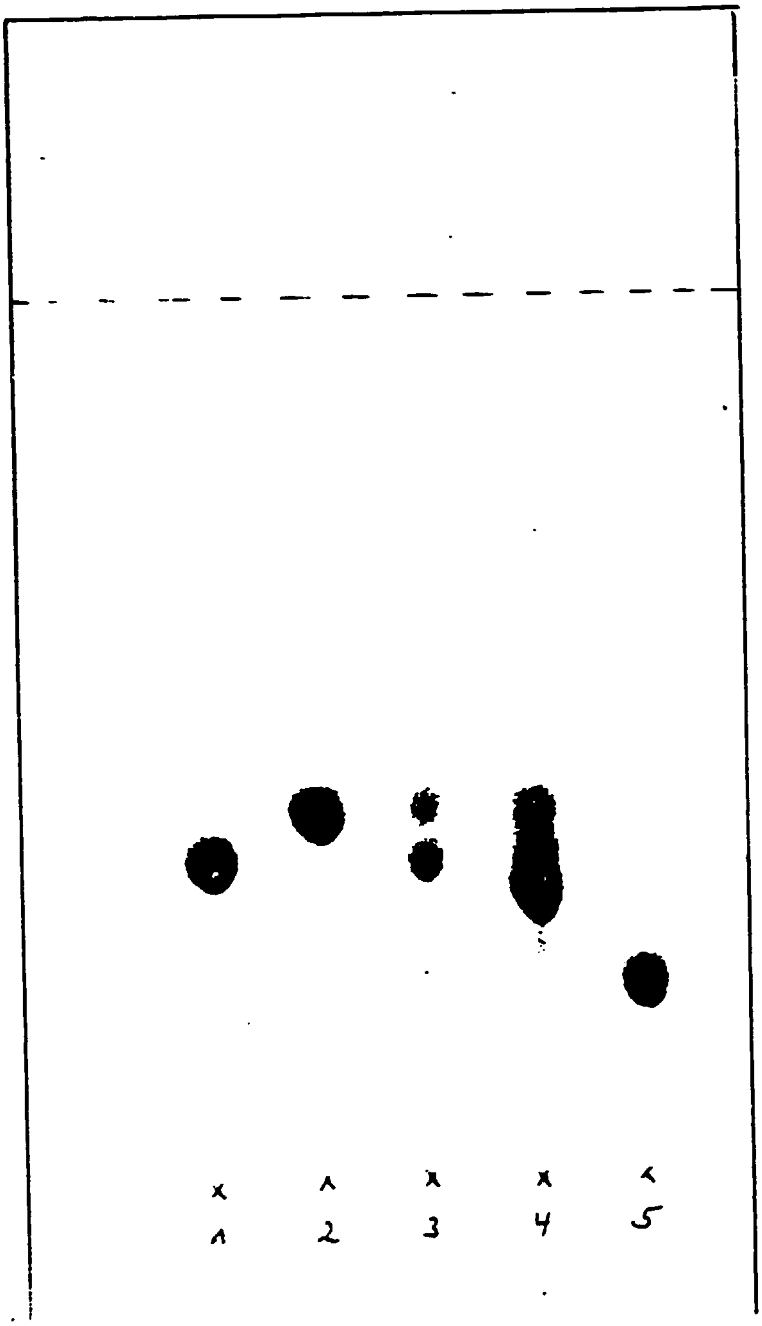
The water soluble carbohydrates [13] were analysed by TLC. 2 spots (s. fig. 8) were seen, indicating a mixture of a disaccharide and a monosaccharide. Higher carbohydrates e. g. trisaccharides or tetrasaccharides could not be detected.

The total raw protein was calculated from the total nitrogen content determined by the Kjeldahl method using the conversion factor 6,25 %. A total raw [illegible]in content of 30 % was determined in the three meal sam[illegible].

In the following table the main components of the investigated RSM is compared to the average values found in literature.

constituent	results found	
	own investig. %	lit. %
H_2O	9.1	8
lipid	6.2	11
sol.carbohydr.	19.9	40
total protein	30.0	29
ash	6.5	5
fibre	8.0	7
Σ	<79,4 %	100 %

Only in the soluble carbohydrates our results differed from the literature values. By microscopical inspection of the RSM, besides of cell fragments, a lot of almost spherical particles (diameter ~ 5-10 µm) were found. Their colour darkened on addition of diluted iodine solution. This confirms the assumption, that these particles consist of starch, which is a water insoluble carbohydrate and therefore has not been assessed together with the soluble carbohydrates. Its amount was determined in the range of 15 - 20 %.



1. 50 µg saccharose
2. 50 µg glucose
3. 50 µg saccharides ex rubber seed
4. 250 µg saccharides ex rubber seed
5. 50 µg raffinose

Fig. 8: TLC of rubberseed carbohydrates
Silica gel, n-butanol : formic acid : H_2O = 33 : 50 : 1
diphenylamin/anilin/phospnoric acid

Starch is a normal constituent of many seeds. It is quite astonishing that in analyses of rubber seed or RSM, reported in the literature, starch never has been mentioned. Presumably in those materials the starch had been converted already into soluble carbohydrates by enzymes. The material investigated was from the beginning of the seed fall period, has been collected immediately and was stored cold. Thus the starch cleaving enzymes may not have had much chance for attack. It should, however, be mentioned that starch could be seen in the seeds even after 5 months at room temperature.

III 5.3. Hydrogen cyanide

The content of hydrogen cyanide in the RSM obtained by hexane extraction of freshly crushed kernels was assessed after 3 months storage of the RSM at -20°C. To liberate the HCN from its glycosidic moiety, the RSM was acidified and the HCN was steam-destilled and assessed via colour formation with picric acid [14].

The amount of 140 mg HCN/kg was remarkably low for a material which had been stored so carefully. But still this content was about 2-3 times higher than reported for RSM stored at normal conditions. Therefore the assessment was repeated with a RSM sample kept for 6 months at room temperature (darkness). An amount of 95 mg HCN/kg was found which shows, that the HCN-content can be reduced by a simple storage procedure. A much lower KCN-content of 20 mg HCN/kg was obtained after heating the RSM with Hydrochlorid acid and steam for 10 min. at 100 °C.

III 5.4. Aflatoxins

Aflatoxins are formed by certain mould varieties (e.g. Aspergillas flavus, Aspergillus niger) which grow on fat containing seeds in warm climates.

In preparing the kernels for production of oil and meal 10 % dark seeds, which could possibly contain mould, were selected out beforehand. In two batches of the selected material the aflatoxin content were determined [15].

	Selected batch Nr. 1	Nr. 2
aflatoxin B_1:	1.85 mg/kg	1.70 mg/kg
" B_2:	0.10 "	0.03 mg/kg
" G_1:	4.25 "	7.10 mg/kg
" G_2:	0.16 "	0.35 mg/kg
Σ :	6.36 mg/kg	9.18 mg/kg

Calculated on the total amount of kernels the amount of aflatoxins is at least 0.65 mg/kg and might go up to 1 mg/kg in the total meal if no selection of the moulded kernels is provided. The aflatoxin content of those kernels which were free of moulded material by manual selection showed sufficiently low figures given below:

aflatoxin B_1	0,002 mg/kg
aflatoxin B_2	< 0,001 mg/kg
aflatoxin G_1	0,002 mg/kg
aflatoxin G_2	< 0,001 mg/kg

III 6. Discussion

In this chapter several important aspects with regard to the composition and the possible utilization of rubber seed is discussed in view of the laboratory work.

III 6.1. Preliminary remarks

It was already known from the literature and was revealed during the field study that the quality of the oil and the meal would be strongly dependent on the freshness of the seeds. Because of the circumstances that compelled us to carry out the laboratory trials within the time schedule of the project and to meet the above mentioned requirements the whole rubber seed used in this laboratory work was collected in the beginning of the seed fall and, as a consequence, may not be a representative product in all aspects. Consequences may be seen with respect to the content of starch, phosphatidic acid and lipase in the seeds. Therefore slight differences in the behaviour of the oil and the meal during the technical production from this seed compared to materials from later seed fall might be possible. But this will not change any of the main statements in the following discussion.

III 6.2. Influence of drying and storing on the oil yield

Although the seeds used for the laboratory trials were dried down to a water content of less than 5 % it was found as mentioned in chapter I.3.1 that there was a significant lipase activity which leads to a fat splitting even when the seeds were stored at 0°C. In the literature [I, 18a] it has been reported that kernels with 3-4 % H_2O could be stored for 24 weeks at 30-37°C without substantial increase in free fatty acid content of the oil. According to another paper

[I, 3] less than 5 % H_2O in whole nuts should be present, to prevent enzymatic attack. Obviously in this investigation it was ascertained, that the hydrolysis was not stopped by low temperature at this water content. Probably it is also important, whether in the predrying step of the nuts such a high temperature is reached, that the lipolytic enzyme is killed. This means that drying conditions with temperatures > 80°C have to be applied in the drying equipment irrespectively whether this is done by solar heated air or in a fire heated drying oven.

As a possible consequence of the enzymatic fat splitting the oil yield in the extraction process is reduced significantly. On a period of four months the oil yield was diminished from 32 to 25 % while the acid value were going up from 6 to 17. A similar observation, however, on a much lower level of acid values has been made by the Rubber Research Institute of Malaysia (private communication of Mr. Nadarajah). The figures obtained in that unpublished study were as follows:

Property	Period of storage (months)					
	0	2	4	6	8	10
% moisture	3.16	2.78	4.07	4.21	3.86	5.07
% oil yield	39.5	39.4	40.0	37.2	31.7	29.3
% free fatty acids in the oil	0.65	0.98	1.12	1.14	1.40	1.86

A similar behaviour is not reported for other oil seeds like linseed and rapeseed, apart from the always occurring small oil yield losses during long storage.

III 6.3. Composition of RSO

The composition the extracted RSO investigated in this study did not show much differences for the triglyceride and fatty acid content in comparison to other highly unsaturated vegetable oils. This is in accordance with the literature.

Fairly new results which have not been reported in previous papers were obtained by the analysis of the unsaponifiable matter and the minor constituent. The most surprising result was obtained in the GPC analysis of the oil. The crude RSO contains about 1 % of a polyisoprene material with a molecular weight of several hundred thousand Daltons. Because of the very good oil solubility of such materials this component could only be reduced partly in a refined procedure. Several attempts failed to remove this polymere material from the oil by applying different absorption materials. The technological problem caused by this material is discussed later (s. III 6.5, p. 92).

As a refined and even a crude RSO has an acceptable taste and does not contain high levels of harmful components the oil may be used for human consumption. In this case the toxicological aspect of the existing polyisoprene in the oil is of importance. The polyisoprene as a very inert material lacking any functional groups may be seen without hesitation with respect to the toxicity. The influence of such a material on the human body, however, if applied over a long period of time cannot be predicted as absolutely safe. Most likely the polyisoprene will pass the intestines without any resorption or metabolisation. Polymeric olefines for instance with a molecular weight of 800 thousand Dalton in a 90 days toxicity test with rats did not show any harmful effects [16, 17].

However, to run no risk more long term toxicological data should precede the marketing of RSO for human consumption. Long term toxicity studies are very expensive. The cost for a long term study with two species will be in the order of several thousand Dollars. In view of these immense costs it should be pointed out clearly that such a study could be carried out only if a serious attempt is made to marketing RSO as an edible oil.

III 6.4. Composition of rubber seed meal

As the protein and carbohydrate content of the rubber seed meel is of really good nutrional value for the use in animal feeds in this study emphasis was laid on the minor components which could cause toxic effects on the animals as described in several papers. As the gossypol content of the rubber seed meal was found to be equal or lower in comparison to linseed or soya meal only the hydrogen cyanide or a possible mould toxin content may have adverse effects in using RSM as animal feed.

The content of hydrogen cyanide in the form of a glycoside will be quite high in the seeds direct after seed fall. 140 mg/kg was found in the meal when they arrived at the laboratory after drying. In the literature contents of 2000 mg/kg is reported. Encymatically splitting of the glycoside in the presence of water during storage reduces the hydrogen cyanide content rather quickly. Under the same condition the lipase activiy will split the triglycerides, and therefore cause bad yield of a low quality oil. If the pre-dried seeds with a low water content are stored, encymatic splitting fails and therefore the hydrogen cyanide release may be somewhat lower. But even in this case hydrogen cyanide content may come down to 10 mg/kg in the meal during the storage or will be lowered to a value of 1 mg/kg by a short steam treatment.

The lethal dosis of hydrogen cyanide for pigs is reported to be the same as for men (1 mg HCN/kg body weight). Assuming that 1/10 of the lethal dosis will not do any harm to the animal then a fifty kg animal may take up not more than 5 mg HCN with one meal. If the HCN content of the RSM contains 10 mg HCN/kg, not more than half a kg RSM could be consumed by the pig per feeding. This calculation shows that the HCN-content could be a limiting factor in the utilization of RSM as feeding stuff, if the storage of the seeds and the meal is not optimized. Optimization of this process has to be done on pilot plant scale.

A much more limiting factor for the usage of RSM as feeding stuff comes from mould toxins, especially aflatoxin. In chapter III, 5.4, p. 86 the results for the aflatoxin contents of the comparable freshly collected and dried seeds used in the study demonstrate the uneasy situation of the RSM. A limiting maximum content of 50 µg/kg aflatoxin in animal feeding stuffs is recommended by WHO and introduced in most countries over the world. Amounts of about 1 mg aflatoxin per kg foodstuffs as determined in this study are very dangerous to young animals especially calfs and have been shown to cause death i. animal husbandry. Because any treatment of the seeds or the meal to remove aflatoxin content is too expensive (most suitable method is ammonia treatment at 120 °C of several minutes), the only possibility to avoid these problems of animal feeding is the manual selection of the moulded mostly dark appearing kernels.

Another way to minimize the problems with HCN and aflatoxins will be the limitation of RSM to 25 % in the total feeding stuff. This is in accordance with what has already been reported in the literature.

III 6.5. Technological aspects

While with the exception of the optimizing procedure mentioned in III 6.4, p. 90 no problems occur with the technical preparation of rubber seed meal, some difficulties in the processing of the RSO particularly the production of a high quality oil have been revealed in this study.

In chapter III, 3.2.2.3 a relatively high phosphor lipid content with a high amount of phosphatidic acid was determined in a crude RSO. The latter cannot easily be removed from the oil by the desliming step, and therefore may cause problems by emulsification of the oil containing high amounts of FFA during the refining step. The only possibilities to remove the phosphatidic acid drastically would be an adsorption column cleaning or ultrafiltration of the oil. Both processes would be far too expensive for processing refined RSO. Therefore the best way to avoid this problem in the neutralization step as mentioned in chapter 3.1, is quickly drying of the fresh collected seeds at temperatures of 80°C to kill the lipolytic enzymes.

A further problem in the raffination of RSO is a low filtering rate, obviously caused by the content of polyisoprenes which are clogging the filter. On page 80 uselessness of filter aids is described. Solvent solution of the oil may be a suitable method, but because the solvent has to be removed after filtration by an evaporation process this is an expensive process. A more practical attempt to overcome the filtering problem would be the mixing of RSO with other vegetable oils before the raffination. The feasibility of this procedure has to be proved on pilot plant scale experiments.

As a consequence of the above outlined difficulties in the raffination of RSO careful considerations are required for a set up of pilot plant trials in order to develop a RSO processing with the aim to obtain a high quality oil on a low cost level.

III 7. Summary

Rubber seeds grown 1985, freshly collected and dried in an air oven at 70-80°C in Sri Lanka, were investigated in the NATEC laboratory (Hamburg) with regard to the composition and technical aspects.

After manual decortification the oil was extracted from the crushed seed with petroleum ether. The acid value in the crude oil increased during storage of the predried seeds (H_2O in kernels 7,6 %) at 0°C for several months because of lipase activity. The crude oil was refined via a standard laboratory method including desliming, neutralization, water glass-treatment, bleaching and deodorization.

The oil composition was comparable to other unsaturated vegetable oils with regard to the fatty [illegible] and the oxidized and dimerisized triglycerides or mono- and diglycerides. Gel permeation chromatography (GPC) indicated a high molecular material (~ 1 % of the oil) which was separated by preparative GPC and identified as 1,4-polyisoprene by ^{1}H-NMR. The molecular weight of this material was about 500 000 D which is comparable to that of polyisoprene in latex.

Tocopherols as natural antioxidants were absent in the oil. HPLC analysis of the oil revealed the existence of toco-trienols which have also been found in latex. Phospholipids in the oil were mainly phosphatidic acid, in lesser amounts also phosphatidyl-choline, -inositol and -ethanolamine.

The crude oil had a brown-yellow colour (iodine colour 40) and a pleasant nutty taste. It could be refined into an slightly yellow (iodine colour 3) oil which was almost tasteless. The colours of both oils were reduced by storage at daylight under air. In view of the linolenic acid content of 18 % the taste stability of the crude and refined RSO were quite acceptable for several months. Additional tocopherols gave a somewhat increasing stability. 20 % crude or refined rubber seed oil in coconut oil were acceptable w. r. t. taste up to 5 months.

Technically filtration of rubber seed oil is a somewhat difficult problem. Obviously the polyisoprene clogs the filter. It was not possible, to remove this polymer by adsorbents or to overcome the difficulties by using other filter material or filtering aids. Addition of 50 % hexane improved the filtration considerably. Also mixtures of 20 % rubber seed oil and 80 % coconut oil were filtered in quite a normal manner, but it has to be checked in pilot plant trials, whether this holds also in technical scale.

In rubber seed meal some 15 - 20 % starch were detected, which has not been reported in other papers before. The water soluble carbohydrates consist only of mono- und di-saccharides, no tri- or tetrasaccharides, which may cause digestion problems, were detected.

The content of toxic hydrogen cyanide of 95 - 140 mg/kg meal was in the known range. In the moulded and rotten part of the kernels, which has been separated before processing, an alarmingly high amount of aflatoxins has been found. Recalculated on the total amount of kernels the aflatoxin content was at least 0,65 mg/kg.

The results of the laboratory investigations are discussed in view of the technical and economical aspects of the utilization of rubber seed, and the key points are lined out which have to be taken into consideration the the set up of pilot plant trials the further progress of this project.

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Recommendations for pilot plant trials

In view of the already available experiences in the utilisation of rubberseeds and taking into account the problems outlined in the study for the production of RSO of high quality and RSM to be used as animal feeding stuff the following route for the set up of pilot plant trials for the oil and meal production is recommended:

1) Collection of seeds to be done by people at the states (children and dependants) organized by the superintendents or their estates managers.

2) Drying of the seeds is to be done on the estates in the smoke-houses or by solar energy equipments. The latter has to be built at the estates.

3) Decortication of the seed is to be done on the estates on a rubber-mill or on a groundnut decorticator. The latter has to be installed, selection of kernels from the hulls is done manually.

4) Milling of the kernels in a chakku mill together with molassis or in an electrical groundmill. Both milling systems have to be installed at the estates. Organisation of this work to be done by the estates superintendents and estate managers.

5) Transport of the raw oil and cake to the oil and feed consuming companies.

6) Refining of the oil should be done in small batches of about 500 kg, the cake should be mixed with other materials to obtain finished animal feed products and stored for several weeks before given to the animals.

As there is a relatively high demand of cheap oil in the paint industry for the preparation of alkyd resins the oil should be shipped from the estates directly to the paint factory. The oil may be used in a refined or unrefined state for the preparation of the finished products.

In this route the low transport costs, the relatively low costs for the investment of the simple machinery for decortication and milling leads to a process which is based on a very profitable system. From the economical point of view it may be worthwhile to do the seed collection, drying, decortication and milling at one estate in an area of several estates so that the machinery equipments have to be installed only at one place.

Interest for this model was mentioned by the general estate manager of the Sri Lanka States Corporation 111. By this model on the one hand with a fairly simple technology a comparable high priced raw material could be produced in a not industrial area. On the other hand only products of improved quality not containing waste-weight materials like moisture or shells have to be transported to the refineries and factories in the industrial area where more sophisticated technologies for the preparation of final products have to be applied.

Appendix 1

Complete References of the Literature Survey
with regard to Rubberseed

This literature retrieval contains citations on

RUBBER/SEED OR HEVEA
and
NUTRITION OR FOOD OR FEED OR EDIBLE (Part 1)
and NOT (NUTRITION OR FOOD OR FEED OR EDIBLE) (Part 2)

Taken into consideration are records retrieved under these key words from the databases

311:Chemical Abstracts Search - 1982-1985
310:Chemical Abstracts Search - 1980-1981
320:Chemical Abstracts Search - 1977-1979
309:Chemical Abstracts Search - 1972-1976
308:Chemical Abstracts Search - 1967-1971
51:FSTA (FOOD SCIENCE & TECHNOLOGY ABSTRACTS) - 1969-1985

Sequence of records is chronological, latest records named first. Owing to retrieval in different databases double entries may occur.

Record # 1
Ord.no. : 311:02077786
CA-No. : 102(9)77786t
Source : Journal
Titel : Plant tissues as indicators of soil nutrient availability for Hevea: glasshouse evaluations
Author : Yew, F. K.; Pushparajah, E.
Location: Malay.
Journal : J. Rubber Res. Inst. Malays.
Date : 1984 ,32(3)171-81
Coden : JRRIAN
ISSN : 0035-953X
Language: English

Record # 2
Ord.no. : 311:01089481
CA-No. : 101(11)89481g
Source : Journal
Titel : Preliminary study on levels of rubber seed meal for broiler rations
Author : Sripongpun, S.; Pralomkarn, W.; Chandumpai, A.
Location: Fac. Sci., Prince Songkla Univ., Thailand
Journal : Warasan Songkhla Nakkharin
Date : 1983 ,5 (2)131-5
Coden : WSNAEV
Language: Thai

Record # 3
Ord.no. : 311:01071537
CA-No. : 101(9)71537v
Source : Journal
Titel : Evaluation of some chemical and nutritional characteristics of rubber tree seed (Hevea brasiliensis)
Author : Selle, Celia Margarita; Gonzalez de Mejia, Elvira; Elias, Luiz G.; Bressani, Ricardo
Location: Univ. Valle Guatemala, Guatemala, Guatemala
Journal : Arch. Latinoam. Nutr.
Date : 1983 ,33 (4)884-901
Coden : ALANBH
ISSN : 0004-0622
Language: Spanish

Record # 4
Ord.no. : 311:01005766
CA-No. : 101(1)5766t
Source : Journal
Titel : Chemical composition and nutritional value of para-rubber seed and its products for chickens
Author : Narahari, D.; Kothandaraman, P.
Location: Dep. Poultry Sci., Madras Vet. Coll., Madras, 600007, India
Journal : Anim. Feed Sci. Technol.
Date : 1984 ,10 (4)257-67
Coden : AFSTDH
ISSN : 0377-8401
Language: English

Record # 5
Ord.no. : 311:00066745
CA-No. : 100(9)66745m
Source : Journal
Titel : The influence of processing and storage on hydrogen cyanide and tannin contents of para-rubber seed and its products
Author : Narahari, D.; Kothandaraman, P.
Location: Dep. Poult. Sci., Madras Vet. Coll., Madras, 600007, India
Journal : Anim. Feed Sci. Technol.
Date : 1983 ,9 (4)319-23
Coden : AFSTDH
ISSN : 0377-8401
Language: English

Record # 6
Ord.no. : 311:00033693
CA-No. : 100(5)33693q
Source : Journal
Titel : Study on the zinc status in rubber growing soils and its effects on rubber trees in China
Author : Wang, Guohong
Location: Rubber Cultiv. Res. Inst., Acad. Trop. Crops South China, Peop. Rep. China
Journal : Turang Xuebao
Date : 1983 ,20 (3)313-21
Coden : TJHPAE
ISSN : 0564-3929
Language: Chinese

Record # 7
Ord.no. : 311:00021985
CA-No. : 100(3)21985y
Source : Konferenz-Bericht
Titel : Legume cover crops as a source of nitrogen in plantation crops in the tropics
Author : Pushparajah, E.
Location: Rubber Res. Inst. Malaysia, Kuala Lumpur, Malay.
Journal : Trans. Int. Congr. Soil Sci., 12th
Date : 1982 ,2,)189-97
Coden : SOPPAH
Language: English
Publish : Ind. Soc. Soil Sci., New Delhi, India

Record # 8
Ord.no. : 311:00004937
CA-No. : 100(1)4937s
Source : Journal
Titel : The saponin content of some Nigerian oil seeds
Author : Achinewhu, S. C.
Location: Dep. Food Sci. Technol., Rivers State Univ. Sci. Technol., Port Harcourt, Nigeria
Journal : Qual. Plant. - Plant Foods Hum. Nutr.
Date : 1983 ,33(1)3-9
Coden : QLPLAN
ISSN : 0377-3205
Language: English

Record # 9
Ord.no. : 311:99157269
CA-No. : 99(19)157269m
Source : Konferenz-Bericht
Titel : Nitrogen cycle in rubber (Hevea) cultivation
Author : Pushparajah, E.
Location: Soils Crop Manage. Div., Rubber Res. Inst. Malaysia, Kuala Lumpur, 16-03, Malay.
Journal : Nitrogen Cycling South-East Asian Wet Monsoonal Ecosyst., Proc. Reg. Workshop
Editor : Wetselaar, Robbert (Ed)^ Simpson, Jeffrey R. (Ed)^ Rosswall, Thomas (Ed)
Date : 1981)101-8
Coden : 50DSAY
Language: English
Date : 790000
Publish : Aust. Acad. Sci., Canberra, Australia

Record # 10
Ord.no. : 311:99052404
CA-No. : 99(7)52404y
Source : Journal
Titel : Evaluation of the suitability of the methods for assessment of nutrients of Datmara soils with respect to rubber plant
Author : Anam, K.; Didar-Ul-Alam; Rahman, Shafiqur; Huq, S. M. Imamul
Location: Dep. Soil Sci., Univ. Dhaka, Dhaka, Bangladesh
Journal : Dhaka Univ. Stud., Part E
Date : 1982 ,30 (2)181-9
Coden : DUBSDX
Language: English

Record # 11
Ord.no. : 311:99004337
CA-No. : 99(1)4337g
Source : Journal
Titel : Relative efficacy of some antifeedants and deterrents against insect pests of stored rice
Author : Devi, D. Ambika; Mohandas, N.
Location: Div. Entomol., Coll. Agric., Trivandrum, 695 522, India
Journal : Entomon
Date : 1982 ,7 (3)261-4
Coden : ENTOD5
ISSN : 0377-9335
Language: English

Record # 12
Ord.no. : 311:98159194
CA-No. : 98(19)159194m
Source : Journal
Titel : Finding of .alpha.-, .beta.- and .gamma.-dehydrotocopherol in wheat germ oil by HPLC and GC/MS - a contribution to tocopherol analysis
Author : Mueller-Mulot, W.; Rohrer, G.; Oesterhelt, G.; Schmidt, K.; Allemann, L.; Maurer, R.
Location: Kontrollabteil., Hoffmann-La Roche A.-G., Grenzach-Wyhlen, Switz.
Journal : Fette, Seifen, Anstrichm.
Date : 1983 ,85 (2)66-72
Coden : FSASAX
ISSN : 0015-038X
Language: German

Record # 13
Ord.no. : 311:97161710
CA-No. : 97(19)161710z
Source : Journal
Titel : Commercial experience in the use of leaf analysis for diagnosing nutritional requirement of Hevea
Author : Kow, Chang Ah; Hai, Teoh Cheng
Location: Harrisons and Crosfield Prang Besar Res. Stn., Selangor, Malay.
Journal : Proc. Rubber Res. Inst. Malays. Plant. Conf.
Date : 1982)220-31
Coden : PMPCDT
ISSN : 0126-9054
Language: English
Date : 810000

Record # 14
Ord.no. : 311:97161595
CA-No. : 97(19)161595r
Source : Journal
Titel : Capability and management of alluvial soils under Hevea in Peninsular Malaysia
Author : Daud, Noordin Wan; Pushparajah, E.
Location: Rubber Res. Inst. Malaysia, Malay.
Journal : Proc. Rubber Res. Inst. Malays. Plant. Conf.
Date : 1982)174-202
Coden : PMPCDT
ISSN : 0126-9054
Language: English
Date : 810000

Record # 15
Ord.no. : 311:97161572
CA-No. : 97(19)161572f
Source : Journal
Titel : Nitrogenous fertilizers for Hevea cultivation
Author : Pushparajah, E.; Huat, Tan Keh; Lock, Chin Siew
Location: Rubber Res. Inst. Malaysia, Malay.
Journal : Proc. Rubber Res. Inst. Malays. Plant. Conf.
Date : 1982)203-19
Coden : PMPCDT
ISSN : 0126-9054
Language: English
Date : 810000

Record # 16
Ord.no. : 311:97022603
CA-No. : 97(3)22603d
Source : Journal
Titel : Effect of rubber seed oil on the development and regression of experimental atherosclerosis of the aorta in rabbits
Author : Liu, Chaoran; Tang, Chaocai; Yang, Liang; Chen, Guozhen
Location: Kunming Med. Coll., Kunming, Peop. Rep. China
Journal : Zhonghua Xinxueguanbing Zazhi
Date : 1982 ,9(1)54-7
Coden : CHHCDF
Language: Chinese

Record # 17
Ord.no. : 311:96033588
CA-No. : 96(5)33588h
Source : Journal
Titel : Gossypol in rubber seed meal
Author : Abdullah, Abdul Salam; Hutagalung, R. I.
Location: Fac. Vet. Med. Anim. Sci., Univ. Pertanian Malaysia, Serdang, Malay.
Journal : Pertanika
Date : 1981 ,4 (1)96-8
Coden : PERTDY
ISSN : 0126-6128
Language: English

Record # 18
Ord.no. : 311:96019162
CA-No. : 96(3)19162h
Source : Journal
Titel : Ultrastructure of mineral deficient leaves of Hevea. III. Quantitative considerations
Author : Binte Hamzah, Samsidar; Gomez, J. B.
Location: Rubber Res. Inst. Malaysia, Kuala Lumpur, Malay.
Journal : J. Rubber Res. Inst. Malays.
Date : 1981 ,29(1)15-23
Coden : JRRIAN
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Language: English

Record # 19
Ord.no. : 311:96005596
CA-No. : 96(1)5596r
Source : Journal
Titel : Ultrastructure of mineral deficient leaves of Hevea. II. Effects of micronutrient deficiencies
Author : Hamzah, Samsidar Bte; Gomez, J. B.
Location: Rubber Res. Inst. Malaysia, Kuala Lumpur, Lumpur, Malay.
Journal : J. Rubber Res. Inst. Malays.
Date : 1980 ,28(1)17-25
Coden : JRRIAN
ISSN : 0035-953X
Language: English

Record # 20
Ord.no. : 310:95041360
CA-No. : 95(5)41360x
Source : Journal
Titel : Some aspects of the mineral nutrition of young hevea trees in Ivory Coast

Journal : Rev. Gen. Caoutch. Plast.
Date : 1981 ,61J,)87-94

Record # 21
Ord.no. : 310:95023463
CA-No. : 95(3)23463d
Source : Konferenz-Bericht
Titel : Manuring in relation to exploitation systems
Journal : RRIM Train. Man. Soils, Manage. Soils Nutr. Hevea
Date : 1981)211-20

Record # 22
Ord.no. : 310:95005614
CA-No. : 95(1)5614z
Source : Konferenz-Bericht
Titel : Fertilizers and tneir efficient use
Journal : RRIM Train. Man. Soils, Manage. Soils Nutr. Hevea
Date : 1981)175-202

Record # 23
Ord.no. : 310:95005613
CA-No. : 95(1)5613y
Source : Konferenz-Bericht
Titel : Variations in leaf nutrient contents and their interpretation
Journal : RRIM Train. Man. Soils, Manage. Soils Nutr. Hevea
Date : 1981)101-14

Record # 24
Ord.no. : 310:95005612
CA-No. : 95(1)5612x
Source : Konferenz-Bericht
Titel : Major nutrients : role and deficiency symptoms
Journal : RRIM Train. Man. Soils, Manage. Soils Nutr. Hevea
Date : 1981)87-92

Record # 25
Ord.no. : 310:95005611
CA-No. : 95(1)5611w
Source : Konferenz-Bericht
Titel : Nutrient cycle in a rubber plantation
Journal : RRIM Train. Man. Soils, Manage. Soils Nutr. Hevea
Date : 1981)76-86

Record # 26
Ord.no. : 310:95005610
CA-No. : 95(1)5610v
Source : Konferenz-Bericht
Titel : Chemistry and fertility of soils
Journal : RRIM Train. Man. Soils, Manage. Soils Nutr. Hevea
Date : 1981)12-23

Record # 27
Ord.no. : 310:93219751
CA-No. : 93(23)219751z
Source : Journal
Titel : Fertilizer value of Mussoorie rock phosphate for manuring of rubber
Journal : Rubber Board Bull. (India)
Date : 1980 ,15 (3-4)67-70

Record # 28
Ord.no. : 310:93219665
CA-No. : 93(23)219665z
Source : Journal
Titel : Effect of air-drying on nutrient concentration in leaves. II. Effect on sun-drying and hot iron-pressing on nutrient concentration in leaves
Journal : Rubber Board Bull. (India)
Date : 1980 ,15 (3-4)57-8

Record # 29
Ord.no. : 310:93219664
CA-No. : 93(23)219664y
Source : Journal
Titel : Effect of air-drying on nutrient concentration in leaves. 1. Studies on preparation of leaf samples of rubber (Hevea Brasiliensis) for chemical analysis
Journal : Rubber Board Bull. (India)
Date : 1980 ,15 (3-4)56-7

Record # 30
Ord.no. : 310:93184952
CA-No. : 93(19)184952m
Source : Journal
Titel : Land-disposal of rubber factory effluent : its effects on soil properties and performance of rubber and oil palm
Journal : Proc. Rubber Res. Inst. Malays. Plant. Conf.
Date : 1979)436-57

Record # 31
Ord.no. : 310:93131112
CA-No. : 93(13)131112f
Source : Journal
Titel : Rubber seed meal as a protein supplement in growing swine rations
Journal : J. Natl. Sci. Counc. Sri Lanka
Date : 1979 ,7(2)101-4

Record # 32
Ord.no. : 310:93069333
CA-No. : 93(7)69333y
Source : Journal
Titel : Effect of feeding rubber seed cake to growing calves on dry matter and nutrient utilization
Journal : Gujarat Agric. Univ. Res. J.
Date : 1980 ,5(2)40-3

Record # 33
Ord.no. : 310:93045002
CA-No. : 93(5)45002p
Source : Journal
Titel : Effect of potassium and aluminum treatments on growth and nutrient uptake of rubber seedlings and on soils
Journal : J. Rubber Res. Inst. Malays.
Date : 1979 ,27(2)92-103

Record # 34
Ord.no. : 310:93044760
CA-No. : 93(5)44760x
Source : Journal
Titel : The copper, zinc, manganese, iron and aluminum contents of soils commonly used for Hevea brasiliensis cultivation. I. Distribution

within soil profiles
Journal : J. Rubber Res. Inst. Malays.
Date : 1979 ,27(2)68-78

Record # 35
Ord.no. : 310:93025044
CA-No. : 93(3)25044a
Source : Journal
Titel : Comparative effects of ashing and acid digestion on the elemental analysis of some tropical tree crops
Journal : Inf. Ser. - N. Z. Dep. Sci. Ind. Res.
Date : 1978 ,134 (Plant Nutr., v1)113-22

Record # 36
Ord.no. : 310:92214154
CA-No. : 92(25)214154b
Source : Konferenz-Bericht
Titel : A radiotracer study on seasonal uptake of phosphorus by mature rubber and oil palm
Journal : Asean Soil Conf., (Proc.), 3rd
Date : 1977)181-91

Record # 37
Ord.no. : 310:92214153
CA-No. : 92(25)214153a
Source : Konferenz-Bericht
Titel : The effects of potassium, calcium and magnesium on cation exchange capacity of Hevea roots
Journal : Asean Soil Conf., (Proc.), 3rd
Date : 1977)167-70

Record # 38
Ord.no. : 310:92127394
CA-No. : 92(15)127394m
Source : Journal
Titel : Evaluation of rubber (Hevea brasiliensis) seed cake for promoting growth in calves
Journal : Indian J. Nutr. Diet.
Date : 1979 ,16 (10)383-9

Record # 39
Ord.no. : 310:92040483
CA-No. : 92(5)40483r
Source : Dissertation
Titel : Extractable minerals in nine Malaysian soils and their effect on the mineral contents of four Hevea clones
Date : 1979)193 pp.

Record # 40
Ord.no. : 310:92021299
CA-No. : 92(3)21299u
Source : Journal
Titel : Response of rubber (Hevea brasiliensis) seedlings to phosphorus sources on a Malaysian clay soil
Journal : Proc. - Soil Crop Sci. Soc. Fla.
Date : 1979 ,38,)81-3

Record # 41
Ord.no. : 310:92005325
CA-No. : 92(1)5325u
Source : Journal
Titel : A review of work done at the Rubber Research Institute of Malaysia on fertilizer and ethephon stimulation in the rubber smallholder sector
Journal : Planter
Date : 1979 ,55 (639)272-8

Record # 42
Ord.no. : 320:91209897
CA-No. : 91(25)209897w
Source : Journal
Titel : Studies on goat nutrition. IV. Comparative evaluation of conventional and unconventional feed for evolving a cheap and economic ration for goats
Journal : Kerala J. Vet. Sci.
Date : 1978 ,9 (2)206-14

Record # 43
Ord.no. : 320:91139742
CA-No. : 91(17)139742r
Source : Buch
Titel : RRIM (Rubber Research Institute of Malaysia) Training Manual on Soils, Management of Soils and Nutrition of Hevea
Date : 1979)234 pp.

Record # 44
Ord.no. : 320:91139715
CA-No. : 91(17)139715j
Source : Journal
Titel : Methods of fertilizer application for mature rubber trees
Journal : Menara Perkebunan
Date : 1976 ,44 (5)221-6

Record # 45
Ord.no. : 320:91004390
CA-No. : 91(1)4390d
Source : Journal
Titel : Discriminatory nutrition for rubber in Java and South Sumatra
Journal : Menara Perkebunan
Date : 1977 ,45 (3)111-20

Record # 46
Ord.no. : 320:90185493
CA-No. : 90(23)185493n
Source : Journal
Titel : Potassium-supplying power of seven soils under rubber
Journal : J. Rubber Res. Inst. Malays.
Date : 1978 ,26(1)13-20

Record # 47
Ord.no. : 320:90102534
CA-No. : 90(13)102534c
Source : Journal
Titel : Residual effect of applied phosphates on performance of Hevea brasiliensis and Pueraria phaseoloides
Journal : J. Rubber Res. Inst. Malays.
Date : 1977 ,25(3)101-8

Record # 48
Ord.no. : 320:90038035
CA-No. : 90(5)38035m
Source : Journal
Titel : Fertilizer elements and gibberellic acid interaction on the buddability of para rubber (Hevea brasiliensis Muell. Arg.) seedlings for green budding
Journal : MIT Res. J.
Date : 1977 ,7 (1)71-80

Record # 49
Ord.no. : 320:89214163
CA-No. : 89(25)214163s
Source : Journal
Titel : Fertility of soils used for rubber in southern Bahia and degree of tolerance of this crop to aluminum
Journal : Rev. Theobroma
Date : 1977 ,7 (4)125-32

Record # 50
Ord.no. : 320:89196117
CA-No. : 89(23)196117y
Source : Konferenz-Bericht
Titel : Manuring of Hevea under ethephon stimulation
Journal : Lect. Notes - RRIM Course Tapping, Tapping Syst. Yield Stimul. Hevea
Date : 1978)196-203

Record # 51
Ord.no. : 320:89178739
CA-No. : 89(21)178739y
Source : Journal
Titel : The efficient use of phosphatic fertilizer in relation to the phosphorus fixing capacities of rubber tree soils
Journal : J. - Rubber Res. Inst. Sri Lanka
Date : 1978 ,54(1, Pt. 2)263-9

Record # 52
Ord.no. : 320:89162250
CA-No. : 89(19)162250w
Source : Journal
Titel : Management of covers under Hevea in Sri Lanka
Journal : J. - Rubber Res. Inst. Sri Lanka
Date : 1978 ,54(1, Pt. 2)291-8

Record # 53
Ord.no. : 320:89162102
CA-No. : 89(19)162102z
Source : Journal
Titel : Nutrition and fertilizer use in Hevea and associated covers in peninsular Malaysia - a review
Journal : J. - Rubber Res. Inst. Sri Lanka
Date : 1978 ,54(1, Pt. 2)270-83

Record # 54
Ord.no. : 320:89103777
CA-No. : 89(13)103777a
Source : Journal
Titel : Characteristics and some fatty acids of para rubber seed oil in Malaysia
Journal : Nettai Nogyo

Date : 1978 ,21 (3-4)201-5

Record # 55
Ord.no. : 320:88189018
CA-No. : 88(25)189018t
Source : Journal
Titel : A note on application of mixed rubber factory effluent to mature rubber
Journal : Planter
Date : 1977 ,53 (619)468-9

Record # 56
Ord.no. : 320:88132912
CA-No. : 88(19)132912a
Source : Journal
Titel : Comparison of various methods of plant material mineralization employed most frequently in Czechoslovakian laboratories
Journal : Sci. Agric. Bohemoslov.
Date : 1977 ,9 (3)157-64

Record # 57
Ord.no. : 320:88119941
CA-No. : 88(17)119941q
Source : Journal
Titel : Natural rubber encapsulated fertilizers for controlled nutrient release
Journal : Proc. Rubber Res. Inst. Malays. Plant. Conf.
Date : 1976)63-74

Record # 58
Ord.no. : 320:87200080
CA-No. : 87(25)200080j
Source : Journal
Titel : Latex flow studies. X. Distribution of metallic ions between phases of Hevea latex and the effects of yield stimulation on this distribution
Journal : J. Rubber Res. Inst. Malays.
Date : 1977 ,25, Pt.
1,)31-49

Record # 59
Ord.no. : 320:87052030
CA-No. : 87(7)52030a
Source : Journal
Titel : Biological evaluation of para-rubber seeds (Hevea braziliensis)
Journal : Nutr. Rep. Int.
Date : 1977 ,15 (5)497-510

Record # 60
Ord.no. : 320:87004557
CA-No. : 87(1)4557f
Source : Journal
Titel : A study on the effect of some trace elements on the growth of rubber seedlings in nursery
Journal : Rubber Board Bull. (India)
Date : 1976 ,13 (1-2)30-2

Record # 61
Ord.no. : 320:87004556
CA-No. : 87(1)4556e
Source : Journal
Titel : A study on the relative efficiency of some nitrogenous fertilizers on the growth of rubber seedlings in the nursery
Journal : Rubber Board Bull. (India)
Date : 1976 ,13 (1-2)11-13

Record # 62
Ord.no. : 320:86015591
CA-No. : 86(3)15591t
Source : Journal
Titel : Feasibility of using rubber seeds as animal feed supplement
Journal : West Afr. J. Biol. Appl. Chem.
Date : 1976 ,19(2)22-4

Record # 63
Ord.no. : 309:85158487
CA-No. : 85(21)158487d
Source : Konferenz-Bericht
Titel : Special problems in tropical humid areas : soil and plant nutrient studies in rubber cultivation
Journal : Int. Symp. For. Fert., (Proc.)
Date : 1975)331-50

Record # 64
Ord.no. : 309:85158463
CA-No. : 85(21)158463t
Source : Journal
Titel : Nutritional and fertilization requirements of Para rubber tree cultivation
Journal : Bol. Tec., Cent. Pesqui. Cacau
Date : 1975 ,33,)32 pp.

Record # 65
Ord.no. : 309:85019998
CA-No. : 85(3)19998h
Source : Journal
Titel : Brown bast and nutrition : a case study
Journal : Rubber Board Bull. (India)
Date : 1975 ,12 (3)83-3

Record # 66
Ord.no. : 309:85004213
CA-No. : 85(1)4213z
Source : Konferenz-Bericht
Titel : Efficient use of fertilizers
Journal : Proc. Rubber Res. Inst. Malays. Plant. Conf.
Editor : Ling,
Sim Lee (Ed)
Date : 1974)102-14

Record # 67
Ord.no. : 309:84003577
CA-No. : 84(1)3577p
Source : Journal
Titel : Manuring practices on rubber estates in North Sumatra
Journal : Rubber Board Bull. (India)
Date : 1975 ,12)54-60

Record # 68
Ord.no. : 309:83176905
CA-No. : 83(21)176905b
Source : Journal
Titel : Glycerol content in Indian vegetable oils
Journal : J. Oil Technol. Assoc. India
Date : 1975 ,7(1)31-2

Record # 69
Ord.no. : 309:83111142
CA-No. : 83(13)111142f
Source : Konferenz-Bericht
Titel : Evolution of mineral nutrition of Hevea in the Ivory Coast as a function of the vegetative cycle according to foliar diagnosis
Journal : Potassium Cult. Sols Trop., C. R. Colloq. Inst. Int. Potasse, 10th
Date : 1974)209-14

Record # 70
Ord.no. : 309:83077458
CA-No. : 83(9)77458b
Source : Konferenz-Bericht
Titel : Progression in fertilizer use, particularly of potassium fertilization in relation with productivity of Hevea
Journal : Potassium Cult. Sols Trop., C. R. Colloq. Inst. Int. Potasse, 10th
Date : 1974)475-83

Record # 71
Ord.no. : 309:81135034
CA-No. : 81(21)135034g
Source : Journal
Titel : Recent developments in the nutrition of Hevea in West Malaysia
Journal : Rubber Res. Inst. Ceylon, Q. J.
Date : 1973 ,50,
Pt. 1-2,)68-83

Record # 72
Ord.no. : 309:80026287
CA-No. : 80(5)26287a
Source : Journal
Titel : Effects of nitrogenous fertilizers on growth of rubber seedlings and leaching losses of nutrients
Journal : J. Rubber Res. Inst. Malaya
Date : 1973 ,23(Pt. 5)356-64

Record # 73
Ord.no. : 309:79135389
CA-No. : 79(23)135389k
Source : Journal
Titel : Simple method for the detection of rubber seed oil in other vegetable oils
Journal : Indian Oil Soap J.
Date : 1972 ,37 (12)311-12

Record # 74
Ord.no. : 309:79090954
CA-No. : 79(15)90954x
Source : Konferenz-Bericht
Titel : Radiotracer studies on phosphorus uptake by Hevea brasiliensis from Malayan soils for determining active root distribution

Journal : Isotop. Radiat. Soil-Plant Relat. Incl. Forest., Proc. Symp.
Date : 1972)465-79

Record # 75
Ord.no. : 309:79004186
CA-No. : 79(1)4186u
Source : Journal
Titel : Urea and the platation crops
Journal : Assam Rev. Tea News
Date : 1972 ,61 (10)469-75

Record # 76
Ord.no. : 309:78042119
CA-No. : 78(7)42119m
Source : Journal
Titel : Pedogenesis and soil fertility in West Malaysia
Journal : Natur. Resour. Res.
Date : 1971 ,No. 11,)129-39

Record # 77
Ord.no. : 309:77138767
CA-No. : 77(21)138767b
Source : Journal
Titel : Assessment of the cation nutrient status of acid soils
Journal : RRIC (Rubber Res. Inst. Ceylon) Bull.
Date : 1971 ,6 (3-4)41-61

Record # 78
Ord.no. : 309:77100124
CA-No. : 77(15)100124b
Source : Journal
Titel : Use of appropriate fertilizer for rubber based on soil and leaf nutrient survey
Journal : Rubber Res. Inst. Ceylon, Quart. J.
Date : 1971 ,48(Pt. 3-4)160-7

Record # 79
Ord.no. : 309:77086992
CA-No. : 77(13)86992z
Source : Konferenz-Bericht
Titel : Soil survey for assessing fertilizer requirement for rubber (Hevea brasiliensis)
Journal : Int. Symp. Soil Fert. Eval., Proc.
Editor : Kanwar, J. S (Ed)
Date : 1971 ,1,)427-35

Record # 80
Ord.no. : 308:75127075
CA-No. : 75(21)127075g
Source : Journal
Titel : Value of 21 amino acids as nitrogen sources for Phytophthora cactorum and P. heveae
Journal : Can. J. Microbiol.
Date : 1971 ,17 (10)1319-25

Record # 81
Ord.no. : 308:75047907
CA-No. : 75(7)47907q
Source : Journal
Titel : Effect of nitrogen-phosphorus-potassium fertilization on the growth

of WR 101 Hevea seedlings in the nursery
Journal : Menara Perkebunan
Date : 1970 ,39 (1-2)15-19

Record # 82
Ord.no. : 308:74110955
CA-No. : 74(21)110955a
Source : Journal
Titel : Organic phosphorus fraction of rubber soils in Ceylon
Journal : J. Indian Soc. Soil Sci.
Date : 1970 ,18 (2)133-40

Record # 83
Ord.no. : 308:73055046
CA-No. : 73(11)55046g
Source : Journal
Titel : Automated determinations of phosphate content of soils under rubber cultivation
Journal : J. Sci. Food Agr.
Date : 1970 ,21 (6)275-8

Record # 84
Ord.no. : 308:72020980
CA-No. : 72(5)20980j
Source : Journal
Titel : Effect of fertilizer applications on latex properties
Journal : J. Rubber Res. Inst. Malaya
Date : 1969 ,21(Pt. 2)181-91

Record # 85
Ord.no. : 308:72020893
CA-No. : 72(5)20893h
Source : Konferenz-Bericht
Titel : Rubber, an example of progress in fertilizer use in tropical agriculture
Journal : Trans., Int. Congr. Soil Sci., 9th
Editor : Holmes, J. W
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Date : 1968 ,4,)77-84

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Ord.no. : 308:72019571
CA-No. : 72(5)19571v
Source : Journal
Titel : Technological and economic aspects of plant protein production
Journal : Voeding
Date : 1969 ,30 (8)447-63

Record # 87
Ord.no. : 308:71021229
CA-No. : 71(5)21229n
Source : Journal
Titel : Potassium status of some soils in the rubber growing areas of Ceylon
Journal : Rubber Res. Inst. Ceylon, Quart. J.
Date : 1967 ,43(Pt. 1-2)19-33

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Ord.no. : 308:70095831
CA-No. : 70(21)95831d
Source : Journal
Titel : Effects of trace elements on Hevea brasiliensis seedlings grown in

the nursery
Journal : J. Rubber Res. Inst. Malaya
Date : 1968 ,20(Pt. 5)217-25

Record # 89
Ord.no. : 308:67089865
CA-No. : 67(19)89865q
Source : Journal
Titel : Detection of rubberseed oil in edible oils by paper chromatography
Journal : Curr. Sci.
Date : 1967 ,36 (15)403-4

Record # 90
Ord.no. : 308:66075280
CA-No. : 66(17)75280n
Source : Journal
Titel : Use of phosphates in the cultivation of Hevea brasiliensis in Malaya
Journal : Outlook Agric.
Date : 1966 ,5 (2)69-73

Record # 91
Ord.no. : 308:66026536
CA-No. : 66(7)26536s
Source : Journal
Titel : Use of tracer elements to improve the productivity of hevea culture
Journal : Meded. Landbouwhogesch. Opzoekingsstn. Staat Gent
Date : 1965 ,30 (2)1165-84

Record # 92
Ord.no. : 51:256253 84-04-n0163
Titel : The saponins content of some Nigerian oil seeds.
Author : Achinewhu, S. C.
Location: Dep. of Food Sci. + Tech., Rivers State Univ. of Sci. + Tech., PMB 5080, Port Harcourt, Nigeria
Journal : Qualitas Plantarum - Plant Foods for Human Nutrition, 1983, 33, (1), 3-9

Record # 93
Ord.no. : 51:236959 83-04-s0615
Titel : Effect of feeding rubber seed meal-based diets on performance and serum thiocyanate level of growing-finishing pigs.
Author : Ong, H. K.; Radem, J.
Location: Poultry + Pig Branch, MARDI, Serdang, Selangor, Malaysia
Journal : MARDI Research Bulletin, 1981, 9, (1), 78-82

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Ord.no. : 51:236810 83-04-q0049
Titel : The use of rubber seed meal in poultry. II. The effect of rubber seed meal in layer diets.
Author : Yeong, S. W.; Syed Ali, A. B.; Yusof, N.
Location: Feed Resources + Anim. Nutr. Branch, MARDI, Serdang, Selangor, Malaysia
Journal : MARDI Research Bulletin, 1981, 9, (1), 92-96

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Ord.no. : 51:150439 78-06-N0254
Titel : (OIL PLANTS OF ZAIRE. III. BOTANICAL FAMILIES PRODUCING OILS OF RELATIVELY HIGH UNSATURATION.)
Author : KABELE NGIEFU, C.; PACQUOT, C.; VIEUX, A.
Location: UNIV. NAT. DU ZAIRE, FAC. DES SCI., KINSHASA II, ZAIRE
Journal : OLEAGINEUX, 1977, 32, (12), 535-537

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Ord.no. : 51:015894   70-04-N0140
Titel : (METHOD FOR SELECTIVE HYDROGENATION OF OILS CONTAINING LINOLEIC ACID AND HIGHER UNSATURATED FATTY ACIDS.)
Titel : VERFAHREN ZUM SELECTIVEN HYDRIEREN VON OELEN, DIE LINOLSAEURE NEBEN HOEHER UNGESAETTIGTEN FETTSAEUREN ENTHALTEN.
Author : DE JONGE, A.; ERKELENS, J.
Location: UNILEVER NV
Journal : WEST GERMAN PATENT APPLICATION, 1969, 1 467 539

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101165997	99135474	98002767
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101127409	99083585	97210302
101126950	99055242	97210301
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101126913	99050232	97195722
101093037	99040058	97183745
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101020752	99034384	97158661
101020751	99002131	97132962
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100053504	98139505	96159546

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96139936
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Factors influencing responses to ethephon

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Relationships between yield and clonal physiological characteristics of latex from Hevea brasiliensis

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Purification and characterization of hevain, a serine protease from Hevea brasiliensis

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A simplified boron diffusion treatment for rubber wood
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A model of the vacuolar system : latex lutoids of Hevea brasiliensis. (Report on 15 years of research done in the Ivory Coast and in France)
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The lysozyme of Hevea brasiliensis latex : isolation, purification, enzyme kinetics and a partial amino acid sequence
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95117337	93240893	92105735
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95092204	93221705	92105687
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95044971	93144510	
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Effect of storage on the properties of high-ammonia-preserved Hevea latex concentrate
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91194932	90156952	89158687
91187853	90153245	89147893
91176426	90123463	89124425
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91022113	90019267	88184466
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91006208	89198849	88116283
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70085190	68075363	67008803
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70029868	68050747	67008694
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262694 84-08-n0409
(Finding of ALPHA -, BETA - and GAMMA -dehydrotocopherol in wheat germ oil by HPLC and GC/MS - a contribution to tocopherol analysis.)
Zur Auffindung von ALPHA -, BETA - und GAMMA -Dehydrotocopherol in Weizenkeimoel mittels HPLC und GC/MS - ein Beitrag zur Analytik der Tocopherole.
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019988 70-08-L0523
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File 5:BIOSIS PREVIEWS 81-85/MAY BA7910;RRM2810
(c.BIOSIS 1985)See File55,255

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~?T 1/6/1

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N A T E C
Institut für naturwissenschaftlich-technische Dienste GmbH

Dr. G.C. Schmerse

Appendix 2 - Contacts during the field study in Sri Lanka

Rubber plantations:

Elpitiya State plantation, Alpitiya, Sri Lanka	Mr. G.N. de Almeida (Superintendent)
Igalkanda Estate, Elpitiya, Sri Lanka	Mr. P.S. de Silva (Superintendent)
Usk Valley State Plantation, Baduraliya, Latpandura, Sri Lanka	Mr. D.H. Ariyaratne (Superintendent)
Dalkeith State Plantation, Baduraliya, Latpandura, Sri Lanka	Mr. N. Ratwatta (Superintendent)
Yatadola State Plantation, Matugama, Sri Lanka	Mr. A.H.V. Perera (Superintendent)
Clyde State Plantation Tebuwana, Sri Lanka	Mr. A.H.N. Wlikala (Superintendent)
Sri Lanka State Plantation Crop. 111 Ceylon Planters, Provident Sciety	Mr. M.R.C. Peiris (Chairman)

Authorities:

Ministry of Rural Industrial Development Colombo	Dr. V. Ramanathan (Additional Scretary)
Department of Small Industries, Government of Sri Lanka, Colombo	Bandula S. de Silva (Director)
Department of Animal Production and Health Director Dr. S.B. Dhanapala Kandy	Dr. G.H.P. Ganegodu (Veterinary Research Institute)

Research Institutes:

Rubber Research Institute of Sri Lanka	Mr. S.W. Karunaratne Acting Director (Head of Rubber Chemistry & Technology Department)
Ceylon Institute of Scientific and Industrial Research (CISIR)	Dr. Mendes Dr. C. Wijesundera (Head of Section Oils & Fats)

M. Nadarajah (Rubber Consultant)
Formerly Head of Rubber Chemistry & Technology Department, RRISL

Industries:

Ceylon Oils & Fats Corp. Mr. D.S.Kalansuriya (Chairman)	oil mill, fats, soaps, chemicals
Adamjee Lumanjee & Sons Ltd. Mr. Adamjee Lukmanjee (Director)	oil mill
Sedawatte Mills Mrs. Amari Deraniyagala (Director)	oil mill
Lever Brothers Ltd. Mr. A. Mangold (Chairman) Dr. I. Ismail (acting Technical Director)	oils, soaps
British Ceylon Corporation Mr. Jerry Perera (Production Manager)	paints, soaps
Paints & Gen. Industries Ltd. Mr. M.D.S. Perera (Director)	paints, soaps
Lankem Ceylon Limited Mr. A.C. Gunasinghe (Managing Director)	paints, soaps, oils

Appendix 3 - Analytical methods

(Literature References see III, page 95, 96)

1. Acid Value [1]: Some 3 - 10 g oil is weighed to the nearest 10 mg into a 200 ml Erlenmeyer flask, dissolved in 50 ml ethanol : ether = 1 : 1 (v/v, titrated before against phenolphthalein with 0.1 N KOH) and titrated with 0.1 N KOH (phenolphthalein as indicator).

$$\text{Calculation: } AV[\text{mg KOH/g}] = \frac{56{,}1 \cdot \text{ml } 0{,}1 \text{ N KOH} \cdot 0{,}1}{\text{weight of oil [g]}}$$

2. Lipase screening test [2]: Principle: An aqueous suspension of the test material reacts for some hours with test kits containing esters and pH-indicators. The acids liberated cause a colour shift in the solutions which is compared with a colour scale.

With rubber seeds: 10 mg in 90 µl H_2O cleave in 4 h at 37°C:

5nmol 2-naphthyl-butyrate
5nmol 2-naphthyl-caprylate
5nmol 2-naphthyl-myristate

3. Fatty acid composition of oil: 200 mg oil is converted into fatty acid methylesters by mixing with 2 ml methanolic $NaOCH_3$(0,25 N). After 5 - 10 min the mixture is extracted with 1 ml iso-octane and 2 ml methanol. 1 - 2 µl of the iso-octane-phase is injected into a gaschromatograph.

Gaschromatographic conditions:

column:	Sil 88, 25 m, fused silica capillary	
temperatures:	injector:	$250^{\circ}C$
	column:	$160 - 220^{\circ}C$, $2^{\circ}/min$
detector:	FID, $300^{\circ}C$	

The integrated areas of the individual fatty acid methylesters are calculated as % of the total area of all peaks.

4. Gel permeation chromatography [3]: The oils are separated on polystyrene columns. Tetrahydrofurane is used as solvent. The eluted substances are detected by a differential refractometer.

Column set for oil analysis: 3 Ultrastyragel columns with 500, 200 and 100 Å pore diameter (Waters), 1 Microgel column, 100 Å pore diameter (Chrompak).

Column set for molecular weight determination of polyisoprene: 3 Ultrastyragel columns with 100, 500 and 10^4 Å pore diameters, 1 micro-Styragel column 10^3 Å, 1 Microgel column 10^6 Å.

Flow rate: 1 ml/min, temp. $38^{\circ}C$

Calibration is done with polystyrene of known molecular weights.

Preparative GPC: Column 300 x 25 mm, Prep Gel 10 µm, 50 Å pore diameter (Latek GmbH). 5 mg unsaponifiable from RSO, injected in 1,9 ml, were separated with THF (p.a., Merck) at a flow rate of 5 ml/min. When the high molecular material appeared, 6,5 ml were collected and evaporated. A blank run was performed without UM and 6,5 ml THF collected after the same time.

5. Assessment of polar fatty acids [4]: Fatty acids, obtained from the assessment of the UM of RSO, were esterified with methanol/BF_3. Ca. 1 g of these methylesters were dissolved in 10 ml petroleum ether : ethyl ether = 87 : 13 (v/v) and given on a column of 10 g SiO_2 (activated at 160°C) in this solvent. Elution with 60 ml of this solvent and evaporation gave the amount of unpolar fatty acid methylester. The difference to 100 % is calculated as amount of polar fatty acid methylester.

6. Unsaponifiable matter [5]: 5 g fat is hydrolised by heating with 50 ml 2 N ethanolic KOH (reflux, 1 h). The cooled solution is diluted with 50 ml H_2O and then extracted with 100 ml petroleum ether (3 times). The combined petroleum ether extracts are washed with 40 ml H_2O and 40 ml ethanol : H_2O = 1 : 1, dried with Na_2SO_4 and evaporated. The residue is dried at 103°C to constant weight.

7. HPLC-Method for assessment of tocopherols: 20 µl of the oil were injected into the following HPLC-system:

column:	Spherisorb Si 60 (Kontron), 250 x 4,5 mm
solvent:	Iso-octane with 0,5 % isopropanol
flow rate:	1 ml/min
detector:	Fluorescence, Ex. 295 nm, Em. 325 nm

synthetic tocopherols are used as test substances

8. Assessment of phospholipids [11]: The phospholipids are separated on a TLC-plate (SiO_2) using the solvent mixture chloroform : acetone : methanol : acetic acid : H_2O = 10 : 4 : 2 :

2 : 1. The amount of phospholipid per spot should be equivalent to 0,1 - 3 µg P. After staining with J_2-vapor the spots are scraped off and transferred into 10 ml centrifuge-tubes (precleaned with H_2SO_4 K_2CrO_4). 0,7 ml 70 % perchloric acid is added (saturated with ammonium molybdate) and then the tube is heated at 160°C for 1 h. After cooling 4,3 ml of a freshly prepared mixture of 2 parts 1 % ammonium molybdate solution and 3 parts of a 2 % ascorbic acid solution are added and heated at 50°C for 1 h. The centrifuged mixture then is measured in 1 cm cuvettes at 820 nm against water. The value is corrected for a blank, containing SiO_2 of a similar size as the spots, but free of phospholipids. Equilibration is done with KH_2PO_4 spotted on TLC-plates and treated in the same way as the phospholipids.

9. Colour stability of RSO: The oil was put in a Petri disk (1 cm layer) and stored at day light (Nov. - Dec. 1985, window to the North side) for several weeks. At certain intervals the iodine colour was assessed in a colorimeter (Hellige-Colorimeter).

10. H_2O-determination in RSM: About 1 g sample is dried at 120°C to constant weight. The loss is calculated as water.

11. Fat content in RSM: 5 g samples are extracted in a Soxhlet apparatus with petroleum ether or ether for 2 h. After evaporation of the solvent the residue is calculated as fat.

12. Soluble carbohydrates [13]: 5 g of the finely ground RSM is mixed in a mortar with 12 ml of a 1 % $HgCl_2$-solution. After dilution with water to 200 ml, the mixture is heated to 50°C for 20 min. At room temperature 5 ml phosphoric acid (20 ml

85 % H_3PO_4 diluted to 1 l) is added, which is afterwards neutralised by a saturated aqueous solution of $Ba(OH)_2$. Then the slightly alkaline mixture is filled up to 250 ml with water and filtered. 50 ml (~ 1 g RSM) are evaporated in a platinum vessel and dried at 103 - 105°C (weight a). After weighing, the residue is ashed at 800°C (weight b). In a separate sample of the water solution the N-content is assessed by the Kjeldal method.

Calculation: % soluble carbohydrates
= (weight a - weight b) • 100 - % N • 6,25

13. Hydrogen cyanide in RSM [14]: 10 g RSM, 40 ml H_2O and 5 ml 5 % trichloroacetic acid were given in a 250 ml 2-necked flask equipped with an air cooler connected with a 10 ml flask. This small flask contained 3 ml H_2O and 1 ml 5 % Na_2CO_3-solution and was cooled with ice. A constant stream of N_2 is blown through the apparatus while the contents of the 250 ml flask is boild for 15 - 20 min.

The content of the small flask then is transferred into a 10 ml measuring flask containing 2 ml of a 1 % picric acid solution in water and filled up to 10 ml. After 3 min in a water bath of 100°C and 1 h at room temperature the adsorbance was measured (1 cm, 500 nm) against a blank, containing 1 ml 5 % Na_2CO_3, 2 ml picric acid solution and 7 ml H_2O. A calibration curve was obtained with KCN.

14. Aflatoxins [15]: 50 g ground sample, mixed with 5 g Hyflosupercel is defatted by shaking with 150 ml petroleum ether. After filtering and washing with 2 x 50 ml petroleum ether the residue is air dried and extracted in a Soxhlet with chloroform : methanol = 90 : 10 (3 h). The solution is evaporated, the residue dissolved in 100 ml petroleum ether : methanol = 1:1-mixture and transferred to a separation fun-

nel. After addition of 5 - 10 ml H_2O the aqueous methanolic layer is washed with 50 ml petroleum ether and diluted with 70 ml water. This liquid is extracted 4 times with 25 ml chloroform, the combined chloroform solutions are evaporated and the residue dissolved in 2,00 ml chloroform.

2 - 20 µl of this solution is spotted on a 20 x 20 cm TLC-plate (Macherey u. Nagel, Sil-G/25-HR) and developed in 2 directions with

1) Ether : methanol : H_2O = 94 : 4,5 : 1,5 (saturation)

2) Chloroform : aceton : methanol = 90 : 10 : 2 (without saturation)

The dried plate is quantitatively assessed with a TLC-scanner (e. g. Spectrodensitometer Model SD 3000, Schoeffel Instr. Corp.), fluorescence mode (Ex 365 nm, Em 435 nm). Standard curves are obtained with pure aflatoxins.