UNIVERSITY OF JAFFNA
SRI LANKA

Prof. Sivapathasuntharam Mageswaran

Memorial Lecture
2002

By
Prof.S.Mohanadas

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Introduction

Late Professor Sivapathasutharam Mageswaran is one of the pioneers of the University of Jaffna. He was one of the most dedicated and dynamic pioneers who played a major role in guiding the development of this University.

Prof.Mageswaran was one of the finest synthetic organic Chemists SriLanka produced. His students and well- wishers have graciously established a fund for conducting a memorial Lecture annually to honour Prof.Mageswaran and to remember his yeoman services.

I am happy to see Prof.Mohanadas, Rector of Vavuniya Campus and an Associate Professor of Agricultural Chemistry of the University of Jaffna delivering this lecture. He has done research extensively in coconut and palmyrah.

I wish Prof. S.Mohanadas every success and for the continuation of activities of this nature.

Prof.P.Balasundarampillai,
Vice-Chancellor,
University of Jaffna,
19th July,2002.

THE PALMYRAH PALM AND THE COMPOSITION OF PALMYRAH FRUIT PULP

1. Introduction

The Palmyrah palms of today are a result of natural selection process over thousands of years and these are one of the natural resources of Sri Lanka.

The palms grow to about 40 – 60 feet in height and the trunk is very coarse rugged and not smooth.

The genus ***Borassus*** according to Beccari (1913), is made up of seven species, although present day taxonomists recognize no more than four (Uhl and Dransfield, 1987). Whatever the true number of species the genus ***Borassus*** is one of the most widely distributed of the palmae, with a range extending in a broad belt from Western Africa to Eastern Indonesia, it is a genus of tropical wet or dry climates.

There are three most important economic species such are ***Borassus aenthiopum*** Mart, occuring in Africa, ***Borassus flabellifer L.,*** found in coastal areas of India, Sri Lanka and mainland of South East Asia and ***Borassus Sundaicus*** Becc., restricted to Indonesia. In each of these geographic areas ***Borassus*** palms are of significant value to local populations.

2. Distribution of Palmyrah

a) Worldwide

There are about 140million palmyrah trees (refer Table-1) distributed worldwide. The palmyrah palm ***(Borassus flabellifer L)*** is widespread in the arid tropics of South America, East America, India, Sri Lanka and South East Asia.

In other words, we can say it grows extensively in the drier regions of SriLanka, India, Burma, Thailand, Vietnam, Malaysia, Nigeria, East Africa, Madagascar and Indonesia.

These are one of the natural resources of Sri Lanka and India, which are potentially useful, but underutilized palms of the world.

Table I Worldwide distribution of Palmyrah

Countries	Palmyrah (In Million)
World	140 (about)
India	60
West Africa	50
Sri Lanka	11.1
Indonesia	10
Madagascar	10
Burma	2.3
Kampuchia	2
Thailand	2

b) Sri Lanka

In Sri Lanka, palmyrah, the palm tree grows abundantly in the Northern and Eastern provinces and in the districts of Anuradhapura, Polannaruwa, Kurunegala, Puttalam and Hambantota to a lesser degree.

It is estimated that at present, there are about 11.1 million trees (refer Table II) in Sri Lanka, with about 3.5 million each in the Jaffna peninsula and Kilinochchi, 3 million in Mannar. The balance is found in the Districts of Mullaitivu, Vavuniya, Batticaloa, Trincomalee, Puttalam and Hambantota.

Table II Distribution of Palmyrah in Sri Lanka

Parts of Sri Lanka	Palmyrah (In million)
North – East Sri Lanka	11.0
Jaffna	3.5
Kilinochchi	3.5
Mannar	3.0
Mullaitivu	0.5
Trincomalee	0.21
Batticaloa	0.20
Puttalam	0.12
Ampara	0.04
Anuradhapura	0.04
Vavuniya	0.008

3. Products of Palmyrah

Mythologically palmyrah is a "wishing Tree" titled "KATPAHATHARU" which means it gives several products of value for the human use. Utility, fancy, novelty, food and nutrition and pharma – medio values take it to lofty heights, which the palmyrah itself is physically blessed and physiologically endowed to sustain human existence and to conserve social forestry.

The Palmyrah Palm provides through its inflorescence sap, fruit pulp and tuber a plethora of rich nutritive foods, beverages and other edibles and through its leaves, leaf stalk and other forms of non – edible provide immense potential for industrial development.

Inflorescence sap products

Sap is the most economically important product. There are so many products by the technique of "tapping", fermented sap (toddy) is a popular and cheap beverage of the palmyrah grown

areas. By distillation of fermented toddy arrack can be obtained. From sweet toddy (unfermented sap), Treacle, jaggery, sugar and other confectionary items are prepared.

Fruit Products

From fruit pulp, cordial, jam, palmyrah crush, pinnattu (sundried fruit pulp), sauce items are prepared. From tuber, boiled and unboiled tuber flour are obtained. In addition to that charcoal, toys items and fancy products are also obtained from palmyrah fruit shell. Nungu, Softfibre are the other products from palmyrah fruit.

Leaf products

Palmyrah palm has gained its popularity in providing wide range of raw material for handicraft items, the chief of which are the tender palmyrah leaves. Mature leaves are used as cattle fodder. In addition leaf petiole is used as a material for fencing and fire wood.

Fibre Products

Palmyrah fibre is one of the world's stiffest and is used in the manufacture of heavy – duty brushes for use in the factories. It is also used in other fibre based utility items for domestic purposes.

Palmyrah cane is extracted from the leaf stalk, which yields cane threads of high tensile strength. These strands could be woven into many fancy and utility items (baskets, chair backs, etc)

Timber products.

Palmyrah timber is strong, sturdy and has unique properties and they are used as rafters, beams, door frames in the construction of buildings.

Further furnitures, fence posts and fancy goods are obtained from palmyrah timber.

3. **Palmyrah fruit pulp**

The fruit of palmyrah is an important component of palmyrah palm. It varies in size, colour and has been classified in more than ten morphological types. More recently four distinct morphological fruit types have been described. A thick leathery pericarp encloses 1 – 3 (frequently 3) seeds embedded in fibre enmeshed with a yellow or more rarely orange fruit pulp. This thick viscous liquid is called palmyrah fruit pulp (PFP).

Palmyrah fruit pulp can be extracted with water (1:1 or 1:2 V/V) either manually or by a fruit extractor. The cost and time of the manual extraction of the pulp from fruit reduced from SLR 1.52 to 0.54 and from 11.9 min. to 0.48 min per fruit respectively by the introduction of the mechanical extractor in 1987. This paved the way for large scale economical use of the pulp industrially.

However, the bulk of palmyrah fruit pulp (PFP) goes to waste on account of

i) Inadequate basic knowledge of processing and
ii) The presence of a bitter principle and bioactive factors now collectively known as flablelliferins. These are steroidal saponins.

On examining the food composition of fruit pulp many utilizable components emerge viz. sugar, dietary fibre, pectin, carotenoids and a newly emerged factor for consideration is a family of steroidal saponins called flabelliferins.

In utilizing palmyrah fruit pulp two main routes of use emerge.

a) Utilization of the material as a whole or by fermentation.
b) Separation of commercially valuable components

Here flabelliferins play a critical role. The obvious route for utilization is by use of the liquid juice or dried material as a sweetmeat as is done traditionally. This is limited by the presence of a bitter flabelliferin (F-II) steroidal saponin tetraglycoside. However this bitterness can be reduced by enzymatic hydrolysis with a cheap enzyme. This debittering can lead to jams, cordials etc. Further details are in section 5.6.

5. Composition of palmyrah fruit pulp (PFP)

Palmyrah fruit pulp consists of 75 – 80% of moisture, under the rest carbohydrates are the major components, consist about 18 – 20%. The following table III shows the composition of PFP.

Table III Proximal composition of PFP

Constituent (100^{-1})	Results I	Results II
Moisture (g)	77.2	79.1
Energy (k cals)	87	not reported
Protein (g)	0.7	2.8
Fat (g)	0.2	1.0
Total carbohydrate (g)	20.7	18.5
Sugars (g)	not reported	14 – 16
Crude fibre (g)	not reported	1.5
Ash (g)	not reported	4.3

Result I reported by Perera and co - workers[1]
Result II reported by Jeyaratnam [2]

5.1 Carbohydrates

The main digestible carbohydrates are simple sugars. Palmyrah fruit pulp consist 16-20% sugars, of which sucrose, glucose and fructose dominate.

Analysis showed the following configurations

sucrose[2]	– 6.6 g/100g
glucose [2]	– 3.5g/100g
fructose[2]	– 3.4g/100g
oligosaccharides[3] (unidentified)	–1.5 g/100g
pectin[2,4]	– 4.4, 6.7g/100g
Rhamnose [3]	– Trace amount

It also consist a branched glucan[5] but its anomeric configuration was not determined.

The corbohydrate moiety of the saponin was investigated by component analysis using alditol acetates and linkage analysis using methylation and conversion to partially methylated alditol acetates, all made with GLC and GLC – MS. This confirmed the presence of a branched structure with two rhamnoses linked 1,2 and 1,4 to glucose which is attached to the sapogenin.[6] (Ariyasena, D.D, &[6] Jansson P.)

Study of chemical shifts and coupling constants of the sugar residues showed that glucose had a β anomeric configuration while both rhamnoses were α.

PFP is a potentially fermentation base because of its constituent of sugar. However, fermentation is fast enough only in same types of pulps not containing significant levels of flabelliferin B, which inhibits yeast and bacteria. Usage of large innoculum of

yeast, while selecting the strain could result in about 90% of the pulps becoming commercially fermentable to give a wine (bitter) or a spirit.

Fermentation of pulp to alcohol is a natural process that comes to mind, as is the isolation of pectin for use in foods.

5.2 Carotenoids

Carotenoids were first reported as 3.2 mg.100g[2]. However the range of 1 – 10mg/100g[4] and most recently 2 – 253mg/100g[7,8] was reported.

Carotenoids were separated by MPLC and analyzed by UV – Visible absorption spectra and identified using spectroscopic date in the literature and standards.

The carotenoids were assumed to be β carotene[2], however spectra using a scanning spectrophotometer on a hexane extract showed that PFP most commonly had a λ max of 422 – 428nm[7] but occasionally a λ max of 434nm and 437nm[7,8] showing that carotenoids are mixtures and probably vary in composition.

Examination of a pooled PFP of ë max 427nm (the common type) separated by medium pressure liquid chromatography showed that the presence of a mixture of four main carotenoids.[9,10] They were α 0 - carotene and β – zetacarotene (structurally pro – Vitamin A) [9,10]. These carotenoids are labile and easily subject to oxygenation[10].

5.3 Mineral constituents

The macro metallic ions reported[2] in palmyrah are as follows (g/kg)

K – 5.7(g/kg)
Na – 0.2 (g/kg)
Mg – 0.6 (g/kg)
Ca – 0.7 (g/kg)

Microelement reported[2] are as follows (mg/kg)

Fe – 22 (mg/kg)
Zn – 17 (mg/kg)
Mn – 95 (mg/kg)
Cr – 1.6 (mg/kg)
Cu – 4.3 (mg/kg)
Co – 0.6(mg/kg)
Ni – 0.8 (mg/kg)
B – 2.6 (mg/kg)
Pb – Trace

5.4 Aminoacids

PFP contains 0.42g/100g free aminoacids, of which lysine, aspartate, glutamate and phenylalanine dominate[2].

5.5 Fatty acids and sterols

Of the fattyacids, oleate, palmitate and linolinate are most common. Among the lipids was reported[2] the free sterols (0.3%) stigmast –5en - 3 β ol (24Et) and lanosterol.

In another study sitosterol, sito 5en - 3 βol (24&Et) was identified as the only free sterol,[11] while other workers claimed that there are no significant quantities of free sterol[8]. Further saponin content has been reported in the range of 0.15 – 0.4mg/100g[2,8].

5.6 Flabelliferins

Palmyrah fruit pulp (PFP) is known to contain a plethora of flabelliferins. These compounds were steroidal saponins and the term flabelliferin was coined from the specific name flabellifer, flabelliferin is a very interesting compound, because it play a key role in the determination of the future modes of utilization of PFP[7].

Studies showed that there was a plethora of at least 14 flabelliferins[7]. Among these were the bitter flabelliferin (F – 11, tetraglycoside), the antimicrobial flabelliferin (F_B – triglycoside), two non –bioactive flabelliferins (F_C, triglycoside and (F_D, diglycoside), a monoglucoside, a

monorhamnoside and tetraglusoside (F – 1)

Separation techniques

Steroidal saponins from palmyrah were first reported in Jaffna by Jayaratnam[2], who identified a monoglucoside and a monorhamnoside of spirost – 5en – 3 β ol. Isolation was achieved using dried palmyrah fruit pulp (PFP), first extracted with petroleum ether (40 – 60°C) and chloroform in a soxhlet extractor followed by methanol extraction and acetone extraction in which acetone fractions were concentrated and cooled in an ice water bath to obtain white solid.

In another study[12,13] (using PFP from Hambantota), 4 flabelliferins were isolated by incorporating a flash chromatography step at the end of the isolation procedure.

The flabelliferins isolated were F – 11 (bitter, tetraglycoside) F_B and F_C (Triglycosides) and F_D(diglycoside) all with rhamnose (rha) terminii.

Other following various techniques were found to be used to achieve separation.

- Selective solvent extraction[11]
  (A good technique for separating small carbohydrate moiety flbelliferins from large ones)
- Solvent chromatotron[11]
  (This is a good separation technique for (F – 11)
- MPLC[11,8,12,4,6] – medium pressure liquid chromatography (most economic, time saving and efficient method which is applicable to nearly all different types of flablliferin profiles)

It is a suitable method to separate five flabelliferins including.

F_B (Triglycoside)
F_D (diglycoside)
F_E (diglucoside)
F_F (monoglucoside)
F_N (MW 884, 1rho, 2glc)

Structural studies on flabelliferins

Aglycone

Using the trymethyl silylation technique after acid hydrolysis and GC/EI/MS, H – NMR, C – NMR and 2D – NMR, the structure of the aglycone was unambiguously found to be β – sitosterol[8,6,14]

The glucose, which was attached to the sapogenin, had a β configuration[6].

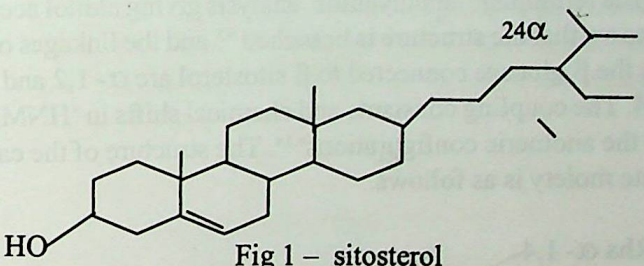

Fig 1 – sitosterol

Flabelliferin I (F – I)

It was isolated only once in PFP and showed to be a tetraglucoside.[15] molecular weight (MW) 1062. Nature of the sugar linkages is not known.

Flabelliferin II (F – II)

This is the bitte flabelliferin, MW 1030 with a rhamnose (rha) terminus[15]. It was reported that it had 2glucose (glu) and 2 rha [15].

When F – II was hydrolyzed by a heat stable α amylase (specific for α - 1 – 4 and to a lesser extent α - 1 – 6)[12,15] the structure of carbohydrate moiety can be deduced as follows.

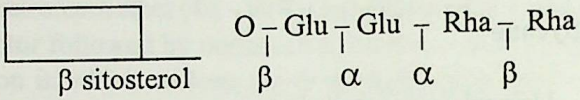

Fig 2 – Probable sequence of the bitter flabelliferin

Methylation analysis confirmed that the carbohydrate chain is not branched [12,15].

Flabelliferin B (F_B)

This is the antimicrobial flabelliferin,[16] MW 868[13] with 2 rha and 1glu (rha terminus)[13]. methylation analysis giving alditol acetates has shown that the structure is branched [5,8] and the linkages of the rha to the β glucose connected to β sitosterol are α- 1,2 and α- 1,4. The coupling constants and chemical shifts in 'HNMR gave the anomeric configurations[8,14]. The structure of the carbohydrate moiety is as follows.

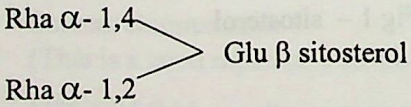

Fig 3. Flabelliferin B

The branched structure is the only one of this type so far elucidated of all the flabelliferins.

Flabelliferin C (F_C)

Its molecular weight is identical to F_B (868) with a rha terminus,[13] but it is not antimicrobial.[16]. It is not susceptible to naringinase and α-amylase hydrolysis.[17] It therefore has no β bonds and no α - 1,4 or α- 1,6 glucose bonds.

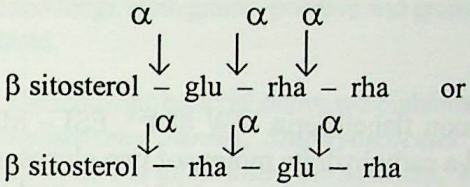

Fig 4: Alternative structures of F_C

Flabelliferin D (F_D)

This was shown to be a diglycoside with 1 rha(terminal) and 1 glu with a MW of 722[13]. The structure of F_D (diglycoside) showed[17] that the sugar chain was linked to β – sitosterol by the anomeric carbon of glucose. Glucose is linked to a rhamnose by α - 1,4 linkages. The anomeric carbon of glucose (β – configuration) was linked to the β– sitosterol of F_F (monoglucoside). Though the linkage position of F_f and F_N could not be determined due to insufficient data.

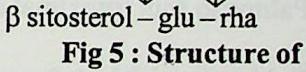

Fig 5 : Structure of F_D

Flabelliferin E (F_E)

This was shown to be a diglucoside of MW 738 of the same aglycone[6,8]. It was found,[8] that F_E having a carbohydrate moiety of two glucoses, both glucoses had a β configuration. The glucoses were linke d by either a β –1,2 or β – 1,4 linkage.

Flabelliferin F (F_F)

It showed a MW of 576 corresponding to a monoglucoside[6,8]. Coupling constants and chemical shifts confirmed a β glucosidic configuration[6,8]. This is probably the naringinase hydrolytic of the contaminant of F_D.[17]

Flabelliferin N (F_N)

This is an uncommon flabelliferin MW 884[6,8]. ESI – MS data showed[6] that it had a carbohydrate moiety of two glucoses and a α rhamnose with a glucose terminus, which was attached to β sitosterol by the second glucose.

β sitosterol – glu – rha – glu

Fig 6: Sequence of F_N

Debittering

PFP has a bitterness due to its constituent of flabelliferin II. Debittering is an important step for utilizing PFP in the form of jams and cordials, etc.

Debittering is done by two methods.

a) Traditional method: Palmyrah fruit (proximal end upward) is heated on hot coals.
b) Scientific method: Debittering is done by using enzymes [naringinase (β glycosidase and β rhamnosidase activity) or heat stable α - amylase[8,12]]

Effect of flabelliferin on microbes and mice

PFP has an antimicrobial effect. Flabelliferin B is the responsible flabelliferin for the antimicrobial effect. This inhibit a number of bacteria and fungi. Both gram – positive and gram – negative bacteria are inhibited.

The following bacterial strains were inhibited[12,16] by flabelliferin B. ***Staphylococcus aureus, Staphylococcus*** **epidermidis, Escherichia coli,** ***Pseudomonas aeruginosa,*** **Proteus rettigeri,** ***Aciinetobacter calcoaceticus*** var Lowffic.

Further, the growth of ***Saccharomyces cerevisiae*** strain S_{11} F_3 was inhibited by F_B at 60 $\mu g\, ml^{-1}$ and F – II at 250$\mu g\, ml^{-1}$ [12,16]. A mixed culture of baker's yeast was also inhibited.[18]

In addition to that flabelliferin causes growth gain and growth retardation effect. For example feeding trials using ICR mice showed that feed containing 10% PFP caused a statistically significant weight loss, compared to control.

The results showed at 10% PFP it was only the bitter PFP that reduced weight gain (P = 0.0 14) compared to control. The non -bitter sample gave increased weightgain compared to control (P=0.8 $x10^{-4}$) And also it was showed that if F – II was present, PFP was probably a nutritious feed for mice.[8]

Fermentation

It has been reported that there was a correlation between rate of fermantation and flabelliferin. That is there is a negative relationships between rate of fermentation and F_B content. Both F_B and F – II produce lag in fermentation at concentrations of 1mg/ml. However fermentation efficiency in all cases, bar one was good, 85 – 95%
It was also reported that exposure to naringinase increased the rate of fermentation,[18] because naringinase hydrolyzed the F_B.

Table IV – Utilization of Palmyrah palm products in some countries

Countries	Handicraft Item Leaf	Timber	Timber for building construction	Fibre	Sweet toddy Items	Fermented Toddy	Nungu	Pannattu	Palmyrah fruit items	Boiled and unboiled tuber
West Africa Eg: Mali Ghana	-	-	+	-	-	-	-	-	-	-
India	+	-	+	+	Sweet toddy Sugar, Sugar candy,	_	freshly eaten	+	-	+
Indonesia	+	-	-	-	Sweet toddy, Sugar, treacle	_	_	-	_	_
Thailand	+	+	-	-		-	packed in tins	-	pittu	-
Sri Lanka	+	+	+	+	Sweet toddy, sugar, Treacle, Sugar candy	Toddy Arrack	freshly eaten	+	confectionary items Panam	+

- Not poduced
+ Produced

Utilization of PFP

The traditional use of palmyrah is given in Table IV. It is seen that Sri Lankan, especially the people of north – east tamils make use of the palmyrah for food and shelter. India comes second. Thailand produces a canned product of nungu, the tender endosperm.

Recently in Sri Lanka new products are being produced based from palmyrah fruit pulp.

PFP consist of diversity of flabelliferin profiles. Flabelliferin II is a bitter flavelliferin and detract from food use. flabelliferin B is an antimicrobial flabelliferin and retard the fermentation.

Utilization could have been benefited, if an easily distinguishable morphological character was correlated to flabelliferin profile. However none of the characteristics studied viz., size, colour of fruit, colour of pulp were of use for such correlations. Although F-II could be detected by taste and F_B by fermentation neither property can find use in a commercial or field scenario.

PEP consist of useful constituents. Pectin is an important component for many utilizable products. It can be isolated from PFP by the calcium salt on alcohol precipitation of acid extract. Pectin had good gel strength because it was reported to have an acetyl value of 3.5 and methyl value of 5.4 This is of value as commercial food pectin.

On the other hand PFP can be depectinized by pectinase to yield on filteration a clear juice. This has been fermented to give a bitter wine.

The presence of flabelliferins (steroidal saponins), which are foam stabilizers, provide an avenue for use of the material as a detergent, value added shampoos and skin lotions.

The antimicrobial flabelliferins, which are also foam producing and stabilizing could be a good component for toothpaste. Here the fermented fruit pulp for toothpaste is a good method. If this tooth paste contain enough pectin and flabelliferins it will help by its physical, chemical and anti – microbial properties.

The enzymatic hydrolysis of these antimetabolic substances has opened the doorway for the use of pulp as a carbon source in industries. Alcoholic, citric acid and lactic acid fermentation studies tend support that the antimicrobial substances can be either destroyed or their effects can be overcome by supplementing the fermentation medium with nutrients.

During distillation of ethanol, carotenoids are oxidized; this facilitate the PFP is to be used as a food colouring agent.

In PFP, where the presence of 14 – 16% sugar, when subjected to aerobic fermentation makes the yield of alcohol sufficiently high to be viable process. Furthermore, in the scenario of palmyrah sap and PFP yielding sugar substrates during different times of the year that are complementary, it will be possible to run a distillery all year round.

Thus utilization of PFP is an important phenominum in the consideration of a country's economic point of view.

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