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A REVIEW OF THE CHEMISTRY AND BIOCHEMISTRY OF SEED SHOOT FLOUR AND FRUIT PULP OF THE PALMYRAH PALM (*BORASSUS FLABELLIFER* L)

E.R.JANSZ*, NADI T. WICKREMASEKARA, AND K.A.V. SUMUDUNI
Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura.

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Abstract: This review with 74 references, focuses on the chemistry and biochemistry of palmyrah seed shoot and palmyrah fruit pulp. While palmyrah fruit pulp is highly underutilized due to its bitterness, there is so far no evidence of its toxicity. On the other hand, palmyrah flour, which has been consumed for centuries, has many reported toxic effects, viz., neurotoxicity, hepatotoxicity, immunosuppression, clastogenic and mutagenic effects. This review discusses the above in relation to studies directed at utilization of palmyrah fruit pulp based on its sugar, pectin and carotenoid content and the effect a group of steroidal saponins termed flabelliferins will have on utilization. The structural studies and bioactivities of these flabelliferins are discussed. Also discussed is the reported toxicity of odiyal flour.

Key words: Palmyrah, shoot flour, odiyal, toxicity, fruit pulp, flabelliferins, bioactivity, utilization, fermentation.

INTRODUCTION

The palmyrah palm (*Borassus flabellifer* L) is widespread in the arid tropics of South America, East Africa, India, Sri Lanka and South-East Asia.[1] It is a feature of the landscape of North-East Sri Lanka where it is called the tree of life. It is estimated that at present there are about 11-12 million trees in Sri Lanka,[2,3] with about 3.5 million each in the Jaffna peninsula and the Wanni districts of Killinochchi and Mullaitivu.[3] In Jaffna, palmyrah represents a major socio-economic factor even at the present time as it is estimated that about 100,000 families are directly or indirectly employed in palmyrah-based industries.[3]

From the point of industry, the sap products are most important, as the influorescence sap (sweet toddy) gives many products [3,4,5,6,7,8] including fresh toddy, fermented sap (toddy), arrack (distilled spirit), treacle, jaggery and palm sugar. In addition, many microbiological studies have been conducted on fermentation of sap.[9, 10] However, these aspects have not been considered to be within the scope of this review, which is mainly chemical and biochemical. Further, any new finding of sap product research and development has not been documented in refereed journals. In addition, techniques have largely been adapted from the coconut industry where methods are well known.

* Corresponding author

Also not considered within the scope of this review are discussions on handicrafts and other products from palmyrah leaf, stalk, etc[8,10] which are traditional techniques, and which though economically important have little or no input from modern science and technology. Also not discussed here are agricultural and agro-economic aspects.

The total production of palmyrah fruit pulp is a fraction of its total potential of 10-15 kilo tonnes annually.[12,13] The production of palmyrah shoot and its products is about 600. tonnes annually.[14]

Palmyrah fruit pulp

Overview: The fruit of palmyrah varies in size, colour and has been classified into more than 10 morphological types.[15] More recently, 4 distinct morphological fruit types have been described.[16,17] A thick leathery pericarp encloses 1 to 3 (frequently 3) seeds embedded in fibre enmeshed with a yellow or more rarely orange fruit pulp.[16,17] A thick viscous liquid called palmyrah fruit pulp (PFP)[8] can be extracted with water (1:1 or 1:2 v/v) either manually or by a fruit pulp extractor.[13]

Composition of PFP

Proximate Composition: PFP consists of 75-80% moisture and the main components comprise carbohydrates (Table 1)

Table 1: Proximal composition of PFP[8,10]

Constituent (100^{-1})	Study1	Study 2
Moisture (g)	77.2	79.1
Energy (k cals)	87	-
Protein (g)	0.7	2.8
Fat (g)	0.2	1.0
Total Carbohydrate (g)	20.7	18.5
Sugars	-	14-16
Crude fibre	-	1.5
Ash	-	4.3

-,Not reported

Amino acids, fatty acids & sterols: PFP contains 0.42g.$100g^{-1}$ free amino acids,[10] of which lysine, aspartate, glutamate and phenylalanine dominate.[10] Of the fatty acids

of lipid, oleate, palmitate and linolinate are most common.[10] Among the lipids was reported[10] the free sterols (0.3%) stigmast-5en-3 β ol (24α Et) and lanosterol. In a subsequent study sitosterol, sito-5en-3βol (24α Et) was identified as the only free sterol,[19] while other workers claimed that there were no significant quantities of free sterol.[20] The latter conclusion was arrived at by a study of more than 10 PFPs from different locations.[20]

There is no controversy on saponin content, which has been reported to be in the range of 0.15 to 0.4 mg.100g^{-1}.[10,20,21]

Carbohydrates: The main digestible carbohydrates are simple sugars[10] of which sucrose, glucose and fructose (6.6,3.5 and 3.4 g-100g^{-1} respectively) dominate. There are traces of rhamnose[8] in PFP and 1.5g.100g^{-1} oligosaccharides[8] (unidentified). Pectin is present and reported as 4.4g.100g^{-1} [10] and 6.7g.100g^{-1}.[22] A branched glucan has also been reported[23] but its anomeric configuration was not determined.

Carotenoids: Carotenoids were first reported as 3.2mg.100g^{-1} [10] but clearly varies considerably as does the colour of PFP. A range of 1-10mg.100g^{-1} [22] and most recently 2-253mg.100g^{-1} [16,20] was reported. The carotenoids were earlier assumed to be β carotene,[10] however, spectra using a scanning spectrophotometer on a hexane extract showed that PFP most commonly had a λ max of 422-428nm[16] but occasionally a λ max of 434nm and 437nm[16,20] showing that carotenoids are mixtures and probably vary in composition. Examination of a pooled PFP of λ max 427nm (the common type) separated by medium pressure liquid chromatography showed the presence of a mixture of 4 main carotenoids.[24,25] They were α-carotene and β-zeacarotene (structurally pro-vitamin A)[24,25] and lycopene and zeta-carotene (non pro vitamin A).[24,25] These carotenoids are labile and easily subject to oxygenation.[25]

Table 2: Production

Food	Amount (kg)
Pamnan paanan (a drink)	3696
Palmyrah crush	254
Cordial	419
Jam	30
Palmyrah pulp	917
Pinnatu	1034

Minor constituents: Vitamin C content has been reported as 28 mg. kg^{-1}.[10] The macro metallic ions reported in palmyrah are as follows (in g. kg^{-1}) K, 5.7;Na, 0.2;Mg, 0.6;Ca, 0.7. Microelements reported in the same study[10](in mg. kg^{-1}) were Fe, 22;Zn, 17;Mn, 95;Cr, 1.6;Cu, 4.3;Co, 0.6;Ni, 0.8;B, 2.6;Pb, trace.

Food Preparations: The fruit pulp provides a number of traditional preparations.[26] These include a type of chewing gum (pinnatu), jams, cordials, sauces, palumellows (cf marshmallow), toffees, and can be a component in ice cream, biscuits, fruit bars and other confectionary.[8,26] The major production data are shown in Table 2.

With a potential for 10-15 k tonnes per annum, it is clear that only a fraction of the PFP is utilized. This is mainly on account of a marketing problem due to the bitterness of PFP.[27,28] While those with an acquired taste prefer bitterness, this attribute is a deterrent to wide utilization to those not familiar with PFP (PDB 1992, Personal Communication) The nature of the bitter compound was not known until less than a decade ago. Until this time suggestions had been that the causative molecule was an alkaloid or fatty acid. (Report GTZ, 1989, unpublished)

The flabelliferins: This family of compounds first attracted attention when one of them was identified as the cause for bitterness.[27] Further studies showed that there was a range of at least 14 flabelliferins[16], one of which was anti-microbial.[21,29] These compounds were steroidal saponins[21,30] and the term flabelliferin was coined from the specific name *flabellifer.*[27] Several lines of study directed at isolating and identifying flabelliferins as well as studies on bioactivity have since been reported. In addition, the flabelliferins appear to play a key role in the determination of the future modes of utilization of PFP.[16,17,20,21]

Separation techniques: Steroidal saponins of PFP were first reported by an extraction of a methanolic extract of dried PFP from Jaffna which had been successively extracted using petroleum ether (40-60°C) and chloroform in a Soxhlet extractor. Only a monoglucoside and monorhamnoside were reported.[10] This was probably due to use of inadequate water in the extracting medium, which is needed to isolate the flabelliferins of longer carbohydrate moiety from PFP.[27] Curiously in that study [10] no reference was made to the bitterness of PFP.[10] The two monoglycosides were separated by pre-prepared TLC of an acetone extract.[10] Eventually, the bitter flabelliferin (subsequently called F-II) was isolated using a methanolic extract of fresh fruit pulp from Kalpitiya. Removal of fat solubles was by petroleum ether and sugars by dry cellulose chromatography and acetone extraction. The flabelliferins crystallized as needles from an acetone extract.[27] Two flabelliferins F-I (a tetraglucoside M.W. 1062) and F-II a (tetraglycoside,M.W. 1030) were isolated.[27] F-II was an intensely bitter flabelliferin.[27] This technique was not good enough to separate more complex flabelliferin mixtures and as would be seen later, flabelliferins from different fruits gave vastly different profiles.[16,17,20] In another study[21,30] 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-II (bitter, tetraglycoside) F_B & F_C (triglycosides) and F_D (diglycoside) all with rhamnose (rha) terminii.[21,30] As flabelliferin profiles increased to show up to 14 [16,17] in certain PFPs, separating them became more difficult. Various techniques were used to achieve separation. These included, selective solvent extraction (which is a good technique for separating small carbohydrate moiety flabelliferins from large ones[19]), solvent gradient chromatography,[19,20,31] the chromatotron,[19,20] (which is a good separation technique for F-II) and MPLC which is the most economic, time saving and efficient method which is applicable to nearly all different types of flabelliferin profiles.[19,20,21,22,33,34] By this technique using a gradient of

Hexane ⟶ CH_2Cl_2 ⟶ ethyl acetate ⟶ methanol

it has been possible to separate 5 flabelliferins including F_B(triglycoside) F_D(diglycoside) F_E (diglucoside) F_F (monoglucoside) and F_N (MW 884,1 rha, 2 glc). With 3 earlier isolates, this brought the total number of flabelliferins isolated to 8, at least another 6 remain to be isolated.[16,17]

A new technique involving direct separation of carotenoids, sugars and flabelliferins without the use of working up techniques has been reported.[35]

Structural studies on flabelliferins

(a) *The aglycone*: Isolated flabelliferins have been hydrolysed by enzymes[21] or trifluoroacetic acid[10,20,32,34] to produce an aglycone (sapogenin). Direct MS analysis [10,32] and GC/MS analysis[32,34] of a silylated derivitive has shown its molecular weight to be 414. After this point there has been controversy, the aglycone was first identified as spirost-5en-3β ol, then as stigmast -5en -3 β ol (24 α Et)[20] then as sitosterol[32] and finally unambiguously as β– sitosterol.[20,33,34] (See Fig 1)

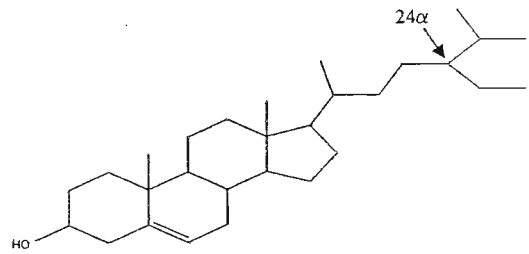

Figure 1: β– Sitosterol

(b) *Flabelliferin I (F-I):* This was isolated only once from PFP and showed to be a tetraglucoside,[27] MW 1062. Although the same component was isolated from

palmyrah flour[36] no further details on the nature of sugar linkages except for 2-β anomeric configurations of glucose (glc) have emerged.

(c) *Flabelliferin II (F-II):* This is the bitter flabelliferin, MW 1030 with a rhamnose (rha) terminus.[27] It has been shown to have 2 glc and two rha.[27] Hydrolysis of F-II with naringinase yields rha[31] a flabelliferin F_X (MW 868) shown to be identical with another flabelliferin F_C,[21] and an aglycone.[21,27] F-II was hydrolyzed by a heat stable α amylase (specific for α-1-4 and to a lesser extent α-1-6) [21,27], from which a tentative structure of the carbohydrate moiety can be deduced to be as follows. (Figure 2)

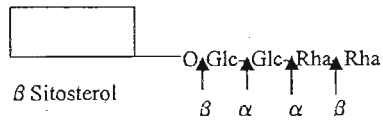

Figure 2: Probable sequence of the bitter flabelliferin

Methylation analysis is needed to be carried out to confirm linkages. The absence of a M-162 peak [21,27] confirmed that the carbohydrate chain is not branched.

A further controversy has been reported. Namely that this may not be the only bitter compound in PFP as only 40% of the bitter PFP is hydrolyzed by α-amylase.[37,38] This seems to show that there are two bitter compounds: (1) very bitter hydrolyzed by both naringinase [21,37,38] and α-amylase and a less bitter flabelliferin not susceptible to hydrolysis by α-amylase (no Glc α Glc bond). Although both have similar R_f, clearly the sugar sequence (in a linear chain) is different. A similar bitter compound has been detected in palmyrah flour.[36]

(d) *Flabelliferin B (F_B)*: This is the antimicrobial flabellifrin,[29] MW 868[30] with 2 rha and 1 glc (rha terminus).[30] Methylation analysis giving alditol acetates has shown that the structure is branched[20,23] 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 ^1H nmr gave the anomeric configurations.[20,33] The structure of the carbohydrate moiety is therefore as follows (Figure 3)

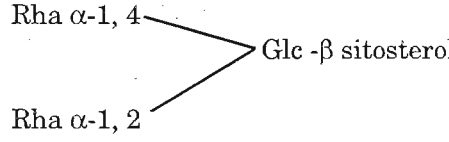

Figure 3: F_B

This branched structure is the only one of this type so far elucidated of all the flabelliferins. Branching was confirmed by the detection of 2 diglycosides and one monoglycoside on exposing the F_B to a mixed culture of yeast.[24,25] The scheme given in Figure 4, shows that only a branched carbohydrate moiety can produce the products formed.

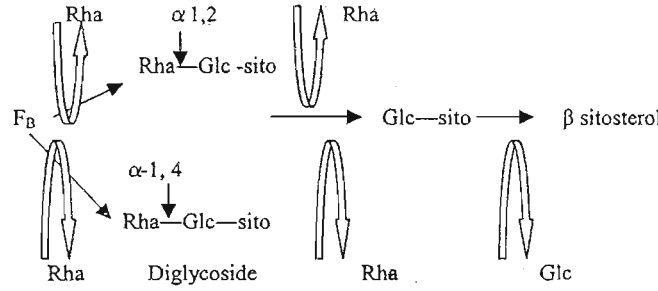

Figure 4: Proof of Branched Structure of F_B

A problem that arose is that the treatment of F_B with naringinase (a β glucosidase and a β rhamnosidase) yielded rha and one diglycoside,[24] which indicated a β rha. This can be explained by the presence of two flabelliferins occurring in different samples: one having 2 α rha links and the other 1 rha α and the other β. Thus showing that there is yet much to be revealed in flabelliferin structures.

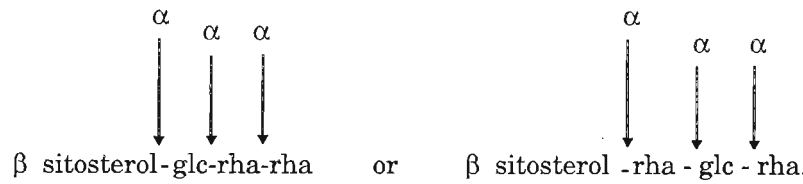

Figure 5: Alternative structures of F_C

(d) *Flabelliferin C*: Not much is known about this structure, as it has not been subject to sugar sequencing. By FAB/MS, its molecular weight is identical to F_B (868) with a rha terminus [30]but it is not antimicrobial.[29] It is not susceptible to naringinase and α-amylase hydrolysis.[31] It, therefore, has no β bonds and no α-1,4 or α-1,6 glucose bonds. There is no evidence of a M-162 fragmentation peak on FAB/MS analysis and therefore does not have a glucose terminus branch. Its most likely sequences are as given in Figure 5.

(d) *Flabelliferin D*: This was shown to be a diglycoside with 1 rha (terminal) and 1glc with a MW of 722 by FAB/MS analysis.[30] This was supported by GC/EI/MS analysis which also gave a MW of 722.[34] Chemical shifts and coupling constants

with ^{1}Hnmr showed that glc was in β configuration and rha was α and methylation analysis showed a 1,4 bond.[34]

Its structure is therefore as given in Figure 6

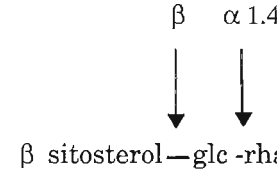

Figure 6: Structure of F_D

F_D releases rhamnose very slowly with naringinase,[31] indicating that some β-1,4 rha terminus may be present as an impurity in a later study.[31]

(f) *Flabelliferin N (F_N)*: This is an uncommon flabeliferin of MW 884.[20,34] On EI/MS analysis it gave fragmentation peaks at M-162 and M-308 indicating a sequence as given in Figure 7.

β sitosterol - glc- rha- glc

Figure 7: Sequence of F_N

Methylation analysis, 1H-NMR and enzyme hydrolysis patterns have not been studied.

(g) *Flabelliferin E (F_E)*: This is a diglucoside with a MW of 738.[20,34] ^{1}Hnmr analysis indicates a β anomeric configurations (chemical shifts and coupling constants). Further data has not been reported.

(h) *Flabelliferin F (F_F)*: EI/MS studies showed a MW of 576 corresponding to a monoglucoside.[20,34] Coupling constants and chemical shifts confirmed a β glucosidic configuration.[20,34] This is probably the naringinase hydrolytic product of the contaminant of F_D.[31]

Debittering

Specifically this refers to the removal of bitterness.

Traditionally, this is done by heating the palmyrah fruit (proximal end upward) on hot coals. Froth emerges which when removed takes with it part of the bitterness (PDB, personal communication)

The only scientific method of debittering has so far been enzymatic, using naringinase (β glycosidase and β rhamnosidase activity) or heat stable α-amylase. [20,21,27,39,40,25] As indicated in section 2.4.2 there is one very bitter compound hydrolysable by both enzymes[38] and a less bitter compound hydrolyzed only by naringinase.[38]

Debittering is a key step for more widely utilizing PFP in the form of jams and cordials.

It should be noted that debittering also leads to hydrolysis of some other non-bitter flabelliferins.[20,39]

Antimicrobial effect: An inhibitor was found to inhibit mixed batches of yeast culture.[41] Inhibitors were also detected in PFP (whole) that inhibit a number of bacteria and fungi. LD_{50} value 1mgml^{-1} was as follows; *Pseudomonas aeruginosa* (0.02) *Klebsiella pneumoniae* (0.13), *Escherichia coli* (0.03) *Bacillus licheniformis* (0.13) *Saccharomyces cervisiae* (0.06) *Aspergillus oryzae* (0.26). Earlier the antibacterial and anti-yeast growth effect was shown to be due to F_B. Both gram-positive and gram-negative bacteria inhibited at about 35μg.disk^{-1} by the Bauer-Kirby method. This sensitivity is comparable to *Ampicillin*. The bacterial strains inhibited were: - *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus rettigeri*, *Aciinetobacter calcoaceticus* var Lowffic.[21,29] This activity was lost when PFP was exposed to naringinase hydrolysis due to the destruction of F_B.[21,39] It has been proposed that this antibacterial effect could be of use in an ointment for topical applications to heal sores and ulcers.[43] Knowledge of the structure of F_B[33] would be of advantage as would the fact that some of the bacteria mentioned above have strains that are pathogenic and resistant to many common antibiotics. Further, growth of *Saccharomyces cervisiae strain* S_{11} F_3 was inhibited by F_B at 60μg ml^{-1} and F-11 at 250 60μg ml^{-1}.[21,29] A mixed culture of baker's yeast was also inhibited.[41] This provides a basis for the observation that PFP does not spoil easily.

Effect of weight gain by mice: Feeding trials using ICR mice showed that feed containing 10% PFP caused a statistically significant weight loss,[44] compared to control WHO breeding feed ($p=0.023$) despite feeds being iso-caloric and feed intake being similar. The effect was reversed by naringinase.[44] However, since naringinase hydrolyses both F11 and F_B, the results were ambiguous. Selecting varieties of PFP containing F-11 and no F_B (bitter) and F_B and no F-11 (non-bitter), showed[45] that at 10% PFP it was only the bitter PFP that reduced weight gain ($p=0.014$) compared to control. The non-bitter sample gave increased weight gain compared to control ($p=0.8\times10^{-4}$), showing that if F-11 was not present, PFP was probably a nutritious feed for mice. The probable mechanism of action of F-11 was inhibition of the Na^+/K^+ ATPase. Only F-11 among flabelliferins inhibited the ATPase of ghost red blood cells.[40] Thus, it is postulated that F-11 reduces glucose uptake, while food intake is

unaffected. This effect can have implications in lowering the glycaemic index of food; a situation, which can be advantageous to diabetics.

Some indicators on biosynthesis of flabelliferins: No biosynthetic enzymes have been isolated. Speculations can be made by considering the structure of the flabelliferins where structures have been elucidated.[20,21,27] The only sugars in the carbohydrate moiety are rhamnose and glucose.[27] Each can be α or β.[20,21] There appears to be no specificity for the acceptor molecules in carbohydrate chain lengthening.[20] Even though the maximum number of residues in the carbohydrate chain has so far been found to be 4, the potential for varied structures is mind-boggling. It is possible to conclude that since rha is a minor component in PFP,[8] that the corresponding nucleoside diphosphotransferase must be very active and / or with a low Km value. Three other such transferases are required to explain the structures so far elucidated.

Utilization of PFP

Diversity of flabelliferin profiles: The key to the wider utilization of PFP is the nature of its flabelliferin profile. PFP can be bitter, containing F-11 and detract from food use.[27] It can be an anti-yeast -growth factor[21,29] and more importantly retard fermentation.[17,21,29,46] The matter is complicated by the existence of a number of flabelliferins (at least 14). On some of these will depend the strategy of utilization. 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.[16] Although F-11 could be detected by taste[16,27] and F_B by fermentation[17,46] neither property can find use in a commercial or field scenario.

There appeared to be some correlation between location and flabelliferin profile but the extent of sampling in the study was insufficient.[16,20]

Fermentation of PFP: Fermentation of PFP has been reported.[17,46] There was a strong correlation in lower rates of fermentation and F_B content. Both F_B and F-11 produce lag in fermentation[21,29] at concentrations of $1 mg.ml^{-1}$. However, fermentation efficiency in all cases, bar one, was good, 85-95%. The sample which did not ferment was collected from Polonnaruwa and had at least 10 flabelliferins.[17,46] This observation was not possible to explain from F_B content alone.

It had been previously shown that exposure to naringinase increased the rate of fermentation.[41] This was not surprising as naringinase hydrolyzed F_B.[21,25,31] PFP has also been used for citric acid fermentation.[47]

Utilization of palmyrah constituents: The useful constituents of PFP had been described as far back as 1967.[48] Pectin can be isolated from PFP by the calcium salt on alcohol precipitation of acid extract.[22,49] Pectin was reported to have an acetyl

value of 3.5 and methyl value of 5.4 and had good gel strength. [22,49] This is of value as commercial food pectin. Alternately, PFP can be depectinized by pectinase[23,47] to yield a clear juice on filtration.[47] This has been fermented to give a bitter wine (Personal communication, PDB). Fermentation and distillation with or without pectinase action will yield a waste-containing, colour and flabelliferins. Without pectinase action the texture, foam characteristics, colour and anti-microbial properties of PFP have been made use of as toothpaste after fermentation of sugar.[43] The colour could have value only as a food colouring agent,[44] since carotenoids are oxidised during distillation of ethanol.[24] The most feasible commercial use is aerobic fermentation, where the presence of 14-16% sugar[10] makes the yield of alcohol sufficiently high to be viable. 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.[16,50]

Conclusions on utilization of PFP

* PFP can be utilized as a traditional product.[26,28]

* Most PFP (90%) can be used as a fermentation base,[17,46] hydrolysis of F_B will increase rate of fermentation.[41]

* Sweet PFP[16] can be used directly for jams and cordials.

* About 40% of bitter PFPs can be debittered with a cheap heat stable α-amylase for food use.[38]

* Less bitter PFP can be utilized traditionally.

* PFP components can be separated and utilized.

Palmyrah seed shoot

Overview: The palmyrah fruit contains 1, 2, or 3 seeds (frequently 3). The seed kernel contains a galactomannan.[8,51] On germination the seed produces a shoot (fleshy food storage scales) which gives rise to the product kottaikilangu, on boiling and drying or on drying alone.[8] This shoot grows to 12-15 cm in height before it is harvested.[52] If the seed shoot is sun dried it is referred to as odiyal. The shoot may be boiled and dried in which case it is called plukodiyal.[8] Odiyal and plukodiyal may be ground and sieved to give palmyrah flour (odiyal flour or plukodiyal flour)

The flour can be made into a number of foods which are used traditionally. The odiyal is usually consumed as a porridge called Khool and a steamed product called pittu. (PDB, personal communication) Plukodiyal is generally converted into oil cakes, and a product called palmyrah cunchy which is a type of biscuit made by

mixing with wheat flour, sugar and margarine rolled to a pastry, cut and baked in an oven.[8] Also made out of plukodiyal is a product called palmposha which again has about 70% palmyrah flour, roasted green gram, rice, soya, sugar, etc.[8]

Composition of palmyrah flour

There have been only two major reports on the subject.[10,52] These findings are summarized in Table 3 along with some other analytical data. With regard to major nutrients and energy value, odiyal flour is comparable to common flours like rice and wheat flour.[52] The main carbohydrate is starch.[8] The starch has a low viscosity and gelatinization temperature but a good setting property exhibiting unique properties as a food starch.[8] The starch is devoid of bitterness inherent in palmyrah shoot and has a large grain size of about 40μm, similar to potato starch.[54] Sugar content is low (< 1%) comprising: sucrose, glucose, and fructose.[10] Odiyal has considerable fibre and should lead to a low glycaemic index.

Table 3: Composition of odiyal[10,52,53]

Parameter	Study 1	Study 2	Study 3
Energy (KJ/100g^{-1})	1423	-	-
Moisture (g.100g^{-1})	10.8	-	-
Protein (g.100g^{-1})	3.1	5.4	6.4
Total carbohydrate (g.100g^{-1})	77.1	-	81.6
Digestable carboydrate (g.100g^{-1})	-	-	69.7
Crude fibre (g.100g^{-1})	5.6	5.0	11.9
Soluble fibre (g.100g^{-1})	-	-	89
Insoluble fibre (g.100g^{-1})	-	-	3.0

-, Not determined

Energy content .100g^{-1} is similar to cereals and tubers (on dry weight). Protein content is lower than in cereals but higher than in tubers. Fat content is low and comparable to cereals and tubers. Lipids comprise mainly of triglycerides of palmitate, oleate and linolinate.[10]

The metal ion content of odiyal flour is given in Table 5. Sodium and calcium content is higher than normal cereal flours, otherwise metallic ion content is normal.

Microelements analysed indicate (in mg. kg^{-1}) Zn, 14.3; Mn, 2.7; Cr, 1.0; Cu, 28; Co, 0.7; Ni, 2.9; Pb, 0.23; and B, 2.9.

On Amino acid analysis, odiyal flour was shown[10] to contain 0.02 g.$100g^{-1}$ free amino acid. This included[10] (g. kg^{-1}): Gly, 0.275; Ala, 0.233; Val, 0.044; Leu, 0.071, Ile, 0.044; Phe, 0.079;Tyr, 0.01; Ser, 0.398;Thr, 0.067; Cys, trace; Met, 0.006; Glu, 0.732; Asp, 0.077;Lys, 0.112; His, 0.053, Arg, 0.073 and Pro, 0.929.

Gln, Asp, Trp were not detected. More significantly, no modified amino acids and amines were detected.[10]

Table 5: Metal ion content of odiyal flour

Metal ion (mg.$100g^{-1}$)	Study 1	Study 2
Na	82	50
K	185	200
Ca	44	20
Mg	27	50
Fe	0.8	2

Of the minor natural products, steroidal saponins (discussed later in review) were detected.[10] Unlike in PFP, Vit C and carotenoids were not detected.[10]

Therefore, composition wise, odiyal flour appears to be a good starchy staple. However, this bright picture is severely challenged by many reports of toxic effects. These are discussed below.

Toxic effects

Neurotoxicity: This is by far the most widely studied toxic effect.
The symptoms of neurotoxicity were first reported in 1971.[55] The authors had interpreted the symptoms to be a secondary effect of hepatotoxicity. Wistar albino rats fed on a diet of kottaikilangu showed within 4-5 days symptoms of ruffled coat and apathetic behavior. They stopped taking food at that stage and on the 6-7 day showed nodding movement of the head, uncoordinated spasms of forelimbs and falling over backward. This progressed to ataxia, immobility of hind limbs, followed by total immobility, laboured respiration and finally death within10 days. All 6 batches of kottaikilangu tested gave the symptoms.[55]

Nearly a decade later, another group of workers[56] highlighted neurotoxicity being the cause for symptoms described previously. In addition they observed malaise, upright posture and salivation as early symptoms. In the study, unlike in the previous study, diet was clearly specified, being a 1:1 mixture of palmyrah flour and MRC 41 B diet. Feeding aqueous extracts by oesophagal tube to Wistar rats and the brine shrimp assay monitored the toxicity of the extract and its fractions. The study[56] reported a 400-fold enrichment of the neurotoxin by methanol: water (1:1) extraction and removal of impurities by lead acetate precipitation. The toxin was purified using absorption on a strong cation exchange resin and elution with aqueous ammonia. Using Sephadex G-25, the molecular weight of the toxin was estimated as 1400. The toxin was reported[56] to be stable in neutral alkaline medium and was hydrophilic in nature. The authors[56] concluded that the toxin had no COOH group but had an ionized quaternary N group. They[56] postulated that the delay of onset of symptoms was possibly due to a necessity for the toxin to undergo metabolic transformation for absorption by the intestine or by the nervous system (the latter seems more likely).

This introduces the concept of a pro-toxin (*cf* pro-drug) rather than a neurotoxin in palmyrah flour. This may give a clue to why neurotoxicity is not reported in major palmyrah flour consumers among humans.

Recent studies[57] have shown that depending on the source of odiyal flour, the time taken for appearance of neurotoxic symptoms can change, viz., Jaffna>Mannar>Kalpitiya.

That is, the Kalpitiya flour showed the most severe neurotoxicity. Further, wet heat (boiling and plukodiyal preparation) did not destroy toxicity.[57] Dry heating of odiyal (80°C, 45 Min) destroyed toxicity.[53] Although the toxin is very soluble in water, washing odiyal did not remove toxicity, but rather reduced it.[57] Steaming as in the case of pittu preparation did not affect toxicity.[57]

The previous[56] conclusions that the toxic compound was a quaternary ammonium salt containing a glycoside was not borne out as the toxic fraction was very water soluble and gave a ninhydrin positive reaction, pointing to a primary amine. Its chromatographic behaviour also pointed out to the possibility of it being an amine. [58]

However, the conclusion that the toxin is a neurotoxin and not a hepatotoxin was substantiated by the fact that the neurotoxic symptoms paralleled the elevation of serum aspartate aminotransferase (AST) and not serum alanine aminotransferase (ALT).[53] This is because ALT is diagnostic of liver disease, and an elevation of AST without increase of ALT is consistent with neurological damage.

It is possible, therefore, that the hepatotoxic symptoms observed in 1971[55] and 1976[59] have no relationship with the neurotoxic effect. The fact that subsequent studies[53,56] had not shown hepatotoxicity indicates that the two causative factors are different.

Regarding the nature of the neurotoxin, it has become clear that the MW 1400 isolate[56] is an adduct or mixture of an amine and flabelliferin[36,58] (contains a glycoside). What is not clear is whether some sort of synergism exists as the mixture gives rise to more toxic effects than the purified amine.[58] It is also possible that the amine in the absence of the flabelliferin is lost to some extent during purification. This is possible as amines not containing a strong ionic group can be volatile.

The hepatotoxic effect: The symptoms of neurotoxicity described above were first ascribed to hepatotoxins.[55] Lines of evidence for a hepatotoxin were two fold. Firstly, *in-vitro* studies on liver mitochondrial enzymes and secondly histopathological studies.[55,59,60]

The *in-vitro* studies involved succinic dehydrogenase and "succinic oxidase". The latter name is now not used and is usually synonymous with succinic dehydrogenase. The source of enzymes was not mentioned, the experimental design did not introduce cofactors and the interpretation of results of 0_2 uptake by Warburg respirometer was open to question. Therefore, the importance of these *in-vitro* studies is difficult to assess.[55]

Histopathalogical evidence was strong;[55,59] this included the observation of swollen liver mitochondria, veno-occlusive lesions,[55,59] hydrotropic degeneration of the centrilobular cells and fatty degeneration of the centrilobular and periportal cells.[55] However, the study reported that there was no parallel between the intensity of mitochondrial disfunction as evidenced by lysosomal acid phosphatase, malic dehydrogenase and α-glycerophosphate dehydrogenase activities with the extent of lesions observed in the liver.[55]

Histopathological studies also showed minimal oedema of the cerebral cortex and congestion of the kidney.[55]

A subsequent study by the same group confirmed veno-occlusive lesions of the liver of rats after prolonged feeding with palmyrah flour.[59] Chronic hepatic lesions included intra-luminal fibrosis of the centrolobular veins, bile duct proliferation and increase in reticular fibrosis.[59] No thrombosis and hepatic megalocytosis was seen.[59] The study concluded that the toxic factors responsible are different from pyrrolizine alkaloids and dimethylnitrosoamine, which produce similar lesions.[55,59]

In another study by the same group,[66] pregnant Wistar rats immediately after parturition were fed on 100%, 50% and 33% palmyrah flour. Studies on 44 suckling

rats showed that the infants did not show neurotoxic symptoms but significant neonatal deaths occurred. When the maternal rats were fed on 100%, 50% and 33% palmyrah flour, the sucklings died in 1-7 days and 2 weeks. Survival was therefore dose dependant.

Histopathalogy[60] of neonate livers showed that the livers had dark patches. Internal examinations showed free pleural fluid and peritoneal fluid and enlarged and pale kidneys. External abnormalities included subcutaneous hemorrhages at extremities of tail and limbs and in thoracic abdominal walls.[60]

The liver showed gross dilation of the sinusoids, foecal hemorrhages and mild to intense centrilobular sinusoidal congestion.[60] The authors concluded that the hepatotoxin of palmyrah flour could be transferred by milk to produce toxicity in suckling rats.[60]

However, another group of researchers found no evidence of a hepatotoxic effect of palmyrah flour, although neurotoxicity was observed. They concluded that hepatotoxin and neurotoxin were different entities.

Recent studies[53] showed that Wistar rats fed on 50% palmyrah flour showed no gross evidence of liver abnormalities, and more significantly, serum alanine aminotransferase activity which is a sensitive indicator for liver cell damage, even sub-clinically, showed no elevation in comparison to that of rats fed on WHO test feed.

That study[53] also suggested that nutrition may play a part in the expression of toxic effect. It was concluded that if results of different studies are to be compared, publications must include details such as feed composition, feed intake and weight gains or losses. The obvious hepatic lesions seen by one group[55,59] and not by others [53,56] is puzzling, especially as the palmyrah kottaikilangu originated from the same location, viz., Kalpitiya in the North -West of Sri Lanka. Further, the group reporting hepatotoxicity had been careful to eliminate chance contamination of feed by aflatoxins and pesticide residues.[55] There is no clue as to the identity of the hepatotoxin.

The immunosuppressive effect

Wistar rats on prolonged feeding with palmyrah flour produced malignant lymphomas.[61,62] This has been attributed to immunosuppressive factors in the flour. It is clear that this factor is not associated with the neurotoxic effect of palmyrah flour.[63] The basic hypothesis[64] was that lymphoid neoplasias arose from a toxic constituent of palmyrah flour. This is manifest by depressed humoral and cell mediated immune response in rats after short term, high dose or long term medium dose feeding of palmyrah flour. In a study where inbred mice were fed with 40%

palmyrah flour, a delayed type hypersensitivity response (DTH) to sheep red blood cells was observed.[65] DTH was noted after 6 days and maximum suppression occurred in the sensitization period. The immunosuppressive effect was transferable to normal mice fed on normal feed by way of viable spleen cells. The cells transferring the effect were T cells with a Ly-1 surface antigen (negative to Ly-2 antigen).[65] No lesions were observed in the liver. The authors concluded that oral feeding with palmyrah induced T suppressor cell generation, which was able to suppress the DTH response to sheep red blood cells.

Using haemaggluttinating antibody titres and haemolytic plate forming count in the spleen following immunization with sheep red blood cells induced another study[64] showing the humoral and cell-mediated response of rats fed with 25% palmyrah flour. The immune competence of animals was depressed significantly in both cell-mediated and humoral response. Lymphocytes from peripheral blood failed to respond to phytohaemagglutinin stimulation. The study[64] concluded that it is possible that these immunological alterations were etiologically related to malignant lymphomas, which develop in rats after prolonged feeding. The authors state [64] that palmyrah flour is the only staple food demonstrating significant alterations in immune competence of experimental rats.

It is widely quoted[66,67,68] that prevalence of malignant lymphomas in the North-East of Sri Lanka (in which palmyrah flour is commonly consumed) is 3-4 times that of the rest of the country. However, there is no access to data from the original epidemiological study.

Unlike the other toxins of palmyrah flour, the immunosuppressive factor has been isolated and its structure elucidated.[68] It was found to be 1[(17α, 23(t)-dammara-20, 23-diene-3β 25 diol] (Fig 8)

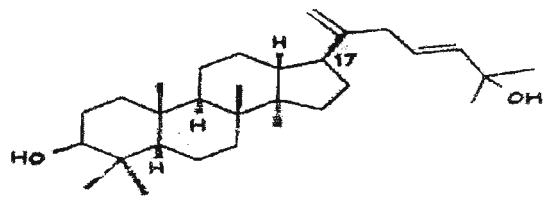

Figure 8: The immunosuppressive agent

It is a triterpenoid and was isolated by selective solvent extraction and purified by chromatographic techniques.[68] The isolation procedure was activity-directed by using the murine mixed lymphocyte reaction (MCR). The yield was 0.5mg.kg^{-1} palmyrah flour. Spectroscopic analysis by ^{1}Hnmr, ^{13}Cnmr and FAB/HRMS on the pure product, revealed the compound to be a dammarane.[68] Dammaranes are widespread in plants but mainly have a 17β substitutuent (not 17α as found in the

study[68]). This is the first time a 17α configuration has been reported for a natural product.

The compound has been made commercially viable by synthesis from a major component of natural "Dammar resin" in only 7 steps giving an overall yield of 4.3%. It is an extensively potent immunosuppressant with an Ic_{50} of 10ng.ml^{-1} on the MLR assay.[68] Of the toxins of palmyrah flour this is the only one characterized.

Mutagenic and clastogenic effects

Palmyrah flour was tested for mutagenic effects[69] on *Salmonella typhimurium* (strains TA 78 and TA 100) and *Escherichia coli* (strains Wp_2, Wp_2uvrA, CM 1881 and CM 891). Both boiled and raw odiyal showed dose related mutagenic response in the base pair sensitive strains. The investigation added mutagenicity to the wide range of toxicity of palmyrah flour discussed in the review. Another study on induction of sister-chromatid exchange[70] in human blood lymphocytes by aqueous extractives of palmyrah flour showed that the phenomenon was dose related but there was no consistency between batches of subjects. Sister-chromatid exchange was found to be proportional to chromosome length. The finding supported incidence of human malignant lymphomas[66] and toxicity in rats[55] and bacteria[69] reported to be due to constituents of palmyrah flour. The effect was mainly on group A chromosomes and resulted in chromatid and chromosomal breaks.[70] Some large and small acentric fragments were also observed.[70] These effects were dose dependent and consistently produced by different batches of palmyrah flour. However, the potency was less than in the mitomycin control. The study[70] suggests that the non-random appearance of chromosome aberrations (also seen in human malignant lymphomas) may be due to a specific tumor-causing agent as observed in the case of non-Burkitts lymphomas (group A, C, D) bleomycin (group C) and adrinomycin (group A, D)

Other bioactivities caused by palmyrah flour

Palmyrah flour mixed with that of other roots, on oral administration to albino rats induced morphological changes in the endometrial surface epithelium of the uterus when viewed through a scanning electron microscope.[71]

The smooth pattern of the endometrical cells changed to haphazardly orientated groups of cells and loss of microvilli. It was postulated[71] that this structural disparity affects the smooth functioning of the nidatory preparation of the endometrium. The experiment lends weight to the use of this preparation traditionally as an oral contraceptive in India.

The anti-microbial activity of palmyrah flour has been reported[42] both in boiled and unboiled odiyal. (Table 3)

Table 6: Antimicrobial effect of palmyrah flour[42]

Microorganism	$LD_{50}(g.ml^{-1})$	
	Boiled	Unboiled
Pseudomonas aeruginosa	0.03	0.02
Klebsiella pneumoniae	0.17	-
Escherichia coli	0.03	0.02
Bacillus licheniformis	0.02	0.08
Micrococcus roseus	0.02	0.01
Staphylococcus aureus	0.01	0.03
Citrobacter freundi	0.02	0.02
Saccahromyces cervisiae	0.03	0.03
Aspergillus oryzae	-	0.07
Aspergillus niger	-	0.07

Some flabelliferins of palmyrah flour are also bioactive

The flabelliferins of palmyrah flour

Saponins were stated to be absent in palmyrah flour in an early study.[56] Later Jeyaratnam[10] reported 2 steroidal saponins in palmyrah flour. They were a monoglucoside and monorhamnoside. Another report[27] indicated the presence of the flabelliferins F_C and F-I in palmyrah flour. Recent studies[36,58] showed the presence of a large number of flabeliferrins in the flour from Kalpitiya. These include F-I, F_B (anti microbial) F_C, F_D, F_E, F_F and a bitter flabelliferin not identical with F-II of PFP. The separations were made by MPLC and Rf determined preparative TLC and structure identification on TLC and fragmentation pattern on enzyme hydrolysis.[36,58]

The bitter flabelliferin presented difficulties in isolation, as it was unusually soluble in water and not in methanol unlike the other tetraglycosides and appeared to be strongly associated with a ninhydrin positive spot (probably an amine). This mixture of flabelliferins and amine produced some of the neurotoxic symptoms on Wistar rats.[72] Removal of the amine from the bitter flabelliferin by cation exchange yielded a flabelliferin very soluble in methanol and a ninhydrin positive white solid, which produced some neurotoxic symptoms of the mixture but to a lesser degree.[58] This flabelliferin was not the same as the very bitter flabelliferin of PFP as it could not be hydrolyzed by heat stable α-amylase and yields rhamnose but no aglycone on hydrolysis with naringinase.

The strong binding of the flabelliferin to other compounds has been observed previously in that they bind to a UV fluorescent volatile compound in PFP.[73]

The role of flabelliferins in toxicity, especially neurotoxicity, is suggestive from results[58] but details have still to emerge. Further, it is reported [42] that palmyrah flour has an antimicrobial factor. This may be F_B as reported previously in PFP[29] and /or some other flabelliferin. Clearly more work has to be done on the bioactivity of flabelliferins.

Other toxins of palmyrah

The root of palmyrah is known to contain a slow acting poison.[10] The pollen from palmyrah is reported to have a 90k Dalton factor responsible for allergic reactions .[74]

CONCLUSIONS

The direction of research on these two basic raw materials from palmyrah, differs vastly. PFP is underutilized and shows little evidence of being toxic to mammals. Odiyal is widely utilized in North - East Sri Lanka as a staple and has many reported toxic effects.

The strategy for PFP is therefore to increase utilization, while in odiyal much remains to be done including identification of the toxic factors, studying the effects of processing on toxicity, detoxification techniques and attempts to utilize toxins for the benefit of man as has been done for the immunosuppressive dammarane.

The full significance of the presence of numerous flabelliferins both in PFP and in flour is worthy of investigation including its significance from an evolutionary standpoint.

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