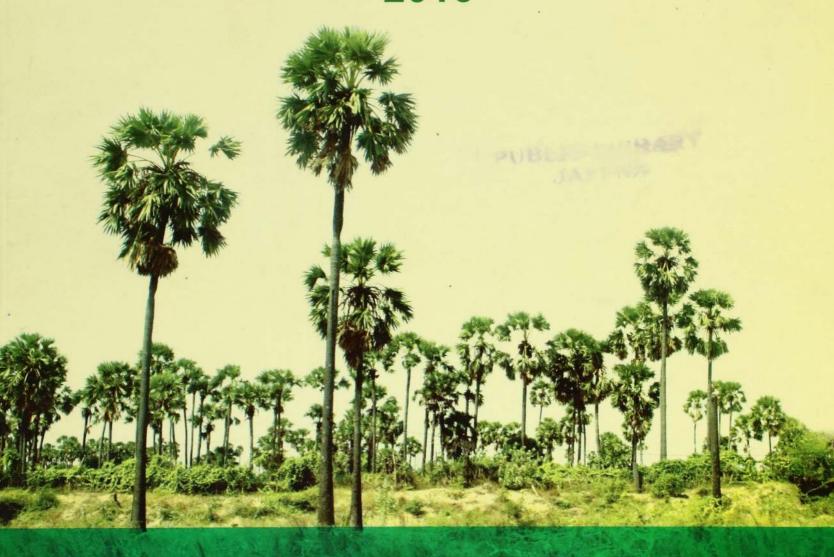




# ENHANCEMENT OF PALMYRAH INDUSTRY THROUGH RESEARCH 2016



Palmyrah Research Institute
Palmyrah Development Board
Ministry of Prison Reforms, Rehabilitation,
Resettlement and Hindu Religious Affairs

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# Enhancement of Palmyrah Industry through Research 2016

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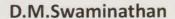
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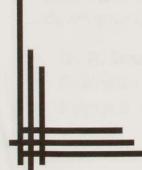
#### Minister's Message

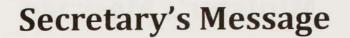
I am indeed proud to convey my message for publishing the book "Enhancement of Palmyrah Industry through Research".

Palmyrah Development Board gives its highest efforts to uplift the quality of Palmyrah products to international level and the participation of Palmyrah Research Institute (PRI) to accomplish this goal is highly appreciated. PRI functions to find the nutrient substances and the medicinal demand of Palmyrah products. This book, "Enhancement of Palmyrah Industry through Research" contains the publications of PRI from 2012 to 2016. I believe that this publication would draw the attention of vast number of food industries and the researchers who are related to this field and it would elevate the demand for Palmyrah products. I congratulate the whole team of researchers of Palmyrah Development Board best of luck on this book launching to achieve the goal of this publication and I hope they will continue to serve to the growth of Palmyrah Development Board through their valuable service.



Minister,
Ministry of Prison Reforms,
Rehabilitation,
Resettlement and Hindu Religious Affairs.





Palmyrah has the iconic impact in the economic, social and cultural life of the people from the ancient time. Even though people have been getting usage from different parts of Palmyrah for a long time, the Palmyrah Development Board is introducing new machineries with the support of well experienced staffs of Palmyrah Research Institute (PRI) to get the optimum benefits from Palmyrah resources. Hard work and dedication of the management and the researchers of Palmyrah Development Board to strengthen the quality of the Palmyrah based products are really commendable. Since the rehabilitation of PRI in 2012, many researches have been done on Palmyrah at the PRI and this publication, "Enhancement of Palmyrah Industry through Research", is the evidence to prove the work of the research team of the board. I congratulate all the researchers of Palmyrah Development Board for this publication and I hope the finding of research will create a positive impact in the production and marketing of Palmyrah products through grasping attention of different palm industries in the country.

#### Mr. W.A.S.B Amarathunga

Secretary (Acting), Ministry of Prison Reforms, Rehabilitation, Resettlement and Hindu Religious Affairs.

#### Words from the Chairman

I am extremely happy to pen few words for this book, "Enhancement of Palmyrah Industry through Research", that contains the publications of Palmyrah Research Institute (PRI).

The Palmyrah Development Board is to develop Palmyrah based sector in a systematic and scientific manner. Our board is giving its maximum effort in various attempts to stand as a successful organization that can directly or indirectly support the growth of Sri Lankan economy. PRI is one of the sectors of Palmyrah Development Board that functions to enhance the capacity of Palmyrah palm and to achieve the above goal of our board.

It is rather hard to get the people excited about the Palmyrah products in these days because of changing modern life patterns/styles of them. However, the researchers of our board are working hard to uplift the quality of Palmyrah products so as to get optimum use from the under-utilized palmyrah resources.

This book is published to expose the researches of our researchers not only in locally but also in globally and to educate the people about the values of Palmyrah resources. We expect that this publication would draw the attention of many people from various fields.

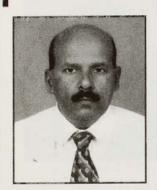
Therefore, as the Chairman of Palmyrah Development Board, I am proud to document this book within the period of four months since I assumed my duty as the Chairman of this board. I believe that this book will create a high impact on Palmyrah based products and will be a gate for new opportunities in the Palmyrah sector. I congratulate the whole team of researchers of Palmyrah Development Board and wish them to achieve more in this field for the development of our board.

#### Dr. R. Sivasangar

Chairman, Palmyrah Development Board.



#### Message from General Manager



It is a pleasure to write this message on the occasion of the publishing the book on 19th, Dec,2016.

The Palmyrah Research Institute (PRI) inaugurated the research activities in 2012 to focus on analytical, food technology and microbiological aspects of Palmyrah palm. P.R.I. scientists have responded effectively to the country's basic research needs and have carried out a substantial amount of work in their respective fields with a special focus, as in the past, on applied research. It is encouraging to note the effort made by P.R.I. scientists to disseminate their findings among the Palmyrah dependent families, co operative societies and related institutes through various programmes.

This book "Enhancement of Palmyrah Industry through Research" to make available production of valuable materials on the technical and other information pertaining to Palmyrah palm restoration and the industries had been a longstanding necessity.

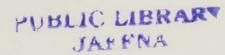
This publication is the outcome of the extensive and exhaustive research work carried out by the P.R.I. and the other research oriented institutes.

I wish to commend the initiative taken by the PRI and to publish their research work in the form of this book.

M.B.Loganathan,

B.Sc. (Agriculture), M.Phil.





### Message from Research Manager

Palmyrah Research Institute was re-established in 2012 with well as a modernized laboratory equipped laboratories and qualified staff. It has been functioning splendidly with vigour since its reestablishment. Launching of the book 'Accomplishments of Palmyrah Research Institute from 2012 to 2016' is a milestone in the path of the development of the institute. The book illustrates the research activities of the institute with reference to the investigations conducted from 2012 to 2016 on nutritional and medicinal properties of Palmyrah, new product development and improving the productivity of Palmyrah based products. It gives me immense pleasure to disseminate the research outputs of the institute via this book launching as I hope this will create awareness about the potential of Palmyrah among the entrepreneurs of Palmyrah industry which will consequently lead to the growth of the Palmyrah sector.



#### Mr. Srithayalan. Srivije indran,

Manager (Research and Quality Control) Palmyrah Research Institute B.Sc (Special in Chemistry), M.I.Chem.C, M.Sc (Industrial Analytical Chemistry), M.A (Administration), PgDMM (Manufacturing).





Staff of Palmyrah Development Board - 2016

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#### Pillars of Palmyrah Research Institute



#### Mr. Srithayalan Srivijeindran

Manager Research and Quality Control

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## Research Scientists Analytical Laboratory



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M.Sc (Pharmacology), B.Sc (Chemistry)



Miss. Diluckmini Bernard

B.Sc (Hons) (spl)

(Food Production and Technology Management)

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Dr. (Ms) Anuluxshy

Balasubramaniyam

PhD (Applied Science),

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B.Sc (Biological Science), PGDip



Mr. Sakthivel Vimalraj
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Diploma in Laboratory Technology

#### **Microbiology Laboratory**



Mrs. Subajini Mahilrajan MPhil (Biochemistry), B.Sc (Hons) (spl) (Botany)



Miss. Robika Kailayalingam

B.Sc (Biological Science)

#### Hi - Tech Laboratory



Mrs. Mary Jeno Winston

M.Phil (Reading)

(Chemical and Process Engineering),

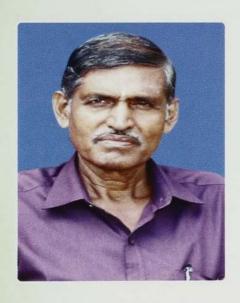
B Sc (Hons) (sp) (Chemistry)



Miss. Gnanasharmala
Ariyaratnam

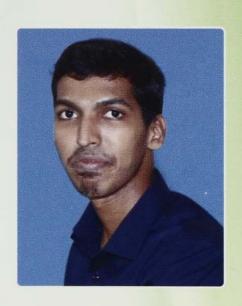
B.Sc (Reading) (Foundation)

#### **Research Dissemination Unit**



Mr.Thiyagarajah
Paneerselvam
Certificate Course in Workers

Certificate Course in Workers
Education programme
Diploma in Labour Studies



Mr.Pathmasivam Sivanathan
B.Sc (Hons) (Computer Network and Security),
CCNA, HNDIT

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# 1. UPGRADING THE LIVELIHOOD OF PEOPLE INVOLVED IN TRADITIONAL PALMYRAH FAVOURED INDUSTRIES.

The Palmyrah Research Institute (PRI), located on the Kandy Road, Kaithady in Jaffna has expanded its functions towards modernizing its research activities, in order to enhance the livelihood of thousands of families in the North, who are dependent on the Palmyrah industry. The PRI, which was established in 2012, remains a blessing to the Palmyrah related research in the North, where the Palmyrah based resources were identified as economically viable substances that could improve the livelihood of Palmyrah dependent people. Various necessary changes were made with regard to the Palmyrah sector, and the establishment of palm products co-operative unions was a milestone in the course of this development. Various institutionalized enterprises were undertaken through these unions to improve the livelihood of people involved in the Palmyrah sector. The Palmyrah Development Board (PDB), which is one of the enterprises established in an important organization registered in history. At the beginning, the PDB was engaged in a limited amount of research only. Later the research activities were conducted appropriately in order to get the maximum benefits from the Palmyrah palm, creating awareness regarding the Palmyrah resources among the people through scientific evidences, with the interest of benefitting them. The necessity for raising the standard of Palmyrah products through quality research had been felt, and this resulted in the establishment of PRI as a separate unit of the PDB. PRI which was rehabilitated and opened in the year 2012 with the financial help of the government of India started functioning with vigour and splendor. There are four important divisions of laboratories in the institute to fulfill its functions: analytical, food and technology, microbiology and hi-tech laboratories. The analytical laboratory conducts activities in relation to enhancing the standard of products and creating an awareness of the nutritional components of the Palmyrah related food products. The food and technology laboratory conducts activities to achieve the aim of utilizing the highly nutritious Palmyrah resources to manufacture delicious food items according to the norm of the Sri Lankan Standard Ordinance Act, craved by the people in this highly modernized world. The microbiology laboratory functions with the objective of confirming the medicinal significance of Palmyrah products and also to investigate the product enhancement th rough the microbiological effects on the products. The hi-tech laboratory functions to identify and determine the quantities of nutritional components, which are traceable amounts in the foods, with the aid of high technology laboratory instruments such as high performance liquid chromatograph and gas chromatograph. Furthermore,

the PRI is involved in a range of activities namely research and exchange activities, rendering laboratory services, and publication and dissemination of research findings. The institute has taken its final steps in obtaining the ISO accreditation so that the test reports supplied by the laboratory will be valid internationally. PRI is planning to set up a new branch for the dissemination and publication of research outputs and to engage in market research, creating possibilities for the Palmyrah products to reach international markets.

People of Sri Lanka are in the custom of carrying out a number of small industries from generation to generation. Together with the various types of small industries to improve their livelihood, they engage in agricultural activities also. In such a tradition, industries relating to Palmyrah are of very ancient heritage. In the northern part of Sri Lanka, even though Palmyrah favored resources can contribute much to the economic development of Sri Lanka, it appears that this field did not attract much significance. Therefore, Palmyrah favoured products remained limited among the other productivities. For example industries like tapping of toddy, an intoxicating drink from Palmyrah palms, producing sugar molasses, treacle, Palmyrah fruit pulp leather together with certain products of handicrafts woven out of strips of dried young Palmyrah leaves like large boxes (containers) mats and winnowing fans were all became confined. Among the traditionally found resources of agricultural and marine favoured resources in the northern part of Sri Lanka, Palmyrah favoured resources also have become very important. However, at the beginning, no efforts were taken to improve the lives of people who were dependent on Palmyrah favoured products for the improvement of their livelihood. In the long run, along with the changes in social politics and economics, Palmyrah favoured resources were identified as a very economically important field to enhance the livelihood of people and to fulfill their basic requirements. Various necessary changes were undertaken relating to this. In the course of this development, palm products co-operative unions can be considered as a milestone. Various institutionalized enterprises undertaken through these unions to improve the livelihood of the people engaged in Palmyrah favoured Industries, which were registered in history. In such a course of history, Palmyrah Development Board is an important organization. At the beginning, the Palmyrah Development Board was engaged only in a limited amount of research. Later the research was utilized in the right manner to get the maximum benefits from the Palmyrah palm, and those benefits were to be given to the people with evidences in a scientific manner in order to create awareness among people about Palmyrah resources. Sensing the necessity of raising the standard of their Palmyrah production the importance of tessearch was felt to be essential in taking the form of the Palmyrah Research Institute as a separate unit of the Palmyrah Development Board.

#### Palmyrah Research Institute

Palmyrah Research Institute was rehabilitated and opened in the year 2012 by the Ministry of Traditional Industries and Small Enterprises Development with the financial help of the government of India and the government of Sri Lanka has started functioning with a new radiance and splendor. At present the Palmyrah Research Institute is functioning with a renewed vigor performing many successful activities according to specified objectives.

#### The vision and mission of the Palmyrah Research Institute.

Since Palmyrah Research Institute developed through small measures of research, it has created its own vision and mission, reaching a maximum level in Palmyrah research So that the field can contribute towards the national economic development of Sri Lanka.

#### Mission

Enhancing the practice of research for the innovative technology transfer to develop, promote, popularize and regulate sustainability of Palmyrah industry to be a significant contributor to the Gross Domestic Product (GDP) of Sri Lanka.

#### Vision

Achieve excellence in the Palmyrah research for enhancing sector cotribution to nation al economic development of Sri Lanka.

#### **Objectives**

On the basis of mission and vision identified at the Palmyrah Research Institute, the fun ctioning is with the following objectives.

- Maintenance and improvement of the quality and standards of Palmyrah based products through Palmyrah products producers.
- Enhancing the promotion and direction of the Palmyrah research institute activities.
- The conducting and furthering of scientific research in connection with the processing and utilization of Palmyrah products.
- The Introduction of new techniques in the processing of Palmyrah products.

- Strengthening the Palmyrah Research institute by collaborating with the research institutes of Sri Lanka and international research institute and the universities of Sri Lanka like Wayamba University, Uvawellassa University, Sabaragamuwa University, Peradeniya University and Jaffna University.
- Providing data or facts based on researches to Palmyrah stakeholders in order to increase the utility of Palmyrah palm.
- Handling research to increase the resources relating to the growth and cultivation of crops and predicting the results.
- Enhancing research activities by the consultation of experts in various scientific field.

#### **Activities of Palmyrah Research Institute and Laboratories**

Palmyrah Research Institute which functions on the basis of above mentioned objectives of far sightedness and statements of duties, collaborate with some universities and some other research institutes to undertake research and also engage in activities to promote the standard of Palmyrah products and the efficiency of productivity, analyze the nutritional value of Palmyrah products and provide technological counseling for this, there are four important divisions of laboratories.

#### **Analytical Laboratory**



In relation to increase the Palmyrah favoured products in a profitable manner, to enhance the standard of products and to create an awareness of the nutritional components of the Palmyrah related food products; the analytical laboratory has undergone many activities. As this laboratory containing much modern equipment, the following activities can be stated as remarkable,

- By analyzing quantities of the nutrient components of the Palmyrah related products and providing the details to increase the demand for the Palmyrah products.
- Undertaking experiments to inspect the standard of the Palmyrah favoured products
  of the manufacturers with small investment, according to the norm of Sri Lankan
  Standard ordinance act and provide report and counseling regarding the findings.
- Finding out the chemical changes that occurred during the process of manufacture
  of the Palmyrah favoured food products and regulate them to make them beneficial
  to the production.
- Changing the Palmyrah favoured products non related to food in a way to increase its worthiness.
- Helping in the academic activities of universities and technical colleges
  - Rendering explanatory lectures regarding the laboratory equipment which are used to analyze the nutrient composition of the Palmyrah favoured products and also favouring the practicability of the equipment.
  - Rendering lectures relating to the chemical nature of the Palmyrah related food products.

#### Food and Technology laboratory

 Undertaking visits to cooperative unions engaged in Palmyrah and coconut favoured productivity, identifying their processes of manufacture of food products, and explicitly giving instructions regarding the correct procedures.



This laboratory carries the following activities to achieve the aim of utilizing the highly nutritious Palmyrah resources to manufacture delicious food items craved by the people in this highly modernized world

- · Inventing new types of food items and familiarizes them in the market.
- To change the structure and standard of the food items already presenting in the market to suit the norm set by the Sri Lankan Standard ordinance act.
- Assisting by rendering explanatory instructions and lectures regarding academic activities of universities and also technical colleges.
  - Rendering explicit lectures regarding the laboratory equipment which is used to analyze the nutrient composition of the Palmyrah related components and also favouring the practicability of the equipment.
  - Carrying out lectures relating to the chemical nature of the Palmyrah related products
- Encouraging and guiding to obtain the standard certificate issued by the Sri Lankan Standard ordinance act, by introducing in the field of industry, exceptionally good manufacturing processes and health related procedures in the production of food items.

#### **Microbiology Laboratory**



Even though the Palmyrah favoured products have been trusted to be beneficial medicinal wise, it appears that there are no scientific evidences to prove the benefits. Microbiology laboratory functions with the objective of confirming the belief and also to find out the enhancement through the microbiological effects on the Palmyrah favoured food products. The following can be mentioned as the important functions of the laboratory

- Undertaking experiments relating to microbiology on the products of Palmyrah foods and provide a report based on the tests and also render counseling.
- Determine the shelf life of Palmyrah favoured products and other new products by research on the growth rate of microorganisms.
- Utilizing microorganisms which are beneficial to raise the standard of the Palmyrah related products and to convert the worthless resources to worthier ones.
- Those who are engaged in the laboratory work through the experiences gained, rendering assistance to academic activities of the universities and technical colleges.

#### Hi – Tech laboratory



The Hi – tech laboratory functions with High technology laboratory equipment or instruments (Hi performance liquid chromatography and gas chromatography instruments) which are one of highly expensive instruments in Sri Lanka. The following can be mentioned as the useful features of the laboratories and also the equipment.

 Identifying and determining the quantities of nutritional components which are traceable amounts in the foods, for example detailed names and quantities of vitamins, sugar and also amino acids. This method will pave way to make a list of the detailed nutritional data. This work is accomplished with the usage of High Performance Liquid Chromatography (HPLC) equipment.





• A detailed study of the components in alcohol and fatty acids is also carried out in this laboratory as a work. The equipment Gas Chromatography (GC) is used for this study.

#### **Activities of Palmyrah Research Institute**

Research Dissemination Program Since, the inception of the Palmyrah Research Institute to date, it has been developed to the level of undertaking various activities.

#### 1. Research and exchange of technique

Upgrading the quality of Palmyrah products, disseminating the significance of medicinal and nutrient values of the products, producing new Palmyrah products based on the demand of modern and healthy food supply are some of the activities which are undertaken by this institute.

Further it has been involved in collaborating with palm development societies which produce Palmyrah related food items. As a part of this activity, this institute provides consultations and training to implement the best manufacturing activities, sanitary activities and quality control activities in production centers. In addition to that PRI carries out certain plans to make accessible the research findings to the public.

#### 2. Laboratory Services

The chemical and microbiological analysis with regard to Palmyrah products is undertaken and reports are submitted. More than hundred analysis reports have been already submitted.

#### 3. Research and Field activities of Universities

The lectures and the practical sessions for the palm and latex technology and value addition degree third year students of Uva Wellassa University are provided by this institute. Apart from this, the opportunities are provided for various university students to complete their industrial training. Also, Laboratory facilities for individual scientific research are provided to final year students of Sri Lankan universities.

4. Publication of research findings, dissemination and exchange of techniques
The research findings are made accessible to proper utilizer through workshops and
seminars. The institute also undertakes activities to disseminate the medicinal, chemical
and other useful characteristics of Palmyrah to other researchers as well as the people in the
science field through research journals and research conferences. And most of the findings
of the research carried out through laboratory activities are with satisfactory conclusions.

### The research carried out in the Palmrah Research Institute during the past:

An initial research plan was created in consultation with the Palmyrah development co-operative societies which are involved in Palmyrah related industries, investors, educationist and various others and research were carried out based on this plan. During the last four years, the problems faced by the industries related to Palmyrah resources have been analyzed and more than thirty researches were carried out. Out of them, the following can be denoted as important studies.

- 1. Upgrading the quality of Palmyrah jaggery
- 2. Identifying the adulterants in the jaggery using modern testing methods
- 3. Deciding the nutrient quality in the jaggery and other Palmyrah products
- 4. Calculating the glycemic index and insulin index of the jaggery and other Palmyrah food products
- 5. Upgrading the quality of Palmyrah treacle
- 6. Preservation of Palmyrah pulp
- 7. Upgrading the quality of Palmyrah toddy
- 8. Extracting pectin from Palmyrah fruit and analyzing its features
- 9. Upgrading the quality of Palmyrah sugar candy
- 10. Upgrading the quality of Palmyrah bottled toddy
- 11. Analyzing the total phenolic content and nutrients in Palmyrah fruit pulp leather and tuber flour
- 12. Creating new techniques for marketing Palmyrah products
- 13. Analyzing the nutrients in processed sweetened Palmyrah fruit pulp leather and producing preserved processed sweetened Palmyrah fruit pulp leather using Palmyrah treacle

#### 14. Estimating the antioxidant bio active components.



#### 15. Research regarding non edible products of Palmyrah

- Using Palmyrah fiber waste producing compost.
- Extracting natural dye from "Black Henna" leaves and coloring Palmyrah leaves and studying the anti-fungal effect of this natural dye.
- Studying the possibility of producing cattle feed from Palmyrah leaves at commercial level.
- Producing activated charcoal from Palmyrah need shell
- Protecting Palmyrah leaves by reducing the microbial effect and coloring of Palmyrah leaves.
- Calculating the time limit to harvest Palmyrah haustorium and Palmyrah tuber simultaneously with their high nutrient values.

#### 16. Other research on new food production

- Yoghurt making using Palmyrah pulp and milk
- Sponge cake making using Palmyrah related rawmaterials.
- Instant Palmyrah soup mix preparation
- Upgrading the quality of "Palmposha" a nutritional cereal
- Preserving ice-apple in Palmyrah sweet sap
- Bakery production using tuber flour and local resources
- Making jelly added with Palmyrah pulp
- Pudding making using tuber flour as an ingredient
- Palmyrah fruit pulp leather based fruit and nut bar preparation

#### Current research and activities

The research plan for the current year is activated under the supervision of the Ministry of Prison Reforms, Rehabilitation, Resettlement and Hindu Religious Affairs. The following are some of the important activities undertaken this year

- Studying the possibility of extracting ethan0ol from Palmyrah waste
- Analyzing the vitamin compounds in Palmyrah jaggery
- Comparing the quality characteristics of Palmyrah treacle with other palm treacle
- Analyzing the nutrient variation between fresh pulp and preserved pulp
- Development of pulp incorporated ice cream
- Analyzing the nutrients in ice-apple and preserved ice-apple
- Nutrient analysis of haustorium and production of preserved haustorium
- Introducing climbing device, pulp extractor and fruit pulp leather dryer and studying the possible usability
- Production of debittering enzyme to remove the bitterness of the Palmyrah fruit pulp.
- The final steps to obtain ISO certificate for the laboratory is also in progress

#### Future plans of Palmyrah Research Institute

- 1. Setting up a new branch to disseminate, publish and encourage the research activities of this institute.
- 2. Involving in the activities of taking the research findings and data obtained by the institute to the producers and the user level.
- 3. Handing over the production with regard to the new invention of the institute to a separate division and also collaborating with other institutions for production.
- 4. Producing and marketing the new invention of the institute by involving in the marketing activities through market research.

- 5. Involving in the activities of creating national and international marketing possibilities using the diversity of Palmyrah products.
- 6. Introducing new technologies and techniques to increase the quality production of Palmyrah products
- 7. Involving in the activities of making PRI a profitable institution
- 8. Involving in international collaboration

The realization of the significance of Palmyrah resources have been gradually increased at present. The interest and concern on Palmyrah products have also been increased. The necessity of research with regard to development and production of Palmyrah products is felt. Thus the Palmyrah Research Institute becomes a significant institution and therefore, its research has now become expanded and multifaceted. The findings of its research are appreciative, successful and encouraging. However, without proper facilities to make the producers accept the research findings and make bulk production for marketing, the institute faces challenges in its future development. Consultation and recommendations in this regard should be taken into consideration to overcome these challenges. If recommendations are considered favourably and activated, not only the PRI but all the fields related to palmyrah production also would be prosperous in the future and they could be contributing favourably to national production and national economy.

# 2. Scientific Publications of Palmyrah Research Institute 2012 - 2016

# 2.1 Publications in International Journals

# 2.1.1 Microbial, Physico- chemical and Sensory Evaluation of Preserved Palmyrah Fruit Pulp

Robika Kailayalingam, Subajini Mahilrajan\*, Srithayalan Srivijeindran and Ponnuchamy Navaratnam

#### ABSTRACT:

Fruits of Palmyrah palm (Borassus flabellifer) are seasonal; therefore their fibrous (mesocarp) fruit pulp (PFP) extracted with water and should be preserved with lengthened shelf life to ensure its availability in local and international market throughout the year. Therefore a study on preservation of PFP was carried out with or without various concentrations of preservatives, Sodium benzoate (SB), Sodium metabisulphite (SMS) and combinations of the both at different ratio. pH of the PFP was adjusted to 3.8 with citric acid, heated in a water bath at 90°C for 20 Sec, preservatives were added, mixed well then bottled pulp was heated at 80°C for 30 min in thermostatic water bath and kept at room temperature (30°C) for 180 days. Initial pH with stabilization has come to about 4.2. Aliquots of them were withdrawn periodically (at 30 days intervals) and were analyzed for microbial, physicochemical and sensory characteristics. PFP alone (without preservatives) was spoiled with increasing pH by showing adverse characteristics (unacceptable odour) before 24hours of storage. All the treatment showed significant (p<0.001) increased in total soluble solid (10.82 - 13.10° brix) and declined in pH (4.42 - 4.14) was observed with a proportional increase in the acidity (0.71-0.91%) for treatments of T1 - T5 (containing SB), T6-T10 (containing SMS) and T11-T15 (containing both SMS & SB) up to 180 days. But no colony (Total Plate Count) was observed in the pulp treated with SMS and with combination of SMS & SB at various concentrations up to 120 days of storage. Among the all treatments the pulp treated with SB were found to be inferior in both colour and flavour characteristics. Even though it was found that PFP treated with SMS, T6-T10 could be stored for extended

period of 180 days without any major changes in chemical, microbiological and sensory characteristics, whereas T7 (with SMS,0.4g/l) was selected as the best treatment based on the overall acceptability.

Keywords: Palmyrah Fruit Pulp, Preservatives, Sensory evaluation

#### Introduction

Palmyrah (B.flabellifer) fruit is the oldest and most important tropical fruit. It is indigenous or naturalized throughout tropical and subtropical South and Southeast Asia. Palmyrah fruit is mostly used as fresh fruit for pinattu (Dried pulp) and oil cakes, but due to its perishable nature it cannot be stored for long period of time. Pulp is yellow in colour due to the presence of carotenoids (Provitamin A). It is a good source of vitamin C and contains appreciable amount of pectin [1]. Jeyaratnam (1986) [2] said that pulp contains appreciable amount of saponin and also believed that pulp provides dermatitis relief.

During peak of harvest season (Aug- Oct) large quantity of fruits are wasted due to limited shelf life in storage. In order to make the PFP available during the off season it has to be preserved with lengthened shelf life. Sales centres of Palmyrah Development Board, Katpakams sell bottled PFP to prepare fruit base edible products. But during storage period colour of the bottled pulp turns to yellowish brown. Despite the fact it has to be developed with favourable chemical treatment for the preservation of PFP. Because of its high fermentable nature under the influence of microbes, it is dried as Pinattu for short term preservation. But it is also preserved by making panampanam (diluted drink), cordial, crush and jam with moderate shelf life.

Sodium benzoate (SB) and Potassium metabisulphite (PMS) are commonly used as preservatives for long term storage of fruit pulp because of their better antimicrobial activity [3]. The maximum level for the use of these chemicals in fruit preservation including pulp and purees as described in the Codex Standards adopted in 2001 and 2006 are 1000 mg/kg SB as benzoic acid and 500 mg/kg PMS as residual  $SO_2$  [4]. Keeping in view these facts, this study was undertaken to find out the inhibitory effect of SB, SMS and both in different ratio with varying concentrations for microbial, chemical, physical and sensory quality of PFP stored at room temperature.

The aim of this study was to extend the shelf-life of the PFP by determining the best proportions of food additives like Sodium benzoate and Sodium metabisulphite (SB, SMS and both in combination) to be applied for preservation of PFP at room temperature (30°C). If storage of pulp can be improved for a long period, both PFP and its based food products will increase earnings in the Sri Lanka domestic and foreign markets.

#### Material and methods

This research was approved by Research and Development division of Palmyrah Development Board.

Determination of Microbial Count

The method of Sri Lankan Standard: 516 Part 1: 1991 [5] was used.

Preparation of Nutrient Agar Plates

Plate Count Agar (PCA) HIMEDIA Laboratory Pvt. Ltd medium (2.35g) in a 250ml conical flask was dissolved in 40ml of distilled water by heating in a water bath, made up the volume to 100ml with the same, plugged with cotton wool well, sterilized at 121°C and 15lb in-2 pressure for 15 min and then allowed to cool to 45°C.

#### Serial dilution

Sample (10g) was transferred into a labeled sterile dilution bottle, made up the volume to 100ml with peptone (HIMEDIA) water (peptone 1g, NaCl (HIMEDIA) 8.5g made up to1000ml with water) under the sterile condition. Aliquots of it were taken after the thorough mixing by using vortex mixer (VELP SCIENTIFICA ZX3) and repeated the same process to obtain required dilution.

# Microbial Count

Diluted sample (1ml) was transferred into each sterile plate. 20ml portion of the medium was poured to each plate in the laminar flow chamber (BIOBASE), mixed gently and allowed it to cool at room temperature. Plates of different dilutions were incubated at 37°C for 48hrs and the appeared colonies in the plates were counted and the total colonies were calculated. This experiment was repeated twice and the mean values (CFU/g) of these were calculated.

Physicochemical analysis

Total Soluble Solids (TSS)

Total soluble solids (TSS) of each sample were determined directly by using Refractometer (HSR500, Japan) at room temperature and expressed in terms of <sup>0</sup>Brix value.

# Acidity

The method of SLS: 730:2010 [6] was used. The acidity of the given sample was determined as citric acid (%w/w) by titrating 10ml of sample against 0.1 N NaOH (SIGMA) using phenolphthalein (SIGMA) as an indicator.

### pH

Homogenized sample (25ml) was taken in a clean beaker (25ml) and its pH was measured by using a digital pH meter (Sension PH 31-Spain) at room temperature.

# Sensory evaluation

The method described by Larmond (1977) [7] was used. Selected sample was evaluated by a panel of judges from Palmyrah Research Institute staff with Oral Consent Scripts for sensory characteristics like colour, flavour, texture, mouth feel and over all acceptability. The judges were provided with prescribed questionnaires to record their observation. The information contained on the performance was 5 = Like very much; 4 = Like slightly; 3 = Neither like nor dislike; 2 = Dislike slightly; 1 = Dislike very much. The panelists expectorated the sample and rinsed mouth using distilled water between samples.

# Preparation of PFP

Well ripened Palmyrah fruits available in plenty in their season at Kaithady, Northern Region of SriLanka, were washed twice with potable water and their tepals (tops) were removed then again washed with potable water and dipped in hot water for few seconds. Then ectocarp (skin) was peeled manually, the remainder (nutlets) was macerated with warm water (nutlet: water in ml = 1:100). Diluted pulp (PFP) was extracted manually using sieve after the removal of seeds and insoluble fibres.

# Adjustment of pH

Initial pH of the PFP was measured with pH meter (Sension+ PH 31-Spain) and then the pH of the pulp was adjusted to proper pH 3.8 with concentrated solution of food grade commercially available citric acid and mixed well.

### Blending

Acidified pulp was blended by using electric blender at low speed for 5 min.

# Effect of heating at 80°C for 30min Pasteurization in the preservation of PFP

PFP (pH3.8) poured into the capped clear glass bottle (100ml, without the addition of preservatives) was heated in a thermostatic water bath at 80°C for 30 minutes and then allowed to cool to room temperature [8,9] and stored for a period of 150 days. Aliquots of them were taken for the analysis.

# Effect of preservatives in the preservation of PFP

Common preservative Sodium Benzoate and Sodium metabisulphate (Food Grade) available in local market were used. The PFP was heated in a thermostatic water bath GEMMYCO at 90oC for 20 sec., preservatives were added according to the TABLE 1, mixed well and they were transferred into clear sterile glass bottles separately and capped well. The bottled pulp was heated in a thermostatic water bath at 80°C for 30 min and then allowed to cool to room temperature [8,9]. They were stored at room temperature for a period of 180 days. Aliquots of them were taken in 30 days interval for the analysis.

**Table 1:** Concentrations of different preservatives used in preservation of PFP, alone or their combination at varying ratio

Treatments	S B %	, SMS%,
Treatments	(w/v)	(w/v)
T1	0.04	
T2	0.08	
T3	0.12	
T4	0.16	-
T5	0.2	-
T6	-	0.03
T7	=	0.04
T8	-	0.05
T9	-	0.06
T10	-	0.07
T11	0.02**	0.015**
T12	0.04	0.02
T13	0.06	0.025
T14	0.08	0.03
T15	0.1	0.035

\*Half of the concentration of each preservative used alone before was used together here.

### Statistical analysis

Results obtained from chemical analysis (pH, brix and acidity) with three replicate were subjected to three way ANOVA. The significant difference among the treatments was tested in Least Significant Difference (LSD) at 5 % level of significance using SAS (version 9) System software.

Friedman non-parametric statistical method was used to analyze the sensory evaluation data based on 5-point hedonic scales. In this data analysis 95% confidence interval was considered, and analysis was done using Minitab 13 software.

#### **Results and Discussion**

Palmyrah fruit and their products have gained considerable importance by contributing significantly to the economy of Sri Lanka. On the other hand freshly extracted pulp is highly attractive in appearance and possesses good taste and aroma, but it deteriorates rapidly in 24h. This is mainly due to fermentation caused by moulds, yeasts and bacteria. The enzymes secreted by them may affect the colour and flavour adversely. Chemicals present in the pulp may react with one another and spoil its taste and aroma. Air coming in contact with the product may react with the glucosidal substances present in it. This deterioration must be avoided by application of the food preservation principle which first involves the prevention or delay of the microbial spoilage.

The present study was carried out to identify a suitable chemical preservative/s such as sodium benzoate, sodium metabisulphate either alone or in combination for satisfactory storage of PFP at room temperature. Efficiency of preservation and storage behavior of fruit pulp is depended on physicochemical characteristics such as acidity, pH and Total Soluble Solids (TSS) and biological parameters. Period of storage had shown a pronounced effect on physicochemical attributes of chemically preserved PFP.

The mean TPC was significantly increased from 0 to 9 cfu/g for T5 to T1. A maximum mean value was recorded in T2 while minimum value was observed in T4. Treatments T6-T15 showed growth of microorganism at 180 days of storage but counts (cfu/g) were in the acceptable range given in SLS 730: 2010. Hence chemical preservatives decreased the microbial load significantly in PFP. These results are in accordance with the findings reported by Hussain et al., (2003) [12] and Hashmi et al., (2007) [13] for mango pulp.

### Microbiological evaluation

Benzoic acid inhibits the growth of mold, yeast [10] and bacteria. It is either added directly or created from reactions with its Sodium, Potassium or Calcium salt. The mechanism starts with the absorption of benzoic acid into the cell.

If the intracellular pH changes to 5 or lower, the anaerobic fermentation of glucose through phosphofructokinase is decreased by 95%. The efficiency of benzoic acid or benzoate is thus depended on the pH of the food [11]. Sodium metabisulphite releases  $SO_2$  gas when added to water,  $SO_2$  kills yeasts, fungi and some bacteria and also it acts as an antioxidant.

. \*Microbial analysis of fresh PFP showed that total palate count (TPC) at the initial time was 2x106 cfu/g and also heat treated PFP at  $80^{\circ}$ C (without the addition of chemical preservatives) was spoiled before 15 days whereas the pulp containing preservatives (T1-T15) exhibited no microbial growth up to 120 days period of storage. At 150 days of storage,

Physicochemical evaluation Acidity and pH

There were interaction between preservatives, concentrations and storage period for acidity values while except preservatives for pH. Significantly higher mean pH was observed for PFP treated with SB (4.32) when compared with SMS (4.24) and both SB, SMS (4.21) and there were significant different (p<0.05) between mean pH of the pulp with the storage period while which was decreased with period of storage. PFP treated with SMS (T6-T10) and both SB, SMS (T11-T15) showed less increase in pH compared with PFP treated with SB (T1-T5). This may be due to either utilization or neutralization of acidic compounds present in the pulp otherwise compound/s secreted by organism. This condition may facilitate

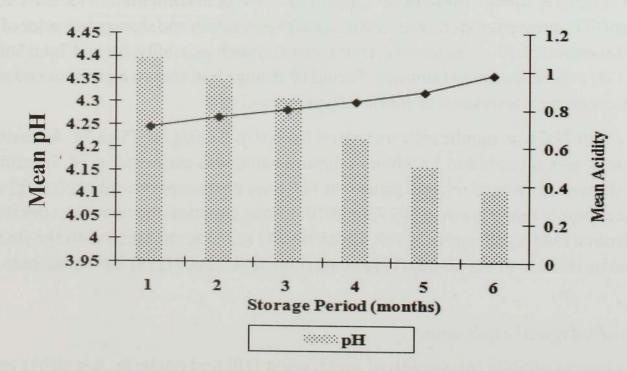


Fig 1: Change in pH and acidity of PFP incorporated with preservatives during the period of storage

organism/s to prolong their growth and thereby leads to deteriorate the pulp. Abbassi et al., (2009) [14] attributed the increase in pH and the decrease in titrable acidity with increased storage period of the mangoes.

The results relating to the increase in acidity and decrease in pH (Figure 1) during the storage of PFP are in complete agreement with other researchers [15]. pH plays dual role in the fruit juices by acting as a flavour promotion and preservation. Decrease in pH of the fruit pulp samples proportional to increase in acidity has been confirmed by several researchers and may be attributed to the presence of SB in the pulp samples [16, 17].

Significantly higher mean acidity was observed for PFP treated with SB (0.88%) while no significance difference between pulp with SMS (0.79%) and both SB, SMS (0.80%). Change in acidity of PFP with the period of storage has been showed in Figure 1 while there were no significant different (p<0.05) between 60, 90 and 120 days of the storage. While that acidity was significantly increased from 30-180 days. PFP treated with SMS (T6-T10) and both SB, SMS (T11-T15) showed less increase in percentage of acidity compared with PFP treated with SB (T1-T5). The increase in acidity may be ascribed to rise in the concentration of weakly ionized acid and their salts during storage and also due to formation of acid by degradation of polysaccharides and oxidation of reducing sugars or by breakdown of pectin substances and uronic acid [19, 17]

### Total Soluble Solids (TSS)

There were interaction between all factors such as preservatives, concentrations and storage period. Amin *et al.*, (2008) reported the effect of time of fruit harvest affects the fruit quality. The variability in TSS in the PFP might be attributed to the alteration occurring in cell wall structure during ripening process. Moreover, various hydrolytic enzymes also affect complex carbohydrates changing them into smaller compounds. Significantly higher mean °brix was observed for PFP treated with both SB, SMS (13.00) when compared with SMS (11.02) and SB (12.15). TSS was significantly increased gradually up to a storage period of 180 days (TABLE 2). While there were no significant difference between 60 and 90 also 120 and 150 days of storage. PFP treated with both SB, SMS (T11-T15) and SB (T1-T5) showed more increase in °brix compared with PFP treated with SMS (T6-T10) while there was significance different between treatments.

About half of the soluble sugars of PFP are mainly composed of fructose (3.4%), with about 6.6% sucrose and 3.5% glucose. The high sugar content of pulps from ripe fruits might be attributed to the transformation of starch into soluble sugars under the action of phosphorylase enzyme during ripening [19, 20] and water soluble pectin from insoluble proto pectin in lime squash and fruit bases, respectively [21, 22].

**Table 2:** Effect of storage on TSS of the PFP (°Brix)

Treatments			1 1614	Stora	age (day	ys)	husha	in the
	30	60	90	120	150	180	Mean	±SD
T1	11.83	11.84	11.89	11.95	11.90	12.00	11.9	0.06
Т2	12.2	11.96	12.01	11.98	11.90	12.00	12.01	0.09
Т3	12.17	12.03	12.11	12.48	12.25	12.50	12.26	0.18
T4	12.26	12.13	12.15	12.5	12.35	12.60	12.33	0.17
T5	12.43	12.17	12.19	12.38	12.25	12.30	12.29	0.09
Т6	10.79	10.93	10.97	10.95	10.98	11.00	10.94	0.07
T7	10.86	10.90	10.93	10.98	10.95	11.00	10.94	0.05
Т8	10.77	10.83	10.9	10.89	10.78	10.80	10.83	0.05
T9	10.86	10.92	11.26	11.45	11.40	11.50	11.23	0.25
T10	10.77	10.83	11.09	11.45	11.40	11.50	11.17	0.29
T11	12.65	12.9	12.91	12.95	12.90	13.20	12.92	0.16
T12	12.84	12.85	12.87	12.90	12.85	13.20	12.92	0.13
T13	12.86	13.03	12.29	13.20	12.95	13.60	12.99	0.39
T14	12.77	12.85	13.00	13.40	12.99	13.60	13.10	0.3
T15	12.71	12.78	13.01	13.30	13.20	13.60	13.10	0.31
Mean	11.92	11.93	11.97	12.18 <sup>b</sup>	12.07ь	12.29a		
±SD	0.86	0.85	0.78	0.88	0.82	1.00	alberadie	SHEW I

Each value in the table is represented as mean  $\pm$  SD (n = 3). Values in the mean row followed by a different letters (a-d) are significantly different (p<0.05).

# Sensory evaluation

Every fruit is selected by its visual appearance because colour of fruit is main attribute for judging the eatable quality of fruit and the same process is applied for the colour of PFP in this research. The values for colour of all the treated samples decreased during storage at ambient temperature. The PFP from various varieties collected from different production sites were not exactly at the similar ripening stage thus they may vary in colour and other sensory characteristics. Aina & Oladunjoye (1993) [23] reported that the colour change in mangoes is primarily associated with several biochemical changes, both degradation and synthesis of various classes of molecules including carotenoids in fruit.

A number of biochemical reactions or metabolic activities are involved in the ripening process of mango fruit such as increased respiration, ethylene production, change

in structural polysaccharides causing softening, degradation of chlorophyll and synthesis of carotenoids, changes in carbohydrates or starch conversion into sugars, organic acids, lipids, phenolic compounds and a number of volatile compounds. All these changes lead to ripening of fruit with softening of texture to acceptable quality. These factors predominantly contribute towards developing a total sensory profile of the mango fruit [24].

The colour of T2 and T3 was spoiled and turned yellowish brown during 90 days of storage interval. Median values of colour score for T7 and T8 is high (44.5) when compared with other treatments while T3 showed very less score of median and also this median value decreased with increase concentration of preservatives (TABLE 3).

**Table 3:** Effect of selected treatments on median value of sensory analysis at 180 days of Storage

	Flavour	Colour	Mouth feel	Texture	Overall acceptability
T2	14.5	14.5	17	19.5	15
Т3	15.5	13	17	19.5	13
T7	42	44.5	37.5	31.5	37.5
T8	42	44.5	40.5	36.5	30
T12	37.5	34	39	41	33.5
T13	37.5	38.5	38	41	32.45

Flavour is comprised of aroma and taste. The score for flavour decreased for PFP during storage at room temperature. Flavour score of T7 and T8 were higher than that of T12 and T13 during 180 days of storage while the scores noted for T2 and T3 were very less. Score of overall acceptability for T2 and T3 was less than other treatments when compared with others and median overall acceptability score at initial time of storage was highest for T7 (Figure 2).

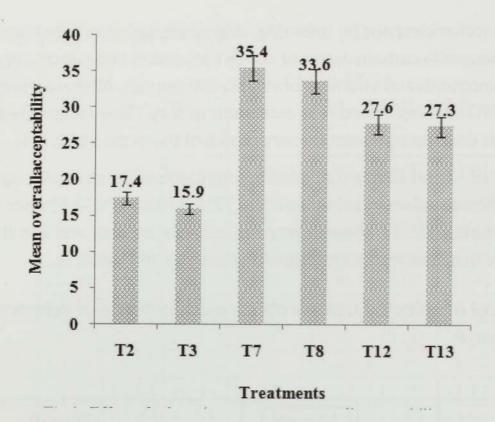


Fig 2: Effect of selected treatments on overall acceptability

Organic acid and sugars ratio primarily creates a sense of taste which is perceived by specialized taste buds of the tongue. Thus, sweetness due to sugar and sourness from organic acids are dominant components in the mouth feel of many fruits [25]. But in PFP mouth feel is due to bitter compounds called flabelliferins which vary with many factors such as place at which Palmyrah tree is grown, type of fruit and stage of ripening at which that fruit is tested. These factors play a major role in the assessment of its sensory qualities and acceptability [26]. In this study, T7 had highest overall acceptability at initial and 180 days of storage. Therefore based on the sensory characteristics the T7 was recognized as relatively better than the selected treatments (Figure 2).

# Conclusion

From this research, it is evident that storage of PFP incorporated with preservatives showed an increase in acidity and brix values besides the decreased level of microflora with time. However, according to the organoleptic evaluation done up to 180 days of period of storage PFP containing SB was rejected by panelists, whereas among the PFP containing SMS alone and combination of SB & SMS, PFP with SMS (0.4 g/l) was selected as better with respect to overall acceptability. Hence it is proved that pasteurization of PFP

incorporated with SMS (0.4 g/l) at pH 3.8 and 80°C for 30min is needed to store PFP for 6months without any loss of acceptable characteristics.

### Competing interests

The authors declare that they have no competing interests.

#### Authors' contribution

PN- made consultancy and revising the manuscript; RK carried out the research activities and revising the manuscript; SM carried out the research activities, statistical analysis and drafted the manuscript; SSV- coordinated & management of research activities. All authors read and approved the final manuscript.

# Acknowledgements

The authors thank Ministry of Traditional Industries and Small Enterprise Development, Sri Lanka for the financial support and also would like to express their gratitude to all the staff of Palmyrah Research Institute, Jaffna, Sri Lanka, for their kind support and assistance in the project.

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IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS) 9(6), pp. 51-57, 2016

# 2.1.2 Comparative Study on Phytochemical and Antimicrobial Activity of Different Solvent Extracts of Pinattu

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#### Abstract

Palmyrah (Borassus flabellifer) fruit is mostly used as fresh fruit, because of its perishable nature it is traditionally preserved as dried fruit pulp as pinattu and constituents of crude extracts of pinattu were evaluated. Samples were collected from three different branches of Palmyrah Development Board and extracted with different solvents as aqueous, methanol, ethyl acetate and petroleum ether then concentrated extracts were used for the study. Alkaloids and tannins were not detectable in all the extracts while aqueous and methanol extracts gave positive results for carbohydrates, proteins, phytosterols, saponins, flavonoids, phenols and amino acids and fats and fixed oils. Spectroscopic determination of total phenolic, flavonoids and saponin content were significantly (p<0.05) different among the solvents and the highest amount was identified, in aqueous extract (19.92±0.5)mg/g, methanol extract (0.18±0.0)mg/g and methanol extract (509.88±4.18) mg/g respectively. Based on their diameter of the zone of inhibition least MIC of aqueous extract for Staphylococcus (1.4±0.1cm), E.coli (1.2±0.0cm), Pseudomonas (1±0.0cm) and methanol extract for E.coli (1.4±0.1cm) and Pseudomonas (1.1±0.00cm) was 0.5mg/ml. The Salmonella and Bacillus was showed 0.75mg/ml for aqueous extract while 0.25 and 1.0mg/ml for methanolic extract respectively. Klebsiella was showed 0.25mg/ml for both aqueous extract (1.1±0.0cm) and methanol extract (1.35±0.0cm). Highest inhibition zone was observed for 1 mg/ml of aqueous extract (2.15±0.2cm) in Protease when compared with positive control (1.9±0.1cm). This finding shows that crude aqueous and methanol extract of pinattu contains high amount of phytochemicals, exhibit significant antibacterial activity with relatively lower MIC (≤ 1mg/ml) when compared with ethyl acetate and petroleum extracts.

Key words: Antibacterial activity, Pinattu, Phytochemicals, Saponin and Solvents

#### INTRODUCTION

Total phenol, total saponin and the total flavonoids content determination in Palmyrah dried fruit pulp leather (pinattu) extracts is interesting helpfulness for global nutritionists due to their valuable effects on human and animal health. Thus, investigation of various phytochemicals present in Palmyrah based product is the noble study in order to understand their health benefits.

Phenolics are one of the key secondary metabolites existing in the plant kingdom. They are low molecular weight compounds (mol. wt. <2000 amu) universally present in all tissues of higher plants and play an important role during the development of a plant. They have multiple biological effects, including antioxidant activity (Gulcin *et al.* 2005), anti-inflammatory activities and metal chelation properties (Rice-Evans *et al.* 1997).

Flavonoids the most common group of polyphenolic that are found universally in plants. These are widely distributed in plant achieving many functions. They are important in plant for normal growth development and defense against contamination and injury (Kähkönen et al. 1999), anti-aging (Hadnick et al. 1998), antioxidant (Croteau et al. 2000), antibacterial and antifungal activities (Hassan, 2010), anticancer, anti-cardiovascular disease and anti-inflammatory (Nijveldt et al. 2001).

Saponins are among several plant compounds which have beneficial effects. Among the various biological effects of saponins are antibacterial, anticancer (Mathers, 2002) and antiprotozoal (Avato, 2006). Saponins are surface-active glycosides with detergent, wetting, emulsifying, and foaming properties (Mitra and Dungan, 1997). The palmyrah fruit pulp contains pectin, sugar, carotenoids in addition to numerous steroidal saponins (flabelliferins) (Thabrew and Jansz, 2004). This was suspected to reduce weight gain in ICR mice (Ariyasena et al. 2002) and also inhibit the increase in blood glucose after a glucose intake, while improving the level of faecal glucose, thus suggesting an inhibition of intestinal glucose uptake (Uluwaduge *et al.* 2005).

Nowadays antibiotics are effectiveness against serious bacterial infections. However, only one third of the infectious diseases known have been treated from these synthetic products. This is because of the emergence of resistant pathogens due to the indiscriminate use, incessant and misuse of antibiotics. One of the methods to reduce the resistance to antibiotics is by using antibiotic resistance inhibitors from plants. Plants are known to produce a variety of compounds to defend themselves against a variety of pathogens. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant pathogens (Sen and Batra, 2012). Palmyrah palm have

been used as traditional treatments for numerous human. Hence, researchers have recently paid attention on biologically active compounds, isolated from plant species used in herbal medicines for the development of novel drugs and functional foods.

Although various studies have been reported on various plant extracts, while there was little research has been conducted on Palmyrah palm. Therefore, this study was conducted to determine the amount of the total phenol, flavonoid and saponin content by using spectroscopic methods and antimicrobial activity of various extracts of Palmyrah dried fruit pulp leather (pinattu).

# MATERIALS AND METHODS

This research proposal was approved by Research and Development division of Palmyrah Development Board and research committee of Uva Wellassa University (6.9826° N, 81.0768° E), Sri Lanka.

# Collection of sample

Palmyrah fruit pulp leather was obtained from the three different branches of Palmyrah Development Board (PDB) then cut into small species and pool together. After that 100 g of sample was weighted in random manner.

# Preparation of plant extracts

Palmyrah fruit pulp leather (100 g) was extracted in a soxhlet extractor for 24 hours with petroleum ether (boiling point 40-60°C, polarity index: 0.1), ethyl acetate (boiling point 76.5°C, polarity index: 4.3), methanol (boiling point 65°C, polarity index: 6.6) and water (polarity index: 9) separately based on polarity index. The extracts were evaporated under reduced pressure using rotatory evaporator (IKA), then extracts stored at 4 °C (Gulcin, 2005). All the extractions were performed in duplicate.

# Qualitative evaluation of phytochemicals

Petroleum ether, ethyl acetate, aqueous and methanol extracts were tested for the presence of phytochemical constituents by performing the standard methods such as Mayer's test, Dragen-dorff's test and Wagner's test for Alkaloids, Molisch's test and Fehling's test for carbohydrates, foam test for saponins, Libermann-Burchard test and Salkowski's test phytosterols, ferric chloride test for phenols., alkaline reagent test and

lead acetate test for flavonoids, Ninhydrin test and Xanthoproteic test for protein and aminoacids, gelatin test for tannins, modified Borntrager's test for glycosides and test for fat and oil.

# Determination of total phenolic content

The total phenolic content of the pinattu extracts was determined using the Folin-Ciocalteu reagent (Maurya and Singh, 2010). The reaction mixture contained 0.5ml of diluted extracts, 2.5ml of freshly prepared 10 % diluted Folin-Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. Mixtures were kept at ambient conditions for 30 min to complete the reaction. The absorbance at 760 nm was measured. Gallic acid was used as standard and the results were expressed as mg gallic acid (GAE)/g pinattu.

# Determination of total flavonoid content

Total flavonoid content was determined using aluminium chloride (AlCl $_3$ ) according to a known method (Ordonez et al. 2006) using quercetin as a standard. The plant extract (3 ml) was added to 5% NaNO $_2$  (0.3 ml). After 5 min at room temperature, AlCl $_3$  (0.3 ml, 10%) was added. After further 5 min, the reaction mixture was treated with 2 ml of 1 M NaOH and the absorbance was measured at 510 nm. The results were expressed as mg quercetin (QE)/g pinattu.

# Determination of total saponin content

Total saponins contents in pinattu extracts were estimated (Hiai et al. 1976). Different extracts of  $0.25\,\text{ml}$  of solution was taken and  $0.25\,\text{ml}$  of vanillin reagent (8%, w/v) was added. Then  $2.5\,\text{ml}$  of 72% (v/v) sulphuric acid was added slowly on the inner side of the wall. After mixing the content tubes were kept in a water bath at  $60^\circ\text{C}$  for  $10\,\text{min}$  then cooled in icecold water bath for  $4\,\text{min}$ . Absorbance was measured at  $544\,\text{nm}$  using spectrophotometer against the reagent blank. Quillaja saponin was used as a standard and the content of total saponins was expressed as mg Quillaja saponin(QS) equivalents /g pinattu.

# Statistical analysis

The results obtained from the four extracts with three replicate were subjected to analysis of variance by complete randomized design (CRD). The significant difference among the extracts was tested in Least Significant Difference (LSD) at 5 % level of significance using SAS software.

#### RESULTS

# Qualitative evaluation of phytochemicals

Phytochemical screening in pinattu (Figure: 1) showed that all the selected tests gave positive results for any of the crude solvent extracts except tannin test (Table 1).

# Quantitative evaluation of phytochemicals

Aqueous, methanol, ethyl acetate, and petroleum ether extracts were prepared to examine the total phenolic, flavonoid and total saponin content of the dried Palmyrah fruit pulp by using spectrophotometry.

Total phenolic content was estimated by using Folin-Ciocalteu reagent. Total phenolic content of pinattu was dependent on different solvent extracts and expressed as milligrams of gallic acid equivalents (GAE) equivalent. Table summarizes that total phenolic compounds in solvent extracts varied widely, ranging from 0.011 and  $19.92\pm0.42$  mg/g expressed as gallic acid equivalents (GAE). Aqueous extract [ $19.92(\pm0.42)$ ] exhibited significantly (p<0.05) highest total phenolic content than methanolic extract [ $5.74(\pm0.06)$ mg/g]. There were no significant different between the extracts of ethyl acetate and petroleum ether. The amount of total phenol obtained from these extract was very less when compared with aqueous extract.

The content of flavonoid expressed as quercetin equivalents. Pinattu extract obtained from water not quantifiable while methanol extract showed  $0.18\pm0.00$  mg/g of flavonoid.

Total saponin contents in pinattu (% w/w) expressed as Quillaja saponin equivalents. Which was significantly different with various extracts such as water, methanol, ethyl acetate and petroleum ether was  $427.08(\pm 7.84)$ ,  $509.88(\pm 4.18)$ ,  $16.62(\pm 0.39)$  and  $34.08(\pm 0.86)$  mg/g respectively. Methanol extract gave highest saponin content among the selected extract.

# Antibacterial analysis

In the trail experiment of antibacterial activity assay, ethyl acetate extract of pinattu was not exhibited inhibition zone for all tested bacteria. It may be due to the low concentration of the extract in the solvent. Therefore, extracts of aqueous, methanol and petroleum ether obtained from pinattu was selected for antibacterial assay studies.

According to the two-way ANOVA results obtained for all test organisms showed p< 0.001 for both extract and concentration. Hence the main effects were highly significant at 99.9% probability level. Since p value for the interaction effect was found as< 0.001 so there is a significant interaction effect between extracts and concentrations showed at 99.9% level.

According to the Table 3 all the test bacteria gave highest inhibition zone diameter for aqueous extract except *Staphylococcus* and *Salmonella* while these two bacteria showed highest inhibition zone diameter for methanol extract in the meantime petroleum ether extracts showed lowest diameter for all the tests bacteria.

The antibacterial activity of both extracts with different concentration was evaluated according to their diameter of the zone of inhibition against various bacteria and the results were compared with the activity of the standard (chloramphenicol (0.1mg/ml) and solvent, serve as positive and negative control respectively.

Least MIC of Staphylococcus was 0.5 mg/ml for aqueous extract however 0.25 mg/ml for methanol extract (Table 5). Inhibition zone of Staphylococcus was showed no significant different between 1mg/ml concentration of aqueous (1.85±0.07 cm) and methanol (1.85± 0.07 cm) extracts. Zone of inhibition from Staphylococcus in the concentration of 0.75 and 0.5 mg/ml methanolic extract were significantly higher than that of 0.5 mg/ml of aqueous extract. E.coli was showed same least MIC (0.5 mg/ml) for both aqueous and methanol extract. Inhibition zone of E.coli was showed significant different between the concentrations (1.0 mg/ml, 0.75 mg/ml, 0.5 mg/ml, 0.25 mg/ml) of aqueous and methanol extracts. Least MIC of Pseudomonas was 0.5 mg/ml for aqueous extract though 0.5 mg/ ml for methanolic extract (Figure 2). Inhibition zone of Pseudomonas was showed no significant different between the positive and  $1 \text{mg/ml} [2.15 \pm (0.07)] \text{ cm}$  concentrations of aqueous extracts and 0.75 mg/ml and 1mg/ml [1.7± (0.00)] concentrations of methanolic extracts. Zone of inhibition for Salmonella were showed no significant different between the 1mg/ml concentration of aqueous [1.5± (0.00)] cm and 0.5mg/ml methanolic [1.5± (0.00)] cm extracts. Inhibition zone of Salmonella was showed significant different between the other concentrations of both extracts. Least MIC of Salmonella was 0.75 mg/ml for aqueous extract (Figure 3), however 0.25mg/ml for methanolic extract. MIC of Klebsiella was 0.25 mg/ml for aqueous extract and methanol extract. Inhibition zone of klebsiella showed 1.6  $\pm$  (0.00), 2.0  $\pm$  (0.14) cm for 1mg/ml concentrations of aqueous and methanolic extract respectively. Protease was showed highest inhibition zone for 1mg/ml of aqueous extract when compared with positive control. There were no significant different between the zones of inhibition obtained from Protease in the concentration of 1 and 0.75 mg/ml methanolic extract and 0.75 mg/ml of aqueous extract and also no significant different between the 0.5mg/ml concentration of aqueous  $[1.35\pm(0.07)]$  and methanol  $[1.3\pm(0.00)]$  cm extracts. MIC of Protease was 0.25 mg/ml for aqueous extract and methanol extract. Least MIC of *Bacillus* was 0.75 mg/ml for aqueous extract whereas 1mg/ml for methanol extract. Zone of inhibition obtained from *Bacillus* for 1mg/ml concentration of aqueous and methanol extract was  $1.55\pm(0.07)$ ,  $1.45\pm(0.07)$  cm respectively (Table 4).

#### DISCUSSION

Manoharan et al. 2014 also reported that phytochemicals screening in the pinattu showed positive results for steroids, triterpenoids, carbohydrates, saponin, flavonoids and proteins in varied amounts in the ethanolic and aqueous extracts while chloroform extract showed negative results for all tested compounds except for carbohydrate while glycoloids, alkaloids and tannins were not observed in any of the extracts. Most of these results agree with our qualitative analysis of pinattu.

Analysis of the total phenol and flavonoids content in plants materials is attracting thoughtfulness for pharmaceutical universal due to their beneficial effects on health of human and animal. Which are rich in phenolics are gradually being used in the food industry because these bioactive compounds prevent the lipids oxidative degradation and improve the quality and nutritive value of foods (Kähkönen et al. 1999). Phenolic compounds are one of the phytochemicals considered as secondary metabolites and these compounds derived from phenylalanine and tyrosine occurs ubiquitously in plants and is diversified (Naczk and Shahidi, 2004). In this experiment aqueous extract contained high amount of total phenolic content because which is in the plant depends on the type of extract, i.e. the polarity of solvent which are used in extraction. The solubility of the phenols is high in polar solvents and delivers high concentration of these compounds in the crude extracts during the extraction (Mohsen and Ammar, 2008). Palmyrah pinattu contained very less amount of flavonoids when compared with other tested phytochemical. These are probably the most important natural phenols and one of the most diverse and general group of bioactive natural compounds. These compounds contained a broad spectrum of chemical and biological activities including antioxidant properties of radical scavenging properties. Several studies reported that bioactive components concentrations are affected by plant species and plant variety (Shiraiwa et al. 1991), degree of maturity, growing environment, agronomic factors such as climate and soil, cultivation year, location grown, season (Oleszek, 1996), and extraction method (Onning, 1993). For example saponins frequently are isolated by boiling in methanol (Oleszek et al. 1992), ethanol (Oleszek, 1990) and n-butanol (Massiot, 1992).

In our study methanol and aqueous extract contained better antimicrobial activity. Duddukuri *et al.*, 2011 (Duddukuri, 1992) also reported that the antibacterial activity of methanol extract of *Borassus flabellifer* L. seed coat (soft outer shell) and examined against Gram positive bacteria i.e., *Bacillus subtilis*, *Staphylococcus aureus* and Gram negative bacteria i.e., *Klebsiella pneumonia* and *Serratia marcescens*. This showed consistently significant inhibitory activity. Furthermore, the minimum inhibitory concentration was ranged between 100µg to 1 mg/ml implying the significance of antibacterial activity.

#### CONCLUSION

This study was showed that among the four extracts, most of the biologically active phytochemicals were present in the aqueous and methanol extracts. Antibacterial activities with relatively lower MIC ( $\leq 1$ mg/ml) values, confirm that methanol and aqueous extracts of pinattu of *Borassus flabellifer* exhibit significant antibacterial activity when compared with other extracts. Therefore it could be considered as beneficial for further investigation on the palmyrah dried fruit pulp.

### Competing interests

The authors declare that they have no competing interests.

### Authors' contributions

WP- Internal supervisor of the research project; SM- External supervisor; SS-Undergraduate research student; SS & SM carried out the antimicrobial activity study, phytochemical studies and carried out statistical analysis; SS, SM &WP - Drafted the manuscript; SSV- coordinated & management of research activities. All authors read and approved the final manuscript.

## Acknowledgments

Authors would like to express their gratitude to the staff of Palmyrah Research Institute, Jaffna, Sri Lanka, for their kind support in the project also kind support given by Ms.K.Robika, is greatly appreciated.

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 Table 1: Phytochemical evaluation for various solvent extracts of pinattu

Phytochemical	Reagents	Results								
Test	used (test	Aqueous	Metha-	Petro-	Ethyl					
	performed)	polarity	nol (6.6)	leum	acetate					
and the same of the		index (9)		either	(0.1)					
		RE-REF		(4.3)						
Detection of alka-	Mayer's Test	Reddish	Yellow							
loids		brown								
	Wagner's Test	Reddish	Reddish	Reddish	Reddish					
		brown	brown	brown	brown					
in in the latest the l										
Detection of car-	Molisch's Test	Purple col-	Purple	Purple col-	Purple					
bohydrates		or ring	color ring	or ring	color ring					
		+++	+++	++	++					
	Fehling's Test	Reddish	Reddish	Blue	Blue					
		black pre-	black		Mashi					
		cipitate	precipi-							
		+++	tate+++		igmina Munica					
	After heated and hydrolysis			Green	Green					
Detection of sapo- nins	Foam Test	+++	++							
Detection of phy-	Salkowski's	++	+++	+	+					
tosterols	Test			1111111111						
makabahandar teri	Libermann	++++	++++	++	+					
harden 13	Burchard's test									
Detection of phe-	Ferric Chloride	Black	Black	Yellow	Yellow					
nols	Test	+	+							

Flavonoids test	In Alkaline Reagent test	++	++		
	In Lead Acetate test		Orange		
Duotoina aud A:	I W d	++	++		
Proteins and Ami-	In Xanthopro-	Reddish	Reddish		yellow
no Acids test	teic test	brown	brown		+
		++	++		
	In Biuret test:				
	Ninhydrin Test				
Tannins test	Gelatin test				
Fats and Fixed oils	Filter paper test	+++	++		
Glycosides test	Brontrager's Reagent test			++	++

**Table-2:** Spectroscopic determination of total phenol, total flavonoids and total saponin content of various extracts of pinattu.

Phytochemicals Solvent	Total phenol (mg/g)	Total flavonoid (mg/g)	Total saponin (mg/g)
Water (aqueous)	19.92(±0.42) <sup>a</sup>	0.00	427.08(±7.84) <sup>b</sup>
Methanol	5.74(±0.06) <sup>b</sup>	0.18±0.00	509.88(±4.18) <sup>a</sup>
Ethyl acetate	$0.11(\pm 0.00)^{c}$	ND	16.62(±0.39)°
Petroleum ether	$0.01(\pm 0.00)^{c}$	ND	34.08(±0.86) <sup>d</sup>

Each value in the table is represented as mean  $\pm$  SD (n=6). Values in the same column followed by a different letter (a-d) are significantly different (p<0.05), ND: Not determined.

**Table 3:** Effect of different solvent extracts of pinattu on inhibition zone (cm) of different bacterial species.

Solvent /Bacteria	Aqueous	Methanol	Petroleum Ether
Staphylococcus	0.94 <sup>b</sup>	1.30 <sup>a</sup>	0.22°
E.coli	0.86a	0.76 <sup>b</sup>	0.00°
Pseudomonas sp	1.08a	0.88 <sup>b</sup>	0.24°
Salmonella	0.52 <sup>b</sup>	1.25ª	0.00°
Klebsiella	1.37a	1.08 <sup>b</sup>	0.00°
Protease	1.24ª	1.14 <sup>b</sup>	0.00°
Bacillus sp	0.53a	0.29 <sup>b</sup>	0.00°

Each value in the table is represented as mean  $\pm$  SD (n = 3). Values in the same rows followed by a different letter (a-c) are significantly different (p<0.05).

Table 5: MIC of different crude extracts of pinattu against bacteria

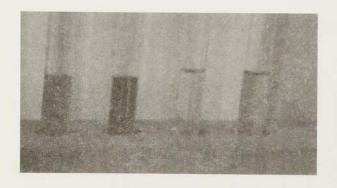
Extracts	Staphylococcus	E.coli	Pseudomonas sp	Salmonella	Klebsiella	Protease	Bacillus sp
Aqueous mg/ml	0.5	0.5	0.5	0.75	0.25	0.25	0.75
Metha- nol mg/ ml	0.25	0.5	0.5	0.25	0.25	0.25	1.0

Each value in the table is represented as mean  $\pm$  SD (n = 3). Values in the same rows followed by a different letter (a-c) are significantly different (p<0.05).

**Table: 4:** Effect of different concentrations of various extracts on inhibition zone (cm)

Concentration (mg/ml)	(+)	1.0	0.75	0.5	0.25	(-)	(+)	1.0	0.75	0.5	0.25	(-)
รทววดวดเกินปพาร	$2.4\pm(0.14)^a$	1.85±(0.07)°	1.45±(0.07) <sup>f</sup>	$1.4\pm(0.14)^{f}$	40	h0	2.3±(0) <sup>b</sup>	1.85±(0.07)°	$1.7\pm(0.00)^{d}$	$1.6\pm(0.00)^{e}$	1.35±(0.07)g	40
E.coli	$2.25\pm(0.07)^a$	1.75±(0.07) <sup>b</sup>	1.35±(0.07) <sup>d</sup>	1.2±(0.00) <sup>€</sup>	<sub>8</sub> 0	90	2.25±(0.07) <sup>a</sup>	1.6±(0.14)°	1.2±(0.00)€	$1\pm(0.00)^{if}$	±0	g0
ds svuowopnəs <sub>d</sub>	2.1±(0.14) <sup>b</sup>	2.15±(0.07) <sup>b</sup>	1.85±(0.21)°	1,4±(0.14)	я0	90	2.3±(0.00) <sup>a</sup>	$1.7\pm(0)^{d}$	$1.6\pm(0.14)^{d}$	$1.1\pm(0.00)^{f}$	ē0	<b>50</b>
ทุๅอนอนบุซร	2.25±(0.07) <sup>b</sup>	1.5±(0.00) <sup>c</sup>	1.1±(0.00) <sup>f</sup>	g0	g0	<sup>80</sup>	$2.4\pm(0.14)^a$	1.9±(0)€	1.75±(0.07) <sup>d</sup>	$1.5\pm(0.00)^e$	1.1±(0.14) <sup>f</sup>	g0
wileisdelia	2±(0.01) <sup>b</sup>	1.6±(0.00)°	1.4±(0.00) <sup>f</sup>	1.3±(0.00) <sup>g</sup>	$1.1\pm(0.00)^{h}$	i0	2.15±(0.07) <sup>a</sup>	2±(0.14) <sup>b</sup>	1.8±(0.14)°	$1.7\pm(0.14)^d$	1.35±(0.07) <sup>f</sup>	,0
Protease	1.9±(0.14) <sup>b</sup>	2.15±(0.21) <sup>a</sup>	$1.7\pm(0.14)^{c}$	$1.35\pm(0.07)^{d}$	$1\pm(0.00)^{e}$	.0r	$2.15\pm(0.07)^a$	1.7±(0.00)€	1.6±(0.00)€	$1.3\pm(0.00)^{d}$	$1. \pm (0.00)^{e}$	JO
ds sullionA	2.7±(0.00) <sup>b</sup>	1.55±(0.07)°	$1.1\pm(0.00)^{e}$	.0 <sub>t</sub>	.00	0 <sub>t</sub>	2.75±(0.07) <sup>a</sup>	1.45±(0.07) <sup>d</sup>	0,	<sub>4</sub> 0	0t	0 <sub>f</sub>

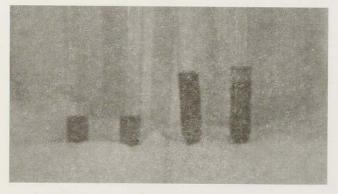
Each value in the table is represented as mean  $\pm$  SD (n = 3). Values in the same rows followed by a different letter (a-i) are significantly different (p< 0.05). (- control): respective solvent, (+ control): chloramphenicol (0.1 mg/ml)



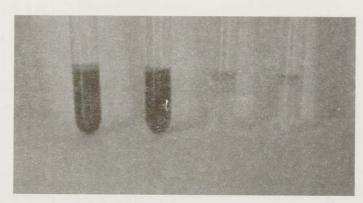
Result of Mayer's Test



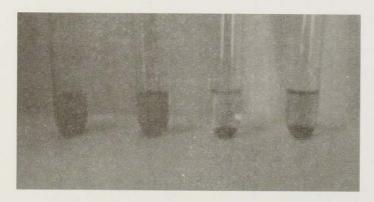
Result of Wagner's Test



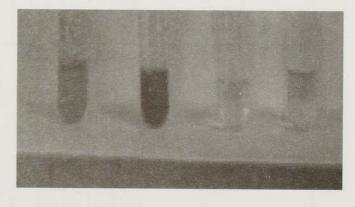
Result of Molisch's Test



Result of Fehling's Test



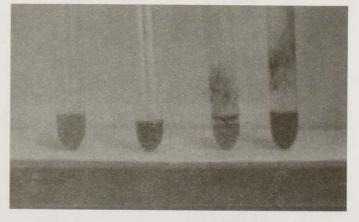
Result of Ferric Chloride Test



In Lead Acetate test



Gelatin test



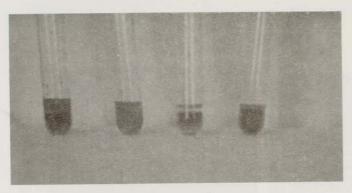
Borntrager's test



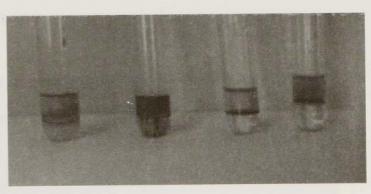
Result of the non-reducing sugar



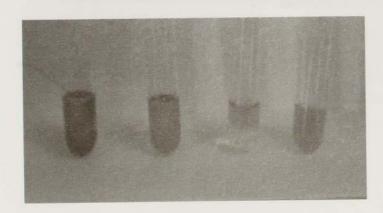
Result of the non-reducing sugar



Result of the non-reducing sugar



Result of the non-reducing sugar

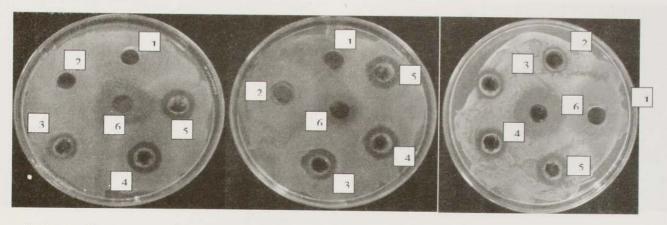


Xanthoproteic test



Biuret test

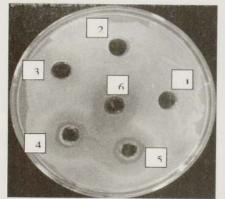
Figure 1: Qualitative phytochemical screening of palmyrah fruit pulp leather or pinattu



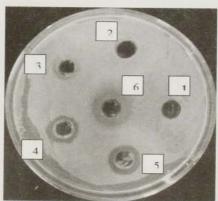
Salmonella spp

Klebsiella spp

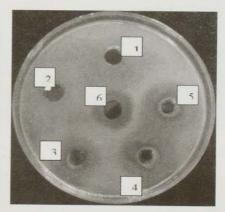
Staphylococcus spp



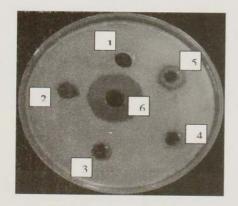
Escherichia coli



Pseudomonas spp

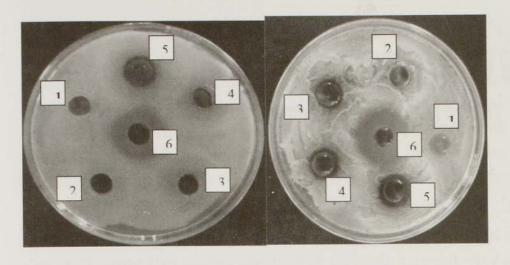


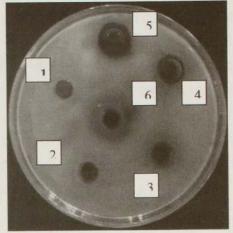
Protease spp



Bacillus spp

Figure 2: Antimicrobial activity of methanol extract of Borassus flabellifer L. dried pulp or pinattu against different bacterial species: (-) control -1; 0.25 mg/ml -2; 0.5 mg/ml -3; 0.75 mg/ml -4; 1.mg/ml -5; (+) control -6

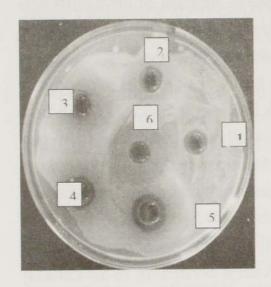




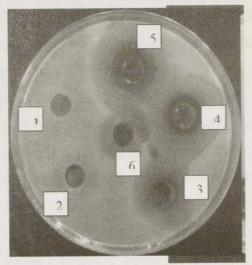
Salmonella spp

Klebsiella spp

Staphylococcus spp



Pseudomonas spp



Protease spp

Figure 3: Antimicrobial activity of aqueous extract of Borassus flabellifer L. dried pulp or pinattu against different bacterial species: (-) control -1; 0.25 mg/ml -2; 0.5 mg/ml -3; 0.75 mg/ml -4; 1.mg/ml -5; (+) control -6

International Journal of Scientific Research in Agricultural Sciences, 3(3), pp. 062-072, 2016

# 2.1.3 Enhance the quality of palmyrah (Borrasus flabellifer) jaggery

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#### **Abstract**

Palmyrah (Borrasus flabellifer) jaggery industry is one of the ancient and large cottage industries in Palmyrah society and it is produced by concentrating the inflorescence sap of Palmyrah palm (Sweet Toddy) to a thick consistency. The Palmyrah jaggery made in household level has bitter taste due to the presence of excess amount of lime with low purity. During the period of May 2013 to June 2013 four field experiments were carried out to optimize the amount of quick lime (CaO) for a suitable Palmyrah sweet sap collection in the areas of Jaffna district, using Randomized Complete Block design. This experiment was carried out with four different concentrations of quick lime such as 5.0, 3.0, 2.5 and 2.0 grams of CaO in liter of sap. The quick lime used in this study was produced from sea shells with 96% of purity at Palmyrah Research Institute laboratory. Existing Palmyrah jaggery available in the market is inferior in its quality as liquefaction and deterioration of color. Through this research attention to be made to eradicate its hydroscopic nature in the existing jaggery to upgrade it. Physicochemical quality parameters of commercially available samples and produced jaggery samples were studied according to Sri Lankan standards for jaggery. Quality of jaggery was enhanced using sweet sap with acceptable lime with higher degree of purity. For suitable jaggery production, 2.508 ± 0.411 grams of lime (96 % purity) per one liter of sweet sap were optimized and this jaggery was scored high score than jaggery from other field experiments.

# Introduction

Palmyrah (Borassus flabellifer) palms are mainly distributed in north and east provinces of Sri lanka and it is known to be a valuable economic plant [1]. There are varieties of Palmyrah products available in the local and international market. Palmyrah leaves are used for thatching, mats, hats and different handicrafts, stalks are used to make fence and black timber is used in constructions.

The young, either male or female inflorescences of Palmyrah palm produces the fresh sap. The fresh sap is a sweet clear watery liquid and it is a good source of vitamins: riboflavin, vitamin B12 vitamin C, thiamine and nicotinic acid and minerals: calcium, iron, zinc, copper and phosphorous [2]. Fresh sap plays a role in the indigenous medicine as anti-diabetic, anti-hyperglycemic and anti-hyperlipidemic agent [3]. This unfermented fresh sap is called as sweet sap or 'pathaneer' [4], it can be consumed freshly or prepare the sap based products. The sweet sap is also allowed to ferment to yield a mild alcoholic beverage which is called "toddy" [5] [6] [7].

The sweet sap based products as jaggery, treacle and sugar candy are made out by concentrating the sweet sap to the suitable consistency in open pans. Palmyrah palm jaggery is the main marketable product and which is more nutritious sap based product, containing 1.04 % protein, 0.19 % fat, 76.86% sucrose, 1.66% glucose, 3.15% total minerals, 0.861 % calcium, 0.052 % phosphorus; also 11.01 mg iron per 100 g and 0.767 mg of copper per 100 g [2]. Jaggery, a sugar rich food product is produced all over the world under different names, such as Gur (India), Desi (Pakistan), Panela (Mexico and South America), Jaggery (Burma and African countries), Jaggery (Sri Lanka), Htanyet (Myanmar), Panocha (Philippines), Rapadura (Brazil), and Naam Taan Oi (Thailand) [8]. It is consumed directly or used for preparation of sweet confectionery items and ayurvedic/traditional medicines [9].

Fermentation of sweet sap is caused by micro organism and occurs during the period of collection right up to processing. The fermenting organisms are dominated by yeasts, particularly *Saccharomyces cerevisiae* [10]. Traditionally quick lime is used to prevent the fermentation of sweet sap in the preparation of jaggery at the cottage industries in Jaffna peninsula [1, 10]. It is generally obtained from furnacing oysters (mostly sea shell used in Jaffna peninsula) using coconut husk charcoal and the temperature is not up to the optimum level to produce pure lime. Therefore, the quick lime shows a high possible for adulteration by impurities such as ash, sand, unheated sea shells. A n o t h e r majori ssue is the Local tappers are using excess amount of Lime which present in sweet toddy. However, there are no studies carried out until now about the composition and purity of quick lime used in local production. Hence this study is to initiate the study on preserve quality for extends the shelf life of palm jaggery and to standardize and improve the techniques of production of jaggery. This leads to increase the local and export market demand for palmyrah jaggery.

# **MATERIALS AND METHODS**

This research work mainly focuses on the physicochemical properties of jaggery samples and enhance their quality to the international grade via purified and optimized the quick lime

# Determine the physicochemical properties of Sweet sap and Jaggery samples

Physicochemical properties of commercially available sweet sap and jaggery samples, which are collected from different areas in Jaffna district, were analyzed for this study and the physicochemical properties of the jaggery samples were compared with Sri Lankan Standards for jaggery [11].

# Evaluate the purity of quick lime obtained from the traditional kiln

The quick lime samples were randomly collected from two different traditional kilns in Anaikoddai area and Pandaitharippu area. These collected quick lime samples were cooled down to the room temperature and packed in a moisture proof container [12]. Moisture free quick lime samples were taken on a clean surface to make cone and it was quartered to get a representative sample of small fragments.  $100 - 200\,\mathrm{g}$  of this moisture free quick lime sample fragment was ground well via using the pestle and mortar and it passes through a No. 7 mesh sieve.  $0.50\,\mathrm{g}$  of sieved quick lime sample was transfer into the  $300\,\mathrm{mL}$  Erlenmeyer flask containing  $20\,\mathrm{mL}$  of CO free distilled water and plugged the flask immediately. The mixture was swirled and boiled for 2 minutes. Then  $150\,\mathrm{mL}$  of distilled water and  $15\,\mathrm{g}$  of sucrose were added to the above mixture. The flask was shaken well for 5 minutes and allowed to stand for 30 - 60 minutes. Then it was titrated with the standard HCl solution by using the phenolphthalein as an indicator and finally the purity of quick lime samples were estimated.

# Prepare the optimum amount of purified quick lime

Naturally occurring sea shell were randomly collected in the coastal area. Two sets of  $10\,\mathrm{g}$  of sea shell samples were taken into the crucible plates and kept into the furnace at  $750\,\mathrm{^oC}$  for  $10\,\mathrm{minutes}$  and  $20\,\mathrm{minutes}$ . The above steps were repeated again for the various temperatures such as  $850\,\mathrm{^oC}$ ,  $950\,\mathrm{^oC}$  and  $1000\,\mathrm{^oC}$ . Then the purity of the produced lime samples was analyzed and find out the optimum purity of quick lime

# Produce the quality improved sweet sap

Matured and healthy female Palmyah palms were selected near jaggery producing areas of Jaffna peninsula in the period of May to June. The well prepared and healthy inflorescences were identified for the sap collection. The selected inflorescences were tapped in the evening between 5 to 6 p.m to ooze out sweet sap and these were collected in to the well hygienic quick lime applied earthen pots. The known amount of 96 % of purified quick lime was applied into the earthen pots to prevent the fermentation under the previous experience of the local tappers. The different amount of quick lime

such as 5g/L, 3g/L, 2.5g/L and 2g/L were applied into the sweet sap collection which are indicate as a field trial such as T1, T2, T3 and T4 respectively

The nylon covering nets were used on pots to avoid the fall/entry of ants, insects and spiders during the collection period of sweet sap. The sixteen hours later, the collected sweet sap samples in earthen pots were quickly transferred into the separate high molecular high density polyethylene (HMHDPE) bottles. Initially, these bottles were washed thoroughly with boiled distilled water, drain out completely and capped immediately to avoid microbial contamination. The sample bottles were placed into a cooler with ice to maintain the temperature below 5 °C and this was immediately transferred to the laboratory in aseptic and sterile condition.

The pH of the sweet sap in each sample bottles was measured directly by using the pH meter (HATH) and Brix was measured by using the hand refractometer. The collected sweet sap samples from various earthen pots in same field trials were pooled together and measured the pH value of this pool sample. Then this pooled sweet sap was transferred into aluminum pan and heated to 120°C to prepare jaggery. These prepared jaggery samples were packed in high density polyethylene (HDPE) bags under vacuumized condition and jaggery samples were labeled as A, B, C and D which are produced from the field trials T1, T2, T3 and T4 respectively. The produced jaggery samples from various field trials were carried out to select best jaggery samples on the basis of sensory attributes as the colour, odor, appearance, flavor and texture. For this sensory evaluation, the five points hedonic scale was used to select the total acceptability of the samples and it were statistically analyzed using the MINITAB statistical analysis package according to the Friedman nonparametric test at 5 % confidence level of significance [12].

# RESULTS AND DISCUSSION

The study of quality characteristic of marketable sweet sap

The most significant quality characteristic of the commercially available sweet sap samples, which is obtained from different areas, were analyzed and tabulated in the Table 1.

This research study clearly indicates that the marketableavailable sweet saps have shown higher pH, pale yellow in colour and bitter taste due to the presence of excess amount of quick lime. This unfavorable colour and taste have given unpleasant condition to produce jaggery without deliming treatment. Personal interviews taken from local tappers during our field visits is clearly notified that they haven't any scientifically knowledge about the amount of lime which is adequate to prevent the microbial activities during the collection of sweet sap.

The minimum amount of quick lime addition at higher degree of purity will ensure that the optimum alkaline pH of the sweet toddy.

Table 1: pH, brix and colour of the commercially available samples

Place	pН	Brix	Colour	Taste
Pandaiththarippu	11.20	11.5	White	Bitter
Pandaiththarippu	11.14	11.0	White	Bitter
Chavakacheri	11.71	13.5	Pale yellow	Bitter
Chavakacheri	11.70	12.5	White	Bitter
Atchuveli	11.20	11.4	White	Bitter
Atchuveli	11.62	12.5	Pale yellow	Bitter
Atchuveli	11.23	11.0	White	Bitter
Kondavil	11.68	13.2	Pale yellow	Bitter
Kondavil	11.55	12.6	Pale yellow	Bitter
Allarai	11.12	12.4	White	Bitter
Allarai	11.59	13.0	Pale yellow	Bitter

Study the chemical constituents of marketable Jaggery

The moisture content, total ash, acid soluble ash, matter insoluble in water and reducing sugars of the marketable jaggery samples obey the limits of specification for jaggery according to the Sri Lankan standards but sugars and non reducing sugar content is exceed the SLS standard level [12]. The most significant chemical constituents for the jaggery samples are listed down in table 2.

Table 2: Chemical constituents of the market available Jaggery

	Moisture	Total ash	Acid	Matter	Reducing	Sugars, non
-	content	percent	insoluble	insoluble	sugars	- reducing
Sample	percent	by	ash percent	in water	percent	sugars
No	by mass	mass	by	percent	by mass	percent by
	(W/W)	(W/W)	mass	(W/W)		mass
			(W/W)			(W/W)
1	6.78 %	1.83 %	0.11 %	1.23 %	6.10%	76.80 %
2	3.39 %	1.45 %	0.12 %	1.65 %	2.32 %	91.09 %
3	6.79 %	2.65 %	0.20 %	1.43 %	2.16 %	88.04 %
4	7.48 %	2.62 %	0.30 %	1.65 %	2.11%	81.20 %
5	7.07 %	2.45 %	0.13 %	1.65 %	5.79 %	82.65 %
9	7.73 %	1.80 %	0.11 %	1.23 %	2.25 %	85.61 %
STS	10 % (max)	3.5 % (max) 0.5% (max)	0.5% (max)	2 % (max)	13% (max)	70 % (max)

The moisture in the products exceeded the SLS limits at ambient temperature before their expiry period, is tabulated in table 3. Therefore, jaggery samples start to melt before the expired.

Table 3: The moisture content in the commercially available jaggery samples

Sample No	Moisture content percent	Moisture content percent by
	by mass in 1st day	mass after 3rd month
1	7.72	23.16
2	8.43	21.82
3	8.19	22.89

Study the purity of commercially available quick lime

This research study clearly point out that the commercially available quick lime which is collected from Anaikoddai kilns shown slightly higher purity rather than the Pandaitharippu kilns. However these two. Kilns are not produce high purified quick lime, which is, tabulated in table 4. This impurity arises from the traditional kiln due to the following drawbacks such as (a) the kiln totally opens to environment and using coconut husk to heat sea shells. The important reaction occurs in the lime producing kiln take place at the optimum temperature (900°C) which is the calcining of limestone.

$$CaCO_3(s) \rightarrow CaO(s) + CO_2(g)$$

However, this heating process cannot be reached the actual temperature of above 900 °C [14]. (b) The sea shells are packed in several layers and between these layers coconut husk are spreaded into the traditional kiln. Therefore, there are the possibilities for adulteration by impurities as soil and coal present in quick lime. According to the results from the research work and the above drawbacks, traditional kiln should be modified in the future to produce a highly purified quick lime.

Table 4: The purity of commercially available quick lime

Place	Purity of lime expressed as CaO (%)
Anaikoddai	$59.99 \pm 0.97$
Pandaitharippu	$42.34 \pm 0.74$

# Study the purity of quick lime in the laboratory scale

A various yield percentage of quick lime was produced as a function of temperature and time. In this study, 800 °C is not enough to produce the lime above 90 % of purity. Other temperature treatments are selected to optimize the time. We can get lime with 96 % of purity in above the 800 °C. Even the purity of lime is increasing with increased the time period and the temperature. Finally, 850°C for 30 minutes treatment was selected as a best consumable method to produce lime with minimal amount of energy required

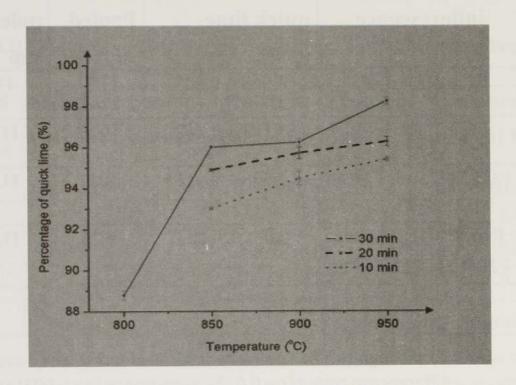


Figure 1: The percentage of quick lime as a function of the various temperature for the treatment of 30 minutes (—— line), 20 minutes (——— line) and 10 minutes (…… line).

# Collection of sweet sap

Different field visits were carry out to optimize the lime for the processing of palm jaggery and all the summarized results were recorded in the table 10. In field visit 1 (T1) field visit 2 (T 2), field visit 3 (T3) and field visit 4 (T4) amount of applied quick lime were 4.668  $\pm$  0.847 g/L, 3.188  $\pm$  0.242 g/L, 2.508  $\pm$  0.411 g/L and 2.051  $\pm$  0.833 g/L and pH of them were 11.51, 10.62, 9.43 and 8.23 respectively. According to Sri Lankan Standard [1] fresh jaggery samples from T1 , T2 and T3 field visits at the time of packaging moisture content of all was below 10 %. Sweet sap samples with pH between 7 and 8 quickly changed into acidic medium with time and this indicates the microbial activity is present considerably. Jaggery from field visits T4 shows melting property and moisture

content was below 12.5 %. It was identified that fermented sweet sap also course melting of jaggery. Therefore pH of sweet sap needs to be maintained above pH 9 in large scale application. For the suitable jaggery production without deliming step 2.508  $\pm$  0.411 g of lime (96 % purity) per one liter of sweet sap was optimized.

Table 10: Summary of all field visits

Filed visit	Name of the inflorescence	Amount of quick lime (g/L)	pH of Pooled sap	Brix of poled sap
T1	F1, F2, F4 & F5	$4.668 \pm 0.847$	11.51	12.8
T2	F6, F7, F8 & F9	$3.188 \pm 0.242$	10.62	11.72
Т3	F11, F12, F13, F14 & F15	2.508 ± 0.411	9.43	11.63
T4	F16, F17, F18, F19, F20, F21, F22 & F23	$2.051 \pm 0.833$	8.23	11.00

# ensory evaluation of produced jaggery

Sensory data obtained through the five point hedonic evaluating test, revealed that there were significance difference in colour (p = 0.012), appearance (p = 0.003) and texture (p = 0.001) characteristic among the jaggery samples produced in the laboratory. However there were no significant difference in flavour (p = 0.296) and mouth feel (p = 0.145) among the samples. The sample C gained the highest rank for the colour, appearance, and texture. Therefore the sample C was selected as the best sample.

Table 11: Sensory attributes of the jaggery

Sensory attributes	P value	Sum of the rank				Best sample
	I LILIONIA I A	A	В	C	D	Valuation of a final of valuation
Colour	0.012	19.5	27.5	29.5	13.5	С
Appearance	0.003	17.5	29.0	30.0	13.5	С
Texture	0.001	17.0	28.5	31.5	13.0	С
Flavour	0.296	18.0	27.5	24.5	20.0	В
Mouth feel	0.145	15.0	24.0	27.0	24.0	В

# Evaluation of moisture content of prepared Jaggery samples

Moisture content of prepared jaggery samples were determined in different time periods as in first day, after first month and after sixth month. Jaggery from fourth field trial melted before one month and this indicates even the pH 8 is suitable to produce jaggery, fermented sap causes the melting of jaggery easily. Jaggery produced from other field trials can be stored for six months.

Table 12: Moisture content of jaggery with average of triplicate readings after 6 months

Field trials	Moisture content percent by mass in 1st day	Moisture content percent by mass after 1st month	Moisture content percent by mass after 6th month
T1	3.52	3.72	8.69
T2	3.98	4.01	8.94
Т3	4.12	4.87	9.45
<b>T4</b>	4.43	25.65	-

# Conclusion

Palmyrah jaggery is one of the most popular sweeteners in north and east provinces of Sri Lanka. The color of the sweet sap was improved with using the proper amounts of applied lime per liter.

Sweet sap should be maintain at pH 9 by using a purified quick lime to stop the fermentation and it can be useful to get quality improved jaggery as high consumer acceptance in the market level. At this optimum pH range jaggery can be produced without deliming step from fresh, unfermented and filtered sweet sap of Palmyrah palm.

# Acknowledgements

Authors like to thank the Department of Chemistry, University of Jaffna, and Palmyrah Research Institute for providing necessary facilities.

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Scholars Research Library Journal of Natural Products Plant Resources, 5 (2), pp. 37-42, 2015

# 2.1.4 Optimization of Palmyrah (Borrasus flabellifer) Fruit Pulp in Different Varieties of Fruit Yogurts

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#### **ABSTRACT**

The research focused on processing palmyrah fruit pulp into a value added product to broaden the utilization of Palmyrah fruit pulp. Set, swiss style, jelly and pulp preserve yoghurts incorporated with Palmyrah fruit pulp were developed. Control yoghurt and plain yoghurt for other preparations were prepared with 13 % sugar, 1% gelatin, 12 % skim milk powder and lactic acid bacteria culture following household method. The fresh pulp was kept at - 25°C for 48 hours and heated up to 85°C for 30 minutes with 5 % cane sugar. 0.6 % tartaric acid, 1 % ascorbic acid and 0.13 % sodium chloride. The treated pulp was added at 5 %, 7.5 %, 10 %, 12.5 % and 15 % into both set and swiss style yoghurts. Fruit jelly was prepared with sugar, gelatin, sodium citrate, citric acid and 20 % pulp. It was then incorporated into yoghurt at 5 % and 6 %. Palmyrah fruit pulp preserve was prepared by heating sugar, pectin and pulp (45. 8 %) until its brix reached 68.5° and it was topped on set yoghurt at 5 %, 7.5 % and 10 %. Sensory evaluation for colour, odour, appearance, mouth feel and texture was conducted with 21 untrained panelists using 5 point hedonic scale and the optimized pulp concentrations for set, swiss style, jelly and preserve yoghurt were 5 %, 7.5 %, 6 % and 10 % respectively (p < 0.05). The overall sensory qualities of all Palmyrah fruit yoghurts were rated as good to very good. There were no significant changes in sensory attributes, brix and pH in storage at 4°C. Shelf life was 18 days for all products at 4°C without any preservatives except set yoghurt as it showed separation of water from the third day of the preparation. Nutritional and microbiological qualities of the products were investigated and compared with control yoghurt. Protein was higher in swiss style (6.12 %) and jelly (7.77 %) yoghurts. Carbohydrate was higher in swiss style yoghurt (36.87 %) and preserve yoghurt contained highest fat content (2.57 %).

Keywords: Carbohydrate, Fruit yoghurt, Palmyrah fruit, Pulp, Protein

#### INTRODUCTION

Palmyrah (*Borrasus flabellifer*) possess a great capacity to yield several products of economic importance and hence it is called "Wishing tree" which means a palm that yields anything and everything. Almost every part of the palm is utilized but most of the products from the palm are made by traditional methods known from the time immemorial.

It is estimated from the statistical data of existing female palms in Sri Lanka that, about 20,000 tons of Palmyrah fruit pulp is available annually during fruit season but around 10000 tons of pulp is thrown in to nature or used for a nimal feeding (Jansz et al,2002) every year because its uses are limited mainly due to the presence of a bitter compound and lack of trials are done to process into various consumer attractive value added products.

Moreover, Palmyrah fruit pulp is nutritious and has a yellow colour due to carotenoids which are precursors of vitamin A and therefore it has potential of being as a source of vitamin A and giving attractive yellow colour to foods. In addition to that, it is revealed that pulp is rich in vitamin C (ascorbic acid) and a good source of pectin which could be used to process the fruits into various products (Theivendrarajah, 2008)

With regard to palmyrah plantation, it is reported that currently 24.260 hectares equivalent with 11 million palms are available of which 3.5 million trees are in Jaffna, 3.5 million in Kilinochchi, 3 million in Mannar and balance scattered all over the country (Palmyrah development board, 2010). Government's Mahinda Chintana 10 - year plan aims to increase the current level of palms from 11 million to 16 million by 2016 and the vision of it for the future strongly emphasizes on the need to promote Palmyrah based products including its fruits. Also the government has given high priority for dairy development (Ministry of livestock and rural community development, 2010). Currently major part of milk production is done by farmers domestically and their life is as same as that of Palmyrah dependents.

The above facts generated the idea to produce a value added consumer attractive product using Palmyrah fruit pulp and milk. As a result the research was conducted with the aims of developing formulae for different kinds of Palmyrah fruit yoghurts, analyzing nutrient contents of the yoghurts and determining shelf lives of them. The research, when industrialized will give the benefits to the Palmyrah dependents of approximately 1/3 rd of the population of the population of Northern and Eastern provinces where Palmyrah occurs extensively by increasing the marketing and utilization of Palmyrah fruit pulp, minimizing the wastage of a nutritive resource and under exploitation of the health aspects of pulp and employment opportunities to produce novel product for consumers.

#### BACKGROUND

Yoghurt is a healthy and delicious fermented milk product due to its nutritive and therapeutic value with a custard-like consistency which differentiates it from other fermented milk products (Perdigon et al., 2002). The Codex Alimentarious Commission (2008) defines it as a coagulated milk product obtained by lactic acid fermentation through the action of Lactobacillus bulgaricus and Streptococcus thermophilus from milk with or without additions and the microorganisms in the final product must be viable and abundant.

Yoghurt is commonly considered as safer product and nutritionists are being considering incorporating inexpensive source of nutrient to make it an almost complete food (Boghra and Mathur 2002). Yoghurt is easily digestible and palatable than milk as it contains less lactose. Further, it is treasured for controlling the growth of bacteria and in curing of intestinal disease, for instance, constipation and diarrhea, lowering of blood cholesterol and anti-carcinogenic effect (Kamruzzaman *et al.*, 2002). Yoghurt is a great source of B vitamins, phosphorus and calcium. Organoleptic properties such as flavour, texture and aroma of yoghurt depend upon so many factors including the raw materials, manufacturing p rocess and the b acterial s trains used (Kumar and Mishra, 2004).

According to the Sri Lankan Standard for yoghurts (SLS 824 Part 2:1989) a standard yoghurt should be smooth, glossy surface, no crack or holes on the top, no whey synersis, no off flavor or odor and clean layer on the surface of the yoghurt. It also says that yoghurt must contain minimum of 3.0 % fat whilst it categorizes the yoghurt that contains be tween 0.5 % and 3.0 % fat as low fat yoghurt and that with less than 0.5 % fat is non-fat yoghurt.

Palmyrah fruit pulp has bitter taste due to flabelliferins and it gives a negative impact on utilization of pulp. Despite that from the point of health, Palmyrah fruit pulp has numerous benefits to us. It is now proved that flabelliferins lowers the glucose absorption into blood, increase the cardiac anti oxidant activity and decrease the blood cholesterol. Recent studies show that pulp is rich in provitamin A (32 ppm), vitamin C (285 ppm) (Jeyaratnam, 1986) and contains lycopene as well (Pathberiya and Chandrika, 2003). Lycopene is an anti oxidant which is beneficial in cardiovascular ailments and cancer.

# MATERIALS AND METHODOLOGY

Materials

Fresh milk was procured from Yarlco, the only dairy production unit in Jaffna peninsula and cream separation was done there using their cream separator. Other ingredients were collected from Jaffna market. Palmyrah fruits were collected from Kopay and Navatkuli.

Reagents used for chemical and microbiological analysis of the developed products were purchased from Sigma Aldrich. All glassware were obtained from ISO certified companies in United Kingdom and Germany and the equipment used for the studies were caliberated and made in United Stated of America and Germany.

# Quality tests and preparation of raw materials

Selection and quality of raw materials are the major determinants of the quality of end products in a processing. Therefore raw materials are analyzed prior to be subjected to the processing to ensure the production of good quality end product. In the study, raw milk was subjected to clot boiling test with 68 % alcohol according to COMESA/East African Standards. Also the pH, titrable acidity as lactic acid, total soluble solid, fat and solid non fat of milk were determined. It was boiled up to 80°C for 15 minutes.

Pest attack free, fresh, black skinned, well ripe fruits were selected to the study. The nutlets were dipped in warm water (45 -  $50^{\circ}$ C) for few seconds and then macerated. The ratio of pulp: added water was 1:0.5 (v/v). Pulp was then strained through a muslin cloth. It was kept in deep freezer at -25°C for about 48 hours.

Palmyrah fruit pulp was treated with 5 % sugar, 0.6 % tartaric acid, 1 % ascorbic acid,

0.2 % citric acid, 0.13 % sodium chloride and 1 % gelatin and the mixture was heated up to 85°C for 30 minutes to incorparate into set and swiss style Palmyrah fruit pulp yoghurts.

For jelly yoghurt, palmyrah fruit pulp jelly was prepared with sugar (84 %), gelatin (11.5 %), tartaric acid (1.06 %), sodium citrate (0.18 %), water and 20 % pulp following the house hold method. Sugar (53 %) and pectin (0.35 %) were he ated together with the pulp (45. 8 %) were heated until brix reached 68.5° to prepare fruit preserve for fruit preserve yoghurt. The preserve was prepared freshy in each preparation, so that it can eaisly be poured on the yogurt easily before it gets set.

# Preparation of yoghurts

For the plain yoghurt for all four products, 13 % sugar, 1% gelatin and 12% skim milk powder were added and the amounts of them were same in all products. The house hold method for yogurt production was used to prepare yoghurt using lacitic acid bacteira culture procured from Jamma. The preparation of yoghurt, its processing conditions and ingredients were kept same in the preparation of all products.

Optimization of pulp in set and swiss style yoghurts Five recipes in each category of yoghurts were developed for Palmyrah fruit pulp incorporated set fruit yoghurt and swiss style yoghurt (Table 1).

**TABLE 1:** Optimization of fruit pulp concentrations in Palmyrah fruit pulp set yoghurt and swiss style yoghurt

alen-iii-		Treatments						
Tunes	1	2	3	4	5			
Treated	5.00	7.50	10.00	12.50	15.00			
fruitpulp amounts	lyte hoves	- uere						
(g 100 ml <sup>-1</sup> )								

Optimization of pulp as jelly in fruit jelly yoghurt Two recipes of palmyrah fruit pulp jelly yogurts were prepared by blending the jelly in different percentages (Table 2). All ingredients except jelly were kept constant in all blends.

TABLE 2: Optimization of palmyrah fruit pulp jelly in swiss style yoghurt

To the	Pulp in the jelly added to the product (g 100 ml <sup>-1</sup> )
Treatment	
1	5.0
2	6.0

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# Optimization of pulp as preserve in fruit preserve yoghurt

Three recipes of palmyrah fruit pulp preserve incorporated set yoghurts were prepared. Table 3 shows the percentage of palmyrah fruit pulp preserve added in each treatment. The preserve was poured on the set yogurt. All ingredients except fruit pulp preserve were kept constant in all blends.

TABLE 3: Optimization of palmyrah fruit preserve in Palmyrah fruit preserve yoghurt

Treatment	Pulp in the product (g 100ml <sup>-1</sup> )
1	5.0
2	7.5
3	10.0

# Selection of the formula of each variety of prepared yoghurts

The formula in each variety of yoghurts which gained most consumer preference was selected via sensory evaluation conducted by 21 untrained panelists using 5 point hedonic scale method and analysis of variance was conducted on the sample means for flavor, colour, mouth feel, appearance, texture and over all acceptability. Statistically significant attributes were further analyzed to see where mean difference existed using Minitab (Friedman test) at 95 % confidence interval (P < 0.05).

# Analysis of selected yoghurts

All selected products were subjected to the following tests and the results were then compared with that of control yoghurt.

# Chemical analysis

pH, total soluble solids, and titrable acidity were a nalyzed according to SLS 824: Part 2,1989. Crude protein, moisture, crude fat, crude fibre, ash and total carbohydrate were determined in accordance with Association of Analytical Chemists Standard (2002). Same was done for commercially available plain yoghurt as control and compared with developed Palmyrah fruit yoghurts.

# Microbiological analysis

Total plate count and yeast and mold were tested for skim milk powder, sugar, milk, water and milk solution prior to the addition of culture. *E. coli*, yeast and mold in the selected products were assessed a ccording to SLS 824 Part 2: 1989. Same was done for commercially available plain yoghurt.

# Shelf life study

For the shelf life evaluation pH, brix and titrable acidity were measured once in in three days for 21 days. At the same time, texture, appearance, colour, smell and air bubble formation on the surface of the product were observed by experienced persons at the station where the research was carried out.

Experimental design and statistical analysis Friedman non parametric statistical method was used to analyze the results of sensory evaluation data at 95 % confidence level was considered.

# RESULTS EVALUATION AND DATA ANALYSIS

# Quality tests and preparation of raw materials

The analysis showed that the raw milk met the quality requirements specified in SLS 824: Part

2: 1989 for yogurt preparation. In Table 4 the fat, solid non fat, density, pH and acidity of the purcehased milk are given in ranges because the milk was procured three times throughout the studies and the tests for fat, solid non fat and density of raw milk were carried out for each batch.

**TABLE 4:** Quality parameters (fat, solid non fat, density, pH and acidity) of raw milk used for the study

Constituent of raw milk	Range
Fat	0.02 – 0.03 g 100g <sup>-1</sup>
Solid non fat	7.5 – 7.6 g 100 g <sup>-1</sup>
Density	28.6 – 29.1
рН	6.6 - 6.7
acidity	0.14 - 0.17 g 100g <sup>-1</sup>

Clot boiling test is carried out to ensure the quality of milk. In the test poor quality milk will coagulate and fine particles of curd will be visible whereas the quality milk will not give so. When the acidity of milk exceeds 0.21% the milk get spoiled and is not good for the production. The raw milk used in the study did not clot in the alcohol test and the pH and acidity were ranged 6.6-6.7 and 0.14-0.17% respectively which fell into the acceptable ranges of pH and acidity for consumption.

# Fruit pulp preparation

It would be better if some parameters such as penetration ability, pH and total soluble solids are standardized for the selection of good quality fruits as it will be more beneficial and easy to the industries to select super grade fruits for the production. Palmyrah fruit pulp was collected and kept in at -25°C till used in order to preserve and minimize the bitterness of pulp. Therefore the pH, brix and acidity were determined soon after the extraction only. The pH, total soluble solids and the acidity by means of ascorbic acid were 4.06, 15.2 % and 0.66 %. Acidity was expressed in terms of ascorbic acid as this is the dominant acid in the fruit. The results showed that the pulp was not spoiled.

Pulp has bitter principle which hinders its exploitation in food industries. However Jansz (2006) stated that the bitterness can be reduced by freezing beyond -20°C. Therefore pulp was kept in deep freezer at -25°C for about 48 hours to minimize its bitter taste and the bitterness of fresh pulp and frozen pulp was compared. It was experienced via tasting that the frozen pulp was less bitter than the fresh.

After the preparation of pulp for set and swiss style yoghurts, its texture, aroma and sweetness were improved and the colour remained as that of fresh pulp. Tartaric acid was added to reduce the pH closer to that of yoghurt. Citric acid and ascorbic acid were added as anti oxidants to prevent discolouration in the preparation. So dium chloride was added as taste enhancer.

Palmyrah fruit pulp jelly prepared for the development of fruit jelly yoghurt was smooth and soft without any bubbles or foam on the top. The final pH of solution was 3.1 which is the most suitable pH for jelly to settlle off.

Palmyrah Fruit preserve was darker than the natural colour of the pulp but the texture was as same as that of jam. No air bubbles or sugar crystals formed and it was smooth and transparency.

# Sensory evaluation of yoghurts

Friedman test in Minitab was used to analyze the results of sensory evaluation to select the most preferred blend of each type of Palmyrah fruit yoghurt.

# Palmyrah fruit pulp set yoghurt

The preference of each sensory attributes of all Palmyrah set yoghurts developed in the study were rated from 5 to 1. Point 5 was given for like very much whilst point 1 was dislike very much.

**TABLE 5:** Sensory evaluation of set palmhyrah fruit yoghur

Sensory	Median score for the products						
attribut e	175	101	115	142	107		
Flavour	4ª(45.5)	4ª(44.0)	3 <sup>b</sup> (31.5)	3 <sup>b</sup> (29.0)	3 <sup>b</sup> (30.0)		
Color	4 <sup>a</sup> (47.5)	4 <sup>a</sup> (49.0)	3 <sup>b</sup> (33.5)	3 <sup>b</sup> (25.5)	3 <sup>b</sup> (24.5)		
Appeara	4 <sup>a</sup> (47.0)	4 <sup>a</sup> (52.5)	3 <sup>b</sup> (29.0)	3 <sup>b</sup> (26.5)	3 <sup>b</sup> (25.0)		
Mouth feel	4 <sup>a</sup> (50.0)	4ª(49.0)	3 <sup>b</sup> (27.5)	3 <sup>b</sup> (26.5)	3 <sup>b</sup> (25.0)		
Texture	4 <sup>a</sup> (52.5)	4ª (47.5)	3 <sup>b</sup> (31.0)	3 <sup>b</sup> (26.0)	3 <sup>6</sup> (23.0)		

Results (Table 5) shows that the products 175 and 101 scored same median 4 for all analyzed sensory attributes and no significant difference existed in-between the products for each attributes. Products 115, 142 and 107 s h o wed significant different be tween the other two and among them, and scored less than 175 and 101. To select the best product between 175 and 101 the sum of rank had to be considered as the medians were same for each attribute. Product 175 has scored higher than the other for flavor, mouth feel and texture whereas the product 101 scored higher for colour and appearance. It would be rather good if the overall acceptability of the product was also a nalyzed. However as 175 scored higher for flavor, mouth feel and texture which indeed play in the success of product product 175 which contained 5% fruit pulp was selected as best.

# Palmyrah fruit pulp swiss style yoghurt

Colour, flavour, appearance, mouthfeel and texture of each blend was rated from 5 to 1 where 5 was assigned to like very much and 1 was to dislike very much.

Same alphabets on means in a row show non-significant differences at the confidence level of 95%. Sum of ranks are given along with the medians in the brackets.

- 175 Set yoghurt with 5% fruit pulp
- 101 Set yoghurt with 7.5% fruit pulp
- 115 Set yoghurt with 10% fruit pulp
- 142 Set yoghurt with 12.5% fruit pulp
- 107 Set yoghurt with 15% fruit pulp

Same alphabets on means in a row show non-significant differences at the confidence level of 95%. Sum of ranks are given a long with the medians in the brackets.

- 215 S wiss style yoghurt with 5% fruit pulp
- 265 S wiss style yoghurt with 7.5% fruit pulp
- 253 S wiss style yoghurt with 10% fruit pulp
- 242 S wiss style yoghurt with 12.5% fruit pulp
- 227 S wiss style yoghurt with 15% fruit pulp

The results of sensory evaluation revealed that the all products have scored the same median 4 for flavor, colour and mouth feel and no significant difference existed among them for each of those attributes. Product 265 scored the highest median and sum rank for appearance, mouth feel and texture. Also it was clear that product 265 scored the best for all sensory attributes. Therefore product 265 containing 7.5% fruit pulp was selected.

From the above results of both types of yoghurts, it could be noted that the selected swiss style yoghurt contained higher percent of pulp (7.5%) than set yoghurt (5%). Also the comments given by the panelists brought out that the consistency and appearance of swiss style yoghurt were better than that of set yoghurt and bitterness could not be sensed in swiss style yoghurt although the pulp was higher in that. About two or three days after the production set yoghurt showed water separation and the appearance became down. Therefore among the selected set and swiss style yoghurts, swiss style yoghurt with 7.5 % fruit pulp was selected for further studies.

# Palmyrah fruit pulp jelly yoghurt

Sensory evaluation revealed that both products scored 4.5 median to flavor, colour and texture and product 307 s cored less than product 321 for appearance and mouth feel. Also the sum of ranks of product 321 were higher than those of 307 for all attributes. Therefore product 321 was selected finally (Table 7).

TABLE 7: Sensory evaluation of palmyrah fruit jelly yoghurt

Sensory attributes	Median score for the products			
	321(5 % of pulp)	307 (6 % of pulp)		
Flavour	4.5 <sup>a</sup> (20.0)	4.5 <sup>a</sup> (19.0)		
Color	4.5a (21.0)	4.5a (18.0)		
Appearance	4.5a (21.5)	3.5 <sup>b</sup> (16.5)		
Mouth feel	4.5 <sup>a</sup> (23.5)	3.5 <sup>b</sup> (15.5)		
Texture	4.5 <sup>a</sup> (20.0)	4.5 <sup>a</sup> (19.0)		

Means in the each row followed by the same letters are not significantly different at p < 0.05. Sum of ranks are given along with the medians in the brackets.

# Palmyrah fruit pulp preserve yoghurt

The results of sensory evaluation brought out that product with  $10\,\%$  of fruit pulp scored 4.5 median to flavor, colour texture and mouth feel and the sum of ranks of product was higher than the other products with  $5\,\%$  and  $7.5\,\%$  fruit pulp and thus it product with  $10\,\%$  of fruit pulp was selected.

# Analysis of selected products

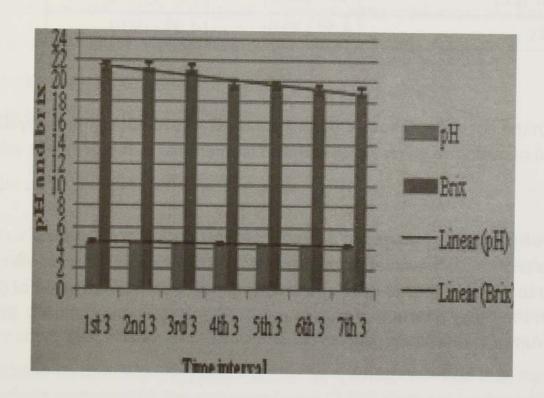
Chemical analysis - pH, Brix and acidity pH, acidity and total soluble solids of the all types of yoghurts selected in the sensory evaluation were analyzed to determine the chemical quality of them.

#### Time interval

SLS 824 Part 2: 1989 says that the acidity by means of lactic acid of standard yoghurt should be between 0.8 % and 1.25 %. Acidity of all products selected in the studiy was in between the limits specified in the standard. In contrast, the pH of the products were slightly higher than the optimum pH of 4.2 for coagulation. It may be due to the acidity of fruit pulp and the organic acids (citric acid, tartaric acid etc) added in preparation of pulp for the product developments.

# pH and brix of yoghurts with storage

The results show that the pH and brix did not change much and they were in acceptable limits even after 18 days. Fruit jelly yoghurt showed the pH above 4.2. The change in pH and total soluble solids of each type of yoghurt were observed for 21 days (Figures 1, 2, 3 and 4).

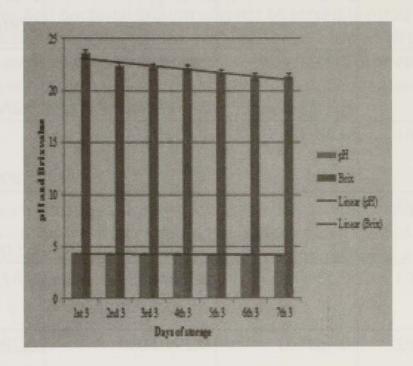


**FIGURE 1:** Study on change of pH and brix of palmyrah fruit pulp swiss style yoghurt for 21 days

Values are means of triplicates. Vertical bars indicate the standard errors.

The milk solids content (including the fat content) for yoghurt ranges from around 9

% for low fat yoghurt to more than 20 % for certain types of concentrated yoghurt. Many commercial yoghurt products have milk solids contents of 14 - 15 % (Tamime and Robinson, 1999). The total solids content of milk can be increased by concentration processes, such as, evaporation under vacuum, and membrane processing (i.e., reverse osmosis and ultra filtration).



Values are means of triplicates. Vertical bars indicate the standard errors.

FIGURE 4: Study on change of pH and brix of palmyrah fruit set yoghurt for 21 days

Although the characterisites of Palmyrah fruit pulp set yoghurt were in the limits, separation of whey and water was observed on the third day from the development.

Nutrient analysis of selected palmyrh fruit yoghurts in the study

Codex Alimentarious Regulations for yoghurt (2008) indicates that the minimum milk protein content is 2.7 % (except for concentrated yoghurt where the minimum protein content is 5.6 % after concentration) and the maximum fat content is 15 %. The results (Table 8) show that the fat content of all products did not exceed 15 % and were between 0.71 % and 2.47 %. Therefore, all Palmyrah fruit pulp yoghurts can be categorized as low fat fruit yoghurts. The protein content of preserve yoghurt was lower than the limit as the preserve added for yoghurt was about 40 % and thus the milk in the yoghurt was reduced drastically and the preserve contained almost 60 % of sugar. It would be more informative about the nutrient value and medicinal benefits of the products if the analysis of crude fibre, dietary fibre and vitamin A is carried out.

TABLE 8: Nutrient contents of developed palmyrah fruit pulp yoghurts

ingraphy to the	Amount of nutrients (g 100g-1)						
Nutrient	Swiss style yoghurt	Jelly yoghurt	Preserve fruit yoghurt	Commercial yoghurt as control			
Protein	6.12±0.05	7.77±0.0	2.48±0.0 3	5.26±0.01			
Crude fat	0.71±0.01	0.90±0.05	2.47±0.03	0.80±0.05			
Moisture	55.59±0.3	66.48±0.	61.38±0.02	65.00±0.15			
Ash	0.56±0.03	0.53±0.01	0.92±0.02	0.81±0.01			
Acid insoluble ash	0.15±0.02	0.46±0.0	0.29±0.01	0.29±0.01			
Carbohydra te + fibre	36.87±0.37	23.86±0.09	32.46±0.08	27.84±0.12			

Palmyrah fruit pulp contains provitamin A (32 ppm), vitamin C (285 ppm) and 190 lycopene as well (Mohanajayelauxmy, 1986). Apart from the nutritional benefits of pulp, it was discovered that Flabelliferin II was preventing the absorption of glucose into the blood stream with no adverse reactions (Janz, 2006). Further it has a nti glycemic a c tivities, anti-microbial activities and hypocholestremic acivities which are benefits for our health. 10-12% pectin in pulp decrease cholesterol by 25-35% by binding bile salts and c holesterol (Jansz, 2006).

# Microbiological analysis

According to SLS824 Part 2: 1989 E.coli, yeast and mold, lactic acid bacteria, and *Salmonella* should be analyzed howbeit since the media was not available at the time when the research was carried *Salmonella* will be checked in future works of the research. Total plate count test was done for s kim milk p o wder, s u gar, milk a n d p o table water according to SLS 516 part 1, 1991. But bacterial growth was in acceptable range in potable water and bacterial growth was not observed in other ingredients. *E. coli* was absent in all products including control yoghurt. Yeast count of all products were not more than 1000 per gram and Mold did not exist in the selected products. Mostly the microbial contamination occurs during the process. Therefore implementation of hygienic practices during process will help reduce microbial contamination.

# Organoleptic analysis.

Texture, flavor and appearance were observed one in three days to determine the shelf life. They remained as fresh throughout the period of 21 days. But since the pH and acidity went beyond accepted level after 18 days observations were not taken as it will not contribute to determine shelf life further.

# Shelf life study

pH and brix were measured once in three days to determine the shelf life of products for 21 days. At the same time, appearance, colour, smell and texture were observed by experienced persons. As discussed above in pH and brix change, the shelf life period was determined as 18 days without any preservatives at 4°C.

# CONCLUSION AND FURTHER WORK

The optimized Palmyrah fruit pulp concentrations for its set yoghurt, swiss style yoghurt, jelly yoghurt and preserve yoghurt were 5 %, 7.5 %, 6 % and 10 % (v/v) respectively. All four p roducts c o mplied with Sri Lankan Standard 824:1989 and the pH, brix and acidity of the final p roducts were acceptable ranges. The shelf life of the product was 18 days at 4°C without any preservatives. However a complete analysis of nutrients including vitamin A and C, phyto nutrients and dietary and crude fibres can give a complete nutrient profile and nutritional value of the developed p roducts. Therefore, in the next season of fruits these constituents will be an alysed. Also, the study on development of yoghurt with chemically preserved pulp will be studied. Consumer preference test and marketing survey should be done to improve its quality.

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International Journal of Multidisciplinary Studies (IJMS) 1 (1): 91-103, 2014

# 2.1.5 Screening the antifungal activity of essential oils against decay fungi from Palmyrah leaf handicrafts

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#### **Abstract**

# Background

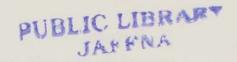
The palmyrah leaves handicrafts are affected by fungal attack on rainy season; this could be prevented by some protective agents. Instead of using expensive and harmful chemicals, it was decided to test the activity of natural plant extracts on fungal attack. Fungi were isolated, purified from affected palmyrah leaves then characterized as *Aspergillus niger*, *Aspergillus flavus* and *Penicillium sp.* and used for this study. Different concentrations of extract prepared from thyme (*Thymus vulgaris* L) flower and commercially available thymol has been evaluated for their antifungal activity against isolated fungi. Minimum Inhibitory Concentration (MIC) and Percentage of Growth Inhibition (GI) of thyme extract and thymol were determined to screen the antifungal activity.

#### Results

There was no significant different on growth inhibition among the fungi species for all the concentrations of thyme and thymol. While growth inhibitions for all concentrations of hot water extract of thyme showed significantly different (p<0.05) and increases with concentrations. The extract of 15ml/dl thyme and 0.5, 1.0, 2.0 ml/dl of thymol showed significantly higher GI (100%) for all fungi species, while 0.1 ml/dl of thymol showed significantly lesser activity when compared with 10ml/dl of thyme extract. Minimal Inhibitory Concentration of thymol was shown to be 0.5 and thyme extract was 15ml/dl respectively.

#### Conclusion

Based on the results thymol in the concentration of 0.5ml/dl could inhibit the fungal growth besides thyme extract of 15ml/dl could be used instead of commercially available thymol. Therefore thyme could be used for the preparation of ecofriendly fungal agent to protect the Palmyrah leaves handicrafts in addition field testing is essential to achieve this in industrial level.



Key words: Extracts, Fungi, Growth Inhibition and Preservation

# Background

Macroscopic filamentous fungi are ubiquitous micro organisms with a great capacity to colonize many kinds of substrates and to develop under humid environmental conditions. Fungi and their air borne spores have been recognized as possible causative agents of various diseases in human [1] in addition they cause discolourations on Palmyrah leaf handicrafts.

Nowadays, application of chemical compounds is considered as the common method and most inexpensive to fungal control. However, their adverse effects on human health and the environment, promoted to produce natural fungicide [2]. Biologically active compounds found in plants appear to be more acceptable and safer than synthetic compounds and exhibit a wealthy source of potential for control the fungal agents [3].

Several studies have shown that thyme oils, particularly those of *Thymus vulgaris* and *Thymus zygis* [4, 5] possess significant antifungal, insecticidal, and antimicrobial activities, it may vary based on the variation on the chemical composition.

The essential oils of more than one hundred species of the genus *Thymus* have been chemically investigated; reveal about 360 different volatile components in total. Among these, the monoterpenes were the most prominent group while sesquiterpenes represent a lower percentage of the volatiles. Generally, plants of the genus *Thymus* are considered as the most common source of the monoterpenoid phenols, thymol and carvacrol [6].

Several *Thymus* species are locally known as "Omum" and the dried herbal parts are used in herbal tea, condiment and folk medicine. The essential oils of some *Thymus spp*. are characterized by the presence of high concentration of the isomeric phenolic monoterpenes thymol and/or carvacrol [7]. Traditionally this has been used as a medicinal plant in the treatment of headaches, coughs, diarrhea, constipation, warts, worms and kidney malfunction [8]. Thyme also possesses various beneficial effects as antiseptic, carminative, antimicrobial and antioxidative properties [9].

The main objective of this work was to characterize the in vitro antifungal activities of hot water extract of thyme and pure thymol, as comparative substance, on different fungus species isolated from Palmyrah leaf handicrafts.

#### Results

The antifungal activity of the different concentrations thyme extracts and thymol with different treatments, in terms of percentage of growth inhibition of mycelia were calculated and tabulated in Table 1. Results showed that the thymol have great potential of antifungal activity against all of the three fungi such as *Aspergillus niger*, *Aspergillus flavus* and *Penicillium sp*.

Table 1: Percentage of growth inhibition with different treatments

F u n g u s species	T <sub>1</sub>	T <sub>2</sub>	<b>T</b> <sub>3</sub>	<b>T</b> <sub>4</sub>	<b>T</b> <sub>5</sub>	<b>T</b> <sub>6</sub>	<b>T</b> <sub>7</sub>	T <sub>8</sub>
A.niger	40.68 <sup>e</sup>	50.13 <sup>d</sup>	76.98b	100ª	59.4°	100ª	100°	100ª
A.flavus	2.01 <sup>e</sup>	2.26 <sup>d</sup>	76.44 <sup>b</sup>	100°	59.4°	100ª	100°	100ª
Penicillium	7.02 <sup>e</sup>	7.27 <sup>d</sup>	75.38 <sup>b</sup>	100ª	66.92°	100ª	100 <sup>a</sup>	100°

Activity of hot water extracts of thyme (T1-T4) was compared with that of pure thymol (T5-T8). All the four different concentrations of pure thymol (T5-T8) showed the 100% of GI for  $A.niger.\ A.flavus$  and Penicillium at 4th day of incubation and minimal inhibitory concentration of thymol was  $0.5\,\text{ml/dl}$  (T6). The hot water extract of (T4)  $15\,\text{ml/dl}$  thyme and  $0.5\,\text{(T6)},\,1.0\,\text{(T7)},\,1.5\,\text{(T8)}$  ml/dl of commercially available thymol were showed significantly (p<0.05) higher GI (100%) for all fungi species, while  $0.1\,\text{ml/dl}$  (T5) of thymol showed lesser activity when compared with  $10\,\text{ml/dl}$  (T3) of thyme extract, significantly.

GI of thyme extract at the concentration of 10 ml/dl (T3) was 76.9, 76.4 and 75.3% and 1 ml/dl (T1) was 40.6, 2.0 and 7.0 for *A.niger*. *A.flavus* and *Penicillium* respectively at 4 th days of incubation (Figure 1) while thymol with the concentration of 0.1ml/dl showed 59.4, 59.4 and 66.9% GI for above stated fungi respectively at  $7^{th}$  days of incubation.

# Discussion

The Palmyrah leaves are long, fan-shaped, 2 to 3 m in length. The first two tender unexpanded whitish leaves and the next 12 young green leaves are used in making various handicrafts. The whitish tender leaves are used for making soft fine handicrafts while the young green leaves are used for making stronger, but coarse textured utility items like mats,

baskets, packaging material, inner lining of heavy duty fibre baskets etc. The mid rib known as eekils can be used to make implements for sweeping the gardens, wall hangers, baskets and a type of carrying cases. Therefore the dried Palmyrah tender leaf was selected and cut into small pieces and inoculated into the PDA plate then incubated at room temperature for 4 days. After incubation colonies were purified by repeated streaking then purified fungi were maintained in agar slants at 4°C throughout the study and used as stock cultures [10].

# Antifungal activity assay

All species of fungi, using of any concentrations caused significant differences (p<0.05) on inhibitory effect of extracts. It could be observed that as extract concentration increases, the inhibitory effect also increased. In other words, the inhibitory effect of the extract was proportionate to its concentration. As stated by Rasooli *et al.*, 2006 [11] and Amini *et al.*, 2012 [12], with the increase of concentrations, the susceptibility of fungi increased.

According to the results, we can consider that strong antifungal activity of thyme hot water extract could be attributed to thymol itself. Rasooli and Owlia (2005) [13] showed that thyme oils were affect cell wall and cell membrane of *A. parasiticus*. The plasma membrane was irregular, dissociated from cell wall, invaginated and associated with the formation of lomasomes, which were found in fungi treated with imidazole components. At low concentrations, phenolic lipophilic compounds such as thymol alter cell permeability of microbes, permitting the loss of macromolecules. Exact cause effect relation for the mode of action of phenols has not been determined, but they may inactivate the essential enzymes, react with cell membrane proteins or disturb genetic material functionality [14]. From this point of view, thyme, which is rich in thymol and other antifungal components, could be used for preparation of disinfecting agent for fungi present in the Palmyrah leaf handicrafts in low concentration.

Rakotonirainy and Lave´- drine (2005) [15] reported on fungi static but not fungicidal activity of linalool (295 and 415 ppm) after 21 days of exposure of mouldy books in glass chamber, volume 10Æ2 l. Comparing with these results, vaporous phase of essential oil of thyme happens to be more effective.

# Conclusion

Hot water extract of thyme in the concentration of 15ml/dl, which is rich in thymol besides other antifungal components, could be used for disinfect fungi and also comparable to pure thymol. Considering the results, we recommend the use of thyme selected for development of new and safe fungicides. Further formulation and field experiments are necessary to achieve this target.

# Materials and methods

# Microbial culture and condition

The thyme extract and thymol were assayed for antifungal activity against the fungal strains *A.niger*, *A.flavus* and *Penicellium sp* isolated from Palmyrah leaf handicrafts which are affected by fungi. These fungi were grown on PDA plate at room temperature and maintained with periodic sub-culturing at 4°C.

# Thymol and Thyme

Thyme flowers obtained from domestic market and Thymol from Sigma was used for this study.

# Preparation of thyme powder

Thyme flowers were dried in oven at 40oC for 24h, ground into fine powder in an electric grinder and sieved to obtain a fine sample. Then sealed in a polythene bag and stored at room temperature until extraction.

# Preparation of extract

Dried powered of thyme (20g) was weighed and macerated with 100 mL of water (boiled for 1/2h - hot water extract) and filtered through Whatman No 1 filter paper. This crude extract was used to screen the antifungal activity of extract.

Screening of thymol and thyme extract for antifungal activity by dilution method PDA medium with different concentrations of thyme extracts and thymol were prepared as Table 2. About 15 mL of the medium with above treatments were poured into each petridish and allowed to settle. Nine mm discs of 5 days old culture of the test fungi from the margin of the plates were incised, placed at the center of the petridishes, and incubated at room temperature for 4-7 days. After incubation the colony diameter was measured in millimetre. For each treatment three replicates were maintained. PDA medium without the extract served as control. Growth zones were measured at 4th and 7th days of incubation.

After incubation the colony diameter was measured in millimetre. For each treatment three replicates were maintained. PDA medium without the extract served as control. Growth zones were measured at 4<sup>th</sup> and 7<sup>th</sup> days of incubation. The fungi toxicity of the extract in terms of percentage of growth inhibition of mycelia was calculated by using the formula

Growth inhibition (%) =  $dc - dt/dc \times 100$ 

Where dc = Average increase in mycelial growth in control,

dt = Average increase in mycelial growth in treatment [16].

The antifungal agent nystatin added to the agar plates (final concentration of 1.0 mg/L) served as a positive control of *Aspergillus niger*, *A. flavus* and *penicillium sp*. Each experiment was repeated to confirm the results.

Authors' contribution

Table 1: Different concentrations of thyme and thymol extracts with different treatments

Treatments	Extracts	Concentrations (ml/dl)
T1	Thyme	1
T2	Thyme	5
T3	Thyme	10
T4	Thyme	15
T5	Thymol	0.1
Т6	Thymol	0.5
T7	Thymol	1.0
Т8	Thymol	1.5

# Statistical Analysis:

The results (percentage of inhibition) obtained from the eight treatments with three replicate were subjected to analysis of variance by complete randomized design (CRD). The significant difference among the treatments was tested in Least Significant Difference (LSD) at 5 % level of significance using SAS software.

# Competing interests

The authors declare that they have no competing interests.

#### Authors' contribution

SM & RK participated in the conception and design of the study, carried out the antimicrobial activity study, SM- Drafted the manuscript. NAM- carried out statistical analysis and SSV- coordinated & management of research activities. All authors read and approved the final manuscript.

# Acknowledgements

Financial assistance given by Ministry of Traditional Industries & Small Enterprise Development is acknowledged.

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# 2.1.6 Optimization of dyeing condition and its dyeing on Palmyrah (Borassus flabellifer) leaves

Subajini Mahilrajan<sup>1\*</sup>, Jeyarani Nandakumar<sup>2</sup>, Robika Kailayalingam<sup>1</sup> and SriThayalan SriVijeindran<sup>1</sup>

#### Abstract

Palmyrah leaf based articles have been dyed mostly with direct dyes till today, which always have problems to dyers is that complications in dye reproducibility. Therefore this study was concluded about standardize the dyeing variables of alkaline dye. The optimum wave length was 600 nm, out of a set of wave length ranging from 400-700 nm on the basis of highest absorbance. Five dye concentrations (0.1-0.5g/l) were tried and 0.3g/l dye concentrations were selected on the basis of dye absorption. Likewise for the good dye absorption optimum temperature, optimum time and optimum leaf: liquor ratio was 100°C, 30 min and 1:20 respectively. Significantly higher dye absorption and wash fastness was observed leaves with beaching than without bleaching while there was no significant different among the property of light fastness. Effect of auxiliary was studied with sodium chloride, sodium carbonate and naphthalene among the auxiliaries naphthalene was selected as the best source for the maximum dye absorption based on the light fatness properties.

Key words: Palmyrah leaf, dye absorption, Naphthalene and Fastness

# INTRODUCTION

History

Dyeing is a complex process, where numbers of variables are involved. Dyeing process is broadly governed by fabric, dye and time, temperature, pH of the fabric and liquor, type of auxiliary used etc. Any minor variation in any of these variables causes problems in dye reproducibility, though it is possible to achieve reproducibility in dyeing results. By standardizing each and every variable consistent reproducible results can be achieved.

#### Literature Review

The temperature of the dye bath affects the affinity of the dye molecules towards fibre, rate of hydrolysis, migration and covalent bond formation, therefore the dyeing temperature selected must be as per the dye type. Percentage of dye absorption increased with increase in temperature and after that, the dye absorption decreased (Taylor, 2001; Bae, et al., 1998).

Auxiliaries play an important role in dyeing of reactive dyes. They help in better exhaustion of the dyes. Alam, et al., 2008; Farha, et al., 2010 reported that the addition of electrolyte to the dye liquor of anionic dye increased the uptake of dye by the fabric. The electrolyte used in dyeing dissociates completely in aqueous dye liquor. For entering into the fabric, the charge on surface (negative in fabric) will have to be neutralized since both anionic dyes and fabric have the same charge. Sodium ion (Na+) from sodium chloride is cationic and in the dye liquor is attracted by the negatively charged fabric. By bonding the sodium cations neutralize the anionic surface charge of the fabric. Now the neutralized fabric can attract the organic dye molecules which have a greater affinity for the fabric than the aqueous solution.

Dyes molecules permanently bind with cellulose based fibers (cotton, rayon, hemp, linen) as well as leaves, when the pH is raised. Soda ash (sodium carbonate) is generally used to raise the pH and is either added directly to the dye or in a solution of water in which garments are soaked before dyeing. (Anonymous, 2007). Because of this reason alkaline dyes were used for this study.

# Problems identified

Leaf based articles have been dyed mostly with direct dyes till today, which always have problems to dyers is that complications in dye reproducibility as well as consumers as these articles fades very easily. So an attempt has been made to standardize the dyeing process for leaves using alkaline dye which have good colour fastness.

# **Objective**

Study the dyeing process to reduce the fade of dyed Palmyrah Leaves

# Specific objective

Optimization of alkaline dye and its dyeing on Palmyrah leaves

# Scope of the study

Increases the uses of dyed Palmyrah leaves when compared with leaves without dye due to improve technique. If dyeing process can be improved Palmyrah leaf based products will increase the earning in domestic and foreign market.

#### **DESCRIPTION OF RESEARCH**

Standardization of dyeing process for Palmyrah leaves with alkaline dye: Experiments were carried out to optimize dye concentration, dyeing time, dyeing temperature and leaf liquor ratio for dyeing of Palmyrah leaf with alkaline dye.

# Determination of optimum wave length

For determining the optimum wavelength 1 ml of dye was diluted to 200 times and absorbance of the solution was taken on a spectrophotometer at different wave lengths from 400 to 700 nm. The wavelength reflecting the highest optical density was selected.

# Optimization of dye concentration

For determining the optimum dye concentration, five different concentrations of reactive dye i.e. 0.1, 0.3, 0.5, 0.75 and 1g/L were taken and samples were dyed at 95-100°C for 30 minutes. Absorbance of dye solutions before and after dyeing was recorded at optimum wavelength. The dye solution giving the maximum dye absorption was taken as optimum dye concentration.

# Optimization of dyeing temperature

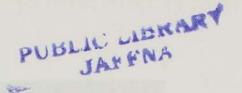
To optimize dyeing temperature, dyeing was carried out using optimum concentration of dye at five different temperatures i.e. 70, 80, 90 and 100°C. The temperature giving maximum dye absorption was taken as the optimum dyeing temperature.

# Optimization of dyeing time

The leaves samples were dyed using optimum dye concentration for five different time durations i.e. 10, 20, 30 and 40 minutes. The optimum dyeing time was selected on the basis of maximum dye absorption.

# Optimization of leaf liquor ratio

To ascertain the optimum dyeing liquor leaf (L: R) ratio, five samples were dyed at the optimum concentration, temperature and time at 1:10, 1:15, 1:20, 1:25 and 1:30. Optimum dyeing leaf liquor (L: R) ratio was decided on the basis of maximum dye absorption.



# Effect of bleaching agent

This palmyrah leaf contained high amount of lignin therefore the leaves samples were heated at 100°C for 10min with hydrogen peroxide (4ml/l) and then dyed using optimum dyeing condition. For control treatment was done without pre heating and hydrogen peroxide. The optimum dyeing was selected on the basis of maximum dye absorption.

# Effect of auxiliaries

Different auxiliaries such as sodium chloride, sodium carbonate and naphthalene (10g/l) were added in dye bath separately. Best auxiliary was decided on the basis of maximum dye absorption.

# Fastness testing

Wash fastness of the leaves dyed without bleaching, dyed with bleaching, dyed with naphthalene, dyed with NaCl, dyed with Na $_2$ CO $_3$  and dyed with bleaching and Naphthalene under optimized condition was tested according to ISO 105 –CO3 method. The above dyed leaves were washed in soap solution (Na salted) for 30 min at room temperature. Half of the dyed leaf was draped with black paper and covered with glass slide then placed on direct sun light for 25h. Then light fastness was tested by colour scale is for assessing changes in colour of leaf in colour fastness tests, for example the leaf consists of five number of colour each representing a visual difference and contrast. The fastness rating goes step-wise from:

Note 5 = no visual change (best rating)

Note 1 = a large visual change (worst rating).

The colour scale has the 5 possible values: 5, 4, 3, 2 and 1

# RESULTS AND DISCUSSION

Basic or cationic dyes on ionization give coloured cations and form an electrovalent bond with the –COOH group of wool and silk. These dyes are applied from neutral to mildly acidic pH. These dyes have poor light fastness.

i. Determination of wave length: Optimum wave length is the wave length at which maximum absorbance was observed. The absorbance was recorded from 400 to 700 nm. The maximum absorbance was observed at 600 nm hence this wave length was selected for further studies (Figure: 1).

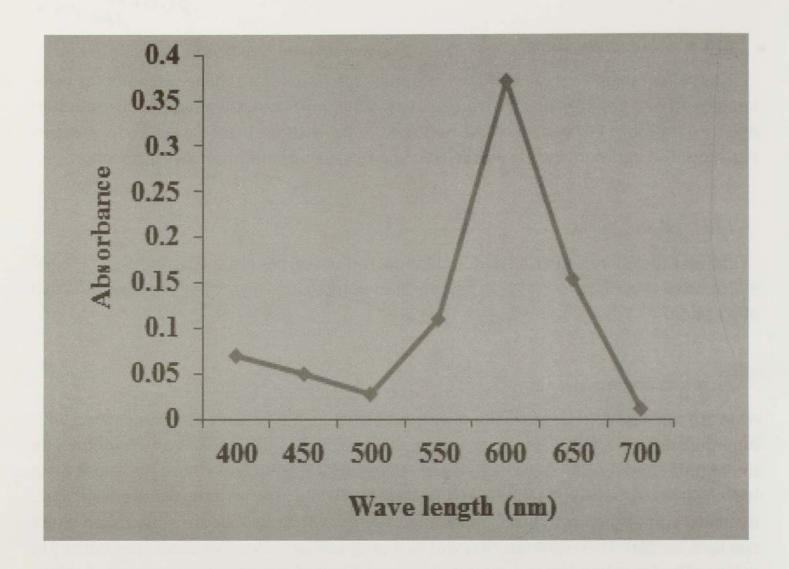


Figure 1: Wave length of alkaline dye (Malachite green).

# Optimization of dye concentration

On the basis of dye absorption to optimize the dye concentration 0.1, 0.2, 0.3, 0.4 and 0.5 g/L dye concentration were taken (Figure 2). There was no significant difference (P > 0.05) between treatments with higher absorbance of dye with the concentration of 0.3, 0.4 and 0.5 g/L. Hence 0.3 g/L dye concentration was selected because mean difference between 0.5 and 0.3g/L is greater than mean difference between 0.3 and 0.4 g/L and also 0.3 g/L is cost effective concentration when compared with higher absorbance concentrations. It is found that dye absorption by cotton fabric increased with the increase in dye concentration in the dye bath because the absolute quantity of the absorbed dye increases while the relative quantity diminishes (Singla *et al.*, 2012).

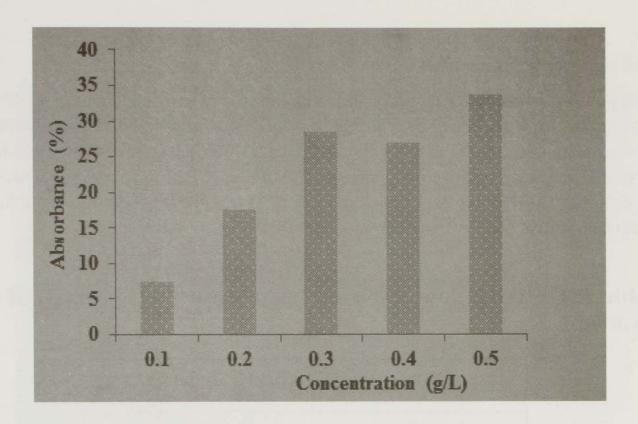


Figure 2: Optimization of dye concentration on the basis of dye absorption

Optimization of dyeing temperature

Dyeing temperature is the temperature that is suitable for dye absorption and fixation of dye on the leaf material. For optimizing dyeing temperature, dyeing was carried out at four different temperatures i.e. 70, 80, 90 and 100°C. The percent dye absorption at different temperatures is given in Table: 3. Mean absorption value for temperature 90 and 100°C is significantly higher than that of temperature 70 and 80°C, while there was no significant difference between the absorptions of temperature for 90 and 100°C. Therefore 90°C was selected as the optimum dyeing temperature. Ali *et al.*, (2008) reported that this increase in dye uptake can be attributed to better dye exhaustion at higher temperature.

Table 3: Optimization of dyeing temperature on the basis of dye

absorption

Temperature (°C)	Absorbance (%)
70	17
80	21
90	64
100	86

# Optimization of dyeing time

Dyeing time is the time required to get the dye fixed on Palmyrah leaves. The effect of dyeing time on absorbance is shown in Table 4. Mean absorption value for time at 30 and 40min is significantly higher than that of time at 10 and 20min, while there was no significant difference between the absorptions of time for 30 and 40min. Therefore 30min was selected as the optimum dyeing time for better exhaustion. Longer the dyeing time create higher absorbance until dye exhaustion attains equilibrium (Ali *et al.*, 2008).

Table 4: Optimization of dyeing temperature on the basis of dye absorption

Time (min)	Absorbance (%)
10	70
20	75
30	86
40	91

# Optimization of Leaf liquor Ratio

The effect of leaf liquor ratio on absorbance is shown in figure 2. Mean absorption value for L: R for1:10, 1:15 and 1:20 is significantly higher than that of L: R for 1:25 and 1:30, while there was no significant difference between the absorptions of L: R for for1:10, 1:15 and 1:20 (Figure 3). Therefore 1:20 was selected as the optimum dyeing L: R for better exhaustion. Higher absorbance value at lower L: R may be explained by crowding of dye molecule at lower L: R resulting in increased dye exhaustion of the leaf (Ali *et al.*, 2008).

# Effect of bleaching agent

The leaves samples were heated at 100oC for 10min with hydrogen peroxide (4ml/l) and then dyed using optimum dyeing condition. For control treatment dyeing was done without preheating (Figure 4). Mean percentage of dye absorption was significantly higher for dyed with bleaching than dyed with non-bleaching method.

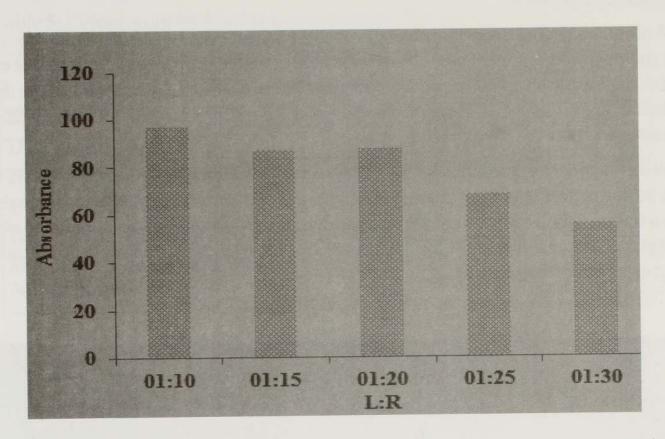


Figure 3: Optimization of leaf liquor ratio on the basis of dye absorption

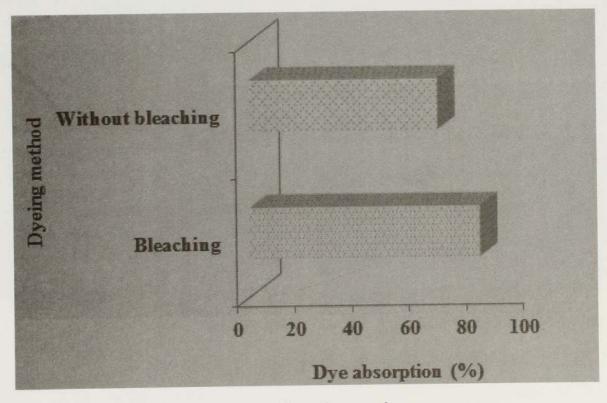


Figure 4: Effect of bleaching on dye absorption

# Effect of auxiliaries

A mordant is more important than the dye itself. Moreover, the ideal mordant for bulk use should produce appreciable colour yield in practicable dyeing conditions at low cost, without seriously affecting physical properties of fibre or fastness properties of the dyes. Different auxiliaries such as sodium chloride, sodium carbonate and naphthalene (10g/l) were added in dye bath separately.

Percentage of dye absorption with  $Na_2CO_3$  was significantly higher than other auxiliary this may be due to de-colouration of dye on alkaline medium. Among the NaCl and naphthalene there was no significant different between on dye absorption while de-colouration to direct sunlight for 24h was higher for NaCl than naphthalene (Figure 5). Therefore Naphthalene was selected for further study.

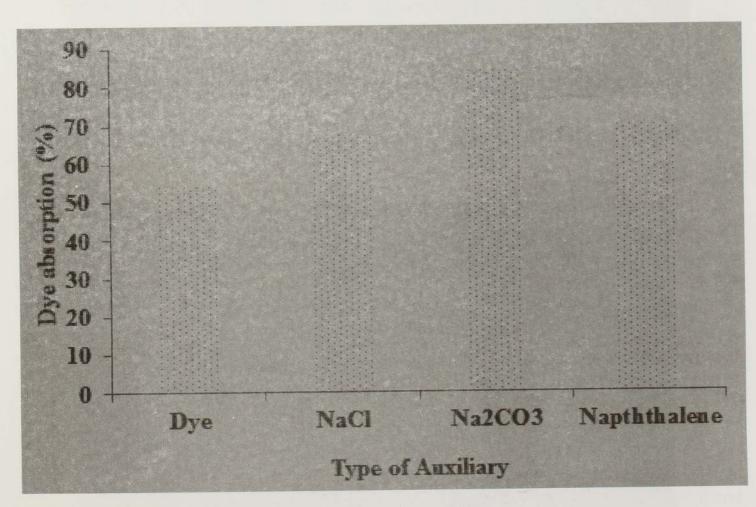


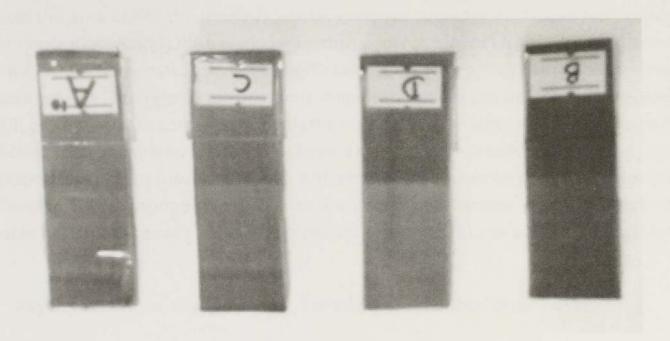
Figure 5: Effect of auxiliary on dye absorption

# Fastness testing

Table 5: Colour score for fastness test of different dyed method

Method of dyeing	Wash fastness	Light fastness
Dyed without bleaching	4	3
Dyed with bleaching	5	3
Dyed with naphthalene	4	4
Dyed with NaCl	3	3
Dyed with Na <sub>2</sub> CO <sub>3</sub>	2	2
Dyed with bleaching and Naphthalene	5	3

Wash Fastness for colour scale for dyed with bleaching and dyed with bleching and Naphthalene was showed higher than other treatments (Table 5) While those treatments showed less light fastness colour scale although dyed with naphthalene showed good light fastness (Figure 4) property.



**Figure 4:** Dyed without bleaching (B), dyed with bleaching (A24), dyed with naphthalene (A10), dyed with NaCl (C), dyed with Na2CO3 (D) and dyed with bleaching and Naphthalene (A25).

#### CONCLUSION

This study could be conclude for optimum dyeing with good dye absorption accordingly leaf materials have to dyed with the concentration of 0.3g/l, Temperature 100°C, Time of dyeing 30 min and leaf: Liquor ratio 1: 20. Besides dyed with bleaching was showed best wash fastness and dyed with naphthalene showed good light fastness.

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IOSR Journal Of Environmental Science, Toxicology And Food Technology 8, (1), PP 47-52, Jan. 2014

# 2.1.7 Glycemic Index and Insulin Index of Palmyrah Based Edible Products Commonly Consumed in Jaffna

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#### Abstract

The objective of this study was to determine the insulin index and glucose index to isoenergetic (1000 kJ) portions of Palmyrah based foods commonly consumed in Jaffna and their correlations between nutrient and phytochemical contents. Subjects were selected those having fasting blood sugar less than 100 mg/dl. Glucose (reference standard) and four test foods such as pinnatu, Jaggery, odiyalpittu and pullukodiyal flour snak were administrated to four groups of 24 subjects and serving weight were calculated based on the energy content. Subjects were fasted for 12h before the administration of foods then venous blood samples were collected at 0 time and every 30 min for two hours after either feeding with glucose and test foods. An insulin score and glycemic score were calculated from the area under the insulin/glucose response curve for each food with the use of reference food. Among the test foods pinattu has low glycemic (52.9%) and low insulin index (36.47%). Carbohydrate, ash and Total Dietary Fiber (TDF) present in the those products were negatively correlated with glycemic index. Insulin index was negatively correlated with TDF, protein and ash contents. Not only the nutrient contents but also the phytochemicals such as total phenols and flavonoids showed negative correlation. All the tested products showed less plasma insulin, although high plasma insulin causes coronary heart disease. Therefore Palmyrah products could be used in order to develop new value added foods, which could have more beneficial health properties regarding glucose and insulin metabolism.

Keywords: Insulin, Glycemic index, Carbohydrate and Total dietary fiber

#### INTRODUCTION

Palmyrah palm has great economic potential and every part of the palm has economic value [1]. The edible palm products are classified into sap, fruit and tuber based products. Among the sap based products 'neera' is obtained by tapping the inflorescences, which is consumed as such after deliming process or converted in to jaggery, sugar or candy [2]. Ripped fruit pulp used in different food preparations at domestic level. Fresh Palmyrah pulp could be extracted manually or mechanically [3]. The fibers are removed by straining through muslin cloths sunlightdried by called 'pinnatu'. Tuber based products such as Palmyrah 'odiyal flour' (dried tuber flour) and 'plukodiyal flour' (boiled, dried tuber flour) are used for traditional food preparations.

Palmyrahfruit pulp has shown antidiabetic [4], anti-inflammatory [5], wound healing, anthelmintic activity [6], analgesic and antipyretic activity [5]. Methanolic extract of the male flowers of *Borassus flabellifer* inhibited the serum glucose levels in sucrose-loaded rats, which may be due to presence of spirostane-type steroid saponins [7]. It also has been documented that Palmyrah flour possesses immunosuppressant property [8]. Even though the Palmyrah has innumerable medical importance [9] it has not received due importance.

Among the Palmyrah-based products, fruit pulp showed caloric value (energy) is 102.83 kcal/100g [10] and also contained more than 5% of soluble fiber [11], which may influence on glycemic and insulinemic response [4]. Lu *et al.*, (2004) [12] have shown that ingestion of arabinoxylan rich fibers decreased postprandial insulin and glucose responses in healthy subjects. Chandalia *et al.*, (2000) [13] have shown that a high dose intake of fibers over six weeks had beneficial effects on type 2 diabetes patients by improving glycaemic control, attenuating hyper insulinaemia. Even though the Palmyrah food based edible products are the traditional foods of Jaffna inhabitants, their nutritive values have not been studied so far.

The aim of this study was to determine the postprandial insulin responses and glucose response to isoenergetic portions of Palmyrah based products such as Jaggery, pinattu, odiyalpittu and pullukodiyalflour snak in non-diabetic subjects.

# **MATERIAL AND METHODS**

Materials

Glucose (Glucolin pure glucose) was from SmithKline Beecham (Pvt) Ltd, Colombo-01. 'Pinattu', 'odiyal' flour and 'pullukodiyal' flour were from 'Katpaham' Sale Center, Palmyrah Development Board, Jaffna, Sri Lanka and the 'Jaggery' was from Pandatharippu Palm Development Society Jaffna, Sri Lanka.

# Preparation of test foods

The foods were selected to represent Palmyrah based natural and processed foods commonly consumed in Jaffna peninsula populates. Foods were prepared in bulk to minimize variations in composition. Each food was served as a 1000kJ portion with 250 mL water.

'Pinattu'

Pinattu cut in to 2.0× 1.0×0.3 cm<sup>3</sup> slabs was used.

'Odiyal pittu'

The odiyal flour (250g) was soaked in 2500ml water for 60 minutes, strained through a muslin cloth and squeezed to wet dry. After mixing with wheat flour (50g) and salt the mixture which was made into fine granules, mixed with scrapped coconut (75g) and steamed for 20minutes.

'Pullukodiyal' flour snak

'Pullukodiyal' flour (200g) was mixed with sugar (20g), scrapped coconut (50g) and water (100ml) and made into small balls.

# Analytical method

Determination of energy contents of the Palmyra based foods

The energy content of each of the food preparations were measured with Bomb Calorimeter (e2k) and serving weight was calculated for each food is have a total energy of 1000kJ [14].

# Nutrient analysis

All the food samples were analyzed for moisture, fat, protein, ash and total dietary fiber contents [15].

# Phytochemical analysis

Food samples (10g) were extracted with aqueous (polarity index: 9) in a soxhlet extractor for 24 hours. The extracts were concentrated using rotator evaporator (IKA), and stored at 4°C. Phytochemicals such as total phenolic content [16] and total flavonoid content [17] were estimated and the results were expressed as mg gallic acid equivalents (GAE)/g at 760nm and quercetin equivalents (QE)/g at 510nm, respectively.

# Estimation of blood glucose level

Plasma glucose concentration was analyzed with glucose hexokinase [18] in a semiautomated biochemical analyzer (TC 3300).

# Estimation of serum insulin level

Serum insulin concentration was measured with insulin ELISA kit (BIOTINA).

# Calculation of Glycemic index and insulin index

After the administration of the reference or test foods blood glucose and insulin levels measured for 2 hrs with 30min interval. Area Under Curves (AUC) for glucose and insulin (gAUC and iAUC) were calculated, based on Simpson's Rule, where fasting concentrations were used as the baseline and truncated at zero. Descriptive data are expressed as mean  $\pm$  standard error mean [14].

Insulin score IS (%) was calculated for each test food by dividing the insulin AUC value for the test food by the insulin AUC value for glucose (reference food), and expressed as a percentage.

IS (%) = Area under the 120 min insulin response curve for 1000 kJ test food \*100

Area under the 120 min insulin response curve for 1000 kJ glucose

# Selection of subjects

Four separate groups of 24 healthy adults [19], [20] age between 18- 40 years were selected. Informed consent was obtained from all of the subjects. The subjects taking prescribed medications and non-diabetic subjects those have fasting blood sugar greater than 100 mg/dl were excluded.

# Administration of test and reference food

A fasting (12h overnight fast) venous blood sample (2.5ml) of each subject was collected. The subjects were administrated with either 62.5g (1000kJ) glucose in 250ml of water or with test food and where glucose was used as reference food. Venous blood samples (2.5ml) were collected at 30, 60, 90 and 120 min after the administration of glucose and test foods and dispensed into two tubes where one contained KF-Na EDTA (for the plasma glucose analysis) and the next tube without clotting agent (for serum insulin analysis). Blood samples were centrifuged at 2500 rpm for 10min and plasma, serum were separated into blood collection tubes and stored immediately at -80°C for analysis.

#### Ethical clearance

The ethical clearance (J/ERC/13/43/NDR/0063) for this study was obtained from the 'Ethical Review Committee', Faculty of Medicine, University of Jaffna.

# Statistical methods and data analysis

Glycemix index (GI) for each food was also calculated by using the same equation with the corresponding plasma glucose concentration. Glycemic and insulinemic indexes of different types of foods were analyzed by Randomized Complete Block Design (RCBD) using SAS software version 9.1.

The results obtained from nutritional and phytochemical analysis with triplicate were subjected to analysis of variance by Complete Randomized Design (CRD). The significant difference among the extracts was tested in Least Significant Difference (LSD) at 5 % level of significance using SAS software.

Linear-regression analysis was used to test the associations between glucose and insulin indexes, correlation between GI and nutritional, phytochemical content, that of between SI and nutritional, phytochemical content using SAS software version 9.1.

#### RESULTS

Freshly prepared Palmyrah based foods samples were tested for their energy content with bomb colorimeter and then serving weights were calculated. Since this study contained four different types Palmyrah based food items, it was decided to select six subjects in a group. Their mean height and weight were  $53.8(\pm 7.7)$  Kg and  $164.3(\pm 6.7)$  cm respectively (TABLE 1).

# Nutritional analysis of test foods

Moisture content of the odiyalpittu and pullukodiyal flour snak was higher than jaggery and pinattu. Carbohydrate content of the jaggery [57.75 ( $\pm$ 6.8)g/100g] was significantly higher than other test foods while there were no significant difference between the carbohydrate content of odiyalpittu and pullukodiyal flour snak. Protein contents of pullukodiyal flour snak was the highest [3.90( $\pm$ 0.2)] and it significantly higher than odiyalpittu [3.25( $\pm$ 0.2) g/100g]. There were no significant difference between fat contents of pullukodiyal flour snak [4.11( $\pm$ 1.3)g/100g], odiyalpittu [5.94( $\pm$ 1.4)g/100g] and very little amount of fat content found in jaggery and pinattu. Pinattu [11.98( $\pm$ 1.9)g/100g] contained highest amount of TDF and it was significantly higher than that in odiyalpittu [10.12( $\pm$ 1.5)g/100g], pullukodiyal flour snak [7.87( $\pm$ 2.7)g/100g] and jaggery [2.15( $\pm$ 2.0)g/100g] (TABLE 2).

TABLE 1: Energy content, serving weights of different palmyrah based foods and means age, Body Mass Index (BMI) of the subjects

Average BMI (kg/m²)	19.90 (±2.32)	19.46 (±2.4)	21.04 (±1.77)	19.47 (±1.82)
Mean Age (Years)	30 (±4.11)	33 (±3.30)	25 (±1.86)	33 (±7.21)
Female (No.)	2	1	2	m
Males (No.)	4	2	4	ю
Total Subjects (No.)	9	9	9	9
Weight of 1000kJ ener- gy test food (g)	76.3	68.6	115.3	93.8
Energy (mJ/kg)	13.092 (± 0.05)	14.577 (± 0.19)	8.670 (± 0.12)	10.658 (± 0.12)
Test	Pinattu	Jaggery	Odiyal piitu	Pullu- kodiyal snak

# Phytochemicals analysis of test foods

Total flavonoid and phenol contents of all the test foods significantly (p<0.05) different from each other. Both total flavonoid and phenol contents were highest in pinattu (TABLE 2).

TABLE 2: Nutrient, phytochemical contents and GI and IS of test

Nutrients/	Nutrients/ Phytochemicals	Jaggery	Pinattu	Odiyal pittu	Pullukodiyal flour snak
Nutrients	Moisture	5.71(±0.014	12.86 (± 0.79)°	56.86 (±0.01)ª	35.02 (± 0.53) <sup>b</sup>
(g/ serving	ydrate	57.75 (±6.8ª	45.82 (±1.5) <sup>b</sup>	38.66 (±8.4)°	41.68 (±8.2)°
weight)	Protein	0.91 (± 0.0)°	1.80 (±0.2) <sup>b</sup>	3.25 (±0.2) <sup>b</sup>	3.90 (± 0.2)ª
	Fat	0.03 (± 0.0b	0.05 (±0.0) <sup>b</sup>	5.94 (±1.4) <sup>a</sup>	4.11 (±1.3) <sup>a</sup>
	Total dietary fiber	2.15 (±2.0)°	11.98 (±1.9)³	10.12 (±1.5) <sup>b</sup>	7.87 (±2.7 ) <sup>6</sup>
	Ash	2.05 (±0.0) <sup>b</sup>	3.15 (± 0.1) <sup>a</sup>	0.46 (± 0.0)⁴	1.24 (± 0.0)°
Phyto- chemicals	Iotal Phenol content	176.5 (±0.03) <sup>b</sup>	315.6 (±0.02)ª	43.7 (±0.01) <sup>d</sup>	126.0 (±0.01)°
(mg/serv-					
ing weight) Total Flave conte	Total Flavoniod content	28.7 (±0.06c	188.1(±0.06)a	0.0	66.6 (±0.02) <sup>b</sup>
(%)		53.77	52.90	52.27	60.33
15(%)		62.50	36.47	68.90	40.17

Each value in the table is represented as mean  $\pm$  SD (n = 3). Values in the same rows followed with a different letter (a-d) are significantly different (p< 0.05).

# Fasting glucose and insulin concentrations

Fasting plasma glucose and serum insulin levels of it four different groups of subjects showed no significant difference between reference and test foods (TABLE 3).

# Relationship between BMI and fasting glucose, insulin concentrations

Mean fasting insulin concentration of each test foods such as jaggery, pinattu, odiyalpittu and pullukodiyal flour snak was negatively correlated with mean BMI values of respective group that variability was 52, 91, 83, and 13% respectively while there were significant different (p>0.05) between fasting insulin concentration and BMI. Likewise there were positive correlation between mean fasting glucose concentrations of groups consuming Jaggery and pullukodiyal flour snak and mean BMI values respective group, while there were negative correlation for mean fasting glucose concentrations of groups consuming pinattu, odiyalpittu BMI values respective group therefore coefficient determination values accounted for 2, 29, 1, and 18% variability in BMI respectively and no significant different between fasting glucose concentrations and mean BMI.

# AUC of glucose and insulin after the consumption of test and reference foods

There was a no significant different in both insulin and glucose AUCs of reference standards of all foods (TABLE 3). Although while there were significant differences between individual AUCs of both test foods and reference standards. Mean insulin AUCs of test foods were showed significant different, that of jaggery (5340.75uIU/ml/min) was showed significantly higher than other three test foods. However; Mean glucose AUC of each test foods showed not significant different (p<0.05).

Insulin AUC values were divided by glucose AUC values which were 8.59, 1.26, 3.37 and 1.28 for Jaggery, pinattu, odiyalpittu and pullukodiyal flour respectively while there were no significant different between pinattu, odiyalpittu and pullukodiyal flour. Therefore Jaggery was a markedly insulinogenic relative to their glycemic effect (Fig: 1). Consequently jaggery was a greatest product to promote the production or release of insulin to their glycemic response followed by other Palmyrah products such as odiyalpittu, pullukodiyal flour and pinattu.

Table 3: Glucose and insulin and AUC for all the Group of test and reference foods

AUC	Insulin	8544.6		5340.7		6085.7		2219.2		1139.8		3012.6		6692.0		2688.4	
IA	Glucose	2381.8		1280.6		3907.0		2066.6		2180.5		4372.3		2968.5		1791.0	
120	Insulin	38.61	(±21.79)	23.47	(±6.40)	29.00	(±28.05)	26.91	(±23.01)	24.45	(±12.19)	26.44	(±10.10)	58.35	(±34.44)	10.77	(±7.81)
	Glucose	91.39	(±16./1)	92.10	(±5.43)	86.40	(±4.57)	86.12	(±8.66)	87.33	(±9.14)	90.13	(±7.81)	88.32	(±6.85)	85.40	(±6.21)
06	Insulin	88.77	(±40.90)	39.46	(±11.87)	57.10	(±42.45)	30.24	(±22.06)	44.68	(±9.28)	26.94	(±10.51)	66.77	(±26.43)	24.38	(±5.73)
6	Glucose	99.99	(±4,12)	69.16	(±9.24)	98.92	(±8.07)	92.30	(±6.55)	98.27	(±5.62)	96.37	(±4.68)	100.58	(±6.34)	94.75	(±6.27)
0	Insulin	101.47	(±40.13)	65.85	(±33.72)	85.38	(±49.97)	42.07	(+29.31)	64.68	(±14.79)	34.24	(±14.99)	88.10	(±21.44)	42.65	(±14.92)
09	Glucose	109.97	(+13.40)	97.06	(±4.83)	117.48	(±8.41)	102.45	(±8.85)	111.57	(±4.63)	103.53	(±7.05)	109.15	(±6.27)	97.27	(±13.39)
30	Insulin	105.21	(CT:077)	72.07	(±42.68)	75.64	(±42.21)	41.47	(±25.65)	61.21	(±14.04)	64.21	(±24.15)	77.54	(±27.38)	52.68	(±39.36)
3	Glucose	114.30	102.20)	102.41	(±5.11)	132.00	(±5.41)	115.55	(±5.56)	127.55	(±3.34)	115.55	(±2.99)	129.17	(±6.41)	118.05	(±8.44)
0	Insulin	14.82	13.00	13.02	(±12.36)	11.10	(+11.84)	16.00	(±14.65)	12.74	(±2.49)	14.387	(±4.87)	12.08	(±6.32)	12.31	(±8.09)
	Glucose	78.31	TC TT	(7.7)	(±6.79)	77.67	(±9.42)	83.25	(±12.73)	90.52	(±5.66)	93.72	(±1.88)	83.40	(±6.13)	85.23	(±10.70)
	Glucose (mg/dl)/ Insulin level (uIU/ml)	Reference food	Taggery	Jagger		Reference		Pinattu		Reference		Odiyal pittu		Reference		pulluko- dival flour	snak
Time (min)	Glucose (mg/c level (uIU/ml)		Groun 1	dinam				Group 2	oolaham			Group 3	موراده	M		Group 4	3544 (d) 1

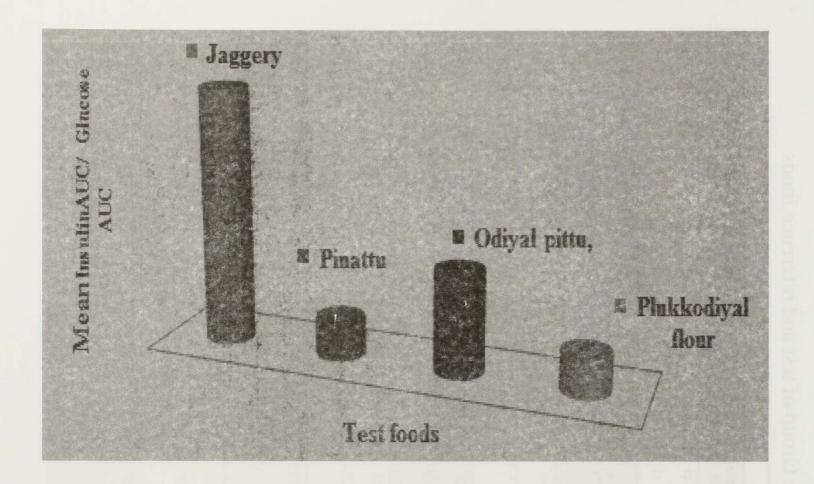


Fig 1: Ratio of insulin AUC to glucose AUC responses.

# Effects of different nutrient on GI values

Linear-regression analysis of carbohydrate was not significant different while other nutritional factors such as fat, TDF, ash and protein showed significant different (p<0.05) among the test foods. Correlation coefficient values of TDF (r=-0.02, p=0.008), carbohydrate (r=-0.03, p=0.055) and ash(r=-0.04, p=0.006) showed negatively correlated with GI values of test foods while positively correlated with protein (r=0.42, p=0.007) and fat (r=0.04, 0.003).

# Effects of different nutrient on IS values

Linear-regression model showed not significant different between SI values of test foods and nutrients such as carbohydrate, TDF, protein, fat and ash. Mean IS values were negatively correlated with TDF (r=-0.18), protein (r=-0.02) and ash (r=-0.39) except fat (r=0.12) and carbohydrate (r=-0.01). The above same correlation pattern was found between nutritional content of composite meals and insulin response by Bao *et al.*, 2009 [21].

# Effects of different phytochemicals on GI and IS value

Linear-regression model was not showed significant different between GI values and phytochemical contents of test foods. There were negativelyweek correlation between total flavonoid (r=0.01), total phenol (r=0.01) and GI values of test food. As well there were negatively correlation between total flavonoid (r=0.65), phenol (r=-0.35) content and IS values of test foods therefore 65 and 35% of variability in IS values of test foods respectively.

# Glycemic and insulin indexes

The raise in blood glucose level and insulin level was less than that of reference food (glucose) (TABLE 2). Among the palmyrah based products pinattu was low GI (52.90) and low IS (36.46) while jaggery (53.77) and odiyalpittu (52.27) were low GI and medium IS and both GI and IS was medium for pullukodiyal flour snak (TABLE 2).

#### DISCUSSION

The glycemic index and insulin index of Palmyrah foods are an important tools used in treat people with diabetes and in weight control. Food with a low GI makes full lengthier and stay satisfied longer, less likely to overeat. The GI helps to know the type of foods which are useful to control the blood glucose level. Insulin index which is less well-known index is more important than the measures of glycemic response [14]. Therefore this study on glycemic index and insulin index was under taken to measure it GI and IS of the Palmyrah based edible products. Nevertheless, no studies have been carried out and reports do not available on GI and IS of Palmyrah based edible products.

In fact, the GI of a carbohydrate-rich food can vary greatly depending on a number of factors including the variety, origin, processing, and preparation of the food, the other nutrients that are consumed with the food and even the time of day in which the GI is measured [22].

Wolever and Bolognesi, 1996 [23] reported that glycemic response data obtained from a test at lunch time, after a standard breakfast, differ significantly from those obtained after an overnight fast. In various in vivo studies test food servings have been determined based on calculated data or data received from food tables and those do not resemble to the actual nutrient composition of the food product. In the present study energy of the consumed test foods were determined by using bomb colorimeter. The energy obtained from pinattu, jaggery, odiyalpittu and pullukodiyal flour snak were used for calculate the serving weight of each test foods.

Gaesser, 2007 [24] actually showed that diets with higher GLs were associated with lower BMIs. In this study there were significantly negative correlation was found between mean fasting insulin concentrations and mean BMI values, same result was reported by Holt et al., 1997 [14] among the six groups of subjects. Jaggery and pullukodiyal flour showed positive correlation while pinattu and odiyal pittu showed negative correlation between mean fasting glucose concentration and mean BMI hence BMI was increased that pinattu and odiyalpittu decreased the mean fasting glucose concentration, this could be due to the amount of dietary fiber content of the test foods because order of the TDF content was pinattu>odiyalpittu>pullukodiyal flour > jaggery (TABLE 2).

Incremental AUC is the area beneath the curve only down to fasting level. If the blood glucose level falls below the baseline, the area below fasting is ignored. Thus, the incremental AUC can never be less than zero [25]. All the test foods showed insulinogenic relative to their glycemic effect base on the ratio obtained from the insulin AUC values were divided by glucose AUC values (Figure 1). In fact, the earlier study on Palmyrah pinattu as an anti-diabetic food component by Uluwaduge *et al.*, (2007) [4] showed in insulin-dependent diabetic patients (IDDM) revealed a lower glucose response to pinattu and there was a significant reduction (p< 0.01, by 15-48%) in blood glucose concentration after a glucose challenge.

Ahmed et al., 1976 found that addition fat to carbohydrate meal enhances insulin secretion even though the plasma glucose response actually decreases and also Holt et al., 1997 [14] found no relation between the postprandial insulin response and the fiber content of a food. Whereas Albrink et al (1979) [26] found that lower insulin response in healthy subjects when consume meals that have high-fiber content than low-fiber content [27]. Palmyrah pinattu contained appropriate amount of dietary fiber. Therefore it should influence the GI and IS of palmyrah pinattu and has low GI and IS (TABLE 2). Glucose and insulin responses decreased after with meals containing soluble fibers, including pectin, Oatrim (oat fiber extract), guar gum, and methyl cellulose fibers, when compared with meals without soluble fiber (Behall et al., 2002). Morgan (1992) [28] reported that three macronutrients stimulate the release of several gut peptides, but to different degrees. Protein and fat are particularly effective in stimulating gut peptide release despite a small glucose effect. Thus, the insulin response to a carbohydrate food varies with the amount of fat, protein, or both.

Palmyrah products generate lower postprandial insulin response than reference standard glucose. This response is not only due to the fiber content of products, but also due to the bioactive components such as total phenol as saponin etc. (TABLE 2) and the physical structure of Palmyrah products. There were significant correlation were observed between phytochemicals and GI values of test foods.

#### CONCLUSION

This present study showed that among Palmyrah based products pinattu showed low glycemic and insulin index. Specifically these products decrease postprandial insulin response compared to reference standard glucose. In other words, less insulin is required for regulation of postprandial plasma glucose concentrations in healthy subjects after intake of all tested palmyrah food products than glucose. High plasma insulin has been shown to be an independent risk factor for coronary heart disease. Therefore palmyrah based food products could be used in order to develop new value added foods, which could have more beneficial health properties regarding glucose and insulin metabolism.

# Acknowledgements

The authors like to thank Palmyrah Research Institute, Palmyrah Development Board, Jaffna, Sri Lanka and Department of Biochemistry Faculty of Medicine, University of Jaffna, for providing the necessary facilities.

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Accepted by IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)

# 2.2 Publications in International conference

# 2.2.1 Effect of Furnacing Time Periods and Temperature on Purity of Quick Lime for the Production of Palmyrah (Borrasus flabellifer) jaggery

S. Mary\*1 and S. Srivijeindran1

#### Abstract

Quick lime is commonly used additive in Sri Lanka during the period of palmyrah (Borassus flabellifer) sweet sap collection to delay sap fermentation by natural yeast and bacteria. There are several Palmyrah sweet sap based products as jaggery, treacle, sugar candy and sugar produced in northern Sri Lanka which shows poor keeping qualities and large variation in physicochemical characteristics among batches. Quality of the above products mainly connects with collection of raw material. Local tappers who are following poor quality control practices as applying excess amount of quick lime with low purity that is adulterated by impurities as sand and ash. In Jaffna two traditional kilns present in Pandaiththrippu and Anaikkoddai are producing quick lime from sea shells using coconut husk charcoal. Purity of quick lime expressed as CaO (g/100g) produced from Anaikoddai kiln was 59.99±0.97 and Pandaitharippu kiln was 42.34±0.74. Quick lime produced from both kilns were not in suitable condition to be used during processing of Palmyrah sweet sap for the production of standardized Palmyrah sweet sap based products. Hence this research was designed as two factor factorial design of experiment. Temperature (750 °C, 850 °C, 950 °C and 1000 °C) and time period (10, 20 and 30 minutes) combinations of treatments were carried out to produce high purity quick lime through furnacing of sea shells. Purities of quick lime obtained were statistically analyzed using SAS package. Significant differences were observed among all selected temperatures and time periods. Significantly highest value for quick lime purity was obtained in 1000 °C and 30 minutes. Application of the treatments 1000 °C for 20 minutes, 1000 °C for 30 minutes, 950 °C for 30 minutes and 850 °C for 30 minutes were produced significantly same and higher purity of quick lime compared to other of treatments. According to high energy consumption 850 °C for 30 minutes treatment was selected to produce quick lime with 96.02±0.06 % of purity.

Key words: Kiln, Palmyrah (Borrassus flabbellifer), Quick lime, and Sweet sap

#### Introduction

Matured male and female inflorescences of Palmyrah palms are tapped to ooze sweet sap. In Sri Lanka earthen pots with inside application of quick lime are used to collect sweet sap (Theivendirarajah, 2008). Barh and Mazumdar (2008) reported that Palmyrah sap is a good source of vitamins such as riboflavin, vitamin B12, vitamin C, thiamine and nicotinic acid and minerals such as calcium, iron, zinc, copper and phosphorous. Jagannadha Rao et al (2009) stated that the pH of fresh Palmyrah sweet sap is 6.8 and total sugar content is 10.93. Hence sweet sap can easily undergo alcoholic fermentation by natural yeast and other microorganisms in the environment. Application of quick lime maintains the sweet sap in basic medium (pH ≥ 8.5) which is susceptible to microbial activity during the period of sweet sap collection. In Jaffna there are two traditional kilns present in pandaiththrippu and anaikkoddai area producing quick lime from sea shells. These kilns are simply made by brick stones on ground and totally open to environment. Coconut husk and fire wood are used to heat sea shells. The above firing materials are spread into several layers between sea shells. The important reaction occurs in lime producing kiln take place at the optimum temperature (900 °C) which is the calcining of limestone (Atkins et al., 2010). In traditional kilns during processing of quick lime local firing materials are produced inadequate heat and temperature is not reached to 900 °C inside of the kiln. Hence sea shells were partially converted into quick lime. Mostly it is continued for one day and next day after it is cooled to environmental condition produced quick lime was packed into polyethylene bags to send to sweet sap based industries without quality checking. Purity of quick lime from traditional kilns was unknown. Hence it should be studied first to enhance the quality of sweet sap processing. The main objective of this research study is to find out suitable temperature and time period combination to produce quick lime with optimum purity. Application of quick lime at higher degree of purity and minimum amount of it will ensure quality control of sweet sap.

# **Materials and Methods**

# Collection of lime samples

Quick lime samples were collected from traditional lime producing kilns build up in two areas of Jaffna namely as Anaikoddai and Pandaitharippu. The kilns were allowed to cool to environmental condition after processing is finished. Produced quick lime samples were randomly collected in different parts of kilns into moisture proof bags and these were transferred to Palmyrah Research Institute laboratory. Collected quicklime was taken on a clean surface to make cone and it was quartered to get a representative sample of small fragments. 100 - 200 g of this was ground by using mortar and pestle and it was passed through a No.7 mesh sieve to sieve the sample. Analysis of the purity of quick lime Sample of quick lime (0.50 g) was weighed accurately and it was applied into a 300 ml Erlenmeyer flask containing 20 ml of CO<sub>2</sub> free distilled water.

Sample containing flask was heated to boiling for 2 minutes. Distilled water (150 ml) and 15 g of sucrose were added. The flask was capped and it was mixed by shaking at intervals for 5 minutes. It was allowed to stand for 30 minutes to 1 hour. After washing the sides of the flask with distilled water, the content was titrated against standard HCI solution (0.1 M) with using phenolphthalein as an indicator. About 90% of the estimated amount of acid was added before shaking the flask and then complete titration, with the final acid being fed slowly until the pink colour disappears. Optimization of conditions for quick lime preparation Natural sea shells were collected by using convenience sampling method in coastal area near to Anaikoddai and Pandaitharippu. Laboratory muffle furnace (Manufacture: Hobersal, Model: JD230 "PAD" and temperature range: 100 °C - 1200 °C) was used to produce quick lime. Temperature and time period (two factors) were taken as variables. Hence experiment was designed in two factor factorial complete randomized design. Four different temperatures (750°C, 850 °C, 950 °C and 1000 °C) and three different time periods (10 minutes, 20 minutes and 30 minutes) were selected to optimize processing conditions (temperature and time period) to produce high purity quick lime. Collected sea shell samples were taken into the crucibles and kept into furnace and produced quick lime samples were cooled to room temperature and immediately packed into air tight glass containers to analyze the purity of quick lime. The study was performed with three replicates for 12 treatments. Statistical analysis All results (purity of quick lime) were analyzed in SAS 9.1 software and the mean separation was done by least significant difference (LSD) at p=0.05. Analyzed entire data obtained during the experiment was expressed as means ± standard deviation.

# **Results and Discussion**

Purity of quick lime produced from traditional kilns Analysis of results revealed that the purity of quick lime expressed as CaO (g/100g) produced from Anaikoddai kiln was 59.99±0.97a and Pandaitharippu kiln was 42.34±0.74 b. It shows high significant difference between produced quick lime samples from two traditional kilns and purity also very low in both kilns. Therefore it is essential to increase the purity factor of producing quick lime in order to maintain quality control during processing of Palmyrah sweet sap which is essential to produce standardized Palmyrah sap based products. Purity of prepared quick lime Statistical analysis of results which were obtained from two factor factorial experiment there were significant differences observed among four different temperatures and among three different time periods. According to the results, a significantly highest lime purity was obtained in 1000 °C for 30 minutes. Since results revealed that temperature and time period are the factors determining the purity of quick lime; results should be statistically analyzed for combination of those two factors. It is better method to get economically viable temperature and time period combination for industrial application. All combination of temperature and time period treatments were completely randomized to perform stall stical analysis and obtained mean and significant values were indicated in Table 1. Among those 12 treatments there were no significant difference were observed among 1000 °C for 20 minutes, 1000 °C for 30 minutes, 950 °C for 30 minutes and 850 °C for 30 minutes. Those four treatments also have got significantly higher value for quick lime purity rather than that of other 8 treatments. Hence 850 °C for 30 minutes could be selected as best temperature and time period combination when compared to 1000 °C for 20 minutes, 1000 °C for 30 minutes, and 950 °C for 30 minutes.

Table 1: Effect of applied temperature and time period combination on purity of quick lime

ncr, Lorn - Hito A.	10 minutes	20 minutes	30 minutes
750 °C	86.58±0.11 <sup>i</sup>	87.92±0.34 <sup>h</sup>	88.78±0.23 <sup>g</sup>
850 °C	93.02±0.06 <sup>f</sup>	94.90±0.06 <sup>d</sup>	96.02±0.06 <sup>ab</sup>
950 °C	94.49±0.34 <sup>e</sup>	95.72±0.28 <sup>bc</sup>	96.25±0.17 <sup>a</sup>
1000 °C	95.42±0.11°	96.28±0.23 <sup>a</sup>	96.32±0.11 <sup>a</sup>

Proceedings of the 4 th International conferences of Eastern University, 2016.

# 2.2.2 Antifungal Activity of Some Plant Extracts Against Decay Fungi

# From Palmyrah Leaf Handicrafts

Subajini Mahilrajan, Jeyarany Nandakumar, Robika Kailayalingam and Srithayalan Srivijeindran.

#### Abstract

The Palmyrah leaves handicrafts are affected by fungal attack; this could be prevented by some protective agents. Instead of using expensive and harmful chemicals, it was decided to test the activity of natural plant extracts on fungal attack. Fungi were isolated from affected Palmyrah leaves by repeated streaking on PDA plates, isolated fungi were purified and characterized as *Aspergillus niger*, *Aspergillus flavus* and *Penicillium sp*. Extract of thyme (*Thymus vulgaris* L) flower obtained in local market and thymol have been evaluated for their Antifungal Activity against isolated fungi by using different concentrations. To screen the antifungal activity, MIC (Minimum Inhibitory Concentration) and Percentage of Growth Inhibition (GI) of thyme extract and thymol were determined. Minimal Inhibitory Concentration of thymol was shown to be 0.5ml/dl. The hot water extract of 15ml/dl thyme and 0.5, 1.0, 2.0 ml/dl of commercially available thymol showed significantly (p<0.05) higher GI (100%) for all fungi species, while 0.1 ml/dl of thymol showed significantly lesser activity when compared with 10ml/dl of thyme extract. Therefore thyme could be used for the preparation of ecofriendly fungal agent to protect the Palmyrah leaf handicrafts also field testing is essential to achieve this in industrial level.

Key words: Extracts, Fungi, Growth Inhibition and Preservation

#### Introduction

Microscopic filamentous fungi are ubiquitous micro-organisms with a great capacity to colonize many kinds of substrates and to develop under extreme environmental conditions. Fungi and their airborne spores in the indoor and outdoor environment have been recognized as possible causative agents of various diseases in human (Nielsen, 2003) and spoilage of Palmyrah leaf handicrafts.

The essential oils of more than one hundred species of the genus *Thymus* have been chemically investigated; reveal about 360 different volatile components in total. Among these, the monoterpenes were the most prominent group while sesquiterpenes represent a lower percentage of the volatiles. Generally, plants of the genus Thymus are considered as the most common source of the monoterpenoid phenols, thymol and carvacrol (Bruneton 1995).

Several *Thymus* species are locally known as "Omum" and the dried herbal parts are used in herbal tea, condiment and folk medicine. The essential oils of some *Thymus spp.* are characterized by the presence of high concentration of the isomeric phenolic monoterpenes thymol and/or carvacrol (Baser, 1995). Traditionally this has been used as a medicinal plant in the treatment of headaches, coughs, diarrhea, constipation, warts, worms and kidney malfunction (Simon et al., 1999). Thyme also possesses various beneficial effects as antiseptic, carminative, antimicrobial and antioxidative properties (Baranauskiene *et al.*, 2003).

Several studies have shown that thyme oils, particularly those of *Thymus vulgaris* and *Thymus zygis* (Bruneton, 1999; Pina-Vaz *et al.*, 2004; Stahl-Biskup & Sa´ ez, 2002), possess significant antifungal, insecticidal, and antimicrobial activities, it may vary based on the variation on the chemical composition.

The main objective of this work was to characterize the in vitro antifungal activities of hot water extract of thyme and pure thymol, as comparative substance, on different fungus species isolated from Palmyrah leaf handicrafts.

# Materials and methods

# Collection of sample

Palmyrah leaf based handicrafts are generally affected by fungus during rainy season. So affected tender leaf of Palmyrah was collected from design center of Palmyrah Development Board during rainy season and used for the isolation of Palmyrah leaf born fungus.

Isolation and detection of Palmyrah leaf born fungi Preparation of Potato Dextrose Agar media PDA plates and slants

Potato Dextrose Agar (3.9g) was dissolved in 40mL of distilled water in a boiling water bath and total volume was made up to 100mL then sterilized at 121°C and 15lb/in² for 15 minutes. After sterilization, the medium was allowed to cool to 50°C and poured in to sterile petridishes (20mL/ Petridish) under aseptic condition. The PDA was prepared as above stated procedure and 7 mL of the medium was poured into boiling tubes. The tubes were plugged with cotton wool and sterilized at 121°C and 15lb/in² for 15 minutes. The tubes were then cooled in an inclined position and used for storage of the fungus.

# Isolation and Purification of fungal strains

Palmyrah leaf was cut into small pieces and inoculated into the PDA plate then incubated at room temperature for 4 days (Robert Koch and Friedrich Loeffler 1884). After 4 days three different colours of the colonies were selected, purified by repeated streaking and transferred to PDA slants and kept at 4°C.

# Identification of isolated fungal strains

Selected fungal colonies were characterized to species level based on macroscopic morphology and microscopic features.

# Thymol and Thyme

Thyme obtained from domestic market and Thymol from Sigma was used for this study.

# Preparation of thyme powder

Thyme flowers were dried in oven at 40°C for 24h, ground into fine powder in an electric grinder and sieved to obtain a fine sample. Then sealed in a polythene bag and stored at room temperature until extraction.

# Preparation of extract

Dried powered of thyme (20g) was weighed and macerated with 100 mL of water (boiled for 1/2h - hot water extract) and filtered through Whatman No 1 filter paper. This crude extract was used to screen the antifungal activity of extract.

Screening of thymol and thyme extract for antifungal activity by dilution method

as Table 1. About 15 mL of the medium with above treatments were poured into each petridish and allowed to settle. Nine mm discs of 5 days old culture of the test fungi from the margin of the plates were incised, placed at the center of the petridishes, and incubated at room temperature for 4-7 days. After incubation the colony diameter was measured in millimetre. For each treatment three replicates were maintained. PDA medium without the extract served as control. Growth zones were measured at 4<sup>th</sup> and 7<sup>th</sup> days of incubation.

After incubation the colony diameter was measured in millimetre. For each treatment three replicates were maintained. PDA medium without the extract served as control. Growth zones were measured at 4<sup>th</sup> and 7<sup>th</sup> days of incubation. The fungi toxicity of the extract in terms of percentage of growth inhibition of mycelia was calculated by using the formula

Growth inhibition (%) =  $dc - dt/dc \times 100$ 

Where dc = Average increase in mycelial growth in control,

dt = Average increase in mycelial growth in treatment (Singh et al., 1999).

The antifungal agent nystatin added to the agar plates (final concentration of 1.0 mg/L) served as a positive control of *Aspergillus niger*, *A. flavus* and *Penicillium sp*. Each experiment was repeated to confirm the results.

# Statistical Analysis:

The results (percentage of inhibition) obtained from the eight treatments with three replicate were subjected to analysis of variance by complete randomized design (CRD). The significant difference among the treatments was tested in Least Significant Difference (LSD) at 5 % level of significance using SAS software.

# Results

Isolated fungi B, G & A were identified as *Aspergillus niger, Aspergillus flavus* and *Penicillium sp* respectively at species level in based on macroscopic and microscopic features (Table 2).

The antifungal activity of the extracts with different treatments, in terms of percentage of growth inhibition of mycelia were calculated and tabulated in Table 3. Results showed that the thymol have great potential of antifungal activity against all of the three fungi tested.

Activity of hot water extracts of thyme (T1-T4) was compared with that of pure thymol (T5-T8). All the four different concentrations of pure thymol (T5-T8) showed the 100% of GI for *A.niger*. *A.flavus* and *Penicillium* at  $4^{th}$  day of incubation and minimal inhibitory concentration of thymol was  $0.5 \, \text{ml/dl}$  (T6). The hot water extract of (T4)  $15 \, \text{ml/dl}$  thyme and  $0.5 \, \text{(T6)}$ ,  $1.0 \, \text{(T7)}$ ,  $1.5 \, \text{(T8)}$  ml/dl of commercially available thymol were showed significantly (p<0.05) higher GI (100%) for all fungi species, while  $0.1 \, \text{ml/dl}$  (T5) of thymol showed lesser activity when compared with  $10 \, \text{ml/dl}$  (T3) of thyme extract, significantly.

GI of thyme extract at the concentration of 10 ml/dl (T3) was 76.9, 76.4 and 75.3% and 1 ml/dl (T1) was 40.6, 2.7 and 7.0 for *A.niger*. *A.flavus* and *Penicillium sp* respectively at 4<sup>th</sup> days of incubation (Figure 1) while thymol with the concentration of 0.1ml/dl showed 59.4, 59.4 and 66.9% GI for above stated fungi respectively at 7<sup>th</sup> days of incubation.

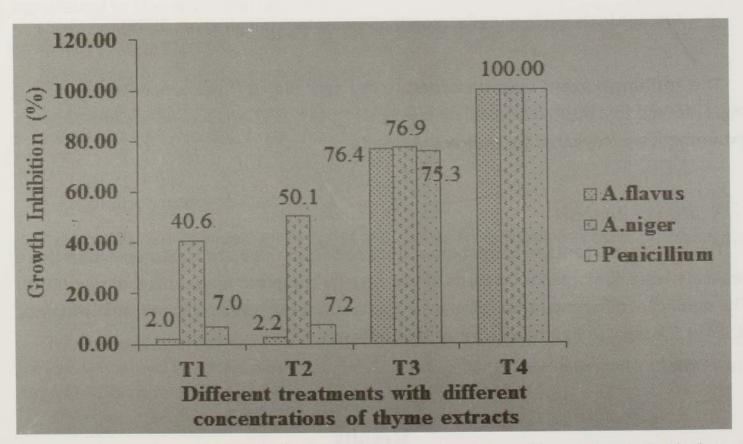


Figure 1: Average growth inhibition of thyme extract for different fungi at 4th days of incubation

#### Discussion

# Isolation of Palmyrah leaf borne fungi

The Palmyrah leaves are long, fan-shaped, 2 to 3 m in length. The first two tender unexpanded whitish leaves and the next 12 young green leaves are used in making various handicrafts. The whitish tender leaves are used for making soft fine handicrafts while the young green leaves are used for making stronger, but coarse textured utility items like mats, baskets, packaging material, inner lining of heavy duty fibre baskets etc. The mid rib known as eekils can be used to make implements for sweeping the gardens, wall hangers, baskets and a type of carrying cases. Therefore the dried Palmyrah tender leaf was selected and cut into small pieces and inoculated into the PDA plate then incubated at room temperature for 4 days. After incubation black (B), green (G) and bluish green (A) coloured colonies were selected for further study. Selected colonies were purified by repeated streaking then purified fungi were maintained in agar slants at 4°C throughout the study and used as stock cultures.

# Antifungal activity assay

All species of fungi, using of any concentrations caused significant differences (p<0.05) on inhibitory effect of extracts. It could be observed that as extract concentration increases, the inhibitory effect also increased. In other words, the inhibitory effect of the extract was proportionate to its concentration. As stated by Rasooli *et al.*, 2006 and Amini *et al.*, 2012, with the increase of concentrations, the susceptibility of fungi increased.

At low concentrations, phenolic lipophilic compounds such as thymol alter cell permeability of microbes, permitting the loss of macromolecules. Exact cause effect relation for the mode of action of phenols has not been determined, but they may inactivate the essential enzymes, react with cell membrane proteins or disturb genetic material functionality (Lopez-Malo *et al.*, 2005).

# Conclusion

Hot water extract of thyme in the concentration of 15ml/dl, which is rich in thymol besides other antifungal components, could be used for disinfect fungi and also comparable to pure thymol. Considering the results, we recommend the use of thyme selected for development of new and safe fungicides. Further formulation and field experiments are necessary to achieve this target.

# Acknowledgements

Financial assistance given by Ministry of Traditional Industries & Small Enterprise Development is acknowledged.

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**Table 1:** Different concentrations of thyme and thymol extracts with different treatments

Treatments	Extracts	Concentrations (ml/dl)				
T1	Thyme	1				
T2	Thyme	5				
T3	Thyme	10				
T4	Thyme	15				
T5	Thymol	0.1				
Т6	Thymol	0.5				
Т7	Thymol	1.0				
Т8	Thymol	1.5				

Table 2: Macroscopic and microscopic features of isolated fungus

Characteristics	В	G	A
Colony colour	Black with	Greenwith	Bluish green with white
	white reverse	cream reverse	revers
Hyphae	Septate hyaline	Septate hyaline	Septate hyaline
Conidial head	Radiate	Radiate	Radiate
Conidiophore	Smooth hyaline	Smooth hyaline	Simple
Vesicle	Globose	Subglobose	
Conidia	Black globose	Globose	Round, Unicellular Unbranch
Phialide	Unisereate	Bisereate	

Table 3: Percentage of growth inhibition with different treatments

Fungus species	T <sub>1</sub>	T <sub>2</sub>	<b>T</b> <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>	<b>T</b> <sub>7</sub>	T <sub>8</sub>
A.niger	40.68 <sup>e</sup>	50.13 <sup>d</sup>	76.98 <sup>b</sup>	100°	59.4°	100°	100°	100ª
A.flavus	2.01 <sup>e</sup>	2.26 <sup>d</sup>	76.44 <sup>b</sup>	100°	59.4°	100°	100ª	100ª
Penicillium	7.02 <sup>e</sup>	7.27 <sup>d</sup>	75.38 <sup>b</sup>	100°	66.92°	100ª	100ª	100 <sup>a</sup>

Proceedings of Jaffna University International Research Conference (JUICE 2014)

# 2.2.3 FORMULATION OF INSTANT SOUP MIX POWDER USING UNBOILED PALMYRAH (Borassus flabellifer) TUBER FLOUR AND LOCALLY AVAILABLE VEGETABLES

N.Niththiya<sup>1</sup>, S.Vasantharuba<sup>1</sup>, M. Subajini<sup>2</sup>, S Srivijeindran<sup>2</sup>

#### **Abstract**

Vegetable and prawn added soup prepared with incorporation of Palmyrah tuber flour known as Odiyal Kool is one of the popular and traditional food of Northern Sri Lankan population. This study was to develop dehydrated instant soup mix to reduce the difficulty in the preparation of soup. An instant dehydrated vegetable and prawn added soup mix were developed using unboiled Palmyrah tuber flour as thickening agent and dried vegetables, salt, spice and tamarind paste as other ingredients. Initially the saponin content of the flour was removed by a pretreatment before adding to the soup. The ingredients such as manioc, long bean, carrot, moringa leaves, onion and garlic were dehydrated in an oven using established procedures. The optimum levels of Palmyrah tuber flour, tamarind paste and garlic powder were found by conducting preliminary trials using five point hedonic scale sensory evaluation tests. Then optimum level of prawn powder was incorporated into prawn added soup mix in addition to other ingredients used in the vegetable soup mix using similar trials. Soup mix: water ratio of 1: 12.5 was selected as the most preferable level for the reconstitution of the soup mix. Proximate analysis revealed that the final vegetable and prawn added soup mix possessed 3.24 %, 4.76 % moisture 1.16%, 1.50% fat, 5.9%, 7.15% protein, 5.58%, 8,66% ash, 4.8%, 5.1% fibre, 79.32%, 72.8 carbohydrate and 5.56, 6.52 pH. The total plate count of fresh vegetable and prawn added soup mix was 2.1x10³ and 2.9 x 10³ cfu/g respectively, which was within the safe range. The result of the shelf life evaluation studies namely moisture content, sensory evaluation and microbial analysis revealed that both soup mixes could be stored for two months without affecting their quality. The results of the cost of production analysis reveals that the unit price for vegetable and prawn added soup mix were 30.11and 51.06 rupees respectively.

Key words: Dehydrated vegetables, Palmyrah tuber flour, Soup mix

#### INTRODUCTION

Soup is the one of the traditional food can be classified as an appetizer, warm food during cold and sick. In the modern world commercially prepared instant soup such as canned, dehydrated, and frozen soups have replaced homemade soup as preparing a soup is a time consuming process. Instant soup can become an alternative food for breakfast because it could fulfill the adequacy of energy and nutrient required by the body, very practical in preparation and taking only short time to serve [1].

Dry soup mix contained vegetables in many forms have several advantages over canned soups [2]. These soup mixes are popular among the people because of easy to make. Among all dehydrated products instant mixes gained popularity in the recent years, by way to providing convenience, hygienic, extensible shelf life and easy to carry. So that it reduces the transportation cost and available in various packages and it requires very less time to prepare food [3].

Palmyrah has an existing potential of producing 3000 metric tons of tuber flour annually [4]. Palmyrah flour is considered a good source of carbohydrates, fiber and some micronutrients such as calcium, magnesium and ferrous ions [5]. The consumption of the odiyal flour can be increased by the introduction of more value added products with good palatability. Odiyal flour was used up to certain level in the instant soup mix. The nutritive and health beneficial components found in the tuber might improve the quality of soup mix.

Vegetables are low in calories and high in fiber content. They are also best source of antioxidants and other phytonutrients. An important advantage in using dehydrated Vegetables are that they are available year round rather than just seasonally [6].

This study was carried out to develop consumer preferable nutritious precooked soup mix from locally available resources and evaluates the nutritional as well as sensory attributes of the developed mix. The objective of this study is to develop a dehydrated instant vegetable soup mix and prawn added soup mix using odiyal flour and prawn added soup mix using odiyal flour and and a mix of compatible ingredients namely vegetables (manioc, long bean, carrot, moringa leaves and onions), rice grits, spices (garlic), salt, chilli powder, tamarind and prawn.

#### MATERIALS AND METHODS

This study was carried out in the laboratory of Palmyrah Research Institute (PRI), Kaithady, Jaffna. Raw materials were procured from the sales centre of Palmyrah Development Board ('Katpaham') and local market. Analytical test were conducted in PRI Laboratory and laboratory of Dept. of Agricultural Chemistry, University of Jaffna. All the chemicals used in the analysis were analytical grade and each experiment was done in triplicates.

### Preparation of unboiled Palmyrah tuber flour (Odiyal flour)

Odiyal flour was sieved using 60-mesh size sieve and soaked in excess water for about two hours to remove soluble bitter compounds, which are present in Odiyal flour. After drained out the excess water wet odiyal flour was mixed with the required amount of tamarind paste according to the soup composition. Then the odiyal flour tamarind paste mix was spread as thin layers and dried in an oven at the temperature of 80°C for 3 hours. The dried flour paste mix was ground by using a mechanical grinder, sieved off, packed and kept in room temperature conditions (28±2°C) until further use.

### Preparation of vegetables

Selected vegetable samples such as long bean, carrot, moringa leaves were blanched for predetermined times and immersed in 0.2% sodium metabisulphite (SMS) solution and dried in an oven for standard time using established procedures Boiled manioc samples also dried using similar procedures. The dried samples were ground and sieved off to a thickness of 0.5mm using NO.35 sieves. Similarly selected onion and garlic samples also dried using established procedures, ground and sieved off to a thickness of 0.25mm using No.60 sieves. These samples were packed and kept in room temperature conditions (28±2°C) until further use [6, 7].

### Preparation of prawns

Fresh and good quality prawn was selected sand their skin was peeled off and cooked with excess 2% salt solution in pan. The cooked prawns were sliced into small pieces and dried in an oven for 10 hours at 65°C and the dried product was ground, packed and kept in room temperature conditions (28±2°C) until further use.

#### Optimization of ingredients

All the ingredients were mixed according to the specified ratio for the development of soup mix. In sensory evaluation, each sample was subjected to five-point hedonic scale test (5-like very much, 1- dislike very much) and acceptability of sample was judged by 30 untrained members. They determine the consumer preference of each sample. The panelists judged the sensory characteristic such as appearance, colour, aroma, texture, taste mouth feel and overall acceptability of the samples of the samples.

In the first trial amount of Palmyrah tuber flour was changed (10, 20, 30, 35 and 40 g) and all other ingredients were kept at the same level. Then a panel of judges tested the developed soup mix samples. The most preferable amount of odiyal flour used for the soup mix (35 g) was selected from results of the above panel.

In the second trial the soup mix was prepared by using different amount of tamarind paste (5, 10, 15 and 20 g) The most preferable amount of tamarind paste used for the soup mix (10 g) was selected from the results of the sensory panel. Similarly the most preferable amount of garlic (0.82 g) was selected in the third trial based on the evaluation of sensory panel. Based on the results of the sensory panel the ingredients needed for the vegetable soup mix was finalized.

Then in the fourth trial prawn added soup mix was prepared by using different amount of prawn powder (6.3, 12.6 and 18.9 g) and maintaining all other ingredients at the same level similar to vegetable soup mix. The most preferable amount (12.6 g) of prawn powder was selected from the results of the sensory panel.

Table 1 gives the optimized amount of ingredients used for the preparation of both vegetable and prawn added soup mix.

### Proximate analysis

Proximate analysis was done for both vegetable and prawn added soup mix. Proximate analysis including moisture, ash, fat and fibre content were analyzed by standard AOAC methods [8]. Protein content was analyzed by Kjeldhal method [9] and carbohydrate content was estimated by difference method. Calorific value was estimated by multiplying the percentages of protein, fat and carbohydrate with the recommended factors. pH value was determined by pH meter.

### Reconstitution of the formulated soup mix

The optimum condition for the reconstitution of formulated soup mix was evaluated by conducting preliminary trials. Initially known weights of soup mix samples (40g) were taken and mixed with different amount water (400, 500 and 600ml) separately and heated for 5 minutes in a hot plate. The appearance and consistency of soup was observed by a panel. The optimum amount of water required for reconstitution was selected based on the evaluation of the panelists.

### Shelf life study

The developed vegetable and prawn added soup mix were packed in high density poly ethylene bags and kept in ambient conditions (28±2°C) for a period of 2 months. The changes in moisture content, microbial load (total plate count and yeast and mould count) and sensory characters were evaluated periodically at monthly interval.

### Cost of production

Cost of production was estimated separately for both type of soup mixes and it was given as cost for unit (40 g) weight.

### Statistical analysis

Friedman non-parametric statistical method was used to analyze the sensory evaluation data based on 5-point hedonic scales using Minitab software. The triplicate data of proximate contents were statistically analyzed by Completely Randomized Design (CRD) using analysis of variance (ANOVA) in SAS statistical software (Version 9.1). The significant differences were compared at 95% confidence interval (p<0.05) using Duncan's New Multiple Range Test (DNMRT).

#### RESULTS AND DISCUSSION

Proximate analysis of vegetable and prawn added soup mix powder was given in Table 2. The average moisture content of the vegetables and prawn added soup mix powder were 3.24%, 4.72% respectively. According to the U.S standard, the moisture content of the dry product must not greater than 5%. Prawn added soup mix (7.15%) had higher total protein than that of vegetable soup mix (5.09%). The two soup mix samples had lower fat content (1.16%, 1.50%) than that of results of Abeysinghe and Illeperuma (2016) [2] for formulation of an MSG free instant vegetable soup mix (4.1%). The ash content of the prawn added soup mix (8.66%) differs from the vegetables soup mix (5.58%). This difference probably exists because that the prawns are good source of minerals and pre drying treatments of the prawns (cooking with 2% salt solution). Singh *et al.*, (2003) [10] also observed higher percentage of ash (13.5%) content during the development of mushroom-whey soup powder. The fiber content of vegetable soup mix (4.8%) and prawn added soup mix (5.10%). The present result of soup mix, fiber content higher than other soup mix which are prepared without using odiyal flour. The reason of this increasing, odiyal flour and long bean consist higher fiber content.

### Reconstitution of the formulated soup mix

Among the different ratio of reconstitution (1:10, 1:12.5. 1:15) the ratio of 1:12.5 was widely accepted by the panel of judges as the optimum level of reconstitution for both vegetable and prawn added soup mixes.

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### Shelf life study

There was no difference in the sensory scores of both soup powder samples stored at room temperature (28±2°C) in high density polyethylene packaging materials for two months. Therefore the developed soup mix samples have acceptable sensory character up to 2 months.

### Estimation of moisture content during storage

The initial moisture content of the vegetable soup mix (3.24%) increased to 3.25% after one month and next month not the change in the moisture content. The initial moisture content of the prawn added soup mix (3.21%) increased to 3.24% after the one month and the next month it was increased to 3.25 percentages. The moisture percentage not exceed 5% within 2 months, this percentage is acceptable level dehydrated vegetables products without spoilage.

### Microbial analysis

Both of soup mix samples showed negative result for the yeast and mold  $10^{-1}$  dilution. No growth molds and yeast detected in two samples of soup mix in fresh form. Heat treatment may be preventing the growth of yeast and mold. The growth of yeast and mold was not observed due to the two months period. Aerobic plate count of the develop fresh vegetable soup mix was  $2.1 \times 10^3$  cfu/g and prawn added soup mix was  $2.9 \times 10^3$  cfu/g. A slight increase in aerobic plate count was observed in both samples up to 2 months.

Jay (1992) [11] reported that the product is microbiologically safe since total microbial count of dehydrated soups should generally be less than  $1x10^4$ cfu/g. The ITI [12] report point out that total bacterial count of their formulated soup is 3800/g. Therefore, the developed soup mixes were within the acceptable limit for safe use for a period of 2 months.

### Cost of production

The unit (40 g) price for vegetable soup mix and prawn added soup mix were 30.11 and 51.06 rupees respectively. This new product will help to open door in Palmyrah related industry in the island through the creation of an opportunity tom offer comparatively low cost instant soup mix powder with higher nutritional and sensory qualities.

#### CONCLUSION

Vegetable and prawn added soup prepared with the incorporation of Palmyrah tuber flour is one of the popular traditional foods of Northern Sri Lankan people. Nevertheless, the popularity of this soup is decreasing due to difficulty in the preparation of quality product. The developed vegetable and prawn added instant dried soup mix have an acceptable sensory, nutritional and microbial quality and it can be stored under ambient condition without affecting the quality characters. The cost of production also found in acceptable level. The developed soup mix is more convenient than traditional product and this will improve its popularity among the younger generation.

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Table1: Amount of ingredients present in the soup mix

Ingredients	Vegetable soup mix	Prawn added soup mix		
Odiyal flour (g)	35	35		
Rice grits (g)	2	2		
Manioc(g)	4	4		
Long beans(g)	4	4		
Moringa leaves(g)	0.25	0.25		
Carrots(g)	0.15	0.15		
Tamarind(g)	10	10		
Chilli powder(g)	1	1		
Salt(g)	5	5		
Onion(g)	0.75	0.75		
Garlic(g)	0.82	0.82		
Prawn powder (g)	0	12.6		

Table 2: Proximate analysis of vegetable and prawn added soup mix powder

Components %	Vegetables soup mix powder (Mean± SD)	Prawn added soup mix powder (Mean ± SD)
Moisture	3.24 (0.75)	4.76(0.35)
Fat	1.16(0.03)	1.5(0.06)
Protein	5.9(0.15)	7.15(0.13)
Ash	5.58(0.140)	8.66(0.33)
Fiber	4.8(0.62)	5.1(0.26)
Carbohydrate	79.32	72.83
Calorific value (kcal/g)	351.32	333.42
рН	5.56(0.00)	6.52(0.01)

(The values in the parentheses are standard deviation)

**Proceedings of Jaffna University International Research Conference (JUICE 2014)** 

## 2.2 4 Going local: Exploring consumer behavior and motivations for Palmyrah based products

Shaliny S., and Srithayalan S.

In recent years, in Sri Lanka, government pays its focus on promoting Palmyrah industry through developing appropriate marketing strategies. Before 1978, this agro-based Palmyrah industry was excluded from the Sri Lankan national economic development plants and programmes compared to Coconut and Kithul based industries. Inherent limitations of the Palmyrah industry influcence the under-development of the sector in relation to socio-economic and cultural nature of the industry With the establishment of Palmyrah Development Board {1978}, improved programmes and actions pertaining to the production and marketing of the Palmyrah industry were developed and contributed to develop as a sub-sector of the Sri Lankan agro-based industries. Due to the various. uncontrollable situations and factors in relation to marketing, the development of Palmyrah industry failed to reach to an optimum level. In light of this trend, study on consumer behaviour and motivation pertaining to Palmyrah based products (PBP) is becoming crucial. The study explored values affect consumer motivation and decision. The stimulus-response model of buyer behaviour was adapted in explaining the consumer behaviour of PBP. The research subjects were people who know and are familar with PDB in traditional market places of North and East provinces, Sri Lanka. Observation Interviews and Questionnaire were used to collect the qualitative data. Findings of the study showed environmental stimuli were stronger than marketing stimuli. Buyers' characteristics strongly inflluence the decision process, mean while problem recognition, information search and alternative valuation were found to contribute minimal.

Keywords: Consumer behaviour, Buyer, Palmyrah products, Motivation

Proceedings of International Conference on contemporary Management -2014 (ICCM- 2014), pp 779-789.

### 2.2.5 Improving the quality of Palmyarh bottled toddy and its partial clarification

Subajini Mahilrajan, Robika Kailayalingam, Brithayalan Srivijeindran and Ponnuchamy Navaratnam

A sample of Palmyrah toddy was obtained from Kaithady Palm Development Co-operative Society, Sri Lanka and allowed for prolonged (22 h) natural fermentation under the ambient laboratory conditions (~30°C). It yielded the highest percentage of alcohol [5.2 %, (v/v)] and the least acidity (0.57). Clarification of toddy carried out by both membrane filtration using 12  $\mu m$  and 0.45  $\mu m$  (control and the treatment, respectively for yeast) filter papers and centrifugation (locally applicable speed, 3000 rpm for 10 min). These clarification methods resulted 494, 42.5 and 55.6 NTU turbidity, respectively, whereas the turbidity of the fresh toddy was 678 NTU. However, membrane filtration method reduced the alcohol content by 40 %. To find out the optimum conditions for the pasteurization, the fresh toddy was bottled and heated at varying temperatures (55, 60, 65, 70, 75, 80 and 85°C) for 20 min. Percentage alcohol content remained the same after the pasteurization conducted with all the temperatures. Alcohol content, acidity and the TBC (CFU/ml) of the toddy sample pasteurized at 80°C for 20 min was satisfactory with values of 5.0, 0.44 % and 18, respectively. Heat treatment (pasteurization) improved the quality (alcohol content, acidity and turbidity) of the final product and its shelf life for 6 months at room temperature.

Financial assistance given by the Ministry of Traditional Industries & Small Enterprise Development is acknowledged.

Proceedings of the Peradeniya Univ. International Research Sessions, Sri Lanka, Vol. 18, p 520.

### 2.2.6 Preliminary phytochemical screening of extracts of Palmyrah fruit pulp

Nilushiny Aloysius Manoharan, Subajini Mahilrajan and Srithayalan Srivijeindran

Palmyrah palm (Borassus flabellifer) has been traditionally used to relieve biliousness. dysentery, gonorrhea, diuretic, anthelmintic, heart burn, dermatitis and given during enlargement of spleen and liver, fever and certain respiratory diseases. The objective of the present study was to screen the different phytochemicals from ethanolic, chloroform and aqueous extracts of fresh Palmyrah fruit pulp and sundried fruit pulp (Pannatu). Ethanolic and chloroform extraction were carried out by soxhlet extraction, followed by rotary evaporation, while the aqueous extraction was carried out by boiling in distilled water and filtration of fresh fruit pulp and sundried fruit pulp. Extracts were tested for phytochemicals, such as steroids and triterpenoids (Liebermann Burchard test, Salkowski's test), carbohydrates (Molisch's test, Fehling's test), glycosides (Keller Killiani test, Borntranger's test), saponins (Foam test), flavonoids (Ferric Chloride test, Alkaline reagents test, Lead acetate solution test), alkoloids (Wagner's test), tannins (Braymer's test, Gelatin test) and proteins (Biuret test). The results revealed that extracts contain potentially beneficial phytochemicals, viz; steroids, triterpenoids, carbohydrates, saponin, flavonoids and proteins in varied amounts in the ethonolic and water extracts. Chloroform extract showed negative results for all tested compounds except for carbohydrate. Glycoloids, alkaloids and tannins were not observed in any of the extracts. The results of the current study suggest that some phytochemicals present in the fresh Palmyrah fruit pulp and sundried fruit pulp possess potential antioxidants that can lead to the isolation of novel beneficial compounds.

Finncial assistance given by the Ministry of Traditional Industries and Small Enterprise Development, Sri Lanka is acknowledged.

Procedding of the Peradeniya University International Research Session, Sri Lanka, Volume 18, pp545.

## 2.2.7 Standardization and enhancement of quality of Palmyrah (Borrasus flabellifer) jaggery

Mary S., Velauthamurty K., Srivijeindran S., and Sashikesh G.

Palmyrah jaggery is produced from concentrated inflorescence sap of palmyrah palm (Sweet toddy) to a thick consistency. For the jaggery production, sweet sap (pH 6) was collected with the application of lime to stop the fermentation caused by wild yeast and bacteria. The existing jaggery available in the market is inferior in its quality as liquefaction and deterioration of color. It shows poor keeping qualities are due to the presents of moisture from hydroscopic inorganic impurities present in less purified lime produced by the local cottage level kiln. Present study was carried out during the period of May to June using Randomized Complete Block Design in Valikamarn area of Jaffna peninsula. To optimize the amount of quick lime (CaO), this experiment was carried out with four different concentrations of quick lime such as 5.0,3.0,2.5, and 2.0 grams of CaO in one litre of sweet sap with three replicates. The quick lime used in this experiment was produced from sea shells with 96% purity. Physiochemical quality parameters of produced jaggery samples in the laboratory and the commercial samples were studied and it was compared with Sri Lankan Standards for jaggery. For suitable jaggery production, the optimam amount of quick lime is found as  $2.508 \pm 0.411$  g of quick lime for one liter of sweet sap. The jaggery produced using optimum amount of quick lime was scored high in sensory evaluation.

Key words: Palmyrah (Borrasus fiabellifer), Jaggery, Quick lime, Sea shell, Sweet toddy

International Conference on Multidisciplinary Approaches 2014, University of Sri Jayewardenepura, pp 123.

# 2.2.8 Assessment of water purification capabilities of activated charcoal prepared from Palmyrah (Borassus flabellifer) kernel shell by different carbonization and chemical activation processes

Mary S., Sashikesh G., Nilushiny A.M., and Srivijeindran S.

The adsorption capability of activated carbon is applied to remove large variety of compound from contaminated water through carbon adsorption. The scope of this study was to investigate the water purification capabilities of prepared activated charcoal from dried Palmyrah (Borassus flabellifer) kernel shell. 18 types of different activated charcoal samples were prepared through the following steps as carbonization, chemical activation and pyrolysis. Carbonized kernel shells which were prepared at 400°C, 500°C and 600 °C for 10minutes and 20 minutes, were chemically activated by base (Potassium hydroxide), acid (Phosphoric acid) and water. Finally activated kernel shells were pyrolyzed at 800°C for 30 minutes. Physico-chemical analysis of collected pond water before and after filtration using prepared activated charcoal was carried out. The pH and conductivity were measured by electrometric method. The calcium and magnesium were determined by ethylene diamine tetra acetic acid complexiometric titration method. Mohr's argentometric method was used to determine chloride. Nitrate and ammonia were determined by Kjeldhal method. The phosphate content determination was carried out by spectrophotometric (Vanadomolybdate colour development) method. All results were analyzed in SAS software and the mean separation was done by LSD at p=0.05. Results of analyzed water quality parameters revealed that the best thermal condition to carbonize the kernel shell was at 600 °C for 20 minutes and basechemical activation was the best than acid or water activation. 41.7 % of nitrate, 54.5 % of ammonia, 58.3 % of calcium, 57.8 % of phosphate and 42.3 % of chloride were removed from collected pond water during water treatment by using activated charcoal produced at best carbonization and activation conditions. The physical characteristics such as colour, odur and taste were analyzed by organoleptic method and these characteristics were also superior in treated pond water than untreated pond water for all the activated charcoal treatments. This study will be a positive sign to prepare activated charcoal by applying different pyrolysis conditions and which is applied to treat water from natural contaminated water bodies and industries.

Key words: Palmyrah (Borassusflabeliffer) Kernel shell, Pyrolysis and Carbonization

Proceeding of International Conference on Multidisciplinary Approaches 2015, pp 201.

# 2.2.9 Determination of nutritional facts of Palmyrah (Borassus flabellifer) sap based products existing in the market of Jaffna peninsula.

Nilushiny A.M., Arachige J.W.M., Mary S., and Srivijendran S.

A study was carried out to determine the nutritional composition of Palmyrah sap based products (Jaggery. treacle and sugarcandy) existing in the market. The study was conducted at Palmyrah Research Institute in order to determine nutritional variation in such products and to display nutrient and proximate composition on food label of the respective products. Since five Palm Development Co-operative Societies (PDCS) out of 16 in Jatfna Peninsula only had produced Palmyrah sap based Productsas jaggery. treacle and sugarcandy in the year 2014, all five were selected for the study. An experiment was performed in Complete Randomized Design (CRD) Protein, fat. carbohydrate (total sugar and reducing sugar). phosphorous, calcium. magnesium and iron contents were determined in AOAC method. All results were analyzed in SAS software and the mean separation was done by LSD at p = 0.05. Protein content of the sap based products has ranged from 0.62% to 0.86%. Wide variation in fat content of the Jaggery was observed among areas and it varied from 0.056% to 0.52%. Percentage of fat in treacle and sugar candy was found 0.012% to 0.018%,. Total sugar content was varied differently in sap based products and jaggery (from 78% to 94.6%), for treacle which is 62% to 67%. and for sugarcandy that is from 85% to 95%. Reducing sugar content of the products was found in very trace amount. Among mineral composition analyzed for the products, calcium content was higher both in Jaggery and treacle and phosphorous content (0.1% to 0.13%) was higher in sugarcandy. Among three products magnesium content was also higher in jaggery (0.04%, to 0.06%.). Iron content ranges from 0.007 to 0.025%, 0.021 % to 0.035% and 0.008 to 0.017%, in jaggery treacle and sugarcandy respectively.

Keywords: Palmyrah, Nutrient Proximate, Mineral, Sap

Proceeding of International Conference on Multidisciplinary Approaches 2015, pp 227.

## 2.2.10 Comparative studies of functional properties of Palmyrah shoot flour with wheat and rice flour

Subajini Mahilrajan, Robika Kailayalingam, Nilushiny Aloysius Manoharan and Srithayalan Srivijeindran.

Palmyrah palm (Borassus flabellifer) produces two types of flour such as odiyal and pulukodiyal flour. Odiyal flour obtained from sundried ground seed shoot of Palmyrah though boiled dried and ground tuber give rise to pulukodiyal flour. Rice and wheat flour with different functional properties yield products with different textural qualities. If this Palmyrah flour has appropriate functional properties it must be selected as a raw material to produce products, such as desserts and noodles. Therefore the present study was carried out to compare the functional properties of different flour, that is, wheat flour (PRIMA), rice flour (ANNA) and Palmyrah flour such as odiyal and pulukodiyal flour. The functional properties (Water absorption capacity, Oil absorption capacity, Foam capacity and foam stability, Bulk density, Swelling capacity and Least Gelation Concentration) and moisture content of flours were evaluated. Wheat flour (4.34%) and rice flour (11.52%) showed significantly (p<0.05) lowest and highest moisture content respectively when compared with other flour. Odiyal flour has significantly higher values for Oil absorption capacity (93.33%) and Foam capacity (40.64%) while pulukodiyal flour showed highest values for Water absorption capacity (320%), Bulk density (0.79g/ cm³) and Swelling capacity (29.00%). Wheat flour was showed highest value for least gelation concentration while rice flour showed lowest value compared to Palmyrah flour. Palmyrah flour have good functional properties compare to wheat and rice flour which enhance the functional ingredients of food products also has a lot of potential in the food industry as thickening agent for desserts preparation in the food systems.

Key words: Functional properties, Palmyrah, Shoot flour

Proceedings of International Conference on Dry Zone Agriculture (ICDA 2015) Faculty of Agriculture, University of Jaffna

## 2.2.11 Formulation and quality assessment of compost prepared with different compositions of Palmyrah resources

A.M. Nilushiny, T. Karunaithan, S.Mary, S.SriVijeindran and A.Jeyakanth

A study was carried out to find the best formulation for compost by utilizing Palmyrah resources and to study the effect of such compost on crop yield. This study was done at Palmyrah Research Institute. The experiment was designed in a complete Randomized Design with seven treatment and three replicates. Palmyrah leaf, Palmyrah coir dust, paddy straw cow dung and commercial compost were used as raw materials for the study. Palmyrah leaf (L) and Palmyrah coir dust (C) were mixed in different ratio while maintaining other raw materials in same percentage each for all treatments. T1(L:C0 40:20), T2 (L:C 10:50), T3 (L:C 20:40), T4 (L:C 30:30), T5 (L:C 40:20), T6 (L:C 50:10), T7 (L:C 60:0). Cow dung, paddy straw and commercial compost were added in 30%, 5% and 5% respectively. A pot experiment was done under green house with test crop of Okra (Abelmochus esculcntus). Four months after formulation different types of compost were applied to test crop and quality of compost was tested according to SLS 1246: 2003. Result were analyzed in SAS software and the mean separation was done by LSD at p = 0.05. All seven composts have met minimum requirement of carbon and nitrogen content. T7 exceeded the limit of C:N ratio and also did not meet the minimum requirement of phosphorous content. Other six treatments obey to SLS in C:N ratio (10 to 25). T3 and T5 did not meet the minimum requirement of magnesium and phosphorous (0.5%), respectively. T1 also had lower phosphorous content than the minimum limit of SLS (0.5%). T4 and T6 did not meet the requirement of magnedsium content. Result of the pot experiment revealed that treatment T2 has given higher crop yield. According to the crop response nutrient composition T2 was indentified as the best compost formulation.

Keywords: Coir dust, Compost, Palmyrah

International Conference on Dry Zone Agriculture-- ICDA 2015, 60.

## 2.2.12 Effect of different heat treatments and additives on sustaining ethanol content of Palmyrah toddy

S. Marya, M. Senthilnanthananb\*, A. M. Nilushinya, S. Srivijeindrana

Palmyrah (Borassus flabellifer) toddy is a naturally fermented inflorescence sap of palm that contains 5-6 % of ethanol and is utilized as an alcoholic beverage and for ethanol production. During January to August every year, toddy is collected by tappers and supplied to vendors; the excess toddy is delivered to distilleries. Even though quality products could be produced from the excess Palmyrah toddy, bulk is thrown away in the local taverns due to lack of suitable techniques available to sustain ethanol content of toddy at an acceptable level until it is processed. Hence, the present study focused on developing a suitable method to maintain ethanol content of Palmyrah toddy at an acceptable level for prolonged period. So that it could be processed into quality products. Different treatment methods, using potential additives such as lime, benzoic acid and sorbic acid and temperatures of 50 °C and 60 °C independently and combined, were applied to the toddy and the time taken to reach around 4 % (w/v) ethanol content was measured at each instance. The application of the above additives to Palmyrah toddy revealed that benzoic acid and sorbic acid are effective additives. The heat treatment of palm toddy demonstrated that heating at 60 °C for 30 minutes retains ethanol content above 4 % (w/v) up to 108 hours and application of benzoic acid at 60 °C and sorbic acid either at 50 °C or 60 °C prevents further fermentation up to 156 hours from the time of collection of toddy. Due to the concerns over the employed additives with respect to their cost and impact on the quality of processed products, heat treatment is recommended to retain ethanol content of Palmyrah toddy at an acceptable level for prolonged period.

Keywords: Palmyrah, Toddy, Fermentation, Ethanol, Additives

Proceddings of the 4th international conferences of Eastern University 2016

## 2.2.13 Potential of Palmyrah (Borassus flabellifer) leaf powder as ruminant feed and its chemical and physicochemical properties

Vasanthakumar Sethurajah, Sangheetha Sriskandarajah, Karunainathan Thirunavukkarasu and Srivijeindran Srithayalan.

Palmyrah (Borassus flabellifer) leaves are utilised as a supplementary animal feed in the Northern and Eastern provinces of Sri Lanka due to its nutritional value and availability throughout the year. However, Palmyrah leaves are currently not utilised as storable animal feed, nor have there been any studies to determine the potential of using Palmyrah leaf powder as animal feed. The objectives of this study were to determine the nutritional value and physicochemical properties of Palmyrah leaf powder and to explore the potential of using powdered Palmyrah leaves as an animal feed, Sundried, chopped and ground Palmyrah leaves were sieved through a mesh (no 18) and the chemical composition and physicochemical properties of this powder were analysed using standard methods, Further, the economic viability of using this powder as ruminant feed was also assessed. The nutrient composition of Palmyrah leaf powder was as follows (w/w percentages): crude protein 10.84  $\pm$  0.45, ether extract 5.36  $\pm$  0.08 g and ash 7.04 g  $\pm$  0.26. The energy content of the powder was 17,65 KJ and it contained 7.47 ± 0.12 w/w % of moisture. Physicochemical properties of the powder are as follows; pH 5.67± 0.01, total soluble solids  $1.62 \pm 0.01$  Brix, inverted sugar  $1.64 \pm 0.01$ , bulk densities  $36.22 \pm 2.75$  g/cm3, water holding capacity  $2.76 \pm 0.06$  g/lg dry sample, oil holding capacity  $1.97 \pm 0.08$  g/lg dry sample and swelling capacity 61.66%. These results show that Palmyrah leaf powder has potential as a feed resource and can be used to feed ruminants especially in the Jaffna peninsula.

Peradeniya University International Research

Session, 2016

## 2.2.14 Consistency of the quality of Palmyrah jaggery produced from Palmyrah treacle during off season at a production facility in Jaffna

Shanmugam Mary, Rajaratnam Shanthini and Srithayalan Srivijeindran.

Palmyrah (Borassusflabellifer) jaggery production from fresh sweet sap is seasonal (February to July). During off season, jaggery is produced from stored treacle which was produced from sweet sap collected during the previous season. In this study, we examined quality of 30 jaggery samples from randomly selected batches made from stored treacle at a community-scale production facility in Jaffna. Sri Lankan standards (SLS) for Jaggery stipulate the following limits for quality jaggery: moisture<10%; total sugar>70%; reducing sugar<13%; ash<3.5%. Estimated means of jaggery samples were 4.6±0.7%, 87.4±2.9%, 8.5±0.5% and 1.7±0.2%, respectively. AD statistic for ash content (p<0.05) rejected the null of normal distribution. Means of calcium, sodium, potassium and phosphorus were  $0.14\pm0.11~\%,~0.39\pm0.04\%,~0.86\pm0.07\%$  and  $0.09\pm0.01~\%,$  respectively, which did not differ appreciably from literature values. Corresponding CVs spanned the range of 7 to 9%. AD statistics confirmed normal distributions for all minerals except for potassium (p<0.05). Means of pH and Brix value were 5.3±0.1 (CV=2%) and 19.5±1.0 (CV=5%). Estimated pH values showed a normal distribution (p=0.605) whereas Brix values did not (p<0.05). We therefore concluded that moisture, ash, total sugar and reducing sugar contents of Palmyrah jaggery samples tested confirmed well with the SLS for Jaggery. AD statistics led to the generalization of the above conclusion to the population of jaggery made during off-season at the said facility, except for ash content which was expected owing to the ad-hoc addition of low quality (40-50% purity) quicklime to sweet sap used for treacle and/or jaggery productions. The narrow range spanned by pH signified effective deliming during processing of every batch. High values of CVs of moisture content and ash content and the non-normal distribution of ash content and Brix value call for tighter controls during jaggery processing to further enhance the consistency of the quality of jaggery produced at the production facility targeting international market.

> Accepted for Peradeniya University International Research Session, Sri Lanka.

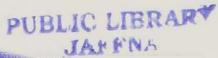
## 2.2.15 Effect of heat treatment on keeping quality of Palmyrah sweet Sap

Surenther S., Mahilrajan S., Chandrasena G., Robika K. and SriVijeindran

The Palmyrah palm (Borassusflabellifer) is grows extensively in Northern part of Sri Lanka. It is called as 'Tree of life' has contributed immensely to the people both as food and shelter and also the base for income generation. The most important product of palm is the sap or juice. It should be extracted from both male and female inflorescences (dioecious plant) by the process known as tapping. Harvested sap should be immediately processed due to the highly perishability as it under goes spontaneous fermentation via air born yeast microflora. The main objective of the study was to identify the optimum temperature and time for preservation of sweet sap and detected the suitable shelf life for bottled sweet sap via the physical, chemical, microbiological and sensory quality of preserved sweet sap. Traditionally guick lime is added to prevent the fermentation; phosphoric acid was selected at pH 8 for the removal of lime as calcium phosphate. Delimed sweet sap was used for the study of thermal treatment in order to increase the keeping quality of Palmyrah sweet sap. Three experiments with different thermal treatments were conducted to preserve the sweet sap. Experiment 1 (preservatives such as citric acid and sodium metabisulphite) and Experiment 2 (thermal treatments of 60, 70 80 and 90°C) were rejected through the microbiology and sensory evaluation. In the 3rd experiment the bottled sweet sap was heated at 105, 110 and 115°C for different time intervals (15 and 30min) and stored at room temperature. There were no significant differences (p<0.05) in chemical, physical and microbial evaluation of selected treatments at 60 days of storage. Based on sensory evaluation, Treatment 5 thermal processing at 105°C for 15 min was selected as the best treatment and it could be stored for 60 days without changing its native characteristics.

Key words: Palmyrah, Preservation Sweet sap Temperature and Time

International Conference on Dry Zone Agriculture-- ICDA 2016, Sri Lanka



### 2.3 Publication in National conference

### 2.3.1 Evaluation of some plant materials as fermentation inhibitor

for Palmyrah (Borrassus flabellifer) sap

K.D.P.P. Gunathilake a \*, S.Nivethinia, S. Mahilrajanb, S. Srivijenthiranb

Palmyrah sap is a sugar rich inflorescent sap which is easily fermented by the air born microbes during collection from the tree. Quick lime addition in the collecting pots is the traditional method to prevent fermentation. However due to some limitations of this method this study was conducted towards other methods to inhibit the fermentation. Lime addition was taken as the positive and toddy (without any addition) was considered as negative control. The sap was collected with different treatment for 24 or 16 hours and parameters Brix, pH, tritrable acidity, alcohol and Reducing sugar were analysed. According these results better method was selected Addition of antimicrobial plant materials was experimented with Launaeacoromandelica, Syzygium cumini dried bark, Syzygium cumini dried seed, garlic, onion cashew leaves. Although tow plants (Launaeacoromandelica and Syzygiumcumini) dried bark were selected to inhibit the fermentation they incorporated colour compounds into the sap. sap collected using colour removed material did not showed inhibition as previous. Total phenol, flavonoid and tannin content were determined for these two barks (normal and washed) and Vateriaacumintabark in methanol and aqueous extraction. Addition of food grade alkaline compound (sodium bi carbonate and sodium carbonate) were tested. Alkaline medium given by two compounds were not sufficient like lime. Usage of Palmyrah leaf bucket in sap collection also did not inhibit fermentation. Finally, cooler system was developed to inhibit the fermentation which was selected as best method.

Physio- chemical properties of collected sap for 16 hours using cooler system

Treatment	Brix pH	Tritrab	le acidi	ty	Reduc	ing suga	r	Alcoho	ol
	Mean SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
	de institution			a Trace	and the		HE		
Toddy	8.303 0.331	3.547	0.34	0.114	0.006	47.233	2.232	1.383	0.053
Lime	12.096 0.381	11.423	0.391	0.006	0.006	8.148	2.568	-0.145	0.061
Cooler	14.254 0.316	5.637	0.324	0.008	0.005	8.16	2.127	0.053	0.05
Cooler	12.215 0.304	4.523	0.312	0.037	0.005	28.657	2.052	0.409	0.048
Control	the birth falling		= a militar						

The indicated means are adjusted means by the covariates volume.

The sap obtained from cooler showed high brix value compare with all treatments. Although there were no significant different in tritrable acidity and reducing sugar between cooler and lime added sap 0.053% of mean alcohol was detected in cooler system. The sap collected from this method was tested for microbial count, sensory analysis and nutritional analysis to compare with the traditional method. The sensory analysis showed the same attribute value to both cooler and lime applied sap.

Wayamba University, Undergraduate Research Symposium - 2016

## 2.3.2 Effect of different precooking and dehydration techniques on selected chemical properties of Palmyrah (Borassus flabellifE!r L) tuber flour

S. Krishnarajah 1\* S. Sangheetha<sup>2</sup> and AM.M.U. Adikari<sup>1</sup>

Palmyrah palm (Borassus flabellifer L.) is widely spread in the arid tropics of North-East Sri Lanka. Its tuber is an important source of starch in the villages of Jaffna Peninsula. Palmyrah tuber flour is used to prepare different food products such as porridge, pittu, biscuits and palmposha. The fresh tubers have a limited shelf life due to its high moisture content thus preventing long distance transportation. Use of proper precooking and dehydration techniques in tuber flour preparation can improve its quality and shelf life. Therefore, the research was conducted to determine the effect of different precooking and dehydration techniques on selected chemical properties of Palmyrah tuber flour and select the most suitable combination in Palmyrah tuber flour preparation. Four months old tubers belonging to the variety of black skin fruit of Palmyrah were selected for the research. The effect of three precooking techniques; autoclaving for 30 minutes, steaming for 45 minutes and boiling for 30 minutes followed by two dehydration conditions; sun drying for 7 days and oven drying at 85°C for 4 hours were evaluated during the experiment. The pretreated sample was sliced into even size pieces (3 mm). Dried slices were ground to a particle size of 0.297 mm, sieved and packed in high density polyethylene bags and were stored at room temperature. Proximate composition, crude fiber, total starch, resistant starch, total sugar and reducing sugar contents of the developed tuber flour types were measured. The results indicated that drying methods have not shown any significant effect (P>0.05) on crude fiber and crude protein content. The flour obtained by steaming and oven drying had the highest carbohydrate 87.31 % (SD± 0.26) content. Significantly high crude fiber content was recorded in steamed and oven dried tuber flour. In the case of starches and sugars, autoclaved and oven dried flour had the highest resistant starch 1.37 mg (SD± 0.13) and the lowest reducing sugar 0.047 g (SD± 0.23) contents. Both flours obtained by boiling and sun drying or boiling and oven drying resulted flour with high total sugar and values were 1.99 g (SD± 0.01) and 1.96 g (SD± 0.02) respectively. All precooking methods resulted nearly same total starch content 1.74 mg (SD± 0.02). The study confirmed that autoclaving followed by oven drying at 85°C for 4 hours is the most suitable precooking and dehydration combination to be used in Palmyrah tuber flour production.

**Keywords:** Dehydration, Palmyrah tuber flour, Precooking, Reducing sugar, Resistant starch

Wayamba University, Undergraduate Research Symposium - 2016

## 2.3.3 Comparative Study of Proximate Composition of Palmyrah Pinattu and Flour (Odiyal, Boiled odiyal)

Srikantharasa S., Mahilrajan S., Wijesinghe P. J. A. W, G. Bandra and S Srithayalan

Palmyrah (Borasslis flabellifer) palm can be considered as a gift of nature as they contribute wide range of vital products for human diet and existence. Among palmyrah products fruit and flour are highly utilized by local population. Fruits are mostly used as fresh, because of its perishable nature it is traditionally preserved as dried frujt pulp leather called as plnattu. Palm produces two types of flour such as odiyal (Dried tubter) and plukodiyal (boiled and dried tuber) flour. in this study the proximate composition of palmyrah pinattu and flour were evaluated. Samples were collected from three different branches of Palmyrah Development Board and used for the analysis their were no significant different between moisture content of the boiled odiyal flour (11.66  $\pm$  0.001) and odiyal flour (10.66  $\pm$  0.001) while pinatu showed (16.6  $\pm$   $0.008) % (10.66 <math display="inline">\pm$  0.001)Protein content of boiled odiyal Flour: [6.51 $\pm$ 0.062J and odiyalflour 6.7813 $\pm$ 0.06) were significantly higher when compared with pinatu [2.23±0.062%] as well as fat content of odiyal flour was 0.43±0.013 g/100g and that was significantly higher than boiled odiyal flour and pinattu. Significantly higher amount of ash was observed in pinattu (0.04±0.001) when compared with flour. Boiled odiyal flour [7.13±0.18)] contained significantly higher crude fiber content than odiyal flour [4.49±0.15) and pinatu (5.06±0.01) g/100g. Carbohydrate content was significantly higher for odiyal flour [77.59±0.5)] when compared WIth pinattu [75.91± 0.61)] and boiled odiyal flour  $74.37 \pm 0.65$  g/100g this study was suggested that proximate composition of pinatu and flour were various and which were good source fiber and protein.

Key words: boiled odiyal flour, Palmyrah, Pinattu

Proceedings of the 6th Research symposium of Uwa wellasa University, Sri Lanka, January 28-29, 2016.

### 2.3.4 Analysis of Biochemical properties of palmyrah leaf flour: Utilize as a commercial cattle feed.

Vasanthakumar S., Sangheetha S, Srivijeindran S. and Sashikesh G

Palmyrah (Borrasus fillabellifer) leaves are used as a substitute cattle feed not only in the North and East province of Sri Lanka but also in some other south Asian countries due to the availability throughout the year and their biochemical compositions. There arc no research studies carried out related to the Palmyrah leaf flour to be used as a cattle feed and also no appropriate methods were adopted to preserve the Palmyrah leafflour as a storable cattle feed for a long time. 'The aim of this research study is analyse the biochemical properties of the Palmyrah leaf flour. Sundried chopped and ground Palmyrah leaves were sieved through the mesh size 0.2 mm and the biochemical composition of Palmyrah leaf flour was determined. the 100 g of Palrnyrah leaf flour contained 10.84 w/w % of crude protein, 5.36 w/w % of ether extracts, 92.53 w/w % of dry matter, 86.02 w/w % of dry organic matter and 7.04 w/w' % of ash content. This research study is clearly shown that the biochemical properties of the Palmyrah leaf flour arc considerably higher than the roughages which are locally available in global market. So this research study strongly recommended that the dried Palmyrah leaf powder can be used as supplementary cattle feed to enhance performance of dairy cows not only in North and East province of Sri Lanka but also in other Asian countries.

Key words: Palmyrah leaf flour, Paddy straw, Cattle feed, Biochemical properties.

Proceedings of the 23 rd Annual Session of Jaffna Science Association, 2016.

### 2.3.5 Root extent and canopy coverage of palmyrah palm in the Jaffna District.

Vasanthakumar S., Karunainathan S. and Srivijeindran S.

Proceeding on National Symposium Agriculture 2015 pp 25-26.

## 2.3.6 Development of palmyrah (Borrasus flabellifer) tuber based precooked supplementary food—"Palmyrah nutrimix"

Piratheepan S., Sangheetha S., Perera O. D. A. N., Srivijeindran S., Jayasinghe C.V. L.

Peer reviewed proceedings of Wayamba International Conference 2014, Wayamba University of Sri Lanka, 2014, 219

## 2.3.7 Optimization of sponge cake formulation with palmyrah food resources and evaluation of its quality characteristics

KasthaniK., Sangheetha S., Vasantharoopa S., and Srivijeindran S. Peer reviewed proceedings of the annual conference - 2014,

Jaffna Science Association, 2014, 70.

### 2.3.8 Effect of packaging materials on shelf life of palmyrah food resource cake

Kasthani K., Sangheetha S., Vasantharoopa S. and Srivijeindran S.,

Peer reviewed proceedings of the annual conference - 2014, Jaffna Science Association, 2014, 71.

## 2.3.9 Effect of palmyrah coir dust on physicochemical properties of potting media and on growth and yield of chilli.

Puthisigamani S., Jayasinghe H.A.S.L,. Gunadasa H.K.S.G, Nilushiny A.M. and Sri Vijeindran S.

5th Research Symposium on Value addition for sustainable development, UwaWellasa University of Sri Lanka, 2014, 40-42

### 2.4 Submitted for Publication

# 2.4.1 Optimizing the harvesting time of Palmyrah (Borassusflabellifer) haustorium to retain its nutrient content without affecting the development of tuber

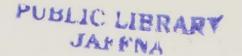
Srivijeindran 1, 2, Champa D. Jayaweera 1 and P. Navaratnam 2

#### **ABSTRACT**

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Palmyrah haustorium, consisting of nutritional values for man kind is generally wasted without consumption. The main aim of this research is to find the best developmental state of Palmyrah haustorium at which more nutrients are retained for the betterment of human health while taking care not to disturb the traditional tuber production. Raised seed beds were made with randomly selected 400 similar seeds (age, size, variety) in four different places in Jaffna peninsula, Sri Lanka. Those four places were Island, Thenmaradchi, Vadamaradchi and Valikamam. Samples consisting of 40 seeds were collected in three week time intervals, starting from sixth week of germination. The haustorium was analyzed for change of mass and amounts of nutrients while starch and total sugar present in tuber were also analyzed. Mass of haustorium in samples of Island and Valikamam increased steadily up to 9th week with values 19.25 and 21.17 g/seed respectively whereas samples of Thenmaradchi and Vadamaradchi showed their maximum having 16.64 and 15.76 g/seed respectively. Sugar content of haustorium was found to be at their maximum at the age of 12 weeks and afterwards their concentrations started to decline. Total phenolic content had almost reached the maximum and an appreciable amount of protein was found in all the four different samples at the end of 12th week. On the other hand a drop in starch content and an increase in sugar content were observed in tubers at this stage. Taking into account the nutritional content of both haustorium and the tuber, it was concluded that harvesting should be done at the end of 12th week.

Key words: Palmyrah, Haustorium, Tuber, Nutritional changes, Locations



### INTRODUCTION

Today, consumers' perception towards the high quality, healthy foods which provide additional health benefits beyond the basic nutritional requirements is becoming greater than ever. With increased awareness of a healthy lifestyle based on consumption of functional foods or other functional ingredients, natural foods have gained greater popularity over artificial foods and this makes scientists to search for plant resources to fulfill the requirements of the consumers.

Palmyrah palm (Borrasus flabellifer) belongs to the family palmae, grows well in the arid zone. The female palms produce fruits and on maturation the ripened fruits automatically fall to the ground. Few fruits along with seeds are collected by people to extract the pasty mesocarp and mix with water to produce a diluted pulp in order to get a sun-dried flat sheet, called 'Pinnattu'. The remaining fruits are heaped up for a short period and then beds are made with three or four tiers of seeds and their moisture level is maintained adequately to form tubers.

As a general practice, Palmyrah (Borassus flabellifer) tubers are harvested at their full maturity stage and the remainder of the seed bed after harvesting is thrown to nature though it has benefits for humans. The residuum of the seed bed contains Palmyrah haustorium which is a delicious white, spongy edible part formed during germination. On germination, the basal part of the embryo enlarges to form the cotyledony structure, and this is called haustorium. The haustorium transfers the nutrients to the embryo. Hence haustorium will be more nutritious containing sugars, essential amino acids and other micro nutrients and bio active compounds which are highly beneficial for our health. People in rural areas consume it raw and fresh. It has been proved through scientific investigations that an increased consumption of this haustorium has several health promoting as well as disease preventing benefits [1]. Haustorium actually transfers the nutrients to tubers which ultimately produce valuable plants. Therefore, in order to get more healthy plants and nutritious tubers the harvesting must be properly practiced. Proper harvesting period has to be clearly identified to minimize the wastage of nutritious resources.

However, the haustorium now harvested at the final stage after digging up the tubers is watery and has no taste whereas the tubers develop sugars and the starch content get reduced resulting in poor quality of tubers for further processing. Therefore analyzing the haustorium for its nutrients and palatability is very helpful in determining the proper harvesting time at which deliciousness and nutrients are retained at optimal level for both haustorium and tubers of Palmyrah, without affecting the development of the tuber. Analysis of the tuber for its important nutrients at different maturity levels will also help to avoid the damage and wastage of these resources.

In this research study, it has been hypothesized that the nutrient composition and organoleptic properties of haustorium vary with its developing (ripping) time and that proper harvesting period may prevent wastage of resources and loss of nutrients. The study in this paper illustratesan effort to determine the proper harvesting period for haustorium containing the highest possible nutrient content. This could pave way to increase the utilization of an under-utilized resource—isting abundantly in Sri Lanka.

#### **MATERIALS AND METHODS**

Selection of study site

In order to analyze the parameters of Palmyrah haustorium in Jaffna peninsula the district was divided into four divisions such as Island, Thenmaradchi, Vadamaradchi, and Valikamam according to the different soil types as shown in Figure 1.

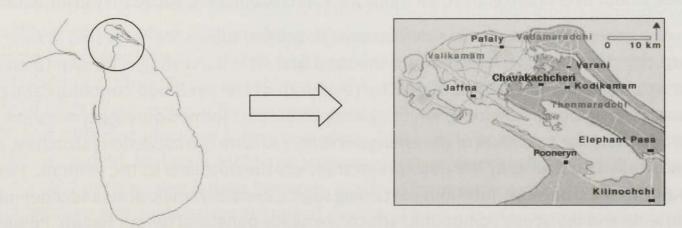


Figure 1. Site map (google map)

Preparation of seed bed for Palmyrahhaustorium and tuber development

Same age and variety of 400 medium sized seeds were collected randomly from study sites during October 2015.

### Sampling technique

At the time interval of three weeks, seeds were harvested by systematic sampling from all four corners and center of seed bed. According to statistical theorems, at least 10 % of the total population of the seed bed had to be sampled and therefore 40 seeds with 40 tubers were harvested at each interval.

### Preparation of samples

Harvested haustorium was mixed well and then the sample size was reduced using quartering method. Samples were dried at 80°C for overnight, ground, homogenized and then packed in air tight containers. The sample bottles were kept at room temperature condition.

### Proximate composition

Association of Official Analytical Chemists (AOAC) methods were used to determine the Moisture [4], crude fat [5], protein [5], dietary fiber [4], starch [2], and total ash [4]. The protein content of the samples was calculated using the factor  $N\times6.25$ . Sugar content was determined by use of an UV visible spectrophotometer (Thermo Scientific GENESYS 10S UV/Vis, USA)[8].

### Mineral content

Potassium and sodium content were estimated using aflame photometer [5]. Calcium and magnesium were determined by EDTA titrametric method [7]. Determination of iron was carried out by a spectrophotometric method using 1, 10- phenanthroline [7].

### Total phenolic content of haustorium

The total phenolic content of Palmyrah haustorium was determined by Folin-Ciocalteu method [6].

### Antioxidant activity using DPPH method

Ascorbic acid was used as the reference standard, to quantify the free radical scavenging capacity, , which was expressed as ascorbic acid equivalent [3]. The inhibition percentage was calculated using the absorbance readings. Finally IC50 value of the standard was calculated.

### RESULTS AND DISCUSSION

The optimal harvesting period was determined by analyzing the haustoruim and tuber harvested at different time intervals.

The study of changes of mass in haustorium and tuber with the time

The average mass of haustorium (g/100 g) dry matter examined is shown in Table 1.According to the results obtained for Thenmarachi and Vadamaradchi samples, the yield slightly increased up to 12th week and then Thenmaradchi sample increased and the other decreased. However all four samples were almost similar in the 12thweek. Among the four, two samples (Island and Valikamam) showed the highest yield in the 9th week and the rest of the two showed their highest yield in 12thweek (Vadamaradchi) and 15thweek (Thanmaradchi). Hence the decision of determining the harvesting period should be made considering the results from further analysis.

Table 1. Average mass of haustorium with definite harvesting time in different selected areas in Jaffna penninsula

	Average mass of haustorium ( g per seed)							
Place	Fixed intervals (weeks)							
	6 <sup>th</sup>	9 <sup>th</sup>	12 <sup>th</sup>	15 <sup>th</sup>				
Island	11.67 ±0.10 <sup>d</sup>	19.25±0.51 <sup>a</sup>	15.04±0.19 b	13.30±0.80 °				
Thenmaradchi	12.20±0.17 <sup>d</sup>	14.89±0.16 °	16.64±0.48 b	17.10±0.29 a				
Vadamaradchi	7.60±0.70 <sup>d</sup>	13.00±0.36 b	15.76±0.45 a	14.80±0.66°				
Valikamam	10.07±0.20 <sup>d</sup>	21.17±1.21 a	15.20±0.38 b	12.00±0.89°				

Each value in the table was represented as mean  $\pm$  SD (n = 3). Values in the same row followed by a different letter (a-d) are significantly different (p< 0.05).

sMass of the haustorium indicates the mass of nutrients. During the germination of seed, haustorium increased in mass rapidly up to 9th week; hence all samples showed the increase in mass (Table 1). The difference observed between samples at 12th week of harvesting could be due to variation of internal and external environment of seed samples. It might be assumed that the function of haustorium at this state mi. ht be high. After 12th week, mass of haustorium decreased probably because nutrients of it are transferred to the embryo as the function of haustorium is to nourish. At this time mass and length of tubers increased (Table 2). All samples from the four different areas showed similar trend in the yield of tubers. Difference in mass of haustorium among the samples is not significant. Yet sudden decrease in mass was observed after 12th week except for the sample collected fromThenmaradchi. However, the percentage increases in the Mass (yield) with the growth fall between 4% and 6 % indicating that the increase of mass within the growth period as slow (figure 2).

Table 2. Average (mass and length) of tuber against the growth period in different areas

Place		s of tuber (g per ber)	Average length of	tuber (cm per tuber)	
Fixed intervals (weeks)		vals (weeks)	Fixed intervals (weeks)		
	12 <sup>th</sup>	15 <sup>th</sup>	12 <sup>th</sup>	15 <sup>th</sup>	
Island	77.34 ±3.81	80.67 ±3.21	34.57 ±1.65	36.17±1.14	
Thenmaradchi	52.00±4.00	54.67±4.51	21.90±1.20	22.80±1.60	
Vadamaradchi	61.67±2.08	65.34±2.51	27.34±1.96	28.84±2.50	
Valikamam	40.34±4.04	42.34±3.79	16.83±1.25	17.67±1.50	

Samples were analyzed in triplicate and the results reported are the mean values  $\pm$  standard deviation (SD).

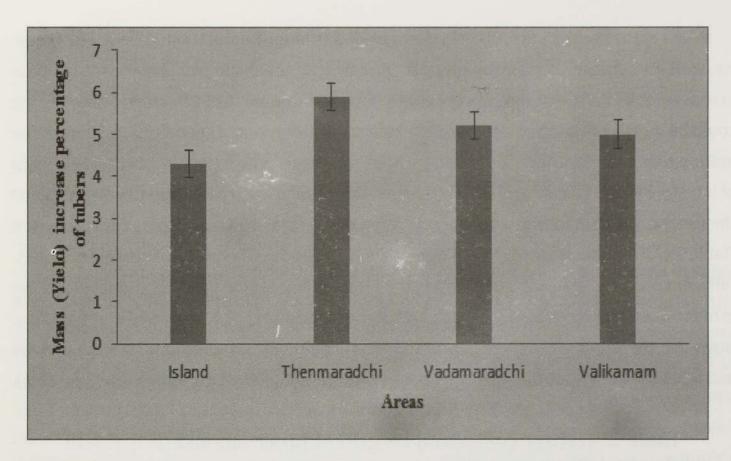


Figure 2: Percentage increase in yield of tubers with time in different areas in Jaffna

The study of sugar content Haustorium with the time

Total sugars and reducing sugars of Palmyrah haustorium are the most desirable characteristics as people prefer haustorium for its sweetness and health benefits rather than its nutritional value. As shown in table 3 reducing sugar of the haustorium in all four different samples significantly increase up to 12th week and maximum concentration of reducing sugar was detected in all four samples at 12th week. Haustorium from Island showed the highest content while the sample from Thenmaradchi showed the lowest among the four different samples at 12th week. However the trend of increase was similar to all. The increment may be due to the accumulation of sugar in the haustorium increases with age while mass of haustorium's tissue increased. Inevitably, the size of the embryo also increased utilizing the nutrient supplied by the haustorium.

Table 3. Reducing sugars in haustorium harvested at fixed time intervals

		Amount of reducin	ng sugars (g/100g)				
Place	Fixed time intervals (weeks)						
	6 <sup>th</sup>	9 <sup>th</sup>	12 <sup>th</sup>	15 <sup>th</sup>			
Island	14.15±0.52 <sup>d</sup>	19.23±0.24°	40.26±0.57 <sup>a</sup>	31.62±0.15 <sup>b</sup>			
Thenmaradchi	9.79±0.10 <sup>d</sup>	25.82±0.07 <sup>b</sup>	32.49±0.05 <sup>a</sup>	17.96±0.08°			
Vadamaradchi	5.72±14.79 <sup>d</sup>	14.79±0.70°	32.83±0.76 <sup>a</sup>	30.91±0.47 <sup>b</sup>			
Valikamam	12.78±0.01 <sup>d</sup>	22.95±0.34°	34.77±0.92 <sup>a</sup>	25.17±0.31 <sup>b</sup>			

Each value in the table is represented as mean  $\pm$  SD (n = 3). Values in the same row followed by a different letter (a-b) are significantly different (p< 0.05).

Total sugar content of haustorium showed a similar trend as reducing sugars. As displayed in table 4, the concentration of total sugars increased with time till 12th week and then decreased and the differences in values between different time points were significant.

Table 4. Total sugars of haustorium harvested at fixed time intervals

	Total sugar content (g/100g) with fixed time intervals						
Place	6 <sup>th</sup>	9th	12 <sup>th</sup>	15 <sup>th</sup>			
Island	66.96±5.92 <sup>d</sup>	84.98±0.26°	89.52±0.52 <sup>a</sup>	73.54±1.01 <sup>b</sup>			
Thenmaradchi	66.13±2.47 <sup>d</sup>	87.82±0.50 <sup>b</sup>	89.83±0.64 <sup>a</sup>	78.65±0.51°			
Vadamaradchi	65.02±0.39 <sup>d</sup>	79.19±0.26°	93.27±1.14 <sup>a</sup>	69.82±0.38 <sup>b</sup>			
Valikamam	78.14±1.16 <sup>d</sup>	80.45±0.77°	95.90±0.38 <sup>a</sup>	68.26±0.25 <sup>b</sup>			

Each value in the table is represented as mean  $\pm$  SD (n = 3). Values in the same row followed by a different letter (a-b) are significantly different (p< 0.05).

The data from table 3 and 4imply that 12th week is the suitable time for harvesting the haustorium as the decline in sugar level after 12th week will affect the taste of it. Also, it would be a suitable time for harvesting the tubers as well.

The study of starch and sugar content of tubers with the time

The suitable mature stage at which tubers should be harvested for processing mainly depends on the starch content. Hence starch was analyzed at two different ages of the tuber.

Starch of tubers from all four areas decreased with time (Table. 5). Palmyrah tubers from Vadamaradchi showed highest content of starch whereas Valikamam tubers showed the lowest.. Amount of starch in Thenmaradchi tubers did not change drastically while the Island tubers showed a sudden decrease with time. However, tubers must be harvested before the declining of the starch content. Therefore, 12thweek should be the best time to harvest the tubers.

Table 5. Change of amount of starch and sugar in Palmyrah tubers with age

Place	Starch content	( g per 100 g)	Total sugar content of tuber (g/100g) with fixed time intervals		
Age of tuber (weeks)		Age of tuber (weeks)			
	12 <sup>th</sup>	15 <sup>th</sup>	12 <sup>th</sup>	15 <sup>th</sup>	
Island	70.35 ±1.65	62.00±1.14	11.21±0.45	15.85±1.86	
Thenmaradchi	60.01±1.20	59.72±1.60	15.51±0.78	18.44±0.42	
Vadamaradchi	73.70±1.96	68.78±2.50	11.39±1.47	17.33±0.13	
Valikamam	53.95±1.25	50.90±1.50	9.41±0.36	11.66±0.92	

According to the results obtained from the analysis of total sugars of tubers, the tubers at the age of 15th week showed the highest total sugar content than the earlier stage which was observed for all four types of samples (Table. 5). The sample of Thenmaradchi showed the highest among all four at both 12th and 15th week of age. Also, the sugar level increased concomitantly as starch content declined. This is because at 15th week starch might have

been hydrolyzed to sugar and then transferred to shoot and root. At this stage new true leaf would grow, therefore utilization of food by the growing plant must be very high.

The study of Total phenolic content in haustoriumwith the time

The total phenolic content of haustorium had a significant change with the growth period as illustrated in table 6. Haustoriums grown in the four different places contain the total phenolic content between  $500\,\text{mg}/100\,\text{g}$  to  $1\,\text{g}/100\,\text{g}$ , indicating that it has a great antioxidant potential.

Table 6. Total phenolic content in haustorium at fixed time intervals

Strict true	Total phenolic content of haustorium with fixed time intervals  (weeks) (mg/100g)					
Place						
	6th	9 <sup>th</sup>	12 <sup>th</sup>	15 <sup>th</sup>		
Island	770.21±2.86 <sup>d</sup>	821.68±14.48°	899.44±2.00 <sup>b</sup>	926.70±12.82 <sup>a</sup>		
Thenmaradchi	597.10±107.67 <sup>d</sup>	599.78±8.66°	822.37±1.91 <sup>b</sup>	824.02±3.59 <sup>a</sup>		
Vadamaradchi	578.43±8.75°	520.98±11.62 <sup>d</sup>	692.17±11.67 <sup>b</sup>	962.88±2.87ª		
Valikamam	640.39±21.35°	639.53±6.75 <sup>d</sup>	946.09±42.51 <sup>b</sup>	950.26±11.80 <sup>a</sup>		

Each value in the table is represented as mean  $\pm$  SD (n = 3). Values in the same row followed by a different letter (a-b) are significantly different (p< 0.05).

As shown in table 6, total phenolic content increased with harvesting period for all four different places. At 12th week, among the four different samples, haustorium grown in Island and Vadamaradchi showed highest and lowest content respectively. At 6th week and 9th week, phenolic content is not significant because haustorium might have been at an immature stage. Synthesis of phenolic content might have happened later. Table 6 reveals that the total phenolic content increased with maturity of haustorium up to 12th or 15th week.

The study of change of the amount of total dietary fibre in haustorium with the time

In contrast to sugars and total phenolic content, a different trend was observed in dietary fibre content with the time of growth of haustorium. All four places hit their minimum at 9th week and slightly increased at 12th week. Then the values suddenly increased to their highest as .5th week. It is clearly illustrated in figure 3.

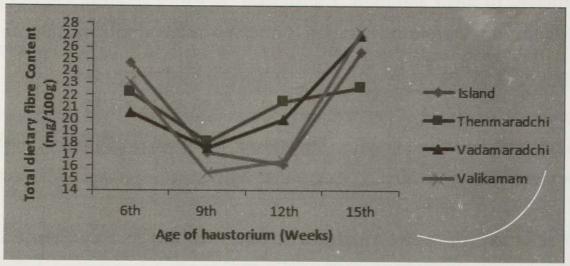


Figure 3. Change of the amount of total dietary fibre in haustorium with time

Initially, haustorium was small in size. This might be due to the high content of dietary fibre. Then other tissues developed, softened and gave a less fibrous nature to haustorium. Later on it had become more fibrous in nature. This might have been the case due to the declining nutrient transfer.

The study of moisture, protein and fat content in haustoriumat 12th week

Moisture content usually decides the taste and palatability of the product. Here the adequate moisture content present in the samples might encourage the consumers to consume the product more and more. The moisture content of Palmyrah haustorium obtained from Vadamaradchi was the lowest and the highest moisture content was shown by haustorium of the Islands as shown in Table 7. However all haustorium samples contained the moisture content value above 80%.

Table 7. Nutritional content of Palmyrahhaustorium at 12th week

Places	Nutritional content (g / 100g)			
Titlees	Protein content	Fat content		
Island	7.90±0.17	0.14±0.47		
Thenmaradchi	7.64±0.32	0.39±0.78		
Vadamaradchi	8.02±0.45	0.13±0.24		
Valikamam	7.49±0.78	0.36±0.18		

If haustorium consists of more protein it will attract the consumers. Table 8 shows that the protein content of the haustorium in the four places lies in the range between 7 to 8 g per 100g, where protein content of Haustorium from Vadamaradchi showed the highest.

According to table 8, the fat content of Palmyrah haustorium from Thenmaradchi and Valikamam showed the highest and almost similar values whereas the other two types of samples showed the lowest values; hence the samples could be categorized into two groups. Also the fat content varied in the samples in comparison with the content of protein. When protein increased the fat in the same sample decreased.

#### The study of mineral content in haustorium with the time

All four places showed significantly higher mineral content at 6thweek and it significantly reduced at the end of 12th week and it again increased at 15th week. At 12th week of harvesting, Thenmaradchi had the highest mineral content and Island haustorium showed the lowest mineral content when compared with the other two places. The ash content decreased with the growth period in all four places and at the beginning, Vadamaradchi haustorium contained the highest mineral content which drastically decreased with age. The results are shown in table 8.

Table 8. Total ash content of haustorium of palmyrah

Place	Ash content (g/100g) of haustorium at fixed time intervals (Weeks)					
	6 <sup>th</sup>	9 <sup>th</sup>	12 <sup>th</sup>	15 <sup>th</sup>		
Island	5.83 ±0.86 <sup>a</sup>	5.01 ±0.32 <sup>b</sup>	3.30 ±0.23 <sup>d</sup>	4.94 ±0.04°		
Thenmaradchi	5.95 ±0.39 <sup>a</sup>	4.19 ±0.16 <sup>d</sup>	4.28 ±0.02°	4.30 ±0.02 <sup>b</sup>		
Vadamaradchi	6.42±0.20 <sup>a</sup>	4.84±0.09 <sup>b</sup>	3.40±0.17 <sup>d</sup>	4.36±0.14°		
Valikamam	5.80±0.08 <sup>a</sup>	3.75±0.03 <sup>d</sup>	3.78±0.08°	4.07±0.06 <sup>b</sup>		

Each value in the table is represented as mean  $\pm$  SD (n = 3). Values in the same row followed by a different letter (a-b) are significantly different (p< 0.05).

Table 9 indicates that potassium was higher in all samples whilst iron content was negligible when compared to the other minerals considered here. Calcium was the second highest mineral in haustorium. Haustorium of Thenmaradchi contained the highest amount of potassium and sodium whereas haustorium from Vadamradchi contained the highest amount of calcium. These differences in mineral abundance might affect the composition of haustorium.

Table 9. Mineral content of Palmyrahhaustorium

Place	Mineral (mg/100g)					
	Sodium	Potassium	Calcium	Magnesium	Iron	
Island	23.88±0.16 <sup>a</sup>	48.36±0.11 <sup>d</sup>	64±0.23 <sup>b</sup>	39±0.66 <sup>d</sup>	0.34±0.42°	
Thenmaradchi	23.86±0.21 <sup>a</sup>	99.86±0.51 <sup>a</sup>	55±0.17°	41.4±0.63°	0.19±0.12 <sup>d</sup>	
Vadamaradchi	20.31±0.06 <sup>b</sup>	85.93±0.18°	71±0.12 <sup>a</sup>	45±0.64 <sup>a</sup>	0.45±0.15 <sup>a</sup>	
Valikamam	15.23±0.07°	93.45±0.54 <sup>b</sup>	65±0.64°	43±0.21 <sup>b</sup>	0.41±0.12 <sup>b</sup>	

Each value in the table is represented as mean  $\pm$  SD (n = 3). Values in the same column followed by a different letter (a-b) are significantly different (p< 0.05).

The study of phenolic content of in Palmyrah haustorium in different areas

Antioxidant activity is due to the phenolic components present in the haustorium. The extraction of antioxidant is the most important factor in their determination. Yet, based on the study by Arunachalam *et al* (2012) [1] acetone was selected as the best solvent for phenolic compound extraction from haustorium.

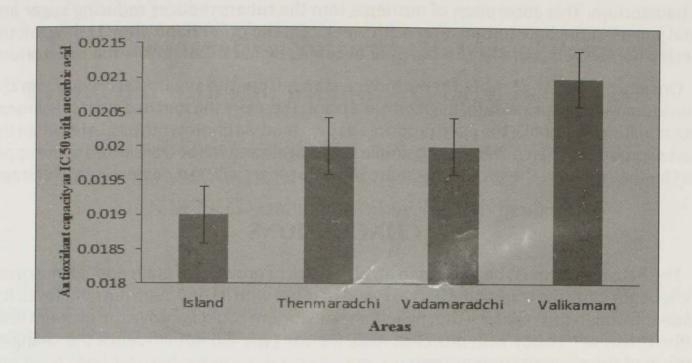


Figure 4. Antioxidant activity of Palmyrahhaustorium in four different areas in Jaffna

As shown in figure 4, Palmyrah haustorium from Valikamam contained the highest antioxidant activity whereas the haustorium from Island had the least among the four different samples. The other two types of samples had almost similar antioxidant activity to each other. However all four had significant amount of antioxidant capacity in terms of ascorbic acid.

Presence of antioxidant activity in the haustorium is beneficial to the health of the consumers. Mainly its aves humans from cancer. Since all four different samples (places) showed the presence of the antioxidant activity, haustorium consumption gives health advantages to humans.

According to the results obtained from the analyzed parameters, the values were at their maximum at 12th week of haustorium's growth, except for the dietary fibre which was at its lowest value at 12th week. In the case of Palmyrah tubers, starch was at its maximum at 12th week and then declined whereas the total sugar showed the opposite trend. This is because of the biochemical changes that occur during the growth of a plant.

When a Palmyrah seed germinates embryo forms two parts namely haustorium and cotyledon. Cotyledon turns to radicle and then forms a new plant while haustorium absorbs the nutrients from endosperm and transfers them to cotyledon. After haustorium penetrates into endosperm it sucks up the nutrients and converts the complex nutrient molecules into their simple forms such as simple sugars, amino acids etc for its growthwhich would take few weeks,. At this stage the abundance of sugar and other essential nutrients, phytochemicals etc of haustorium will be higher. However the growth of cotyledon occurs after 6 weeks when it starts to get nutrients from the haustorium. At 12th week the tuber is formed and it starts growing as a new plant. Therefore tubers start absorbing the nutrients

in haustorium. This absorption of nutrients into the tubers reduces reducing sugar and total sugars in the haustorium after 12th week. On the other hand after 12th week the dietary fibre which could not be digested becomes higher in content in the haustorium.

Once tubers start growing themproduce starch from the sugars absorbed from the haustorium and save it inside for its growth as a plant. Therefore the starch content of Palmyrah tubers will increase while the plant produces its own food via photosynthesis. Later when the leaves sprout, starch is converted into simple sugars again and will be transferred to the upper part for their growth. Therefore the starch content in tubers will start decreasing at this stage.

#### CONCLUSIONS

The haustorium must be harvested at 12th week of growth to retain enough nutrients without affecting the traditional harvest yield of tuber with its optimum nutrient level. It is evident through this research that Palmyrah haustorium could improve the nutrient level in the consumer's body with proteins. Also, the soil type did not influence the changing pattern of the nutrient composition.

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# 2.4.2 Comparative Nutrient Analysis of Palmyrah Fruit Pulp with and without Artificial Preservative

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The study presents the results of a small scale laboratory research carried out to examine the effect of the chemical preservative, sodium meabisulphite (SMS) on the nutritional characteristics of the pulp with time. Palmyrah fruits of same maturity and size from the same palm were subjected to manual extraction of pulp. pH of the pulp was measured and it was adjusted to 3.8 using food grade citric acid. The pH adjusted pulp was then heated to 90 °C for 30 minutes using open pan heating and was allowed to cool down to 60  $^{\circ}$ C. Food grade SMS was added to one portion of the pulp in the ratio of 0.4gL-1 and the other portion of the pulp was not mixed with SMS. Both pulp portions were hot filled into sterilized glass bottles and stored at 4°C in a refrigerator. Continuous nutrient analysis was carried out and the results were subjected to Mann Whitney U test using the software SPSS16. Results of the study exhibits that within two months there is a decline in titrable acidity, Na level, moisture level and there is a rise in pH in the pulp with SMS. Furthermore, the abundance of the sugars, protein, fat and K level remained stable throughout the study which lasted for two months. In the pulp without SMS, there is an increase of reducing sugars and titrable acidity and a decline in pH, and total soluble solids levels with time and the differences between the values obtained periodically were significant. Moreover, the microbial colony count shows that the chemical preservation treatment is effective since the colony count is zero in the pulp with SMS at the end of 2nd month, whereas pulp without SMS shows prominent growth of microorganisms and the total plate count here is 55cfu/ml at the end of 2nd month. Based on the results of this study there is no adverse effect of SMS on the nutritional composition of the pulp. Addition of SMS shows a strong preservation activity when combined with refrigeration whereas refrigeration alone can be employed to preserve the pulp for up to one month.

Key words: Palmyrah fruit pulp, Preservation, Physicochemical properties

### 2.4.3 Bioethanol Production Using Palmyrah Waste Materials

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Ethanol is now the most important renewable fuel in terms of market value. Nowadays it is produced from sugar and starch based materials such as sugarcane and corn. However, the second generation of alcohol production also derived from lignocellulosic materials is now being tested in some plants. In this study palmyrah waste material such as molasses, expired pulp and coir dust was selected and among the selected materials molasses. expire pulp used for the primary alcohol production and coir dust used for the secondary alcohol production which is used for the alcohol production after the pretreatment process. Pretreatment of coir duct was carried out with different alkaline solution (NaOH and Ca(OH)<sub>2</sub>) and acid solution (H<sub>2</sub>SO<sub>4</sub>, HNO<sub>3</sub> and HCl) at 121°C for 15 min and H<sub>2</sub>SO<sub>4</sub> was selected as best hydrolysis agent and used for further study. For the H2SO4 pretreatment two factors such as concentration (3%, 5% and 7%) and times (15min, 30min and 45min) were optimized in three levels. Among the nine treatments 3% of concentration and 45min were selected as optimum condition for hydrolysis or production of reducing sugar from coir dust. Coir dust hydrolyzed solution; molasses (°15 initial brix) and expired palmyrah fruit pulp (°15 initial brix) were used for the fermentation. Fermentation was carried out with bakery yeast inoculated with Peptone, yeast extract and nutrient medium at room temperature and pH 5.0. Therefore, pH of the coir dust hydrolyzed medium obtained from acid hydrolysis was adjusted to pH 5.0 and then allowed to incubation. Significantly, highest alcohol production was observed for coir dust H2SO4 hydrolyzed medium (0.4%), molasses (8.6%) and pulp (5.5%) at 4, 6 and 4th day of fermentation respectively. During the fermentation there were significant different in acidity, pH, reducing sugar and total sugar between tested days. Total sugar content was significantly decreased during the fermentation for all waste materials. Although bioethanol production could be greatly enhanced by enzyme technology and also developing more efficient pretreatment technologies for coir dust and integrating the optimal conditions of fermentation such as inoculum size, volume of initial fermentation medium, into economic ethanol production.

Key Words: Palmyrah, Bioethanol, Fermentation

# 2.4.4 Comparative Study on Quality Characteristics of Different Palm Treacle and It's Antioxidant Activity

Akeeshan B<sup>1</sup>, Subajini Mahilrajan<sup>2</sup>, W.A.J.P. Wijesinghe<sup>1</sup>, Srithayalan Srivijeindran<sup>2</sup>

Treacle is any uncrystallized syrup made during the production of sugar. The most common forms of treacle are golden syrup, a pale variety, and a darker variety known as black treacle. Black treacle or molasses has a typically strong, slightly bitter flavour, and a richer colour than golden syrup. Black treacle is obtained from palm such as palmyrah, coconut and kithul, which are available from Jaffna market used as the main sweetening agents for cake, curd, tea and coffee. There is a competition for the selection of nutritionally best treacle for the customers. Therefore this research was design to evaluate the best treacle this contained good physico chemical and nutritional characteristics. Triplicate treacle samples were obtained from one batch of respective production centers and used for the analysis. All the treacle was obeys SLS specifications while there were significant different among the tested parameters between the treacle. Significantly highest (p<0.05) total sugar (63.90%) and ash content [0.075(±0.002)] % was obtained for palmyrah treacle, while protein content was significantly highest for coconut [0.410 (±0.0040] than kithul[0.570 (±0.0120] and palmyrah [0.028 (±0.0070)] %.Total phenolic content was significantly highest for coconut [0.807 (±0.004)] while there were no significant different between palmyrah [0.547 (±0.001)] and kithul [0.545 (±0.002)] mg/100g. DPPH scavenging ability with the IC50 values of palmyrah, coconut and kithul were 0.0169 (±0.003), 0.029 (±0.004) and 0.021(±0.004) respectively. Total phenolic contents exhibited significantly positive correlation (0.79, p=0.011) with the IC 50 values of different treacle. The results of this study showed that palmyrah treacle showed highest amount of minerals and antioxidant activity when compared with other palm treacle.

Key words: Palm treacle, Antioxidant activity

## 2.4.5 Comparative Nutritional Analysis of Fresh and Preserved Palmyrah Young Fruit Kernel (Ice Apple)

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The present study was carried out with fresh and preserved palmyrah young fruit kernel to evaluate nutritional quality with respect to protein, minerals, sugar content and fiber components, vitamin C and total phenols. The objective of the research was study the effects of the palmyrah sweet sap on the characteristic of palmyrah young fruit kernel preservation through the comparison of the chemical and nutritional characteristics of fresh and preserved palmyrah young fruit kernel. Palmyrah young fruit kernel was immersed into the palmyrah sweet sap with the brix 25, pH 4 and sterilized at 121°C for 15 minutes. The nutritional analysis of fresh fruit kernel has shown (89.270±0.241) g/100 g moisture, (0.8375±0.007) g/100 g protein and (4.9382 ±0.0905) mg/100 g vitamin C content significantly higher than preserved samples. Comparative analysis of macro and micro nutrient composition showed that preserved sample has significantly (p < 0.05) higher (0.083±0.002)]g/100 g fat content and (0.4250±0.006) g/100g ash content than fresh sample of the palmyrah young fruit kernel while there were no significant different between the storage period. (13.8438±0.054) g/100 g total sugar, (3.4632±0.191) g/100 g reducing sugar and minerals such as Ca, Na, K and P was significantly higher for preserved sample than fresh sample while there were no significant difference between storage periods. There were no significant differences in all the parameters except (0.8709  $\pm 0.010$ ) g/100 g fiber and (0.0578  $\pm 0.0006$ ) g/100 g total phenol content between the storage periods. The results indicate that the Palmyrah young fruit kernel is endowed with essential nutrients required for human consumption and can be used in health promoting benefits. This study will help the food producer or the confectionary manufacturer to select the appropriate concentration of palmyrah sweet sap solution for making palmyrah young fruit kernel and at the same time consumers could prefer palmyrah young fruit kernel by preserving with sweet sap because which is nutritious.

Keywords: Palmyrah young fruit kernel, Palmyrah sweet sap, Osmotic dehydrated,

### 2.4.6 Development of Palmyrah (Borrasus flabellifer L) Fruit Pulp Incorporated Ice Cream

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Incorporation of fruits into the ice cream is one of the choices to increase the nutrition value of the product, flavorings and palatability. The study was focused to formulate a nutritionally rich ice cream by incorporating Palmyrah fruit pulp. Four levels of Palmyrah pulp (9%, 12%, 15% and 18%) were tested for the formulation by 30 semi trained sensory panelists using 5-point hedonic scale. Results of the sensory evaluations revealed that 15% Palmyrah fruit pulp as the best incorporation level. Proximate composition and other nutrients of the selected sample were analyzed. Microbiological quality was evaluated using Total aerobic plate count, yeast and mold count, Coliforms and Salmonella counts of the selected product. Accordingly selected Palmyrah fruit pulp ice cream contained 66.01% moisture, 9.89% fat, 1% ash content, 0.77 % dietary fiber, 21.36% total sugar, 4.3% reducing sugar, 17.38 % Non reducing sugar, 101.50 mg per 100g Vitamin C, 49.75 mg/100g Calcium, 36.74 mg/100g Sodium, 183.67 mg/100g Potassium. The product was complied with Sri Lanka Standards and was safe for consumption up to 10 weeks at -4 °C without any artificial preservative. In conclusion, ice cream with acceptable consumer preference can be developed by using Palmyrah fruit pulp. Future studies are needed to analysis of antioxidant activity of developed ice cream and increase the storage period.

Keywords: Palmyrah fruit pulp, Ice cream, Nutritional analysis, Microbial analysis

### 2.4.7 Reformulation of palmyrah

# (Borrasus flabellifer L) fruit ready to serve drink and modification of its process to improve some of its selected properties

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Although palmyrah fruits are seasonal they have excellent chemical and physical properties for the development of food and beverages. Though there is a demand for palmyrah ready to serve beverage, existing commercial products are failed due their poor quality. Hence this study was conducted to improve the quality of palmyrah ready to serve beverage by reformulation and modification of process. Pectin and citric acid were selected as stabilizer and acidulant through ranking test with 11 semi trained panellists. Using general full factorial design, 18 treatments were carried out to optimize the levels of fruit (5 %, 8.5 %, 12 %), sugar (10 %, 12.5 %, 15 %) and pH (3.5, 4.0) in the final formula. The final formulation was evaluated through 31 sensory panelists using 9 point hedonic scale. The formulated beverage contained 12 % fruit pulp, 12.5 % sugar and pH of 4.0. The level of pectin was adjusted to 0.66 % and fruit pulp was subjected to homogenization (30000 rpm, 5 min). Chemical and nutrient analysis of final product and commercially available drink revealed that the reformulated product was significantly better that existing product in nutrients. It contained 0.14 % crude protein, 0.78 % crude fat, 0.41 % crude fibre, 0.17 % ash and 11.97 % total sugar. The developed product did not show any growth of yeast and mold and did not result total bacterial count though out the shelf life study for 10 weeks. Hence the developed palmyrah fruit ready to serve drink showed better quality in the analyzed properties and scored the most in the sensory evaluation than the commercially available product.

Key words: palmyrah, antioxidant, homogenization, preserved pulp, ready to serve drink

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