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# Industrial Technology Institute



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## **Industrial Technology Institute**

# **Proceedings of the Annual Research Symposium 2013**

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# **Contents**

Bio Technology	01
Chemical Microbiology	07
Electro Technology	09
Environment Technology	11
Food Technology	14
Herbal Technology	21
Intellectual Property	29
Materials Technology	30
Noise and Vibration	38





### Cloning of potato SBgLR gene with rice Glb promoter to produce lysine-rich rice

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Rice which is the staple food in Sri Lanka is deficient in lysine which is one of the eight essential amino acids. As a result, protein deficiency disorders are particularly severe where rice is the major staple food. Genetic engineering is one of the possible ways to resolve this nutritional problem through enhancing the total lysine and total protein content in rice. The objective of this study was to increase both the lysine and total protein content of the rice seeds by introducing a pollen-specific *Solanum tuberosum* genomic lysine-rich (*SBgLR*) gene from potato (*Solanum tuberosum* L.) into rice seed under the control of the rice seed specific globulin (*Glb*) promoter.

Total RNA from potato pollens was reverse transcribed with oligo dT primers and PCR amplified using \$SBgLR\$ gene-specific primers. The amplified product (which is 650bp in size) was purified and cloned in pCR®2.1-TOPO® vector. Recombinants were screened by colony PCR and putative colonies were subjected to restriction digestion with \$Eco\$ RI. A fragment of the expected size (650bp) was observed. The clone was sequenced and analyzed using a sequence analyzing program. A search of the non redundant nucleic acid database at National Center for Biotechnology Information (NCBI) revealed significant similarity (95%) to a previously cloned sequence (Acc. No. AY377987.1) which had been used to design \$SBgLR\$ gene-specific primers. The deduced amino acid sequence of the \$SBgLR\$ protein in this study displayed a high degree of similarity (94%) to the deduced amino acid sequence of the \$SBgLR\$ sequence cloned previously.

In order to clone the *Glb* promoter, DNA was isolated from rice leaves (*Oryza sativa indica*) and was amplified by PCR using gene-specific primers for *Glb* promoter. The amplified product (which is 1000bp in size) was cloned in pCR®2.1-TOPO® vector. The colonies were screened using a rapid screen method and colony PCR to identify the recombinant colonies. Recombinant clones were subjected to restriction digestion with *Eco* RI. A fragment of expected size (1000bp) was observed confirming the cloning *Glb* promoter.

At present, work is in progress to clone the *SBgLR* gene under the control of the *Glb* promoter in pCAMBIA for *Agrobacterium* mediated transformation.

Key words: SBgLR cDNA, Globulin (Glb) promoter, Molecular cloning sidsenara@iti.lk





### Cloning of soybean ferritin gene towards iron-fortification of rice

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Iron deficiency is a severe problem encountered by around 30% of the world's population, especially in the third world countries such as Sri Lanka. Most individuals suffering from iron deficiency are below the poverty line and depend on their food staples as the sole source of nutrition. Rice which is the staple food in Sri Lanka is deficient in iron. Increasing the iron content of rice by genetic engineering may help to solve the problem of dietary iron deficiency. Ferritin is an iron storage protein which stores upto 4500 iron atoms in the central cavity. The objective of this study was to increase the overall iron content of rice by increasing its ferritin content through transformation of soybean (*Glycine max*) ferritin gene to rice seeds. A seed specific globulin (Glb) promoter will be used to drive the expression of the ferritin gene.

Total RNA was isolated from 5 day old soybean cotyledons and cDNA was synthesized by reverse transcription using olgo(dT) primers. The synthesized cDNA was amplified using ferritin gene specific primers. The amplified product was purified and cloned in pCR®2.1-TOPO® vector. Recombinants were screened using colony PCR. Recombinant clones were also subjected to restriction digestion with *Eco*R I. A restriction fragment of expected size (800 bp) was observed, confirming the successful cloning of the ferritin gene. The clone was then sequencedand analyzed. A search of the non redundant nucleic acid database at National Center for Biotechnology Information (NCBI) with the cloned sequence revealed significant similarities to a number of sequences cloned previously (Acc. No. AK 245798.1, BT097559.1, EF05589.1). A soybean clone (Acc. No. BT097559.1) displayed the highest degree of homology both at the nucleotide (99%) and deduced amino acid levels (100%).

Currently, work is underway to clone the ferritin gene under the control of the seed specific globulin promoter in pCAMBIA1391Z for *Agrobacterium* mediated transformation into rice.

**Key words:** ferritin gene, Iron-fortification, transgenic rice

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### Detection of dog meat adulteration in mutton using a PCR assay

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In many developing countries the identification of species origin for meat is a considerably difficult task. Therefore, providing proof of fraudulent substitution for more expensive meat is a challenge. The aim of this study was to establish a qualitative Polymerase Chain Reaction (PCR) based method to detect dog (Canis lupus familiaris) meat adulteration in mutton. In this study, cytochrome b (Cyt b) gene was used to detect the source of meat. Primers were designed to amplify 809 bp and 157 bp regions of dog and goat (Capra aegagrus hircus) Cyt b genes respectively. Dog meat samples used in this study were authenticated by the veterinary research institute, university of Peradeniya. Mutton containing 1%, 5% and 20% (w/w) of dog meat samples in raw and cooked (45 minutes boiling in distilled water) form were used in DNA extraction and subsequent PCR analysis. Results indicate that adulterations as low as 1% (w/w) dog meat in mutton were detected by PCR. Moreover, a clear relationship was observed between the intensity of PCR products and percentage of dog meat in mutton. Hence, this method could be used as a semi quantitative PCR technique. The high sensitivity of PCR, which facilitates accurate and more reliable analysis of meat adulteration, is shown in this study. Therefore, the qualitative PCR based method used in this study can be used as a pragmatic solution to detect dog meat adulteration in mutton.

**Keywords:** meat adulteration, PCR assay

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# Development of a novel multiplex Polymerase Chain Reaction (PCR) assay for the diagnosis of Whooping cough (Pertussis disease)

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Whooping cough or pertussis disease is caused by two Bordetella species; B. pertussis and B. parapertussis. Although the disease has been under control after vaccines were introduced, it has re-emerged in some countries within the last two decades causing around 300,000 deaths globally per annum. Culture, PCR and serological methods are used to detect pertussis infection. Although culture is considered to be the gold standard for it's diagnostic specificity, PCR is widely used due to it's high specificity and sensitivity. In Sri Lanka, only the culture method is currently used for pertussis detection. However, due to high intake of antibiotics by patients with symptoms of pertussis infection, the culture method often gives false negative results. Therefore, in this study, a multiplex PCR method was developed to detect clinically relevant B. pertussis and B. parapertussis, using the repetitive insertion sequences IS481 and IS1002 of the two species. Both insertion elements IS481 and IS1002 are present in the genome of B. pertussis while, only IS1002 is present in B. parapertussis. For each insertion sequence, two pairs of primers were designed with one set of primers nested within the other primer pair. Partial sequence of each insertion element containing the target region was PCR amplified using genomic DNA isolated from killed B. pertussis vaccine (DTwP) and cloned into pGEM®-T easy vector system. The nested primer pair from each element was used for multiplex PCR. The sensitivity of the multiplex PCR assay was determined by using serial dilutions of linearized cloned plasmid from each insertion element. The PCR assays amplified 142 bp and 266 bp fragments from the insertion element IS481 and IS1002 respectively. The individual PCR assays were capable of detecting 200 copies of IS481 and 1 copy of IS1002. A multiplex reaction that amplified both fragments was also developed by optimizing the MgCl<sub>2</sub> concentration and the use of a PCR enhancer. The assay was capable of detecting 200 copies of IS481 and 10 copies of IS1002 insertion elements. In B. pertussis, the IS481 and IS1002 insertion elements are present in 200 and 10 copies per genome respectively while in B. parapertussis, IS1002 insertion element is present in 10 copies per genome. Therefore, the multiplex PCR assay has the potential to detect a single organism of either B. pertussis or B.parapertussis. Currently, work is underway to test clinical samples using the optimized assay.

**Keywords:** Whooping cough diagnosis, PCR assay

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# Identification of bacteria immobilization medium to use as a vehicle to carry hydrocarbon degrading bacteria for bioremediation

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The hydrocarbon resources are the principle energy source used in Sri Lanka. Due to the foremost petroleum usage there are many hydrocarbon contaminated sites in Sri Lanka. Hydrocarbon contamination of soil and water give rise to many environmental issues. Cleaning of these sites can be achieved through chemical, physical and biological methods. However, biological method is preferred over the other methods as it provides an environmentally friendly, efficient and costeffective solution. Bioremediation of hydrocarbon contaminants using immobilized hydrocarbon degrading bacteria is one of the wildly used biological methods. Although several countries have already used this method for bioremediation of petroleum spills, it has not been previously used in Sri Lanka. Development of a bio-product for bioremediation of hydrocarbon contaminants requires firstly, isolation and identification of different hydrocarbon degrading bacterial types and secondly to develop a bacteria immobilization media to use as a vehicle to carry bacteria. Both the bacteria and the immobilization medium should be environmental friendly. The potential immobilization medium should be able to carry a large number of bacteria, protect them from desiccation and mechanical stress besides contributing to the effectiveness of hydrocarbon degradation of bacteria. In a previous study, several bacterial types were isolated and identified from petroleum contaminated soil. The purpose of this study is to identify and developan immobilization medium for the hydrocarbon degrading bacteria. Two bacteria samples from the earlier study were used in our study. For bacterial identification; DNA was extracted from overnight pure cultures of bacteria by Phenol-chloroform extraction method, purified and used for 16s rRNA sequencing. Bioedit version 7.2.0 and NCBI blast were used for sequencing analysis and species identification. Results showed that both bacterial samples contained Raoultella ornithinolytica strain SG-5. In this study Calcium alginate was tested as a potential bacterial carrier for bioremediation purposes. Sodium alginate was known as a good hydrocarbon degrading bacterial carrier for bioremediation applications. In this regard as the first stepwe extracted Sodium alginate from Sri Lankan brown seaweed by alkaline extraction method. Over night bacterial cultures were then suspended in sterile sodium alginate (3%) solution after centrifugation and sterilization with saline. For the formation of the calcium alginate beads immobilize with bacteria, sodium alginate/bacteria cell mixture was dripped into cold CaCl<sub>2</sub> using a syringe. The surface strength of the beads was increased by placing them in fresh CaCl<sub>2</sub> solution for 2 hours. The bacteria immobilization in beads was then confirmed by placing the beads on Luria broth (LB) agar plates and observing the bacterial growth. This study provides insight into potential use of calcium alginate as a bacteria carrier to develop a bio-product for bioremediation of hydrocarbon. Currently work is underway to assess the bio-product for its bioremediation potential in petroleum contaminated water and soil samples.

**Keywords:** Bioremadiation, hydrocarbon contamination

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### α-Amylase production from *Manihot esculenta* (cassava)

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The starch liquefaction process in the brewing industry uses two key industrial enzymes, αamylase and amylo glucosidase. The high cost of production in Sri Lanka has led to importation of these enzymes from countries such as Denmark and Singapore, resulting in loss of foreign exchange. This study focused on using locally produced low cost cassava (Manihot esculenta) roots as a primary substrate to produce α-amylase using Bacillus subtilis subsp. spizizenii str. ATCC 6633. Cassava root extract was produced by milling cassava roots obtained from the local market with distilled water. Cassava root extract (20% w/v) was supplemented with yeast extract (0.5% w/v), pH adjusted to 6.7, autoclaved and inoculated with 500 µl of overnight liquid culture of Bacillus subtilis. Fermentation of cassava root extract was carried out at room temperature (RT) in shaker incubators (180 rpm) and α-amylase activity was analysed at 37 °C and 55 °C using the Dinitrosalicylic colorimetric method. The enzyme activity at 37 °C and 55 °C were 1.21 ± 0.011 U and 1.47 ± 0.006 U respectively. Optimization of fermentation conditions indicated 120 hours of incubation and pH 8.5 for culture medium resulted the highest enzyme activity. Furthermore, α-amylase produced in this study had optimum activity at 55 °C and pH 5. The findings in this study suggest that amylase produced by Bacillus subtilis using cassava root extract is suitable for low temperature (55 °C) starch hydrolysis for brewing industries. Further optimization in fermentation conditions, purification and characterization studies of the enzymes may lead to improving the potential for cost effective production of these enzymes. Therefore, we propose locally produced cassava root extract as a substrate for cost effective production of industrial enzymes in Sri Lanka.

Keywords: Amylase, Bacillus subtilis, Starch, Detergents, Tapioca

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### Heavy metal impurities in inorganic fertilizers used in Sri Lanka

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Conventional inorganic fertilizers used in agriculture may cause an inadvertent addition of heavy metals to the soil, which are contained as impurities. Regulations also have been proposed for phased-in limits on maximum heavy metal concentrations permitted in fertilizers, or they are already in effect. These heavy metals may accumulate in soil with repeated fertilizer applications. Cadmium (Cd) is the heavy metal of most concern because it may affect human health. Other heavy metals of possible significance are arsenic (As), lead (Pb), and mercury (Hg).

Fertilizers used in Sri Lanka were analyzed from year 2010 to 2013, to determine the concentrations of Cd, As, Hg and Pb depending on the fertilizer type. Mean level of Cd in triple supper phosphate (TSP) was 1.7 mg kg<sup>-1</sup> (n=50) and was well below the maximum level permitted in national standard. However, few consignments (n=5) had Cd level of 4.5 mg kg<sup>-1</sup> with one exceptional sample having a value of 25.4 mg kg<sup>-1</sup> which is far above the control level. Mean arsenic levels of the monitored TSP samples were 23.8 mg/kg (n=5). Single super phosphate (SSP) had mean Cd level of 0.5 mg kg<sup>-1</sup> (n=4) and As level of 14.0 mg kg<sup>-1</sup>. Rock phosphate contained mean As level of 19.9 mg kg<sup>-1</sup> (n=6) while Cd was not detected (<0.2 mg kg<sup>-1</sup>). Other fertilizers analyzed, were Zinc Sulphate (n=9), Murate of Potash(n=3), and liquid fertilizers (n=10) and did not contain heavy metals levels exceeding the maximum levels stipulated in the relevant standards.

In conclusion, concentrations of heavy metals varied considerably among the same type of fertilizer lots as well as among the different fertilizer types. Levels of Cd and As in some analyzed materials were significant relative to those naturally present in soils. Few samples of TSP analyzed have significant amount of As, and therefore it is recommended to establish control limits for As in phosphate fertilizers. Continuous fertilization of soils could increase the heavy metal contents exceeding natural abundances in soils, and transfer of these metals to the human food chain must not be overlooked.





# Analysis of volatile components of *Caryota urens L.* (Kithul palm) treacle by headspace-solid phase microextraction - gas chromatography-mass spectrometry (HS-SPME-GC-MS)

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In Sri Lanka, traditionally, treacle made from *Caryota urens* L. (Kithul palm), sap is used as a natural sweetener. The present Sri Lanka Standard for treacle (SLS 772:1987) is a common standard that covers treacle made from Kithul, Coconut, Palmyrah, and Sugar cane. The parameter available for identification of the type of treacle in this standard is organoleptic assessment (specific taste and the aroma) of the different treacle, which is subjective. This is one of the major drawbacks in promoting these unique products in the international market as a trained sensory panel is mandatory for differentiation purpose. Therefore, it is important to develop an instrumental technique for the purpose of identification of treacle types, in development of quality standards.

Treacle is prepared by concentrating the respective sap or juice obtained from inflorescence or stoke, using heat. As a result of this treatment, the non-enzymatic heat-induced browning reactions, namely Maillard and caramelisation reactions, take place leading to the formation of volatile aldehydes, ketones and heterocyclic compounds. These volatiles contribute to the characteristic caramel-like, slightly burnt-sugar aroma and flavour of treacle. However, the complete identification of these volatile aroma compounds of the Kithul treacle has not yet been reported.

The headspace volatiles Kithul treacle aroma was subjected to solid phase microextraction with a 50/30µm divinylbenzene / Carboxen / polydimethylsiloxane coated 2cm stable-flex fibre, and subjected to gas chromatography mass spectrometry for the identification of the aroma constituents with a Carbowax column.

Aroma volatile components were different for the four types of treacle and can be used as a fingerprint of the respective treacle type. Volatile component of Kithul treacle was rich in ethanol (70.66%), ammonia (10.60%), isoamyl alcohol (4.95%), bis- (2-ethylhexyl) hexane dioic acid ester (4.10%), 2-methyl -1-propanol (3.94%), benzeneethanol (1.78%), ethyl acetate (1.06%), 3-methyl-1-butanol (0.55%), phenol (0.35%), 3,5-dimethyl pyrazine (0.34%), 2,6-dimethyl pyrazine (0.28%), methyl pyrazine (0.24%), pyrazine (0.06%), as major constituents. Several furanone derivatives, furan derivatives and phenolic asters were also present as minor constituents. This is the first report of the volatile aroma profile of the Kithul treacle.

**Key Words:** Caryota urens, Kithul, aroma, volatiles, HS-SPME-GC-MS

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### Music induced hearing impairment

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This report discusses a new approach of hearing impairment .it is better that is introduced as "Music induced Hearing impairment" .In this work a same MP3 audio file was executed in different mobile phones to measure the output of an A-Weighted sound pressure level with the setting of 50% and 90% of their maximum volume. It was revealed that above 85dB (A) limits could be reached with the normal use of ear phones in day today life and the safe limit is the 50% of maximum volume setting of the mobile phones.

**Keywords:** Hearing impairment, hearing loss, noise limits, Exposure, sound pressure, Music





### Street Light Controlling System Using Existing Radio Broadcasting

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This research was conducted to design and evaluate a street light management system using existing radio broadcasting network. Coded audio signals transmitted through an existing radio station were used as control signals. Coded audio signal pattern was identified based on the frequency, duration and the order of the tones. PIC microcontroller was used to detect and identify the signal and to trigger power control module which controlled the street lamps. Using an existing radio station as the transmitter reduced the implementation cost while providing an optimal coverage and security for the signals.

Extra precautions were taken to reduce the miss triggering and to increase the robustness against the factors such as power failures and power surges. Units were tested in the laboratory and are currently being tested for the outdoor environment and observed to be functioning properly under average conditions.

Keywords: Automated, Street lights, PIC, Frequency, Radio broadcast, Audio signal





### An Evaluation of Coating Material Dependent Toxicity of Silver Nanoparticles

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Silver nano particles (AgNPs) synthesized using numerous types of coating materials may exhibit different toxicity effects. The study evaluated coating material dependent toxicity by selecting 3 types of AgNP synthesis methods with different coating materials (citrate, polyvinyl pyrrolidone, and branched polyethyleneimine, coated AgNPs as citrate-AgNPs, PVP-AgNPs, and BPEI-AgNPs respectively). Two acute aquatic toxicity tests were performed; 48hr *D. magna* and MetPLATE *E. coli* toxicity tests. Significantly different toxicity effects were observed in *D. magna* test exhibiting lethal median concentrations (LC<sub>50</sub>) for citrate-AgNPs, PVP-AgNPs, and BPEI AgNPs respectively as, 2.7, 11.2, and 0.57  $\mu$ g/L. Median inhibitory concentrations (EC<sub>50</sub>) for MetPLATE tests were 1.27, 1.73, and 0.31mg/L respectively with significant different toxicity effects. Silver ion fractions were detected in the range of 2.4 -19.2 % in tested NP suspensions. Study suggests the toxicity effects are due to the cumulative action of ionic and nano particle fractions in the suspension.





### Review of industrial air pollution control technologies that can be adapted to Sri Lanka

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Air pollution sources of industry can be found as either stationary sources or as fugitive sources. Usually combustible products such as  $CO_2$ ,  $SO_2$ ,  $NO_x$ , VOC and other gases are emitted from stationary sources such as chimneys, stacks. Fugitive emissions can be found in mineral crushing plants, solvent vapor in paint using industries, odor causing VOC from food industries etc. Industrial air pollution has significant local and regional impacts such as acid rain, global warming, nuisance and health impacts. The impact dimensions are determined by the volume and concentration of contaminated air emanated from the industry. There are two types of controls that can be taken to mitigate industrial air pollution.

The regulatory controls stipulated by central environmental authority such as national ambient air standards and standard for emission from stationary sources are important. However effectiveness of regulatory controls also depends on instrumental capacity and availability of technically skilled personals.

Technological solutions are available for dust control, nuisance odors and solvent vapors. These technologies can be adopted for local conditions and can implement successfully to mitigate air pollution by industries.

A case study will be presented for designed air pollution control system for Poultry farms. In this study it was observed the volatile organic compounds and gases emanated from this farm have drastically reduced. ITI is ready to adopt this system to other farms in the country.

Key words: air pollution, air pollution control technology, odor control, poultry farms

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# Advanced Air Dispersion Modeling (ADM) for predicting atmospheric impacts to accommodate air polluting industries in proposed Investment Promotion Zone (IPZ), Puttalam

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It is of great importance to study the existing status of ambient air quality (AAQ) and its dispersion ability, which depend on background emission sources, topography and climatic conditions before locating new plants/industries. This data can be effectively utilized in predicting atmospheric impacts to accommodate air polluting industries in most suitable location of the proposed IPZ.

The scope of this study was to assess air dispersion ability and AAQ status with respect to potential locations for new industries/plants of proposed IPZ. Mitigation measures such as; maximum allowable stack gas emission rates for a single stack, fuel consumption for any industry and number of stacks were studied in this regard. This would be useful for environmental regulatory authorities, policy makers and other interested parties in accommodating air polluting industries in the proposed IPZ, Puttalam.

This ADM study was carried out for three air quality parameters namely Nitrogen Oxides ( $NO_X$ ), Sulphur Dioxide ( $SO_2$ ) and Particulate Matter (PM) using US EPA recommended "ISC-AERMOD View" Version 6.6 software. Emission inventory data of large-scale point sources within 25 km study area were used to run the model. Time averaged  $SO_2$  and  $NO_X$  and PM concentrations in the ambient air were mapped within the selected study area for each case. Model output was validated by comparing predicted emission concentrations (by ISC-AERMOD View software) with actual measured values (by ambient air sampling).

Model output demonstrated that the maximum allowable stack gas emission rates for a single stack are  $SO_2$  13.5 g/s,  $NO_X$  16.5 g/s and PM 26 g/s. The respective maximum allowable fuel (< 3% sulphur) consumption for any industry is 1500 l/day and maximum allowable number of stacks recommended for the proposed site is only 42.

**Key words:** Air Dispersion Modelling (ADM), Ambient Air Quality (AAQ), Proposed Investment Promotion Zone (Proposed IPZ), SO2, NOx, PM, stack

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# Clarifying and separation of phenolic constituents of beetroot juice using membrane filtration technology and process modeling

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In food processing industries, application of conventional methods such as juice concentration, require high thermal load and are known to destroy the antioxidant capacity of the fruit, including total phenolic compounds. To overcome these problems, non thermal methods such as membrane filtration technology is used. The aim of this study is to clarify and separate the phenolic constituents of beetroot juice using membrane filtration technology and process modeling.

Beetroot purchased from the local market was used in this study. The edible portion of the beetroot tuber was sliced, and introduced in to a universal mixer with water (1:1 ratio w/v), juice from the ground material was filtered through a muslin cloth. This juice was used in the experiments to study the application of cross flow micro filtration technique. The total phenolic constituents of the juice were quantified in permeate and retentate at different pressure settings (2.1 x 10<sup>6</sup>, 3.5x10<sup>6</sup> Nm<sup>-2</sup>). Phenolic constituents were quantified using Folin-Ciocalteu reagent, by spectrophotometry (765 nm). The phenolic content in the permeate flux and retentate varied depending on the pressure created near the membrane. The flow rates of permeate and retentate were calculated using the measured volume and time. The increase in temperature of the bulk solution was observed with time. The results were fitted into the film model. The film model equation was derived by taking mass balance across concentration polarization layer which was formed during the process. According to the results obtained the mass transfer coefficient K is 3.65 x 10<sup>-5</sup>, while the true solute (phenolic content) removal near the membrane is 0.773. Therefore, the solute transport or mass transfer through the membrane is dominated by convection, since the observed solute removal decreases with increasing permeate flux. The increase in temperature from 31.4 °C to 37.6 °C, may have an effect on the mass transfer coefficient and for the true rejection of solutes. The deviations in the repeatability of the experiments may be attributed to the variability in the raw material.

Key words: membrane filtration, microfiltration, phenolic constituents, permeate, retentate

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### Comparison of the potential of producing ethanol from locally available cassava varieties

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Ethanol production from starch based substrates plays a pivotal role in the production of bio fuel and alcoholic beverages. Cassava (*Manihot esculenta*), also known as manioc or tapioca plays a vital role in ethanol manufacturing in some countries. Both the ethanol yield and the cyanide content of the final product are important in producing alcoholic beverages after distillation. The objective of this study was to select the variety which best suits for ethanol production with an acceptable level of cyanide content. Identity confirmed three varieties *Kiri Kawadi*, *Sudu Peradeniya* and *Kalu Peradeniya* were selected for the comparison of both ethanol yield and the cyanide removing potential while processing. Drying of chips, grinding, liquefaction, enzymatic hydrolysis and fermentation were the basic stages selected for ethanol production. Total sugar content in prepared flour, hydrolyzed samples and fermented samples were compared. Cyanide content after selected processing stages such as drying, enzymatic hydrolysis and fermentation were calculated. Variety *Sudu Peradeniya* yielded the highest average ethanol content of 7.99 (v/v) % while having the lowest level of cyanide content, which is 2.1 ppm in the fermented product. Results further confirmed that cyanide content in final fermented products were within standard levels for consumption.

Key words: Cassava, Cyanide, Ethanol production





# Application of sulphur dioxide, pasteurization and low temperature storage for shelf life extension of amla (*Phyllanthus emblica* (Linn.) pulp

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The fruit of *Phyllanthus emblica* (Linn.) has high potentiality in processing functional food due to proven medicinal properties. High seasonality prevents continuous industrial processing therefore the study was conducted to evaluate the effect of sulphur dioxide, pasteurization and low temperature storage (10  $^{\circ}$ C  $\pm$  2) to preserve amla pulp for minimum three months. Single and combined treatments of ambient storage (28 $^{\circ}$ C  $\pm$  2), refrigeration (10  $^{\circ}$ C  $\pm$  2), pasteurization at 85 $^{\circ}$ C and addition of 1000 ppm sodium metabisulphite were applied on pressed amla pulp. Samples were analyzed for microbiological, chemical and sensory parameters of a processed beverage at three weeks intervals during storage.

Control deteriorated after three days with unacceptably high total plate counts, yeasts and mold counts and significant dark browning. Counts were not detected in treated samples. Samples which were not pasteurized, initially indicated significantly higher (p<0.05) overall acceptability. ascorbic acid content, antioxidant activity and lightness compared to pasteurized samples. The above parameters reduced significantly (p<0.05) during storage. Titratable acidity and TSS increased while pH, and tannin content reduced without significant effect. Reduction of antioxidant activity was related to loss of both ascorbic acid and tannins. Refrigeration and pasteurization alone could preserve amla pulp only up to three and six weeks respectively, with acceptable taste and overall acceptability. Sulphur dioxide had a preserving effect (p<0.05) on lightness, ascorbic acid and antioxidant activity whether stored under ambient or refrigerated conditions. Refrigeration could substitute pasteurization when combined with sulphur dioxide and resulted in a significantly better product at the end of storage. Application of 1000 ppm sodium metabisulphite and refrigeration (10 °C ± 2) is most suitable to extend storage life of amla pulp up to 15 weeks. The treatment resulted in significantly highest (p<0.05) organoleptic acceptability, lowest titratable acidity (5.16 % as anhydrous citric acid ± 0.1), highest tannin content (0.918 % as gallotannic acid ± 0.038), highest antioxidant activity (IC<sub>50</sub> value 407.22 ± 4.9), and lightest colour (70.95 ± 0.8). The TSS content was 7.27 °Brix ± 0.2 and the vitamin C content was 294.53 mg/ 100 g ± 8.25.

**Key words:** Amla pulp, pasteurization, sodium metabisulphite, ascorbic acid, refrigeration udayani@iti.lk





### **Enhancement of rate in Production of Natural Vinegar using Rotating Bed Generator**

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Vinegar is an acidic liquid in which the key ingredient is acetic acid (ranging from 4-18 % (w/v)). It is mainly used as a flavouring agent in food pickling and in medicine. Natural vinegar is produced by aerobic fermentation in the presence of acetic acid bacteria. Natural vinegar can be produced by using several methods. In this study a novel method of producing natural vinegar has been introduced. This method can be utilized for a wide range of applications. This vinegar generator can be used to produce natural vinegar from wine prepared using coconut water and natural fruit juices. The rate of acetification in this generator has been enhanced with facilitated aeration by rotating a packed bed. This packed bed consists of halmilla wood shavings. During the process Acetobacter bacteria are attached to wood shavings, which facilitate conversion of ethyl alcohol in to natural vinegar. The generator is rotated two hours per day; one hour per each time. A mix culture of Acetobactor converts the alcohol (7-10 %) to 4-6 % acetic acid. This process of manufacturing acetic acid is a faster method and it takes less than 10 days for acetic acid fermentation.

Key words: Acetic acid, fermentation, vinegar, acetification, Acetobactor





# Suitability of Locally Grown Tomato Varieties for Processing with special reference to Process Technique and Lycopene Content

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Sri Lanka being a tropical island yields an annual production of 65,157 MT of tomato and nearly half of the produce is subjected to post harvest losses during the glut period. In addition to the limited availability, insufficient technology and peoples' belief in poor colour development and inferior quality of local tomatoes has led to import tomato pulp and juice from many countries. Lycopene, a carotenoid and also a natural antioxidant that imparts redness to tomatoes can undergo degradation during processing and storage. Thus, the study was aimed to determine the lycopene content in five local tomato varieties (Thilina, Tharindu, Bathiya, Maheshi and Rashmi), in laboratory processed and imported tomato pulps. Further, to explore the optimum pulp concentrating technique and to determine the relationship between colour and lycopene content in concentrated pulps. Lycopene estimation was done using two different methods and pulp concentration was achieved by vacuum pan and open pan techniques at 60 °C. Local varieties, Tharindu and Rashmi showed highest and lowest lycopene content respectively while in all five varieties lycopene level ranged from 4 to 12 mg/100 g. However, significant difference was observed in lycopene content of the same variety of tomatoes in different harvests (P<0.05) and similar results were obtained for all five varieties. Among concentrated pulps, Tharindu var. showed the highest lycopene content and it was evident that open pan pulp concentration was superior to vacuum pan technique in terms of lycopene level in pulps.

Key Words: Lycopene, tomato, postharvest loss





# Value added products from locally grown ginger: selection of suitable variety, maturity & location

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Ginger, Zingiber officinale is a unique spice which is used in fresh, preserved & dry forms. Ginger rhizomes impregnated with sucrose and glucose by an osmotic process and subsequently dried, coated with a thin transparent coating of icing sugar or coated with crystals of sugar (sucrose) are called candied, glazed or crystallized ginger. The specific objective of this study was to identify the most suitable variety, location and the stage of harvesting ginger for processing.

Ginger rhizomes were harvested between five to ten months from rhizome initiation and three varieties namely Chinese, Rangoon and Cochin (Local) in two locations (Alawwa & Makandura) were selected. In the preparation of pickled and glazed or crystallized ginger, the pieces of ginger are boiled and immersed in a series of sugar solutions with Brix range of 25° to 75° at pH 3.5-4.0 in the air tight containers at room temperature for 5 days. The final products were obtained by adjusting the required levels of Brix and pH of the sugar solutions.

Sensory evaluation results of the final products indicated that the appearance, taste/ flavor and the texture was significantly better (p < 0.05) in Rangoon variety harvested at the six months stage. The overall acceptability was almost same in the final products of the ginger obtained from Alawwa & Makandura.

Water activity (a<sub>w</sub>) of Rangoon variety ginger preserve and ginger crystals of the six months stage of maturity harvested from Alawwa & Makandura were between 0.60- 0.75. Results of the total plate counts and yeast and mould counts of the final products revealed that it is microbiological acceptable.

Key words: Osmotic dehydration, ginger, Chinese variety, Rangoon and cochin varietie

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# Study on underutilized fish species *Sardinella longiceps* (Indian oil sardines) with special reference to nutritional value

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Most of the underutilized fish species available in the sea around the country are used as fish baits and production of fish meals. Large quantities of these species are caught as by-catch of edible fish species. *Sardinella longiceps* which is also known as Indian oil sardines is one of the main by-catches of trammel net, trawlers and seines. *Sardinella longiceps* is rich in omega -3-fatty acids (n-3). Due to high fat content of this fish, palatability for human consumption is very low. Therefore, this study was conducted to develop an omega-3-fatty acid rich food product from this species by enhancing the palatability. Thereby, to increase the economical values and the demand of the Indian oil sardines.

Three processing techniques such as cooking, frying and pressure cooking were used to preprocess the sardine. The method of pressure cooking was selected as the best processing method and pressure cooked sardine was used for the product development. Sardine incorporated fish burger and snack was developed and quality analyses such as nutritional composition, sensory and microbial quality were tested.

Processing of raw sardines with the high pressure and control acidity gave the best performance to the product development. Process sardines were used to make fish burgers and fish incorporated extruded snacks. However, results of sensory attributes and nutritional composition of both products revealed that the fish snack has to incorporate low percentage of fish than fish burger. The amount of enriched omega -3-fatty acids is also high in fish burger as the amount of fish in the burger is comparatively high. In addition, fish burger can be eaten as a food in main meal while initial cost of production is also comparatively low. Present results confirmed that this underutilized fish species could be use to develop an omega-3-rich fish burger with a commercial value.

**Key words:** omega-3-Fatty acids, *Sadine Sp*,Fish burgers

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# Anti-diabetic properties of some traditional red rice (*Oryza sativa* L.) varieties of Sri Lanka

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Rice is the staple food in Sri Lanka and rice cultivation in the country has a documented history of more than 2300 years. The country holds thousands of different traditional rice varieties which had been in the diet for centuries and reported to have a wide variety of health benefits in the traditional knowledge of Sri Lanka. However, no comprehensive studies have been conducted in the country to date to investigate the bioactivities of these varieties. Present study evaluates the anti-diabetic properties of some traditional rice varieties. Masuran, Dik Wee, Goda Heeneti and Sudu Heeneti traditional red rice varieties were used in this study. Freeze-dried 70 % rice bran ethanolic extracts were investigated for anti-amylase (AA, n=6), anti-glycation (AG, n=6), glycation reversing (GR, n=6), acetyl-cholinesterase (Ach, n=4) and butyryl-cholinesterase (Bch, n=4) inhibitory activities in vitro. These varieties were also studied for in vitro starch digestion rate (indication of glycemic index) using in vitro dialysis method (n=4). The results demonstrated significant (P<0.05) and dose dependent AA, AG, GR, Ach and Bch inhibitory activities. IC<sub>50</sub> for AA, AG, Ach, Bch and EC<sub>50</sub> for GR among selected varieties were ranged from  $58.28 \pm 3.17 - 102.65 \pm 7.01$ ,  $4.92 \pm 0.24 - 12.06 \pm 0.36$ ,  $37.00 \pm 0.68 - 291.00 \pm 3.54$ ,  $18.50 \pm 0.60 - 96.60 \pm 0.56$  and  $44.25 \pm 0.62 - 82.66 \pm 3.34 \mu g/ml$  respectively. Rice bran of Masuran showed the highest AA and AG activities whereas brans of Sudu Heeneti exhibited the highest GR, Ach and Bch inhibitory activities. The results of in vitro starch digestion rate showed Sudu Heeneti, Masuran and Dik Wee had low in vitro starch digestion rate while Goda Heeneti had intermediate digestion rate compared to white bread as the reference. In conclusion, these selected Sri Lankan traditional red rice will be good natural sources for applications in management of diabetes and related complications. Further, this is the first report of AG and GR for any rice variety worldwide and AA, Ach, Bch inhibitory activity and in vitro starch digestion rate for Sri Lankan rice varieties.

**Key words:** Anti-diabetic, Sri Lankan traditional rice, red rice bran

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# Antibacterial activity of decoction and methanolic extract of leaf of *Myristica fragrans* against MRSA

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The Myristica fragrans (English: nutmeg; Sinhala: sathikka; Tamil: sathikkai) is an evergreen tree, belonging to the family Myristicaceae. In Traditional medicine (Siddha medical system) the leaves are used as one of the ingredients of Parankikilangu choornam, Periyapatpam, Vellaruku patpam, Astabirava kulikai, Thankaellathi mathirai, kakkuvan lehiyam, Impooral lehiyam, Karisalai lehiyam and Brinhamila thylam. These medicines are used to treat skin diseases, respiratory diseases and arthritis. Very few studies have been carried out on the leaves of M. fragrans when compared to the seed and mace of this plant. Antimicrobial activity of common bacteria has been carried out but no report on the activity against Methicillin Resistant Staphylococcus aureus (MRSA). The decoction and methanolic extracts were screened against six bacterial isolates (Staphylococcus aureus - NCTC 6571, and five wild strains of Methicillin resistant Staphylococcus aureus (MRSA)). The aim of the study is to determine the antibacterial activity and Minimum inhibitory concentration (MIC) of decoction and methanolic extract of leaf of M. fragrans against these pathogens. The plant leaves were collected from Kandy, cleaned and ground to a coarse powder to prepare the decoction (40 g of coarsely powdered leaves boiled in 480 ml distilled water until the volume was reduced to 60 ml and further reduced to 30 ml using a reduced flame). Methanolic extract was obtained using Soxhlet extractor. Screening of the antibacterial activity of decoction and methanolic extract was performed by the cut well diffusion using Mueller – Hinton Agar (MHA). The MIC was detected using Agar dilution method. The mean ± SD of the diameter of inhibition zone of decoction against S. aureus NCTC 6571  $(18.6 \pm 0.5 \text{ mm})$  and all 5 MRSA (range  $16.0 \pm 0.0$  to  $18.0 \pm 0.8$  mm) was almost equal to the diameter of the inhibition zone of methanolic extract against these organisms (range from 18.0 -19.0 mm). In agar dilution method this decoction showed activity against S. aureus NCTC 6571 and 5 MRSA in 1/10 dilutions. MIC of the methanolic extract for S aureus NCTC 6571 was 1.0 mg/ml. These values are similar to all tested 5 MRSA strains (1 mg/ml). In order to check the active ingredients phytochemical screening was carried out for leaves and the results revealed the presence of alkaloids, tannins, steroids, flavonoids, glycosides and triterpenoids in this plant. The ability of the decoction and methanolic extract of M. fragrans leaf to inhibit the growth of bacteria is an indication of its antibacterial potential which may be employed in the management of bacterial infections.

**Key words:** Antibacterial activity, MIC, *M. fragrans l*eaf, decoction, methanolic extract. selvaluxmy@iti.lk

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# Acute and sub – chronic effects of Trichosanthes cucumerina Linn. hot water extract and its fractions on blood glucose levels in Type 1 diabetic rats

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Diabetes Mellitus is a chronic metabolic disorder affecting approximately 4% population worldwide and is expected to increase by 5.4 % in 2025. Trichosanthes cucumerina Linn (Family: Cucurbitaceae) is one of the major ingredient in several polyherbal preparations that are prescribed to diabetic patients. However, the antidiabetic potential of T. cucumerina grown in Sri Lanka has not been validated by scientifically controlled investigations. Therefore, the aim of this study was to investigate the antidiabetic potential of hot water extract (HWE) and its fractions of T. cucumerina aerial parts, using streptozotocin (STZ) – induced Type 1 diabetic rats.

Acute and sub acute (28 days) antidiabetic effects of HWE and its fractions [pet – ether fraction (PEF), ethyl acetate fraction (EAF), methanol fraction (MEF) and remaining aqueous fraction (AQF) obtained by sequential fractionation of the HWE] were evaluated using 750 mg/kg and 75 mg/kg doses respectively. Glibenclamide (0.6 mg/kg) was used as the reference drug. Rats in control groups were received 1 mL of distilled water or 1 mL of Tween 80 (1% in distilled water) respectively.

There was no immediate antidiabetic effect observed either with the HWE or glibenclamide. However, with continuous administration of the HWE there was a gradual reduction in the fasting blood glucose levels observed after 28 days of treatment (by 57 % on day 14 and on day 28 by 64 %). Similarly, with continuous administration of glibenclamide, there was a gradual reduction in the fasting blood glucose levels observed after 28 days of treatment (by 59 % on day 14 and on day 28 by 64 %). A drastic body weight drop was observed in diabetic controls from end of the 3rd week while the HWE and glibenclamide treated rats significantly gained body weight from end of the 2nd week compared to the initial body weights of these rats. Similarly, there was a gradual reduction in the fasting blood glucose levels of EAF, MEF and AQF treated rats compared to the respective control groups. Apart from the EAF, other two fractions caused a significant reduction in plasma glucose levels accompanied by a significant increase in insulin levels of these rats. However, EAF caused a significant reduction in plasma glucose levels only without affecting the serum insulin level. Among the tested fractions, AQF showed the maximum reduction in plasma glucose levels (on day 14 by 25% and on day 28 by 64%) in STZ – induced Type 1 diabetic rats with highest increase in insulin level. In conclusion, these findings rationalize the traditional usage of T.cucumerina as an antidiabetic agent

**Key words:** Trichosanthes cucumerina Linn, diabetes, blood glucose

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### Antioxidant and low glycemic properties of *Caryota urens* L. (Kithul) treacle.

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Palms, a large group of monocot plants including about 2500 species belonging to family Arecaceae are predominantly found in South American, African and Asian regions. However, comparatively more palm species are still left unused or underutilized due to their highly limited uses at certain geographic and ethnology boundaries. Therefore, scientific investigation into the traditional palm based foods is important to introduce them into modern market as functional foods. *Caryota urens* L. (Kithul) has been tapped for its sweet phloem sap for many centuries and concentrated syrup has been used as a traditional sweetener in Sri Lanka. "Kithul treacle" is traditionally claimed as health food but no scientific studies are reported. Therefore, in this study, antioxidant and low glycemic activities of *Caryota urens* treacle were studied using *in vitro* assays and animal models in *vivo*.

Freeze dried authentic Kithul treacle samples (12) were used for *in vitro* antioxidant assays. Fresh authentic Kithul treacle was used in animal experiments as treatment and artificial treacle prepared in the laboratory with same sugar composition as in treacle was used as control. Antioxidant activities was estimated using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS<sup>+</sup>) radical scavenging assays, ferric reducing antioxidant power assay and ferrous ion chelating assay. Two groups of male Wister rats (8 rats/group) were fed with Kithul treacle and artificial treacle added diet separately for 28 consecutive days and antioxidant activity of blood serum was determined.

Three groups (7 rats/group) of overnight fasted (16 h) Male Wister rats (250 to 275 g) were orally administered with Kithul treacle and the other three groups with artificial treacle at three different loads (2.75, 3.75 and 4.75 g/ kg body wt) using 50 % (w/v) solutions in saline. In other experiment, two groups of rats were deprived from food immediately before they were orally administered with Kithul treacle and artificial treacle at a load of 2.75 g/kg body wt. Serum blood glucose levels were recorded immediately before (0 min.) and 30, 60 and 120 min. after oral administration.

Trolox Equivalent Antioxidant Concentration values of Kithul treacle for DPPH, ABTS and FRAP assays were 0.15 $\pm$ 0.01, 0.58 $\pm$ 0.07 and 2.65 $\pm$ 0.22 mmole Trolox Equivalents (TE) per 100 g of Kithul treacle. Also it showed metal ion chelating activity (IC<sub>50</sub> was 19.60  $\pm$  3.90 mg/ml). In rats, serum antioxidant activity of Kithul treacle supplied group (3.70 $\pm$ 0.13 mmole TE/l) was significantly (P<0.05) higher than control (2.91  $\pm$ 0.16 mmole TE/l).

Incremental Area Under the Curve (IAUC) of glycemic response curves of Kithul treacle fed groups at 2.75, 3.75 and 4.75 g/Kg body wt. loads were 23.7±5.0, 39.1±1.2 and 59.7±2.8 mg\*min./dl respectively whereas artificial treacle fed group showed significantly higher respective IAUC values (43.9±4.0, 54.4±2.6 and 71.9±2.2 mg\*min./dl). Under non-fasted condition at 2.75 g/kg body wt load, Kithul treacle showed significantly lower IAUC (15.7±1.5 mg\*min./dl) compared to control (29.2±4.6 mg\*min./dl). Kithul treacle has showed marked (46 %) reduction in glycemic response at 2.75 g/kg body wt. load at both fasting and non-fasting. 2.75 mg/kg body wt. load was a equivalent of average human consumption of Kithul treacle (15 to 20 g/serving) in Sri Lanka.

Kithul treacle showed antioxidant activity at both *in vitro* and in *vivo* and low glycemic response at both fed and fasted blood glucose levels supporting traditional claims and potential of Kithul treacle as a bioactive natural sweetener at global level.

**Key words**: Caryota urens L., bioactivity, antioxidant

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### Biobutanol as an alternative biofuel to cater the energy demand in Sri Lanka

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In recent years, crude oil prices have been rising overall and have experienced sustained levels of higher prices. In addition, burning of fossil resources leads to an increase in greenhouse gas emissions, with a dramatic impact on the world's climate change with global warming. Thus, alternative fuels are required, as mobility is a major part of the world's energy system. Among several liquid alternative fuels, Biobutanol has shown great promise because of its very similar properties to gasoline. Up to date no reports on any research work yet implemented to produce Biobutanol using agricultural by products in Sri Lanka: Therefore this review provides an overview of research activities on other countries in acetone-butanol-ethanol (ABE) fermentation over the past two and a half decades and the possibility to produce alcoholic biofuels mainly the Biobutanol as an alternative to fulfill the current energy demand in Sri Lanka. The possibility and the importance of agricultural by products such as rice straw as the lignocellulosic biomass which can be used as the sustainable feedstock for the Biobutanol production is addressed here. The Anaerobic fermentation of rice straw hydrolyzate using Clostridium acetobutylicum ATCC 824 has been found to be highly predominant culture strains in different fermentation methods for the Biobutanol production by most other researchers. For the product recovery, new and efficient processes have been developed for in situ removal and recovery of the ABE solvents by different research groups. The higher solvents yield and significant sugar utilization makes rice straw a potential feedstock for biofuels production in Sri Lanka. Research efforts in science, engineering, and economics of ABE fermentation by different research groups have brought biobutanol close to commercialization as liquid alternate fuel to date in the world. Now it is the time Biobutanol has to make a promising role in the Sri Lankan scenario for an alternative biofuel to cater the energy demand.

**Key wards:** Biobutanol, Rice Straw Hydrolyzate, Lignocelluloses, ABE fermentation *Clostridium acetobutylicum* 

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# Ethnopharmacological survey on raw materials used in traditional systems of medicine in Sri Lanka: A case study

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Demand for herbal products worldwide has being increasing at an annual rate of 8%. The estimated value of the global herbal market in the year 2050 will US\$ 5 trillion by the year 2050. In the present survey, information on raw materials, source of materials, plant parts used, heavily used and rare materials, quality of materials, locally cultivable plant species, government involvement, problems faced and potential helpful organizations were investigated by using pretested questionnaire. Diverse range of materials including 297 species (65%) of dried plant materials, 59 (13 %) fresh plant materials, 69 (15 %) types of minerals, 18 (4 %) animal materials and 12 (3 %) other materials have been listed. Altogether 309 plant species belonging to 106 families dominated by family Leguminosae were found and out of them 46 heavily used (more than 10,000 kg/year) belonging to 35 families were recorded. The main source of herbal material was local origin (71%), followed by imported (26%) while 3 % was obtained either direct importation or from local collection. Leaves were the most frequently used plant part (47 %). High price, poor quality, absence of continuous supply and insufficient supply, adulteration, poor processing techniques, high amount of impurities, wetness and microbial content have been identified as main constraints Decreasing of available medicinal plants, lack of proper cultivation and processing protocols, incorrect identification and lack of proper quality control methodologies were identified as major challenges in the industry. Implementation of proper programmes for identification, cultivation and postharvest techniques including quality control and standardization of herbs are very important while focusing much attention on research and development programmes were suggested. Information generated through the present study could be incorporated for the future development programmes related to medicinal plant industry.

**Keywords**: Ethnopharmacological survey, Medicinal materials, Sri Lanka, Traditional medicine dharma@iti.lk





# In vitro anti-diabetic properties of bark and leaf of Ceylon cinnamon (Cinnamomum zeylanicum Blume)

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Ceylon cinnamon (CC) (*Cinnamomum zeylanicum* Blume) known as 'true cinnamon' in the world has been used as a spice in Sri Lanka for centuries. Cinnamon bark is reported to have many biological activities including some anti-diabetic properties (AD) worldwide. However, there are no conclusive evidence to confirm that these reported activities are from authenticated CC. Further, very limited research has been conducted worldwide on cinnamon leaf to date. Therefore, the present study evaluates the anti-diabetic properties of bark and leaf of Ceylon cinnamon *in vitro*.

Freeze dried ethanol and Dichloromethane: Methanol (DCM: M) bark and leaf extracts of CC were used in this study. AD properties were evaluated using *in vitro* anti-amylase (n =4), anti-glucosidase (n=4), anti-glycation (n=4) and glycation reversing (n=6) assays.

Both bark and leaf of CC had significant anti- amylase, anti-glycation and glycation reversing activities (p < 0.05). However, both extracts did not show anti-glucosidase activity (400  $\mu$ g/ml). Among the studied AD properties bark showed significantly high activities compared to leaf (p < 0.05). Further, ethanolic bark extract demonstrated significantly higher anti-amylase (IC<sub>50</sub>:215  $\pm$  18  $\mu$ g/ml) and anti-glycation (IC<sub>50</sub>:19.42 $\pm$  1.26  $\mu$ g/ml) activities than DCM:M extract. While DCM:M bark had higher glycation reversing activity (IC<sub>50</sub>:94.33  $\pm$  1.81  $\mu$ g/ml) (p < 0.05). Moreover, among the studied leaf extracts ethanolic extract showed significantly higher activities (p < 0.05) than the DCM:M extract for all the studied AD properties (IC <sub>50</sub> for anti-amylase, anti-glycation and glycation reversing: 943  $\pm$  48, 15.22  $\pm$  0.47 and 121.20  $\pm$  2.01  $\mu$ g/ml respectively).

In conclusion, this is the first Sri Lankan study to report AD properties of bark and leaf of authenticated CC collected from an original cultivation in southern Sri Lanka. Further, findings of this study indicate the potential of using Ceylon cinnamon bark and leaf in management of diabetes and related complications.

**Key words**: Ceylon cinnamon, Anti-diabetic properties, bark and leaf extracts gasp@iti.lk

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### Standardization of Asamodagam spirit from *Trachyspermum ammi*

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Asamodagam spirit is a herbal preparation which is given specially to children as a remedy for gastro-intestinal problems. The water distillate obtained by the hydrodistillation of *Trachyspermum ammi* (Family: Apiaceae; Sinhala name: Asamodagam; Tamil name: Omum; Sanskrit name: Ajamoda) fruits is used to prepare Asamodagam spirit. The main ingredient of Asamodagam spirit, thymol has antimicrobial and antiseptic properties and if it is taken in excess than the recommended dosage it ultimately affects the central nervous system. Some drug manufactures dissolve thymol crystals in water and market as "Asamodagam spirit" without proper quality control. The present study is focused on the development of a quality control system (quantification of thymol content, pH and specific gravity) for the market samples and the standardization of prepared natural Asamodagam spirit.

The quality control methods including quantification of thymol content by GC and TLC densitometric techniques, pH and specific gravity were carried out to analyze seven brands of market samples of Asamodagam spirit. Asamodagam seeds were purchased from a reliable herbal drug shop, washed well, dried and used for the preparation of standard Asamodagam spirit. The apparatus similar to Clevenger apparatus described in the Sri Lankan Ayurveda Pharmacopoeia was used. Asamodagam seeds (300 g) were soaked overnight in water (4.5 L) and hydrodistilled to obtain 2,25 L of distillate. After the oil separated the water distillate was used to prepare the Asamodagam spirit. The distillate was diluted with equal amount of water (1:1) to obtain the standard Asamodagam spirit. Thymol content, pH and specific gravity were obtained for the standard samples.

The thymol content in the market samples ranges from 300 - 1000 ppm; pH value: 6.4 - 7.0; and the specific gravity: 0.9998 - 0.9999 at room temperature. The standard Asamodagam spirit produced by similar apparatus as Clevenjeri apparatus contains thymol: 400- 650 ppm; pH: 6.6 - 7.0; and the specific gravity from 0.9999 - 1.0002 at room temperature. It was reported that the thymol ranges from 800-1000 ppm when it is produced using Nadiyanthra which is not commonly practised now. Since Asamodagam spirit is mainly consumed by children, preparation and standardization of good quality Asamodagam spirit will be helpful to the Asamodagam spirit manufacturers as well as to users.

**Keywords**: *Trachyspermum ammi,* GC, TLC, thymol, hydrodistillation

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### Compulsory Licensing Of Patents: Unnecessary Evil or Formidable Tool?

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A patent is a set of exclusive rights granted by the State to anyone who invents new and useful process, machine, article of manufacture or composition of matter, or new and useful improvement thereof, for a limited period of time in exchange for a public disclosure of the invention.

The policy in granting a Patent is also to encourage inventors and to ensure that the inventions are worked on a commercial scale and to the fullest extent that is reasonably practicable without undue delay.

Patents, although potentially providing barriers to the rest of the world, are not granted merely to enable patentees to enjoy a monopoly on their patents. They impose an obligation on a patentee to work the patent on a commercial scale and to its fullest extent which could be achieved through licensing and assigning of patents.

A Patent system will normally not contain anything that fetters a Patentee's power to act as a monopoly. Many counties have felt the urge to qualify the right of a Patent holder in the name of political objectives such as local working of the invention or the satisfaction of consumer demand. This is generally achieved through compulsory licensing, which can prevent a patentee from acting as the sole producer. In such a system the Patentee will be obliged to a face direct competition although he will receive a "reasonable" royalty from its sales.

Compulsory licence is an exception to rights created by a Patent, usually justified as an intervention by Government to correct a market failure or a perceived abuse of monopoly rights created by a Patent holder. The legal basis of compulsory licensing and developments under the Trade Related Intellectual Property Rights (TRIPS) Agreement, the issues under it, limitations to exploit it and the assistance it could provide developing countries will also be discussed in the paper. The rationale of compulsory licensing and an examination of how it mitigates, cures or recuperates the perils of anticompetitive activities is also discussed.

While Developed countries tend to focus more on efficient IP protection regimes and enjoy monopolies created by IP, the developing and least developed countries are sometimes faced with issues due to monopoly rights enjoyed by IP holders.

Compulsory licensing can be used effectively to handle most of the antitrust concerns of developing countries including excessive pricing, high royalty payments and unfair refusal to license patents.

**Key words:** Abuse, compulsory, exclusive, interest, monopoly, voluntary

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### Applicability of standard classification of corrosive environment for Engineering design

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The operating environment is the most important factor which determines the corrosion of materials. Therefore the service life of metal structures such as bridges, towers, buildings are determined by the nature of atmosphere and how the materials behave in these environments. Internationally researches have conducted great amount of studies to understand this phenomena and many approaches have been taken to understand the corrosivity of atmosphere. In many instances these researches have followed the mass loss variation method to evaluate the corrosivity of atmosphere which is exposing a test coupon to the atmosphere and determine the mass loss after a predetermined period of time. And it has considered the mass loss as an indication of corrosivity of atmosphere.

But in Engineering design the service life of a component is determined by the actual strength loss. Therefore it is important to understand whether mass loss represents actual strength loss or how the atmospheric variables effect on strength of material at each atmosphere. Therefore In this paper the authors discuss about behavior of metal under different atmospheric conditions by means of strength loss and mass loss.

**Key words:** Atmospheric corrosion, Corrosivity, Corrosion management

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# Comparison study of absorbent properties in banana pseudo stem fibers with commercial wood pulp for using in personal care products

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Currently in Sri Lanka, personal care products such as sanitary napkins, diapers and wet tissues are being produced using imported commercial wood pulp. The present research work was carried out to develop a process that utilizes the absorbent properties of banana fibers in personal care products. Suitability was studied in five common banana varieties ("Kolikuttu", "Alu Kesel", "Seeni Kesel", "Ambun" & "Ambul Kesel") in Sri Lanka. The process involved cutting, mechanical extraction, bleaching, washing and finally mechanical smoothing. Absorbing behavior in artificial blood and urine solutions were studied in comparison with commercial wood pulp which is currently being used in personal care products. Processed banana fibers had absorption properties ranging from 700-1200% and 700-950% as determined using the initial weight of fibers in artificial blood and artificial urine solutions respectively. The observations made in the study concluded that the absorption property of banana fiber was superior to the commercial wood pulp. The major advantages of this invention are having personal care products with underutilized biodegradable materials at low cost, saving foreign exchange by reducing imports, new job opportunities and farmers will be able to harvest the waste material in return for a profit.





### Estimation of standard absolute entropies, S°<sub>298</sub>, from formula mass and atomic number: Organic and inorganic liquid compounds

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For the first time, the general relationship(s) of formula mass (M) and atomic number (Z) to the standard absolute entropies ( $S^{\circ}_{298}$ ) of organic and inorganic liquid compounds has been found enabling approximate estimation of  $S^{\circ}_{298}$  of numerous compounds only knowing M and Z.

For compounds M < 100;

$$S_{298}^{\circ}(JK^{-1}mol^{-1}) \approx 4.184 \times 2 \times \left[\frac{M-Z}{logZ}\right]^{\frac{2Z}{M}}$$

For compounds M > 100:

$$S_{298}^{\circ}(J \ K^{-1} mol^{-1}) \approx 4.184 \times 10 \times \left[\frac{z}{log z}\right]^{\frac{1}{N}}$$

The results were compared with reference values of S°<sub>298</sub> appeared in literature using 226 data points indicating;

For organic liquid compounds M < 100;

Average unsigned % error = 10.9

For organic liquid compounds M = 100-200;

Average unsigned % error =12.1

For organic liquid compounds M > 200;

Average unsigned % error = 25.3

For inorganic liquid compounds, average unsigned % errors are 24.9, 11.5 and 4.0 for compounds with M < 100, M = 100-200 and M > 200 respectively

The advantages of this new method can be summarized as follows;

- \* This method appears to be used for wide range of compounds those are not covered by earlier reported correlation methods and group additivity methods, only by knowing M and Z, for approximate estimation of  $S^{\circ}_{298}$  values.
- \* For new, unstable or even hypothetical liquid compounds, we can utilize this procedure to predict  $S^{\circ}_{298}$  values as well as for liquid macro molecules and to couple with various software in order to predict values of  $S^{\circ}$  and  $G^{\circ}$ .

Example Polysorbate 80 (Tween 80), C<sub>64</sub>H<sub>124</sub>O<sub>26</sub>, (M=1310,Z=716), S<sup>o</sup><sub>298</sub>≈857 JK<sup>-1</sup>mol<sup>-1</sup>

Although some %errors reported above are >10%, the finding is still very important, as it simplifies present use of super computers with extreme high capacity that is essential to calculate standard absolute entropies for even moderately large molecules.

**Key words:** entropy, formula mass, atomic number, estimation

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### Purification of natural graphite of Sri Lanka utilizing acid treatments

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Sri Lanka has been well known for possessing a variety of economically useful minerals, which are mainly being exported as low priced raw materials although they play a major role in current manufacturing industry globally. Graphite is a mineral which has a vast potential in value addition. Purity levels of 90 and 99% graphite are in great demand for electro technical applications worldwide. As the quality of graphite from current market leaders of the graphite industry improves, the Sri Lankan graphite industry needs to add value to its material in order to remain competitive. There is a huge demand for high-purity graphite powders worldwide in many applications.

The present work focused on purification of natural graphite up to 99.00 % using different physical, chemical and thermal methods.

Graphite of 97% purity level was used in the study. Firstly graphite was analyzed for constituents. Acid treatment method was included with leaching, water washing and drying in the purification process. Ash content, Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction analysis (XRD), chemical analysis, and scanning electron microscopy (SEM) were used to characterize the purified samples.

Maximum purity level obtained presently is 98.7% by removal of Iron bearing compounds. XRD spectra showed that graphite from Bogala is highly crystalline and crystallinity remained unchanged during the purification process (Or after purification).FTIR spectra do not reveal considerable changes in functionalities upon acid treatment. Attempts are being made to further purification of graphite to higher levels using acid-base and/or acid mixtures.

**Key Words:** Graphite Purification, Characterization, acid treatment

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### The fluoride removal ability of proto type red clay pot-filters

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Excessive fluoride (F<sup>-</sup>) in drinking water should be removed, but simple, low cost methods of fluoride removal are not readily available. Red clay based pot-type filters are being used in some countries including Sri Lanka for the purification of bacteria contaminated water, but not scientifically proven for the partial defluoridation of drinking water. The present study examines the fluoride removal ability of proto type, red clay pot-filters. A series of pot-filters were made using red clay collected from the Western province of Sri Lanka and fired at different temperatures between 400 - 800 °C. The flow rates of all the filters except for those fired at approximately 600 °C, were low and were between 3-4 ml/ min. Though filters fired at 400 °C showed the maximum fluoride removal, the quality of the collected filtrates were poor. Temperatures over 700 °C caused a decline in defluoridation ability. According to the results, the performance of the filters fired at approximately 600 °C was superior in terms of flow rate, fluoride removal and the quality of the filtrates for the particular clay type used in this study. The filters could be used up to 6 filter cycles before regeneration was required. The findings indicate that at optimized conditions, the red clay pot-filters investigated in this study has the potential of being an inexpensive and simple method for partial defluoridation of drinking water.

Key words: fluoride removal, red clay, water filter





# Characterization of Graphene Synthesized from Microwave Assisted Thermal Methods Using Graphite Oxide Prepared from Sri Lanka Graphite

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Graphene (Gr), Graphite oxide (GO) and reduced Graphite Oxide (rGO) are highly value added products synthesized from graphite which are used in numerous high-end technical products. In this effort, GO was synthesized from the Sri Lanka graphite(obtained from Kahatagaha mine) using a laboratory modified version of the common Hummer method, from which Gr was synthesized using microwave assisted hydrothermal methods followed by heat-treatment in inert atmosphere. The prepared products (Gr, GO & rGO) were characterized using XRD and FTIR techniques. XRD shows the conversion of GO to rGO after the microwave treatment and to Gr after the heat-treatment of rGO (2θ peak of graphite oxide at about 11° and appearance of broad peak at about 26° characteristic for Gr). FTIR peaks of GO at around 3200 cm<sup>-1</sup> and 1700 cm<sup>-1</sup> are attributed to stretching of O–H and C=O in the structure respectively, and the disappearance of these peaks in the spectrum of the final product obtained from the microwave treatment of GO followed by heat-treatment indicates the conversion of GO into Gr.

**Keywords:** Graphene, Graphite oxide, Reduced graphite oxide, Microwave synthesis, Modified Hummer method, Value addition.





# Characterization of particle (powder) size of commercially available graphite powder on the improvement of the synthesis process of graphene oxide

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Value addition to graphite has been a prevailing interest among the researchers as of its remarkable properties. Graphene oxide (GO) and graphene are the value added products of very high importance in the present research and development of electrochemical applications. The most common synthesis method of graphene oxide and graphene is based on process of oxidation-reduction of graphite. Therefore, it is in paramount important to investigate to which extent the parent materials and the reaction conditions influence the synthesis process and the properties of the resulting products. In the present study we have tried to understand the effect of the particle size of graphite powder on the properties of graphene oxide. Two methods have been employed in reduction/dispersion of particle size, viz. ultra-sonication, and ball-milling. It has been observed that a considerable influence on the reduction of particle size of ball-milled graphite showed well reduced size than that of the ultra-sonication. The particle size (d50) of commercially available graphite powder is about 111 μm, whereas graphite powder ball-milled for at least 3 h is about 37 µm and the powder with ultra-sonication at least for 30 mins is about 53 μm. Therefore, based on the reduced particle size of the two methods, the ball-milling was chosen for the synthesis of graphene oxide. The properties were analyzed using Laserdiffraction technique, UV-Vis spectroscopy, XRD, SEM and FTIR methods. The absorption near UV range (~ 233 nm) and the oxygen functionalities (OH stretching, C-O etc) have increased in GO synthesized using ball-milled graphite powder than GO synthesized from commercial graphite. The intensity for characteristic graphite oxide peak at 20≈ 11° is increased in ballmilled graphite powder than GO synthesized from commercial graphite. The SEM images also showed the layer structure of the GO where, split graphene oxide layers are conspicuous from ball-milled graphite than that of commercial graphite. Our findings will have profound influence upon practical synthesis of graphite oxide and graphene for applications in electrochemical devices in the future.

**Keywords:** Particle size, graphene oxide (GO), graphene, ball-milling, UV-Vis, XRD, FTIR, SEM

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# Optimization of Process Parameters for Manufacturing Light Weight Thin-Walled Red Clay Decorative Articles

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Local red clay based ceramic industry in Sri Lanka is mainly based on manufacturing of potteries, bricks, roofing tiles and ceramic blocks. Main manufacturing technique of traditional red clay pottery items especially decorative article, is a hand throwing method. However In general, the quality of the red clay based ceramic products is often poor. Slip casting on the other hand is able to produce ceramic products that are superior in homogeneity, low in thickness (light weight) and attractive. The lack of knowledge in this new technique in fabrication of red clay products has kept ceramic ware manufacturers using obsolete techniques which consumes large amount of manpower and produce low quality products. Therefore by introducing slip casting method for red clay is a new product line for traditional red clay industry.

This research work describes controlling of parameters in manufacturing light weight thin-walled decorative articles made from red clay using slip casting process incorporating optimum conditions of body compositions and firing temperatures.

Biyagama red clay deposit was selected for this research study which is currently used for pottery industry. Clay bodies were synthesized from red clay mixed with quartz and feldspar. Body slip was prepared by wet grinding method employing ball mill and a mechanical stirrer. The test samples were cast using plaster of Paris moulds. The samples were fired at about 500°C.

It can be concluded that slip casting method could be effectively used as a fabrication technique for Biyagama red clay to manufacture a light weight thin walled (3mm) decorative articles.

**Key words:** Red clay, decorative articles, slip casting.

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# Measurement and evaluation of noise levels at the vicinity of ceramic factory: A case study

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A study was carried out to determine the existing noise levels in and around ceramic factories. A total of twelve factories were selected for this work. The different types of the ceramic industry encompass a wide range of raw materials and different manufacturing techniques but common process involved for ceramic sector. but all involve the different selection of clays or other mainly inorganic materials which are processed then dried and fired.

Noise generating in the several steps during the manufacturing process of ceramic products. Mainly environmental noise depend on following factors, location of the land, land area and machinery install locations. Identify effected noise souses are crusher plant, ball mill, spray dryer, generator, kiln, and blower area in the ceramic sector.

The study reveals that for noise levels at crusher plant, breaker and generator working area exceeded prescribed levels for WHO regulation based on 85 dB(A). In addition, very high noise levels were observed in the ball mill, dust plant, air compress, cutting machine, kiln and spray dryer area.

The noise levels exceeded 55dB (A) level for seven industries out of 12 measured ceramic industries (58.3 %) exceeded maximum permissible level at the boundaries. The study indicates the most of ceramic necessity for awareness, warning signs and enforcing existing noise regulations to uplift the current standards.

Key word: ceramic, noise, measurement

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# Predicted Noise levels and noise mapping for proposed Colombo Katunayake Expressway (CKE)

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The purpose of this noise map is to evaluate the potential traffic noise impacts to the residential at selected locations of the proposed Colombo Katunayake Expressway (CKE) and propose a suitable noise mitigation action in order to reduce the noise levels due to traffic volume.

Twenty five receiver points were selected along the CKE, When consider the noise levels without noise barriers at receiver points the predicted noise levels during the day time is less than the threshold value of 65dB(A) at 23 receiver points and during the night time is less than the threshold value of 55dB(A) at 15 receiver points. Considering the noise levels with noise barriers at the receiver points the predicted noise levels during the day time and night time is higher than the threshold value of 65dB(A) and 55dB(A) at one receiver point.

According to our calculation using noise modeling tool, the recommended noise barrier length is 834m and 800m length of vegetation belt.

**Key word:** noise mapping, noise barrier, predicted noise



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