Determination and characterization of microplastics in selected cosmetic products in Sri Lankan market

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Abstract

Microbeads are a type of primary microplastics, out of thousands of types of ingredients that are currently applied in personal care and cosmetic product (PCCP) formulations around the world. Non-degradable nature, hydrophobic behaviour and small size are the main problems associated with microbeads in the environment. The present study is aimed to determine and characterize the different types of microplastics present in the selected facial wash and facial scrubs like cosmetics available in local supermarkets. Ten samples (7 face wash and 3 face scrub samples) were purchased from leading supermarkets located in Colombo and the suburbs. Moreover, samples were categorized as 3 imported and 7 locally manufactured products. Water-insoluble microparticles were separated from other ingredients by filtration through Whatman No. 541 filter papers after dissolving in water at 45 ℃. The dry weight of the water-insoluble particles was determined with an analytical balance. The shape of the particles was defined using a compound light microscope. Determination and identification of microplastics were done using Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy and polymer libraries. Insoluble microparticles were found in all face wash samples except one (Fairness face wash) and all face scrub samples tested. Only the particle size ≥ 22 μm (pore size of Whatman No. 541 filter papers) could capture by this method. All the captured particles were ≥ 22 μm and < 850 μm in size. The quantity of microplastics presents in the imported products was ranged from 1.33± 0.42 to 133.44 ± 3.05 mg g^{-1} while in the locally manufactured products ranged from 1.88 ± 0.50 to 42.70 ± 0.04 mg/g (dry weight basis). The isolated particles were white, green, red, orange, blue, brown and grey in colour, while white was dominated. Seven samples contained more than one colour of microplastics. All the microplastics were spherical, granular or irregular in shape. Microplastics such as polyethylene, polypropylene, thermoplastic polyurethane, and high-density polyethylene (HDPE) were present in the tested samples. This study confirmed the presence of microplastics in face wash and face scrubs and indicates the urgent requirement of implementation and enforcement of regulatory mechanisms for banning the use of synthetic microbeads in the production of PCCP or replacing them with natural particulate matter.

Green synthesis of silver nanoparticles using post distillation water and residual Cinnamon waste

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Abstract

There is an increasing commercial demand for nanoparticles due to their wide applicability in various areas. The development of a reliable and eco-friendly process for the synthesis of metallic nanoparticles is an important step in the field of application of nanotechnology. As an alternative to physical and chemical approaches, renewable plant-mediated synthesis of nanoparticles offers enormous benefits. The present study emphasizes the feasibility of rapid green synthesis of silver nanoparticles (AgNPs) by employing plant waste; cinnamon hydrosol and freeze-dried cinnamon residual water which is discarded after cinnamon bark oil distillation at an industrial scale, without adding external surfactant and capping agent. The effect of the key factors governing the synthesis of AgNPs, including incubation temperature, reaction pH, different volumes of added cinnamon extract, concentration of silver nitrate, and reaction time was used to investigate. The bio reduced AgNPs were characterized by ultra violet -visible spectrophotometer (UV-Vis), X-ray diffraction (XRD), Fourier Transform Infrared spectroscopy (FTIR) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Aqueous silver (Ag^+) ions were reduced and formed silver nanoparticles with a maximum yield at the optimum condition; silver nitrate (1.0 mM, 10 ml), cinnamon extract volume (0.2 ml), pH (11.0), incubation temperature (80 °C) and incubation time (2 h). A visible color change was observed from faint yellow to brown, upon the bioreduction process. The UV–Vis spectrophotometric analysis revealed the characteristic SPR peak at λ_{max} 402 nm of AgNPs and the synthesized AgNPs were stable in the aqueous phase for a long period of about 120 days. The X-ray diffraction confirmed the crystalline nature of the biosynthesized AgNPs from the XRD spectrum which showed four intense diffraction peaks at scattering angles (2θ) of 37.9°, 44.2°, 64.5°, and 77.3° with an average crystalline size of 11.32 nm calculated by using Scherrer equation. The major phytochemicals contained in the waste cinnamon extracts; cinnamaldehyde, eugenol, cinnamyl acetate and other phenolic compounds were characterized by Gas Chromatography–Mass Spectrometry (GC-MS) and Folin-Ciocalteu methods. The FTIR spectrum of waste

cinnamon revealed the characteristic absorbance of the O–H groups at 3296.16 cm^{-1} indicating the presence of phenolic compounds and the other strong band at 1680.23, 1591.65, 1388.16, 1034.11 cm^{-1} which can be assigned to corresponding chemical bonds such as –C=O, C=C, –C–OH, –C–O–C in carboxyl, aromatic ring and ester compounds which are expected to be present in cinnamon bark. The FTIR spectrum of the green synthesized AgNPs showed a shift of the above-mentioned absorption bands and a new band was observed near 1143.98 nm wavelength which confirms the possible influence of phytochemicals for fabrication and stabilization of silver nanoparticles. The waste cinnamon extract acts as a good substitute for harmful reducing and stabilizing reagents such as tri-sodium citrate and sodium borohydride which is used for chemical synthesis of nanoparticles.

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Quality assurance and risk assessment in commercially available cosmetics products in Colombo *district*

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Abstract

The usage of personal care products was rapidly increased and the local market is crammed with numerous products. However, the quality and content of these products have generated issues in both consumer safety and the environment. The aim of the study was to investigate the selected chemical and microbiological parameters of branded cosmetic products of selected categories available in the Colombo district. Total of ninety samples of commercially available cosmetics products including both local (n=45) and imported (n=45) of five common brands of baby shampoo, baby bath, shampoo, conditioners, body wash, shower gel & shower cream were selected from supermarkets in Colombo suburbs and chemical and microbiological analysis were done based on SLS standards. There was an aerobic mesophilic bacterial growth in 70% of products and yeasts & molds growth in 66.7% of the total samples tested. Body wash had the highest percentage (80%) of microbial contamination whereas conditioners, baby baths and shower cream/ shower gel showed a subsequently high percentage of contamination (60%). However, these counts had not exceeded the specified microbiological limit of 1000 CFU/ml. Pathogenic organisms were rarely detected in samples meanwhile *Staphylococcus aureus* was present in one batch of shampoo and *Candida albicans* in two samples of baby products. *E. coli* and *Pseudomonas aeruginosa* were not detected in any tested sample. Anionic surfactant content of all hair and baby shampoo were conformed to the specifications in SLS 1346 and SLS 1342 (≥5% and ≥3% respectively) and in other products, detected anionic content was in the range of 2.2-12.8 % m/m. pH of all samples of baby and hair shampoo were within the limits specified in standards (pH 5-7 & 4-8 respectively) while baby bath, body wash, shower gel/cream and conditioner which were not specified in SLS standards had mean pH in the ranges of (4.7-6.2), (5.9-9.1), (5.6-7.2) and (3.4-5.5) respectively. It was observed that relatively higher variation (SD≥3.5) within test values of active ingredient content and pH among different batches of the same products (baby bath, body wash, shower gel/cream and conditioner) which are not included in the scope of the SLS specifications. Further, yeasts and molds counts were not specified in available hair shampoo and baby shampoo standards. Therefore, it is required to regulate the quality of those products considering public health.

Determination of two alcohol types in liquid hand sanitizers in Sri Lanka using rapid gas chromatographic-flame Ionization (GC-FID) technique

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Abstract

The new Covid-19 pandemic has created high concern among the public on human hands' hygiene more than ever considering the reduction in transmission of infections either by direct or indirect contacts. Alcohol-based hand sanitizers are considered more effective and become more popular than other hygiene products globally as well as in Sri Lanka. Two alcohols; ethanol and isopropyl alcohol (IPA, 2-propanol) are recommended to use in hand sanitizers in effective concentration by the World Health Organization, and Sri Lanka Standard for hand sanitizers (SLS1657:2020). Alcohol percentages are specified in liquid hand sanitizers as 75-85 v/v % for ethanol and 70-80 v/v % for IPA. Considering the new local start-ups to produce hand sanitizers, evaluation of the quality and efficacy of the product is highly required. Therefore, to contend with this new demand for chemical analysis, a study was designed with the objective of developing a rapid analysis method using gas chromatography-flame ionization detector (GC/FID) for commercial laboratories with minimum sample preparation steps and also to evaluate the compliance of alcohol content of final products with the SLS 1657 specification. The developed GC/FID method (capillary column, supercool) using n-propanol (1-propanol, sigma) as the internal standard was linear over the ranges of 0.02-90 % v/v and 0.05-90 % v/v for ethanol and IPA respectively with the r^2 of 0.999. The precision of the method was determined in three levels of 20, 50 and 90 % (v/v) for both alcohols and the percentage relative standard deviations (% RSD) were in the range of 0.70-2.61 and also recovery at three concentration levels were within the range of 99.3-100.5 %. The limit of quantification of the method for ethanol and IPA were 0.02 and 0.06 % (v/v), respectively. Liquid sanitizer samples (n=303) received to ITI from 51 different brands were used for the study and except for three samples which contained 1-propanol, all other samples have detected alcohol type as either ethanol or IPA. All sanitizers with ethanol, except 22 samples conformed to the SLS specification of (75-85 %). IPA content of 112 samples from a total of 153 samples complied with the required specifications (70-80 %) while 25 % of samples with IPA did not conform to the standard and also higher standard deviations (SD>5) of replicates was detected in four brands. The majority of samples (79 % of total samples tested) conformed to the required levels of alcohol content. Since a considerable amount (21%) of hand sanitizers were not conformed to the SLS specification, continuous quality evaluation is an essential requirement to ensure public health.

Degradation of glyphosate in different microbial and pH conditions

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Abstract

The environmental fate of amine salt of Glyphosate (GLY), the common form of widely used herbicide residues in nontargeted environmental compartments, is a complex process. This process contains mainly degradation, adsorption, percolation and desorption through the runoff. Amongst the degradation, microbes play a significant role. Aminomethylphosphonic acid (AMPA) is the major metabolite due to microbial degradation. Therefore, this study was planned to evaluate the effect of different microbial populations and its' density on degradation. Research was conducted at the Dehiaththakandiya MOH area. Altogether, 7 representative water samples were collected from open dug wells (1,2,3 and 6), community RO plant (4), river Mahaweli (5), and lake outlet of the Girigalla lake (7). The pH of the samples was measured onsite. All samples and an ultrapure water control sample were spiked at 30 mg/L of GLY. The samples were kept at room temperature (27 ± 2 °C) under scattered light conditions for 184 days. A validated liquid chromatographic and tandem mass spectrometric (LC-MS/MS) method with no derivatization was used to analyze the GLY and AMPA concentrations in water. The Aerobic plate count (APC) was obtained to calculate the density of the bacteria present in samples along with the residual GLY. The pH of the samples was varied from 6.25 to 8.68 while the highest pH was reported from sample 5 (8.68). Initial APC of sample 4 and the control reported the lowest ($6.65e^2$, $4.30\ e^2$ CFU respectively), and sample 1 had the highest ($1.57e^4$CFU) followed by sample 3($1.17e^4$CFU) and 6 ($1.36e^3$CFU). Continuous growth in APC was observed in samples 6, 3 and 7, with the highest APC in sample 6 ($2.40e^7$CFU) at the end of the study, followed by samples 3 (2.06 e^7CFU) and 7 (7.68 e^6CFU). The reduction of the initial concentration of GLY was insignificant ($p > 0.05$) in sample 4 (27.9 mg/L) compared to the control (29.0 mg/L). While the highest decline was observed in sample 7 (8.2 mg/L) demonstrating a significant reduction with a $p < 0.05$ compared to the control. The remaining samples demonstrated an approximately similar slower reduction. In contrast, the formation of

AMPA initially demonstrated an inconsistency that changed to a continuous increment in the later stage representing the continuous degradation of GLY. In this study, a significant effect from pH and on the degradation of GLY was not observed. Further, a significant difference ($p<0.05$) in the degradation of GLY with the initial density of microbes also was not observed. However, a significant difference in degradation of GLY in six samples except sample 4 was observed compared to the control. Therefore, different types of microorganisms common to water play a major role in the degradation of GLY with different contributions.

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Study on edible frying oil quality during industrial scale open pan batch frying

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Abstract

The present study was carried out to test the quality of cooking oil in repeated frying cycles in order to identify the possible harmful compounds. Three oil samplings were carried out at export and supermarket-oriented food frying companies to identify the changes in edible frying oil quality during intra-day repeated frying cycles. Optimum frying temperatures used by the food processors varied between 165 ^{o}C -185 ^{o}C for blended palm olein and 145 ^{o}C for refined-bleached-deodorized (RBD) palm olein. With blended palm olein up to 6-8 repeated cycles of frying were carried out with sliced onion as the food matrix in locations 1 and 2 and with RBD palm olein up to 10 cycles in location 3 with the same food matrix. The type of frying oil used were identified based on the label information as well as through GC-FID fatty acid composition analysis. Each sample of oil was tested against 10 analytical measurements; peroxide value (PV), iodine value (IV), moisture and volatile matter, free fatty acid content (FFA), saponification value, unsaponifiable matter, viscosity, Lovibond color (red/yellow), *p*-anisidine value (AV), thiobarbeturic acid value (TBA), and *trans*-fat content in the fatty acid profile. Frying oil quality was evaluated against Codex CXS 210-1999 reference values and regulatory limits given in Food Act No.26 of Sri Lanka for respective oil variety (palm, coconut) and oil type (refined, crude) for PV, moisture and volatile matter, IV, FFA content, saponification value, unsaponifiable matter and *trans*-fatty acid content. Analytical parameters such as AV, TBA value, viscosity, Lovibond color (red/yellow) and total oxidation products (TOTOX value) with no standard reference values were used to further visualize the physico-chemical changes in frying oil quality. According to the tested parameters in all samples collected from 3 locations, results were found to be within the reference and regulatory limits except PV value. PV too had not shown significant variation compared to the reference values except in 7th and 10th frying in Location 2. Furthermore, *trans*-PUFA isomers C18:2(c9, t12) and C18:2(t9, c12) present in fried oils did not change significantly from the initial amounts. In summary, in all settings under investigation, no undesirable oil quality degradation was observed. Thus, Palm oil and blended palm-coconut oil, the most commonly used frying oil for commercial purposes in Sri Lanka found to be providing desirable frying conditions during open pan intra-day batch frying up to 10 frying cycles when used with less complex food matrices at temperatures close to smoke point.

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Preliminary survey on the level of histamine in salted dry fish and Maldive fish in Sri Lankan retail market

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Abstract

Histamine is a biogenic amine involved in immune responses in animal tissues. Exposure to a high level of histamine may trigger inflammatory responses. Histamine can be found in fish tissues resulting from the breakdown of histidine during the putrefaction caused by a lack of proper sanitation and storage facilities. Scombroid fish contains a high level of histidine; consequently, a high level of histamine may be found in fresh or processed food. The salted, dried fish (SDF) and Maldive fish (MF) products are popular island-wide due to their long shelf-life and the ability to store under non-freezing storage conditions. In Sri Lanka, "Katta"/double-spotted queenfish, "balaya"/skipjack tuna, "keeramin"/smooth-belly sardinella and "thalapath"/sail fish are leading marine SDF varieties used other than the MF. Considering the inconsistency in the putrefaction in fish tissues, Sri Lanka Standard specification (SLS 643:2007) declares a maximum permitted limit (MPL) of 100 mg/kg for SDF while an MPL of 200 mg/kg of histamine has been declared by SLS 811:1988 for MF, respectively. This study presents the histamine levels observed in 120 samples of SDF (Queenfish n=26, sprats n=30, sardinella n=11, Tuna n=10, sailfish n=10) and MF(n=33) samples collected during the period from 2019 to 2021. A validated, solvent-liquid extraction method followed by Liquid Chromatography-Tandem Mass Spectrometric (LC/MS-MS) analysis was used in this study. Approximately 88% of Queenfish, 80% of Sprats and 73% of Sardinella samples lower than the MPL. In comparison, approximately 50% of the sailfish had histamine levels greater than 100 mg/kg. The average value of histamine in sprat and queenfish fish were 53 mg/kg and 38 mg/kg, while tuna and sailfish had average histamine levels greater than 200 mg/kg. The small size of the fish may facilitate faster drying leading to a low level of histamine in the final product. Despite the peppery flavour and higher potential of causing food allergies, "tuna" has the second-highest consumption rate in Sri Lanka, subsequent to the "sprats". In this study, in 80% of the "tuna", which is a scombrid fish, the histamine level was higher than 100 mg/kg, while 60% of "the tuna" samples had histamine levels greater than 200 mg/kg. Even though "tuna" is used in MF production, only 3% of the

MF samples were above the regulatory limits. This study observed a significant difference ($p<0.05$) on histamine in scombroid SDF and non-scombroid SDF. The histamine level in 81% of SDF of non-scombroid fish was less than the SLS limit, and in contrast, 80% of the scombroid SDF reported histamine above the SLS limit, making SDF of non-scombroid fish safer for consumption.

Validation of a modified QuEChERS method for analysis of 2-methyl-4-chlorophenoxyacetic acid (MCPA) residues in tea using Liquid Chromatography - Tandem Mass Spectrometry (LC-MS/MS)

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Abstract

Sri Lanka is one of the top-ranked countries among quality tea producers and exporters to the world tea market. 2-methyl-4-chlorophenoxyacetic acid (MCPA) is a selective herbicide which is frequently used in the local tea industry. Internationally, a maximum residue level (MRL) in the range from 0.01 – 0.10 mg/kg has been declared for MCPA in tea. With the detection of MCPA residues above the MRL, the local tea industry was faced with the challenge to produce quality tea free from MCPA. Therefore, there was an urgent requirement of the establishment of an accurate and precise method to determine MCPA in tea. Thus, in the current study attempts were made to establish an accurate, precise and validated method for quantification of MCPA residues in tea using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The MCPA residues in tea were extracted using the modified quick, easy, cheap, effective, rugged and safe (QuEChERS) sample extraction approach with acetonitrile containing citrate buffers. The clean-up of the extract was carried out using a combination of primary secondary amines (PSA), C18 and graphitized carbon black (GCB). The detection was performed using LC-MS/MS with electron spray ionization (ESI) in negative polarity. Chromatographic separation was achieved on a synergy 4u Fusion column (50 mm x 2.00 mm x 3 µm) using gradient elution. The validated method had a wide linear working range of 0.005 - 0.250 mg/kg with a correlation coefficient (R^2) of 0.996. The precision evaluated in terms of repeatability and reproducibility expressed as percentage relative standard deviation (%RSD) at three different fortified levels (low, mid and high) was below 20%. Recovery percentages indicated that the validated method is accurate over the entire working range and were 112% for low, 106% for mid, and 115% for high concentration levels respectively. Selectivity of the method was achieved using the mass library comparison of samples against the reference standards. The validated method indicated high sensitivity with a limit of detection (LOD) and limit of quantification (LOQ) values of 0.002 mg kg^{-1} and 0.005 mg/kg respectively. MCPA analyzed using the validated method which included more than 500 number of tea samples indicated that locally produced tea contained MCPA in the range 0.005 – 0.020 mg/kg. The performance

characteristics of the method complied with the international method validation guideline requirements available for pesticide residue analysis. This indicated that the validated method is accurate and precise and can successfully be applied for quantitative analysis of MCPA residues in tea.

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Volatiles and organic acid profiles to use as a chemometric tool to differentiate natural and synthetic coconut (*Cocos nucifera* L) toddy

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Abstract

Toddy is an oyster white, translucent alcoholic drink produced from the sap obtained from various species of palms around the world, including Sri Lanka. The inflorescence of the palm is cut to collect the fresh sap and left to ferment by microbes such as airborne wild yeast and inoculums of previous batches. The resultant beverage is consumed as a drink or used to produce other alcoholic drinks in large quantities. However, there is no scientific method to distinguish between natural and synthetic toddy. Therefore, the objective of this study is to develop a chemometric method using volatiles and organic acid profiles to differentiate natural and synthetic toddy. Ten samples of fully fermented sap of coconut (*Cocos nucifera)* from tapping trees in Kalutara district and 5 bottled toddy market samples were collected under the supervision of the Excise Department. Fifty milliliters of each sample was extracted into 50 ml of ethyl acetate by liquid-liquid partitioning and concentrated into dryness by rotary evaporation. The dried sample was washed with n-hexane, filtered and used for the determination of volatile compounds using Gas Chromatography-Mass Spectrometry (GC-MS, Thermoscientific Trace 1300) with fused silica capillary column (Agilant DB-wax). The residue of the samples was dissolved in water and used to determine organic acid profile using Ultra-High-Performance Liquid Chromatography (UHPLC, Agilent 1260 infinity II) with C18 column (4.6 x 150 mm, 5 µm). All samples were replicated two times. Overall, 109 volatile compounds were detected from both natural and bottled toddy market samples. Following the Principal Component Analysis (PCA), illustrated with clarity, significant grouping of toddy samples collected from tapping sites and bottled toddy indicated significant deviations in volatile compound composition. The best fitted PCA model gave 67% from Principle Component-1 and 16 % from Principle Component-2 in the score plot. According to the analysis Hydrazinecarboxylic acid- phenylmethyl ester, Ethyl hydrogen succinate, 2,4-Di-tert-butylphenol, 1-Hexadecanol and 2-Hexadecanol were the key volatile compounds that contributed to differentiate the two types of coconut toddy samples. UHPLC analysis showed overall 69 separated compounds and according to PCA, clear clustering of site collected and bottled toddy were observed. This study confirmed the usability of volatiles and organic acid profiles of coconut toddy as a chemometric tool to differentiate natural and synthetic toddy. It is recommended to include additional samples representing different locations as well as authentic synthetic samples to improve this model. The study will continue to meet these requirements.

Determination of optimum tempering period of matured coconut to extract coconut water for bottling

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Abstract

Coconut water was recognized as a naturally healthy and isotonic beverage with the same level of electrolyte equilibrium that can be found in human blood. The major properties of isotonic beverages are higher levels of potassium and sugar with a low level of fat. Since coconut water bottling is mainly carried out as a by-product, not much attention is given to the quality of coconut water at the time of extraction. This study was mainly aimed to determine the optimum tempering period of plucked matured coconut to extract coconut water for bottling and to determine the alterations of physical and chemical parameters of coconut water and kernel during the tempering period. Fully matured coconut (12 months) samples from the "Sri Lankan Tall" variety, were collected from the Coconut Research Institute, Lunuwila, Sri Lanka and were analyzed in triplicates for selected chemical parameters for a period of 22 days. In the present study, pH value, electrical conductivity, fat content (%), protein content (%), potassium content (ppm), sugar content (fructose, glucose, sucrose), and Brix value were determined in the coconut water. According to the results, the highest levels of sugar and potassium along with the lowest level of fat were found between the 8th and 10th day of tempering period in the coconut water. The fat level is in compliance with the SLS 98:2013 (Specification for Desiccated Coconut) between the 8th and 10th day of tempering, which encourages the desiccated coconut producers to engage in desiccated coconut (DC) production and bottling of coconut water simultaneously, without holding the DC production for 21 tempering days. An increase in sucrose content was observed in both kernel and water, after the 8th day of tempering. The variation in potassium content (ppm) and electrical conductivity (µS cm^{-1}) showed similar patterns over the tempering days. The pH value, electrical conductivity and protein content in both coconut kernel and coconut water did not show significant variations ($p > 0.05$) throughout the tempering period. In conclusion, between the 8th and 10th day of the tempering period was found to be the optimum time to extract coconut water from matured coconuts for bottling.

Contamination of aflatoxins B1, B2, G1, and G2 in crude and refined coconut oils in Sri Lanka by Liquid Chromatography and Tandem Mass Spectrometry- A preliminary approach

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Abstract

Aflatoxins (Aflatoxin B1, B2, G1 and G2) are toxic secondary metabolites produced by certain fungi of the genus *Aspergillus*, which has a predominant growth on cereals legumes tree nuts; when the conditions are favorable for fungal growth. Coconut oil is predominantly used in Sri Lanka, mainly extracted from dried coconut kernels to cater to the domestic market. Therefore, if *Aspergillus* spp colonizes the coconut kernel, the extracted oil may be contaminated with Aflatoxins. International legislations on Aflatoxins have been imposed considering the toxicity and thermally stability to protect consumers. The maximum permissible levels (ML) of 5.0 µg/kg for aflatoxin B1 and 10.0 µg/kg for total aflatoxins in coconut oil has been declared in Sri Lanka Standards specifications for coconut oil; SLS 32:2017. This study was designed to analyze the contamination of Aflatoxins in 30 physically refined (n=6) and unrefined (n=24) from local and imported coconut oil samples collected from the retail market(n=9) and oil mils(n=21). The analysis was carried out with Liquid Chromatography-Tandem Mass Spectrometric (LC/MS-MS) system. All four aflatoxins were extracted with 80% acetonitrile, followed by Solid-Phase Extraction (SPE) cleanup before the LC-MS/MS analysis. Spike recovery studies assured the accuracy of the method, and matrix-matched calibration was used to address the matrix effect. The limit of determination of the method for all four Aflatoxins was 0.8 µg/kg, and spike recovery was greater than 60% for all four Aflatoxins in each sample batch. In the present study, oil extracted from locally dried kernels was considered "Local", and the oil refined from imported crude oil was considered "Imported". All local samples (n=8) were unrefined, and the Aflatoxin B1 and total Aflatoxins complied with the SLS MLs in 7 samples. Aflatoxin B1 greater than 5 µg/kg was detected in 27% (n=8) of all samples while 6 were unrefined and 2 were refined. Total aflatoxins greater than 10 µg/kg were detected only in 4 samples. All 4 samples were imported, while two were unrefined and two were refined. A significant difference ($p>0.05$) in the level of Aflatoxins between refined and unrefined samples could not be

observed; therefore, a comprehensive study is required to evaluate the level of contamination. Further, the fatty acid composition was studied in selected samples, and the compositions were matched with coconut oil. Moreover, the colour and the opacity of the oil were visually inspected, and a positive relationship could not be observed with the level of aflatoxins.

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Detection and quantification of mycotoxins in black tea by Liquid Chromatography- Tandem Mass Spectrometry

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Abstract

Tea products probably get contaminated with different toxigenic fungi via tea (*Camellia sinensis* L.) leaves and during the manufacturing process. Different types of mycotoxins can be produced by these organisms and it may remain in the final product although the fungus has already been destroyed during the process. This study was aimed for the detection and enumeration of seven different mycotoxins i.e. aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), ochratoxin A (OTA), fumonisin B1 (FB1) and fumonisin B2 (FB2) in black tea samples collected from different agro-climatic regions in Sri Lanka. Tea samples (100) were collected from 28 factories representing different tea grades namely dust, pekoe, OPA (orange pekoe A), BOP (broken orange pekoe) and BOPF (broken orange pekoe fannings). The method employed was solid-liquid extraction pursued via solid-phase purification and followed by Liquid Chromatography coupled with Tandem Mass Spectrometric analysis (LC-MS/MS). Since the regulatory Limits of determinations (LOD) of mycotoxins in tea has not been specified in Sri Lanka, the LOD of the method for each analyte was decided by considering the regulatory limits imposed for coffee by the European Union and the sensitivity of the analytes. The LOD values for both aflatoxins and ochratoxin were assigned as 4 µg/kg whereas FB1 and FB2 were assigned as 0.4 mg/kg and 0.6 mg/kg respectively. The accuracy of the method was assured by spike recovery values obtained for AFB1 (92.6 %), AFB2 (91.3%), AFG1 (81.8 %), AFG2 (83.2 %), OTA (97.2%), FB1 (82.3%) and FB2 (85.6%). This selected method is suitable for the detection and quantification of the above seven mycotoxins simultaneously. Although yeasts and moulds were present, the mycotoxins were not detected in any of the tested tea samples. Therefore, it was also concluded that the fungal communities present in tea samples were not composed of aflatoxigenic fungi.

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GC-MS characterization of oil of *Canarium zeylanicum* (Retz.) Blume (Kekune) bark

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Abstract

Sri Lanka is rich in biodiversity. The plants containing essential oils with specific chemicals show insect repellent effect and the fatty acids from the seeds are mainly used for the edible purpose and other uses. *Canarium zeylanicum* (Retz.) Blume is an endemic plant to Sri Lanka (Family: Burseraceae; Sinhala: Kekune) which grows in low land wet evergreen forests in South West Sri Lanka. The bark of this tree produces oleoresin or gum resin which has fragrance and is used for religious purposes. The seed oil is an excellent source of essential fatty acids (linoleic acid: 49.35 ± 0.35% and; linolenic acid: 19.09 ± 0.01%) while this oil is mixed with *Madhuca longifolia* and *Azadirachta indica* seed oils to be used in light traps to repel paddy bugs (*Leptocorisa acuta*) as an environmentally friendly pest control method called "kem method". The light petroleum ether extract of leaf and seed showed mosquito larvicidal activity. In the present study, the compounds in the bark oil were focused to check the possibility of using them as an insect repellent. The coarsely powdered bark was hydro-distilled in a Clevenger apparatus for 8 hrs to obtain the oil and the volatile components and the fatty acids were analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) technique. The GC-MS results showed that the oil contains α-copaene (20.1%), n-hexadecanoic acid (13.1%), Cadina-1(10),4-diene (10.6%),(Z)-3-Phenylacrylaldehyde (6.7%), β-maaliene (5.6%) and Viridiflorol (5.6%) as major volatile compounds and palmitic acid (24.0%), Linoleic acid (15.8%), oleic acid (12.9%) and stearic acid (4.2%) as fatty acids. The compounds such as palmitic acid and oleic acid showed mosquito larvicidal activity against *Aedes aegypti* and *Culex quinquefasciatus* while palmitic acid, linoleic acid, oleic acid and stearic acid showed mosquito biting deterrent effect. In conclusion, the bark of *Canarium zeylanicum* (Retz.) Blume containing mosquito repellent compounds can be used as an active ingredient in mosquito repellent products.

Study on migration of Poly Aromatic Hydrocarbons (PAHs) accumulated in smoked Fish (*Thunnus albarcares*) under different storage conditions

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Abstract

Poly Aromatic Hydrocarbons (PAHs) are highly associated with wood smoke used for fish smoking and known to be highly carcinogenic/mutagenic compounds. Therefore, the objective of this study was to assess the migration behavior of PAHs accumulated in smoked "Yellowfin Tuna" fish (*Thunnus albarcares*) under different storage conditions with time. Marinated "Yellowfin Tuna" steaks having a thickness of 4 ± 0.5 cm were smoked using coconut husks for 4 hrs at 80 ± 5°C and stored at refrigerated (4 °C) and frozen (-18 °C) conditions, separately. Stored smoked fish steaks were sliced to separate 5 mm thick outer surfaces which were exposed to smoke and middle core from 10 mm away from the outer surfaces for analysis. Sliced outer and middle core layers of refrigerated and frozen smoked fish samples (n=9) were minced, mixed separately and tested for PAHs content, moisture content (%), pH, water activity, firmness (kg) and colour at the 1-day interval for 9 days and 1-week interval for 3 weeks, respectively. PAHs analysis was done using "Agilent 1260 Infinity HPLC" with "Agilent ZORBAX Eclipse" column, diode array UV and florescence detectors. Freshly smoked fish samples showed significantly higher ($p < 0.05$) total PAHs content at the surface (4498 ±991 µg/kg) compared to middle parts (638 ± 154 µg/kg) of the fish steaks. Results revealed that the total PAHs content in the middle layers of smoked fish was significantly increased ($p < 0.05$) up to 1743 ± 74 µg/kg during frozen storage, while no significant PAHs migration was detected during refrigerated storage. Furthermore, Naphthalene (817 ± 27 µg/kg) and Fluorene (419 ± 28 µg/kg) were found to be most prominently migrated PAHs from surface to middle layers of smoked fish steaks. However, the BaP which is identified as the "Marker PAH" for carcinogenicity was not significantly migrated (11 ± 2 µg/kg) to the middle layers of fish steaks. Studied physico-chemical parameters of refrigerated and frozen samples were not significantly changed during storage. The study concluded that the PAHs accumulated in smoked fish steaks are more prone to migrate from surface to middle layers under a longer period of frozen storage.

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Effect of vacuum cold plasma and atmospheric pressure gliding arc discharge cold plasma as sterilization techniques on piperine and volatile oil content of black pepper seeds (*Piper nigrum L.*)

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Abstract

Plasma technique under low and atmospheric pressure conditions are being investigated as novel non-thermal sterilization methods for spices. Therefore, the objective of the present study was to evaluate the effect of vacuum cold plasma and atmospheric pressure gliding arc discharge plasma treatment as non-thermal sterilization techniques on piperine and volatile oil content of Black pepper (*Piper nigrum L.*) seeds. Raw black pepper (matured in 6 months) was harvested from a farm located in Gampaha district, Sri Lanka. Seeds were washed, blanched in boiling water for 2 minutes and dehydrated using an electrical hot air dehydrator at 55 ^{o}C, until the moisture content reached 10 ± 0.2 % (w/w). Cold plasma treatment was given using an atmospheric gliding arc discharge treater (760mmHg, 250V) and a vacuum cold plasma unit (0.165 mmHg, 300W) unit for 20 minutes. Volatile oils were extracted by hydro-distillation. The components of the extracted volatile oil were analyzed using Gas Chromatography-Mass Spectrometry (GC–MS) and Gas Chromatography - Flame Ionization Detector (GC-FID). Pepper oleoresin was extracted from the hydro-distilled residues of the samples by solvent extraction and piperine was isolated from oleoresin by recrystallization. Piperine content was estimated by UV spectrophotometric method. The results indicated that the volatile oil content of atmospheric gliding arc discharge plasma treated (7.39 ± 0.00%) sample was not significantly different ($P > 0.05$) compared to the volatile oil content of vacuum cold plasma treated (7.24 ±0.00%) sample. The most important phyto-constituent in pepper is piperine, which is correspondent to its pungency taste. The piperine content was not significantly different ($P > 0.05$) in APGAP treated (39.37 ± 0.59%) and VCP treated (38.91 ± 0.68%) samples. The volatile oil of pepper is a mixture of a large number of chemical compounds, which contributes to its characteristic aroma and flavor. The relative contents of the principal monoterpenes were decreased in vacuum plasma-treated samples compared to atmospheric gliding arc discharge plasma-treated sample. However,

caryophyllene content (more dominant sesquiterpene) was increased in vacuum cold plasma-treated sample compared to the atmospheric gliding arc discharge plasma-treated sample. The results disclosed that there is no significant effect on piperine content and volatile oil content of vacuum cold plasma treated black pepper sample compared to atmospheric gliding arc discharge plasma-treated black pepper sample.

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Investigations of *Canarium zeylanicum* (Retz) Blume stem bark

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Abstract

Canarium zeylanicum Blume is the only member of the family Burseraceae, available in Sri Lanka claimed to be having rich phytochemicals in the stem bark according to our previous studies. In the present study, efforts have been taken to establish additional data to identity and quality of the stem bark by (a) screening preliminary phytochemicals, (b) development of Thin Layer Chromatography (TLC) fingerprints and (c) identification of volatile compounds using Gas Chromatography-Coupled Mass Spectrometry. Plant material was collected from three locations as per a standard protocol and the collected material was composited and used for the study. Preliminary phytochemicals were screened using hexane, dichloromethane, and methanol extracts separately and found polyphenols, flavonoids, terpenoids and sterols. Fractions in which sterols and terpenoids probably may have come from oleoresin cells present in the bark which later ooze out from the bark. TLC fingerprint was developed for each of the extracts obtained from solvent extraction. These TLC fingerprints can be used for the standardization of chemical constituents in the stem bark of *C. zeylanicum*. The yield of the essential oil present in the bark powder of *C. zeylanicum* was 0.28% v/w (dry weight basis). GC/MS studies were carried out for the essential oil and the results revealed that the presence of 35 compounds out of which major compounds were; α-copaene (28.3%), β maaliene (21.8%), β-cadina-1(10), 4-diene (10.4%), α gurjenene (5.4%), **α**-phellandrene (2.4%), α-pinine (2.2%) γ gurjenene(1.9%), viridiferol (1.6%), β-caryophyllene oxide (1.5%), and α-muurolene (1.1%). The characteristic odor of the bark may be due to the presence of α-copaene and β-cadina-1(10), 4-diene. According to a previous study, cadinene has demonstrated larvicidal activity (LC_{50}: 9.03 µg/ml) against *Aedis aegypti*, vector of dengue Therefore, efforts are being taken to develop mosquito repellant formulation using essential oil of *C. zeylanicum*. In addition, **α**-copaene is an oily liquid hydrocarbon, which has strong repellent activity towards coleopteran pests and therefore, a new research area will be opened up to develop a bio-pesticide as a value-added product from the plant.

Acknowledgement: Financial assistance is given by ITI, Treasury Grant (TG 19/175)

Microwave-assisted extraction of volatile aroma compounds from Pineapple (*Ananas comosus* L.) and its applications in the development of a moisturizing cream

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Abstract

Pineapple (*Ananas comosus* L.) is a tropical fruit that is highly relished for its unique aroma and sweet taste. During the food processing, a large amount of pineapple peel and the core are discarded as waste into the environment causing many public concerns. This unused peel and the core contain important volatile aroma compounds (VACs) that can be used in the cosmetic industry as fragrance ingredients. Therefore, the present study was carried out to extract VACs from different parts of pineapple fruit by solvent-free microwave-assisted extraction (SFME) and to characterize volatile components for the development of a moisturizing cream. Pineapple fruit **(**Mauritius variety**)** were collected from the Divulapitiya area, Gampaha district in Sri Lanka. Peel, flesh, and core of pineapple were subjected to solvent-free microwave-assisted extraction with three different microwave power levels and times of 300 W, 500 W, and 700 W for 15 min, 10 min, 5 min, respectively. The volatile composition of the highest yielding sample was analyzed using Gas-Chromatography-Mass Spectrometry (GC-MS). A moisturizing cream was formulated incorporating VOCs extracted from the pineapple peel extracts as a natural fragrance and was tested for its characteristic properties such as organoleptic (colour, physical appearance, phase separation, homogeneity, immediate skin feel), pH and spreadability. This experimental data suggested that SFME at 500 W was the most effective and economical compared to 300 and 700 W resulting in a yield of 28.27%, 47.20% and 38.92% from the peel, core and flesh of pineapple, respectively. Heptadecane, γ-dodecalactone,γ-decalactone, Hexadecane and decanal were the major compounds identified from GC-MS analysis. The organoleptic properties of the moisturizing cream revealed that it was a white colour, non-greasy cream with no phase separation. The pH of the moisturizing cream was 6.32 ± 0.01 at 27±2 °C. The spreadability of

the moisturizing cream was 4.76 ± 0.11. This study revealed that SFME can be effectively used to extract volatile aroma compounds from Pineapple which shows potential in incorporating in skincare products as a natural fragrance.

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Comparison of antioxidant capacity of locally cultivating *Zingiber officinale* Roscoe

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Abstract

Ginger; *Zingiber officinale* Roscoe, belongs to the family Zingiberaceae, is a herbaceous perennial plant, widely cultivated for its rhizome, in intermediate zones of Sri Lanka. It is bestowed with many medicinal properties which are because of the secondary metabolites, essential oils, and oleoresin, contain in its rhizome. Ginger oleoresins and essential oils are recognized as potential antioxidants and are widely used in the confectionery, fragrance, and beverage industries. Despite the growing demand, the cultivation of local ginger variety has been replaced by a Chinese variety due to easy cultivation practices. Therefore, the objective of the current study is to compare antioxidant capacities of locally grown local (Beheth inguru) and Chinese ginger varieties, as there is a growing concern of farmers to cultivate Chinese variety over local ginger variety. The local and Chinese ginger rhizomes were air-dried (room temperature: 27-32°C; relative humidity 65-70%) for 3 days and soaked in 96% ethanol overnight to obtain ethanol extracts. The extracts were evaluated *in vitro* for antioxidant capacities using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging assay, oxygen radical capacity (ORAC), total phenolic content (TPC), total flavonoid content (TFC). The results were analyzed using a One-Way Analysis of Variance (ANOVA). The local ginger variety exhibited significantly higher DPPH free radical scavenging activity (IC_{50}: 75.991±4.279 µg/ml), TPC (79.155±3.612 mg gallic acid equivalents/g of extract), TFC (58.297±0.811 mg quercetin equivalents/g extract) compared to those of Chinese variety (DPPH assay:IC_{50}:86.044±1.579 µg/ml; TPC: 50.937± 1.998 mg gallic acid equivalents/g of extract and TFC: 24.783±0.715 mg quercetin equivalents/g extract).This study unveiled a significantly higher *in vitro* antioxidant capacity in Beheth inguru, compared to the locally grown Chinese variety and has a high potential for product development on an Industrial scale.

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Availability of essential minerals in rice varieties of Sri Lanka with seasonal variation.

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Abstract

Rice (Oryza sativa L.) is the backbone of Sri Lankan agriculture which fulfill the daily nutritional requirement of Sri Lankans being the staple food. This study was conducted to investigate the availability of twelve essential minerals in selected twenty-six different rice varieties consumed in Sri Lanka. These selected rice varieties were cultivated during Maha (2018/2019) and Yala (2019) seasons and were analyzed for selected elements using intra-laboratory validated test methods. The mean concentration pattern of selected elements in studied rice samples was decreased as, P> K> Mg> Ca> Zn> Mn> Fe> Na> B> Cu> Mo> Se. In accordance with the results of ANOVA, significant differences ($p < 0.05$) were observed in the content of K, Mg, Na, Ca, Mn, Fe, and Cu due to seasonal variation. There were no significant differences ($p > 0.05$) in the content of P, B, Zn, Mo, and Se of analyzed rice varieties due to the seasonal variation. The present study revealed that selected rice varieties in Sri Lanka to be a potential source of valuable minerals with a significant impact on seasonal variation. The investigated Sri Lankan traditional and newly improved rice varieties can be used without performing bio-fortification for Fe and Zn deficiencies. Furthermore, these rice varieties may also be used as good sources for breeding or genetic engineering processes to increase the micro elemental levels in rice grains.

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