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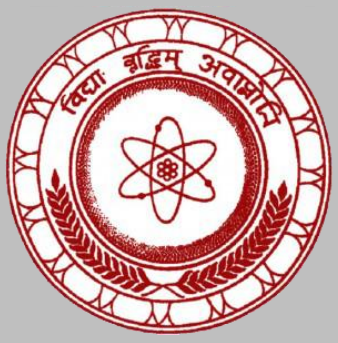

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The Knowledge and Attitude of Medical Laboratory Technologists in the Southern Province on Medical Laboratory Accreditation

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ABSTRACT

Accreditation is a procedure by which an authoritative body gives formal recognition that an organization is competent to carry out specific tasks. The general objective of this study was to study the knowledge and attitudes of medical laboratory technologists in both government and private sector laboratories in the Southern Province towards quality laboratory essentials and the accreditation process.

A descriptive cross-sectional study was conducted using self-administered, pre-tested questionnaires with the participation of 52 medical laboratory technologists in the Southern Province, Sri Lanka. The results were analyzed using SPSS software version 21.Study participants who obtained scores of less than 50%. 50-75%, 75-90%, and more than 90% for the knowledge score were categorized as poor, average, good, and excellent, respectively. Themean (SD) knowledge score among the participants was 40.33(29.35). The Medical Laboratory Technologists' attitudes towards laboratory accreditation were satisfactory. Among the study participants, 82.69% (n=43) are of the view that medical laboratories should be accredited. However, several misunderstandings regarding the same aspect were observed.

The study concluded that the overall knowledge of MLTs on medical laboratory accreditation is not satisfactory. The study was conducted in and around Galle. Therefore we assume that knowledge scores will be poorer among the MLTs who work in the rural laboratories since they possess less opportunity for participation in continuous education, compared to those working for urban employers. However, the overall attitudes of Medical Laboratory Technologists regarding accreditation is satisfactory. The value of educational and training programs on medical laboratory accreditation and evaluation of their effectiveness is emphasized.

Key Words: Accreditation, Attitude, Knowledge, Medical Laboratory Technologists, ISO 15189 standard

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INTRODUCTION

Accreditation is a process in which trained external peer reviewers evaluate the compliance of an organization with pre-established performance standards. ISO 15189 is the gold standard for accreditation of medical laboratories. ISO 15189, was first published in 2003 and revised in 2007 and 2012.The goal of ISO 15189 is the continuous improvement of the laboratory, and to provide necessary information to its workers in order to perform their jobs(Richardson, 2002).By accrediting, a medical laboratory can gain international recognition which proves its quality management system, technical competence, and the proficiency of its personnel to generate an accurate and precise test result for each test(Frost, 2004).

ISO 15189 had become a part of the mandatory medical laboratory accreditation requirements in about 60 countries by the year 2015(Schneider, Maurer & Friedberg, 2017). Each of these countries has its own national accreditation body, which is responsible for granting accreditation (Frost, 2004).

The medical laboratory accreditation standard, ISO 15189, was initiated in Sri Lanka in the year 2005 under the Sri Lanka Accreditation Board for Conformity Assessment Act No.32 of 2005. The Sri Lanka Accreditation Board for Conformity Assessment (SLAB) is the National Accreditation Authority for Sri Lanka. It promotes accreditation activities and provides the necessary accreditation services to facilitate conformity assessments in Sri Lanka. Although there are thousands of medical laboratories functioning in every nook and corner of the country, only about 20 of them have gained the ISO15189:2012 Accreditation Certificate in Sri Lanka (2020).

The aim of this study was to investigate the knowledge and attitudes of medical laboratory technologists in both government and private sector laboratories in the Southern Province towards quality laboratory essentials and the accreditation process.

METHODS AND MATERIALS

Ethical approval for the study was obtained from the Ethical Review Committee, Faculty of Allied Health Sciences, University of Ruhuna, Sri Lanka. A descriptive cross-sectional study was performed. The study was carried out using a purposive convenient sample of 52 medical laboratory technologists who were working in both private sector and government sector laboratories in Southern Province. Medical Laboratory Technologists in 2 teaching hospitals, 3 general hospitals and 5 private sector laboratories in Southern Province (in and around Galle) were included in the study. Questionnaires were distributed among Medical Laboratory Technologists who consented to participate. The questionnaire consisted of 3 sections. Demographic data was collected in section I. In section II, the knowledge of the Medical Laboratory Technologists' regarding the accreditation concept, accreditation body, ISO 15189 manual, quality policy, quality manual, and quality management system was obtained. The attitudes of the Medical Laboratory Technologists on accreditation were assessed using section III. Data was collected using a pre-tested self-administered questionnaire. Demographic data were analyzed using descriptive analysis. Group comparisons were done using t- test and one-way ANOVA by SPSS version 23.

RESULTS

A total of 52 medical laboratory technologists from both private sector and government sector laboratories in Southern Province were included in the study. Demographic characteristics of the sample is indicated in the table I.

Table I: Demographic characteristics of the medical laboratory technologists who participated in the study

Demographic characteristics		Percentage of Medical Laboratory Technologists
Gender	Female	59.6%
	Male	40.4%
Age (years)	21-30	23.1%
	31-40	36.5%
	41-50	19.2%
	51-60	19.2%
	>60	1.9%
Profession	Medical Laboratory Technologist	59.6%
	Medical Laboratory Scientist	28.8%
	Senior MLT	9.6%
	Superintend MLT	0%
	Laboratory Manager	1.9%
Educational level	Diploma	55.8%
	Graduate	42.3%
	Post Graduate	1.9%
Additional qualification	Yes	82.7%
	No	17.3%
Certificate courses	Yes	17.3%
	No	82.7%

Workshops	Yes	69.2%
	No	30.8%
Training programs	Yes	32.7%
	No	67.3%
Conferences	Yes	26.9%
	No	73.1%
Working Organization	Government	86.5%
	Private	13.5%
Working Experience	1-10 years	57.7%
	>10 years	42.3%
Monthly Income	Not mentioned	3.8%
	Less than Rs.50,000	19.2%
	Rs.50,000– Rs.100,000	65.4%
	Rs.100,000– Rs.150,000	11.5%

Participants who obtained scores of less than 50%. 50-75%, 75-90%, and more than 90% for the knowledge score were categorized as poor, average, good, and excellent, respectively. The mean knowledge score was 40.33 (SD±29.35). Among the study participants only 5.8 % (n=3) were in the excellent level, while 13.5 % (n=7) were in the good level, 13.5 % (n=7) were in the average level, and 67.3% (n=35) were in the poor level.

The mean knowledge scores (±SD) of the different groups are given in Table II

Table II: Group wise knowledge scores

Groups		Mean±SD
Gender	Female	35.94±29.97
	Male	46.81±27.84
Age (years)	21 – 30	54.08±24.06[a]
	31 – 50	39.00±30.42

	>50	28.82±28.31
Profession	Medical Laboratory Technologist	27.19±25.12
	Medical Laboratory Scientist	65.20±21.01[b,c]
	Senior MLT	36.00±15.70
	Laboratory Manager	96.00±0.00
Educational level	Diploma	25.72±23.31
	Graduate	57.05±25.15[d]
	Post Graduate	96.00±0.00
Working Organization	Government	37.93±29.83
	Private	55.71±21.85
Working Experience	1-10 years	50.70±27.81[e]
	>10 years	26.18±25.71

[a] p=0.040, [b] p=0.000, [c] p= 0.019, [d] p= 0.000, [e] p= 0.002

There was a statistically significant difference for the knowledge scores in the Medical Laboratory Technologists in different age groups as determined by one-way ANOVA. The knowledge score was significantly higher in Medical Laboratory Technologists between 21 and 30 years of age (54.08±24.06) compared to the Medical Laboratory Technicians more than 50 years old (28.82±28.31).

A statistically significant difference was observed in the knowledge scores of Medical Laboratory Technologists in different professions as determined by one-way ANOVA. The knowledge score was significantly higher in Medical Laboratory Scientists (65.20±21.01) compared to the Medical Laboratory Technicians (27.19±25.12, p=0.019) and Senior Medical Laboratory Technicians (36.00±15.70, p=0.000). There was one laboratory manager included in the study with a post graduate qualification. The knowledge score of that participant was significantly high (96.00).

There was a statistically significant difference for the knowledge scores in the Medical Laboratory Technologists in different education levels as determined by one-way ANOVA. The knowledge score was significantly higher in Graduates (57.05±25.15) compared to diploma holders (25.72±23.31, p=0.000). There was only one study participant with post graduate qualifications. The knowledge score of that participant is significantly high (96.00) indicating the value of higher education on the aspect of laboratory accreditation.

A statistically significant difference was observed in the knowledge scores of Medical Laboratory Technologists with different work experience as determined by one-way ANOVA. Medical Laboratory technologists with less than ten years of experience(50.70±27.8) have a significantly higher knowledge score when compared to Medical Laboratory technologists with more than ten years of experience (26.18±25.71, p=0.002)

There was no statistically significant difference in knowledge between Medical Laboratory Technologists with respect to their gender (p =0.193) or working organization (0.137).

Attitude of Medical Laboratory Technologists on Medical Laboratory Accreditation

Among the study participants, 82.69% (n=43) were of the view that medical laboratories should be accredited. About 88.46%(n=46) of the sample agreed that they needed more information about accreditation.

Among the study participants,75%(n=39) believe that medical laboratories can gain international recognition by being accredited; 82.69%(n=43) of the participants think that accreditation is a way to laboratory errors, 57.69%(n=30) think that waste generated in the laboratory can be reduced by accreditation, 69.23%(n=36) think that accreditation improves the competency of the laboratory, and 78.84%(n=41) think that quality of results and laboratory service can be improved by accreditation.48.07 % (n=25) think that turnaround time (TAT) for a test can be reduced by accreditation while75%(n=39) think that standardization of the laboratory processes could be done by accreditation.

Among the study participants 42.31 % (n=22) believe that accreditation increases the workload. At the same time 38.46% (n=20) think that it will be difficult to apply for accreditation since they already have heavy workloads. About 48.0%(n=25) think that accreditation will cause financial problem to the organization, while 50%(n =26) think that accreditation increases paper work. About 30.78%(n=16)of the sample think that obtaining accreditation for their laboratories will be difficult, since it is difficult for them to adapt new procedures. Nearly half of the participants (48.0%,n=25) think that it will be difficult for them to apply for accreditation for their laboratories, since there are not enough staff members. More than half of the sample (51.92%, n=27) are of the view that there are not enough resource persons to educate them on accreditation. About 46.15 % (n=24) think that lack of laboratory equipment will be an obstacle and 42.31 % (n=22) think that lack of reagents and consumables will be an obstacle. 40.38%(n=21) agree that they have a poor knowledge on accreditation, 38.46% (n=20) think that Medical Laboratory Technologists have a poor attitude about accreditation, and59.61% (n=31) agree that they do not have sufficient support from the higher authorities to apply for accreditation.

DISCUSSION

Laboratory services are an integral part of clinical decision making. Also, laboratory services play a vital role in diagnostic and therapeutic decisions for patients, and disease monitoring and prevention. Medical Laboratory Accreditation ensures the validity of laboratory system management and to promote continuous quality improvement.Although the accreditation concept was

introduced to the Sri Lankan Medical field in the year 2005, only a limited number of Medical Laboratories have obtained ISO 15189 certification up to date(2020).Lack of knowledge regarding accreditation and poor attitudes of the medical laboratory personnel towards accreditation are the mainobstacles for obtaining ISO 15189. Previous Sri Lankan literature about knowledge and attitude of Medical Laboratory Technologists toward accreditation is extremely sparse.

According to the present study the mean knowledge score of the study participants was 40.33 ± 29.35. This is a very poor level. However, the knowledge score was significantly higher in Medical Laboratory Scientists (65.20±21.01) compared to Medical Laboratory Technicians (27.19±25.12) and Senior Medical Laboratory Technicians (36.00±15.70).The knowledge score was also significantly higher in Graduates (57.05±25.15) compared to diploma holders (25.72±23.31), and that of the study participant with post-graduate qualifications was very high (96.00). This may be due to the inclusion of novel concepts such as accreditation into graduate and post-graduate curricula. Further, the knowledge scores of newly recruited Medical Laboratory Technologists (50.70±27.81) are higher than those of the experienced Medical Laboratory Technologists (26.18±25.71).This reflects that education and new knowledge have a greater impact on knowledge about accreditation.

Among the study participants, 82.69% (n=43) were of the view that medical laboratories should be accredited, while88.46% (n=46) of the sample agreed that they need more information about accreditation. Most of the study participants think that gaining international recognition (n=39), reducing laboratory errors (n=43), standardization of the process (n = 39), reduction in waste (n=30), improving the competency of the laboratory (n=36), improving quality of results (n=41) and reducing turnaround time (n=25) are advantages of accreditation. However, some misunderstandings of the Medical Laboratory Technologists were identified. Among the study participants, 42.31% (n= 22) believe that accreditation increases the workload, 38.46%(n=20) think that it will be difficult to apply for accreditation since they already have heavy workloads, 48.0%(n=25) think that accreditation will cause financial problems to the organization, 50.0%(n=26) think that accreditation increases paper work, 30.78%(n=16)think that obtaining accreditation to their laboratories will be difficult since it is difficult for them to adapt to new procedures, 48.0%(n=25) think that it will be difficult for them to apply for accreditation for their laboratories, since there are not enough staff members, 51.92%(n=27) are of the view that there are not enough resource persons to educate them on accreditation, 46.15 % (n=24) think that lack of laboratory equipment will be an obstacle, and 42.31% (n=22) think that lack of reagents and consumables will be an obstacle;40.38 % (n=21) agree that they have a poor knowledge about accreditation, 38.46 % (n=20) think that Medical Laboratory Technologists have a poor attitude about accreditation, and 59.61% (n=31) of the participant agree that they do not have sufficient support from the higher authorities to apply for accreditation.

Astudy, which was conducted in Ethiopia on perception and attitude of laboratory professionals, shows similar findings to the present study. According to that study, about 85% of the laboratory professionals emphasized that accreditation is important for a quality laboratory process(Lulie*et al*., 2014).But a survey which was conducted among laboratory personnel in Belgium and the Netherlands showed conflicting results. In that study, 87% of the study participants did not think that the accreditation process improved the quality of the laboratory results. Also, most of the study participants preferred to work in non-accredited laboratories (Verstraete, van Boeckel, Thys&Engelen, 1998).In a survey which was conducted among Clinical Pathology laboratories, 75% of

laboratories agreed that accreditation improved laboratory services by introducing more documentation and better health and safety training procedures (Gough & Reynolds, 2000).

There are no published data about the knowledge and attitude of Medical Laboratory Technologists towards accreditation in Sri Lanka. So comparison of the results obtained fromthe present study is not possible.

CONCLUSIONS

The mean knowledge score among the study participants was 40.33 ±29.35, which is poor when compared to studies done elsewhere. So workshops, conferences, or awareness programs should be introduced to fill the knowledge gap. The knowledge score was significantly higher among Medical Laboratory Scientists compared to Medical Laboratory Technologists. Further, the knowledge scores of newly recruited Medical Laboratory Technologists are higher than those of experienced Medical Laboratory Technologists. The accreditation concept was introduced into the Medical Laboratory Curriculum recently, so newly passed out Medical Laboratory Technicians have a considerably better knowledge than experienced Medical Laboratory Technicians. The attitude of Medical Laboratory Technologists towards accreditation is significantly good. The present study was conducted in and around the Galle District. We assume that knowledge scores will be much poorer in the rural laboratories since they possess fewer opportunities for continuous education compared to urban laboratories.

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Evaluation of histidine-tagged DARPin G3 variants for molecular imaging of HER2 expression

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ABSTRACT

HER2 is a transmembrane receptor with tyrosine kinase activity. It is over expressed in about 20-30% of breast cancers and 6-35% in gastric cancers. Therefore, HER2 is targeted in many therapeutic approaches including trastuzumab in breast cancer. Molecular imaging (nuclear medical imaging) of HER2 expression may help in patient selection for such anti-HER2 therapies. Designed ankyrin repeat proteins (DARPins) are recombinant proteins where variant libraries can be established. DARPin G3 is a variant which binds to HER2 with a picomolar affinity. Histidine-containing tags simplify purification of proteins and allow site-specific labeling with $[99mTc(CO)_3]^+$. However, the position and composition of histidine-containing tags might have an effect on the biodistribution of targeting proteins. In this study two variants of DARPin G3 bearing a hexahistidine (His6) or a histidine glutamate (HE)3 tag at the N-terminus were evaluated. Proteins were directly radiolabeled with iodine-125. Binding specificity, affinity and cellular processing of radiolabeled DARPins was evaluated in HER2 expressing cell lines. Labeling of both DARPins with iodine-125 was efficient and the labeled compounds were stable. Binding was specific towards HER2 in HER2-expressing cell lines for both variants. Affinity of both DARPins to HER2 expressing cells was in the picomolar range. A decrease in cell-associated activity at 24 h post-addition was observed that might indicate internalization of DARPins. In conclusion, the translation of both G3 variants to in vivo biodistribution studies is feasible. As DARPin G3 and trastuzumab bind to different epitopes of HER2, imaging of HER2 using G3 could potentially be used to monitor the effectiveness of trastuzumab therapy.

Keywords: DARPin, HER2, histidine tags, molecular imaging, nuclear medicine

INTRODUCTION

Human tumorigenesis is a multistep process that is initiated by genetic alterations in cells that lead to their malignant transformations. During this development cancer cells acquire various functional capabilities, such as growth signal autonomy, tissue invasion and metastasis (Hanahan & Weinberg, 2000). Sustained growth, or chronic proliferation, of cancer cells is achieved by proliferative signalling in several ways. Signaling through receptor proteins is commonly deregulated by increasing the number of receptors on cancer cell surface.

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Human epidermal growth factor receptor-2 (HER2/erbB-2) is a part of the HER family of transmembrane receptors with tyrosine kinase activity (Yarden, 2001). It is involved in cell growth and proliferation and its overexpression in cancer is associated with poor prognosis (Arteaga *etal.*, 2012). HER2 is over expressed in about 20-30% of breast cancer patients (Yarden, 2001)and 6-35% in gastric cancer patients (Bang *et al*., 2009). Several therapeutic approaches to target HER2 in cancer have been developed, *e.g*., monoclonal antibodies (trastuzumab) and antibody-drug conjugates (T-DM1) (Lambert & Chari, 2014; Nahta & Esteva, 2007).

The World Health Organization (WHO) has identified breast cancer in females as the cancer with the highest incidence, with 1.81 million females diagnosed worldwide(World Health Organization, 2018). Breast cancer is primarily treated with surgery, radiotherapy, chemotherapy, hormonal therapy, and targeted therapy, or a combination (Curley *et al*., 2018).Detection of HER2 expression in breast cancer is crucial for selection of patients for targeted anti-HER2 therapies and assessment of response (Wolff et al., 2013).

Tumor biopsy followed by immunohistochemical and fluorescence in situ hybridization (FISH) analysis is the method recommended by the American Society of Clinical Oncology for molecular characterization of HER2 expression (Wolff et al., 2013). However, tissue sampling is an invasive technique that has several disadvantages, such as patient's discomfort and pain, sampling errors, and the difficulty of taking multiple and repeated biopsies (Kurdziel *et al*., 2008). Moreover, differences in antigen expression within the tumor or between primary tumor and metastases are often observed in the same patient (Bedard *et al.*, 2013; Zidan *et al.*, 2005). Therefore, a non-invasive technique, like molecular imaging, would offer several important advantages, such as whole-body imaging, that could be performed repeatedly over the course of therapy.

Radionuclide molecular imaging is based on detection of ionizing radiation emitted from unstable nuclides during radioactive decay. These nuclides are attached to a molecule (imaging probe), which binds to a specific tumor antigen. In Single-Photon Emission Computed Tomography (SPECT) emitted gamma rays are detected. In Positron Emission Tomography (PET) annihilation photons from interaction of positrons with matter are detected (Levin, 2005). Combination of molecular imaging techniques (PET, SPECT) with anatomical imaging techniques, such as Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) provides high diagnostic accuracy (Bockisch *et al.*, 2009; Herrmann *et al.*, 2013).

High sensitivity and specificity of an imaging probe are important for achieving high contrast between tumor and normal tissues in radionuclide molecular imaging. Therefore, a number of parameters should be assessed and validated in a series of *in vitro* and *in vivo* studies (James & Gambhir, 2012). It has been demonstrated that labeling chemistry has a significant influence on cellular processing, biodistribution, and tumor-targeting properties of radiolabeled peptides and proteins (Altai *et al.*, 2013; V. Tolmachev & Orlova, 2010).Therefore, selection of an optimal labeling approach is essential for the development of imaging probes.

The Food and Drug Administration (FDA) of the USA has approved more than 8 intact IgG monoclonal antibodies (150 kDa) as probes for radionuclide imaging (Olafsen & Wu, 2010). However, the major downside of using monoclonal antibodies is long residence time in blood, which

results in poor signal-to-background ratio. In clinical studies an imaging window of 4- 7 days post injection has been found optimal (Dijkers *et al*., 2010; Even *et al.*, 2017). On the other hand, with proteolytic antibody fragments like (Fab')2 (110 kDa) and Fab (55 kDa) imaging is possible on the day of injection (Freise & Wu, 2015). Apart from disadvantages, such as lower affinity towards its target than that of monoclonal antibodies, those fragments show the possibility of increasing imaging contrast by reducing the size of the protein. With protein engineering size reduction has been performed to generate antibody fragments with preserved affinity and specificity, *e.g*.,scFv (25 kDa), diabodies (50 kDa), and minibodies (80 kDa) (Olafsen & Wu, 2010).

Since size reduction of immunoglobulins below 15 kDa (size of VHH, nanobody) is impossible, molecular engineering allows creation of proteins with lower molecular weight, which are based on non-immunoglobulin scaffolds. Molecular display selection techniques allow selection of binders against various targets from large libraries (Nygren & Skerra, 2004). Out of many scaffold proteins affibodies (6-7 kDa) have been thoroughly studied,and provide valuable information for the development of imaging probes based on novel scaffold proteins (Vazquez-Lombardi *et al.*, 2015). For tumor targeting, short peptides and their analogues have also been actively studied (Reubi, 2003).

Designed ankyrin repeat proteins (DARPins) are recombinant non-immunoglobulin scaffolds with established variant libraries. DARPins consist of a number of repeat modules (33 amino acids) that form a structural unit made of antiparallel α-helices followed by a β turn. DARPins usually consist of four to six repeats, including N-terminal and C-terminal capping repeats(Binz *et al*., 2003). Their high thermal stability, solubility, and aggregation resistance allowed wide applications of DARPins in research. DARPins against human vascular endothelial growth factor VEGF-A have shown good safety and efficacy in macular degeneration diseases in clinical trials (Souied *et al.*, 2014).

DARPin G3 (~14.5 kDa) is a variant that binds to domain-4 of HER2 with a picomolar affinity (90 pmol/L) (Zahnd *et al.*, 2010). Histidine-containing tags encoded in recombinant proteins allow purification using immobilized metal ion affinity chromatography (IMAC) and site-specific labeling with tricarbonyl technetium $[^{99m}Tc(CO)_3]^+$(Waibel *et al.*, 1999). It has been shown that both position and composition of a histidine-tag in the targeting protein can have a large influence on itsbiodistribution properties (Hofström *et al.*, 2013). It has been shown that anti-HER2 affibody molecules labeled with 99mTc using a His6-tag had elevated uptake in liver compared to the same affibody molecules without a His6-tag (Ahlgren *et al.*, 2009). Unspecific radioactivity uptake in liver is undesirable because liver is a common metastatic site of cancer. Studies with several affibody molecules showed that the use of (HE)3-tag improved biodistribution and lowered hepatic uptake of $[^{99m}Tc(CO)_3]^+$labelled affibodies (Orlova *et al.*, 2013). In this study two DARPin G3 variants with histidine tags (His6- and (HE)3 were used (Table 1).

Table 1. DARP in G3 variants used in this study.

Variant	Position of the tag	Theoretical molecular weight (Da)
H6-G3	N-terminus	14596.47
(HE)3-G3	N-terminus	14572.38

Selection of an optimal radionuclide for labeling depends on several factors, such as half-life of a radionuclide, emission profile, and application of radiolabeled protein, *e.g.*, for a biodistribution study, imaging or therapy. In this study iodine-125 ($T_{1/2}$= 59.4 d, E_γ = 35.49 4 keV) was selected as a label due to its availability, safety, and long half-life. Iodine-125 is the chemical analogue of iodine-123 ($T_{1/2}$= 13.3 h, E_γ= 159 keV), used for SPECT imaging, iodine-124 ($T_{1/2}$= 4.2 d, E(annihilation quanta)= 511 keV), used for PET imaging, and iodine-131 ($T_{1/2}$ = 8.02 d, E_γ = 364.5 keV, $E_{\beta(max)}$ = 606.3 keV), used for radionuclide therapy.

The labeling chemistry of iodine has been well studied(Behr *et al*., 2002). Direct iodination of polypeptide- and protein-based imaging probes with iodine is performed by oxidation of radioiodide and electrophilic substitution at the ortho position of the activated phenolic ring of tyrosine residues.

The aim of this project is to evaluate two variants of theDARPin G3 protein bearing a hexahistidine (His6) or a histidine glutamate (HE)3 tag at the N-terminus for radionuclide molecular imaging of HER2 *in vitro*.

METHODOLOGY

Materials

Na125I was purchased from Perkin Elmer Sverige AB (Sweden). SKOV-3, BT474 and DU145 cells were purchased from the American Type Culture Collection (ATCC) and were cultured in complete RPMI medium (with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 IU/ml penicillin and 100 µg/ml streptomycin) in a humidified incubator with 5% CO2 at 37 °C. High quality Milli-Q water was used for preparing buffers.

Instrumentation

Instant thin-layer chromatography (iTLC) analysis was performed using iTLC silica gel strips (Varian, Lake Forest, CA, USA). Strips were eluted with acetone: water (4:1). In this system radiolabeled DARPin molecules remain at the application point, while free iodine migrates with the solvent front. Radioactivity distribution along iTLC strips was measured by a Cyclone storage phosphor system (Packard) and OptiQuant image analysis software was used for analysis. The radioactivity in fractions from cell assays was measured using automated gamma-spectrometer with NaI(Tl) detector (1480 Wizard, Wallac, Finland).

Statistics

Data from cell specificity assay was analysed by an unpaired, two-tailed t-test using Microsoft Excel Office 365 in order to determine significant diffe *H M S K Herath et al., JSLAAS, Vol. 3, Issue 1 (2021) 13-26*

Radiolabeling

Labeling of DARPins with 125I was performed by direct iodination as previously described (Tolmachev, Orlova&Andersson, 2014). The same protocol was used for labeling of both DARPin variants. Briefly, to a solution of DARPin (40 µg, 2.74 nmol) in PBS (40 µl), NaI (1.10 nmol, 8.25 µl of 0.02 mg/ml) and Na125I (2 µl, 7.4- 7.9 MBq) were added. After five minutes, a freshly prepared solution of Chloramine-T (71 nmol, 20 µg, 20 µl of 1 mg/ml in PBS) was added. After 60 seconds, a

freshly prepared solution of Na2S2O5 (211 nmol, 40 µg, 20 µl of 2 mg/ml in MQ H2O) was added. Labeling yield was evaluated using iTLC.

Stability test

DARPinslabeled with 125I were incubated at room temperature in 1 M solution of KI for 3 h. The experiments were performed in duplicate. In the control groups radiolabeled DARPins were incubated only with PBS. Loss of radioactivity from DARPins was analysed using iTLC.

In vitro binding specificity of radiolabeled DARPins

These studies were performed using HER2 expressing cell lines SKOV3 (1.6 × 106 receptors/cell) (Tolmachev et al., 2012),BT474 (1.2 × 106 receptors/cell) (McLarty et al., 2009)and DU145 (5 × 104 receptors/cell) (Malmberg et al., 2011). Cells were seeded in 3 cm petri dishes (ca.106 cells per dish) and for each group three dishes were used.

HER2 binding specificity was performed as previously described (Wållberg & Orlova, 2008). Per each cell line two sets of dishes were used. Control group was incubated with 100-fold molar excess of unlabeledDARPins (100 nM) to saturate the HER2 receptors 30 minutes before addition of labeledDARPins (1 nM). After a 60 min of incubation at 37 °C in a humidified incubator, the medium was collected. Cells were washed with 1 ml of fresh medium and 1 ml of 1 M NaOH was added to lyse the cells. After 30 minutes of incubation the lysate was collected. Activity was measured in each fraction and the percent of cell-associated activity was calculated. Average cell number per each dish was calculated and cell associated activity per 106 cells was determined.

Affinity measurement

Binding affinity of radiolabeled DARPin G3 variants to living SKOV3 cells was measured using LigandTracer Grey (Ridgeview Instruments, Vänge, Sweden) as described previously (Tolmachev, Orlova&Andersson, 2014). Kinetics were recorded at room temperature in real time. Increasing concentrations of each radiolabeled DARPin (0.5 and 2 nM) were added to cells for measuring association phase. Then the media was changed to measure the dissociation phase. Signal curves were analysed by TraceDrawer software (Ridgeview Instruments, Vänge, Sweden).

Cell processing of radiolabeled DARPins

Cellular processing of radiolabeled DARPin G3 variants by SKOV3 cells was studied by the acid wash method (Wållberg & Orlova, 2008). Cells (ca. 106 cells per dish, three dishes per data point) were incubated in a humidified incubator at 37 °C with 1 nM of radiolabeled DARPins. At 1, 2, 4, 8 and 24 h after addition, the medium from a set of dishes was collected and cells were washed with 1 ml of serum free medium. Then the cells were treated with 1 ml of 0.2 M glycine buffer with 4 M urea (pH 2.0) for 5 minutes on ice and the membrane bound DARPin was collected. Cells were once again washed with 1 ml of the acid buffer. To collect the internalized conjugate cells were lysed by incubating for 30 minutes with 1 ml of 1 M NaOH. Cells were collected and additionally washed with 1 ml of NaOH. Activity was measured in each fraction; the activity in acid fractions was considered as

DARPin bound to the membrane and activity in base fractions was considered as internalized DARPin.

RESULTS

Labeling

DARPin His6-G3 was labeled with ^{125}I with a 94.9% yield. The labeling yield for DARPin (HE)3-G3 was 98.9%.

Table 2. In vitro stability of ^{125}I-His6-G3 and ^{125}I-(HE)3-G3. Samples were incubated in PBS or in 1 M solution of KI. Analysis was performed in duplicates.

	Protein-associated radioactivity, %			
	1 h		3 h	
	PBS	1 M KI	PBS	1 M KI
^{125}I-His6-G3	97.5 ± 0.4	96.8 ± 0.0	97.1 ± 0.1	96.4 ± 0.6
^{125}I-(HE)3-G3	97.9 ± 0.4	98.9 ± 0.0	98.5 ± 0.1	99.0 ± 0.4

A challenge with 1 M KI did not reveal any significant radioactivity release from the ^{125}I-labeled DARPins in comparison to control (Table 2).

Binding specificity of ^{125}I-labeled DARPins*in vitro*

Binding specificity assay was performed on three cell lines. SKOV3 and BT474 cells have a high level of HER2 expression (> 1 million receptors/cell), while DU145 have a low level of expression (ca. 50 thousand receptors/cell). To determine if the ^{125}I-labeled DARPins bind to living HER2 expressing cells, cells were incubated with the labeled proteins. To determine if the binding was specific, one set of cells was preincubated with an excess of non-radiolabeled DARPins to saturate HER2 receptors. The results are displayed in Figure 1. A significant difference ($p< 0.0002$) in cell-bound activity was observed between blocked and non-blocked groups for both variants, ^{125}I-His6-G3 and ^{125}I-(HE)3-G3, in all cell lines which demonstrates that the binding of radiolabeled DARPins was mediated by HER2 and was specific. Cell-associated activities for high HER2-expressing SKOV3 and BT474 cell lines were higher than that for low HER2-expressing DU145 cells.

Affinity measurement

The data concerning binding affinity of the G3 variants to HER2 expressing SKOV3 cell line are presented in Table 3. The binding curves of ^{125}I-His6-G3 and ^{125}I-(HE)3-G3 to living HER2-expressing SKOV3 cells (Figure 2) were fitted best using a one-to-two interaction model, which suggests that there were two types of interactions of radiolabeled DARPins with HER2. The values for the first interaction (KD1) are in the picomolar range (from 106 to 120 pM), the values for the second interaction (KD2) are in the low nanomolar range (from 3.1 to 4.4 nM) (Table 3).

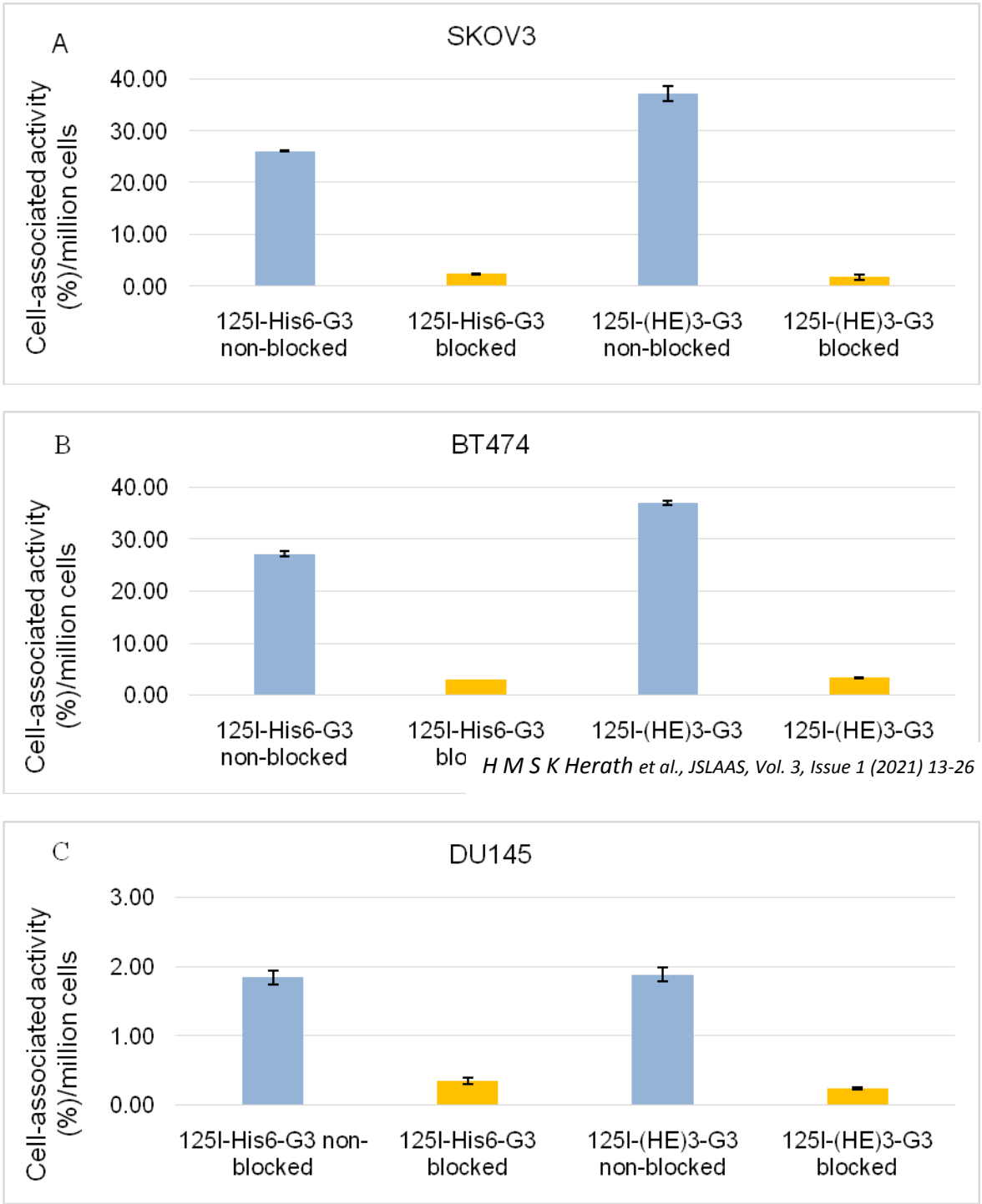

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Figure 1. Binding specificity of 125I-His6-G3 and 125I-(HE)3-G3 to HER2 expressing (A) SKOV3 cells, (B) BT474 cells, and (C) DU145 cells. For pre-saturation of receptors a 100-fold molar excess of non-radioactive G3 variant was added. The data are presented as mean values with standard deviation from three cell dishes. The difference between uptake by non-blocked and blocked cells was highly significant ($p < 0.0002$).

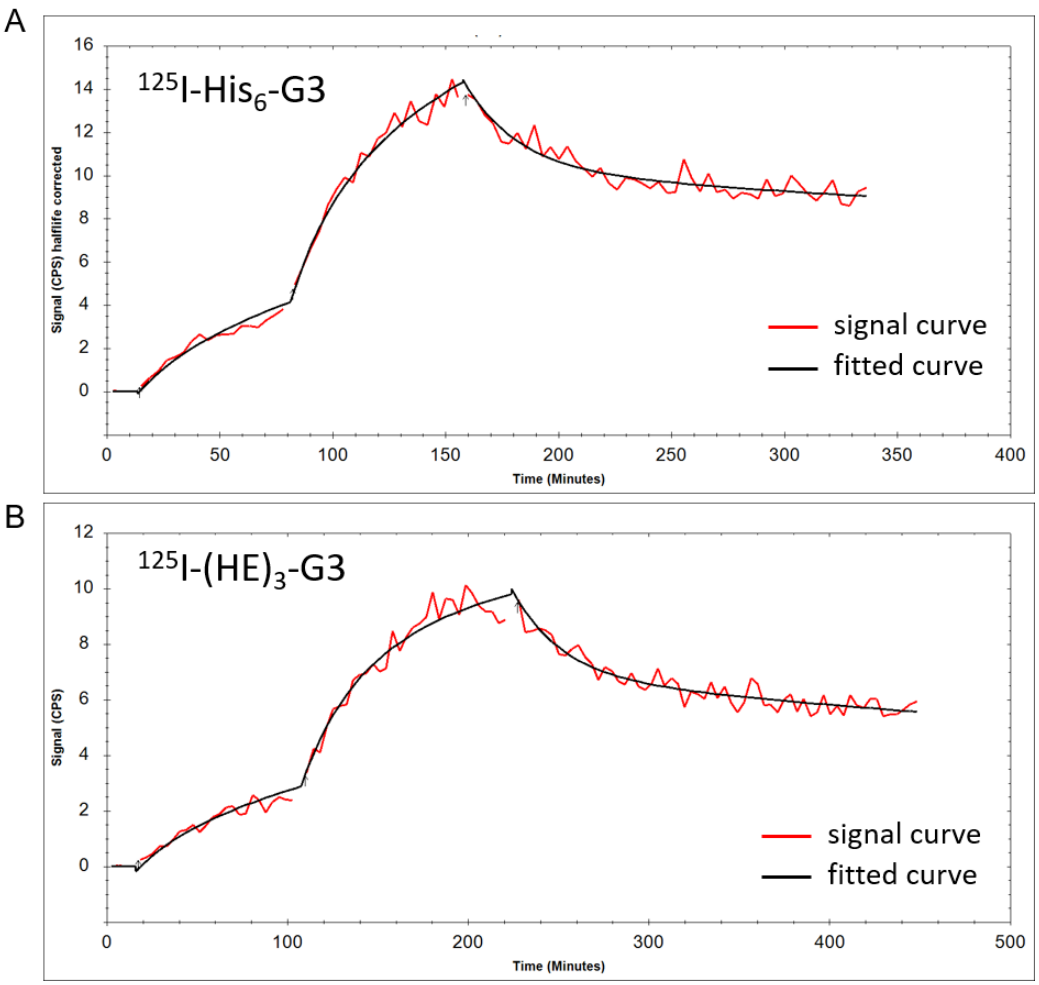

Figure 2. Representative binding curves of Ligand Tracer measurements of (A) 125I-His6-G3 and (B) 125I-(HE)3-G3 to living HER2-expressing SKOV3 cells.

Table 3. Dissociation Constants (KD1 and KD2) for the interaction of radiolabeled G3 variants with HER2 expressing SKOV3 cells.

Variant	KD1 (pM)	KD2 (nM)
125I-His6-G3 (n=2)	120 ± 9	3.1 ± 0.1
125I-(HE)3-G3 (n=4)	106 ±36	4.4 ± 2.1

The cellular processing of radiolabeled G3 variants is presented in Figure 3. Both radiolabeled DARPins showed rapid binding to cells followed by a plateau up to 8 h. The amount of internalized activity at 24 h was in the range of 2.9-3.4%. A decrease in cell-associated activity at 24 h was observed.

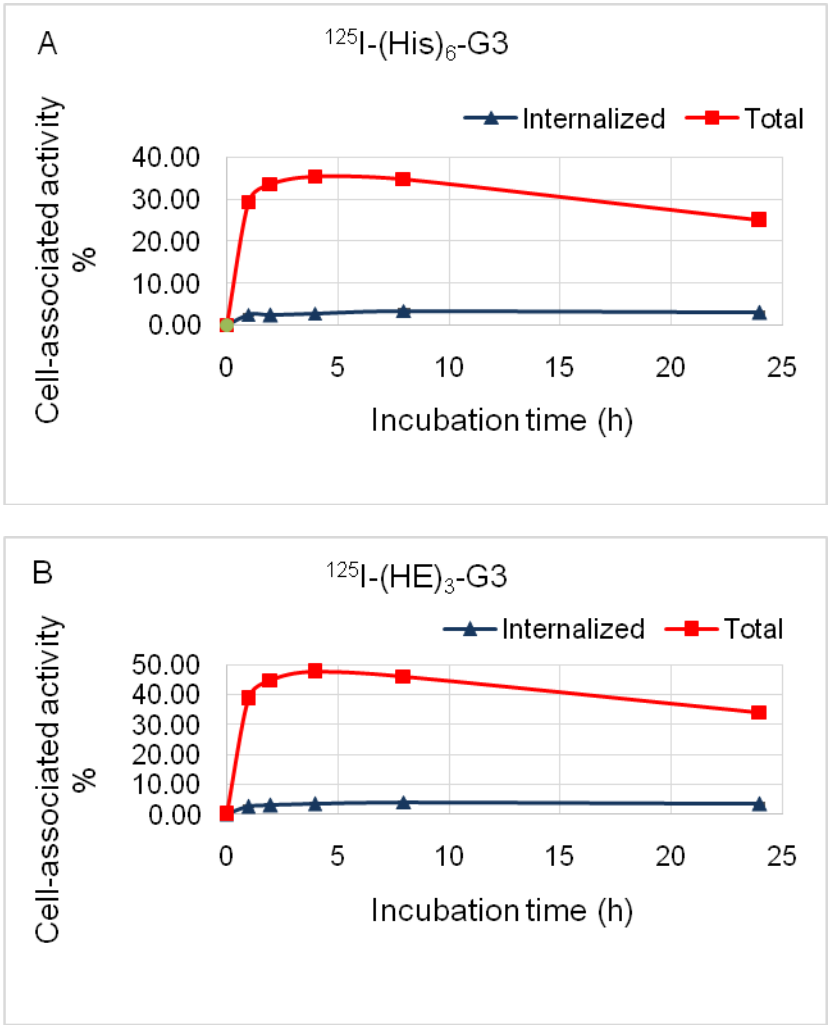

Figure 3. Cellular processing of (A) 125I-His6-G and (B) 125I-(HE)3-G3 after binding to HER2-expressing SKOV3 cells. The data are presented as mean values with standard deviation from three cell dishes.

DISCUSSION

A major advantage of scaffold proteins is their small size, which increases both the extravasation rate from blood to extracellular fluid and diffusion in extracellular fluid. At the same time small size ensures a rapid renal clearance of proteins. High affinity in combination with the properties discussed above could allow high contrast imaging shortly after injection. To develop an imaging probe with a low uptake in normal tissues and a high uptake in tumor, it should have low off-target interactions. To achieve good specificity the binding affinity towards the targeted receptor should be high. Modification of charge and lipophilicity in a purification tag might also influence off-target

interactions of protein with normal tissues. From the previousstudies with affibodies (Hofström et al., 2013)it was hypothesized that the (HE)3 tag at the N-terminus of DARPins could reduce the hepatic uptake in vivo while preserving the purification efficiency during protein production.

DARPin G3 has two tyrosine residues that could be iodinated. Iodine labeling efficiency for both G3 variants was high. Radiolabeled DARPins were stable upon the challenge with a large excess of cold iodide. The binding of both radiolabeled G3 variants ^{125}I-His6-G and ^{125}I-(HE)3-G3 was specific to HER2 in living cells which demonstrated that the labeling method did not affect the binding specificity of G3. Two types of interactions were identified between G3 variants and HER2-expressing SKOV3 cells. The presence of two interactions is commonly observed for anti-HER2 binding proteins, such as affibodies and ADAPTs. The second interaction could be explained by homo- and heterodimerization of HER2 receptors on the cell surface. A similar case was previously studied for EGFR receptors(Björkelund *et al.*, 2011). Cellular processing of both labelled DARPins was characterized by a rapid binding and a plateau. A decrease in cell-associated activity at 24 h post-addition could be accounted for diffusion of iodinated lipophilic radiocatabolites through cellular membrane after lysosomal degradation of DARPin.

CONCLUSION

The translation of both G3 variants to *in vivo* biodistribution studies is feasible and low hepatobiliaryuptake of (HE)3-G3 at the N-terminus of DARPin is anticipated. Clinical application of G3 labeled with other iodine isotopes, *e.g.*,^{123}I for SPECT or ^{124}I for PET imaging of HER2 expression could be envisioned. DARPin G3 and trastuzumab bind to different HER2 epitopes, therefore, imaging of HER2 using DARPin G3 could be used to monitor the effectiveness of ongoing trastuzumab therapy.

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Analysis of the Land Use and Land Cover Changes Using Satellite Remote Sensing in Bolgoda Lake, Sri Lanka: Spatial Monitoring with the Use of Remote Sensing

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ABSTRACT

The rapid utilization of remote sensing (RS) satellites and techniques has provided a reliable and effective platform to characterize terrestrial properties. Precise extraction of water bodies by using RS is significant at present. Multiple methods including supervised classification (Support Vector Machine (SVM)) and vegetation index method (Normalized Difference Water Index (NDWI), Modified Normalized Difference Water Index (MNDWI), and Normalized Difference Vegetation Index (NDVI)) were utilized in the investigation.

Bolgoda is considered the largest freshwater lake in Sri Lanka and is enriched with high biodiversity. But the urbanization and development of the Bolgoda area during the past decades has posed a serious threat to the presence of ecological systems, unambiguous water bodies which play a crucial part in supporting life. Numerous pressing problems have been identified in the Bolgoda area, that could lead to a decline in the quantity and quality of the habitat, create an environmental disproportion, affect biodiversity, and basically impair the overall health of the ecosystem.

RS Satellite images can play a significant role in the investigation, dynamic monitoring, and planning of natural surface water resources. Landsat-5 Thematic Mapper (TM) imagery and Landsat-8 Operational Land Imager (OLI) have high spatial, temporal, and multispectral resolution and therefore provide precise data to detect vicissitudes in the extent of water bodies.

This study has been conducted to detect the changes in water extent and quality during the period 2008 to 2017. It reveals a considerable reduction in water area,of 6.95%, and in vegetation area, of 8.95%, over the last nine years.

Key Words: Landsat, MNDWI, NDVI, NDWI, SVM

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INTRODUCTION

Remote sensing-based approaches play a major role in current environment-related monitoring and modeling, particularly in vegetation and water area extraction. In view of the tremendous momentary inclusion and period repeatability of satellite remote sensing, rapid and precise separate water data obtained by remote detecting has turned into an essential mechanical tool in water resource surveys, water resource monitoring, wetlands security, and disaster anticipation and reduction. Water data abstracting strategies, for example, supervised classification and threshold methods have advanced continuously. Spectral Ratio-based water list strategies, for example Normalized Difference Water Index (NDWI), Modified Normalized Difference Water Index (MNDWI), and Normalized Difference Vegetation Index (NDVI)) play an advanced role in water area detection, extraction, and monitoring.

Spectral knowledge is the most critical for separating Remote Sensing thematic data. Satellite Remote Sensing images record the impression of electromagnetic waves by ground objects and their very own outside radiation data. In contrast to different items, the water body demonstrates a powerless reflectivity, showed in the wavelength scope of noticeable light. Since the water body has the component of solid assimilation in the near Infra-Red (IR) band and mid-IR band, this wavelength range can be utilized to differentiate water from the soil, vegetation, structures, and other ground objects (Gulcan Sarp, Mehmet Ozcelik, *et al*., 2016).

Water on the Earth's surface is a fundamental piece of the hydrological cycle. Having the capacity to get to the spatial dissemination and geological degree data on water bodies continuously has extraordinary noteworthiness in limnology and for understanding relations between local hydrology and environmental change. The surface water element,predominantly freshwater lakes,is a vital part of the water cycle. It is also noteworthy in various scientific disciplines of water body extraction, and in the valuation of present and future water resources. It is also important for humans, food crops, and ecosystems. Therefore, precise extraction of the water surface is quite critical.

The objective of the study was to find any spatial changes in the land use and land cover (LULC) in the study area due to the recent development of nearby areas and further to assess the variation of water area over the other LULC types.

Study Area

Bolgoda covers almost two thirds of the Kalutara district, extending from Anguruwatota to Piliyandala. It is also the largest natural freshwater lake in Sri Lanka. It is located at longitudes 79˚55’ – 79˚58’E and latitudes 6˚40’ – 6˚48’N with adepth range of 20-50 feet. The lake has a catchment of 374 km^2. The Bolgoda Lake system consists of two small lakes, Bolgoda Lake North and Bolgoda Lake South and some streams that deliver fresh water to the Bolgoda Lake system, which finally discharges water into the ocean (Ranwella*et al*., 1995).

The Bolgoda Wetland, known for its natural beauty and wildlife habitat values, also naturally offers many water quality enhancements and controlling services. It is closely connected with the lives of the public in the area and hence many community-bio interactions exist. It is a rich fishery, on which many people depend for a living. An additional noticeable characteristic is the opportunity for the

hotel and hospitality industry, which is developing speedily upward in the area, which also depends on the wetland and its excellence. Protecting and restoring marshlands and their maintainable development can, therefore, contribute to the economic health, public safety, and quality of life of the public.

METHODSAND MATERIALS

Methodology of the Study

Methodology was based on support vector machine classification, vegetation indices (NDVI (Normalized Difference Vegetation Index), NDWI (Normalized Difference Water Index), and GNDVI (Green Normalized Difference Vegetation Index)), and analysis of the land use and land cover changes for 2007 to 2018. The overall experimental process in full detail is explained in Figure 1.

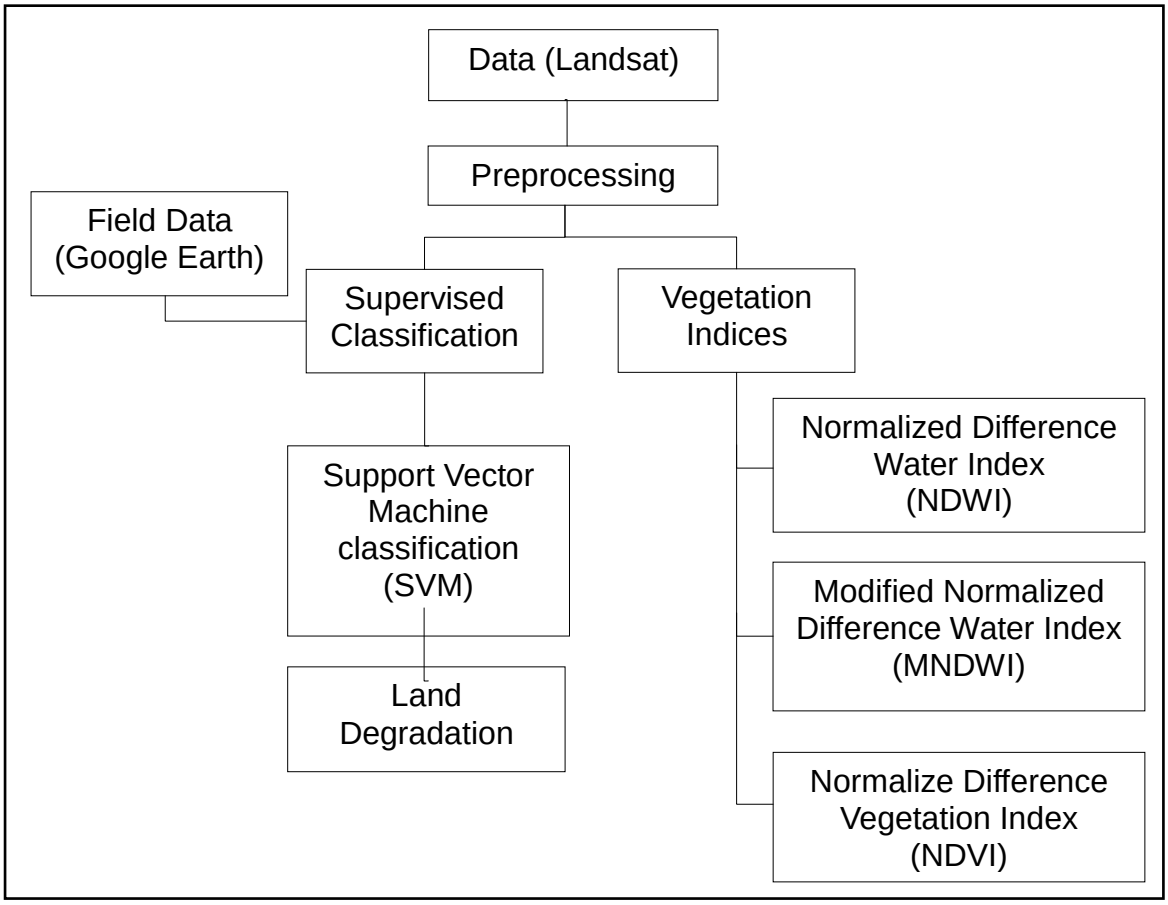

Figure 1: Complete workflow of the experiments conducted under the study

Data

The Landsat program is the longest-running innovation for acquisition of satellite imagery of the Earth. Itprovidesa platform for remote sensing applications on a medium resolution scale. In thisstudy Landsat-5 and Landsat-8 data were used as the data source.

Pre-processing

Landsat images of the lake for 2008, 2014, and 2017 were first radio metrically corrected and then atmospherically corrected using Fast Line-of-sight Atmospheric Analysis of Hypercubes (FLAASH). Processing

Supervised Classification

The training samples were collected from Google Earth, with unambiguous representatives for each class, namely Water, Vegetation, Buildings, and Sand. Support Vector Matching (SVM) supervised classification methods used for classification, and regression tasks that originated from statistical learning theory were implemented over the image.

SVMs are particularly appealing in the remote sensing field due to their ability to successfully handle small training data sets, often producing higher classification accuracy than the traditional methods. The underlying principle that benefits SVMs is the learning process that follows what is known as structural risk minimization. Under this scheme, SVMs minimize classification error on unseen data without prior assumptions made on the probability distribution of the data (Giorgos Mountrakis, Jungho Im, Caesar Ogole *et al*., 2010).

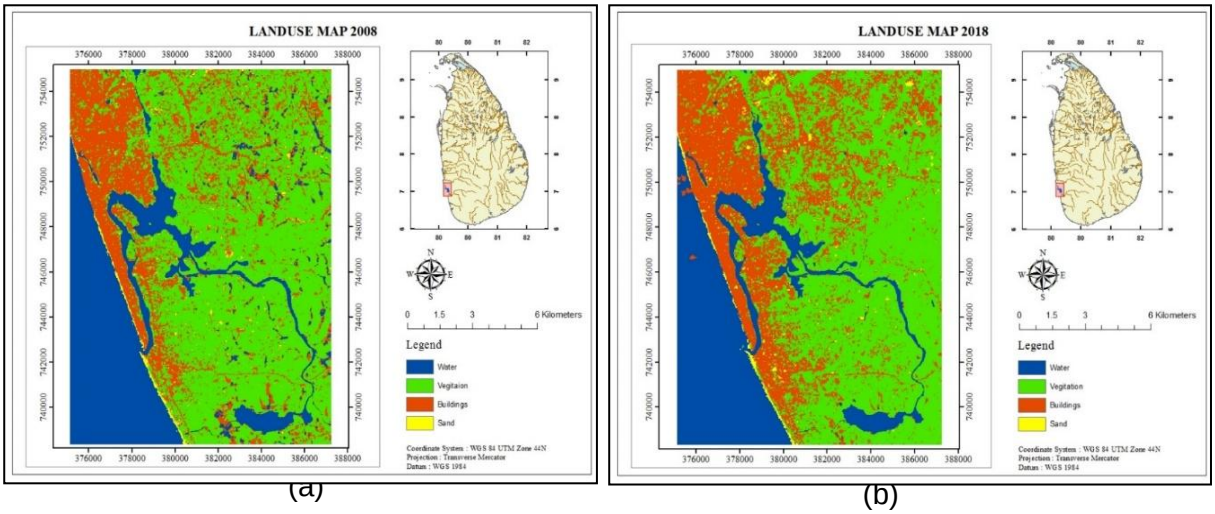

Figure 2: Support vector matching (SVM) classification resulted maps of Bolgoda lake and river network in (a) 2008 and (b) 2017.

Vegetation Indices

Several vegetation indices have been created by linear combination or proportions of RED, GREEN, SWIR,and close infrared spectral bands. Vegetation lists are more sensitive than individual bands to vegetation parameters.

Normalized Difference Water Index (NDWI)

NDWI is sensitive to changes in liquid water content of vegetation canopies. Since water has strongest absorption while vegetation has strongest reflectivity at near infra-red, Mcfeeters(1996) proposed the method of NDWI to highlight the water body, Equation 1.

"NDWI=" (("NIR- SWIR"))/((" NIR+SWIR"))

(01)

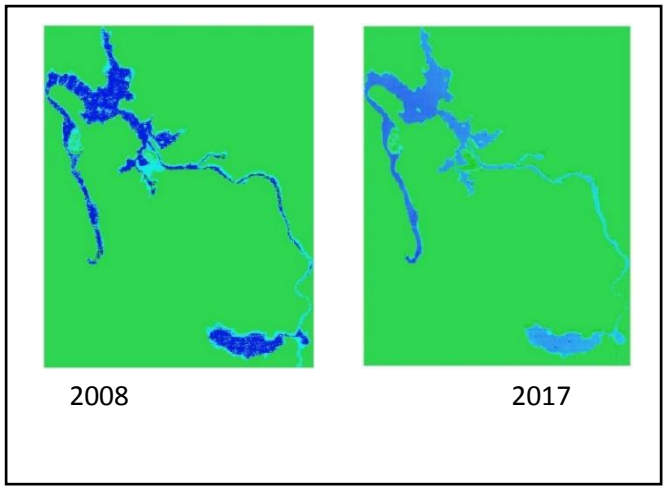

Figure 3: NDWI resulted images of Bolgoda lake and river network in 2008 and 2017.

Modified Difference WaterIndex (MNDWI)

The MNDWI method suggested has been commonly used and is a powerful index that can extract water bodies. The resulting values representing the water features have positive values because of their higher reflectance in band 2 than in band 5, and non-water features have negative MNDWI values, Equation 2,(Xu *et al.*, 2006).

"MNDWI=" (("GREEN- SWIR2"))/((" GREEN+SWIR2"))

(02)

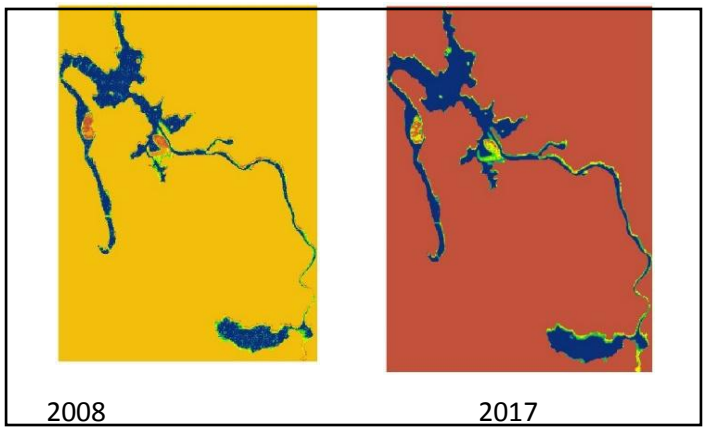

Figure 4: MNDWI resulted images of Bolgoda lake and river network in 2008 and 2017.

The Normalized Difference Vegetation Index (NDVI)

Evaluating the potential of NDVI for watershed monitoring and water quality studies is significant in acquiring an increased understanding of landscape water quality associations. Because of its ability to integrate both land cover and biophysical conditions, NDVI can be helpful in assessing regional watershed conditions that affect water quality and stream condition,Equation 3, (Roderick, M., R. C. G. Smith, and G. Ludwick, 1996).

"NDVI=" ((" NIR-RED"))/((" NIR+RED")) (03)

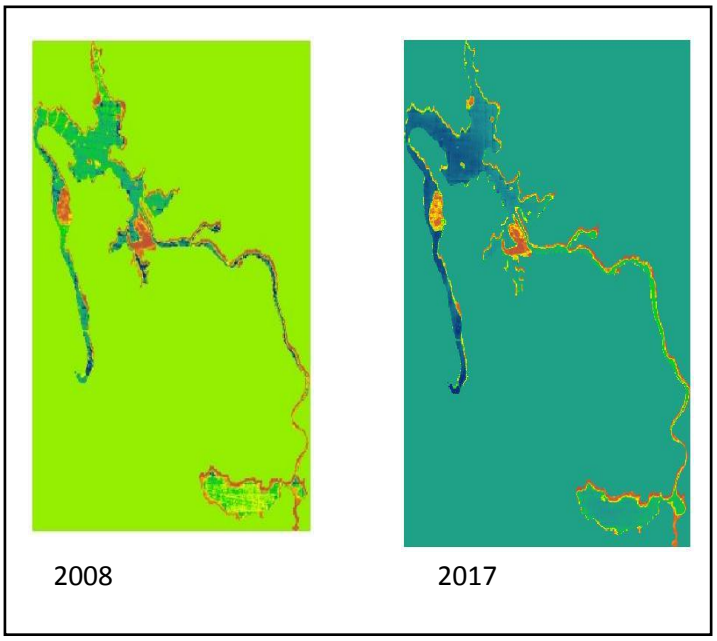

Figure 5: NDWI resulted images of Bolgoda lake and river network in 2008 and 2017.

RESULTS AND DISCUSSION

The study shows a considerable decrease in surface water area in the Bolgoda lake area during the past 09 years. The lake water area was extracted using SVM classification and according to the SVM algorithm, as a reference, demonstrates a reduction of about 3,400,000 m^2 which is equivalent to a diminution of 6.95%. The different features (water, vegetation, building, and barren land), areas, and number of pixels extracted from data of 2008 and 2017 using supervised classification is shown in Table 1. As evident from the table, water features and vegetation areas get diminished with the time while buildings and barren lands were expanding at the same time.

Urbanization is the process of the rapid growth of settlements and populations in urban areas. The Bolgoda basin has frequently encroached on grasslands and agricultural lands, which have been rehabilitated into commercial purposes due to urbanization. The study area has become significantly

urbanized within this time period, and an urbanized cluster has developed to the southern and western part from the main lake, as shown in Figure 6.

Table I: Supervised Classification Result of the study area

Feature	2008 km^2 (Pixels)	2017 km^2 (Pixels)	Change
Water	48.85(54274)	45.45(50500)	6.95% (Decrease)
Vegetation	107.48(119423)	97.86(108732)	8.95% (Decrease)
Buildings	43.32(48134)	55.80(62002)	28.81% (Increase)
Barren Lands	2.51(2793)	3.05(3390)	21.37% (Increase)

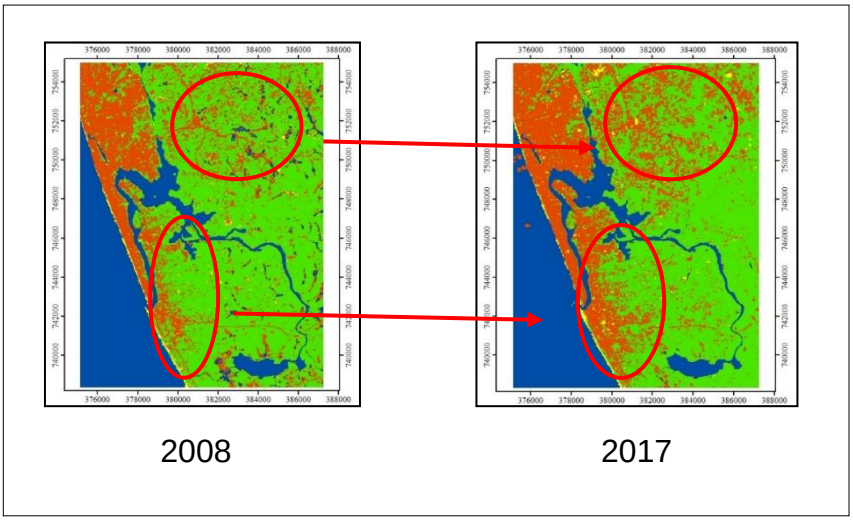

Figure 6: Expansion of urbanize cluster from 2008 to 2017

Moreover, according to the results of vegetation indices the quality of the water body also declined during this period due to the various threats in the Bolgoda area. There are several pressing problems identified in the Bolgoda area, that could lead to deterioration of the quantity and quality of the habitat, create an environmental imbalance, affect biodiversity, and basically impair the overall health of the ecosystem. Additionally, if these conditions continue it would obviously affect the social and environmental well-being of the society dependent on the ecosystem, and negatively influence sustainable development (SLWP *et al*., 2016). Some identified unhealthy interventions are:

- Cleaning of vegetation, especially mangrove areas for developmental activities
- Encroachment and illegal construction
- Filling parts of wetlands for developmental activities
- Loss of sensitive habitats

- Pollution from solid waste dumping, industrial effluents, and household sewage

Difficulties and Limitations of the Study

The major problem of working with optical images is that clouds and ground cover may cast shadows on the target. The mapping of the submerged vegetation using optical remote sensing from airborne sensors is a bit more difficult. It is not easy to find cloud-free images in this area. Hence the experiment was limited to using Landsat Images each year.

The subsequent factor remains atmospheric effects. The atmosphere absorbs and scatters light in a wavelength-dependent fashion. This absorption and scattering have several important implications that cause difficulties for sensor imaging. Atmospheric absorption, scattering, transmission, reflection, and refraction play a major role. FLAASH atmospheric correction is applied to correct the atmospheric errors.

All classification techniques have advantages and disadvantages, which are more or less important according to the data which are being analyzed, and thus have a relative relevance. SVM can be a useful tool for insolvency analysis, in the case of non-regularity in the data; for example when the data are not regularly distributed or have an unknown distribution, SVM provides virtuous result. SVM is an efficient classification method with clear ground truth samples. A clear Region of Interest (ROI) separability index may provide a good outcome.

It is better to use ground truth collection for supervised classification. Ground sample collection also helps to identify the current situation of the study area. In this study some areas of the water body were covered with algae and water plants. Consequently it was difficult to identify them with Google Earth images.

CONCLUSION

The Bolgoda area is home to many threatened species and is enriched with very high biodiversity, and therefore it is difficult to measure the value and the importance of this wetland area. It also works as a community protector in flood level balancing.

On the other hand, its natural beauty has great potential in an area bordering on Colombo for development, tourism, and fisheries, but it is gravely threatened by industrial pollution, with industrial chemicals, effluents from the hotel industry, pollution from sawmills, and the destruction of wetlands and mangrove swamps being some of the worst problems faced by the area. Eutrophication caused by pesticides and fertilizers used in agriculture and domestic waste also contaminates the lake This pollution has affected nearby drinking water and led to the growth of the weeds, which are slowly suffocating the lake´s fauna and flora.

If these conditions continue, we will lose this special ecosystem.This would indirectly affect the social and environmental well-being of society, and negatively influence sustainable development. As humans, we all have a responsibility to protect this treasured lake and river network for future generations, and it is time to take action to stop this destruction around the Bolgoda area.

ACKNOWLEDGEMENTS

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Production of single cell protein using pineapple, sour orange, and sour mango peel

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ABSTRACT

Protein deficiency has become a major challenge worldwide with the fast growing world population, thus, it is important to explore novel alternative methodologies to produce protein to meet the nutritional demand. In this backdrop, locally available pineapple, sour orange and sour mango peel wastes were studied for their suitability to produce single cell proteins using natural palmyrah (Borassusflabellifer) toddy yeast under liquid state fermentation system. Pineapple, sour orange and sour mango peel were collected, cleaned, washed and blended separately and their physico-chemical properties such as total soluble solid (TSS), pH, reducing sugar, moisture content, protein, fat and ash content were determined. The extract of fruit peels were filtered and diluted to 10% and autoclaved separately. The sterilized peel extracts were inoculated with 5 mL natural palmyrah toddy yeast ((1.625±0.15) × 10^6 cells/mL) and allowed for fermentation at 100 rpm for 48 h. The sediments were collected by centrifugation, oven dried and the dry weight was measured to determine the protein content. The biomass yield ranged from 8.61-9.40 g/L with the least biomass yield was observed with sour mango while the maximum yield was observed with pineapple. Pineapple peel generates significantly higher amount of protein (49.7± 1.3%) followed by the sour orange and sour mango peel (29.5 ± 1.2% and 24.6 ± 0.2% respectively). It is concluded thatnatural and locally available pineapple peel waste are good substrate for the production of protein-rich cell biomass using fermentation by natural toddy yeast of palmyrah**.**

Key words: Liquid state fermentation, Palmyrah toddy yeast, Pineapple peel, Single cell protein, Sour mango peel, Sour orange peel

INTRODUCTION

Single cell protein (SCP) is the dead, dried microbial cell protein or total protein extracted from pure microbial cell cultures, such as algae, bacteria, filamentous fungi, or yeasts which grow on different substrates. SCP has applications in animal feed and human food supplements. It is used for the production of animal feed for fattening calves, poultry, pigs, and fish. It is widely used in the food industry as aroma carriers, vitamin carriers, emulsifying aids, and to improve the nutritive value of baked products, soups, ready-to-serve meals, and diet recipes (Suman, *et al.*, 2015).

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Microbial protein has become popular for its high protein content (60-82% of dry cell weight), high efficiency in substrate conversion, high productivity, and requirement forless space (Nasseri, *et al.*, 2011). Besides high protein content, SCP also contains fats, carbohydrates, nucleic acids, vitamins, and minerals. SCP has a good nutrition profile, as it is rich in certain essential amino acids such as lysine and methionine, which are limiting in most plant and animal foods (Suman, *et al.*, 2015). High nucleic acid content and low cell wall digestibility are the two most important factors limiting the nutritional value of yeast for human consumption. Purine in nucleic acid is metabolized into uric acid, and its high concentration may result in gout and renal calculi. Therefore nucleic acid content of SCP must be reduced below 2% (Nasseri, *et al.*, 2011).

Microorganisms used for the production of SCP are bacteria (*Cellulomonas and Alcaligenes*), algae (*Spirulina and Chlorella*), molds (*Trichoderma, Fusarium, and Rhizopus*), and yeast (*Candida and Saccharomyces*) (Nasseri, *et al.*, 2011). Though bacteria has high protein content and short generation time over yeast and mold, it has own limitations such as poor public acceptance, difficulty in harvesting, and high nucleic acid content. Yeast is suitable for SCP production because of its good nutritional profile, easeof harvesting, and ability to grow at low pH, and has a long history of use in traditional fermentation (Nasseri, et al., 2011; Qiang & Iong-zong, 2016).

A wide variety of raw materials can be used for SCP production. Whey, orange peel residue, sweet orange residue, sugarcane bagasse, paper mill waste, rice husk, wheat straw residue, cassava waste, sugar beet pulp, coconut waste, grape waste, and mango waste are some examples (Mensah & Twumasi, 2017). Carbohydrate based substrates are the most widely used raw materials for SCP production due to the fact that sugars are natural microbial substrates (Ugalde & Castrillo, 2002).

Nearly 40% of fruit production goes into waste in Sri Lanka, mainly due to improper supply chain and value chain activities (Institute of Post Havest Technology , 2014). The non-edible portion of fruits, which accounts for about 10–60% of the total weight of the fresh produce,is discarded as waste. Postharvest losses of some fruits stand even higher (*e.g*., mango 30-50%, banana 20%, orange 30-50%) (Ruvini, *et al.*, 2018). Improper management of these wastes constitutes a public health risk and an environmental problem. SCP derived from various waste materials via microbial fermentation can play a vital role in waste management (Spalvins, *et al.*, 2018). There have been very few studies reported exploring the use of fruit wastes for SCP production. This study was aimed at selecting the best substrate for liquid state SCP production among pineapple, sour orange, and sour mango peel wastes.

METHODOLOGY

Materials

Pineapple, sour orange and sour mango peel were collected from local markets in Jaffna, Sri Lanka. Palmyrah toddy was collected from mature palm in Thikkam, Jaffna, Sri Lanka.

Physico-Chemical Analysis of Pineapple, Sour Orange, And Sour Mango Peel

Cleaned and washed pineapple, sour orange and sour mango peels were weighed and the moisture, protein, fat, and ash content were determined using AOAC (2006) methods. The total soluble solids

(TSS) and pH were determined by refractometer and pH meter respectively. The reducing sugar content was determined by a spectrophotometric method (Miller, 1959).

Preparation of Fruit Waste Medium

The collected pineapple, sour orange, and sour mango peels were separately cleaned with distiled water, macerated using the blender into a slurry, and filtered through Whatman No 1 filter paper. The solid content and the pH of the extracts were determined using a refractometer (Atago – DR-A1, Japan) and a digital pH meter (Ohaus - Starter 2100, USA) respectively. The extracts were diluted to 10% using distilled water and sterilized in an autoclave at 121^0C at 15 psi for 15 min, seperately. These sterilized diluted peel extracts were designated as Fruit Waste Medium (FWM),and was used as substrate to select the best fruit peel for the SCP production. Fresh palmyrah toddy samples were used as the source of natural yeast, *Saccharomyces cerevisiae*. The viable cell count was determined using a haemocytometer.

Production of SCP in Liquid State Fermentation

Sterilized pineapple, sour orange, and sour mango peel substrates (50 mL) were transferred into pre-sterilized conical flasks in triplicate under sterile conditions. Then the each 50 mL of sterilized medium was inoculated with 5 mL of fresh palmyrah toddy sample ((1.63 ±0.15)×10^6cells/mL) and allowed for fermentation in shaking incubator (Lab companion SI-600, USA) at a speed of 100 rpm for 48 h at 28°C. After 48 h, the content was centrifuged (4000 rpm for 20 min) and the residue was oven dried (50°C for 16 h) and weighed. Protein content was determined on the basis of total nitrogen content (N×6.25) using Kjeldahl method as per the protocol explained in AOAC, 2006 (Dhanasekaran, et al., 2011).

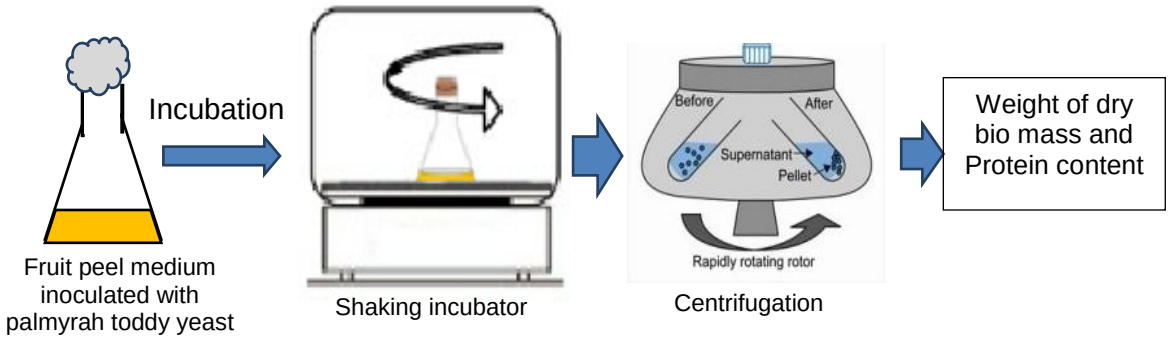

Figure 1. Flow diagram for SCP production from fruit peel

Statistical Analysis

All the experiments were carried out in triplicates and the results were presented as the average of triplicates ± standard deviation. Minitab 17 (Minitab Inc., State College, PA, USA) statistical package was used to execute all statistical analysis. The data were analyzed using ANOVA. Tukey's multiple comparison test was used to determine significant differences at $p < 0.05$.

RESULTS AND DISCUSSION

The physico-chemical properties such as pH, the total soluble solid, reducing sugar, moisture, fat, protein, and ash content of the pineapple, sour orange, and sour mango peel have been presented in the Table 1.

The composition of all the waste samples was significantly different for tested parameters such as moisture, protein, fat, ash, and reducing sugar content. Pineapple peel exhibited the highest moisture content, protein and reducing sugar content and less fat as compared to other peel sample. Sour orange contains higher amounts of protein (7.2±0.1%), while sour mango contains higher amounts of total soluble solids (16.8%) compared to other wastes. Overall results of compositional analysis indicated that the selected pineapple, sour orange, and sour mango peel can be used as potential substrates for microbial growth, due to their reasonable carbohydrate and mineral content.

Table 1: Physico-chemical properties (on dry weight basis) of pineapple peel, sour orange and sour mango peel.

Physico-chemical property	Fruit peel		
	Pineapple	Sour mango	Sour orange
pH	3.7	4.1	4.1
TSS (%)	10.8	16.8	12.3
Reducing sugar (g/L)	27.8 ± 0.9^{a}	25.3 ± 2.6^{a}	12.3 ± 1.3^{b}
Moisture content (%)*	84.7 ± 0.9^{a}	76.2 ± 0.2^{b}	74.8 ± 2.5^{b}
Ash content (%)*	$4.5 \pm 0.3^{a,b}$	4.2 ± 0.5^{b}	6.1 ± 1.0^{a}
Fat content (%)*	0.9 ± 0.1^{c}	2.5 ± 0.3^{a}	1.4 ± 0.1^{b}
Protein content (%)*	6.9 ± 0.1^{a}	6.2 ± 0.2^{b}	7.2 ± 0.1^{a}

Results are expressed as mean of triplicate ± standard deviation.

*Values are in dry weight basis

The values in the same row with different superscripted letters are significantly different (P≤0.05).

Most of the findings of the present study for physico-chemical composition of fruit wastes are similar to the results of various other studies (Imran, *et al*., 2013; Moraisa, *et al*., 2017). The variation in the findings of the present investigation may be due to varietal, cultivar, environmental, and soil condition differences (Barta, 2002).

Selection of the Best Substrate

This study was planned to assess the potential of various fruit wastes for cost effective biomass production. Pineapple, sour orange, and sour mango peel extract were used as fermentation media to harvest dry biomass. The dry mass and protein content were recorded (Table 2).

There is no significant difference in the dry biomass yield and the results obtained were in the range 8.61-9.40 g/L (Table 2). The least biomass yield was obtained for sour mango and the maximum yield was obtained for pineapple. The highest crude protein yield was observed in the medium containing

pineapple (49.7 ± 1.3%), followed by sour orange (29.5 ± 1.2%) and sour mango (24.6 ± 0.2%) (Table 2). Based on the results, pineapple peel extract can be a reasonably suitable substrate for SCP production. The higher reducing sugar content in pineapple peel waste compared to other media favors the higher yield of SCP (Table 1).

Table 2: Dry biomass content and crude protein content of SCP from pineapple, sour orange and sour mango peel medium.

Peel type	Dry biomass (g/L)	Crude protein content (%)
Pineapple	9.40 ± 0.53[a]	49.7 ± 1.3[a]
Sour orange	9.13 ± 0.64[a]	29.5 ± 1.2[b]
Sour mango	8.61 ± 0.90[a]	24.6 ± 0.2[c]

Results are expressed as mean of triplicate ± standard deviation

Means in the same column that do not share the same superscripted letter are significantly different.

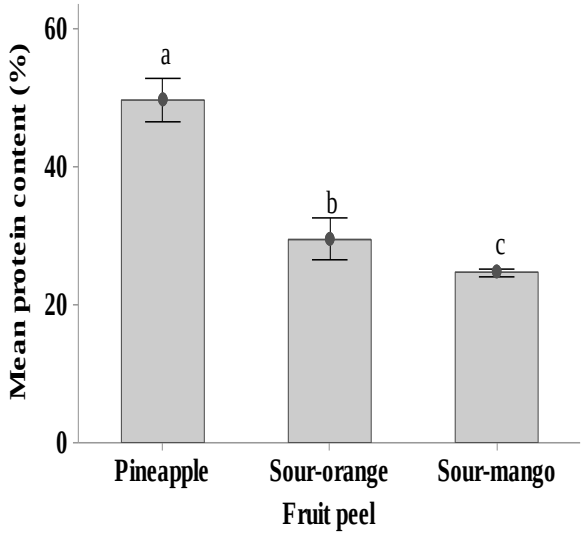

Figure 2: Crude protein content of SCP from pineapple, sour orange, and sour mango peel medium

Some of the previous studies carried out adding supplements such as nitrogen, carbon, and glucose sources were used for the biomass production on waste materials (Mondal, *et al*., 2012). No such supplements were used in the present study to grow yeast culture on selected fruit peel, thus this process of SCP production can be considered cheaper. The above results established that pineapple, sour orange, and sour mango peel extract and palmyrah toddy yeast can be used to produce SCP. This biomass could be recommended as a food or feed after appropriate food quality testing. Since fruit waste were used as substrate in the present study, it plays a vital role in waste management (Suman, *et al*., 2015).

CONCLUSION

Locally available pineapple peel waste can be suggested as the best substrate among the pineapple, sour orange, and sour mango peel for the production of SCP using natural palmyrah toddy yeast through fermentation. SCP produced using pineapple peel as the substrate resulted in 49.7 ± 1.3% of protein with a yield of 9.40 ± 0.53 g/L.

ACKNOWLEDGEMENT

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I. GENERAL INSTRUCTIONS

1) Research presented in the manuscript could be in any field of science. 2) The research work should not have been published or submitted for publication elsewhere. 3) A corresponding author who will be responsible for all communications with the SLAAS Office should be identified. 4) Submission of manuscripts: Manuscripts can be submitted by e-mail or regular mail to the Editor – SLAAS on or before 31st July, 2018. In case of submissions made through regular mail, The authors should forward three (03) hard copies of the manuscript and an electronic copy on a compact disc (CD) along with all other required documents. Authors are advised to mail the documents at least three (03) weeks prior to the deadline. 5) Certificate of authenticity: Declaration form attached hereto should be duly filled, signed by all authors and sent along with the manuscript. The authors who transfer the documents electronically should send the Certificate of Authenticity by regular mail. 6) Information of corresponding author: Duly filled form should be sent by regular mail. 7) Submissions that involve human or animal trials should provide evidence of approval obtained by an ethics review committee.

II. SPECIFIC INSTRUCTIONS TO AUTHORS

1. Documentto be submitted ! Manuscript in MS Word (2003 or 2007 for Windows or later)format. ! A compact disk (CD) containing an electronic copy of the manuscript (for submissions by regular mail only). ! Duly filled and signed 'Certificate of Authenticity' form. ! Duly filled 'Information of Corresponding Author' form.

2. Format for typesetting
   - Paper size: A4 (210 x 297) typed single sided only.
   - Margins: Top, bottom and right margins of 25 mm and a left margin of 30 mm. 2
   - Line spacing: 1.5 (18 points) throughout the text.
   - Length: Length of the manuscript including text, tables, figures and references should not exceed I5 typed pages.
   - Page and line numbering: All pages should be sequentially numbered using Arabic Numbers. All lines should also be numbered sequentially starting from the top to the bottom of each page.
   - Font: Arial font, size 12. ! Language/spelling: UK English only.
   - Software: Authors may use either MS Word 2003 or 2007/2011 for Windows or the Macintosh equivalent.
3. Title Page: Title page should include the following Information;
   - Title and running title (less than 25 Characters). They should be in bold faced letters
   - Name/s and affiliation/s of author/s
   - Email address, mailing address and contact numbers of the corresponding author. Note: Identified the corresponding author by placing an asterisk after the name.
4. Abstract

   Should be limited to a maximum of 250 words.

   Up to a maximum of the five (05) key word should be identified, arranged in alphabetical order, included immediately after the abstract.

   Abstract should be typed in italics. Scientific names in the abstract should be underlined.

   No reference, tables, or figures should be included in the abstract.

5. Body
   - Introduction: Justification of the research work, objectives and hypotheses should be included in the introduction.
   - Methods and Materials/ Methodology: All materials, chemicals, clinical, subjects and samples used should be identified. Analytical, survey and statistical method should be explained concisely. Common analytical methods need not be elaborated.
   - Results and Discussion: Can be combined.
   - Conclusions: Should be concise.
   - Headings: All headings should be in bold capital and centered, e.g., INTRODUCTION
   - Subheadings: All subheadings should be in bold and in title case, e.g., Preparation of Land.
   - Non-English terms: All non-English terms should be italicized, e.g., et al., i.e., viz., except "etc."
   - References: Use APA style 3
6. Table and Figures
   - Should be included in the exact place within the text
   - Tables should be numbered sequentially using Arabic numerals. The titles should be selfexplanatory and placed above the tables.
   - Tabled should not contain any vertical lines
   - Illustration, Line drawing and photographs, if any, should be clear, properly numbered and captioned and ready for reproduction. They should be of high and resolution such as minimum of 300 dpi and saved in .tif or .bmp formats. Please do not use .jpeg or similar formats that do not reproduce well.
   - All lettering, graph lines and points on graphs should be sufficiently large and bold faced to permit reproduction for inclusion in the Journal.
   - Artworks and illustrations should be of appropriate thickness. Please note that thin lines do not reproduce well. Please note that the illustrations, line drawings and photographs should be placed in the appropriate location of the electronic file and numbered sequence with other figures.
7. Units
   - SI units should be used.
   - A single space should be left between the numerical value and the unit.
8. Acronyms and Abbreviations
   - All acronyms should be written in full at the first time of appearance. Abbreviations can be used subsequently.
   - The full stop should not be included in abbreviations. Where abbreviations are likely to cause ambiguity or may not be readily understood by readers, the units should be mentioned in full.
9. On being informed of the acceptance, the manuscripts should be revised as per the reviewers' suggestions and re-submitted to the Editor – SLAAS. The accepted manuscripts will be published in the inaugural Journal of the SLAAS. Manuscripts that do not confirm to the above guidelines will not be accepted.
10. Acknowledgements Only the essential individuals and/or organizations/institutes should be include

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