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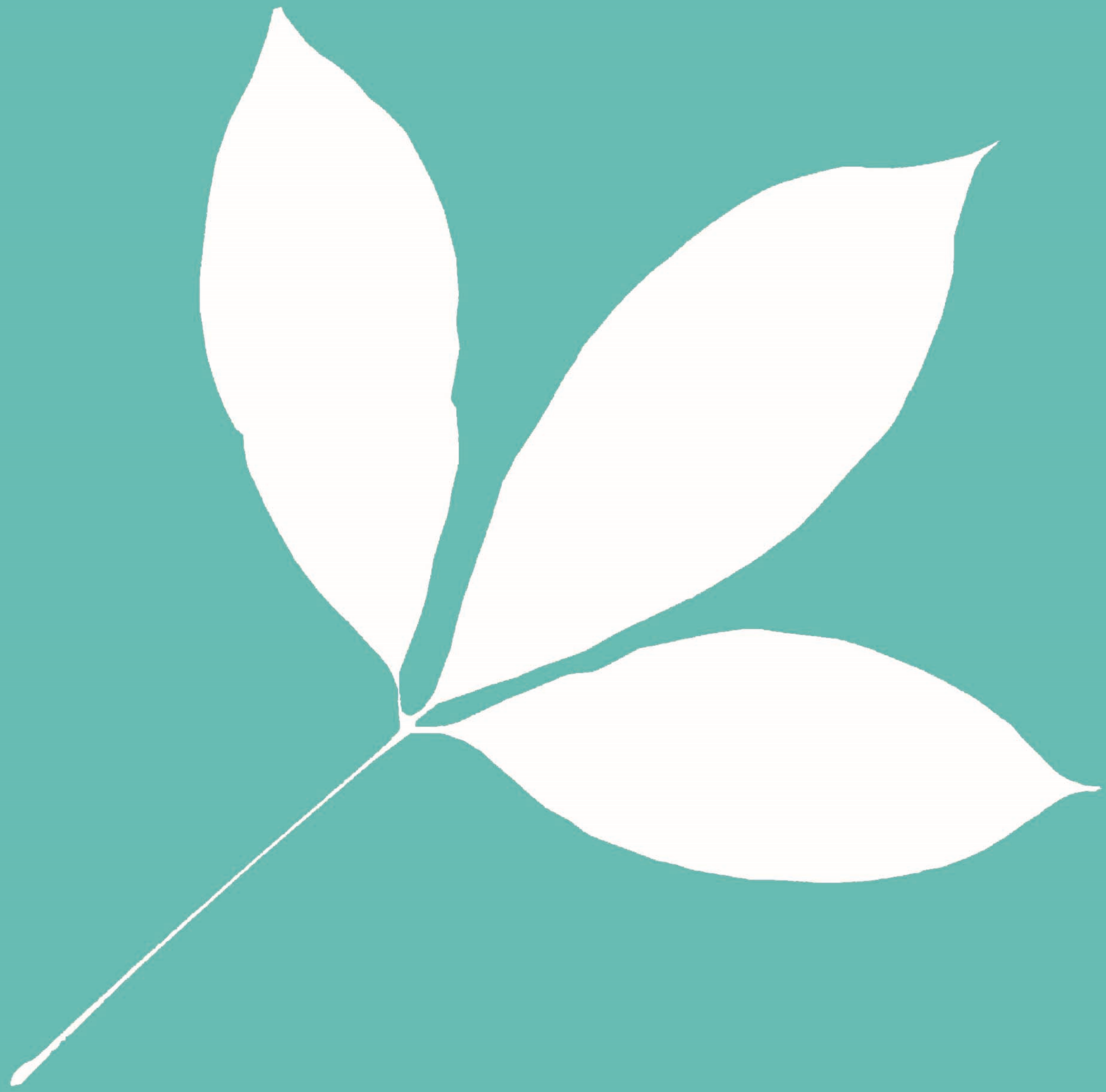

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CONTENTS

JOURNAL OF THE RUBBER RESEARCH INSTITUTE OF SRI LANKA VOL. 95 – 2015

JOURNAL OF THE RUBBER RESEARCH INSTITUTE OF SRI LANKA VOL. 95 - 2015

JOURNAL OF THE RUBBER RESEARCH INSTITUTE OF SRI LANKA VOL. 95 – 2015

JOURNAL OF THE RUBBER RESEARCH INSTITUTE OF SRI LANKA VOL. 95 – 2015

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Effect of latex coated coconut fibre on cure characteristics and physico-mechanical properties of natural rubber-coir composites

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Abstract

A wide variety of natural fibres such as coconut fibre (coir), bagasse, banana fibre, sisal, etc. *can be used to reinforce polymers. Natural fibre reinforced, biodegradable composites are eco-friendly. However, the interfacial adhesion between natural fibre and most of the biodegradable polymers is not adequate. The objective of this study was to modify readily available coir by coating with natural rubber (NR) as well as synthetic rubber latices with the aim of improving adhesion between coir and rubber in order to develop a coir filled NR based composite suitable in manufacture of tyre treads.*

Coir was coated with different compounded latices namely, neoprene, nitrile and NR and with uncompounded NR latex. Thereafter, composites were produced by mixing 15 phr of the latex coated coir fibres with virgin NR according to a tyre tread formulation and properties of the composites were evaluated and compared. Results revealed that processing safety of the compounded NR latex coated coir filled rubber composite is higher than that of the other three composites, whereas uncompounded NR latex coated coir filled rubber composite showed a similar processing safety to that of the two synthetic latex coated coir filled rubber composites. However, the two NR latex coated coir filled rubber composites were faster curing than the synthetic latex coated coir filled rubber composites. Minimum torque results indicated that compounded NR latex coated coir filled rubber composite has a higher processability when compared to the other composites. However, the latter composites indicated a higher state of cure and cross-link density in comparison to the former composite. Tensile strength, elongation at break, resilience and abrasion volume loss of the compounded NR latex coated coir filled rubber vulcanisate are superior to those of the other three vulcanisates.

In overall cure characteristics and mechanical properties, especially abrasion resistance of the composite based on compounded NR latex coated coir indicates that it could be used in manufacture of tyre treads.

Keywords: coir, natural rubber composites, natural rubber latex, neoprene latex, nitrile latex

Introduction

Natural fibres are generally lignocellulosic in nature, consisting of helically wound cellulose microfibrils in a matrix of lignin and hemicellulose. Natural fibres have been used to reinforce materials for over 3,000 years. More recently they have been employed in combination with rubber and plastics (Sakdapipanich *et al.*, 2007). Many types of natural fibers have been investigated for use in rubber and plastic including coconut fibre (coir), bamboo fibre, oil palm fibre, isora fibre, banana fibre, sisal, *etc*. Natural fibres have the advantage that they are renewable resources and have marketing appeal. They are increasingly used in automotive and packaging materials.

Natural fibres degrade faster than synthetic fibres in natural environments, minimizing environmental pollution. However, biodegradation of natural fibre can decrease the life time of fibre reinforced polymer composites. To prolong their performance in natural environments, natural fibres are treated to protect from any circumferential agents. Hydroxyl groups from cellulose and lignin are natural substrates for modification. In addition, coating of fibres often reduces water absorption, protection from bacteria and fungi attack. The treatment methods which have been used for fibre reinforced polymer composites are not suitable for protection of the fibres in contact with soils (Farshid *et al.*, 2011).

Coir is a versatile lignocellulose fibre obtained from the coconut tree (*Cocos nucifera*), which grows extensively in tropical countries. Because of its hard-wearing quality, durability and other advantages, it is used for making a wide variety of floor furnishing materials, yarn and rope. However, these traditional coir products consume only a small percentage of the potential total world production of coconut husk. In the past, composites of coconut fibre/natural rubber (NR) latex were extensively used by the automotive industry (Geethamma *et al*, 1998) and presently mattresses, pots, *etc.* are widely produced using this combination. Apart from the conventional uses of coir as mentioned above, research and development work has been conducted to find new applications for coir, including utilization of coir as a reinforcing material for plastics (Owolabi *et al.*, 1985).

Coir is an inexpensive fibre among the various natural fibres available in the world. Furthermore, it possesses the advantages of a lignocellulose fibre. It is not toxic and possesses no waste disposal problem. Unfortunately, the performance of coir as a reinforcement in polymer composites is unsatisfactory and not comparable even with other natural fibres. This inferior performance of coir is due to various factors such as its low cellulose content, high lignin and hemicellulose content, high microfibrillar angle and large and variable diameter (Geethamma *et al*., 1998).

The hydrophobic NR matrix and hydrophilic cellulose fibre can be made compatible through modification of polymer or fibre surface. The extent of adhesion is usually increased by the use of bonding agents and chemical modification of fibres. The effects of a silane coupling agent (Si69) on curing characteristics and mechanical properties of bamboo fibre filled NR composites were studied (Ismail *et al.,* 2002). The mechanical properties of composites such as hardness, modulus, tensile and tear strengths improved with the addition of Si69. Chemical treatment of cellulose fibres usually changes the physical and chemical structure of the surface. The use of maleic anhydride grafted copolymer and alkaline treatment of the natural fibre are the most used techniques with the aim to improve the fibre-matrix interfacial bonding (Ku *et al*., 2011). Arumugam *et al*. (1989) evaluated mechanical properties of NR composites prepared with pristine and sodium hydroxide treated coconut fibers. They added the same coupling agents as Ismail *et al*. (2001) in order to improve rubber-filler interactions. Results of the compounds prepared with pristine and treated fibres were compared with those of untreated fibres and an enhancement in the mechanical properties was observed. It was reported that alkali treatment on coir fibre enhances the thermal stability and maximum moisture retention (Mahato *et al*., 1993). Prasad *et al*. (1983) reported that the use of alkali treatment on coir fibres improves the mechanical properties of coir-polyester composites.

Jacob *et al*. investigated the effect of chemical modification of banana fibre in NR (Communicated). Modification of banana fibre resulted in superior mechanical properties. Chemical modification of both sisal and oil palm fibres was imperative for increased interfacial adhesion and resulted in enhanced properties (Jacob *et al*., 2004). The influence of alkali treatment on oil palm fiber reinforced NR composites was investigated by Joseph *et al*. (2006). They observed superior mechanical properties for the composites after chemical modification due to better adhesion. Effects of alkali, silane coupling agent and acetylation on oil palm fibres have been studied by Mahato *et al*. (1993). De *et al*. (2006) were able to improve adhesion between grass fiber and NR matrix by alkali treatment.

Chemical modification of pineapple leaf fiber in NR was investigated by Lopattananon *et al*. (2006). Sodium hydroxide and benzoyl peroxide were used to treat the surfaces of fiber. It was found that all surface modifications enhanced adhesion and tensile properties. The effects of different chemical treatments, including mercerisation, acetylation, benzoylation and treatment with toluene diisocyanate (TDI) and silane coupling agents, on isora fibre properties and mechanical properties were analyzed. Isora fibre was seen to have immense potential as reinforcement in NR. Acetylation and TDI treatments gave higher tensile strength values compared to other treatments (Mathew *et al*., 2004). Maize stalk fibres were also chemically treated with acetic anhydride (acetylation) to enhance their compatibility with the hydrophobic rubber matrix (Chigondo *et al*., 2013). The NR-maize stalk fibre filled composites showed good processing safety and when compared with untreated NR-maize stalk fibre composites, the acetylated composites exhibited higher mechanical properties, reduced moisture absorption and higher resistance to hydrothermal aging.

The effect of plasma treatment on cellulose fibers in NR composites was investigated by Ahlblad *et al*. (1994). Chemiluminescence analysis was used to indicate the grafting on the surface of the cellulose fibres and also to estimate the effect of the plasma on the cellulose fibres. The results indicated the possibility of obtaining a surface layer on the fibres, which would lead to improvement of mechanical properties of rubber composites.

The use of chemical treated natural fibres such as coir in composites has increased due to their benefits such as high strength with very low weight and recyclable nature. However, no work on latex coating of natural fibres has been reported in the past. Hence, this research was conducted to study the effect of latex coating of coir on properties of NR-coir composites.

Experimental

Materials

Neoprene and nitrile latex compounds were supplied by Dipped products Plc., Sri Lanka. Compounded NR latex, uncompounded NR latex and virgin NR (RSS 2) were purchased from a local supplier. Zinc oxide (ZnO), stearic acid, carbon black (N 330-high abrasion furnace black), rubber processing oil (Dutrex R), IPPD (N-isopropyl, N’-phenyl paraphenylene diamine), TMQ (2,2,4 trimethyl 1,2-dihydroquinoline), TBBS (N-t-butyl-2 benzothiazole sulphenamide) and sulphur were purchased from the local market. Coconut fibre (coir) was supplied by a local supplier and was used as received.

Method

Coconut fibre was coated with compounded neoprene latex, compounded nitrile latex, compounded NR latex and uncompounded NR latex. Neoprene and nitrile latices were chosen as coating materials for coir as these are readily available and existence of some similarities between each of the structures of the two synthetic rubbers and the structure of NR. Further, better adhesion between each of the two polar synthetic rubber latices and coir would be expected due to the presence of hydroxyl groups in cellulose and lignin of coir. Four composites were produced by mixing the latex coated fibres with virgin NR according to the formulations given in Table 1.

Table 1. *Formulations of latex coated coir filled rubber composites*

Ingredient	**Formulation**			
	S1	**S2**	**S3**	**S4**
Natural rubber (RSS 2)	100	100	100	100
Zinc oxide	5.0	5.0	5.0	5.0
Stearic acid	1.5	1.5	1.5	1.5
Processing oil	3.0	3.0	3.0	3.0
Sulphur	2.5	2.5	2.5	2.5
Flectol H	1.0	1.0	1.0	1.0
MBT	1.5	1.5	1.5	1.5
Uncompounded NR latex coated coir	15	00	00	00
Compounded neoprene latex coated coir	00	15	00	00
Compounded nitrile latex coated coir	00	00	15	00
Compounded NR latex coated coir	00	00	00	15

Properties of the composites containing compounded latex coated fibres were evaluated and compared with those of the composite containing uncompounded NR latex coated fibres.

The mixing cycle used in the preparation of latex coated coir filled rubber composites is given in Table 2. The first stage of mixing was carried out using a Baker Perkins Engineers, Petersborough and London, laboratory Banbury and the second stage was carried out using a David Bridge & Co. Ltd. Castleton Rochdale, England, laboratory two-roll mill (6” x 13”).

Table 2. *Mixing cycle used in the preparation of latex coated coir filled rubber composites*

Stage 1: Internal mixer	**Total time (min.)**
Added NR (RSS 2)	0
Added ZnO + Stearic acid + Flectol H	2
Added ½ (latex coated coir + oil)	4
Added remaining ½ (latex coated coir + oil)	6
Dumped	7
Stage 2: Two-roll mill	
Added MBT	8
Added sulphur	9
Sheeted out	10

Determination of cure characteristics of the latex coated coir filled rubber composites

Cure characteristics such as the minimum torque (M_L), maximum torque (M_H), scorch time (TS_2), optimum cure time (T_{90}), cure rate index (CRI) and delta cure (M_H-M_L) of the four rubber compounds were obtained by a MDR 2000 Moving Die Rheometer (M/S Alpha Technologies, USA) at 150°C.

Preparation of latex coated coir filled rubber vulcanisates

The four latex coated coir filled rubber composites were placed in test piece moulds and pressed between the platens of a hydraulic press (Yeji Corporation, Taiwan). The samples were cured at 150°C temperature and at an applied pressure of 20 MPa according to respective optimum cure times obtained from the rheographs. After curing, the test pieces were removed from the moulds and immediately cooled under tap water to prevent further curing.

Measurement of physico-mechanical properties of the latex coated coir filled rubber vulcanisates

A GotechTesting Machines Inc. tensile testing machine was used to measure the tensile properties of the rubber vulcanisates in accordance with ISO 37: 2011 at room temperature (27±2°C) at a grip separation rate of 500 mm/min. Tear strength of the vulcanisates was measured using angle (Die B) test pieces with the aid of the same machine as per ISO 34-1:2010. Hardness of the vulcanisates was measured by a “Digi Test” hardness tester for hardness in the IRHD N-scale as per ISO 48:2010. Resilience of the vulcanisates was measured by a Wallace Lupke pendulum in accordance with ISO 4662: 2009. Abrasion volume loss of the vulcanisates was determined using a DIN abrasion tester in accordance with DIN 53516.

Determination of aging properties of the vulcanisates

Tensile and tear strengths were evaluated after aging. Aging was carried out in an air circulating oven at 100°C (Sanyo Gallenkamp, UK) for 22 h according to ISO 188: 2011.

Results and Discussion

Cure characteristics of the latex coated coir filled rubber composites

Cure characteristics of the four rubber composites are given in Table 3.

Minimum torque (M_L) is an indication of the processability and is related to the stock viscosity of the compound. The minimum torque of compounded NR latex coated coir filled rubber composite (S4) is lower and hence the processability is higher than that of the other composites (Table 3) and it may be due to better distribution of the compounded NR latex coated coir in the NR matrix. Maximum torque (M_H) indicates the state of cure and delta cure (M_H-M_L) is an indication of the cross-link density. M_H and delta cure of compounded NR latex coated coir filled composite is lower than those of the others. Neoprene latex coated coir filled composite (S2) has the highest crosslink density compared to S1 and S4 composites (Table 3).

Table 3. *Cure characteristics of the latex coated coir filled rubber composites*

Sample No	Minimum torque (M_L) dNm	Maximum torque (M_H) dNm	Delta cure (M_H-M_L) (dNm)	Scorch time (ts_2) min.	Cure time (t_{90}) min.	Cure rate index min^{-1}
S1	2.25	9.34	7.09	0.31	2.27	51
S2	2.39	12.90	10.51	0.29	3.32	33
S3	2.42	11.48	9.06	0.31	3.11	36
S4	1.62	7.66	6.04	1.13	2.34	83

S4 composite containing compounded NR latex coated coir has the highest scorch time (ts_2) or in other words the highest processing safety. However, there is no significant difference between the scorch time of the composites S1, S2 and S3. Table 3 indicates that composites S1 and S4 containing NR latex coated coir are faster curing than the other two composites. In S1 and S4 composites, there are more crosslinking positions due to the presence of a greater number of double bonds and this is probably the reason for faster cure of the composites.

Physico-mechanical properties of latex coated coir filled rubber vulcanisates

Hardness variation of the four rubber composites is shown in Figure 1. This figure shows that there is no significant difference between the hardness of S2 and S3 vulcanisates and they are markedly higher than that of the S1 and S4 vulcanisates. When synthetic latices are coated on coir it becomes stiff due to reduced elasticity and this is probably the cause for higher hardness shown by the S2 and S3 vulcanisates compared to S1 and S4 vulcanisates.

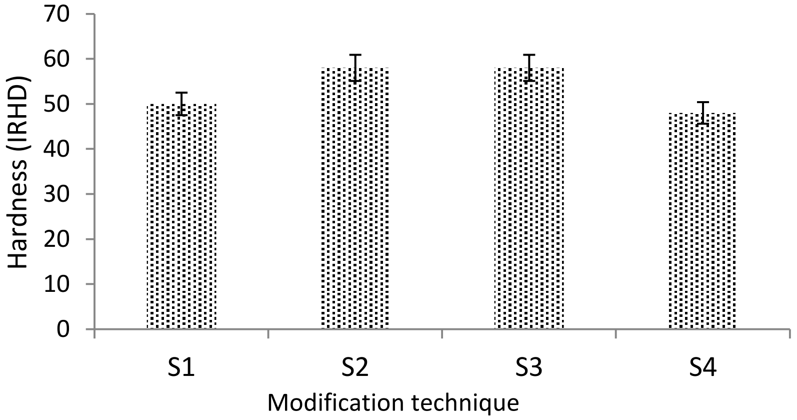

Fig. 1. Variation of hardness of latex coated coir filled rubber vulcanisates

Similar to hardness, modulus at 300% elongation of synthetic latex coated coir filled vulcanisates is markedly higher than that of the NR latex coated coir filled vulcanisates (Fig. 2).

As expected, tensile strength of the NR latex coated coir filled vulcanisates is greater than that of the synthetic latices coated coir filled vulcanisates (Fig. 3). This indicates good adhesion between virgin NR and NR latex coated coir. In other words, NR latex coating has improved adhesion between NR and coir. Jacob & Anandjiwala (2009) reported that when fibres are aligned parallel to the stress direction, tensile strength develops a characteristic drop with increasing fibre volume content until a critical fibre level is reached. Further, it is important to ensure homogeneous fibre dispersion in order to achieve maximum strength and performance of the composite materials (Visakh *et al.*, 2012).

There is no significant difference between the elongation at break of S1 and S4 vulcanisates (Fig. 4). The lower hardness shown by the S1 and S4 vulcanisates reflects higher elongation at break for the same compared to S2 and S3 vulcanisates. However, elongation at break of all the four vulcanisates is at a level acceptable for tyre treads. Generally, higher homogeneity or compatibility indicates higher elongation at break. Since S1 and S4 vulcanisates are more homogeneous, higher elongation at break has resulted for the same.

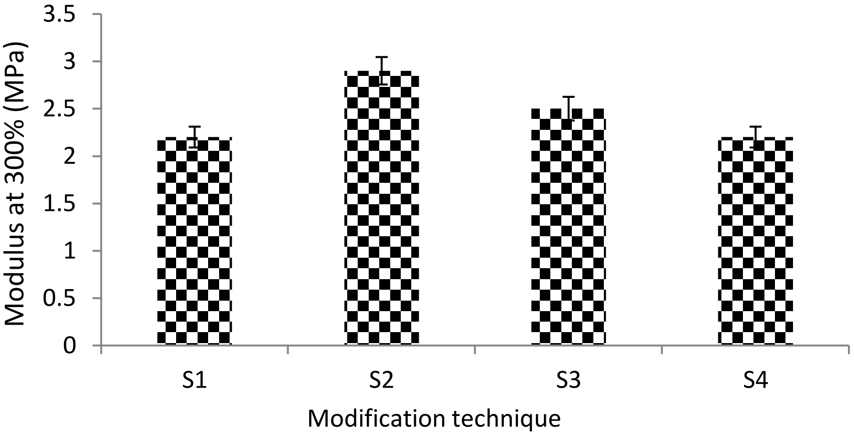

Fig. 2. Variation of modulus at 300% elongation of latex coated coir filled rubber vulcanisates

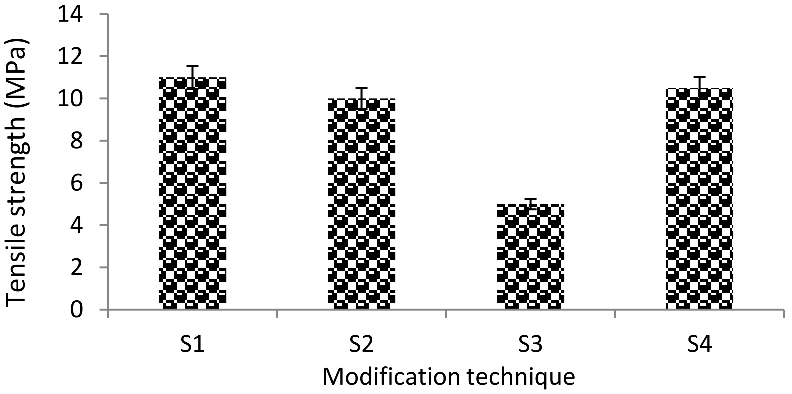

Fig. 3. Variation of tensile strength of latex coated coir filled rubber vulcanisates

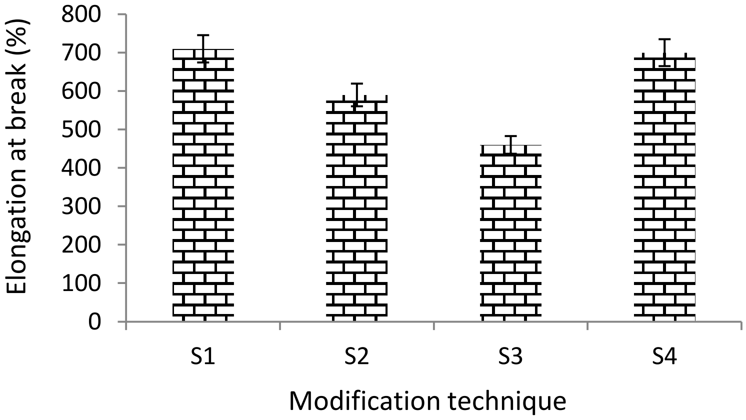

Fig. 4. Variation of elongation at break of latex coated coir filled rubber vulcanisates

Tear strength of the vulcanisate S1 is higher than that of the other vulcanisates (Fig. 5). This indicates that S1 is the most homogeneous vulcanisate. The initiated crack would tend to propagate through the interface and the extent of propagation depends on interfacial adhesion between the latex coated coir and NR matrix. NR latex coated coir improves its interfacial shear strength in polymer matrix (Yousif & Tayeb, 2007). With NR latex coated coir, fiber is exposed more to the polymer matrix, leading to proper interaction between the surfaces (Khalid *et al*., 2008). Additionally, since S1 contains coir coated with uncompounded NR latex, crosslinks would be evenly distributed between the latex coating and NR matrix unlike in the case of composite S4. Hence an interface would not exist between coir and the matrix. This results in higher tear strength for the S1 composite.

The ratio of energy returned to the energy applied is termed as the resilience (Fig. 6). When the deformation is an indentation due to single impact, this ratio is termed "rebound resilience". If the elasticity is higher, then less deformation energy is dissipated as heat. Heat build-up is a measure of the amount of energy that is absorbed. The highest resilience is shown by the S4 vulcanisate and is in agreement with the results of elongation at break. The resilience of nitrile latex coated coir filled vulcanisate (S3) is very much lower than that of the other three vulcanisates. The lower elasticity of the S3 vulcanisate could be attributed to lower elongation at break of the same.

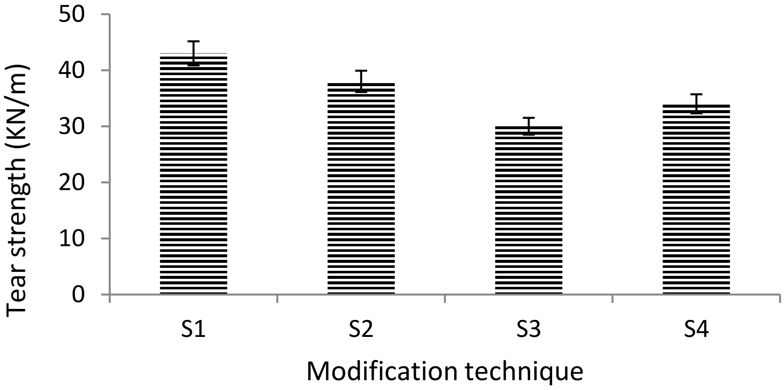

Fig. 5. Variation of tear strength of latex coated coir filled rubber vulcanisates

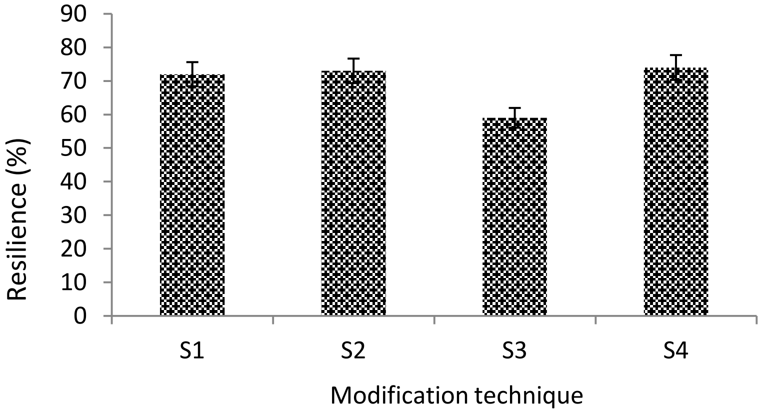

Fig. 6. Variation of resilience of latex coated coir filled rubber vulcanisates

The structure of neoprene is somewhat similar to NR and hence there is not much of a difference between the resilience of S1, S2 and S4 vulcanisates. Figure 7 shows that there is a marked difference between the abrasion volume loss of the three compounded latex coated coir filled rubber vulcanisates S2, S3 and S4. S4 is the highest abrasion resistance vulcanisate.

Abrasion volume loss results also indicate good adhesion between NR latex coated fibre and the NR matrix. The vulcanisate S3 containing nitrile latex coated coir shows the highest abrasion volume loss and can be attributed to poor adhesion or incompatibility between nitrile latex coated coir and the NR matrix and is in agreement with the results of tensile strength, elongation at break and tear strength.

Aging properties of the latex coated coir filled rubber vulcanisates

Table 4 indicates that there is no marked difference between tensile strength and elongation at break, before and after aging at elevated temperatures. Generally, results of properties of vulcanisates after ageing are low in comparison to results of properties before aging due to conversion of polysulphidic linkages to short mono and disulphidic linkages at elevated temperatures. Formation of a large number of these mono and disulphidic crosslinks would increase the crosslink density and hence modulus increase as indicated from Table 4.

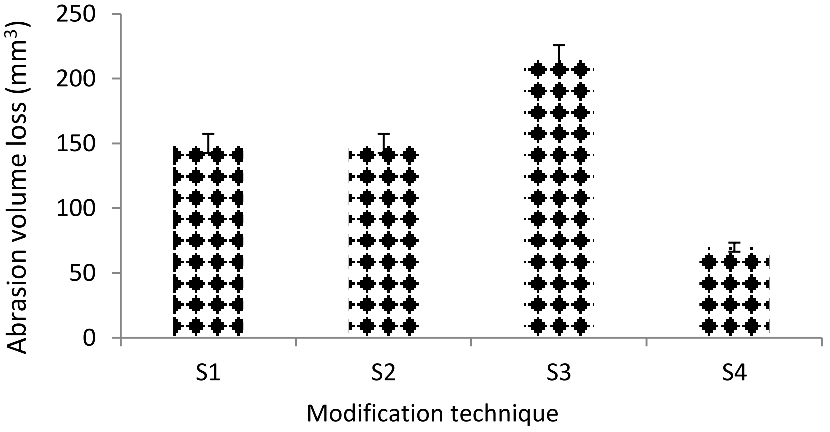

Fig. 7. Variation of abrasion volume loss of latex coated coir filled rubber vulcanisates

Table 4. *Percentage retention of properties of the vulcanisates after aging at 100^{o}C for 22 hrs*

Sample	Modulus at 300% (MPa)	Tensile strength (MPa)	Elongation at break (%)	Tear strength (KN/m)
S1	111	92	92	86
S2	110	95	95	110
S3	102	84	100	100
S4	131	90	87	109

Conclusions

Processing safety of the S4 composite is higher and it is faster curing compared to the other composites. Further, most of the physico-mechanical properties of this composite are superior to those of the other composites and are at a level acceptable for tyre treads. Hence, the compounded NR latex coated coir filled rubber composite could be used in the manufacture of tyre treads.

Acknowledgements

The authors would like to thank Laugfs Corporation (Rubber) Ltd., Horana, Sri Lanka for testing tensile properties and tear strength of the four composites.

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Journal of the Rubber Research Institute of Sri Lanka (2015) **95**, 14-23

Phenotypic diversity of Sri Lankan rubber clones at their immature stage

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Abstract

Systematic identification of morphological markers useful in the characterization of rubber clones has played a vital role in the crop management system. Although they do not exhibit highly distinct variations, there are certain minor, but more or less stable differences, which can be used for identification. This study was undertaken to estimate morphological diversity present in the Sri Lankan rubber clones at their immature stage and to determine the importance of morphological markers in categorizing different clones into distinct groups. Thirty clones were characterized using 29 standardized morphological descriptors. Principal component analysis (PCA) and Cluster analysis based on first twelve principal components that accounted for 85% of the total phenotypic variation distinguish 30 clones into six major clusters. Out of 29 descriptors, 27 were informative and contributed significantly to the variation among the clones selected. This study identifies the morphological descriptors that are most important for characterization of rubber clones grown in Sri Lanka.

Keywords: characterization, morphological descriptors, principal component analysis, rubber clones

Introduction

Genetic improvement in *Hevea* has been described as one of the outstanding success stories in agriculture of the 20th century. The rapid adoption of indigenously bred new clones has contributed substantially to attain higher yield both in the estate as well as in smallholding sectors of the country. Though the handfuls of improved high-yielding new clones are available, the genetic base of the existing population is very narrow.

Identification of these clones plays a vital role in the crop management system and research. Knowledge of relatively consistent characters of a clone either singly or in combination will enable to identify different clones recommended for planting. Although clones do not exhibit highly distinct variations, most of them possess certain minor, but more or less stable morphological features, which can be used upon for identification. However, the expression of those characters is the result of the interaction of genotype with an environment in a different magnitude. Hence, it is always desirable to consider those traits that are least influenced.

Use of morphological markers is cost effective when compared to the use of biochemical and molecular markers for preliminary characterization of a large number of accessions (Martinez *et. al*., 2003). All the clones recommended for planting need to be characterized using a standard set of characters. Morphological classification for rubber is done based on the set of descriptors first suggested by Dijkman (1951), then followed by Jayasekara *et.al.* (1984), and Mercykutty *et. al*. (1991). However, the use of minimum but important list of descriptors would help save resources. Therefore, it is necessary to identify highly discriminating descriptors relevant to the clones selected in order to minimize the list of descriptors.

Principal component analysis and cluster analysis are the commonly used multivariate techniques for characterization and genetic diversity analysis of perennial crops such as tea (Wickramaratne, 1981 and Gunasekara *et. al.,* 2001) coconut (Kumaran *et.al*., 2000). However, in rubber, numbers of morphological studies were reported in all rubber producing countries (De Silva and Sachuthananthavale, (1961), Paardikooper, (1965), Jayasekara *et. al*., (1984), Mercykutty *et. al.,* (1991, 2002), Penot and Rasidin, (1994) and Michel and Beningo, (1994)) and all of them were confined to collection of morphological data for identifying the clones. There is no evidence for statistical analysis for characterization of clones based on their morphological descriptors. Therefore, the objective of the present study was to identify key morphological features contributing to the total phenotypic variation among the Sri Lankan rubber clones at their immature stage.

Materials and Methods

Thirty of the Sri Lankan rubber clones in the 100 series, 200 series and 2000 series were selected (Table 1) (Attanayake, 2001).

Table 1. *Recommended clones and their parentages*

	Clone	Parentage
01	RRIC 100	RRIC 52 x PB 86
02	RRIC 102	RRIC 52 x RRIC 7
03	RRIC 121	PB 28/59 x IAN 45/873
04	RRIC 130	IAN 45/710 x RRIC 45
05	RRIC 133	IAN 45/710 x RRIC 52
06	RRISL 201	RRIC 103 x HP 8501
07	RRISL 203	RRIC 100 x RRIC 101
08	RRISL 205	82 HP x 82 HP
09	RRISL 206	82 HP x RRIC 101
10	RRISL 208	RRIC 101 x RRIM 600
11	RRISL 210	RRIC 101 x RRIM 600
12	RRISL 211	RRIC 101 x RRIM 600
13	RRISL 215	Illegitimate
14	RRISL 216	Illegitimate
15	RRISL 217	PB 28/59 x RRIC 121
16	RRISL 218	PB 28/59 x RRIC 121
17	RRISL 219	PB 28/59 x RRIC 102
18	RRISL 220	PB 28/59 x RRIC 121
19	RRISL 221	RRIC 52 x PB 28/59
20	RRISL 222	RRIC 102 x IAN 45/710
21	RRISL 223	PB 28/59 x IAN 45/710
22	RRISL 225	RRIC 102 x PB 28/59
23	RRISL 226	RRIC 102 x PB 28/59
24	RRISL 2000	RRIC 100 x RRIC 101
25	RRISL 2001	RRIC 100 x RRIC 101
26	RRISL 2002	RRIC 100 x RRIC 101
27	RRISL 2003	82 HP x RRIC 101
28	RRISL 2004	82 HP x PB 86
29	RRISL 2005	PB 28/59 x IAN 45/710
30	RRISL 2006	PB 28/59 x RRIC 36

The clones raised in the polybags were transplanted into the field with the spacing of 3’ x 3’ in a completely randomized block design. Characters were recorded at the plant at 12 to 18 months aged from fifteen plants five each randomly selected from three replicates. Data were collected from the top most nature leaf whorl.

All informative morphological data were selected based on the previous studies carried out by early researchers. As the descriptors used in the study consisted of quantitative and qualitative characters such as;

- Nodes - axillary buds and leaf scars
- Leaf storey - shape, separation and external appetence of leaf storey
- Petiole and petiolule - pulvinus, shape, size, orientation, angle and size of petiole
- Leaves - colour, lusture, texture, shape and size, cross-section and longitudinal section appearance, leaf apex, leaf base, leaf margin and degree of separation

were collated.

Non-parametric data were converted on a scale of numeric data to enable them to be used in statistical analysis. Principal Component Analysis (PCA) was carried out using means of quantitative and modes of qualitative morphological characters. Average linkage cluster analysis was performed subsequently based on the significant number of Principal Components (PC) aiming at grouping of the 30 rubber clones. The analysis was performed using SAS – Version 9.2 (Annon, 2008).

Results and Discussion

Correlation matrices obtained from the PCA of the 29 morphological descriptors shows that Eigenvalues of the first 12 principal components (PCs) were greater than one and they were significantly contributed to the variation existing in the clones studied. Furthermore, those 12 PCs accounted for the 85% of the total variation (Table 2).

Then the eigenvectors generated by the first 12 PC shows that some of the variables (which are highlighted) are comparatively higher than the other variables (Table 2). Although all the twenty-nine variables have contributed to a certain degree to deciding the position of each of first twelve principal components, it is clearly evident that some of the variables play comparatively significant role in selecting the position of each PCs indicating that they are the main contributors in each component.

When explaining the above patterns of the 29 variables, it was clearly evident that 27 variables have contributed significantly to decided the positioning of the first eleven PCs and ultimately to the variation (Table 3). Only two variables were found comparatively less significant. They were the orientation of pulvinus and the nature of veins.

Through the PC analysis, it was able to reduce the number of descriptors (or combinations of descriptors) in the form of principal components. To achieve the main objective of grouping the accessions, cluster analysis was performed based on average linkage on the first twelve principal components. The dendrogram (Fig. 1) indicates that the 30 rubber clones used in this study were grouped into six well-defined clusters at the average distance of 1.0.

Table 3. *Main contributor descriptors for each principal component (PC)*

PC	Main descriptors
1	Leaflet colour, Leaflet luster, Leaflet texture
2	Petiolue orientation, Petiolule size, Shape of leaf base
3	Shape of leaf scar, Petiole shape, Leaf margin
4	Separation of leaf storey, External appearance of leaf storey
5	Shape of leaf storey, Size and width of leaf storey, Petiole size, Leaf area
6	Petiolule angle, Leaflet shape, Leaflet thickness, Shape of leaf apex
7	Leaflet shape, Cross-sectional appearance
8	Petiole orientation, Petiolule angle, Longitudinal sectional appearance, Colour of veins
9	Axillary bud, Longitudinal sectional appearance
10	Petiolule junction appearance, Leaflet luster, Degree of leaflet separation, Shape of leaf base
11	Cross-sectional appearance, Leaflet shape
12	Leaf scar, Degree of leaflet separation, Colour of veins

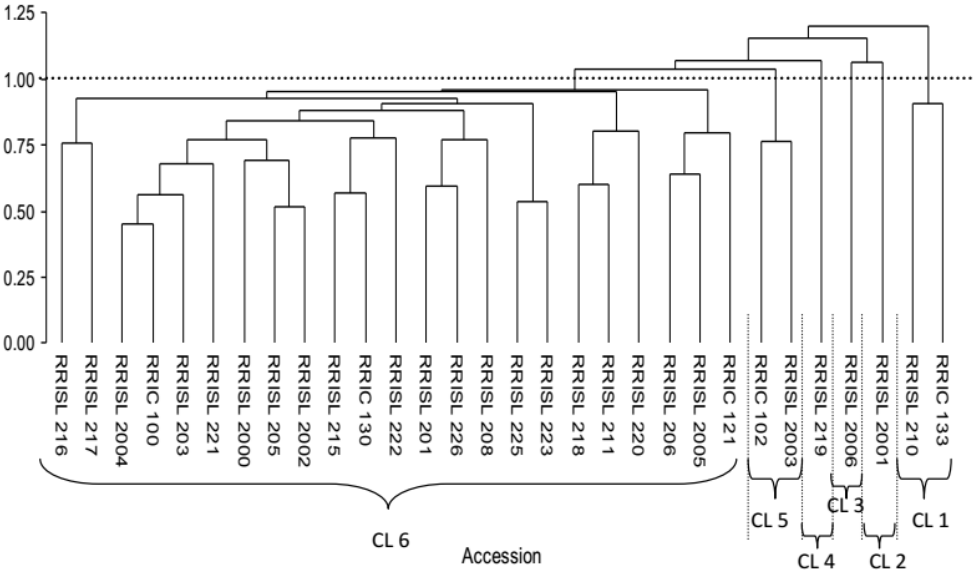

Fig. 1. Dendrogram for 30 rubber clones under studied based on average linkage cluster analysis using 29 morphological descriptors (CL 1 to CL 6 is the different clusters at average distance of 1.0)

Cluster 1 consists of two clones, RRIC 133 and RRISL 210. They possess longer petiolule, dark colour leaflets, glossy luster and smooth texture than other clones that help in the form of a separate cluster.

In cluster 2, consist only one clone RRISL 2001 and it was clearly separated from others because it had a unique morphological feature like circular shape leaf scars and it process some other very important features like broad elliptical shape leaflets, smooth leaf margin, arched shape petiole, apiculate leaf apex and it had leaflets with “S” shape in the longitudinal sectional appearance. Though these characters are not unique to the clone RRISL 2001, by a combination of these characters together it is easy to separate out this clone from others.

The cluster 3 consists only one clone RRISL 2006, and it possessed features of conical shaped leaf story, light green colour leaflets and aristate type leaf apex. The combination of these characters together helped to identify clone RRISL 2006 separately from others.

Cluster 4 includes only one clone RRISL 219 which differs significantly from the upward orientation of petiloule, broad elliptical shape leaf storey, straight appearance of the petiolule junction and touching habit of the leaflet in leaflet separation character. Although those characters are not common to RRISL 219, combinations of them together helped to identify this clone easily.

Cluster 5 includes two clones RRISL 2003 and RRIC 102, and both have common features of conical shape leaf storey, broad elliptical shape leaflets and thin (thickness) leaflets that help to form a separate cluster. Within the cluster, those two can separated from their shape of the petiole, degree of leaflet separation, the shape of the leaf apex and leaf base.

The remaining 23 clones formed a major cluster (Cluster 6) which shows more resemblances in their morphology. This is because that most of the clones possess similar traits due to lower genetic diversity as they were derived from common ancestors.

The present analysis of morphological characters provides the basis for broad classification of rubber clones and PCA identifies the variables contributing to most of phenotypic diversity while clustering helps in grouping of clones based on their degree of relationship to each other based on their common morphological features.

Conclusions

This study classified the 30 rubber clones recommended to grow in Sri Lanka into well-defined phenotypic groups. Principal component analysis (PCA) using 29 morphological descriptors and cluster analysis based on first 12 principal components (PCs) group all 30 clones into six major clusters. Among all 29 descriptors measured, 27 descriptors highly contributed to the phenotypic diversity of the clones. Although clones do not exhibit highly distinct variations, most of them possess certain minor, but more or less stable morphological features, which can be used upon for identification. Analysis of morphological characters provides the basis for broad classification of rubber clones, and PCA identifies the variables contributing to most of phenotypic diversity of the clones.

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Table 2. *Eigenvectors and eigenvalues of the first twelve principal components of twenty-nine quantitative traits of 30 Sri Lankan rubber clones*

Characters	**Eigenvectors**											
	PC 1	**PC 2**	**PC 3**	**PC 4**	**PC 5**	**PC 6**	**PC 7**	**PC 8**	**PC 9**	**PC 10**	**PC 11**	**PC12**
1	-0.1383	-0.1875	-0.2483	-0.0027	-0.0945	-0.2132	0.2801	0.0120	**0.4183**	0.1542	0.1855	-0.0330
2	-0.1003	0.2320	-0.1700	0.0451	-0.1039	0.2478	0.0688	0.2613	0.2030	-0.1586	0.0703	**-0.4351**
3	0.0578	0.0706	**-0.4895**	0.0747	0.0961	0.1149	-0.2057	-0.0403	-0.0132	-0.0229	-0.2346	0.2717
4	-0.1593	-0.0768	0.2220	0.0973	**0.4359**	0.1200	-0.1923	-0.1258	-0.0473	0.0836	0.0583	-0.1127
5	-0.2259	-0.1829	0.0283	**-0.3552**	0.1279	0.0836	0.0112	-0.2205	0.0372	0.0898	0.1219	-0.0073
6	0.1482	0.1799	0.0739	**-0.4603**	0.0414	-0.2085	0.1217	-0.0514	-0.0098	-0.1599	0.2341	-0.0999
7	0.0950	0.2420	-0.2380	0.2657	**0.3297**	0.1277	0.1764	-0.0680	0.1230	0.0393	0.0823	-0.0619
8	0.2406	-0.2095	0.1173	0.0611	0.2992	-0.1127	0.2790	0.1880	0.0741	0.0255	-0.0611	0.0242
9	-0.1353	0.1162	**0.3607**	0.2583	-0.1918	0.1979	0.0153	0.1357	0.2249	0.0421	-0.0934	-0.0589
10	0.2580	0.1217	0.1022	0.0623	**0.3672**	0.0364	0.1949	0.0752	0.2730	0.2160	0.0049	0.1461
11	-0.1821	0.2611	-0.0013	0.0496	0.2185	-0.0590	0.1541	**-0.3611**	-0.2693	-0.0669	0.0935	0.2183
12	-0.0148	**-0.4186**	0.0432	0.0438	0.1892	-0.1076	-0.0810	0.2006	0.1539	-0.0145	-0.2297	0.0365
13	0.0178	0.1724	0.1021	0.1810	0.0844	**-0.3065**	0.1124	**0.4047**	-0.3412	0.0936	0.1443	0.1927
14	0.1340	**0.3411**	0.0843	-0.2690	0.0677	-0.0578	-0.1534	0.0662	0.2688	0.1480	0.1457	0.0760
15	0.2306	-0.1851	0.2350	0.0652	0.0962	0.2663	-0.0475	-0.1664	0.1811	**-0.3471**	-0.0465	0.1579
16	**0.3440**	-0.0264	0.2171	-0.1801	-0.0639	0.1006	0.0697	0.1896	0.0114	-0.0050	0.0950	-0.0734
17	**0.3033**	-0.0416	-0.1349	-0.2170	-0.0804	-0.0919	-0.2628	-0.0775	-0.0706	**0.3096**	-0.1793	-0.2123
18	**0.3551**	-0.0705	-0.0752	0.0380	-0.0757	0.1058	0.0933	-0.1514	-0.1329	0.0854	**-0.3155**	-0.2046
19	0.1627	0.0444	0.1113	0.3228	0.0856	**-0.3398**	**-0.3482**	-0.1187	-0.0559	0.0031	-0.0134	-0.1904
20	0.2208	-0.0827	0.1749	0.1380	**-0.3754**	-0.1042	0.1884	-0.1120	-0.1856	0.1827	0.1076	0.1684
21	0.1915	0.0719	-0.0945	0.2804	-0.2527	**0.3383**	0.0403	-0.1024	0.0177	0.1273	0.2901	0.1566
22	-0.1782	0.2656	**0.3979**	-0.0717	-0.0184	0.1744	-0.0324	0.0259	-0.0962	0.0054	-0.2751	-0.0962
23	0.2076	0.1184	-0.0513	-0.1438	-0.0612	-0.0082	-0.1084	0.1236	0.0770	**-0.5501**	-0.0920	**0.3779**
24	-0.1324	0.0792	0.0521	-0.1375	-0.0909	-0.0023	**0.4500**	-0.1578	0.0622	0.1514	**-0.5142**	0.1972

Characters	**Eigenvectors**											
	PC 1	**PC 2**	**PC 3**	**PC 4**	**PC 5**	**PC 6**	**PC 7**	**PC 8**	**PC 9**	**PC 10**	**PC 11**	**PC12**
25	-0.0582	-0.1114	0.1982	0.1019	-0.1694	-0.1718	-0.1838	**-0.3344**	**0.3557**	0.0208	0.1835	0.1960
26	0.1341	-0.1647	0.0247	-0.1825	0.1287	**0.4479**	0.0054	-0.0527	-0.2188	0.2094	0.2426	0.0583
27	-0.0858	**-0.3379**	-0.0294	0.0595	-0.0032	0.0468	0.2363	0.0681	-0.2454	**-0.3234**	0.1694	-0.1360
28	-0.2065	-0.1158	-0.0295	-0.1048	-0.0462	0.1688	-0.2456	**0.4121**	-0.0430	0.2756	0.0248	**0.3657**
29	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Eigen value	3.958	3.097	2.411	2.201	2.163	1.739	1.681	1.628	1.438	1.308	1.142	1.037
% of total variance	14.1	11.0	8.6	7.8	7.7	6.2	6.0	5.8	5.1	4.6	4.0	3.7
% cumulative variance	14.1	25.2	33.8	41.6	49.4	55.6	61.6	67.4	72.5	77.2	81.3	**85.0**

1 - Axillary bud,
2 - Leaf scar
3 – Shape of leaf scar
4 - Shape of leaf storey
5 -Separation of leaf storey
6 - Ext. appearance of leaf storey
7 - Size and width of leaf storey
8 – Pulvinus
9 - Petiole shape
10 - Petiole size
11 – Petiole orientation
12 – Petiolue orientation
13 – Petiolule angle
14 – Petiolule size
15 – Petiolule junction appearance
16 – Leaflet colour
17 – Leaflet luster
18 – Leaflet texture
19 – Leaflet shape
20 – Leaf area
21 – Leaflet thickness
22 – Leaf margin
23 – Degree of leaflet separation
24 – Cross-sectional appearance
25 – Longitudinal sectional appearance
26 – Shape of leaf apex
27 – Shape of leaf base
28 – Colour of veins
29 – Nature of veins

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Evaluation and utilization of the *Hevea* germplasm collected from 1981 IRRDB expedition to the Amazon; a review

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Abstract

Rubber industry in Sri Lanka that extends approximately around 133,000 ha, is largely based on Wickham's genetic base. Use of genetically improved high yielding planting materials is perhaps the only and cheapest way to increase the yield per unit area. However, due to directional selection carried during past Hevea breeding, the genetic diversity in the Hevea *breeding pool has started to narrow down. Non Wickham's genetic collections can provide genetic diversity and possible desirable genes for plant breeders to overcome this situation. Currently around 8000 accessions of Hevea germplasm have been collected and conserved in Sri Lanka. Majority of them came from the collection of 1981 International Rubber Research Development Board expedition in the Amazonian habitats. The characterization and evaluation of this germplasm was started in early 1990's and continue until today. Two direct selections to the RRISL clone recommendation has been made in 1994 as GPS I and GPS II. Large number of accessions were used in nearly nine hand pollination programmes carried since 1995 and resulted in development of around 1075 new genotypes which now in various evaluation steps. Ten selected genotypes from 1995 hand pollination programme are now in the advanced stage of evaluation, before the recommendation. The process of characterization, evaluation and maintenance of ex–situ germplasm collection with a wide range of genetic materials for useful trials is in progress.*

Key words**:** breeding, genetic diversity germplasm, *Hevea*

Rubber (*Hevea brasiliensis*) is the 3rd largest plantation crop in Sri Lanka next to the tea and coconut. The total extent of cultivated rubber has been estimated to be around 133,000 ha (Anon 2012) and the rubber industry provides employment, both directly and indirectly to about 500,000 persons (Anon 2012). Nearly 65% of the rubber is owned by smallholders and majority of them fall into low income groups. The only way to increase this low income is by increasing the yield per unit area of land. Use of genetically improved high yielding planting material is perhaps the only and also the easiest way to achieve this. Thus a sound, efficient and result oriented *Hevea* breeding programme is vital to the survival of rubber industry in Sri Lanka especially in the face of increasing cost of production.

For several years, Geneticists and Plant Breeders have emphasized the need to conserve *Hevea* genetic resources and to widen the genetic base of breeding populations used by the natural rubber producing countries. Rubber breeding in Sri Lanka is largely based on a small population of about 1919 seedlings introduced in 1876 from Wickham collection. It is believed that Sri Lanka was the centre of distribution of rubber plants to other South East Asian (SEA) countries. Further, introduction of new material from natural habitats has been restricted due to the fear of accidental introduction of South American Leaf Blight (SALB) to this region. Therefore, genetic base in the original population is narrow.

Directional selection during last 80 years of breeding for few economically important characters such as yield, vigor and disease tolerance and also extensive use of clonal vegetative propagation has led to further erosion of genetic variability. Consequently, problems related to breeding and selection of *Hevea*, such as inbreeding depression which lead to declining yield response are becoming more apparent.

Most *Hevea* breeders believe that they have exploited the maximum genetic variability of Wickham’s original introduction and have reached the threshold point with respect to economically important characters such as yield and vigor. *Hevea* breeders felt the need for exploration, collection and conservation and use of *Hevea* genetic material to widen the genetic base of present breeding populations. Necessity for such action becomes even more important as natural stands of *Hevea* in Amazon region are endangered by extensive felling of jungle land for agricultural purposes.

Expedition to collect wild *Hevea* germplasm

In 1981 member countries of the International Rubber Research and Development Board (IRRDB) funded a project to collect new *Hevea* germplasm. The first discussion on the importation of wild *Hevea* germplasm, was held at the Colombo meeting of IRRDB in 1976. Following these, the basic preparations for the proposal of IRRDB project “Collection and conservation of *Hevea* planting materials from south America” were arranged at IRRDB “Plant Breeders” workshop at Kuala Lampur, Malaysia in 1977. Then in 1978, a preliminary mission was done in various South American countries such as Bolivia, Brazil Colombia, Guyana, French, Guiana, Venezuela and Thailand with the aim to arrange scientists and governmental support and contacts. The collection team consisted of eight scientists from Malaysia, Thailand, Indonesia, the Peoples' Republic of Code d'Ivory (Ivory Coast) and Nigeria. Three scientists also joined from Brazil. This collection is now maintained in The Peoples' Republic of Code d'Ivory and Malaysia which serve as distribution centers for African and Asian member countries of IRRDB. Rubber Research Institute of Sri Lanka (RRISL) has spent more than US $ 87,500 for the collection and maintenance of these materials at the Rubber Research Institute of Malaysia (RRIM). Sri Lanka has received about 10,000 genotypes from the RRIM and some of genotypes maintain in the African centre too.

Location of expedition

Three main areas were selected in the Western state of Brazil (Table 1), namely,

1. Acre – better quality rubber, Vigorous and high yielding
2. Rondonia – vigorous and high yielding, variation in the species *Hevea brasiliensis*
3. Mato Grosso – variation in the species *Hevea brasiliensis*

Sample collected trees were very old and their girth ranged from 3m – 6m at 1½ m height from the ground. Also these trees had been already subjected to tapping by native tappers using a multiple cutting system. It showed large volumes of latex around 1½ L to 4L per tapping.

Table 1. *Exploited locations based on districts and states*

States	Districts	Number of location
Acre	Brasileia	6
	Feijo	4
	Sena Madureira	6
	Tarauaca	4
	Xapuri	2
Mato Grosso	Aracotuba	5
	Cartriquacu	11
	Itauba	7
	Vila Bela	1
Rondonia	Ariquemes	1
	Calama	2
	Costa Marques	3
	Jaru	2
	Jiparana	1
	Ouro Preto	1
	Pimenta Bueno	2

Germplasm code: eg AC/ B/1-5: (Acre state/ Brasileia district/ Location number 01- 05th Accession or genotype)

The expedition group collected a total 64,736 seeds and 1522 m of bud wood from 194 presumably high yielding mother trees.

Dispatch of germplasm

This collection was first dispatched to the National Centre of Rubber and Oil Palm Research in Brazil in Manaus (CNPSD) and subjected to a whole procedure of very strong phytosanitary measures (Fig. 1).

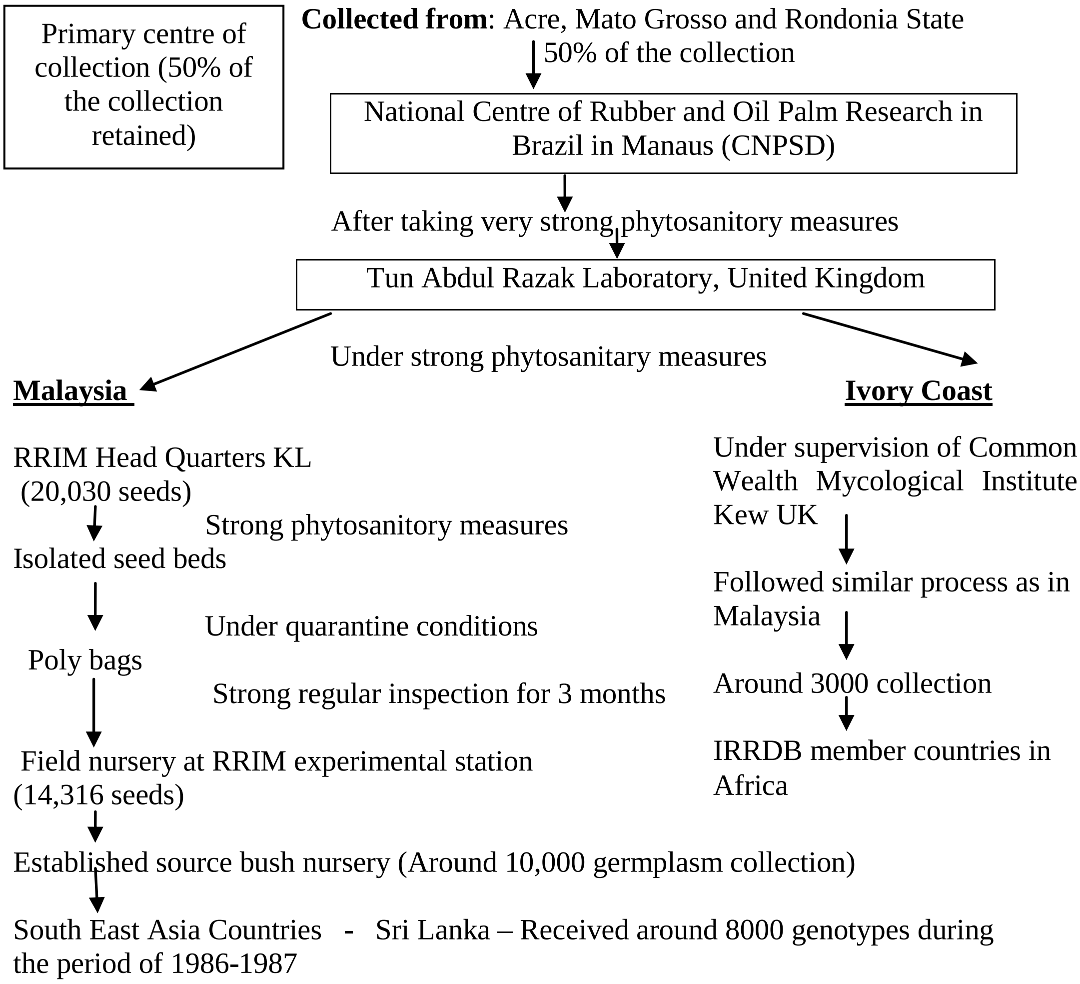

Fig. 1. Dispatch Procedure of germlasm collected from: Acre, Mato Grosso and Rondonia States

Establishment and attempts taken to evaluate and utilize the *Hevea* germplasm in Sri Lanka

Sri Lanka received its first consignment of 100 germplam clones in 1984 and further 8564 genotypes, periodically during the period of 1985-1989. This *ex*-situ collection of non-Wickham germplasm was established at Dickhena division, Neuchatle estate plantation, Kalutara district, in an area around 13.5 ha. From each genotype four plants were multiplied and planted in close (1m X 1m).

Evaluation of first consignment of 100 germplasm clones at Kuruwita Sub station for direct selections

First consignment of 100 germplasm clones was established at Kuruwita substation under small scale level and Morris - Man test tapping was started at the end of year 1989 (Jayasekara, 1989).

Five girth measurements recorded from 1988 to 1992, inclusive of both years, were used to work out the growth rates by regressing individual tree girth on two years taking years 1,2,3,4 and 5 as independent variable (Jayasekara, 1991).

Analysis of variance performed on growth rates of individual trees indicated highly significant differences between clones and between classes, when clones were classified according to their origin, indicating differences in growth rates of clones of different origins. Duncan Multiple Range Test carried out resulted in 17 groups with considerable overlapping. This made it extremely difficult to group them according to their vigour.

In group *a*, with a highest mean girth of 8.52 cm all 3 clones with Wickham origin were included.

There were germplasm clones that had higher growth rates than some of the Wickham derivatives such as RRIC 102 and RRIM 600. RRIC 121 had the highest mean (9.940 cm) followed by a germplasm clone MT-C-1 -1 with a mean girth of 9.45 cm. This indicated that germplasm clones could be a good resource material to incorporate new genetic variability with respect to vigour (Jayasekara, 1992). In addition to the study of the latex vessel rings of bark samples collected from this trial, test tapping was carried out (Jayasekara, 1993).

Other than above evaluation, around 300 plants in this nursery with a girth of 60 cm and above were marked at 90 cm above the bud union for test tapping, the clones that did not give promising yields were discontinued and another batch of 300 plants that had reached the required girth of 60 cm and above were brought into tapping. This second batch and the promising clones selected from the first batch were test tapped to evaluate the yield potential.

Two highly promising clones which yield more than 100 g/t/t have been identified for future testing in large scale trials (Jayasekara, 1993).

The two promising genotypes that were identified in 1993 were test tapped in 1994.Yield of one genotype (GPS II) dropped while the other genotype (GPS

1) continued to give high yields. Mean yield of these two genotypes and the number of test tappings done in 1994 are shown in Table 2 (Jayasekara, 1994).

Test tapping of plants, led to identification of four genotypes which can yield more than 30 g per tree per tapping. Test tapping yield, both average over first three years and the fourth year average are given in Table 3 (Jayasekara, 1994).

Utilization of germplasm in breeding programs in Sri Lanka during 1995-2014

This *ex-situ* collection has been used in hybridization programmers frequently from year 1995 onwards aiming to improve the genetic diversity of rubber plantations in Sri Lanka. These progenies are now being evaluated in various steps successfully (Table 4).

Table 2. *Two promising genotypes selected from evaluation of first consignment of 100 germplasm clones at Kuruwita sub station*

Clone/germplasm code	Yield (g/t/t)	No. of test tapping
GPS 1 (AC/S/12-559)	91.7	40
GPS II (AC/F/6A-471)	40.2	36

Table 3. *Four promising genotypes yielded more than 30 g per tree per tapping, selected from evaluation of first consignment of 100 germplasm clones with at Kuruwita sub station*

Clone/germplasm code	Average yield (g/t/t) for first 3 years* (1992 to 1994)	Average yield (g/t/t) the forth year*
22-137 (RO/JP/3-137)	41.65 (77)	33.41 (156)
GPS II (AC/F/6A -471)	46.36 (73)	42.72 (151)
GPS 1 (AC/S/12-559)	77.65 (73)	99.85 (156)
44-24 (RO/CM/10-24)	48.01 (77)	46.83 (153)

*In parenthesis indicates the number of test tapping on which the average yield is based.

Table 4. *Use of germplasm clones in hybridization programme during 1995-2014 and performance of their progenies*

HP year	Clone/germ plasm code of female parent	Germplasm code of male parent	No. of new genotypes produced	Evaluation stage/ performances of new genotypes	
1995	RRIC 100	(GPS 1) AC/.S/12-559	42	Selected HP entries 95-33 and now at RRI/ECTs* evaluation. However, this selection was grouped with some of recommended clones (Fig. 3) and evaluate (RRI/ECT) collaborative trial 2011-Yatadola	
1996	RRIC 121	GPS 1/AC/.S/12-559	46	Four HP entries (96-58, 96-14, 96-8 and 96 -15) performed well above the control clone RRIC 121 and taken to RRI/ECTs *evaluation	
2000	BPM - 24 RRIC 121	(GPS 36 - 104) AC/F/6A-104	19 48	Small Scale Clone trials	
2001	RRIC 100	GPS 1/AC/.S/12-559	15	Small Scale Clone trials	
2002	IAN 48/875 RRISL 2001 PB 261	GPS 1/AC/.S/12-559	11 02 01	Small Scale Clone trials	
2007	RRIC 130			Small Scale Clone trials - According to sixth year girth data, the progeny RRIC130 X GP 1-2 showed significantly higher girth value.	
		(GPS 1-2) 07.02.81- 02	29	Mean girth at 5th year	61.5a
		(GPS 44-24) RO//CM/10-24	1		52^{ab}
		(GPS 22-137) RO/JP/03-137	262		50.9^{ab}
		(GPS 21-163) AC/F/05-163	124		49.98^{ab}
		(GPS 10-154) MT/C/02-154)			41.17^{b}

HP year	Clone/germ plasm code of female parent	Germplasm code of male parent	No. of new genotypes produced	Evaluation stage/performances of new genotypes
2008	PB 28-59	(GPS 36-147)	27	Small Scale Clone trials - Higher elevation
	RRIC 121	AC/F/6A-147	48	
	RRIC 100	GPS 21-163	39	
		GPS 22-373	4	
		GPS 1-4	2	
		GPS 36-160	27	
		IAN 45/873	4	
		GPS 1-47	5	
		GPS 22-271		
2012	RRISL 2005	GPS 22-16	26	Established at mother plant nursery Genetic diversity analysis of selected parental materials were completed by using RAPD markers (Fig.3). Selected female parents of RRISL 2005 and 2006 were grouped together indicating their genetic resembleness. The greatest genetic distance was between GP 22/137 and GP 22/500 and also the presence of high number of sub clusters indicate the higher genetic variability. Therefore, genetically diverse clones can be obtained from the 2012 HP programme (Madushani *et al.*, 2014)
	RRISL 2006	GPS 22-493	41	
	Hevea nitida	GPS 22-4	41	
		GPS 11-76	06	
		GPS 22-500	15	
		GPS 22-137	16	
		IAN 45/710	09	
2014	(GPS 44-24) RO//CM/10-24	RRISL2100	165	Poly bag nursery
		RRISL 2001		
		RRISL 2006		
		RRIC130		
		RRIC131		

*-RRI/ECTs- Rubber Research Institute/Estate collaborative trials

Evaluation of genetic diversity of germplasm

Importance of germplasm in order to broaden the Hevea *gene pool*

Recently, we have carried out a molecular study to evaluate the genetic diversity of recommended clones. Microsatelite molecular markers were used to determine the genetic diversity of 14 clones which represent all the generations of recommended clones grown in the past few decades; *i.e.*; early seedling selections of RRIC 100 series, RRISL 200 series and RRISL 2000 series.

According to the result, the Dendrogram clearly showed (Fig. 2) that the most of the recommended clones group together regardless of their generation~~s~~ indicating that they are genetically very similar. Such a narrow genetic base is a danger to the rubber industry. In this analysis the clone RRISL 2005 which had been derived using non Wickham parental materials (germplasm) showed highest genetic distance to the other clones showing the urgent need of the use of the new germplasm material in future *Hevea* breeding programs.

Being a member country of International Rubber Research Development Board (IRRDB), maintenance, multiplication and evaluation of this non-Wickham germplasm material is a responsibility of a Rubber Research Institute as well as of the government of Sri Lanka. However, currently, the conservation and utilization of this germplasm have become a problem due to lack of financial and human resources.

Genetic diversity of selected genotypes from year 2008 *Hevea* (*Hevea brasiliensis*) hand pollinated progeny using SSR markers

Twenty three *Hevea* genotypes developed from year 2008 Hand Pollinated progeny, which had used diverse male parents from non-Wickham's germplasm (Table 4), were subjected to SSR analyses for genetic diversity with their three mother parents. Eight *Hevea* SSR markers were used for this study. All these genotypes and mother parents were produced two distinguishable alleles which detected for each of the SSR loci. Three mother clones *i.e*. RRIC 100, RRIC 121 and PB 28/59 were grouped together by showing their close genetic relatedness probably due to their Wickhams genetic base. Sixteen progeny genotypes deviated from mother parents and nine genotypes out of them showed more than 0.5 genetic distances from all three mother parents indicating a greater level of genetic diversity of the progeny because of the use of non Wickham male parents. These genetically diverse clones from 2008 hand pollination progeny can be added to the *Hevea* cultivation in the future (Amaratunga *et al.*, 2013).

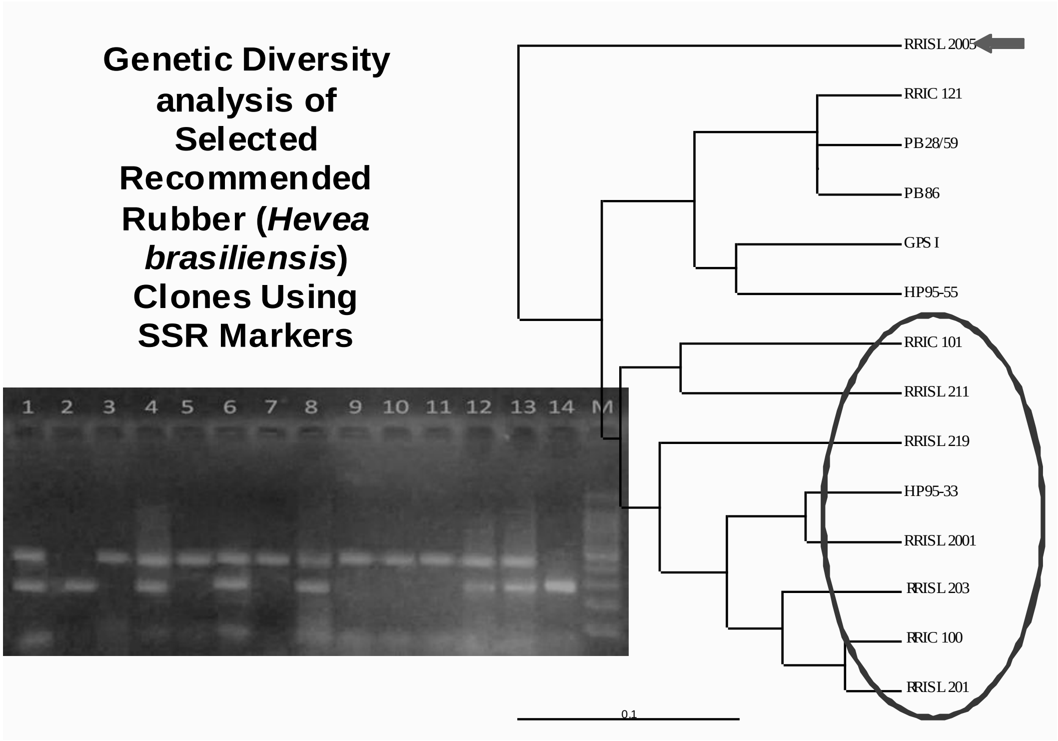

Fig. 2. Molecular analysis of genetic diversity analysis by Microsatellite molecular Markers

```
                                                  ----.085----- [.085] RRISL2005  ⎫     ⎫
                                          --------.12------|0                     ⎬ c   ⎪
                                          |                 -.039-- [.039] GP22/137 ⎪   ⎪
                              -------.112-----|1                                  ⎪     ⎪
                              |           -----.097----- [.097] RRISL2006        ⎭     ⎪
                   ---.064--|3                                                          ⎪
                   |          |        -----.101----- [.101] GP22/493      ⎫           ⎬ A
           ---|5          ---.066--|2                                        ⎬ d         ⎪
           |  |                  -----.107------ [.107] GP11/76            ⎭           ⎪
           |  |                                                                         ⎪
---- |6 -------------.224-------------- [.224] GP22/4                                   ⎪
|     |                                                                                 ⎪
|     |        -------------------.302-------------------- [.302] GP22/16   ⎫          ⎭
|     --.052-|4                                                              ⎬ e ⎫
|              ---------------.235--------------- [.235]  H. nitida          ⎭   ⎬ B
---------------------------.439----------------------------[.439]GP22/500       ⎭
```

Fig. 3. Genetic diversity analysis of selected parental materials of 2012 HP programme using RAPD markers

Evaluation of performances of new genotypes developed using the germplasm of *Hevea* obtained from 1981 IRRDB expedition to the Amazon (RRI/Estate collaborative trial 2011-Yatadola estate)

The IRRDB'81 collection exhibited generally very poor profiles in agronomical characteristics, especially latex productivity. Therefore direct use of this collection for latex production purpose may have limited potential. But progenies of their crosses with Wickhams high yielding clones could produce better clones while expanding the genetic variability.

Ten genotypes selected from 1995 hybridization programme where non Wickham clone GPS 1 was used as a parent, were established at Yatadola estate, Namunukula Plantations PLC. Fifty trees from each genotype were established with control clone RRISL 203. Evaluation of their commercial performances under estate management conditions, was carried out to select genetically diverse clones to be incorporated in RRISL clone recommendation. Performances of all genotypes were well, compared to control clone with respect to the third year girth. Genotypes 95HP29, 95HP41, 95HP19 and 95HP13 obtained higher girth values than control clone RRISL 203 whereas genotype 95HP29 (Fig. 4) showed a significantly higher girth (Table 5). These genotypes were selected for propagation in bud wood nurseries in order to establish a few more RRI/ECT trials. However, all genotypes need to be evaluated further before making a decision on recommendations.

Table 5. *Mean girth of first three years and their DMRT ranks of selected genotypes from 1995/1981 hand pollination progenies under estate management conditions (RRI/ECT collaborative trials)*

Clone	1st year mean girth (cm)	Clone	2nd year mean girth (cm)	Clone	3rd year mean girth (cm)
95HP19	8.49^{a}	95HP19	14.46^{a}	95HP29	25.5^{a}
95HP13	8.21^{a}	RRISL 203	14.36^{a}	95HP41	23.39^{b}
95HP29	7.55^{b}	95HP41	14.28^{a}	95HP19	23.17^{b}
95HP41	7.51^{b}	95HP21	14.24^{a}	95HP13	22.66^{bc}
95HP1	7.34^{bc}	95HP23	13.81^{ab}	RRISL203	22.26^{bcd}
95HP21	7.08^{bc}	95HP13	13.79^{ab}	95HP21	21.98^{bcde}
RRISL203	7^{bc}	95HP29	13.66^{ab}	95HP23	21..04^{cde}
95HP23	6.95^{bc}	95HP1	13.1^{bc}	95HP1	20.47^{de}
95HP33	6.81^{dc}	95HP33	12.55^{c}	95HP33	20.22^{e}
95HP55	6.27^{d}	95HP55	11.37^{d}	95HP55	16.23^{f}
81HP69	6.2^{d}	81HP69	10$^{.e}$	81HP69	14.29^{g}

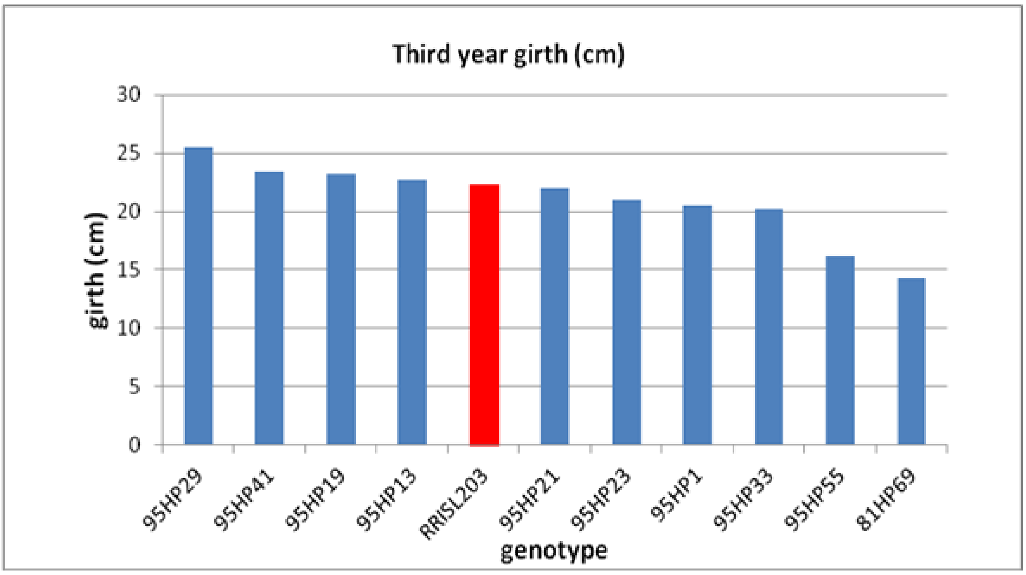

Fig. 4. Third year girth compared with control clone RRISL 203

Current Status of *ex- situ* collection of germplasm in Sri Lanka

The germplasm collections in Sri Lanka are now nearly 30 years old. Vigorous clones have over grown to the extent of hindering the less vigorous ones. This leads to loss of some of these less vigorous genotypes in the collection. Therefore, it is important to fill the vacancies that occur as a result. Having all the genotypes in one location is also a risk. Hence, the duplication of collection in other locations is also important and it is advisable to duplicate the population at least in two more locations, preferably one in wet area and the other in dry area with normal spacing used as in commercial plantation. This will enable us to do future evaluations of economically important trait as well as the maintenance of material without any loss. Further, maintenance and scientific evaluation of this IRRDB germplasm collection and judicial use of promising genotypes will increase the efficiency of the present breeding programme by providing more genetic variability for the breeder to select upon.

Therefore, the project on **Multiplication/Establishment and scientific evaluation of the *Hevea* germplasm collection was commenced in the year 2014 with the aim of enhancement of productivity through genetic improvement and management of genetic resources of**

Hevea**.** (Under new development proposal for Annual Budget, 2014);

Specific objectives are:

- Establishment and maintenance of the 1981 IRRDB germplasm collection obtained from 1981 IRRDB expedition to the Amazon
- Scientific evaluation of the *Hevea* germplasm collection to classify the genotypes according to genetic parameters and by using molecular marker s to identify promising genotypes for future breeding programme
- Incorporation of promising genotypes to *Hevea* breeding programme.

First year: 2014

Four thousand fifty seven (4057) trees that belong to 1478 number of accessions were selected (Table 6) and pollarded at the height of 4½ft above the ground level to prepare bud woods (Fig. 5). Poly bag nursery was raised with 16,000 plants and ground nursery was established with 1000 plants. Three planting sites Nivitigalakele substation (9.2 ha), Polgahawela substation (2 ha) and Monaragala substation (1ha) were selected and land preparation was done. Scientific evaluation and characterization of accessions were started (Fig. 6) (Withanage 2014).

Fig. 5. Preparation of bud wood of selected accessions for multiplication at Neuchatle estate

Table 6. *Selected accessions and their germplasm code prepared to establish in the year 2014- 2015 season*

Germplasm code	Number of selected accessions	Bed No.
RO/PB/1	103	02
RO/PB/2	210	03
RO/OP/4	118	20
RO/JP/3	209	22
AC/F/5	157	21
AC/T/2	45	04
AC/T/1	91	05
AC/T/3	21	06
AC/T/4	54	13
MT/C/1	40	18
MT/C/10	38	17
MT/C/2	90	10
MT/C/6	54	11
MT/C/5	76	12
MT/C/8	07	14
MT/C/9	17	15
MT/C/3	17	16
MT/C/4	98	07
MT/C/7	09	08
MT/C/11	24	09
7/02/81	20	01
Total	1478	

Fig. 6. Establishment of selected accessions for characterization at Neuchatle estate

However, many germplasm collections in many crops are being lost worldwide. The erosion of the genetic variability of *Hevea* plantations in all over the world is under a constant threat of sudden out breaks of a native as well as exotic diseases while complicating the situation with changing climate. Therefore, it is important that the potential uses and values of this genetic resources need to be developed to improve and facilitate productive utilization in *Hevea* breeding in future. Maintenance and conservation of the core collection is also very important.

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Raw rubber and viscoelastic properties of organoclay filled latex crepe rubber

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Abstract

Organoclay (OC) filled natural rubber (NR) latex crepe was prepared in view of investigating the effect of organoclay on raw rubber properties and viscoelastic properties by incorporating aqueous dispersion of OC clay into NR latex followed by conventional acid coagulation procedure.
Raw rubber properties of OC filled fractionated bleached (FB) and unfractionated unbleached (UFUB) crepe rubber were evaluated to characterized the composite samples in comparison to standard crepe rubber whilst process based characterization was carried out with Moony viscosity and Mooney relaxation data. Raw rubber properties have shown that OC acts as a pro-oxidant and as a result, Initial Plasticity (Po) and Plasticity Retention Index (PRI) which measures the thermo-oxidative degradation were negatively affected. Viscoelastic behaviour, as measured with low shear strain rate flow properties, is significantly affected by the addition of OC into the NR latex. Reduction of Mooney viscosity of OC filled crepe rubber has been explained with thermal oxidation as a result of metal impurities in the OC and plasticization caused by the organic modifier of the OC. Mooney stress relaxation has shown that lower clay loading provides a better processability and dispersion of OC within the crepe rubber.

***Key words*:** crepe rubber, Natural rubber, organoclay (OC), Mooney viscosity, Mooney stress relaxation, raw rubber properties, thermo-oxidative degradation

Introduction

Natural Rubber (NR) obtained from *Hevea brasiliensis* is one of the most important engineering raw materials for different industrial applications. Among the different types of NR grades, NR latex crepe is considered as the purest form of NR. After removing non-rubber substituents from the field latex by fractionation and bleaching processes, fractioned bleached (FB) crepe rubber can be obtained whilst without following these two steps un-fractioned unbleached (UFUB) crepe rubber is produced (Senevirathne and Kumara, 2003). Latex crepe is mostly utilized to manufacture of pharmaceutical and surgical articles, infant toys and food contact articles.

NR is widely used to manufacture of engineering products due to its excellent mechanical properties (Onyeagoro G.N., 2013) and due to some of the other advantages such as superior dynamic properties, low heat build-up and excellent tack (De Silva K.G.K., 2003). Although NR has a superior green strength, less stiffness and poor dimensional stability obstruct NR applications in engineering products. Therefore, in the manufacture of rubber products, reinforcing fillers are used to improve the physico-mechanical properties and to reduce the cost of the products. Reinforcing fillers such as carbon black and silica are widely used but carbon black causes environmental pollution (Alwis *et al.*, 2014). Silica is widely used to manufacture of lighter coloured rubber products (Chonkaew *et al.*, 2011).

Recently, polymeric nanocomposites have attracted a greater interest in academia and industry due to its unique properties than conventional polymer composites (Ratnayake *et al.*, 2008; Alwis G.M.C. *et al.*, 2014). Further, NR nanocomposites have shown a significant improvement in physico-mechanical properties at lower loading levels of nanomaterial as filler over conventional rubber composites containing carbon black and silica. Among the nanomaterial/nanofiller, montmorillonite (MMT) clay is one of the widely researched reinforcing filler for rubber applications due to its higher aspect ratio of 50-300 and higher specific surface area of 750-800 m^2/g.

The clay platelets/layers in MMT clay particles are separated by a gallery space, which consists of hydrated cations such as Na^+, K^+ and water molecules. It has been identified that several active sites such as a few positive charges, hydroxyl groups and negative charges are present on the surface of MMT clay crystals (Utracki, 2004). MMT clay crystals are made out of a large number of individual clay platelets/layers with a 1 nm thickness and a few microns in other dimensions (Brindley and Brown, 1980; Ray and Okamotoa, 2003). The space between two successive layers of hydrophilic MMT is too small to diffuse polymer molecules into the gallery space (Utraki, 2004) and, as a result, it is a challenge to incorporate hydrophilic MMT into hydrophobic NR (Alwis *et al.*, 2014). However, organic modification of MMT with the use of quaternary ammonium salts produces organonophilic montmorillonite clay (OMMT), in which compatibility with NR has been improved (Gatos and Kocsis, 2010).

Incorporation of MMT clay into rubber matrix can be achieved in different ways such as melt mixing, latex compounding and *etc.* (Gatos and Kocsis, 2010). However, incorporation of MMT clay at the NR latex stage and followed by coagulation is an easy and economical compared to that of other methods as reported by Li-Qun Zhang, 2010. However, to achieve a better reinforcement and processability, MMT clay should be exfoliated/delaminated with in the rubber matrix into individual platelets, a 1 nm thickness (Alwis *et al*., 2014).

Preparation of MMT clay filled NR latex crepe rubber and its effect on properties have not been reported in the literature. Therefore, developing a novel NR latex crepe rubber with different characteristics would be advancement in the crepe rubber industry.

The objective of the study reported here is to prepare organoclay (OC) filled latex crepe rubber with an optimum dispersion of organoclay and to investigate the effect of nanostructures of OC on raw rubber properties and viscoelastic properties of the crepe rubber.

Materials and Methods

Materials

Natural rubber (NR) latex with 30% dry rubber content (DRC) obtained from Dartonfield rubber factory, Rubber Research Institute was used to manufacture organoclay (OC) filled NR latex crepe rubber. Montmorillonite (MMT) clay (Cloisite 20A) modified with dimethyl dihydrogenated tallow ammonium salt and with an ignite weight loss of 43-45% supplied by Southern Clay Products, Inc. Co. Ltd. was used to prepare organoclay (OC) filled latex crepe rubber. Other general compounding chemicals used were of commercial grade.

Characterization of Organoclay

Metal ions such as iron and copper were analyzed to find out the metal impurities available within the OC. Concentrated hydrochloric acid was added to ashed organoclay samples and they were kept on a water bath for 20 minutes for the digestion at 60 ºC. Digested samples were analyzed for metal ions with an Atomic Absorption Spectrophotometer (AAS), model Avanta series CAN 005471686.

Preparation of organoclay (OC) filled NR latex crepe rubber

Organoclay filled fractionated bleached crepe rubber (OFBC) and unfractionated unbleached crepe rubber (OUFUBC) were prepared by incorporating organoclay dispersion into NR latex followed by standard manufacturing procedure of NR latex crepe rubber (Senevirathne and Kumara, 2003). Table 1 illustrates the organoclay loading for each type of crepe rubber.

An aqueous dispersion of 5% (w/w) organoclay was prepared by mixing organoclay (OC) in water in the presence of small amount of anionic surfactant and 10% (w/w) potassium laurate soap.

Table 1. *Organoclay (OC) filled NR latex crepe rubber samples*

Organoclay amount (phr)	Fractioned bleached (FB)	Unfractionated unbleached (UFUB)
0	OFBC -0	OUFUBC -0
2	OFBC -2	OUFUBC -2
4	OFBC -4	OUFUBC -4
6	OFBC -6	OUFUBC -6
8	OFBC -8	OUFUBC -8

The last digit shows the amount of organoclay loading and ‘0’ denotes the reference sample where it contains no clay.

Characterization of raw rubber properties

Ash content in the OFBC and OUFUBC was measured to evaluate the Aluminosilicate (*i.e.* inorganic material) content in the rubber according to the standard test method of SO 247: 1990 (E) (Test method A) using Gallenkamp muffle furnace (Size 2/Tactical 308).

Clay recovery percentage in both crepe rubber types was computed to assess the amount of organoclay (OC) retained in both crepe rubber samples. It was calculated based on the ash content present in the OC filled crepe rubber samples as per the below equation.

$$\textbf{Clay recovery\%} = \frac{(\mathbf{M1} - \mathbf{M2}) + \mathbf{M3}}{\mathbf{M}} \times \mathbf{100\%}$$

Where, M is the actual added amount of organoclay into 1 kg of rubber, M1 is total ash content of organoclay filled crepe rubber (layered aluminosilicate plus inorganic metal oxides), M2 is ash content of unfilled crepe rubber (metal oxides in the control samples) and M3 is theoretical organic modifier content (43-45%) which has been burnt off during the ashing process.

The effect of dispersed clay structures on stiffness of both OFBC and OUFUBC composite samples was measured with Initial Plasticity (P_o) of the raw rubber. P_o of the OC filled crepe rubber samples was performed by Wallace Rapid Plastimeter, MkV model at 100°C ± 1°C as standard test method of ISO 7323:2006 (E).

Plasticity Retention Index (PRI), which measures the resistance to thermo-oxidative degradation of NR un-vulcanized raw rubber was used to evaluate the effect of organoclay on thermo-oxidative stability of OC filled latex crepe rubber composites. PRI was measured by analyzing the P_o before and after ageing at 140°C for half an hour using the same plastimeter as per the ISO 2930:2009 (E). PRI of the organoclay filled crepe rubber was calculated as per the below standard equation.

$$\mathbf{PRI} = \frac{\mathbf{P_{30}}}{\mathbf{P_0}} \times \mathbf{100\ \%}$$

where, P_{30} – aged rapid plasticity number after aging the sample for 30 minutes 140 °C and P_0 - unaged initial rapid plasticity of rubber at room temperature.

Measuring of nitrogen content in organoclay (OC) filled latex crepe rubber composite samples is important to identify the effect of ammonium modifier in organoclay (OC) on the raw rubber properties and viscoelastic properties. Kjeldahl method as in ISO 1656:2014 (E) was carried out in order to estimate the nitrogen content in organoclay (OC) filled latex crepe rubber composites.

Volatile matter content represents mostly the amount of moisture in crepe rubber and low molecular weight volatile compounds. Higher amount of moisture have the potential to behave as a plasticizer for NR. ISO 248 Part I: 2011 (E) standard test method was used to determine the volatile matter content.

Characterization of viscoelastic behaviour

Mooney viscosity, a measure of resistance to flow at low shear strain rate, of OC clay filled NR latex crepe rubber was investigated to study the effect of OC clay structures on processability of the NR composites. Initial peak of Mooney curve indicates the viscosity at the on-set of the test; it is a function of the green strength of rubber (ASTM D 1646 – 04). The investigation of Mooney viscosity and initial peak of the organoclay filled crepe rubber composites was performed according to the ISO/R 289-1: 2014 (E) using Ektron Mooney viscometer, model EKT 2001 M.

Mooney stress relaxation (MSR)

Mooney stress relaxation, which correlates to the elastic behaviour of rubber, was analyzed to study how dispersed organoclay structures affect the elastic behaviour of the NR composites. Mooney stress relaxation curve was obtained by stopping the rotor immediately after the Mooney viscosity test and recording Mooney values in 20 seconds intervals for 2 minutes. Stress relaxation of rubber can usually be explained with by power law model,

$$\mathbf{M= k(t)^a}$$

where M is torque value in Mooney units and a is the rate of stress relaxation (Malac 2011)

$$\mathbf{\log M = a \log t + \log k}$$

The slope, (a) of log-log plot of power law model is one method of quantifying uncured rubber elasticity and a steep slope indicates less uncured elasticity result in better processability (Malac J., 2009). Also the stress relaxation is the response to a sudden cessation of deformation, when the rotor of the Mooney viscometer stops. The rate of stress relaxation can correlate with molecular structure characteristics such as molecular weight distribution, chain branching and gel content (Rogers M. B. *et al*, 2005).

Results and Discussion

Characterization of organoclay

The analysis of ash content shows that inorganic content in the organoclay is 52% - 58% indicating the balance of 48 – 42% is organic modifier percentage and it is in agreement with the supplier information. This ash content represents the amount of aluminosilicates and other inorganic metal oxide present in the organoclay. Total copper and iron concentration in the organoclay as impurities shown in Table 2 are 1.54 ppm and 540 ppm respectively. Higher amount of these metal ions, known as pro-oxidants, are likely to affect the raw rubber properties and processability of the organoclay filled crepe rubber.

Table 2. *Metal ion content in organoclay*

Metal ion	ppm
Total copper	1.94
Total iron	540.56

Raw rubber properties of organoclay filled crepe rubber

Both organoclay filled fractionated bleached crepe rubber (OFBC) and organoclay filled un-fractionated un-bleached crepe rubber (OUFUBC) samples were compared with the average raw rubber properties of the NR latex crepe rubber (Hand Book of Rubber Processing Technology, RRISL). Table 3 presents raw rubber properties of OFBC-0 and OUFUBC-0 samples containing no OC. According to the results obtained for each raw rubber property, average raw rubber properties of the both samples are comply with standard specifications shown by Senevirathne *et al*., 2003 in Hand Book of Rubber Processing Technology, RRISL. According to De Silva, K.G.K., 2003, it has been reported that the volatile matter content of UFUB crepe rubber can be higher than that of FB crepe rubber because of the higher percentage of proteins along with other non-rubbers absorb moisture.

Table 3. *Average raw rubber specifications of crepe rubber composites*

Property	Specification	OFBC -0	OUFUBC -0
Volatile matter% (w/w)	0.5 % (max)	0.23	0.30
Ash content% (w /w)	0.20 (max)	0.16	0.07
P_0 (Wallace units)	30 (min)	38.6	44.15
PRI (Wallace units)	60 (min)	62.52	78.2
Nitrogen content% (w/w)	0.35 (max)	0.31	0.34
Mooney viscosity	75 – 85	75.85	76.9

Mooney viscosity of OFBC-0 and OUFUBC-0 are not significantly deviated from the recommended range but it is high in OUFUBC-0 sample because non–rubbers can influence to resist the thermo-oxidative degradation of NR at the testing temperature of 100 ºC at low shear strain rate. In addition, non-rubbers especially proteins improves the stiffness of NR by acting as filler which can affect to increase the P_o and Mooney viscosity.

Organoclay recovery percentage

Clay recovery percentage was calculated based on the ash contents in both organoclay filled latex crepe rubber composite samples (OFBC and OUFUBC) to determine the effective clay percentage retained within the crepe rubber. As shown in Table 4, clay recovery percentage decreases with the increase of organoclay loading in both types of the crepe rubber composite samples. As expected, small percentage of organoclay has been washed off with the serum during processing. However, slightly higher organoclay recovery percentage was resulted in FB crepe rubber than UFUB crepe rubber.

Non-rubber substances which are lower molecular weight macromolecules such as proteins may have a potential to intercalate into the interlayer spacing of the organoclay better than rubber molecules due to their smaller sizes. This could be the reason for removing more clay with the serum during processing of OUFUBC than OFBC composites.

Table 4. *Clay recovery percentage in organoclay filled latex crepe rubber*

Sample code	Clay recovery (%)	Sample code	Clay recovery (%)
OFBC-0	-	**OUFUBC-0**	-
OFBC-2	97.30	**OUFUBC-2**	96.53
OFBC-4	97.82	**OUFUBC-4**	89.11
OFBC-6	94.66	**OUFUBC-6**	89.46
OFBC-8	93.78	**OUFUBC-8**	87.94

Raw rubber properties of organoclay filled crepe rubber

Raw rubber properties of both organoclay filled latex crepe rubber (OFBC and OUFUBC) with increasing organoclay loading are shown in Table 5 and 6 where compared with that of average raw rubber properties of the crepe rubber. As expected, volatile matter content (VM %) in both OFBC and OUFUBC composites increases with the filler loading.

Volatile matter content of OUFUBC composites is higher than that of OFBC composites because of the presence of proteins along with other non-rubbers substances results in absorption of water (De Silva, K.G.K., 2003). However, volatile matter content has not exceeded the critical value of 0.5%. Therefore, volatile matter, mostly the absorbed, moisture could not have a significant effect on physical properties of the clay filled crepe rubber.

Nitrogen content in both OFBC and OUFUBC composites has shown an increasing trend with the increase of organoclay loading as organic modifier, dimethyl dihydrogenated tallow ammonium contains nitrogen. Ratnayake *et al.*, (2009), has reported that maximum critical limits for copper and iron ions in NR latex crepe rubber are 1.6 ppm and 30 ppm respectively. As shown in Table 5 and 6, both OFBC and OUFUBC composite samples, especially samples prepared with higher dosages of organoclay, contained higher concentration of iron and copper due to the higher amount of copper and iron ion contaminations in the organoclay.

Table 5. *Raw rubber properties of OFBC composites*

Sample code	VM%	Ash%	N%	Total iron ions (ppm)	Total copper ion (ppm)
OFBC-0	0.23	0.16	0.31	17.98	1.48
OFBC-2	0.27	1.22	0.34	30.47	1.49
OFBC-4	0.33	2.28	0.36	42.96	1.51
OFBC-6	0.36	3.28	0.39	55.45	1.53
OFBC-8	0.42	4.28	0.44	67.94	1.55

Table 6. *Raw rubber properties of OUFUBC composites*

Sample code	VM%	Ash%	N%	Total iron ions (ppm)	Total copper ion (ppm)
OUFUBC-0	0.30	0.07	0.34	20.12	1.43
OUFUBC-2	0.31	1.04	0.38	42.61	1.44
OUFUBC-4	0.34	1.92	0.41	55.10	1.46
OUFUBC-6	0.39	2.77	0.43	67.59	1.49
OUFUBC-8	0.43	3.61	0.47	80.08	1.51

As shown in Figure 1, Initial plasticity (P_o) of FBC-2 and OUFUBC-2 has reduced by 21.7% and 31.78% respectively at 2 phr organoclay loading. As expected, P_o of OUFUBC is higher than that of OFBC composites in each clay loading and, in both cases, when organoclay loading increases more than 4 phr, P_o has not significantly changed. According to these results the initial stiffness of NR has reduced significantly. This could be due to either thermal degradation of NR by the higher amount of iron and copper ions present in the organoclay or plasticization effect created as a result of organic modifier in the organoclay at 100 ºC testing temperature.

In the preparation of OFBC samples the bleaching agent was added. It is also known that disulphide in bleaching agent has a tendency to reduce the molecular weight of NR by acting as a peptizing agent (Jana *et al*., 2006; Jana and Das 2005; Pipat *et al*., 2010). This disulphide in the FB crepe rubber could be a possible cause to reduce the stiffness of the rubber, resulting in reduced P_o values (Ratnayake U.N., 2011). Poor interaction of NR molecules with OC stacks can also have a potential to plasticize the OFBC and OUFUBC composite samples. Plasticity of NR directly correlates with the molecular weight and the molecular weight distribution of NR. Therefore, it can be predicted that iron and copper ions have acted as a pro-oxidant for organoclay filled latex crepe rubber composites.

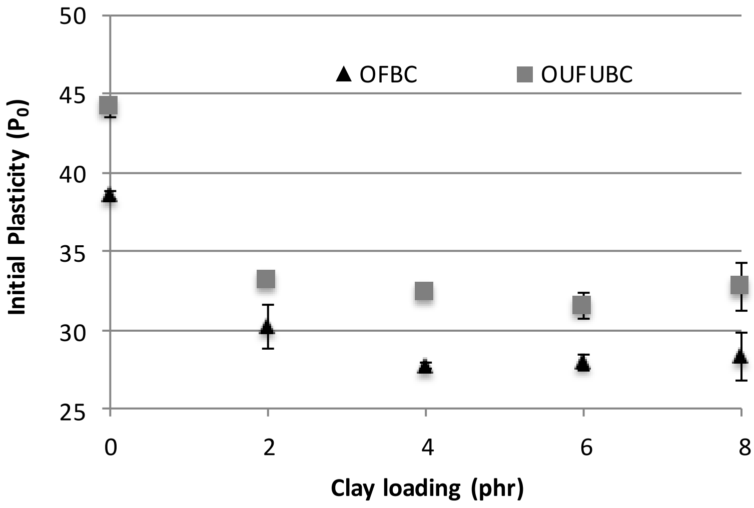

Fig 1. Initial plasticity of organoclay filled latex crepe rubber composites

Plasticity Retention Index (PRI), which measures the resistance to thermo-oxidative degradation, of the crepe rubber samples was analyzed to evaluate the effect of OC clay on thermo-oxidative degradation. As shown in Figure 2, both OFBC and OUFUBC composites have indicated a significant reduction of PRI values compared to that of control samples of both when organoclay loading was increased.

OFBC-2 and OUFUBC-2 composites have shown 76.4% and 71.45% reduction of PRI respectively, indicating that clay accelerates the thermo-oxidative degradation. However, PRI values were not significantly changed beyond the 2 phr loading of OC. PRI of OUFUBC composites is higher than that of the OFBC composites because OUFUBC composites contain natural antioxidants. As reported in Table 5 and 6, total iron concentration in both composites is significantly higher compared to the critical value for crepe rubber (Ratnayake *et al*., 2009) and, as a result, marked reduction of PRI. Nicolaas Maria Huntink, (2003) has reported that metal ion catalyze decomposition of rubber when higher concentrations of copper and iron ions are present. The higher amount of iron and copper ions in the organoclay filled latex crepe rubber composites have a greater potential to accelerate the thermal degradation of NR and reduce the molecular weight by acting as a pro-oxidant.

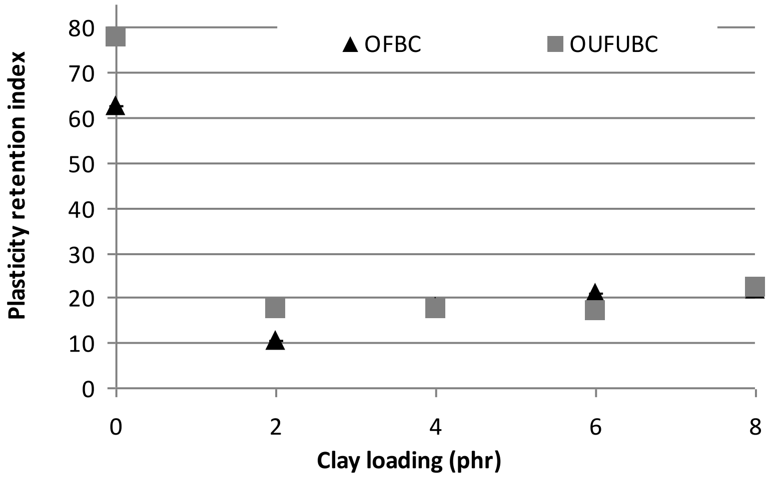

Fig 2. PRI of organoclay filled latex crepe rubber composites

Fe^{3+} ions in the NR latex has the ability to oxidize part of thiol which is used as bleaching agent in manufacturing of fractioned bleached crepe rubber. Fe^{3+} reduces the bleaching power of mercaptan thiol, simultaneously, it makes disulphides and it leads to oxidation or chain break of NR molecules (Michael and Swindell, 2000). Fe^{3+} ions are more effective in this action compared to Fe^{2+} as reported by Ratnayake *et al.*, 2011. Higher amount of copper ions in the crepe rubber also has the same effect to degrade the NR by thermo-oxidation.

Viscoelastic properties of organoclay filled latex crepe rubber

Mooney curves obtained for organoclay filled latex crepe rubber composites are shown in Figure 3 and 4. Initial peak value which can be correlated to green strength of NR, Mooney viscosity and uncured elasticity of both types of crepe rubber containing OC were analyzed to study effect of OC on viscoelastic behaviour.

Figure 5 presents the Mooney viscosity of gum NR and organoclay filled crepe rubber samples. Incorporation of 2 phr of organoclay has reduced Mooney viscosity of OFBC and OUFUBC by 12.9% and 13.8% respectively. Thereafter, Mooney viscosity has significantly reduced with the increase of organoclay loading levels and, as a result, marked deviation from the standard value of crepe rubber as reported by Seneviratne *et al.*, 2003 in Handbook of Rubber Processing Technology, RRISL, indicating a significant effect on processability.

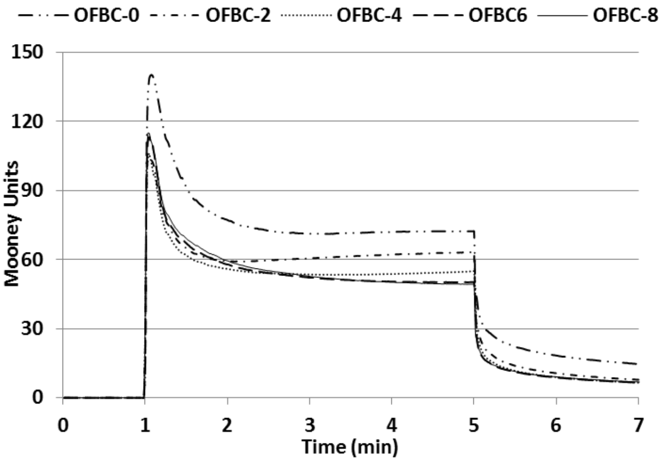

Fig 3. Mooney viscosity curves of OFBCR composites

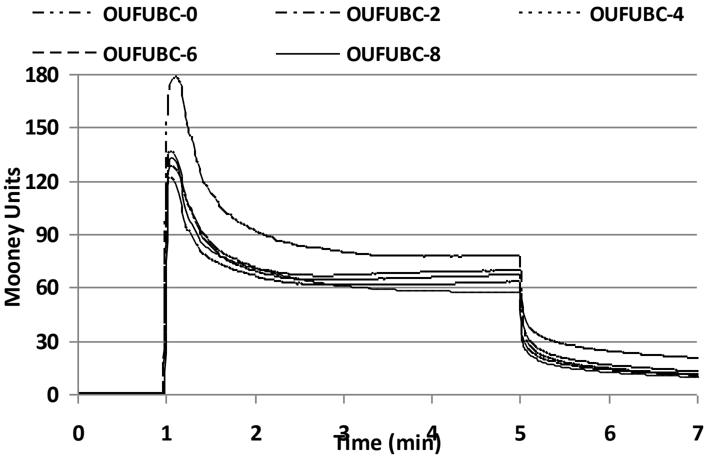

Fig. 4. Mooney viscosity curves of OUFUBC composites

The reason for the reduction of Mooney viscosity when increasing the organoclay loading could be the plasticization of NR molecules by organic modifier in the organoclay because smaller organic non-volatile molecules can act as plasticizers. Ratnayake & Ketipearchchi (2012), have shown that organic modifiers used to modify MMT have a significant effect on thermo-oxidative degradation. Thermal oxidation can also be accelerated at the testing temperature of 100 °C as a result of containing higher concentration of metal ions, especially iron and copper ions.

Organoclay filled crepe rubber consists of higher amount of iron and copper ions that are higher than the critical limits and therefore accelerates the thermo-oxidative degradation as reported before. This would suggest that higher concentration of iron and copper ions in the organoclay filled crepe rubber has reduced the molecular weight of NR by thermal degradation and, therefore, reduce the Mooney viscosity.

There are some other reasons such as organoclay can absorb moisture because it is a highly hydroscopic material which swells in the presence of water and the moisture can plasticize the rubber and reduce the Mooney viscosity (Faheem, 2008). On the other hand, there may be a possibility of chain slippage over the clay platelets due to shear action by the rotor of Mooney viscometer (Cassagnau & Barres, 2010).

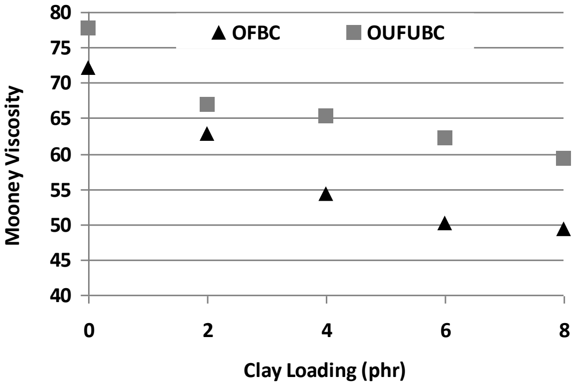

Fig 5. Mooney viscosity of organoclay filled latex crepe rubber composites

The reason for higher Mooney viscosities of OUFUBC composites compared to OFBC composites is the resistance to thermo-oxidative degradation by the natural antioxidants present in OUFUBC composites and some non-rubbers such as proteins act as filler for NR as reported in ASTM D 1646 – 04 standard.

Kader *et al*. (2010) showed Mooney viscosities that decreases between pure polymer and composites and that go through a minimum upon increase of filler concentration. Some arguments for explaining this effect, which can be summarized as follows: the reduction of viscosity upon increasing nanofiller concentration might be partly due to breakdown of platelets stacks and subsequent dispersion and exfoliation under shear, to alignment during flow and to some plasticizing action from the presence of the long chain organic modifier in the clay, possibly causing chain slippage over the clay platelets. As it is clearly shown here, the flow behavior of prepared organoclay filled crepe rubber composites does not exhibit a simple trend because of the miscellaneous and complex influences of polymer- organoclay interactions, degree of dispersion and orientation-related features during flow.

The initial Mooney peak obtained from the Mooney curves of uncured organoclay filled latex crepe rubber composites has shown a greater reduction with the increase of organoclay loading as shown in Figure 6.

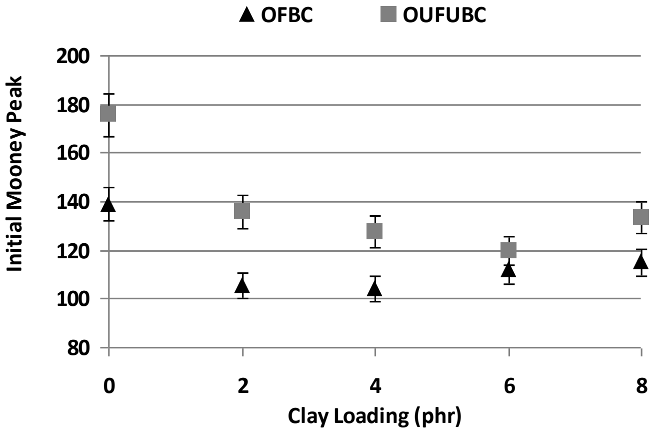

Fig. 6. Initial Mooney peak of organoclay filled latex crepe rubber composites

In both OFBC and OUFUB composites, initial Mooney peak has reduced up to 4 phr clay loading level and thereafter there is no indication of significant change with the increase of organoclay. As reported by the Malac (2010), the initial Mooney peak value can be used to describe the green strength of uncured NR. As shown in initial Mooney peak values, organoclay has a significant effect on green strength of organoclay filled latex crepe rubber. Plasticization effect of low molecular weight modifiers in the organoclay, the oxidation of NR by the copper and iron metal ions in organoclay and slip of molecules on the surface of layered aluminosilicate platelets/stacks could be the main reasons to reduce the green strength of organoclay filled latex crepe rubber.

In addition to Mooney viscosity, Mooney stress relaxation (MSR) data which provides the information on elasticity at low shear strain rate of the uncured rubber. Log-log plots obtained by the power law model for MSR are shown in Figure 7 and 8.

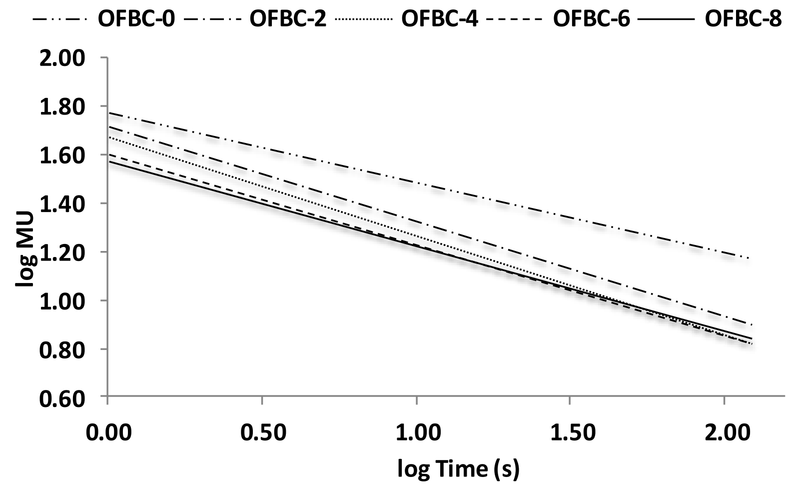

Fig 7. Log – log plot for Mooney stress relaxation OFBC composite

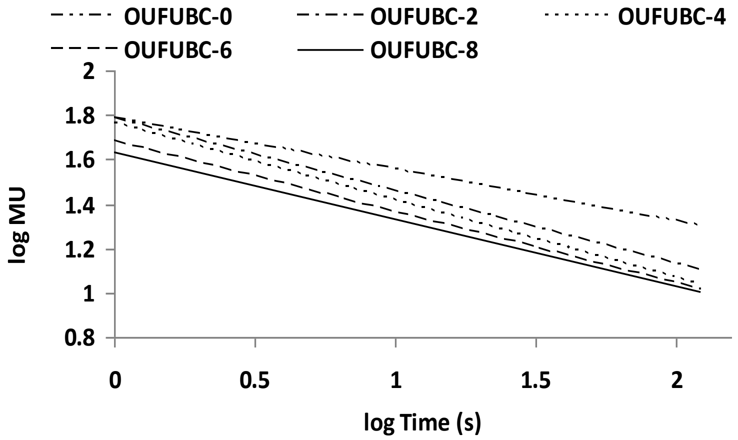

Fig. 8. log – log plot for Mooney stress relaxation of OUFUBC composites

Mooney stress relaxation coefficients for the prepared organoclay filled latex crepe rubber composite samples are shown in Table 7 and Table 8. As reported, the MSR coefficients of both OFBC and OUFUBC composites are initially increased up to 4 phr of organoclay loading level and then it reduces slightly when the organoclay loading is increased beyond 4 phr. Also the MSR coefficients of OFBC-0 and OUFUBC-0 are the minimum and therefore, these samples have the higher elasticity compared to other composite samples filled with organoclay. From 0 to 4 phr of OC loading, the elasticity of both OFB and OUFUB crepe rubber composites is reduced and then the elasticity increases when the organoclay loading increases because the exponent is inversely proportional to the elasticity of uncured composites of NR.

The stress relaxation behavior of natural rubber is a combination of both elastic and viscous response. Viscosity and stress relaxation behavior do not depend on molecular weight and non-rubber constituents in the same way. The rate of stress relaxation has been found to correlate with rubber structural characteristics such as molecular weight distribution, chain branching and gel content (Malac, 2009).

Long relaxation times and high amplitudes of stress relaxation suggest that the mechanism of structural relaxation involving large-scale displacements of isolated clusters of nanoparticles within the rubber matrix and vice versa may be the reason for the stress relaxation behavior of composites in this study according to Sukhy *et al.* (2010). Therefore, according to the MSR coefficient values maximum processability/mixing can be achieved at organoclay loading level of 4 phr with a minimum elasticity.

Table 7. *Mooney stress relaxation of OFBC composites*

Sample code	MSR coefficient (a)	Constant (k)
OFBC-0	0.287	1.773
OFBC-2	0.390	1.719
OFBC-4	0.407	1.673
OFBC-6	0.373	1.604
OFBC-8	0.349	1.574

Table 8. *Mooney stress relaxation OUFUBC composites*

Sample code	Exponent (a)	Constant (k)
OUFUBC-0	0.232	1.786
OUFUBC-2	0.330	1.788
OUFUBC-4	0.348	1.761
OUFUBC-6	0.320	1.683
OUFUBC-8	0.302	1.634

When compare the OFBC and OUFUBC composites, the higher elasticity has been achieved by OUFUBC composites. This may be due to natural anti-oxidants in the OUFUBC composites that can resist the thermal oxidative degradation of NR molecules by metal ions in crepe rubber prepared using organoclay. This resistance is less in OFBC composites and it has shown less elasticity when increase the organoclay loading level as molecular weight reduction is high due to oxidation. Also dispersion of organoclay can be predicted by the Mooney stress relaxation data. According to the results in Table 8, minimum elasticity of both uncured composites has been achieved at 4 phr organoclay loading level. Therefore according to the Malac J this level would be the best processable organoclay loading level.

Conclusion

Organoclay filled fractionated bleached (OFBC) and unfractionated unbleached (OUFUBC) latex crepe rubber composites were successfully prepared by the addition of 5% w/w aqueous dispersion of organoclay in view of enhancing the degree of dispersion of organoclay with a clay recovery percentage more than 80%.

Characterization of raw rubber properties revealed that initial plasticity (P_o) and plasticity retention index (PRI) are adversely affected by the addition of organoclay. Organoclay accelerates the thermo-oxidative degradation of crepe rubber because of it contains higher concentration of iron and copper ions, even with 2 phr loading level accelerates the thermo-oxidative degradation. As a result, higher concentration of metal ions in the organoclay limits the organoclay applications in natural rubber.

Processability, as measured with low shear strain rate Mooney viscosity, has affected by the addition of organoclay. Significant reduction of Mooney viscosity of both grades of organoclay filled latex crepe rubber composites is caused due to combination effect of thermal oxidation and plasticization effect induced by the clay. However, elasticity, as correlated by the Mooney stress relaxation, information have proven that optimum dispersion of organoclay in NR can be achieved with 4 phr clay loading level with a minimum elasticity and better processability.

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Insight into prevention of enzymatic browning and increase of antioxidant potential of crepe rubber with ethephon stimulation

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Abstract

The effect of different ethephon concentrations 1%-5% (w/v) on colour of crepe rubber and plasticity retention index (PRI) was studied. The study was conducted with the RRISL 121 clone using S/2, d3 harvesting system. The results revealed that ethephon stimulation affects strongly the antioxidant and polyphenol oxidase (PPO) inhibitory potential. Further, thiol content and PRI values were increased while giving low Lovibond colour index upto 3% ethephon concentration and the properties were found to be inferior above 3% concentration. A probable reaction mechanism is proposed for colour improvement with optimum concentration of ethephon.

Key words: Ethephon stimulation, Lovibond colour index, plasticity retention index Polyphenol oxidase, Thiol content

Introduction

Latex crepe is the purest form of natural rubber available in the market and Sri Lanka is the world's leading producer of pale crepe and sole crepe for the export market (Seneviratne *et al.*, 2003). Latex crepe is widely used in rubber teats, balloons, condoms, infant items and toys. However, dark colour developed in dry rubber is a major issue in some industries which require a pale colour raw material. Therefore, colour is one of the most important raw rubber properties in latex crepe. The colour of the rubber depends on non-rubber constituents of latex. Raw natural rubber contain about 94% hydrocarbon (cis 1- 4, Poly isoprene) and 6% non rubber constituents such as proteins, lipids, carbohydrate (Allen, 1963). It has been reported that carotenoids, tocotrienols, polyphenol oxidase, fatty alcohol esters, unsaturated fatty acids, fatty alcohols, di-glyceride and mono - glyceride in cause the discoloration natural rubber (NR) (Sakdapipanich, *et al.*, 2007). The concentration of polyphenols, proteins, carotenoids are 2 X 10^{-2}, 1 and 3 X 10^{-5} (w/w) respectively and their contribution to the total absorptivity in the yellow brown region are 2.3, 0.1 and 0.01% respectively. Out of the total absorbance 90% is attributed to the polyisoprene in rubber molecule (Yusree *et al.*, 2012). The enzymatic discoloration is caused by the naturally occurring phenols and amino phenols in latex (Coupe *et al.*, 1972). Discoloration due to ethephon stimulation has been reported (Yapa, 1976), however there is no evidence for the effect of ethephon concentration on colour of crepe rubber. Polyphenol oxidases are the key enzymes in the natural coagulation and darkning of latex (Brozozowska-Hanower, 1978) and they are located inside the frey-wyssling particles. Frey-wyssling particles are mainly lipid in character and are not present in ammonia preserved concentrated latex. Apparently, they are either eliminated during centrifuging or they have got dissolved in the serum when the latex is ammoniated (d'Auzac, 1989). The polyphenol oxidase is responsible for the darkening of coagulated rubber on exposure to air or oxygen (Wititsuwannakul *et al.*, 2002). With the presence of polyphenol oxidase, phenols and amino phenols combine with oxygen from air to form orthoquinones. These orthoquinones react with naturally occurring amino acids and proteins in latex, giving coloured compounds resembling melanin (Mason, 1955). Both polyphenol oxidase (PPO) and phenolic substances are present in latex but do not react with each other intact laticifers. However, in the process of fractionation and coagulation, the Frey-Wyssling particles are disrupted and the enzyme released into the latex serum. This subsequently leads to discolouration of latex (Schadel and Walter, 1981). Stimulation by ethephon greatly reduces latex polyphenol oxidase activity which is known to be correlated positively with plugging index, which results in increase of phenolic substances in stimulated latex. Significant increase in phenolic content reported (after first & second tapping) after ethephon treatment is due to decrease in phenol oxidase activity (Cretin, 1978). Brozozowska-Hanower *et al., 1978* showed a definite lowering of o-diphenol oxidase activity in lattices at the first tapping after stimulation. Phenolic compounds have been reported to react, after enzymatic oxidation with thiols such as cysteine (CSH) and glutathione (GSH) to form colorless product (Mason, 1955). The presence of thiols in high concentration in latex act as an antioxidant hence, it can be used to improve colour (Yapa, 1976).

Glutathione is an endogenous antioxidant that plays a major role in reducing reactive oxygen species (ROS) formed during cellular metabolism (https://en.wikipedia.org/wiki/Glutathione). Therefore a clone with high phenolic content can still yield a colorless or light colour rubber, provided that it contains sufficient thiols. Glutathione is known to play a major role in reducing ROS formed during cellular metabolism. Therefore, the objective of the present study was to investigate the effect of ethephon concentration on latex discolouration and underpinning reasons.

Materials and Methods

Experimental design and location of the study

Six experimental blocks comprising 75 healthy trees from mono clonal (RRISL 121) mature rubber trees were selected from the Galewatta Division, Dartonfield Estate, Agalawatta, Sri Lanka for the experiment. Each block was divided into three replicates comprising 25 trees. Trees in each block were stimulated with 1.6g of 1%, 2%, 3%, 4% and 5% (w/v) ethephon applied on the tapping panel using an ¼" paint brush and tapped with half spiral based once in three days harvesting system by keeping one block without stimulation as the control. All the experimental tapping blocks were tapped by the same tapper in order to avoid tapper variability.

Freshly tapped latex collected into vessels, immersed in ice and then send to the laboratory immediately for the analysis of sucrose and thiol content without preservation and the other fraction was processed into un-fractioned un-bleached crepe rubber (UFUB).

Determination of thiol content in natural rubber latex

Analysis of thiol content was carried out by using colorimetric method developed by Boyne and Ellman, 1972 with three replicates. Serum was extracted by coagulating 1g of latex with 2.5% tri-chloro acetic acid (TCA) and volumed up to 25mL. To 2mL of extract, 0.1mL of dithio- bis -nitrobenzoic acid solution (10 mM) and 2mL tris (0.05M) was added and the optical density was measured at 412nm within 30min using a Jenway 6405 UV/Vis spectrophotometer. The amount of thiol was estimated against a standard curve prepared using reduced glutathione.

Determination of Lovibond Colour Index

The test was carried out according to ISO 4660 (2011) with three replicates. Three test pieces were cut from a homogenized UFUB sample using test piece punch and pressed them together (3.2-3.6 mm thick). The test pieces were then laminated and pressed in a mould between two sheets of transparent polyester film, with mould covers superimposed at a pressure of not less than 3.5 MPa for 5min at 150 ^{0}C±3^{0}C. The 3 test pieces (1.6 mm thick) were retained in the mould and it was compared with standard Lovibond colour glasses (4/19A) under diffuse daylight against a matt white background.

Determination of plasticity retention index

The test was carried out according to ISO 2930(2009) with three replicates. The test pellets were cut from a homogenized sample of UFUB in order to get sample thickness of 3.4 mm. The rapid plasticity number of unaged test pieces and test pieces aged at 140 ± 0.2ºC for 30 min were measured using parallel plate plastimeter.

Infra-red Spectroscopy

The rubber samples were extracted with acetone for 16 hours and acetone extract was sandwiched between two NaCl cells and Fourier transform infrared spectroscopy (FTIR) spectra were recorded on a Nicolet 380 spectrophotometer with 32 scans to improve signal to noise ratio.

Data analysis

The experimental design was complete randomized design and data was analyzed using Minitab 16 statistical software. One-way ANOVA was performed for basic parameters together with mean separation by Tukey method.

Results and Discussion

With s/2 d/3tapping system, the thiol content (antioxidant) and plasticity retention index increases up to 3% and above that concentration the properties were drastically reduced with increasing ethephon concentration (Table 1). It is evident from the results that highest antioxidant activity showed at 3%, hence maximum PRI value also obtained at 3% stimulant level. Above 3%, the thiol content decreased. This may be due to enhanced in vitro synthesis of reactive oxygen species (ROS). These ROS groups are oxidized glutathione (GSH), leading to decrease of their content in latex.

Table 1. *Variation of Thiol content, Plasticity Retention Index and Lovibond colour Index for raw rubber samples treated with 1%-5% ethephon*

Ethephon concentration (%)	Thiol content (mmol/g)	Plasticity Retention Index	Lovibond Colour Index
0	0.67^{e}	59.59^{d}	1.5
1	0.78^{bc}	69.06^{c}	1.0
2	0.81^{b}	76.6^{b}	1.0
3	0.88^{a}	83.13^{a}	1.0
4	0.73^{cd}	71.74^{bc}	1.5
5	0.71^{de}	70.43^{c}	1.5

*Means that do not share same letter are significantly different P<0.05

According to Lovibond colour index 1%-3% ethephon treated samples showed lighter colour than the control sample. 4% and 5% samples are very bright yellow colour with comparison to other samples (Fig. 1). It is evident from the results that with increasing ethephon concentration up to 3%, the Lovibond colour index is reduced, with simultaneous increase in thiol concentration. The lighter colour observed in rubber with ethephon treatment may be due to prevention of phenolic discolouration with thiol compounds. Therefore thiol concentration above a critical level allow a permanent protection against enzymatic browning (Richard *et al.* 1991). However, this level may often be dependent on the physiological condition of the tree. This is in agreement with the explanation given by Mason and Pterson, 1965 who explain that "in a system containing o-diphenol and glutathione, the total oxygen consumption increases sharply with increasing thiol content and this sharp break is accompanied by the marked difference in the colour of the final product".

The probable reaction mechanism of catechol with glutathione is given below (Fig. 2).

The o-quinone which leads to phenolic discolouration is blocked by glutathione (GSH) and it is reduced o-quinone back to catechol. If GSH is not present O-quinone can react with other phenolic compounds or amino acids (in protein) or it can self- polymerize into melanin like brown pigment (Gacche *et al.* 2006). The proposed mechanism is confirmed by FTIR spectrum of acetone extract of rubber sample.

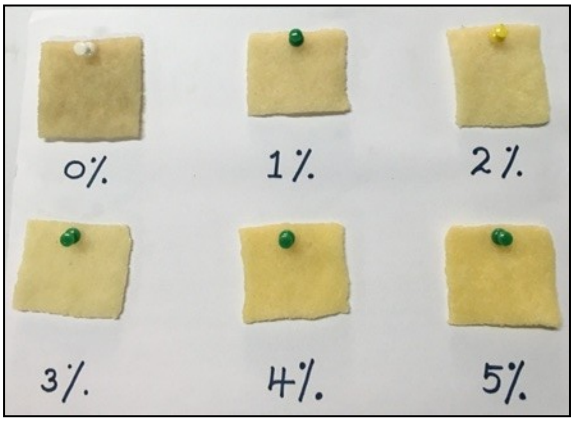

Fig. 1. Visual appearance of rubber samples from the trees treated with 1%-5% concentrations of ethephon

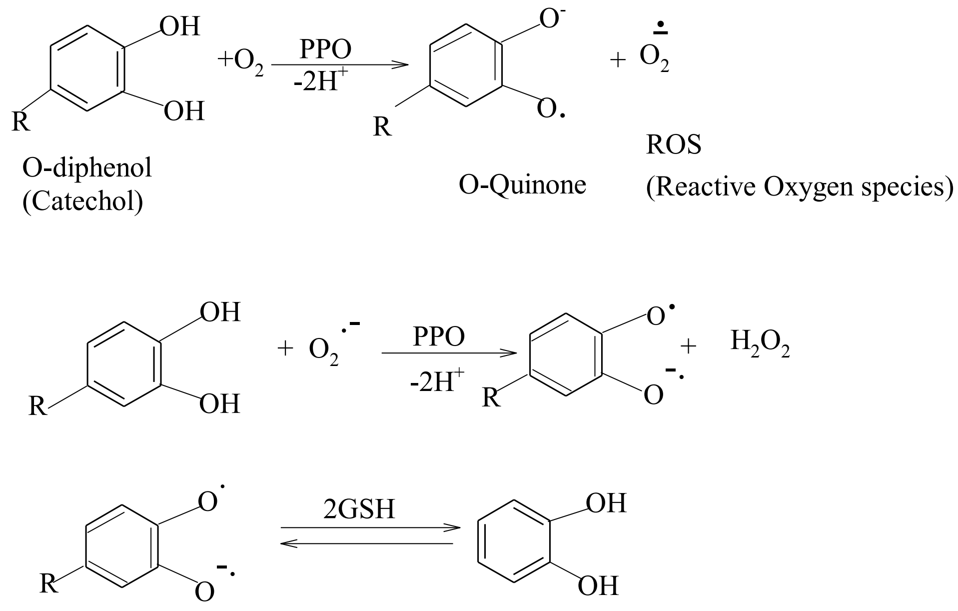

Fig. 2. The probable reaction mechanism for oxidation of O-diphenol in the presence of glutathione

Characteristic semi-quinone C----O stretching band appear around 1510-1475cm^{-1} (Berthomieu *et al.,* 1990). In 0%, 4% and 5% ethephon treated samples showed prominent semi-quinone peak, while 3% and 2% shows very weak band and they disappear in 1% ethephon treated sample (Fig. 3). Therefore it can be assumed that most of the orthoquinones are reduced by glutathione (GSH) and converted to catechol in 1%, 2% and 3% ethephon treated samples.

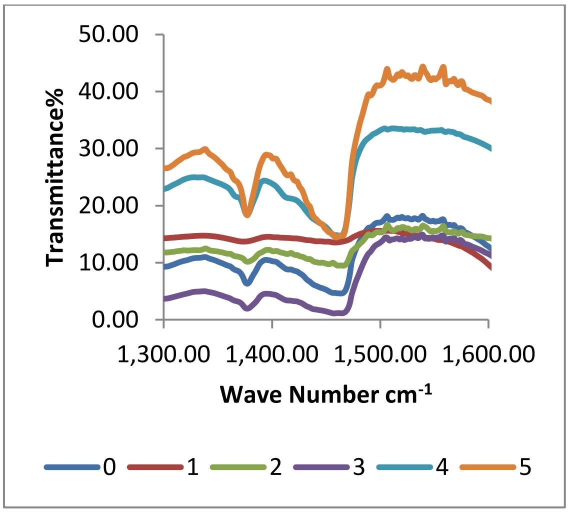

Fig. 3. FTIR spectrum of acetone extract of rubber obtained from the trees treated with different concentrations of ethephon

Conclusions

With S/2, d3 low frequency harvesting system, increase in ethephon concentration resulted in an increase of thiol content and PRI value up to 3% ethephon. Above that level PRI value decreased as a result of decrease in thiol content. Ethephon treated 1%-3% samples showed significantly low Lovibond colour index (lighter colour) and transient increase in colour observed for 0%, 4% and 5% samples. This is a biologically controlled method that can be used to improve the colour of crepe rubber with low frequency harvesting systems.

It is evident from the results that ethephon concentration directly affects the raw rubber properties. To achieve best raw rubber properties, application of recommended dosage is important as over dosage may lead to discolouration. This method can be used to achieve lighter colour rubber by adding glutathione in latex stage as an alternative to apparently toxic, bleaching agent.

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