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Odourless Natural Rubber (ONR)

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Unpleasant odour produced by natural rubber, in particular during mastication of Standard Malaysian Rubber (SMR 20) is objectionable to some workers. The issue of unpleasant odour of natural rubber has long been raised both by the rubber industry and the public. However, until today there seems to be no effective solution to overcome the problem. In view of this situation, work was initiated to develop Odourless Natural Rubber (ONR). This paper discusses the work to investigate and identify microorganisms that are responsible for unpleasant odour. Samples of odourous cuplumps were taken from the SMR factory of Malaysian Rubber Board in Sungai Buloh. Microorganisms isolated from cuplumps were identified by means of VITEK® 2 System. The odourous compounds produced were collected using solid-phase microextraction (SPME) before being subjected to Gas Chromatography Mass Spectrometry (GCMS) to analyse the odour. Samples were analysed on a day to day basis for six days to determine the most odourous compounds. The volatile compounds detected at early incubation period were mainly hydrocarbons. Other volatile compounds produced throughout the incubation were low molecular weight compounds such as volatile fatty acids, sulphurous compounds, amino compounds, esters and alcohols. Three different antimicrobial agents were used to suppress the growth of odourous microorganisms during storage of cuplumps before processing. The SPME/ GCMS analysis provided experimental evidence that sodium hypochlorite and formaldehyde were able to inhibit the growth of microbes and suppressed the unpleasant odour of rubber. This rubber is now called Odourless Natural Rubber (ONR). Mixing was conducted on ONR to evaluate cure characteristics and physical properties of the vulcanised rubber based on ACS 1 mix formulation. ONR enhanced the cure rate and physical properties such as tensile strength, hardness and resilience which were higher than the control (SMR L).

Keywords: natural rubber odour; odourless NR; bacteria inhibition; processing safety; cure characteristics; tensile strength

Hevea brasiliensis, a commercial rubber tree from the family *Euphorbiaceae* produces natural rubber (NR) latex (*Figure 1*). *Hevea* trees convert inorganic nutrients from the soil and carbon dioxide from the atmosphere, into organic carbohydrates which are then turned into rubber latex. Latex of *Hevea* plants contain about 30% of poly (*cis*-1, 4-isoprene) and is harvested by means of a tapping procedure after the bark reaches maturity[1].

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The fresh latex is collected as liquid and then transported to rubber processing mills for the production of rubber goods[2]. In the absence of adequate preservatives, field latex becomes a rich medium for microbial growth and causes auto-coagulation of latex through microbial action[3]. Auto-coagulation is commonly assumed to result from microbial contact with fresh rubber latex during its collection and handling[4]. This auto-coagulation process produces tree lace and cuplumps (*Figure 2*). From the population of bacterial growth, some of the bacteria responsible for auto-coagulation of latex caused a malodorous effect[5] to the tree lace and cuplumps as a consequence of bacterial action on the non rubber constituents. Organic contamination in rubber could support microbial growth even if the rubber hydrocarbon itself was not metabolised[6,7]. Susceptibility of natural rubber towards a microbial attack is a well-known phenomenon and has been sufficiently reviewed[8,9].

The unpleasant odour may also arise during mastication of natural rubber. When these non rubbers (proteins, phospholipids, free fatty acids, furanoid fatty acid, sterol esters *etc*.) are subjected to high temperature during mastication, they break down to give intermediate derivatives with obnoxious smell.

Certain fatty acids are also pungent and when combined with amines the resulting mixture produces malodour which can cause complaints[10]. High temperature mastication is recognised as one of the predominant factors causing workers in the rubber manufacturing industry and members of the public who live in the vicinity of rubber factories to express concern regarding odour released during the manufacturing process. It is a common practice to install an extraction unit at the feed hopper of the internal mixer to channel the odour through the extraction unit. In view of this predicament, work was initiated to develop odourless natural rubber (ONR). Some preliminary work was conducted to identify the types and quantity of microorganism present in the tree lace and cuplumps since these two auto-coagulated rubbers are the main constituents of SMR 20.

EXPERIMENTAL

Samples Collection

The collection of samples was done at Felda Kg. Awah, Pahang and some of the cuplumps were provided by the SMR factory of Malaysian Rubber Board in Sungai Buloh. Five different types of samples were collected and given specific designations abbreviated as follows; cuplumps (LUMP), tapping site on tree bark (TREE LACE), area within the collection cups (CUP), water surrounding the heap of cuplumps (L(H)) and the rubber plantation environment (ENV). All collected samples were cultured on nutrient agar (OXOID) and isolated to pure colonies before the microorganisms were classified according to its morphological characteristics and microscopic observation[5]. For the rubber plantation environment, a nutrient agar plate was left opened for 1 h and was incubated to observe growth. Several preliminary analyses were done such as Gram staining, morphological characteristics and sniffing plate technique. Pure cultures were tested for their ability to grow on sterilised rubber in the absence of any preservatives to analyse the reaction between microorganisms and rubber that may produce a foul smell. Sterilised rubber was prepared by pouring field latex without preservatives into a 90 mm glass Petri dish and autoclaved at 121ºC for 15 minutes. The sterilised rubber was inoculated with pure isolates and incubated before the odour production was determined qualitatively. As a control, the non inoculated sterilised rubber

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Figure 1. Hevea brasiliences producing natural rubber latex immediately after tapping.

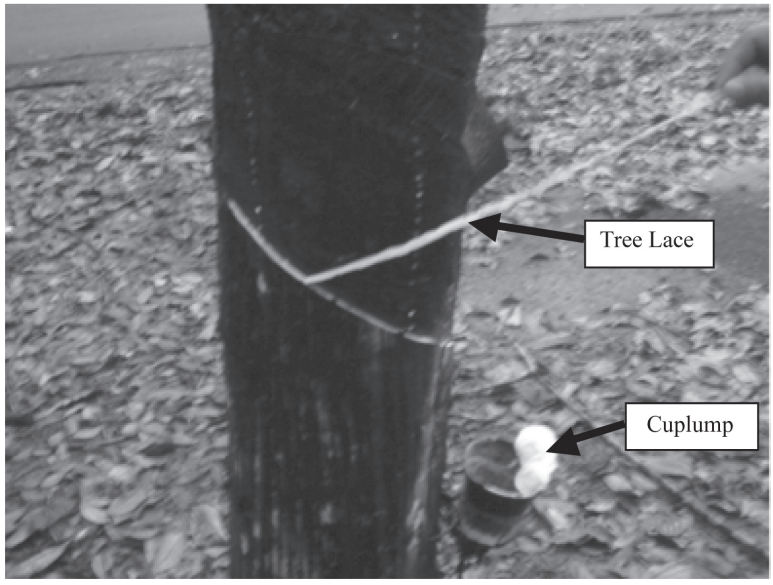

Figure 2. Tree lace being stripped off from the bark of the tree and cuplumps removed from the cup. Tree lace and cuplumps are produced as a consequence of the auto-coagulation process due to microbiological action on non rubber constituents of unpreserved latex.

was used to compare the odour produced with and without the presence of microorganisms.

VITEK® 2 Analysis for Identification of Isolated Microorganisms

Microorganisms were prepared by inoculation of pure culture from a nutrient agar plate to tryptic soy agar plate (TSA) and incubated for 18 to 24 h at 37ºC. TSA media were used in the development of product database identification for optimal performance. Three mL sterile saline was aseptically transferred into the test tube before several pure colonies were inoculated by using a sterile cotton swab. The density of the suspension was checked using a calibrated VITEK® 2 DensiCHEK™ Plus which is equivalent to 0.50 - 0.63 McFarland standard for both GP-identification and GN-identification card. Suspension tubes and cards were placed in the cassette accordingly for not more than 30 min of the suspension age. Then, the cassette was loaded into the instrument by referring to the appropriate Instrument User Manual for instructions on data entry. The results were observed accordingly after 8 to 10 hours.

Preliminary Test for Odour

The pure colony was allowed to grow on sterilised non preserved coagulated NR latex. Sterilisation was done by pouring non preserved NR field latex into a 90 mm glass Petri dish and placed in an autoclave at 121ºC for 15 minutes. The sterilised rubber was inoculated with pure colony and incubated before the odour production was determined qualitatively by sniffing. After 24 h of incubation at room temperature, sniffing test was carried out to check for foul smell or odour. If foul smell was detected, this indicates that reaction had taken place between the microorganisms and the non preserved coagulated NR latex. As a control, the non inoculated sterilised rubber was used to compare the odour produced with and without the presence of microorganisms.

Analysis of Odour Production

Odour produced from the sniffing test sample was analysed using Solid Phase Micro Extraction (SPME) and Gas Chromatography Mass Spectrometry (GCMS) which enabled the gas profile produced from the odour to be analysed. The pure colony was inoculated from nutrient agar to nutrient broth and incubated for 16 to 18 h before they were used for GCMS analysis. The SPME sample was prepared whereby a piece of sterilised non preserved coagulated NR latex measuring 1 cm × 1 cm was suspended into five mL of phosphate buffer saline (PBS) and one mL of the overnight culture was added into the vial. The prepared samples were incubated for six days at room temperature. Analysis of the gas produced from the odour was carried out daily by using SPME. In this test, the vial was heated in an electrical oven to 50ºC for 30 min to allow the gas to evaporate. Polydimethylsiloxane (PDMS) fibre was used for SPME and injected into a vial for 15 min to extract the volatile compound produced. In order to determine the volatile compound that caused offensive odour, odorous cuplump was used as positive control and sterilised non preserved coagulated NR latex was used as negative control. The volatile components were collected and analysed using the GC system by Agilent Technologies 6890N with a capillary column (0.25 mm × 30 m × 0.25 um) Agilent 19091S-433 HP-5MS. Helium gas was used as carrier with flow rate of 1.4 mL/min. The injector temperature was set at 280ºC and the GC temperature was programmed to increase from 50ºC to 170ºC with an increment of 10ºC/min within 15 minutes.

Preparation of Antimicrobial Agent

The antimicrobial agent was diluted with sterile distilled water at different concentrations of 0.5%, 1.0%, 3.0% for sodium hypochlorite; 0.5%, 1.0% for formaldehyde and undiluted HISKA® solution which is the coagulant and density enhancer for rubber latex. Each of these concentrations were prepared in 10 mL solution.

Treatment of Rubber Odour

Twenty mL of freshly tapped latex was poured into a glass Petri dish (90 mm) autoclaved at 121ºC for 15 min and cut into 1 cm × 1 cm dimension. Five hundred grams of cuplump were cut into small pieces to increase the surface area and soaked in antimicrobial agents for 24 hours. Then, the antimicrobial agent was discarded and the treated rubber crumb was allowed to dry until it reached a constant weight which indicated that most of the moisture was eliminated before transferring it into a sterilised vial to be analysed by SPME/GCMS. This was done to determine effectiveness of the treatment in eliminating the foul odour. This treated rubber is now called odourless natural rubber (ONR).

Preparation of ACS 1 (Gum Mix)

All seven ACS 1 gum mix compounds shown in *Table 1* were prepared by mixing on a two-roll mill in accord with the mixing procedure laid down by *ISO 2393*. The surface temperature of the mill rolls was maintained at 70±5ºC throughout the mixing process. The rubber was homogenised in accord with ISO 1795. About 10 g was taken from each finalised mix to determine the cure characteristics by means of rotorless moving die rheometer MDR 2000P at 140ºC.

Moulding and Preparation of Test Pieces

All moulding of test pieces was done in an appropriate compression mould suitable for the test pieces to be prepared. Each moulding was done at 140ºC, where the ACS 1 rubber mix was vulcanised to its optimum state of cure as indicated by its optimum cure time, t_{95} determined from the rheometer chart.

TABLE 1. ACS GUM MIX FORMULATION

Ingredient	Quantity of mix (p.p.h.r.)						
	(1)	(2)	(3)	(4)	(5)	(6)	(7)
SMR L (control)	100						
HISKA®		100					
ONR - Sodium hypochlorite (0.5%)			100				
ONR - Sodium hypochlorite (1.0%)				100			
ONR - Sodium hypochlorite (3.0%)					100		
ONR - Formaldehyde (0.5%)						100	
ONR - Formaldehyde (1.0%)							100
Sulphur	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Zinc oxide	6	6	6	6	6	6	6
Stearic acid	0.5	0.5	0.5	0.5	0.5	0.5	0.5
2-Mercaptobenzothiazole (MBT)	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Physical Characteristic Tests

Hardness test. Hardness test was done in accord with the *ISO 48*. Hardness is a measure of reversible deformation when an indenting force is applied on the rubber surface of a cylindrical test piece of 8 mm thickness at a specified time. The reading must be taken within the 30 s specified time after applying the indenting force. At least five readings at different locations on the rubber surface were recorded and the mean value was taken. The test was repeated on a duplicate test piece. The mean hardness of the two test pieces was reported.

For a perfectly elastic isotropic material, indentation (D) was expressed in hundredths of milimetre and Young's modulus (E) was expressed in megapascals, *viz*[11]. The results are expressed by the formula:

$$D = 61.5\, R^{-0.48}[(F/E)^{0.74} - (f/E)^{0.74}] \quad \ldots 1$$

Where,
F is the total indenting force, in Newtons;
f is the contact force, in Newtons;
R is the radius of the ball, in milimietres.

Rebound Resilience. Rebound resilience test was carried out using the Dunlop Tripsometer *(BS 903: Part A8 – 1973)*. The test piece had the dimensions of 44.6 mm diameter and 4.0 ± 0.1 mm thickness. Two test pieces were used. The test piece was placed in a sample holder and the pendulum was released from a vertical position (45º) to strike the test piece.

The results are expressed by the formula[12]:

$$\text{Rebound resilience (\%)} = [1 - \cos(\Theta - \sigma_2)] / [1 - \cos(\Phi - \sigma_1)] \times 100 \quad \ldots 2$$

Where;
Θ = rebound angle
Φ = angle of drop (45º)
σ_2 and σ_1 the damping corrections for rebound and drop angles respectively. The rebound resilience was recorded in the data computer. The mean values from the two test pieces were recorded.

Tensile Test. Tensile test was done in accord with *ISO37* at 23ºC using an electromechanical tensile machine. Five dumbbell test pieces (Type II) were used. Tensile strength is defined as the force at break (F_B) per unit cross-sectional area measured in the unstrained state (A_o)[12].

$$\text{Tensile strength} = F_B / A_o \quad \ldots 3$$

Apart from tensile strength, the stress at 300% strain denoted as M300, and elongation at break (E_B) were also recorded. The results reported here represent the mean value of five test pieces.

RESULTS AND DISCUSSION

Isolation and Characterisation of Microorganisms

During isolation and purification of colonies, 15 aerobic microbes were isolated from five different sampling sites and given a specific designation. The isolates were characterised according to the Gram stain and morphological characteristics of the colonies on nutrient agar. The preliminary study (sniffing technique) was used as a preliminary qualitative analysis to observe which microorganism produced offensive odour. *Table 2* shows the designation of the isolates, the characteristic shape as well as odour and non odour producing microorganisms.

The microorganisms that produced foul smell were identified using VITEK® 2 system. Five isolates from Gram negative were identified as *Comamonas testeroni* (L(H)2), *Brucella melitensis* (L(H)5), *Alcaligenes faecalis* (ENV4 and LUMP10) and an

TABLE 2. CHARACTERISTICS AND IDENTIFICATION OF AEROBIC MICROORGANISMS ISOLATED FROM DIFFERENT SAMPLING SITES.

Sampling sites	Designation	Gram stain	Shape	Odour	Identified microorganism
Environment	ENV2	Positive	Spherical		*Staphylococcus sciuri*
	ENV3	Positive	Spherical	√	*Micrococcus luteus/lylae*
	ENV4	Negative	Rod	√	*Alcaligenes faecalis*
Tree lace	T.LACE1	Positive	Spherical	√	*Staphylococcus sciuri*
	T.LACE2	Positive	Rod	√	*Kocuria kristinae*
Collection cup surface	CUP1	Positive	Spherical	√	*Dermacoccus nishinomiyaensis*
	CUP3	Positive	Rod	√	*Kocuria kristinae*
	CUP4	Negative	Spherical		Unidentified microorganism
Cuplump	LUMP2	Positive	Rod		Unidentified microorganism
	LUMP7	Positive	Rod	√	*Kocuria kristinae*
	LUMP9	Positive	Rod	√	*Granulicatella elegans*
	LUMP10	Negative	Rod		*Alcaligenes faecalis*
Water around cuplumps	L(H)2	Negative	Rod	√	*Comamonas testosteroni*
	L(H)3	Positive	Rod		Unidentified microorganism
	L(H)5	Negative	Rod	√	*Brucella melitensis*

Note : √ indicates foul odour production

unidentified microorganism (CUP4). In the case of Gram positive isolates, they were ten isolates identified as *Kocuria kristinae* (TL2, CUP3, and LUMP7), *Staphylococcus sciuri* (TL1 and ENV2), *Dermacoccus nishinomiyaensis* (CUP1), *Micrococcus luteus/lylae* (ENV3), *Granulicatella elegans* (LUMP9) and unidentified microorganisms (L(H)3 and LUMP2).

Gas profile analysis using Solid Phase Microextraction (SPME) and Gas Chromatography Mass Spectrometry (GCMS)

In this study SPME/GCMS was employed to characterise volatile compounds of obnoxious odour from natural rubber due to microbial reaction. Odour produced by microorganisms isolated from the rubber plantation environment was used to determine the correlation between rubber odour and microorganisms. GCMS results produced several volatile compounds including siloxane derivatives originating from the PDMS fibre which led to predominant peaks observed in the chromatogram during injection as shown in *Figure 3*. The compounds detected by GCMS from odourous cuplumps are shown in *Table 3* and will be used as a reference against the isolates. The compounds produced by each isolate were compared against the reference (*Table 3*) and are summarised in *Table 4*.

Gas Profile of Treated Rubber Detected from SPME/GCMS Analysis

Table 5 summarises the results of odourous compounds from raw rubber and also treated rubber as identified by GCMS.

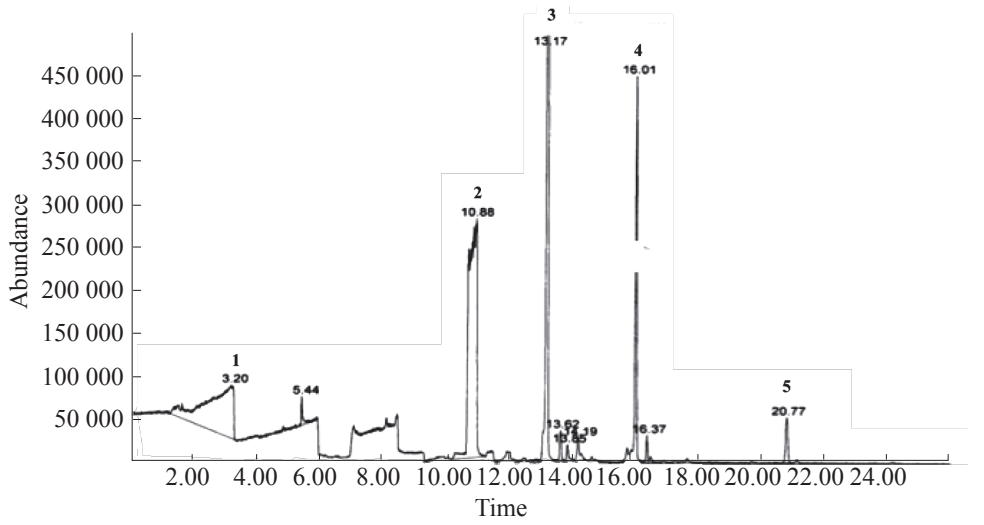

Figure 3. Chromatogram obtained from GCMS analysis showing siloxane derivatives. 1) Cyclotrisiloxane; 2) Cyclohexasiloxane; 3) Cycloheptasiloxane; 4) Cycloheptasiloxane; 5) Tetrasiloxane.

Eighteen compounds were detected as odourous from NR cuplumps. From the observation, 3-cyclohexene-1-methanol, and α, α, 4-trimethyl-, (S) were found in all treated rubber at retention time of 10.37 minutes. Fewer compounds were detected when cuplumps were treated with sodium hypochlorite as the concentration of sodium hypochlorite was increased from 0.5% to 3.0%. There were two compounds detected at 0.5% concentration of sodium hypochlorite, 7H-Dibenzo[b,g] carbazole,7-methyl (4.31 minutes) and Benzene,1-(chloromethyl)-2-methoxy- (14.20 minutes). At 1.0 and 3.0% of sodium hypochlorite concentrations, the compounds detected were not found compared to those produced by odourous cuplumps. In other words, there was no unpleasant odour. These results indicate that sodium hypochlorite was an effective antimicrobial agent to eliminate obnoxious rubber odour.

Meanwhile, camphene was detected at retention time of 10.34 min in all concentrations from 0.5% to 2.4% when the rubber was treated with chloroxylenol. Other than that, 5H-Naphto[2,3-c] carbazole,5-methyl- was detected at retention time of 4.31 min at 1.0% concentration of chloroxylenol. In this study, chloroxylenol was not found to be effective in reducing odour.

There were three compounds detected when the rubber was treated with 0.5% of formaldehyde. The compounds were 5H-Naphto[2,3-c] carbazole, 5-methyl- and 7H-Dibenzo[b,g] carbazole, 7-methyl detected at the same retention time which was 4.31 min whereas, 5-Cholestan-2-one, oxime was detected at retention time of 14.73 minutes. The treatment of rubber using 1.0% of formaldehyde showed a lesser number of odorous compounds. In this case only one compound was detected namely, 5H-Naphto[2,3-c] carbazole, 5-methyl- at retention time of 4.31 minutes. This result indicated that formaldehyde was an effective antimicrobial agent at 1.0% concentration to reduce or eliminate the obnoxious odour.

TABLE 3. COMPOUND DETECTED BY GCMS FROM ODOUROUS CUPLUMPS

Incubation period	Compound Detected	Retention time (min)
Day 1	1) N,N-Dimethyl-N'-(10-propyl-10H-acridin-9-ylidene)-benzene-1,4-diamin	6.97
	2) 5,6,7-Trinitro-1,4-benzodioxane	7.29
	3) 3-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-	9.28
	4) Limonene	9.61
	5) Trans-1-chloromethyl-2-methoxymethylcyclohexane	10.02
	6) 3-cyclohexene-1-methanol,α,α,4-trimethyl	10.37
	7) 5-Hexyn-1-ol	12.69
	8) 5α-Cholestan-2-one,oxime	14.73
Day 2	1) 4-pyridinamine,N-methyl-N,3-dinitro-	7.16
	2) N,N-Dimethyl-N'-(10-propyl-10H-acridin-9-ylidene)-benzene-1,4-diamin	8.55
	3) Butanamide, 3-spiro[2,3]hex-1-ylcarbonyl hydrazono-N-(4-nitrophenyl)-	9.23
	4) Spiro{6,6-dimethyl-2,3-diazabicyclo[3.1.0]hex-2-ene-4,1'-cyclopropane}	9.57
	5) Imidazole-4-propionic acid,ethyl ester	9.98
	6) 3-Thiabicyclo[3.3.1] nonane S-oxide	9.98
	7) Camphene	10.34
	8) 3-cyclohexene-1-methanol,α,α,4-trimethyl-,(S)-	10.34
Day 3	1) 3-Dimethylamino-2-(4-chlorophenyl)-thioacrylamide	2.83
	2) 1-Benzyl-2-ethoxy-6-oxo-1,2-azaphosphinane-2-oxide	7.30
	3) 2-Amino-4-hydroxy-7-[2-phenylethyl] pteridine	7.30
	4) 6-(3,5-Dimethyl-1H-pyrazol-1-yl)-N-benzyl-1,2,4,5-tetrazine-3-amine	7.30
	5) Dimethyl-3-(2-methoxycarbonyl-3-methyl-1-butyl)-2,5-thiophenedicarboxylate	8.52
	6) Pyrazole-1-acetamide,3-nitro-N-(4-iodophenyl)-	9.28
	7) Camphene	10.37
	8) Benzene-1,2-diol,4-(4-bromo-3-chlorophenyliminomethyl)-	12.46
	9) 6H-Benzo[g]-1,3-benzodioxolo[5,6-a]quinolizine-12-methanol,5,8,13,13a-tetrahydro-10,11,14-trimethoxy-,(S)-	14.01
	10) 2-chloro-4,5-dimethylphenol	14.26
	11) Carbanolate	14.26
Day 4	1) Imidazole-4-carboxamide, 5-nitro-N-(4-nitrophenyl)-	1.93
	2) 4-phenyl-3,4-dihydroisoquinoline	3.78
	3) 5,8-Dimethoxy-6-[4-diethylamino-1-methylbutylamino] quinazoline	4.82
	4) Butanedinitrile	5.87
	5) Quinazolin-4(1H)-one,2,3-dihydro-3-(4-dimethylaminophenyl)-2-phenyl	9.55
	6) Benzene,1-(chloromethyl)-2-methoxy-	13.85

TABLE 3 (CONT.). COMPOUND DETECTED BY GCMS FROM ODOUROUS CUPLUMPS

Incubation period	Compound Detected	Retention time (min)
Day 4	7) 1,1'-(4-methyl-1,3-phenylene) bis [3-(5-benzyl-1,3,4-thiadiazol-2-yl)urea]	14.20
	8) 3,3'-Dinitro-4'-chlorodiphenylsulphone	14.71
	9) 2-methyl-6-(5-methyl-2-thiazolin-2-ylamino)pyridine	17.36
	10) 4-dimethylamino-3,5-dinitrobenzoic acid	19.43
	11) Methyl 3-(1-pyrrolo) thiophene-2-carboxylate	22.95
Day 5	1) Benzo[h]quinoline,2,4-dimethyl-	1.34
	2) Propenenitrile,2-(2-benzothiazolyl)-3-(2-thienyl)-	7.25
	3) 2-[P-Fluorophenyl]-6-methylcinchoninic acid	9.23
	4) Cyclopropane,1,1-dimethyl-2-(3-methyl-1,3-butadienyl)-	10.33
	5) Acetonitrile,(2,7-dibromo-1-naphtyl)-	11.66
	6) 9H-Carbazole,3,6-dibromo-	11.66
	7) 2-chloro-4,5-dimethyl phenol	14.20
	8) 3,5-xylenol,4-chloro-,acetate	14.20
	9) 5,5'-Di(ethoxycarbonyl)-3,3'-dimethyl-4,4'-dipropyl-2,2'-dipyrrylmethane	15.05
	10) 2-Nitro-4-(trifluoromethyl) phenol	15.05
	11) 2-methyl-6-(5-methyl-2-thiazolin-2-ylamino) pyridine	19.15
	12) Benz[b]-1,4-oxazepine-4(5H)-thione,2,3-dihydro-2, 8-dimethyl-	19.15
Day 6	1) 2-Amino-4,46,6-tetramethyl-4,6-dihydro-thieno [2,3-c] furan-3-carbonitrile	2.76
	2) 1,2-benzenediol,3,5-bis(1,1-dimethylethyl)-	3.18
	3) 5H-Naphto[2,3-c] carbazole,5-methyl-	4.31
	4) Trans-4-(2-(5-Nitro-2-furyl)Vinyl)-2-quinolinamine	4.31
	5) 7H-Dibenzo[b,g] carbazole,7-methyl	4.31
	6) 1H-1,2,4-Triazole,1-benzyl-3-benzylthio-	7.25
	7) Cyclohexene,1-nitro-	9.25
	8) Cyclohexa-2,5-diene-1,4-dione,2-methyl-5-(4-morpholinyl)-	9.56
	9) Cyclohexanol, 2-methyl-5-(1-methylethenyl)-, acetate,(1α, 2β, 5α)-	10.33
	10) 9-(4-butyl-benzyl)-acridine	11.73
	11) 6H-Dibenzo[ce]1,2-thiazin-9-ol,10-(4-methylphenylsulfonyl)-,5,5-dioxide	12.69
	12) Phenol,2-methylthioacetyl-	14.20
	13) 4-Bromo-3-chloroaniline	18.71
	14) Benzenamine,4-bromo-2-chloro-	18.71
	15) 1H-2-Benzopyran-3-one,7-ethoxy-4-hydroxy-4-methylcarbonyl	18.71

TABLE 4. COMPOUNDS DETECTED BY GCMS FROM REACTION OF ISOLATES WITH RUBBER

Gas profile	Retention time(min)	Isolates used for GCMS analysis														
		ENV 2	ENV 3	ENV 6	TL 1	TL 2	CUP 1	CUP 3	CUP 4	LUMP 2	LUMP 7	LUMP 9	LUMP 10	L(H) 2	L(H) 3	L(H) 5
Benzo[h]quinoline,2,4-dimethyl-	1.34		√	√	√	√	√				√		√		√	
1,2-benzenediol,3,5-bis (1,1-dimethylethyl)-	3.18							√	√	√			√			
5H-Naphto[2,3-c] carbazole, 5-methyl-	4.31									√	√		√	√		√
7H-Dibenzo[b,g] carbazole, 7-methyl	4.31		√		√		√	√		√				√	√	√
N,N-Dimethyl-N'-(10-propyl-10H-acridin-9-ylidene)-benzene-1,4-diamine	6.97					√	√	√	√				√			
1H-1,2,4-Triazole,1-benzyl-3-benzylthio-	7.25				√						√					
2-Amino-4-hydroxy-7-[2-phenylethyl] pteridine	7.30								√			√				
Spiro{6,6-dimethyl-2,3-diazabicyclo[3.1.0]hex-2-ene-4,1'-cyclopropane}	9.57					√						√	√			
3-cyclohexene-1-methanol, α,α,4-trimethyl-,(S)	10.37				√							√	√			
Benzene-1,2-diol,4-(4-bromo-3-chlorophenyliminomethyl)-	12.46					√	√			√	√					
Benzene,1-(chloromethyl)-2-methoxy-	14.20							√					√			

Note : (ENV) rubber plantation environment; (TL) tapping site on tree bark; (CUP) area within the collection cups; (LUMP) cuplumps and (L(H)) water surrounding the heap of cuplumps.

TABLE 5. THE COMPOUNDS DETECTED BY GCMS FROM RAW RUBBER (CUPLUMP) AND TREATED RUBBER

No.	Gas profile	Retention time(min)	Raw rubber	Treated rubber 1	2	3	4	5	6	7	8	9
1	Benzo[h]quinoline,2,4-dimethyl-	1.34	√									
2	1,2-benzenediol,3,5-bis(1,1-dimethylethyl)-	3.18	√									
3	5H-Naphto[2,3-c] carbazole,5-methyl-	4.31	√					√		√	√	
4	7H-Dibenzo[b,g] carbazole,7-methyl	4.31	√	√						√		
5	N,N-Dimethyl-N'-(10-propyl-10H-acridin-9-ylidene)-benzene-1,4-diamin	6.97	√									
6	1H-1,2,4-Triazole,1-benzyl-3-benzylthio-	7.25	√									
7	2-Amino-4-hydroxy-7-[2-phenylethyl] pteridine	7.30	√									
8	Spiro{6,6-dimethyl-2,3-diazabicyclo[3.1.0]hex-2-ene-4,1'-cyclopropane}	9.57	√									
9	Camphene	10.34	√				√	√	√			
10	3-cyclohexene-1-methanol,α,α,4-trimethyl-,(S)	10.37	√	√	√	√	√	√	√	√	√	√
11	9H-Carbazole,3,6-dibromo-	11.66	√									
12	9-(4-butyl-benzyl)-acridine	11.73	√									
13	Benzene-1,2-diol,4-(4-bromo-3-chlorophenyliminomethyl)-	12.46	√									
14	6H-Dibenzo[ce]1,2-thiazin-9-ol,10-(4-methylphenylsulfonyl)-,5,5-dioxide	12.69	√									
15	Benzene,1-(chloromethyl)-2-methoxy-	14.20	√	√								
16	Carbanolate	14.26	√									
17	5α-Cholestan-2-one,oxime	14.73	√							√		
18	Benz[b]-1,4-oxazepine-4(5H)-thione,2,3-dihydro-2,8-dimethyl-	19.15	√									

Note : 1) 0.5% of Sodium hypochlorite, 2) 1.0% of Sodium hypochlorite, 3) 3.0% of Sodium hypochlorite , 4) 0.5% of Chloroxylenol, 5) 1.0% of Chloroxylenol, 6) 2.4% of Chloroxylenol, 7) 0.5% of Formaldehyde, 8) 1.0% of Formaldehyde and 9) HISKA®. (√) indicates compound detected.

Cure Characteristics of ACS Gum Mix Base on ONR and SMR L

The next important part of the research was to test the processability of ONR. Processability is the terminology used in rubber technology that covers processes such as mastication, mixing, calendering, extrusion and moulding. Each of these processes require rubber to flow, especially during shaping processes encountered in calendering, extrusion and moulding. If total flow of the rubber compound is prevented, shaping will not take place completely. One of the common methods to evaluate processability of a rubber compound is to determine cure characteristics of the rubber compound based on the torque – time chart produced from a rotorless moving die rheometer MDR 2000P. *Figure 4* shows the histogram that compares cure characteristics between SMR and ONR ACS 1 gum mix compounds. The scorch time denoted as t_2 indicates the time at which chemical crosslinking starts to take place. The scorch time indicates the safety limit of the rubber compound during processing. The shorter the scorch time, the lower the safety limit of the rubber compound during processing since chemical linking may occur prematurely. If this premature crosslinking occurs, the rubber compound cannot be processed anymore because complete flow of the rubber is prevented and thus shaping is incomplete. From the histogram, the control sample based on SMR gave better processing safety than ONR since the scorch time of the former was about two to three times longer than the latter. In this situation, it appears that ONR has poorer processing characteristics than SMR.

However, this problem can easily be overcome by choosing an alternative accelerator such as CBS (N-cyclohexyl benzothiazole sulphenamide) which can provide longer and better processing safety than the currently used MBT (Mercapto benzothiazole). Apart from choosing a delayed action type of accelerator, the alternative method to minimise the risk of premature crosslinking is to control the temperature during processing by turning on the cooling system of the processing equipment.

The second important information obtained from the cure characteristics data is the optimum cure time denoted as either t_{90} or t_{95} which indicates the time necessary for vulcanisation reaction to complete to 90% or 95% of its maximum state of cure. ONR has a shorter cure time than that of SMR. In this area ONR is better than SMR because a shorter cure time brings higher output than a longer cure time. It appears that the type and quantity of antimicrobial agents employed in the ONR affected the scorch time more than they affected the optimum cure time of the rubber compound. However, it is beyond the scope of the present investigation to discuss how these antimicrobial agents have affected the chemistry of vulcanisation of NR. But it has certainly provided a new area worthy of further investigation.

The third important information is concerning the difference in torque denoted as ΔT (Maximum torque – Minimum torque) which reflects the crosslink concentration of the rubber network. A high ΔT value reflects high concentration of crosslinks to form the rubber network. The histogram shown in *Figure 5* indicates that ONR produced higher ΔT than that of SMR L.

This result implies that the number of crosslink concentration is higher in vulcanised ONR than in vulcanised SMR L. It appears that the antimicrobial agents used in ONR had improved the vulcanisation efficiency as indicated by the increase in ΔT which reflects an increase in the number of crosslinks in the rubber network. This is another advantage of ONR over SMR L where the amount of

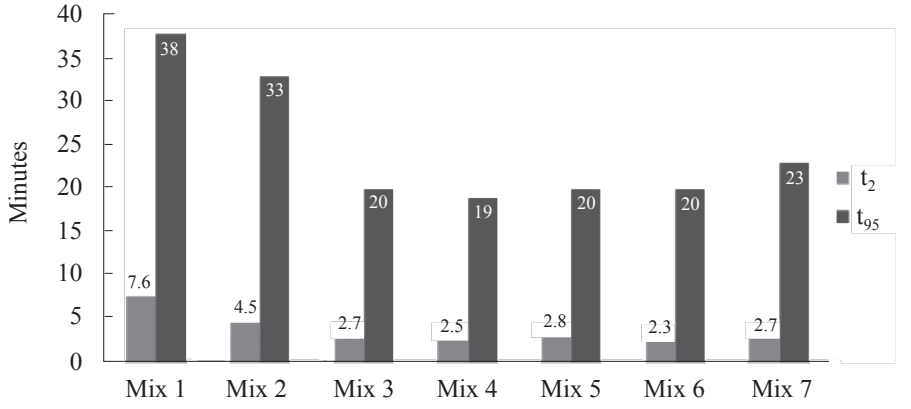

Figure 4. Comparison of cure characteristics between SMR L and ONR based on ACS 1 Gum Mix formulation. t_2 = scorch time; t_{95} = optimum cure time at 140ºC.

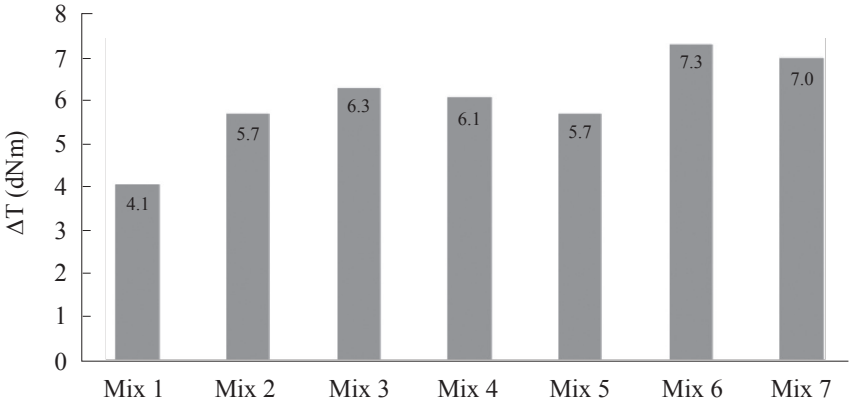

Figure 5. Comparison of the difference in rheometer torque (ΔT) between SMR L and ONR based on ACS 1 Gum Mix compounds.

accelerator and sulphur can now be reduced yet still obtain the same crosslink concentration as that of SMR. As a consequence of low dosage of sulphur and accelerator concomitantly, the compounding cost is reduced as well. However, how the antimicrobial agents have improved the vulcanisation efficiency is not entirely clear at this stage. It is believed that the non rubber constituents play an important role in the chemistry of sulphur vulcanisation of natural rubber. This is a significant area that requires further investigation.

Physical Properties of Treated Rubber

Table 6 shows the overall physical properties investigated in this work. Discussion of physical properties will begin with hardness, followed by resilience and tensile properties.

Hardness. *Figure 6* shows a histogram which compares the hardness of vulcanised ACS 1 Gum Mix compound. The vulcanised ONR gave higher hardness than that of vulcanised SMR L. Since no filler was added into both SMR L and ONR apart from the compounding ingredients necessary for sulphur vulcanisation, the difference in hardness might be attributed to the crosslink concentration of the rubber network. The hardness of vulcanised ONR was higher than that of vulcanised SMR L. This might be attributed to higher crosslink concentration of the former than the latter as a consequence of improved vulcanisation efficiency brought about by the antimicrobial agents used in ONR discussed above. The improvement in vulcanisation efficiency is reflected by the increase in ΔT. *Figure 7* shows the relationship between hardness and ΔT, where hardness increases almost linearly with ΔT. Thus, the results shown in *Figure 7* provide experimental evidence that the antimicrobial agents in ONR enhanced the vulcanisation efficiency by increasing the number of crosslink network.

Rebound Resilience. The effect of antimicrobial agents used in ONR on rebound resilience is shown in *Figure 8*. Mixes 6 and 7 which employed formaldehyde at 1.0% and 4.0% respectively gave the highest resilience. It appears that other antimicrobial agents did not increase the resilience markedly. Apart from hardness, resilience is also sensitive to changes in the crosslink concentration of the rubber network. However, hardness test was more discriminative than the resilience test since the former was able to detect small variations in ΔT than the latter. *Figure 9* shows the relationship between rebound resilience and ΔT where the resilience increased progressively as ΔT was increased. The increase in ΔT was attributed to the increase in the number of crosslinks within the rubber network as a consequence of the increase in

TABLE 6. TENSILE STRENGTH, HARDNESS AND RESILIENCE OF TREATED RUBBER

Antimicrobial agent		Tensile strength (MPa)	Hardness (IRHD)	Resilience (%)
Control (SMR)		13.8±1.0	33.0±0.1	78.0±0.4
HISKA®		23.0±0.4	38.0±1.0	76.0±0.1
ONR - Sodium hypochlorite (%)	0.5	21.3±0.6	38.0±1.0	78.0±1.0
	1.0	18.3±3.4	39.0±1.0	79.0±0.3
	3.0	18.6±1.6	36.0±0.5	76.0±0.5
ONR - Formaldehyde (%)	1.0	22.9±0.6	43.0±0.5	89.0±1.0
	4.0	21.9±0.4	44.0±1.0	88.0±1.0

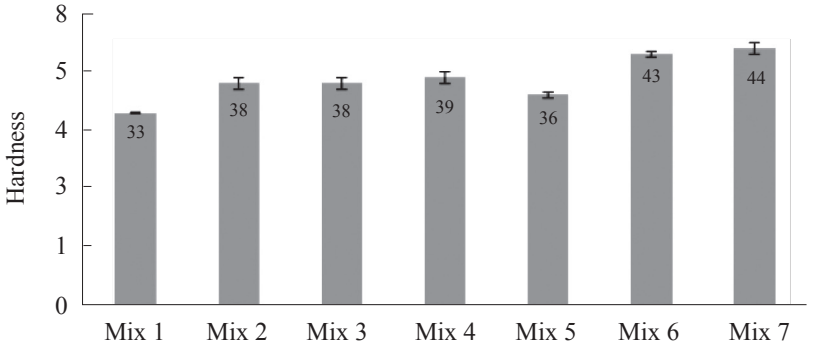

Figure 6. Comparison of hardness between SMR L and ONR ACS 1 Gum Mix vulcanisate .

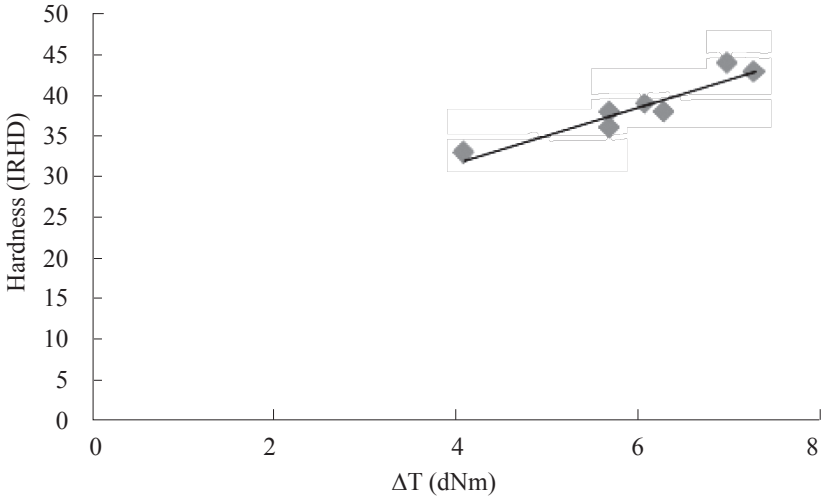

Figure 7. Relationship between hardness and difference in torque, ΔT.

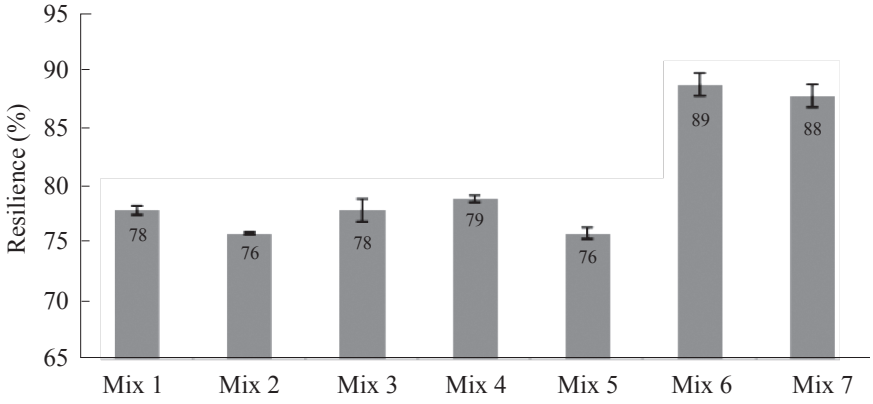

Figure 8. Comparison of resilience between SMR L and ONR vulcanised ACS 1 Gum Mix compound.

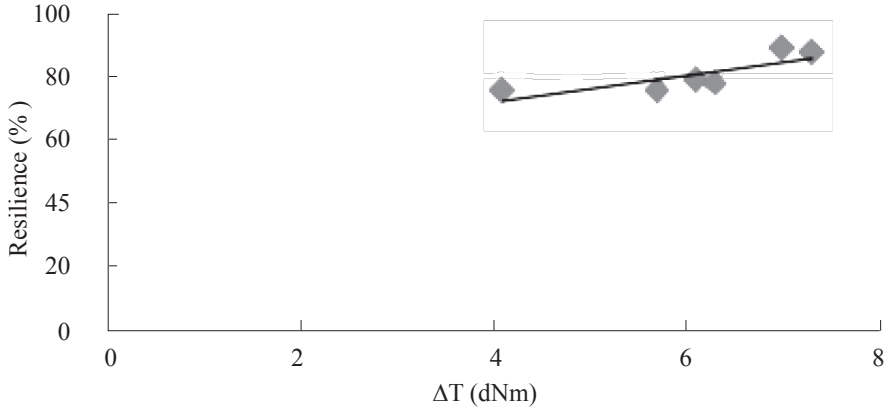

Figure 9. Relationship between resilience and difference in torque ΔT.

vulcanisation efficiency brought about by the antimicrobial agents. This experimental evidence supports the suggestion that the antimicrobial agents used in ONR enhanced the vulcanisation efficiency of sulphur vulcanisation.

Tensile Properties. (i) M300. Tensile properties such as M300, elongation at break and tensile strength are more discriminative and sensitive to compound changes than hardness and resilience tests since the former are destructive tests and the latter two are non destructive tests (*Figure 10*).

Figure 10 shows a histogram comparing the modulus denoted as M300 between SMR L and that of ONR vulcanised ACS 1 Gum Mix compound. Modulus is defined as the ratio of stress to strain, but in this case modulus refers to tensile stress (force/cross-sectional area) at 300% strain. The M300 of vulcanised ONR was higher than that of SMR L. The M300 results are in agreement with the hardness results shown in *Figure 6*. This close agreement is expected because hardness is closely related to the Young's modulus. *Figure 11* shows a linear relationship between M300 and ΔT, thus this result supports the hypothesis that antimicrobial agents used in ONR enhanced the vulcanisation efficiency of sulphur vulcanisation as discussed in the hardness results section.

(ii) Tensile Strength. The tensile strength of all vulcanised ONR gum mix compounds were higher than that of vulcanised SMR L indicating that all the antimicrobial agents used in ONR did not produce a deletirious effect on the mechanical strength. Indeed all these antimicrobial agents enhanced the tensile strength which might be associated to the enhancement in sulphur vulcanisation efficiency discussed earlier. Among the antimicrobial agents, formaldehyde (mixes 6 and 7) and HISKA® (mix 2) gave the highest tensile strength as shown in *Figure 12*.

(iii) Elongation at Break (EB). *Figure 13* shows the comparison of elongation at break between SMR L and ONR vulcanised Gum Mix compound. There is a progressive decrease in elongation at break as we move down from mix 1 to mix 7. This is the

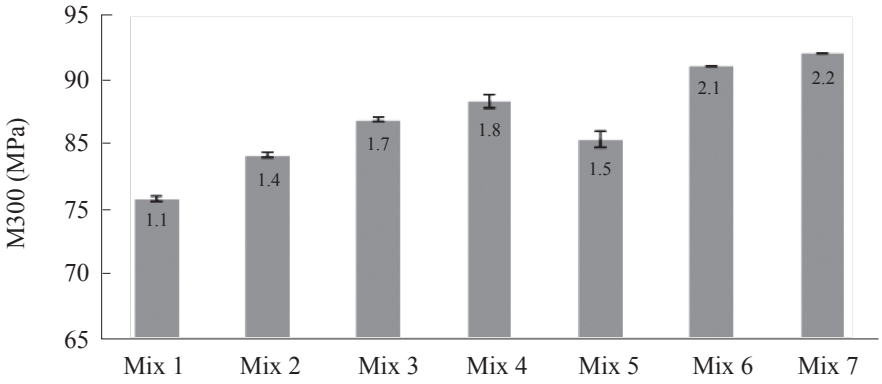

Figure 10. Comparison of "modulus" M300 between SMR L and ONR vulcanised ACS 1 Gum Mix compound.

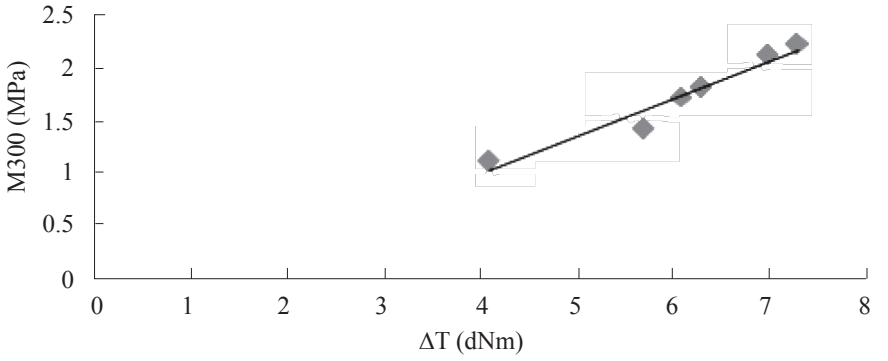

Figure 11. Plot of M300 vs ΔT of vulcanised ACS 1 Gum Mix compound.

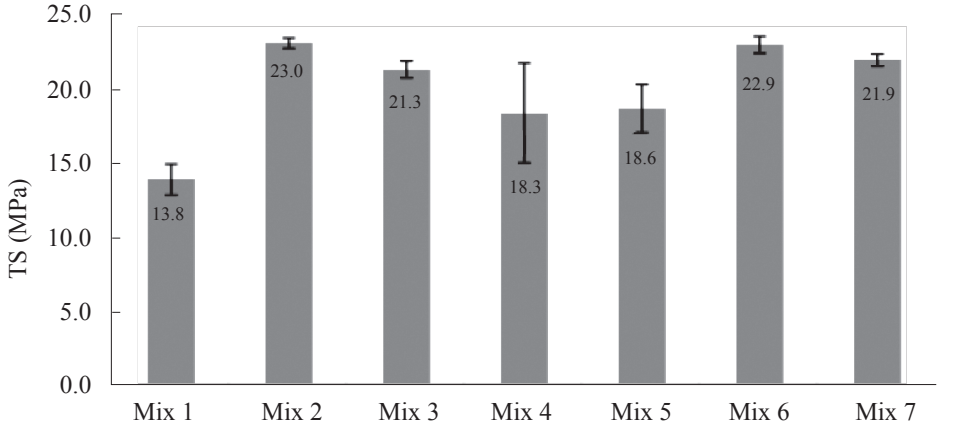

Figure 12. Comparison of tensile strength between SMR L and ONR vulcanised ACS 1 Gum Mix compound.

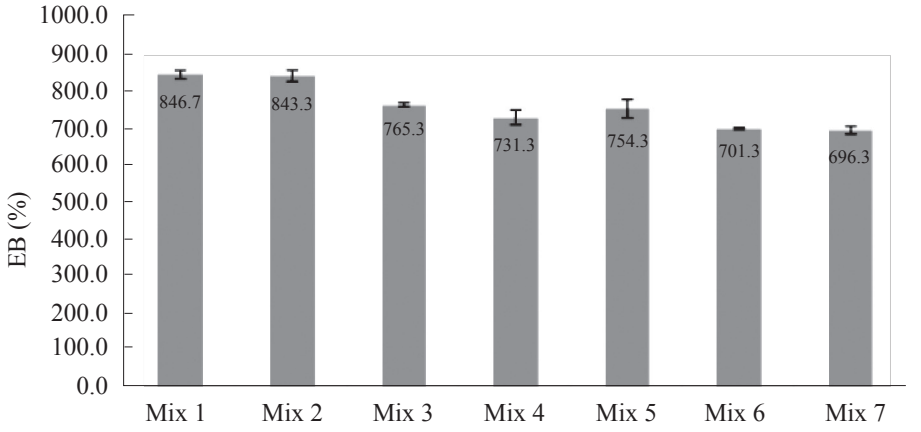

Figure 13. Comparison of elongation at break (EB) between SMR L and ONR vulcanised ACS 1 Gum Mix compound.

expected trend since the extent of elongation would decrease as the number of crosslinks in the rubber network increased as indicated by the increase in hardness, resilience and M300 discussed earlier.

CONCLUSIONS

There were ten Gram positive and five Gram negative microorganisms that were responsible for the production of unpleasant odour. The most predominant compound responsible for the unpleasant odour was 7H-Dibenzo [b,g] carbazole, 7-methyl. This compound was consistently produced by most isolates in this study. The most effective antimicrobial agent was 1.0% formaldehyde, followed by 1.0% sodium hypochlorite. The antimicrobial agent shortened both the scorch time (t_2) and the optimum cure time (t_{95}) of the ACS 1 Mix ONR compounds. Vulcanised ONR ACS 1 Mix compounds gave higher tensile strength and hardness than vulcanised ACS 1 Mix SMR L.

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