Effects of Specific Micro-Organisms on the Technological Properties of Rubber

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Thirteen bacteria and yeasts isolated from Hevea *latex were added to sterile field latex of clone RRIM 501 to study their effect on the technological properties of the resulting rubber. The latex containing at least 10^9 cells per ml was incubated at 30°C for 24 hours. Tests on the rubber showed no significant effects on technological properties of the rubber, though a small increase in tensile strength and modulus values was occasionally noticeable and the Plasticity Retention Index (PRI) decreased slightly. As the cultures used were a fair cross-section of the microbial population of natural rubber latex obtaining in Malaya, the results may well reflect the behaviour of field latex generally when bacteria and yeast are present.*

Latex within the untapped and undamaged *Hevea* tree is sterile, but the latex arriving at the factory 5–6 hours after tapping contains approximately 10^7 bacteria and 10^4 yeasts per ml, the microbes proliferating mainly at the expense of non-rubber substances (BATEMAN, 1963) and also destabilising the latex (JOHN, 1966a and b). Although the microbial utilisation of the rubber hydrocarbon in latex or vulcanisates has been studied (SPENCE AND VAN NIEL, 1936; ROOK, 1955; LEEFLANG, 1963 AND DICKENSON, 1965), little is known about the effects of micro-organisms on the technological properties of the rubber from field latex. A study was therefore made on the effects of a number of pure cultures of bacteria and yeasts, inoculated into sterile latex, upon the technological properties of the dry rubber.

MATERIALS AND METHODS

Organisms

Ten bacterial cultures were used—*Streptococcus faecalis, Micrococcus sp, Enterobacter cloacae, Staphylococcus aureus, Serratia marcescens* (non-pigmented), *Bacillus subtilis, Brevibacterium sp, Pseudomonas sp, Bacillus mycoides and Acetobacter oxydans* (NCIB 9013). The first five are strong acid producers, the next two are late acid producers, the following two are biochemically inactive and the last one oxidises alcohol. All except *A.oxydans* were isolated in Malaya from *Hevea* latex; *A.oxydans* was obtained from the Torry Research Station, Aberdeen. Three yeast cultures isolated in Malaya from *Hevea* latex, using the method of JOHN AND TAYSUM (1963), were included in the study but were not identified separately.

Media

Bacterial cultures except *A.oxydans* were grown in Oxoid nutrient broth No. 2; *A.oxydans* was grown on a medium suitable for the production of acetic acid, containing 1% Oxoid yeast extract, 2% ethanol, 2% re-precipitated $CaCO_3$, and 2.5% Oxoid agar No. 3. The pH of the medium was maintained at 7.4 in all cases. Yeast cultures were grown in Oxoid 0.3% malt extract broth.

Preparation of Washed Cells

After 20 hours incubation at 30°C on a reciprocating shaker, cells were harvested and washed aseptically by repeated centrifuging at 3000 rev/min for 20 minutes (JOHN, 1966a).

Sterile Latex Collection

Latex was collected aseptically from trees of clone RRIM 501 using special extractors (MCMULLEN, 1951). This operation was not always successful, but samples were tested for sterility immediately on collection and the non-sterile portions were discarded. The maximum

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quantity of latex collected was about 300 ml, containing approximately 100 g dry rubber.

Inoculation of Sterile Latex

Separate samples of sterile latex were inoculated with washed cells of each of the first nine bacterial cultures; an uninoculated control sample was also prepared. The experiments were triplicated to provide 27 treated samples and an equal number of control samples. In a further series, sterile latex was inoculated with washed cells of each of the three yeast cultures in the absence and presence of *A.oxydans*, with the usual control patterns for comparison. The inoculations of *A.oxydans* were carried out at various time intervals: in one series, *A.oxydans* and yeasts were inoculated concurrently; in another, the bacterium was inoculated 12 hours after the yeast inoculation. The inoculum was standardised to give an initial population of approximately 10^9 cells per ml. When double inoculations using bacterial and yeast cultures were carried out, each of the organisms was adjusted to this level. Both inoculated and uninoculated latex samples were incubated at 30°C for 24 hours. As far as possible an inoculated test sample and an uninoculated control sample were obtained from the sterile latex collection of the same day.

Preparation of Raw Rubber Crepe

Raw rubber samples were prepared for technological testing from the uninoculated latices by coagulating at pH 5 using 1% acetic acid, pressing the wet coagula and drying at 70°C for 3–4 days in an oven. Samples from inoculated latices were similarly prepared except that adjustment to pH 5 was made to the serum, after biological coagulation, before pressing. Because of the difficulty in collecting a sufficient quantity of sterile latex on the same day, it was occasionally necessary to mix raw rubber crepe prepared on different days to make a set of test and control samples.

Technological Testing

Samples of dried rubber were mixed on an open mill in the ACS 1 compound (rubber 100; zinc oxide 6; stearic acid 0.5; MBT 0.5 and sulphur 3.5). Because of the small size of the samples, tests were limited to tensile strength (BRITISH STANDARDS INSTITUTION, 1956), elongation at break, modulus and PRI (BAKER, *et al.*, 1966; BATEMAN AND SEKHAR, 1966). For economy of material, moulded rings were used instead of rings from vulcanised sheet. Vulcanisation was carried out for 40 minutes at 40°C.

RESULTS

Effect of Bacteria on Technological Properties of Dry Rubber

Three samples were prepared from each of the 9 bacterial treatments and their controls. The results (*Table 1*) show that the bacteria had hardly any effect on the properties of the dry rubber; sometimes slightly higher tensile strength and modulus were observed, suggesting a faster curing rubber. Most samples inoculated with bacteria showed a marginal reduction in PRI.

Effect of Yeasts on Technological Properties of Dry Rubber

From the results of tests using pure and mixed yeast cultures (*Table 2*), it would appear that none of the three yeasts, with or without *A.oxydans*, have had any marked effect on the properties of the rubber.

DISCUSSION

Experience suggests that a delay in coagulation or in processing the coagulum has a considerable effect on the properties of the resulting rubber, though it is not known whether this is due to the metabolic activity of the microbial population or due to latex enzyme systems activated by substances liberated from the lutoids (RUBBER RESEARCH INSTITUTE OF MALAYA, 1965). Natural rubber latex contains a large and diversified microbial population comprising hundreds of species, many of which may become important under particular conditions (TAYSUM, 1957). To obtain a true picture of the cumulative effect of these organisms in large naturally-occurring mixed populations would be an immense task. In this study, ten bacteria and three yeasts were tested for their effects upon the technological properties of

TABLE 1. TECHNOLOGICAL PROPERTIES OF RUBBERS OBTAINED FROM STERILE LATEX INCUBATED IN THE PRESENCE OF BACTERIAL CULTURES

Bacterial culture		†Tensile strength (lb/in²)				Elongation at break (%)				Modulus (lb/in²), 300%				Modulus (lb/in²), 600%				PRI			
		Replication			Mean	Replication			Mean	Replication			Mean	Replication			Mean	Replication			Mean
Streptococcus sp.	T	2330	2650	2330	2437	770	800	780	786	242	213	242	232	1140	1100	1070	1100	86	73	78	79
	C	2330	2330	2080	2248	760	810	780	784	213	213	256	227	1320	1050	1050	1143	100	83	97	93
Micrococcus sp.	T	2380	2460	2430	2423	750	780	820	786	256	213	285	251	1390	1240	1010	1214	96	86	81	88
	C	1540	2310	2260	2034	700	780	800	761	242	213	242	232	1200	1110	1100	1133	93	89	87	90
Enterobacter cloacae	T	2430	2500	2250	2395	780	780	790	781	256	242	242	247	1240	1270	1100	1200	83	86	80	83
	C	1910	2380	2380	2219	680	810	790	760	213	242	285	247	1570	1050	1110	1243	100	91	90	94
Staphylococcus aureus	T	2590	2420	2180	2394	810	810	800	806	213	213	242	223	1140	1020	1010	1057	85	94	65	81
	C	2160	1950	2010	2039	740	770	780	763	213	213	256	227	1320	1050	1020	1133	88	91	75	85
Serratia marcescens	T	1540	2260	2310	2034	670	730	760	719	285	256	256	266	1390	1480	1310	1394	84	76	89	83
	C	1710	2260	2310	2091	740	750	830	772	256	199	213	223	1020	1370	970	1119	97	91	97	95
Bacillus subtilis	T	2380	2480	2220	2357	790	820	790	799	213	213	242	223	1140	1010	1050	1067	89	86	83	86
	C	2220	2420	2180	2271	770	800	830	797	213	199	242	218	1200	1140	850	1062	90	97	97	95
Pseudomonas sp.	T	2350	1710	2260	2105	790	700	760	750	242	256	213	237	1070	1220	1280	1190	86	89	92	89
	C	2050	1740	2210	1996	790	710	790	766	242	213	199	218	930	1280	1070	1091	91	95	94	93
Brevibacterium sp.	T	2380	2130	1860	2125	760	740	740	747	256	242	256	251	1320	1320	1140	1261	95	95	86	92
	C	2050	2180	2130	2119	750	740	810	768	256	213	213	227	1150	1350	940	1147	94	94	91	93
Bacillus mycoides	T	2480	2310	2270	2348	770	770	810	683	242	199	285	242	1270	1240	970	1157	85	92	93	90
	C	2420	2130	2090	2214	810	800	780	789	213	213	213	213	1020	1070	1100	1062	87	92	97	92
S.e. of Mean					±139				±18				±14				±75				±3.6
Min. sig. diff. ($p=0{\cdot}05$)					400				52				39				216				10

T=Treated sample
C=Control sample

†Observed data for tensile strength, elongation and modulus (but not the Means) are rounded off to the nearest ten.

TABLE 2. TECHNOLOGICAL PROPERTIES OF RUBBERS OBTAINED FROM STERILE LATEX INCUBATED IN THE PRESENCE OF YEASTS AND ONE BACTERIAL CULTURE

Treatment			†Tensile strength (lb/in²)				Elongation at break (%)				Modulus (lb/in²), 300%				Modulus (lb/in²), 600%				PRI			
Organism(s)	Sequence of inoculation		Replication			Mean	Replication			Mean	Replication			Mean	Replication			Mean	Replication			Mean
Yeast 1		T	2330	2030	2030	2134	760	690	730	725	242	299	256	266	1270	1620	1320	1404	94	87	93	91
		C	2350	2310	1960	2205	800	770	710	760	213	242	256	237	1070	1200	1440	1233	98	95	94	96
Yeast 2		T	2250	2250	2210	2234	800	760	800	785	213	242	213	223	1020	1280	1010	1105	94	97	94	95
		C	2420	2260	2180	2285	800	810	800	803	213	199	213	208	1100	1020	980	1034	95	95	94	95
Yeast 3		T	2550	2550	2630	2575	770	770	780	772	256	285	256	266	1390	1390	1370	1385	77	80	82	80
		C	2480	2430	2290	2399	780	790	790	786	242	256	213	237	1240	1280	1110	1209	83	85	86	85
Yeast 1+*A.oxydans**		T	2420	2210	2430	2352	790	760	770	774	242	256	256	251	1140	1270	1280	1228	83	90	89	87
		C			2518				790				242				1223				81	
Yeast 2+*A.oxydans*	Concurrently	T	2220	2390	2310	2305	780	790	770	778	242	242	256	247	1140	1180	1240	1186	84	94	97	92
		C			2163				752				271				1273				94	
Yeast 3+*A.oxydans*		T	2380	2560	2560	2499	780	780	810	790	242	242	242	242	1180	1200	1140	1171	78	80	79	79
		C			2077				719				299				1536				83	
Yeast 1+*A.oxydans*		T	2480	2460	2430	2456	770	780	790	779	256	256	242	251	1370	1240	1150	1252	91	94	91	92
		C			2418				769				256				1351				97	
Yeast 2+*A.oxydans*	*A.oxydans* was inoculated 12 hrs after the yeast inoculation	T	2520	2420	2480	2470	770	760	770	765	256	285	256	266	1350	1390	1350	1365	83	91	94	89
		C			2248				715				299				1579				92	
Yeast 3+*A.oxydans*		T	2380	2330	2380	2362	780	790	810	793	242	242	213	232	1180	1150	1020	1119	84	97	79	87
		C			2390				784				242				1223				89	
S.e. of treatment means of 3 observations						±62				±12				±9.5				±61				±2.4
Min. sig. diff. (p=0·05) between T & C in the absence of *A. oxydans*						182				35				28				179				7
Min. sig. diff. (p=0·05) between T & C in the presence of *A.oxydans*						258				49				253				253				10

T = Treated sample
C = Control sample
* = When *A. oxydans* was used as a secondary organism only one control sample was prepared against 3 test samples.

†Observed data for tensile strength, elongation and modulus (but not the Means) are rounded off to the nearest ten.

the dry rubber when a heavy population was incubated for 24 hours. The cultures were chosen to form a fair cross section of the population naturally occurring in latex and thus the results may well be close to that occurring in field latex.

Since latex contains carbohydrates and a variety of yeasts and bacteria, it was thought that there might be a 'vinegar' type of reaction in which the carbohydrates are fermented to alcohol through the agency of yeast enzymes, and the alcohol oxidised by bacteria to acetic acid, which might speed up coagulation and possibly influence the technological properties of the resulting rubber.

The addition to sterile latex of pure cultures of bacteria and yeasts had no effect on the technological properties of the rubber; the same result was also obtained with yeasts and *A.oxydans* combination. Small apparent increases in tensile strength and modulus values of rubber from certain inoculated latices, suggestive of slight alterations in cure characteristics did not reach significance at the 5% level even though the tests were carried out with the ACS 1 mix which emphasises differences in cure characteristics.

The results here recorded are in general agreement with recent studies (JOHN, 1966c) which have shown that rubbers coagulated by biological means have faster curing characteristics while being in no way inferior to those obtained by acid coagulation.

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