PREPARATION OF DEPROTEINISED NATURAL RUBBER USING A NOVEL PROTEOLYTIC ENZYME

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Proteolytic enzymes are commonly used as a hydrolytic agent for the reduction of protein content in natural rubber (NR). In the present an attempt has been made to standardize a method to reduce the protein content of NR latex using a novel proteolytic enzyme. The NR latex was treated with very small quantities of the enzyme and then creamed for six hours. The cream portion was removed and coagulated and this rubber had nitrogen level similar to conventional deproteinised natural rubber (DPNR), but with higher initial plasticity (P_0) and plasticity retention index (PRI). Though the technological properties of the DPNR produced were slightly low, they were well within the process limits. The advantage of this method is production of DPNR with better properties in a short period of time.

Key words: Deproteinisation, *Hevea brasiliensis*, Latex creaming, Natural rubber latex, Plasticity retention index, Proteolytic enzyme

INTRODUCTION

Presence of non-rubber components especially certain proteins in natural rubber (NR) can cause allergy to some people using NR products (Aprem, 2002; Boonme *et al.*, 2014). Vulcanized NR has very high tensile strength, modulus and resilience and hence is used for making large number of latex and dry rubber based products (Ma'zam *et al.*, 2002). Though NR exhibits very good dynamic mechanical properties, this can be further improved by removal of the proteins present in it (Tanaka *et al.*, 1992; Sansatsadeekul *et al.*, 2011). This modification also imparts lower water absorption and better resilience properties (Boggs *et al.*, 1937). There are many engineering applications where water absorption needs to be lower together with stringent dynamic mechanical properties such as lower creep and stress relaxation (Ariyawiriyanana *et al.*, 2013). In such applications, deproteinised NR, is a better choice than the conventionally prepared NR though the plasticity retention index (PRI) is generally low for conventional DPNR (Pichayakorn *et al.*, 2012).

DPNR is a purified form of NR with very low nitrogen (N) and ash contents (George *et al.*, 2000). The special virtue of DPNR products is their low water absorption and electrical stability, which are highly relevant for under water applications (George *et al.*, 2001). DPNR based products offer reduced creep and precision modulus that enable them to be used in engineering applications. Physical or chemical means are common for

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reducing protein content of NR (Chaikumpollert *et al.*, 2012). The removal of proteins in most cases is done in latex stage, since the proteins exist on the surface of rubber particles dispersed in water (Eng *et al.*, 1992). The major physical processes used for deproteinisation are multiple centrifugation or membrane filtration of diluted latex (Moonprasith *et al.*, 2017). The chemical treatment involves hydrolysis of proteins using a proteolytic enzyme or displacement of adsorbed proteins using a surfactant and the subsequent purification of the treated latex by centrifugation or creaming (George *et al.*, 2007, Kawahara *et al.*, 2004, Yappa, 1977). In the present study, a method of producing DPNR by enzymatic deproteinisation using a novel enzyme was standardized. Generally DPNR produced by conventional methods have a low initial plasticity (P_0) and plasticity retention index (PRI) while the present method resulted in DPNR of high P_0 and PRI.

MATERIALS AND METHODS

Field latex collected from the research farm of Rubber Research Institute of India was used as the starting material and a few novel enzyme was used for the hydrolysis of proteins present in latex. Required quantities of 20 per cent solution of a surfactant were used with the enzymes for hydrolysis. All other chemicals used were of laboratory reagent grade. The deproteinisation was carried out using the novel proteolytic enzymes along with creaming using a creaming agent.

Field latex was first preserved with 0.6 per cent ammonia. The latex thus preserved was mixed with the enzymes in different proportions along with different surfactants in required proportions. Ammonium alginate at 0.25 per cent concentration on volume of latex as a creaming agent was also added as a three per cent solution to the latex. The treated latex was stirred well and kept undisturbed at room temperature for different periods. The cream fraction was removed and coagulated using a coagulant to produce DPNR. The DPNR thus produced was dried at 70^0C and compounded using standard formulation. The properties were compared with that of a control (normal dry rubber).

The cure characteristics were studied using RPA 2000. The plasticity parameters (P_0 and PRI) were measured using a Wallace Rapid Plastimeter MK V-P14 and MRPRA ageing oven as per ASTM standards (ASTM 2005, 9.01 and 9.02). The Mooney viscosity [M_L (1+4) 100^0C] was measured using Mooney viscometer model V-MV 3000. The heat buildup was determined using Goodrich flexometer. Tensile properties, tear strength, heat buildup, compression set and hardness were tested as per the respective ASTM standards D 412, D 624, D 623, D 395 B, and D 792 / 2240.

RESULTS AND DISCUSSION

The field latex ammoniated to 0.6 per cent on volume basis was treated with a protease enzyme EP at varying concentrations in alkaline pH and the treated latex was coagulated using a coagulant. The coagulum was washed and dried at 70^0C. The DPNR thus obtained was analyzed for raw rubber properties and N content, as the indicator of protein content. The results are shown in Table 1.

The results showed that the N content could be reduced from 0.43 per cent for the control samples to as low as 0.14 per cent for the enzyme treated samples. Raw rubber properties *viz*. P_0, PRI and Mooney viscosity were retained to a reasonably good level in the enzyme treated samples. However, the N level did not reach to a level that is required

Table 1. **Raw rubber properties of DPNR produced using protease enzyme EP**

Condition (Processing after 24 hrs.)	Nitrogen (%)	P_0	PRI	$M_L(1+4)\ 100^0C$
Control without NH_3	0.43	46	91	80
Control with NH_3	0.40	50	86	82
EP 0.5 phr without NH_3	0.16	51	73	85
EP 0.5 phr with NH_3	0.18	46	78	83
EP 1 phr without NH_3	0.18	51	82	87
EP 1 phr with NH_3	0.19	49	86	86
EP 1.5 phr without NH_3	0.17	51	80	85
EP 1.5 phr with NH_3	0.19	49	86	85
EP 2 phr without NH_3	0.16	51	78	87
EP 2 phr with NH_3	0.14	51	78	87
EP 2.5 phr without NH_3	0.14	49	80	89
EP 2.5 phr with NH_3	0.16	46	83	86

for the DPNR. Hence, the experiments were repeated in an acid pH. The results are shown in Table 2. It was observed that the N could be reduced from 0.44 to 0.13 per cent and P_0, PRI and Mooney viscosity were also retained to a considerably good extent. However, the N level did not reach to the required DPNR level of 0.12 per cent in acid pH also. Thus it can be inferred that mere acid as well as alkaline medium alone is not sufficient for the enzyme EP to hydrolyze the proteins to bring the level down to that is required for DPNR.

The latex hence was treated with two different enzymes (EA and EB) and their combinations and also in the presence of different surfactants and coagulated and processed after 24 hours. The conditions and results are shown in Table 3. The results showed that these conditions were also not sufficient for producing DPNR of the required low N level.

The latex was therefore treated with enzymes EA and EB at different doses and coagulated using acid and processed after 24 hours and the results are shown in Table 4. From the results it is noticed that in this experiment also the N level did not reach to the level that is required for the DPNR. However, the enzyme EB in smaller doses showed some positive results. Further experiments were conducted with enzymes EA and EB under different concentrations to

Table 2. **Raw rubber properties of DPNR produced using protease enzyme EP in acid pH**

Sample (Processing after 24 hrs.)	Nitrogen (%)	P_0	PRI	$M_L(1+4)\ 100^0C$
Control	0.44	49	92	80
EP 0.5 phr	0.20	46	72	80
EP 1 phr	0.16	44	41	78
EP 1.5 phr	0.13	48	71	80
EP 2 phr	0.13	50	76	83
EP 2.5 phr	0.13	49	69	82

Table 3. **Nitrogen content of DPNR produced using different enzymes and combination of enzymes with and without surfactants**

Sample (Processing after 24 hrs.)	Nitrogen (%)
Control	0.49
EP 0.5 phr	0.17
EP 0.5 + EA 0.1 (phr)	0.17
EP 0.5 + EB 0.1 (phr)	0.17
EP 0.5 phr + Surfacant 1 at 0.8 phr	0.16
EP 1.5 phr + Surfactant 2 at 0.5 phr	0.14
EP 1.5 phr at pH 10	0.14
EA 0.5 phr	0.19
EB 0.5 phr	0.15

Table 4. **Nitrogen content of DPNR produced using enzyme EA and EB followed by acid coagulation**

Sample (Processing after 24 hrs.)	Nitrogen (%)
EA 0.1 phr	0.36
EA 0.5 phr	0.27
EA 1 phr	0.20
EB 0.1 phr	0.24
EB 0.5 phr	0.14
EB 1 phr	0.12
Control	0.41

find out the minimum required enzyme level. The results are shown in Table 5.

Deproteinization with the enzyme EB at 0.5 phr could bring down the N level to 0.12 per cent which is in the DPNR level. However, as the N level is still on the brim, it has to be reduced further to a comfortably low level. Experiments were continued with enzyme EB to reduce N level further. In the next step varying holding times were given to the enzyme treated latex and the results are shown in Table 6. For comparison, other two enzymes were also included in the study. From the results it can be seen that enzyme EB at 0.5 phr at 48 hours or above holding time could produce DPNR with N level

Table 5. **Nitrogen content of DPNR-Treatment with EP, EA and EB under different concentrations**

Sample (Processing after 24 hrs.)	Nitrogen (%)
Control	0.39
EP 0.5 phr	0.27
EP 0.75 phr	0.18
EP 1 phr	0.14
EA 0.25 phr	0.31
EA 0.5 phr	0.31
EB 0.25 phr	0.20
EB 0.5 phr	0.14

below 0.12 per cent. Even though the N level was well below the standard level, further attempts were made to bring the N level down to the level of international limits. For further improvement in deproteinisation other additives like urea were also attempted. Creaming of the latex after

Table 6. **Nitrogen content of DPNR: Effect of enzymes and different holding times**

Sample	Nitrogen (%)
Control	0.39
EP 0.5 phr, 24 hrs	0.27
EP 0.75 phr, 24 hrs	0.18
EP 1 phr, 24 hrs	0.14
EA 0.25 phr, 24 hrs	0.31
EA 0.5 phr , 24 hrs	0.31
EB 0.25 phr, 24 hrs	0.20
EB 0.5 phr, 24 hrs	0.14
EP 0.5 phr, 48hrs	0.24
EA,0.25 phr, 48 hrs	0.18
EA,0.5 phr, 48 hrs	0.13
EB,0.25 phr, 48 hrs	0.12
EB,0.5 phr, 48 hrs	0.11
EA,0.25 phr, 72 hrs	0.18
EA,0.5 phr, 72 hrs	0.17
EB,0.25 phr, 72 hrs	0.11
EB,0.5 phr, 72 hrs	0.10

Table 7. **Nitrogen content of DPNR made with enzyme EB: Effect of creaming and urea treatment**

Treatment	Nitrogen (%)	Duration (hr)
EP 0.5 phr, 3 phr surfactant, creaming	0.10	24
EB 0.5 phr, 3 phr surfactant, creaming	0.08	24
EB 0.5 phr, +0.1 phr urea, 1:2 dilution, 24 hr retention, no creaming	0.14	24
EB 0.5 phr, +0.2 phr urea, 1:2 dilution, 24 hr retention, no creaming	0.15	24
EB 0.5 phr, +0.3 phr urea ,1:2 dilution, 24 hr retention, no creaming	0.14	24
Control (No enzyme)	0.39	24

Table 8. **Effect of deproteinisation using enzyme EB at different surfactant levels**

Treatment	Nitrogen (%)	Duration (hr)
EB 0.5 phr, 3 phr surfactant, creaming	0.09	24
EB 0.5 phr, 4 phr surfactant, creaming	0.08	24

Table 9. **Nitrogen content of DPNR: Effect of simultaneous deproteinisation and creaming**

Sample	Nitrogen (%)
EB 0.5 phr, 1:2 dilution, 3 phr surfactant and 24 hrs creaming	0.08
EB 0.5 phr, 1:2 dilution, 3 phr surfactant and 12 hrs creaming	0.09
EB 0.5 phr, 1:2 dilution, 3 phr surfactant and 6 hrs creaming	0.08

enzyme treatment was tried to bring down the N level and the protein level in turn. The effect of surfactant was also studied. The results are shown in Table 7. From the results it can be seen that treatment of urea could not bring down the N to that of DPNR level. However, treatment of enzyme EB at 0.5 phr level combined with the use of three phr surfactant could bring the N level as low as 0.08 per cent. In order to find the repeatability of the process, the enzyme treatment followed by creaming was repeated with enzyme EB with different doses of the surfactant. The results are shown in Table 8. From the results it can be seen that deproteinisation with creaming in presence of four phr surfactant gave low N levels required for DPNR.

In order to reduce the time for the preparation of DPNR, the creaming agent and the enzyme EB were added so that deproteinisation as well as the creaming took place simultaneously in the presence of four phr surfactant. The results are shown in Table 9. It can be seen from the results that the N content after six hours of simultaneous deproteinisation and creaming could produce DPNR of N content as low as 0.08 per cent. This is because the enzyme has hydrolyzed the proteins by the time creaming took place. As the PRI of DPNR is also a concern the raw rubber properties of the DPNR thus produced by the above method were analyzed and compared with that of the commercial DPNR samples. The results are shown in Table 10. From the results it can be seen that the DPNR produced by the above method exhibited a good PRI and P_0 compared to the commercial DPNR samples and also exhibited low N content.

The technological properties of the DPNR produced by the above method were studied by preparing compounds with a standard formulation shown in Table 11. Cure characteristics and technological properties

Table 10. **Raw rubber properties of the DPNR produced using enzyme EB**

Sample	Nitrogen (%)	P_0	PRI	M_L (1+4) 100^{0}C
EB 0.5 phr,6 hrs creaming	0.08	41	54	83
Commercial DPNR sample 1	0.16	38	18	84
Commercial DPNR sample 2	0.07	31	9	59

Table 11. **Formulation of the compound**

Rubber (DPNR)	100
ZnO	5
Stearic acid	1
Antioxidant	1.2
SRF black	40
Napthenic oil	2
CBS	0.9
Sulphur	2.5

were studied. The cure characteristics are shown in Table 12 and it can be seen that the cure torque is slightly higher for control compound than that of DPNR.

The technological properties are shown in Table 13. The results showed that though the technological properties of the DPNR produced were slightly lower they are comparable to that of the control. The ageing studies of the DPNR produced by the above method was also conducted. From the results it can be seen that the deproteinisation did not adversely affect the ageing properties of DPNR and was almost on par with that of control (Table 14).

Table 13. **Dynamic, mechanical and physical properties of the vulcanisate made using DPNR**

Sample	Control	DPNR
Tensile strength, MPa	26.4	26.1
Tear strength, Nmm^{-1}	117.8	106.4
Modulus 100 %, MPa	3.4	2.8
Modulus 200 % , MPa	9.0	7.0
Modulus 300 %, MPa	15.4	12.5
EB %	470	523
Abrasion loss, mm^3	94.2	101.3
Heat build-up, ^{0}C	18	19
Hardness, Shore A	60	61

Table 12. **Cure characteristics of DPNR**

Sample	Particulars				
	$S'M_L$	$S'M_H$	Tan δM_L	Tan δM_H	T90
Control	1.97	11.96	0.78	0.11	6.91
DPNR	1.61	11.28	0.83	0.086	7.35

Table 14. **Ageing resistance of the vulcanisates made of DPNR**

Sample (Aged at 100^{0}C for 72 hours)	Control			DPNR		
	Before ageing	After ageing	% retention	Before ageing	After ageing	% retention
Tensile strength, MPa	26.4	15.7	59.5	26.1	14.8	56.8
Tear strength, Nmm^{-1}	117.8	44.8	38	106.4	40.2	37.7
Modulus 100%, MPa	3.4	4.7	136	2.8	5.1	180.1
Modulus 200%, MPa	9.0	11.3	124.7	7.0	11.9	169.2
EB %	470	266	56.6	523	248	47.4

CONCLUSION

The present study was an attempt to develop a method to produce deproteinised NR through enzyme hydrolysis within a reasonably short time. After trying three enzymes a novel enzyme was identified for deproteinisation. The results showed that the enzyme (EB) can effectively reduce the protein content of NR to the specifications of DPNR. Use of the enzyme EB at 0.5 phr level combined with creaming produced DPNR with N content below 0.1 per cent. Moreover the PRI of the DPNR produced by the above method was very good compared to that of the commercial DPNR. It was also observed that six hours of creaming was sufficient to reduce N content to the level specified for DPNR and the enzyme EB can also be used simultaneously. The study showed that this method can be adopted for the preparation of DPNR with good properties within a reasonably short time (6-8 hours) compared to conventional methods which need long duration for the process.

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