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SOME ASPECTS OF BIOCHEMICAL CHANGES DUE TO MATURATION OF ACID AND PAPAIN TREATED *HEVEA* LATEX

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ABSTRACT

Biochemical changes that occur due to maturation of acid and papain treated Hevea *latex were investigated. An increase in bacterial population in latex of both acid and papain treated, was observed during maturation. The increase in papain treated latex was significantly higher than that in acid treated latex. Higher microbial activity correlated with decreases in carbohydrate and amino acid levels in serum. Maturation of papain treated latex, enhances protein digestion and reduction in ash content without any adverse effect on major raw rubber properties.*

Key words: deproteinization, maturation, papain, rubber

INTRODUCTION

Deproteinization of *Hevea* latex is now widely known to improve the technological and dynamic properties of resulting rubber (Yapa & Yapa, 1984). Papain is one of the proteolytic enzymes that has been reported to yield a low protein rubber with improved properties when latex was treated with the enzyme (Yapa *et al.*, 1980). Attempts have also been made successfully to improve the properties of papain treated rubber by maturation of the coagulum (Fernando *et al.*, 1984, Yapa *et al.*, 1984). Although the enhancement of properties due to maturation has been attributed to microbial activity, no investigation has been carried out to study the actual factors that play a role in such improvement in properties.

The objective of this study was to throw light on some biochemical changes that take place during maturation of papain treated latex, leading to an enhancement of properties of the resulting rubber.

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MATERIALS AND METHODS

Materials: All laboratory scale trials were carried out at the Dartonfield factory of the Rubber Research Institute at Agalawatte. The latex used was multiclonal but mainly that of clone PB 86. Commercial papain (white, Grade 1) was used in all trials.

Papain treatment: Fresh field latex was diluted 1:2 with water prior to addition of papain at 0.05% w/v on latex. Papain was dissolved in a small amount of water prior to addition. Enzyme treatment brought about both coagulation and digestion of proteins. Samples were allowed to mature for periods of 24h, 48h and 72h after which they were milled into thin laces, dried, homogenized and tested for various properties.

Analysis: Nitrogen content of dry rubber as well as that of rubber serum was determined by the micro Kjeldahl method (Tong, 1992). Wallace Plasticity and the Plasticity Retention Index (PRI) were determined using a Wallace Plastimeter (Tong, 1992). Ash content of dry rubber was determined by the method of Tong (1992).

Carbohydrate content of serum was determined by the method of Dubois *et al.* (1956) using phenol- sulphuric acid method. 0.02 ml of serum was used for each determination.

Analysis of amino acids was carried out using two dimensional paper chromatography on Whatman No 1 paper with n-butanol: acetic acid: water (120:30:50 v/v) as the first solvent followed by run in phenol-ammonia solvent system (Smith, 1968).

Determination of the bacterial count of the serum was carried out by the method of Harrigan *et al.* (1976)

RESULTS

pH of serum: The pH of serum after maturation of acid and papain treated *Hevea* latex is given in Table 1. Acid coagulation of latex yielded a serum with a low pH (4.13) which increased gradually and slightly during maturation. In contrast papain treatment yielded a serum with a significantly higher pH (6.24) than that of acid coagulation ($P < 0.01$) and it gradually decreased during the period of maturation.

Nitrogen content of serum: The nitrogen content of serum of both acid and papain treated latex showed a gradual decrease during maturation (Table 2). Serum of papain treated latex had a significantly higher nitrogen content ($P<0.05$) initially, 0.14% compared to 0.11% of acid treated latex.

Table 1. *pH of serum after maturation of acid and papain treated* Hevea *latex*

Treatment	Maturation period		
	24h	48h	72h
Acid	4.13±0.38	4.18±0.55	4.40±0.60
Papain	6.24±0.68	5.92±0.58	5.82±0.49

n = 3, P values < 0.01 for all 3 periods of maturation

Table 2. *Nitrogen content of serum after maturation of acid and papain treated Hevea latex*

Treatment	Maturation period		
	24h	48h	72h
Acid	0.11 (±0.001)	0.098 (±0.00005)	0.087 (±0.0003)
Papain	0.14 (±0.0008)	0.11 (±0.0005)	0.109 (±0.0004)

n=3, acid vs papain P< 0.05 in all 3 maturation periods

Plasticity Retention Index (PRI): The PRI of rubber resulted from acid coagulation of latex was found to be low and it decreased further during the maturation period. Papain treatment on the other hand, gave a rubber with a higher PRI which too decreased on maturation (Table 3). However, it remained at satisfactory levels in papain treated rubber.

Ash content: Ash content of acid coagulated rubber was found to be lower than that of papain treated rubber. On maturation ash content decreased in rubber of both acid and papain treated latex (Table 4). Ash content of papain treated rubber was close to that of acid coagulated rubber, after 72h of maturation.

Table 3. *PRI of resulting rubber after maturation of acid and papain treated* Hevea *latex*

Treatment	Maturation period		
	24h	48h	72h
Acid	47 ± 20	41 ± 16	38 ± 25
Papain	71 ± 22 (P=0.25)	61 ± 17 (P=0.24)	52 ± 24 (P=0.52)

n=3, P values acid vs papain

Table 4. *Effect of maturation on the ash content of acid and papain treated* Hevea *latex*

Treatment	Maturation period		
	24h	48h	72h
Acid	0.08±0.05	0.09±0.06	0.06±0.03
Papain	0.14±0.07 (P=0.27)	0.11±0.06 (P=0.72)	0.08±0.03 (P=0.34)

n=3, P values acid vs papain

Nitrogen content of rubber: The nitrogen contents of the dry rubber obtained from acid and papain treated *Hevea* latex are given in Table 5. The nitrogen contents of rubber of both treatments were found to decrease with maturation. At 24h, the reduction in papain treated rubber was significantly lower than that of rubber from acid treated latex (P=0.05). At 48h too, (*i.e.* 24h of maturation), the reduction in nitrogen content rubber from papain treated latex was significant (P=0.01).

Amino acid analysis: Paper chromatographic analysis of amino acids showed glutamic acid, threonine, alanine, glutamine, valine and lysine to be the major amino acids present in serum of acid treated latex whilst glycine, threonine, valine, leucine, phenylalanine were the major amino acids present in papain treated latex (Table 6).

Amino acid content in serum of both acid and papain treated latex decreased gradually during maturation with the exception of phenylalanine, glutamic acid and leucine which did not show any noticeable change during maturation (Table 6).

Table 5. *Effect of maturation on the nitrogen content of acid and papain treated* Hevea *latex*

Treatment	Maturation period		
	24h	48h	72h
Acid	0.36±0.08	0.31±0.01	0.27±0.06
Papain	0.19±0.01 (P=0.05)	0.17±0.03 (P=0.013)	0.17±0.02 (P=0.11)

n=3, P values acid vs papain

Table 6. *Amino acid composition of serum after maturation of acid and papain coagulated* Hevea *latex*

Amino acid	Acid			Papain		
	24h	48h	72h	24h	48h	72h
Glu	+++	++	+	+	+	+
Asp	++	+	+	-	-	+
Gly	++	+	-	++++	++	+
Ser	+	-	+	-	-	-
Thr	+++	+	+	+++	++	+
Ala	+++	++	+	++	+	-
Glu NH_2	+++	++	+	-	-	-
His	+	-	-	-	-	-
Arg	+	-	-	-	-	-
Tyr	+	-	-	-	-	-
Lys	+++	++	+	+	++	++
Ph al	-	-	-	++	++	++
Leu	-	-	-	++	++	++
Val	+++	++	++	++++	+++	+++

Carbohydrate content: Carbohydrate content of serum of both acid and papain treated latex decreased noticeably during the period of maturation (Fig 1). In papain treated latex the carbohydrate content of serum that stood at 18.13 μg/ml at 24h dropped to 0.75 μg/ml after 72h of maturation whereas in acid coagulated latex the carbohydrate content of 33.23 μg/ml after 24h reduced significantly to 2.7 μg/ml after 72h.

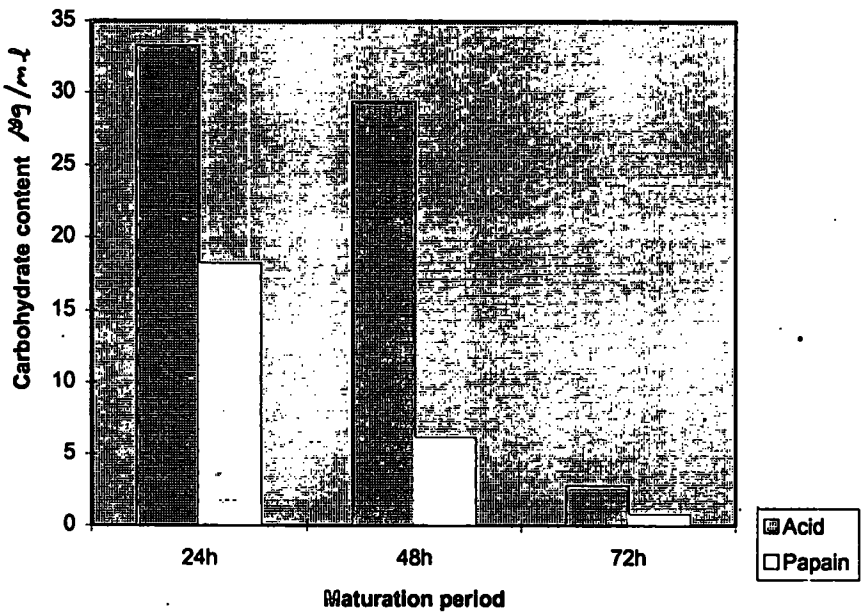

Fig. 1. Effect of maturation on carbohydrate content in latex serum

Bacterial population: The bacterial population of serum of both acid and papain treated latex increased significantly during the maturation period (Fig 2). In papain treated latex serum, the bacterial population increased significantly from 144 x 10^7 per ml at 24h to 179 x 10^7 per ml after 72h whilst in acid coagulated latex too it increased significantly from 179 x 10^2 per ml at 24h to 236 x 10^2 per ml after 24h. Bacterial population of serum of papain treated latex was significantly higher than that of acid coagulated latex serum.

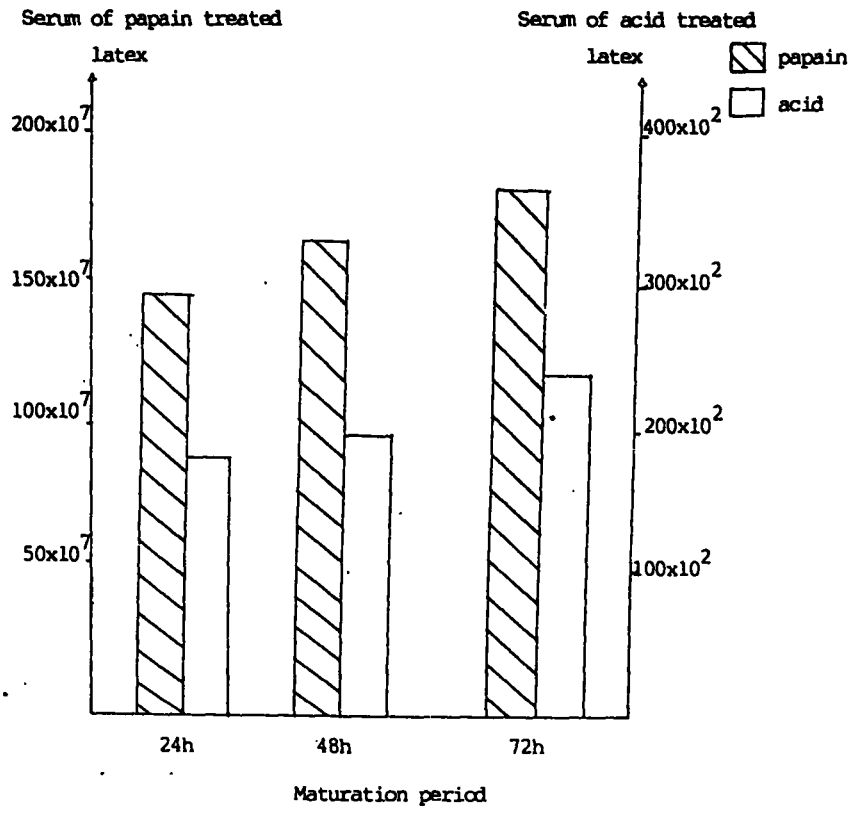

Fig. 2. Variation in bacterial count (per ml) in serum of acid and papain treated latex at different periods of maturation

DISCUSSION

The results of this study throws some light on biochemical changes that takes place during maturation of *Hevea* latex. The contention made in several previous reports by Fernando *et al* (1984) and Yapa *et al* (1980) that microbial activity plays a key role in enhancement of technological and dynamic properties of rubber subjected to maturation is supported by the findings of this study.

The results of this study in general indicate higher microbial activity during maturation of latex and the conditions for such higher activity are more favourable in papain treated latex than that of acid treated latex.

For instance, the gradual decrease in the levels of amino acids and carbohydrates during maturation is indicative of higher microbial activity. This is further evidenced by the increased bacterial population during maturation. Although the amino acids and carbohydrate levels of acid treated latex also increases during maturation, the increase in papain treated latex is significantly higher. The bacterial population of papain treated latex was also significantly higher than that of acid treated latex. The slightly higher nitrogen content on the serum of papain treated latex can be attributed to increased level of hydrolysis of proteins during maturation compared to acid treatment. This is supported by the reduced nitrogen level of final rubber obtained from papain treatment.

The resistance to oxidation of dry rubber which is measured as PRI, is affected by maturation in acid treated latex. However, in papain treated latex, PRI is affected only slightly and remained at satisfactory levels even after 72h of maturation. At 48h, the recommended period for maturation, PRI remained above the minimum specification level of 60 for DPNR. It is therefore evident that maturation of papain treated latex enhances deproteinization process without adversely affecting the major raw rubber properties. A reduction in ash content was observed in both acid treated latex as well as papain treated latex, on maturation. Although the exact factors contributing for this reduction are not known, the reduced ash content, is an added advantage of papain treated and matured latex.

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