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THE USE OF PAPAIN AS A BIOLOGICAL COAGULANT FOR NATURAL RUBBER LATEX

BY

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SUMMARY

The use of papain as a general coagulant for natural rubber latex in conjunction with formic acid for raw rubber manufacture should give a superior rubber as it will have a lower nitrogen content. This method of coagulation would be of value in the manufacture of partially purified crepe and in coagulating of skim latex to get rubber of acceptable quality. Papain fetches a high price but rubber estates could produce it for their own use at reasonable prices by intercropping papaya between immature rubber trees. This can be conveniently done as the period during which papain can be commercially exploited (3 to 4 yr) coincides with the period during which it can be successfully grown as an inter-crop.

INTRODUCTION

Papaya cultivation in Sri Lanka

Papain is dried latex obtained by tapping the skins of the immature fruit of papaw, *Carica papaya*. Tapping consists of making longitudinal incisions (five to six) in the fruit skin. Tapping continues at intervals of 5 days until the fruit is mature. The tree bears tappable fruits within twelve months of planting and a fruit can be tapped for 3 months. The yield would be about 100 to 200 lb an acre. Tapping would not usually be practiced for more than 2 to 3 yr owing to the increased height and reduced yield and vigour of the trees (Cheshire, 1966). It may be mentioned that in 1954 approximately 219 tons of papain were exported from Sri Lanka, whilst the export for 1972 is approximately 10 tons. The reason for this drop is the instability of papain prices and to the advent of the mosaic disease.

Instability of price levels

The most notable feature of the trade in papain is the instability of price levels. This is due to the relative ease with which the supply can be in creased and decreased and because the uses of papain are very limited (Cheshire, 1966). Although low prices may induce buyers to buy more in order to stockpile, the low prices do not basically increase the utilisation of papain.

Uses of Papain

There are two major uses of papain. The first use is for tenderising meat. The meat can either be dipped in papain solution or dusted with papain before cooking or cattle can be injected with a papain solution just before slaughter. The second use is as a beer stabilising agent in light non draught beers.

The above two uses are limited, and if there are proposals to expand papain production, it is important to find a sufficiently large new demand, which would create a new market. The commercial use of papain as a natural rubber latex coagulant would be a new use for papain. Further, it would be advantageous if papain could be produced from papaya grown as an intercrop between the immature rubber trees.

Intercropping of papaya between rubber trees

Senanayake (1968) has suggested papaya as being adaptable for intercropping between rubber trees. Papaya trees appear to be suitable for inter planting with immature rubber, because the normal period during which papain can be commercially extracted from the papaya plant is only within 4 yr of planting and therefore papaya may be grown as an intercrop in immature rubber for about 4 years. Senanayake (1968) also states that if intercropping is adopted, greater importance should be attached to soil conservation because of the additional soil disturbance during the second and third years. The cost of soil conservation may increase, but it could be recovered partly from the returns of the intercrop. Further, intercropping would have the added advantage of reducing the existing high weeding costs, while permitting the growers to raise a crop.

Use of papain as a latex coagulant

The coagulants commonly used for natural rubber latex are acids which bring down the pH of the latex to near that of the isoelectric point of the proteins which protects the natural rubber latex particles (mainly α globulin). The pH of coagulation is approximately 4.6. The acids commonly used as coagulants are formic, acetic, oxalic or sulphuric acid. Recently biological methods of coagulation of natural rubber latex have been introduced. These depend on acids formed by bacteria in the latex from sugars added to natural rubber latex (John, 1966) or from added specific bacteria reacting with naturally present sugars in the latex to give the necessary acids (Satchuthananthavale *et al.*, 1971).

Since fresh *Hevea* latex owes its stability to a large extent to the presence of proteins, it can be destabilized, by treatment with proteolytic enzymes like papain, resulting in coagulation (Resing, 1953). A Patent has been filed on application of this in Sri Lanka (Nadarajah *et al.*, 1972). For several years the rubber plantation industry has been endeavouring to produce a commercial grade of rubber which gives a low protein, ash and acetone extract with low water absorption for specialised use in industry.

A method of doing this is by diluting centrifuged latex to less than 10% and coagulating with formic acid, when a partially purified rubber low in nitrogen content (about 0.2%) and low in ash content (about 0.06%) is obtained (NRPRA Technical Information Sheet No. 12). This rubber fetches a premium of approximately 2½ new pence (approximately 40 Sri Lanka cts) above RSS 1 in the world market.

Recently the Rubber Reserach Institute of Malaysia has produced deproteinised rubber on a commercial scale by enzyme deproteinisation of NR latex. This paper discusses the coagulation of NR latex with papain to give in a simple manner various grades of low protein rubber.

EXPERIMENTAL AND RESULTS

Of the two grades of papain (white and brown) available in the market oven dried papain which is white in colour was used in all the experiments reported in this paper. It was added as an emulsion in a small volume of water. The semi-micro Kjeldahl method was used in all the nitrogen determinations.

Use of papain in raw rubber manufacture

Papain at 500 ppm on the latex causes rapid coagulation and at 250 ppm causes overnight coagulation. Investigations were carried out on the suitability of papain as the coagulant for RSS, pale crepe and skim rubber manufacture.

Use of papain in RSS manufacture

It was found that the drying time when RSS was manufactured was unduly long when papain was used as the sole coagulant. Perhaps, during the enzymatic coagulation a non-porous structure is formed by the degradation of the protein so that diffusion of moisture is retarded.

The isoelectric point of papain is at pH 8.75. Papain has only limited solubility above pH 7, and its optimum pH of activity is generally between 6 & 8. However the optimum pH of commercial papain depends on the substrate and for serum globulin it is 4.9 Since α-globulin is the main protein of *Hevea brasiliensis*, a mixture of papain and an organic acid should be the best coagulant.

Papain was used by us as coagulant of RSS manufacture to replace 50% of formic acid *i.e.* a mixture of papain at 1½ oz and formic acid at 3 oz per 700 lb d.r.c. was used, the two substances being added consecutively. The nitrogen content of RSS using papain as the coagulant are given in Table 1.

TABLE 1

PAPAIN IN RSS MANUFACTURE

Coagulant	Nitrogen %
Papain at 500 ppm	0.12
Papain at 250 ppm	0.27
Formic acid-control	0.36

The PRI, ash and nitrogen values of RSS using papain or formic acid or a mixture of formic acid and papain are given in Table 2.

TABLE 2

PAPAIN IN RSS MANUFACTURE

Treatment/milling	Properties of RSS		
	PRI	Ash%	N%
500 ppm papain only			
same day milling	83	0.21	0.24
overnight milling	87	0.12	0.21
Papain 250 ppm + ½ HCOOH dosage			
same day milling	77	0.24	0.13
overnight milling	89	0.15	0.47
Papain 125 ppm + ¾ HCOOH dosage			
same day milling	71	0.18	0.32
overnight milling	85	0.12	0.47
Control/HCOOH only			
same day milling	72	0.22	0.47
overnight milling	87	0.04	0.39

It will be noted that the use of papain alone lowers the nitrogen content but if a mixture of papain and formic acid is used, then same day milling should be practiced to obtain a lower nitrogen content.

Use of papain in latex crepe manufacture

In latex crepe manufacture the use of oxalic acid at 6 oz and papain at 1½ oz per 100 lb d.r.c. gave a good quality pale crepe. The values obtained for N, PRI, Po, ash and MOD values when papain and oxalic acid (OA) are used as the coagulant are given in Table 3.

TABLE 3

PAPAIN IN LATEX CREPE MANUFACTURE

Coagulant	N%	P_o	PRI	Ash%	MOD
Formic acid (Control)	0.43	47	75	0.08	5.1
Papain 250 ppm OA 6 oz/100 lb	0.33	51	75	0.11	5.6
Papain 300 ppm OA 3 oz/100 lb	0.25	51	77	0.10	5.7
Papain 350 ppm	0.24	50	74	0.12	5.9

The nitrogen content is lower in the papain coagulated rubber, the ash content is higher and the rubber is faster curing than the control. Papain and formic acid could also be used as coagulants in latex crepe manufacture.

When papain and acid are used as the coagulant, the pH of coagulation is higher than when acid is used as the sole coagulant. Ong Chong Oon (1972) has found that the higher the coagulation pH, the better would be the colour. The texture of the lace where papain is used as part coagulant is much better than when acid is used as the sole coagulant. Hence in thin crepe and in sole crepe maunfacture, the smooth roller millings could be considerably reduced if papain is used as part coagulant.

The serum obtained from papain coagulation was analysed for amino acids by paper chromatography; the serum was applied on Watman No. 1 filter paper and run first in n-butanol-acetic acid - water (120 : 30 : 50 v/v), followed by a phenol-ammonia solvent system. A considerable increase in the concentration of 4 amino acids namely tryptophan, valine, phenylalanine and leucine was observed. Amino acids in the serum were also observed to increase corresponding to the concentration of papain used.

The results using raw papaw milk as a coagulant in latex crepe manufacture are given in Table 4.

TABLE 4

USE OF RAW PAPAW MILK AS A COAGULANT

Amount of raw papaw milk/ml/½ gal.	Time taken for coagulation	PRI	N%	Ash%
Control/HCOOH	5 min	84	0.50	0.10
0.25	Overnight	90	0.33	0.13
0.50	Overnight	89	0.26	0.12
1.0	Overnight	84	0.16	0.16
2.0	1 h 30 min	76	0.21	0.14
4.0	15 min	78	0.26	0.16

It will be noted that the nitrogen content is lowered with increasing papain concentrations and with overnight coagulation.

A drawback in the coagulation of field latex with papain is that the ash content increases with papain concentration. Fig. 1 shows the relationship between the papain concentration, ash and nitrogen contents.

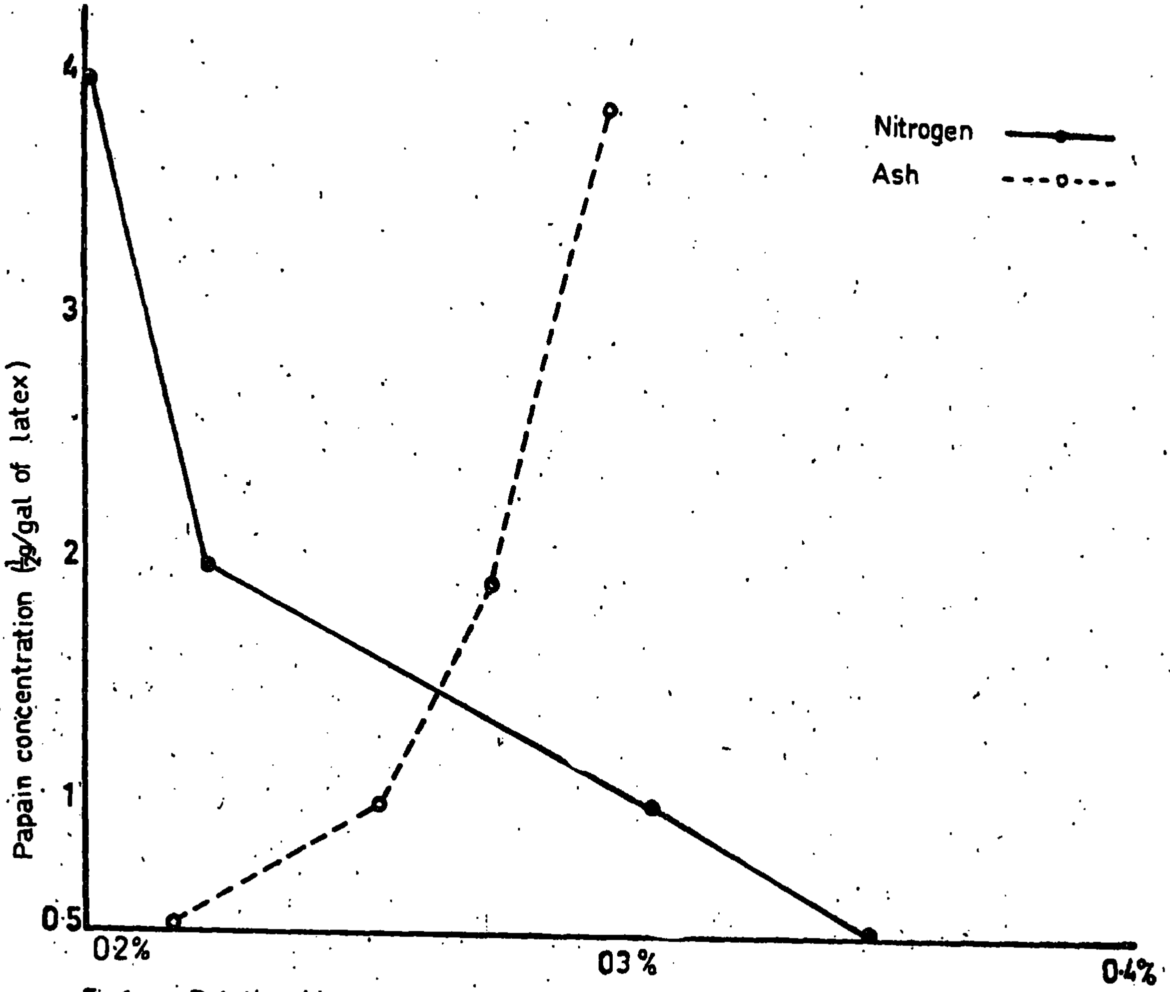

Fig 1 Relationship between papain concentration with nitrogen and ash contents

The lowering of the ash content as well as the nitrogen content is achieved by considerable dilution of the latex before coagulation. Table 5 gives the results obtained by diluting the latex with four volumes of water before coagulation.

TABLE 5

EFFECT OF DILUTION ON NITROGEN AND ASH CONTENTS OF CREPE

Coagulant	Dilution	N%	Ash%
Papain 500 ppm	no dilution	0.27	0.35
Papain 500 ppm	1:4 dilution	0.21	0.15
Papain 250 ppm +HCOOH 500 ppm	no dilution	0.30	0.33
Papain 250 ppm +HCOOH 500 ppm	1:4 dilution	0.17	0.14

Still further dilution of the latex should give a still greater reduction in the ash and nitrogen contents. The taking of a first fraction (yellow fraction) before dilution should also lead to a greater reduction in the ash and nitrogen contents.

Use of papain in skim rubber manufacture

Skim latex autocoagulates after seeding with bacteria in about 3 to 4 days. The rubber has a high nitrogen content and has a foul odour. A rubber with acceptable PRI and nitrogen content and with an acceptable smell can be obtained by taking skim latex, which has been seeded with bacteria, allowing it to stand for two or three days and treating with papain as a suspension at 1½ g per gal and oxalic acid as a solution at ½ g per gal. Fresh skim latex can also be coagulated with papain to give rubber with an acceptable nitrogen content, but dilute sulphuric acid (2%) solution has to be added initially to neutralise the excess ammonia present. Fresh skim latex needs about 850 ml of 2% H_2SO_4 solution per gal for coagulation.

The N, PRI, and MOD values obtained with papain as coagulant are given in Table 6.

TABLE 6

PROPERTIES OF SKIM RUBBER OBTAINED BY PAPAIN COAGULATION

No. of days storage of skim latex	Coagulants per gal	Nitrogen content %	PRI	MOD value
0	½ g oxalic acid, 7½ ml conc. H_2SO_4, 1½ g Papain	0.42	67	6.3
2	1½ g Papain	0.47	36	6.8
2	3 g Papain	0.47	32	6.8
3	control (auto coagulation)	0.93	69	9.3

The PRI should be above 50 to satisfy the SCR 20 specifications and over 40 to satisfy SCR 50 specifications. It is noted from Table 6 that papain used as the sole coagulant for skim latex gives PRI values below 40, whilst the control with autocoagulation had a PRI value of over 60. Skim latex contains high amounts of copper and the H_2S formed during autocoagulation reacts with the copper and renders it inactive. Hence a complexing agent for copper ions has to be used and Table 6 and 7 show that oxalic acid is a suitable complexing agent to be used in conjunction with papain to give acceptable pH values. The results presented in Table 7 refer to 2-day old skim latex which had been seeded with bacteria and had a pH of 7.

TABLE 7

PROPERTIES OF SKIM RUBBER OBTAINED BY PAPAIN AND OXALIC ACID COAGULATION

Coagulants for 1½ gal of skim latex			Properties	
Papain (g)	Oxalic acid (g)	2% H_2SO_4 (ml)	N%	PRI
1.5	0.5	0	0.35	52
1.5	0.5	10	0.33	56
1.5	0.5	20	0.35	54
1.5	0.5	40	0.28	51

DISCUSSION

With 200,000 tons of rubber being produced annually in the world as centrifuged latex, and with a separating efficiency of 85%, about 35,000 tons of rubber as skim latex are produced annually. The dry rubber content of commercial skim latex can lie between 2.5 and 10%. Commercial skim rubber may contain 70 to 85% NR hydrocarbon, between 5 and 10% fatty material and between 9 and 18% of protein compared with average figures for RSS of 95% hydrocarbon, 3% fats and 2% protein. Much of the non rubber material is proteinaceous in nature and leads to offensive odours, and to rapid curing and scorching of the compounded rubber in the course of manufacture.

The high non-rubber content of skim rubber arises from two basic causes. First the serum phase of skim latex contains all the proteins and dissolved matter as the field latex but the rubber content has been drastically reduced and second the rubber particles remaining in the skim are the smaller particles and thus having a relatively larger surface area bearing adsorbed material. The problem of improving the properties of skim rubber is one of drastic reduction of the protein content of the dry rubber and ways of treating skim latex for this purpose have been studied by several workers over many years.

Rubber with properties comparable with those of normal smoked sheet can be obtained by suitable treatment of skim latex with proteolytic enzymes such as trypsin (Morris, 1952), or alkaline hydrolysis of the proteins and lipids after bacterial coagulation of skim latex (Firestone Tire & Rubber Co., 1955). The costs involved in such treatments are important and many latex producers let the serum ferment by bacteria until spontaneous coagulation occurs. Resing (1960) has stated that controlled bacterial degradation with efficient removal of non rubbers is one of the most promising methods of dealing with skim rubber.

We have used both bacterial degradation and treatment with the proteolytic enzyme, papain, in our approach towards the treatment of skim latex to obtain good qualityrubber.

Papain could be used at approximately half the dosage of formic acid to effect coagulation for normal grades of raw rubber. The use of organic acids in RSS and pale crepe manufacture, not only agglomerates the rubber particles by lowering the pH but also precipitates serum proteins which become occluded in the rubber coagulum. The serum proteins do not confer any advantages to the finished product. There is a need to find an economic and practical method of coagulating rubber particles from latex, without precipitating the serum proteins. The use of papain in conjunction with acid is a practical method of precipitating rubber particles from latex without the concomitant precipitation of serum protein. It is known that for certain applications *e.g.* in truck tyre manufacture, resilience and heat build up are important. In these applications a purer rubber with a lower nitrogen content is preferable, and by the use of papain and organic acid as the coagulant, it should be possible for Sri Lanka to produce RSS with nitrogen values well below 0.4%. The present specification for nitrogen is 0.65%. Latex crepe is a special quality, light coloured rubber of high purity and Sri Lanka is the world's largest producer of this type of rubber. Latex crepe where a fraction is taken and not bleached would be a high quality pale crepe to be used in surgical products, products coming into contact with foodstuffs and in the manufacture of rubber derivatives. If papain is used as the coagulant in the manufacture of latex crepe after removing a fraction, a higher quality pale crepe would be obtained because it is purer, due to a lower nitrogen content.

The method of obtaining rubber low in nitrogen by using centrifuged latex (NRPRA Technical Information Sheet No. 12) has the defect that the rubber obtained has a low PRI because of the leaching by the ammonia of alkali soluble antioxidants *e.g.* tocotrienols present in the rubber particles (Nadarajah *et al.*, 1971). This defect does not occur with papain deproteinised rubber.

Partially purfied crepes are of value in the production of modified rubbers where the presence of the non rubber constituents is liable to interfere with the necessary modifications (Leveque & Stokes, 1962). The immediate value of papain coagulated latex crepe in Sri Lanka is in the manufacture of cyclised rubber (Nadarajah *et al.*, 1973).

The reduction on nitrogen content of the final rubber obtained by papain deproteinisation is supported by the fact that there is an increase in amino acid content in the serum after papain coagulation; the increase in amino acid content is due to the release of acids by hydrolysis of proteins by papain; it is also possible that free amino acid in papaw milk may have contributed to this increase. However, the increase in concentration of such amino acids as tryptophan which was not detected in raw papaw milk suggest that they have come from hydrolysis of proteins by papain.

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