

extremely bitter taste qualities and may contribute to the bitterness of roasted products like roasted malt and coffee.

Registry No. 1, 5724-81-2; 2, 123-75-1; 3, 3760-54-1; 4, 4030-18-6; 5, 54151-38-1; 6, 97073-14-8; 7, 97073-15-9; 8, 97073-16-0; 9, 97073-17-1; 10, 61893-12-7; 11, 61480-99-7; 12, 61481-02-5; 13, 80873-59-2; 14, 97073-22-8; 15, 97073-23-9; 16, 97073-24-0; 17, 97073-18-2; 18, 97073-19-3; 19, 6103-92-0; 20, 97073-20-6; 21, 97073-21-7; 22, 91999-33-6; 23, 91999-34-7; L-proline, 147-85-3; rhamnose, 3615-41-6; arabinose, 147-81-9; 2,3-butanedione, 431-03-8; 2,3-pentanedione, 600-14-6; D-glucose, 50-99-7; maltol, 118-71-8.

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A Viscometric Method for Determining Rubber Content in Guayule (*Parthenium argentatum* Gray)

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The viscosity of cyclohexane solutions of guayule rubber increased with rubber concentration. The relationship allows measurements to be made of the rubber content of guayule material by a viscometric technique that is simple and inexpensive. This viscometric method gave results comparable to other methods currently in use to determine rubber content, such as the gravimetric and infrared spectrophotometric methods. Oxidative degradation of the rubber solutions can affect viscosity but these effects can be minimized by avoiding light, by use of appropriate antioxidants, and by storage of samples under refrigeration.

There have been several techniques developed for determining the rubber content of guayule (Spence and Caldwell, 1933; Traub, 1946; Mehta et al., 1979; Willits et al., 1946; Tysdal and Finlayson, 1981; Visintainer et al., 1981; Banigan et al., 1982). Similarly, there are several laboratories engaged in guayule research each employing one, or a variation of one of the techniques, to evaluate the progress of their breeding efforts, to assess the effectiveness of bioregulators, and to measure the rubber content of plants under various physiological and environmental stimuli. Because of the disparity of methods employed by these various laboratories, comparison of data and interpretation of results are made difficult and open to question.

What is clearly needed then, is a relatively inexpensive and simple technique that allows consistent and accurate measurements of rubber content to be made. Also a method is required, that if adopted by the laboratories involved in this research, could standardize the procedure used to analyze for rubber content.

The viscometric method, described in this paper, promises to be a useful method for determining the rubber content of guayule plant material and one which meets the criteria of ease, cost effectiveness and consistency, while being relatively accurate. A comparison is made with other

forms of analysis and some of the factors that can affect the viscosity of guayule rubber solutions are presented.

EXPERIMENTAL SECTION

Plant Material. Material for rubber extraction and viscometric work was collected from three-year-old mixed variety plants grown at Berrenda Mesa Farms near Lost Hills, CA.

Extraction of a Rubber Sample. Shrubs were coppiced at varying heights and shredded with a hammermill at fieldside. The coarsely ground material was then sealed in plastic bags and stored in the dark over ice for transportation to the laboratory.

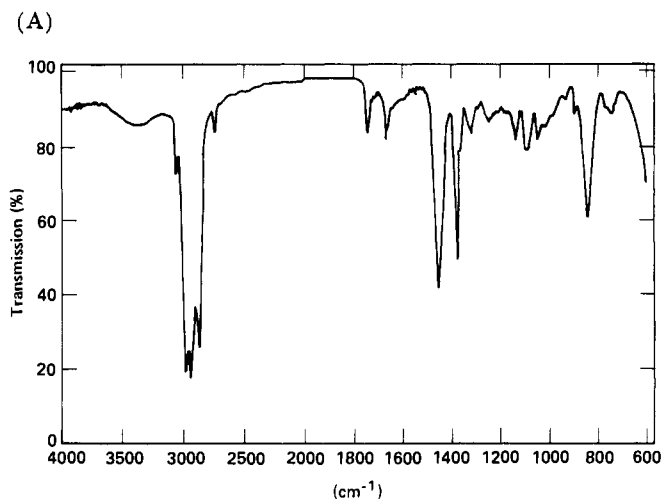
The material was then frozen in liquid nitrogen and passed through a Wiley mill twice, first with a 4 mm screen and subsequently a 2 mm screen. The finely ground samples were stored at -20 °C until they could be processed further.

Approximately 100 g fresh weight of material was placed in a 5-L Erlenmeyer flask, wrapped with aluminum foil to exclude light, and 2 L of cyclohexane added. The flask was placed on an orbital shaker for 2 h at 150 rpm. The rubber solution was then sieved through a 90- μ m stainless steel screen into 1-L centrifuge buckets, and centrifuged at 5000 rpm for 30 min. Finally, the solution was vacuum filtered through Whatman no. 2 filter paper. By this stage, particulate matter had been removed and the green, viscous rubber solution was transparent.

A 2-fold volume of methanol was then added and the contents shaken. The rubber immediately precipitated and could be collected and used as a sample in subsequent studies. The cream colored rubber sample was stored in

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(B) $M_n = 273\,400$
 $M_w = 1\,009\,600$
 $M_z = 1\,986\,200$
 peak mol wt = 905 700
 poly dispersity = 3.69
 intrinsic viscosity = 5.64
 viscosity av = 925 900

Figure 1. Characterization of guayule rubber sample. (A) Infrared spectrum of guayule rubber sample. The rubber was redissolved in cyclohexane and a film cast on a sodium chloride block. (B) Gel permeation chromatographic analysis of guayule rubber sample (ARCO Chemical Company, courtesy Gary Martinie).

methanol in a blackened vial, within a refrigerator. An IR spectrum and a gel permeation chromatographic analysis of the rubber sample is shown in Figure 1. The gel permeation chromatograph used in this work was a Waters 244. The column was a 2-Micropak TSK-GMHG (7.5 mm \times 60 cm) and tetrahydrofuran was used as the solvent with a flow rate of 1 mL/min at 35 °C. A refractometer was the sensing device. Standard polystyrene samples obtained from Waters Associates were used to calibrate the columns.

Viscometric Determinations. Finely ground guayule material was extracted in cyclohexane by the procedure outlined above, however, 1-g samples were used and placed in covered 125-mL Erlenmeyer flasks with 10 mL of cyclohexane. After agitation for 2 h at 150 rpm on an orbital shaker, the rubber solution was sieved through a 90- μ m mesh into a sealed and covered test tube to await determinations. Another 1-g sample was dried at 80 °C for 24 h so that rubber content could be expressed as a percentage of the dry weight of the tissue.

A series of Cannon-Fenske Routine Viscometers was used. The size number used depended on the viscosity of the solution, as speed and accuracy of the measurements were taken into account. For viscosity measurements from ground guayule samples, size numbers 100 and 150 were most suitable.

All measurements were taken at precisely 26 °C. After a series of measurements the viscometers were soaked in a chromic acid solution to remove all rubber residues.

Photooxidative Degradation of Guayule Rubber Solutions. Rubber solutions were prepared by dissolving approximately 100 mg of freshly precipitated rubber in 10 mL of cyclohexane in sealed and covered vials. Vials were then exposed to fluorescent lights with a light intensity at the vial surface of 3000 lux and a temperature of 26 °C. The depth of solution in the direction of light was 15 mm. Butylated hydroxytoluene (BHT) and 1,4-diazabicyclo-[2.2.2]octane (Dabco) were used as antioxidants. Viscosity was monitored over a period of time.

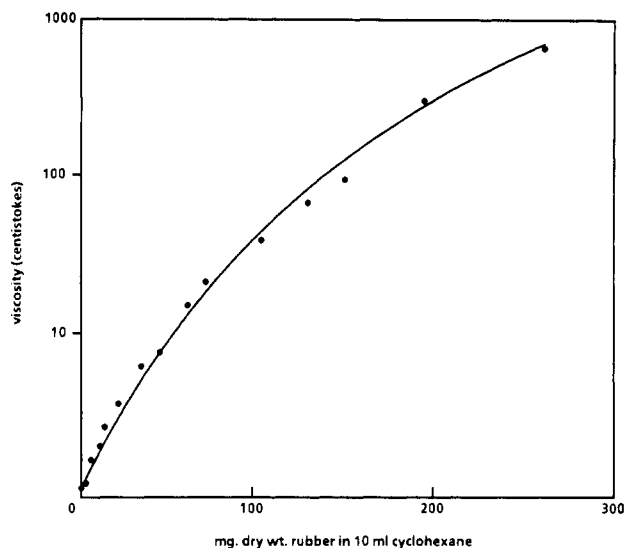


Figure 2. Guayule rubber standard curve.

RESULTS

A guayule rubber standard was prepared. With an increase in the weight of rubber dissolved in cyclohexane there was a rapid increase in the viscosity of the solution (Figure 2). The relationship can be described by the empirical formula, $[R] = ax - b(1 - e^{-cx})$, where $x = \log(\eta_8) - \log(\eta_0)$.

Rubber concentration, $[R]$, is given as mg 0 dry weight rubber in 10 mL of cyclohexane with η_8 as the viscosity of the rubber solution and η_0 as the viscosity of the solvent, cyclohexane. The value of the constants are $a = 312.01$, $b = 1569.40$, and $c = 0.17824$.

Two hours of extraction in cyclohexane was adequate for dissolving the majority of the rubber particles, as the viscosity of the solution did not increase appreciably beyond this time. The existence of the fine particulate matter in the viscous rubber solution also did not interfere with viscometric determinations. The viscosity of solutions was unaltered after further centrifugation and filtration steps. Although these final two steps were necessary in recovering good quality rubber by methanol precipitation, they were not necessary when assaying for rubber content in shrubs. Having established that viscosity measurements could be made relatively easily and that they accurately reflected the amount of rubber dissolved in cyclohexane, attention was placed on comparing this method with other methods currently in use.

Two alternative methods of determining rubber content were used and analyzed independently in separate laboratories. The first method was a modification of the gravimetric technique (Garrot et al., 1981), and the second method was the infrared spectrophotometric technique developed by Banigan et al. (1982). The gravimetric procedure was developed by Spence and Caldwell (1933) and has been used in various modified versions ever since it was first reported (Hammond and Polhamus, 1965; for review). The IR method is a more recent innovation and has been thoroughly tested with the gravimetric, the turbidimetric (Perry and Naqvi, 1980), and the ^{13}C NMR method of analysis (Visintainer et al., 1981).

When replicate finely ground samples were used, a linear correlation existed in rubber content as determined between the viscometric method and both the gravimetric and IR methods (Figure 3). The best comparison existed between the viscometric and IR method ($r = 0.99$) with a poorer correlation for the gravimetric and viscometric method ($r = 0.94$). However, for absolute amounts of

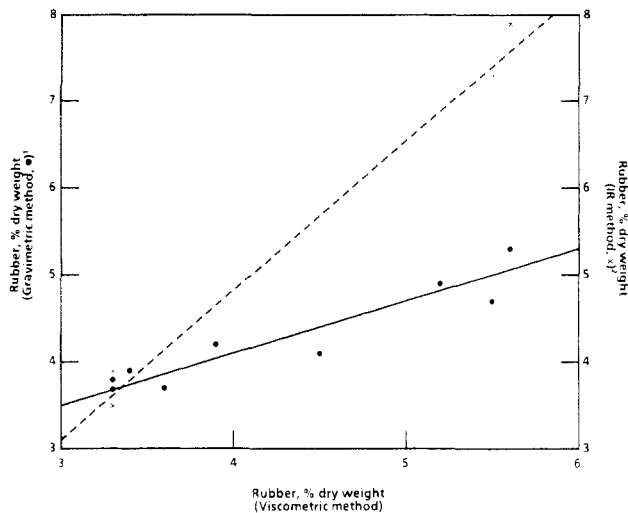


Figure 3. Rubber analysis method comparison. ¹ARCO Solar Industries, BioEngineering Center, courtesy of M. Lindorfer. ²Anver Bioscience Design, Inc., courtesy of A. J. Verbiscar.

rubber, the gravimetric method was in better agreement than the IR method.

Having established that viscosity was a reliable indicator of rubber content in guayule extracts, attention was focused on factors affecting viscosity. The most notable were the effects of light and oxygen on viscosity.

Within an hour's exposure to light, there was a decrease in viscosity of the rubber solution and the degradation process was complete after only 5 days' exposure. Even in the dark there was a loss of viscosity and the use of various antioxidants (10 mM Dabco, 10 mM BHT) were useful in slowing the process in the light and virtually stopping the process in the dark (Figure 4).

DISCUSSION

It has long been known that when increasing weights of rubber are dissolved in certain organic solvents the viscosity of the solution changes (Busse (1937), for review), the viscosity being a function of the amount of rubber dissolved in a given volume of solvent. The relationship suits itself ideally to rubber determinations in guayule material as the rubber is dissolved from finely ground samples and viscometric measurements are compared with a standard to estimate the percentage of rubber in a sample.

The viscometric method correlated well with the other two forms of rubber analysis, particularly the IR method, although for absolute amounts of rubber the gravimetric method was in better agreement (Figure 3). Estimates of rubber content are lower for the viscometric and gravimetric methods, as compared to the IR method, probably because the IR method uses high speed tissumizer milling of the sample and is able to recover a high percentage of the rubber present in the sample. Tissumizer milling of the sample in cyclohexane was not done because the shearing of rubber molecules in solution would be expected to substantially lower the viscosity of the solution (Busse, 1937). The point of interest, from Figure 3, is the high yielding samples could be distinguished from the low yielding samples by using the viscometric method and this was substantiated with both the gravimetric and IR methods. Once high yielding shrubs are identified in this manner, they could be multiplied by an *in vitro* propagation method (Smith, 1983) and rubber extracted from the whole shrub from a proportion of the population. This would provide accurate yield data and the remainder of the population could be used for plant breeding purposes

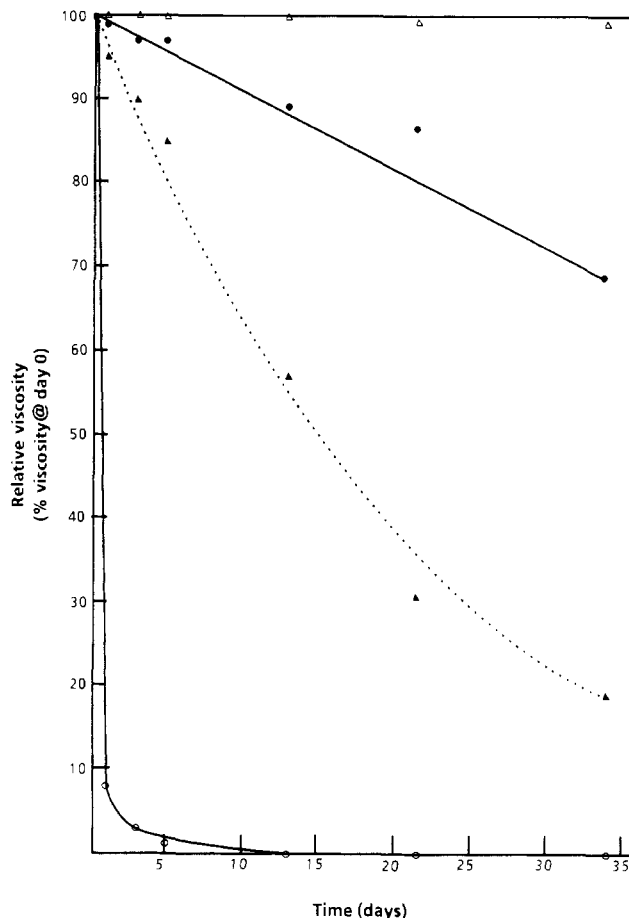


Figure 4. Photooxidative degradation of guayule rubber solutions. Exposure to light (open circle); exposure to light with 10 mM BHT and 10 mM Dabco (closed triangle); dark (closed circle); dark with 10 mM BHT and 10 mM Dabco (open triangle).

to further increase the rubber yield and improve agronomic performance of the crop.

The use of the viscometric method has certain advantages over those of other methods currently in use. These include the following: (1) It is a simple and inexpensive method (shaker platform, viscometers and controlled temperature bath, Erlenmeyer flasks, and cyclohexane). (2) It is sensitive and specific for rubber. (3) Only a small sample is required. (4) The process can be easily scaled-up to run many samples. Some of the disadvantages include the following: (1) The sample must be finely ground. (2) The viscosity of rubber solutions is very sensitive to molecular weight of the rubber.

The grinding of the plant material prior to solvent extraction is the rate-limiting step, but the same is true of all other methods of analysis and is related to the cellular localization of the rubber (Polhamus, 1962). Unlike *Hevea* rubber, where molecular weight and polydispersity are influenced by clone type, guayule rubber has been found to exhibit a fairly uniform molecular weight and was similar between four different geographical regions (Eagle, 1981). Therefore, to the extent that the assumption that molecular weight and variety remain relatively constant is valid, the viscometric method will give a measure of the yield. Also the degree of gel, which could complicate a viscometric analysis, is low in guayule at 1–3% (Eagle, 1981). Factors that can affect molecular weight of guayule rubber, such as oxidative chain scission (Keller and Stephens, 1982), as influenced by heat, light, and oxidation initiators (Cotton, 1937), require care to be taken in the harvesting, storage, and processing of the samples.

Changes in the molecular weight of guayule rubber can be minimized by avoiding prolonged postharvest storage without refrigeration, avoiding light and use of anti-oxidants. Of course, all steps in the determination of rubber content must be standardized, including the age and type of material to be harvested.

Another feature of the viscometric method is the omission of an acetone extraction prior to dissolution of rubber in cyclohexane. Acetone extraction was to remove resins and other substances which affect the rubber determinations in methods such as gravimetric and IR techniques. Only the presence of dissolved rubber affected the viscosity.

Acetone solubles, either redissolved alone in cyclohexane or together with rubber, did not immediately affect the viscosity of the solution. They did, however, accelerate the time dependent degradation of rubber solutions, as measured by a loss of viscosity. Keller and Stephens (1982) state that the unsaturated fatty acids in guayule resin are primarily responsible for the accelerated degradation of rubber by oxidizing to form peroxide compounds which function as oxidation initiators. Acetone extraction of plant material did not prevent the degradation. Conversely it also acted to accelerate the loss of viscosity. Therefore, its inclusion in the extraction of rubber was further unwarranted.

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Relationship between Surface Functional Properties and Flexibility of Proteins Detected by the Protease Susceptibility

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The relationships between the surface properties and the flexibility of proteins were investigated. An attempt to detect the flexibility of protein structure was made by the protease digestion method. Ovalbumin and lysozyme were not susceptible to protease, suggesting rigid or folded molecules, while κ -casein, β -lactoglobulin, and bovine serum albumin were susceptible to protease, suggesting flexible molecules. The digestion velocity of proteins by α -chymotrypsin and trypsin was closely correlated. Good correlations were observed between the foaming power and emulsifying activity and the digestion velocity of proteins. These results suggest that the flexibility of protein structure detected by protease digestion may be an important structural factor governing the foam formation and emulsification.

INTRODUCTION

A number of studies have been done on the relationship between protein structural and functional properties such as emulsification and foam formation (Keshavarz and Nakai, 1979; Kato and Nakai, 1980; Nakai, 1983; Townsend and Nakai, 1983; Shimizu et al., 1983). Summarizing the information obtained from these studies, the most im-

portant structural factor of functional properties seems to be the protein hydrophobicity. It is reasonable to assume that the surface hydrophobicity of proteins plays a governing role triggering emulsification and foaming (Kato and Nakai, 1980; Nakai, 1983; Kato et al., 1983a). Amphiphilic proteins possessing high surface hydrophobicity are forcefully adsorbed at the interface between oil or air and water and cause a pronounced reduction of interfacial or surface tension that readily facilitates emulsification and foaming (Kato et al., 1983a; Shimizu et al., 1983). However, the functional properties of proteins can not be ac-

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