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Purification of Hypoallergenic Latex from Guayule*

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INTRODUCTION

Guayule (*Parthenium argentatum* Gray, Asteraceae) is a woody desert shrub (Whitworth and Whitehead 1991) which is under commercial development as a domestic source of hypoallergenic natural rubber (Siler and Cornish 1994; Carey et al. 1995; Cornish 1996, 1998; Cornish et al. 1996, 2001; Schloman et al. 1996; Siler et al. 1996; Cornish and Lytle 1999). To expedite commercialization, factors affecting yield and stability of latex, in the harvested shrub prior to processing, must be understood in order to optimize and establish post-harvest storage conditions to maximize high quality latex yield (Cornish et al. 1999, 2000, 2001). Little is known about the effect of harvest season or post-harvest conditions on latex quality. Although latex quantification (Cornish et al. 1999) and large-scale (up to thousands of kilograms) purification methods are established (Cornish 1996), the investigation of parameters affecting latex quality require the development of methods suitable for the purification of latex from relatively small amounts of plant material. In this paper, we discuss latex allergies and describe latex purification methods for small (0.01–1.6 kg) and medium (1.6–30 kg) samples.

LATEX ALLERGY

Natural rubber is used in over 40,000 different products, including more than 400 medical applications. High-value dipped and extruded products are manufactured directly from *Hevea* latex. Latex products had been safely used for many decades with only infrequent contact reactions (Type IV) occurring, generally in response to chemical additives, such as the thiurams, carbamates, and mercaptobenzothiazoles.

However, in the 1980s, Type I IgE-mediated allergy appeared (Slater 1989). This life-threatening allergy is triggered by *Hevea* latex proteins, and a hypersensitive individual must take care to avoid contact with all products made with *Hevea* latex. Once sensitized, very little protein is required to induce an allergic response. Allergic reactions include local and systemic urticaria, rhinitis, conjunctivitis, edema, bronchospasm, tachycardia, anaphylaxis, and death (Morales 1989; Slater 1989; Tomazic et al. 1992). The surge of Type I latex allergy coincided with sudden world-wide increased demand for latex gloves in response to the institution of universal precautions to prevent the spread of diseases, such as AIDS and Hepatitis B. New and inexperienced glove manufacturers entered the market, and reduced or eliminated the leaching step normally used to wash the latex products (Bodycoat 1993; Russell-Fell 1993). The washing process removes soluble latex components, as well as chemical additives, and these new underwashed products contained high levels of *Hevea* latex proteins. The use of high protein latex products, especially single-use latex gloves, as well as their extensive deployment throughout society, led to widespread development of Type I latex allergy. The problem was compounded by requirements for health-care workers to frequently change gloves. Proteins in gloves can bind to glove powder. When gloves are removed, the powder becomes a source of air-borne, respirable latex allergens which then expose lung membranes (Tarlo et al. 1994; Tomazic et al. 1994). Also, recognizable latex antigens persist in the respirable fraction of tire dust (Miguel et al. 1996, 1999). Most, but not all, severe allergic reactions occur from direct patient contact with latex gloves and other devices during medical and dental procedures. The first incidents of latex allergy in the United States were reported in 1988, but by 1994 at least 17,000,000 adults in the general US population are affected by Type I latex allergy, 10% to 40% of health care workers, and up to 60% of multiple surgery cases such as spina bifida children (Alenius at al. 1993; Hamann 1993). The actual incidence may be considerably higher than this (Reinheimer and Ownby 1995). Many other countries around the world have serious problems with latex allergy.

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LATEX ALLERGY CIRCUMVENTION

Whereas it is possible to produce low protein latex products that should moderate the incidence of new allergy cases, even trace amounts of allergen still can induce serious systemic reactions in a hypersensitive person. Thus, production of latex products safe for use by allergic individuals requires the elimination of *all* latex allergens. Even if this could be accomplished with *Hevea* latex it would be extremely difficult to prove. Also, complete removal of the rubber particle-bound proteins would necessitate drastic treatment, e.g. with proteases and/or detergents and would adversely affect the performance characteristics and quality of the resulting latex products. We have shown that enzyme-treatment does not lower overall protein content in the whole latex (Cornish et al. 2001). It does appear to solubilize a substantial portion of the rubber-particle bound protein which can then be washed away from the now low protein washed rubber particles to generate a low protein, purified latex (Table 1). However, this treatment may be prohibitively expensive and certainly alters the properties of the latex. For example, purified, enzyme-treated latex very readily coagulates, probably because of damage to the rubber particle membrane (Cornish et al. 2001).

Some manufacturers have introduced *Hevea* latex gloves into the market with lowered protein levels, but tests demonstrated that many contain substantial amounts of proteins that bind IgE antibodies from sera of *Hevea*-hypersensitive patients (Yunginger et al. 1994). Such gloves would be unlikely to induce new allergies but are *not* safe for use by people who are already *Hevea*-hypersensitive. However, since different rubber-producing species contain proteins distinct from those of *Hevea*, it seemed possible that other rubber sources may provide a source of hypoallergenic rubber (Siler and Cornish 1992). The need for an alternative, nonallergenic source of natural rubber created an excellent opportunity for the development of a new, natural rubber crop, and led to the discovery and development of hypoallergenic guayule latex.

GUAYULE LATEX

Latex extraction from guayule shrub is entirely dependent upon the presence of discrete rubber particles suspended in the aqueous cytoplasm of the parenchyma cells. The dry shrub has no extractable latex because the rubber particles are no longer suspended. This dried rubber can be extracted with appropriate organic solvents (Whitworth and Whitehead 1991), but the rubber cannot be used to manufacture high-value, hypoallergenic latex products, which require the rubber to be in latex form (an aqueous suspension of rubber particles). Investigation of the effects of different treatments and conditions, during cultivation and post-

Table 1. Protein content of latex from guayule and *Hevea brasiliensis*, determined using a modified micro-BCA assay (method described in Siler and Cornish 1995).

	Protein content of guayule latex (μg/g dw)	
Treatment	Whole latex	Purified rubber particles
Guayule Low ammonia (2%)	N/A (1)	0.10 ± 0.10 (20)
Hevea Buffered (non-ammoniated)	4.02 ± 0.26 (2)	0.61 (1)
Hevea High ammonia (6%)	4.33 (1)	0.20 ± 0.09 (3)
Hevea Low ammonia (2%)	3.21 (1)	0.13 ± 0.06 (4)
Hevea Enzymatically deproteinized	3.29 (1)	0.11 ± 0.04 (5)

The protein concentration in each sample was the mean of four protein quantifications (with a SE of a mean of four quantifications of 0.63 for whole latex and of 0.041 μg protein/g dw rubber for washed rubber particles). Where possible the protein in each sample type is shown as the mean of the different samples ± SE. The numbers in parentheses after the protein concentration are the number of different samples that were quantified to generate the means ± SE shown. The guayule latex value is a mean of 20 latex samples prepared throughout a year from bimonthly harvests of different lines. *Hevea* latex samples were obtained from CentroTrade, Virginia Beach, Virginia, although the enzymatically deproteinized material is Allotex from Tillotson Rubber Company.

harvest, on latex content, and of the properties of guayule latex, requires the ability to extract, quantify, and purify the latex from guayule. The remainder of this paper describes such methods.

Latex Extraction

Small Samples. Filtered homogenates were prepared from guayule branch samples using a minor modification of the Waring blender method previously described (Cornish et al. 1999). The samples were ground for 2 min in 1:2 w:v shrub:extraction buffer (AAO: 0.2% ammonia, as NH_4OH, 0.1% Na_2SO_3, pH 10) using Waring blenders of ± 1 L capacity, and then filtered through a 1 mm steel mesh, sometimes using a small hand-powered press. The plant material (bagasse) retained by the filter was reground in AAO and refiltered. The two filtrates were pooled. Homogenates were adjusted to pH 10 with NH_4OH and then were stored in sealed glass bottles at 4°C until quantification of their latex content (Cornish et al. 1999). Using this method, grinding shrub samples shipped overnight from Phoenix, Arizona, we have measured latex contents ranging from 39 to 85 mg dry latex/g dry weight (Cornish et al. 1999, 2000).

Medium Samples. At the US Water Conservation Laboratory, Phoenix, Arizona, whole plants (1.6 to 30 kg) were chipped. The chipped shrub was collected into a water-based solution (AAO) consisting of 0.1% Na_2SO_3 as an antioxidant adjusted to pH 11 with NH_4OH. The plants were ground in a hammer mill-type chipper at a 1:1 w:v ratio. Harvested and chipped guayule branches were filtered through a 0.25 inch screen, bagged and shipped to Albany, California (T. Coffelt and F. Nakayama, pers. commun.).

Chipped shrub was then homogenized in one-gallon Waring blenders in 1:1.2 w:v wet chipped shrub:AAO. Homogenates were adjusted to pH 10 with NH_4OH and then were stored in sealed glass bottles at 4°C until quantification of their latex content (Cornish et al. 1999). The homogenate was filtered through a 1 mm steel mesh filter using a home-made hydraulic press. The bagasse retained by the filter was reground in 1:0.8 w:v wet bagasse:AAO and refiltered. The filtrates from the two grinds were pooled.

Chipping and processing shrub without leaves gave a higher yield per weight of ground shrub (Fig. 1), as would be expected because the leaves do not contain rubber. Also, it should be noted that latex levels, in shrub prepared as described above, are about 10% of the amount in shrub directly ground in Waring blenders without chipping before shipping (see section on Small Samples, above).

Latex Quantification

Latex content of all homogenates and purified latex samples were quantified using the 1 ml quantification method previously described (Cornish et al. 1999), where 3 × 1 ml aliquots of homogenate are centrifuged to float the latex, which is then coagulated with glacial acetic acid, harvested, rinsed, dried, and weighed.

Latex Purification

Small Samples. The latex fraction was purified from the homogenates, after quantification of their latex contents. The homogenates were adjusted to 15% glycerol to enhance separation and reduce latex coagulation. The homogenate-glycerol mixture was then centrifuged six times, using 250 ml containers or SS-34 tubes in a bucket rotor, to obtain the latex fraction as an uncoagulated rubber particle suspension. The exact spin speeds and times are dependent upon the stability of the latex in the particular homogenate. However, the first spin is the slowest and shortest (e.g. 1750 × g for 5 min), with spin speeds and times increasing with subsequent spins to the sixth spin (e.g. 9400 × g for 45 min). After each centrifugation, the latex layer that floated to the tops of the tubes was scooped off, with a spoon-shaped spatula, into 5 ml of extraction buffer. The non-latex fine solids component of the homogenate precipitated into the bottom of the centrifuge containers. No more latex floated to the homogenate surface after the sixth spin. The volume of latex was measured and 3 × 1 ml aliquots were quantified to determine the latex concentration (Cornish et al. 1999). Total latex content of each homogenate was then calculated.

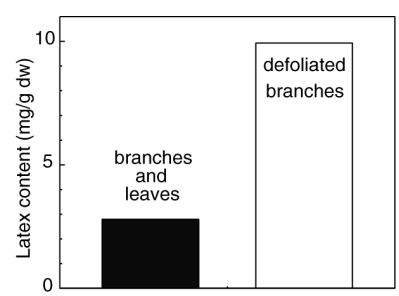

Fig. 1. Defoliation of guayule shrub led to a greater latex yield per g of shrub than shrub processed with leaves still attached.

Each latex sample was further purified by a series of wash/flotation steps using the creaming agent ammonium alginate (A/RN, supplied by

Monsanto, San Diego, California). The latex was diluted 1:5 v:v with creaming agent buffer (AAO with 0.1% ammonium alginate, pH 10) and placed into solid phase reaction vessels without frits (50 to 250 ml, Lab Glass, Vineland, New Jersey) or into separatory funnels. After the latex had floated into a surface layer, the subnatant was drained from the flask and the latex was resuspended in fresh creaming agent buffer. This procedure was repeated 5–10 times, two washes beyond when the subnatant was free of color and of detectable protein (see below for full description). All washes after the first two used creaming agent buffer containing 0.05% ammonium alginate instead of 0.1%. The concentrated latex from the final wash was overlaid with N_2 and stored at 4°C until analyzed for quality parameters. Subsamples were overlaid with N_2 and stored at –80°C until molecular weight analysis.

When we attempted to cream latex directly from homogenate using ammonium alginate buffer, we were unsuccessful because of the high non-latex solid content suspended in the unclarified homogenate. However, we were able to reduce the suspended solids to an acceptable level by centrifuging the homogenate at 1000 × g for 5 min. The small amount of latex that floated to the surface was gently stirred back into the homogenate which was then decanted into a separating vessel and mixed with alginate buffer. Latex then was successfully purified with a series of washes (see below for full description).

Medium Samples. Antifoam A (50 µl/L) was added to the homogenate to reduce foaming and the filtered homogenates then were clarified to reduce the non-rubber fine solids component by passage twice through a basket centrifuge (Marstech Model CF 35 M Clarification System, with Microseparator Model TSK 30 M Centrifugal Clarifier, Bazell Technologies Corporation, Concord, California). It was necessary to reduce the quantity of fine solids (which cannot be filtered away without also removing the latex rubber particles) because they would otherwise rapidly block the latex separator described in the next purification step. The first pass through the basket centrifuge removed approximately 50% of the fine solids component and a second pass removed another 10%. Additional passes did not remove any more material. However, the basket centrifuge did not remove any of the latex from the homogenate during clarification (Fig. 2) irrespective of the guayule line used, or the initial latex concentration in the homogenate. The volume of clarified homogenate was measured, and 3 × 1 ml aliquots were quantified to determine the latex concentration. Total latex content of each homogenate was then calculated.

The clarified homogenate was adjusted to 10% glycerol (to enhance separation and reduce latex coagulation) and 0.01% SDS (to reduce coagulation) and separated into light and heavy phases using an Elecrem Separator (Model 1, Vanves, France) run at full speed. This centrifuge has a disc stack with upward rising channels. Guayule rubber particles have a specific gravity of 0.94–0.96 and are concentrated in the light phase during the separation. We tested different concentrations of latex and glycerol using latex from *Hevea brasiliensis* (Guthrie Latex, Tucson, Arizona) because of its ready availability, its similar specific gravity, and we had previously shown that it behaves very similarly to guayule latex in our different purification procedures (data not shown). Results suggest that lower concentrations of latex may require higher glycerol concentrations to maximize latex separation in the Elecrem Separator (Fig. 3).

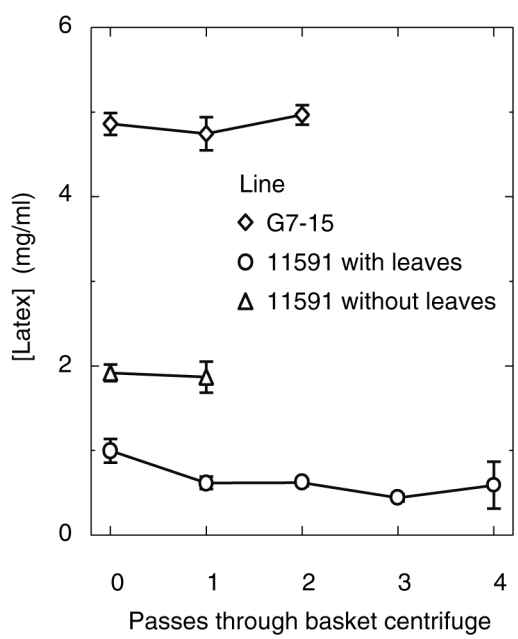

Fig. 2. Filtered homogenates made from three lines of *Parthenium argentatum* were clarified in a Marstech basket centrifuge, with 2–4 passes, for line G7-15, and line 11591 with and without leaves. Latex quantifications are the mean of 3 ± SE. Clarification did not affect latex concentration.

The creaming screw must be carefully adjusted, by trial and error, to optimize the separation of latex from guayule homogenate. The latex fraction becomes enriched in the light phase of the separation. However, the separator is actually a cream separator designed to separate cream from cow's whole milk in which the cream is at a ≥10-fold higher concentration than the latex concentration in guayule homogenate. Thus, even when optimized to maximize the separation, the Elecrem Separator is unable to sepa-

rate all of the latex from the homogenate into the light phase, and substantial quantities remain in the heavy phase. The heavy phase from the separation repeatedly was passed back through the separator, and we determined that seven passes were sufficient to remove almost all of the "extractable" latex (Fig. 4). The light phases were then pooled. It should be noted that although the equipment is suitable for latex purification, it cannot be effectively used for latex quantification because some latex remains in the heavy phase.

Also, defoliation is definitely preferable prior to processing guayule shrub on a laboratory scale because the equipment employed is not designed to completely separate the latex from the homogenate. Homogenate made from leafy shrub contains a lower latex concentration than defoliated shrub homogenate (see Fig. 1) and so a greater percentage of the total latex is left behind in the heavy phase.

Although the pooled light phase can be purified by dilution and reseparation using the Elecrem Separator, leading to higher concentrations of cleaner latex in the subsequent light phases, latex again remains in the heavy phase and so losses occur at each reseparation. Therefore, on a laboratory scale, we normally stop centrifugal separation after the first light phases from the six passes of the first heavy phase are accomplished. The latex in the pooled light phases then is purified using the creaming agent ammonium alginate, A/RN, which allows complete recovery of the latex at each washing stage. The light phase was mixed with creaming

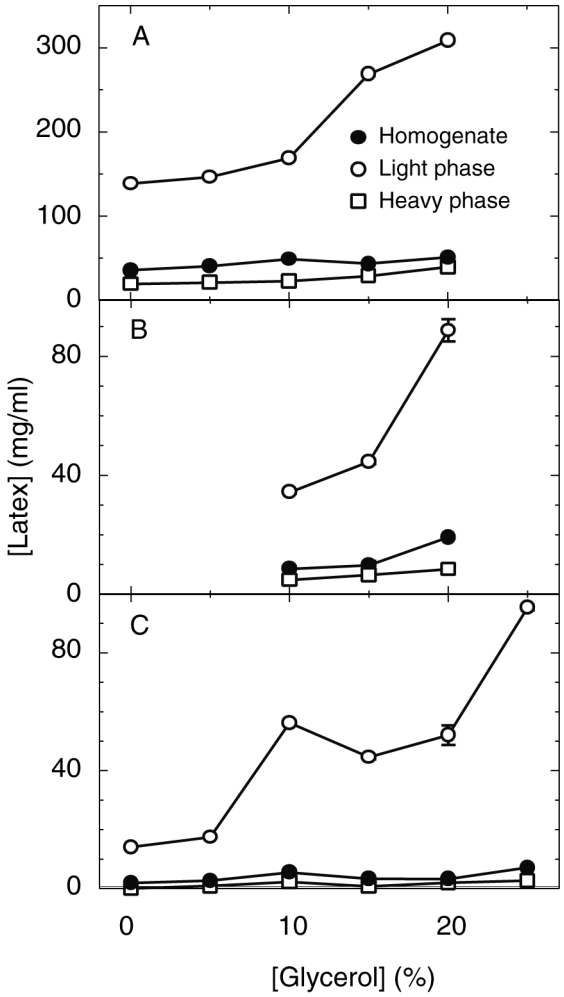

Fig. 3. The effect of glycerol concentration on the concentration of latex separated into the light phase from three different starting concentrations of *Hevea brasiliensis* latex: (A) 50 mg dw/ml; (B) 10 mg dw/ml; and (C) 5 mg dw/ml. The concentration in the homogenate and in the light and heavy phases separated from each homogenate are shown.

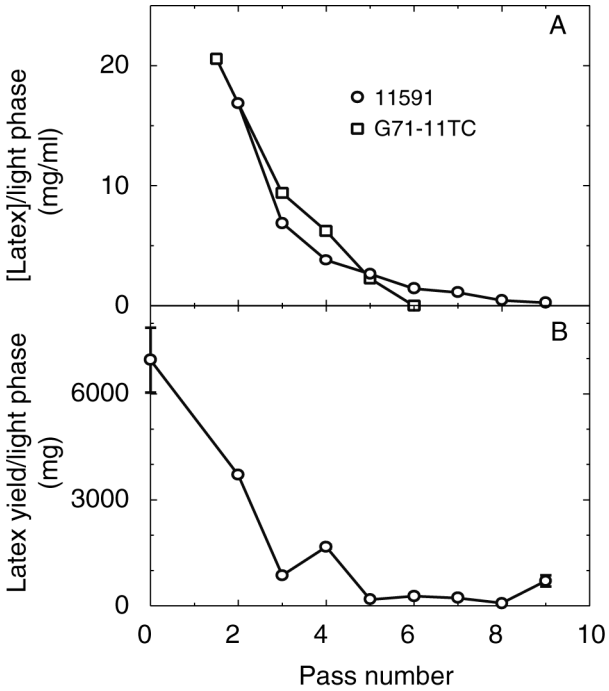

Fig. 4. Guayule latex was extracted from the homogenate, produced in the medium-scale method using a small cream separator. The light phase is enriched with the latex fraction. The heavy phase was passed back through the separator multiple times. (A) The concentration of latex in the light phase separated at each pass in two guayule lines (11591 and G71-11TC) declines with pass number until no more can be harvested. (B) The amount of latex in each light phase of 11591 was calculated by multiplying the volume of light phase after each pass by the latex concentration.

agent buffer (minimizing dilution). Tests proved that the addition of 0.01% SDS had no effect on final protein concentration of the purified latex (data not shown) and had no effect on coagulation. Aspirators up to 15 gallons in capacity were used to cream the latex, depending upon the sample size. The latex fraction floated to the top of the vessel and the subnatant was drained. The latex fraction was rewashed several times with either a 10:1 or 5:1 ratio of creaming agent to latex. The first two washes used 0.1% ammonium alginate, the remainder 0.05% (with the other ingredients remaining constant). Washing continued twice beyond when the subnatant appeared free of detectable pigment or protein (as quantified by the Pierce micro-BCA assay) (Fig. 5). The concentrated latex from the final wash was overlaid with N_2 and stored at 4°C until analyzed for quality parameters. Subsamples were overlaid with N_2 and stored at –80°C until molecular weight analysis.

Concentration of Guayule Latex

Guayule latex from the final creaming step in 0.05% ammonium alginate buffer was allowed to reach a maximal concentration by draining the latex-free buffer from the separatory vessel bottoms until no additional separation into two phases was observed. Samples were taken and determinations were made of final concentration and various other parameters, including particle size distribution, qualitative and quantitative protein components, and molecular weight profile.

Guayule latex achieved a final concentration of 46% solids content, on a dry weight basis. When centrifugation, in the absence of creaming agent, was used to concentrate the latex, it was observed that the latex underwent a phase change at just over 50% solids content. This change was not coagulation: in effect, the latex turned into whipped cream and was no longer fluid. In contrast, latex from the Brazilian rubber tree, *Hevea brasiliensis*, can achieve a higher concentration without losing fluidity and is usually shipped at around a 60% solids content.

Protein Concentration

Protein concentration is an important parameter for guayule latex quality control because commercial efforts are directed towards medical products that can be safely used by patients suffering from life-threatening Type I latex allergy. The allergy arose because a change in processing led to high protein levels in latex products made from *H. brasiliensis* latex. If high protein levels (as are found in guayule homogenates) are allowed to remain in the guayule latex products, they are likely to induce a widespread allergy of their own. This must be avoided at all costs.

We quantify latex proteins using the Pierce micro-BCA assay Siler and Cornish (1995) to ensure that all proteins not associated with the rubber particles are removed from the purified latex (Fig. 5). Guayule latex contains much less protein than *H. brasiliensis* latex (which contains 1%) (Table 1). For example, a recent sample extracted and purified using the medium-scale method described here contained only 0.06%.

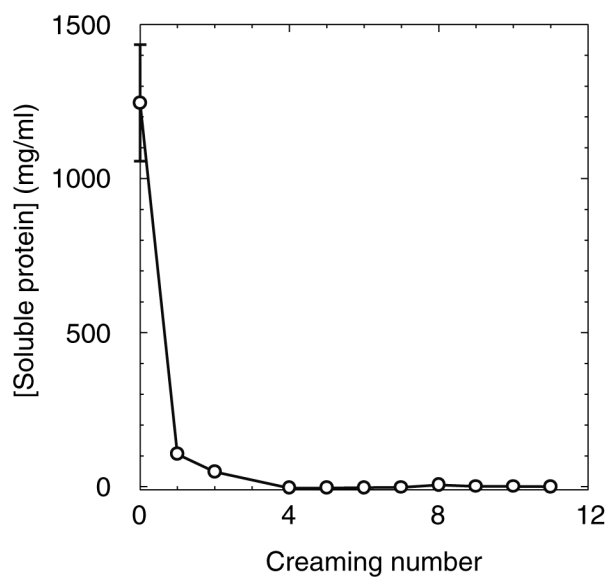

Fig. 5. The crude latex (pooled light phases) was creamed with alginate buffer, which caused the latex to float to the surface. The subnatants were drained from the bottom for the vessel and the latex resuspended in clean alginate buffer. The protein content of subsamples of the subnatants was determined after each wash using the Micro-BCA assay.

CONCLUSIONS

It is possible to purify guayule latex, from a wide range of sample sizes, yielding a consistent product. Homogenate filtration and clarification are essential pretreatments. The purification techniques described allow the effect on latex quality of genetic, environmental and post-harvest factors to be investigated.

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