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REVIEW

Valorisation of Proteins from Rubber Tree

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Abstract

Purpose The objective of this study was to identify the availability, possible applications, and economic potential of proteins that are present in different parts of the rubber tree. Proteins from non-food sources can be used in e.g. animal feed or biochemicals production with no or little competition with food production, rendering them important biobased feedstock. Rubber tree is primarily grown for its latex that is used in rubber production. Indonesia has the largest rubber plantation area that is mostly owned and run by smallholder farmers. Using non-latex fractions from the rubber tree may generate additional income, and increase the economics of rubber plantations in general.

Methods Several biomass streams from the rubber tree and subsequent latex processing were considered. Data were compiled from literature, a case study, and interviews with researchers, smallholder farmers, and managers at rubber processing plant and plantation.

Results Latex waste streams, seeds, and leaves were considered to have the highest potential based on the amount of available proteins, and processes to isolate proteins from these streams were proposed. Isolation of specific functional properties from natural sources requires complex (and expensive) separation processes and therefore only economically feasible when specific use of the protein(s) for high value applications can be identified. Purification of many interesting proteins from latex fractions has already been described. Processing of seeds and leaves may also yield useful proteins for food, other purposes, and also still unknown high value applications.

Conclusions A biorefinery concept can be applied to obtain multiple products from the seeds and leaves, and protein extraction can be performed with available knowledge and technology. Small scale processing can be more beneficial for the farmers, especially if the products are used locally for feed.

Keywords Biorefinery · Protein · Rubber latex · Rubber seeds · Rubber leaves · Indonesia

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Introduction

Proteins are essential components in human diet. Driven by population growth and increasing wealth, global protein consumption for food is estimated to increase from 355 million tonnes/year in 2005 to 748 million tonnes/year in 2050 [1]. Next to food applications, proteins can also be applied for technical applications and biochemical productions. However, the use of proteins for non-food applications should not interfere with food applications. Agricultural residues are important protein sources because they do not compete with protein applications for food. Processing of agricultural residues requires a biorefinery approach that enables the use of all biomass fractions for their optimal economic values [2, 3].

Rubber tree (*Hevea brasiliensis*) is an industrial crop from *Euphorbiaceae* family that grows in tropical climates.

Rubber tree latex is processed into natural rubber in a well-known industrial process. In order to increase the economics of natural rubber production, studies have been performed on optimising latex production that considers genetic improvement, physiology of latex flow, environmental factors, cropping system, disease prevention, and resource conservation [4].

Currently, utilisation of non-latex fractions from rubber tree e.g. wood or rubber seed oil is not a priority, but it potentially becomes important in the future [4–6]. Protein, being one of the main constituents of biomass and the most valuable part in some cases, can potentially increase the overall economics of rubber plantations. The proteins in rubber tree latex are well-identified [7–15], although some are characterised for their allergenic properties [8, 9, 15]. Less attention is given on proteins in the seeds and the leaves of rubber tree [16, 17]. Rubber seed has been identified as a potential protein source, particularly after oil separation for biodiesel production [18, 19].

More than 70 % of world's rubber is produced in Southeast Asia countries, the location of 78 % of rubber harvesting area (Fig. 1a, b). Indonesia is the second largest rubber producer, but owns 3.5 million hectares rubber harvesting area that is the largest in the world [20]. The rubber case in Indonesia represents a major part in world's rubber production that shares similar characteristics to common rubber production practices in Southeast Asia.

Rubber is a predominantly smallholder crop in many major producing countries (Fig. 1c). Definition of smallholding varies between countries, but generally plantations smaller than 40 ha are considered smallholdings [21]. In Indonesia, plantations smaller than 25 ha are considered smallholdings and these plantations constitute of 85 % of the rubber harvesting area. Around 60 % of smallholdings in Indonesia are jungle rubber (agroforests), the rest are plantations owned by farmers who operate the plantation themselves and sometimes employ daily workers for tapping [21–23]. As a commodity, rubber price is prone to fluctuation [22]. Additional income from proteins will benefit most to farmers whose daily income depend on latex tapping, and might come in handy when rubber price is low. Due to the large percentage of smallholder rubber production, a small (local) biorefinery processing may be the best approach to valorise proteins, as this approach gives more room for innovations and presents most benefits to the farmers [24].

The objective of this study was to identify the availability and economic potential of proteins that are present in different parts of the rubber tree. Possible applications in

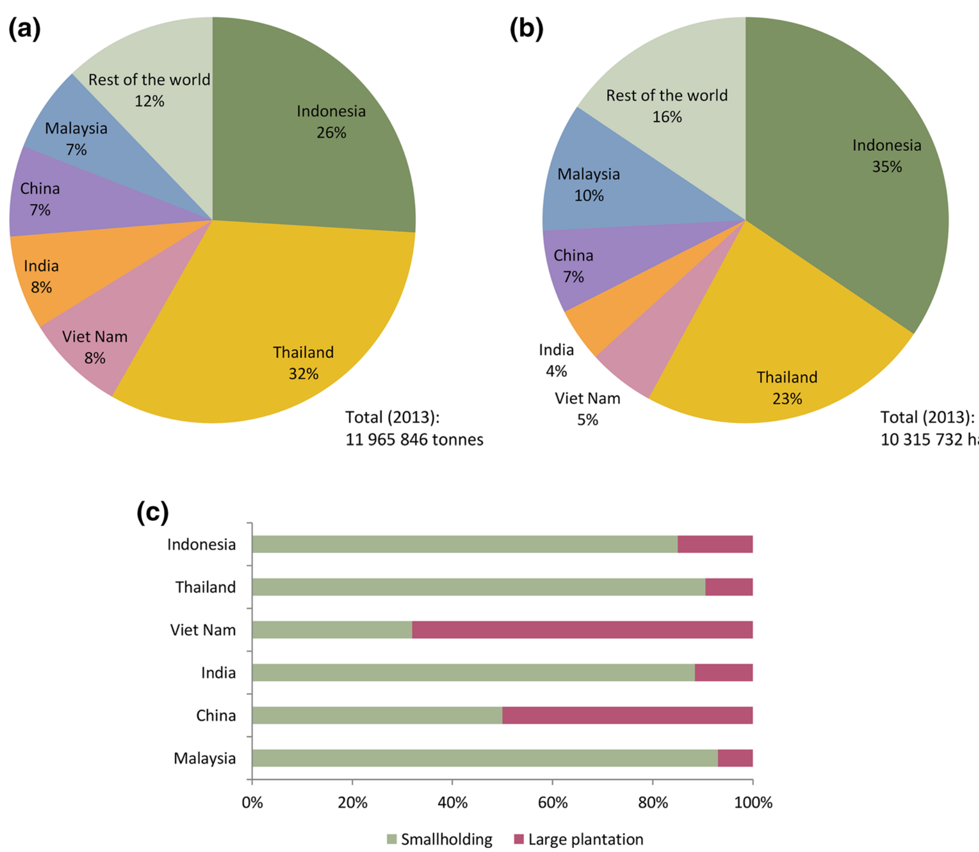


Fig. 1 Overview of global rubber production (**a**), rubber harvesting area (**b**), and types of plantation in major producing countries (**c**) [20, 21]

industrial and rural settings are discussed. For rural setting, local applications in Indonesian rural area are discussed.

Methods

Data on Indonesian rubber production were compiled from literature and interviews with researchers at Rubber Technology Research Centre, West Java; plant and plantation managers at PTPN 8 Cikumpay processing plant and plantation, West Java; and smallholder farmers at Subang (West Java), Palangka Raya and Pulangpisau (Central Kalimantan), and Banjarbaru (South Kalimantan). These locations were selected to represent different types of rubber plantation. As a case study, we also gathered data from a pilot seed refinery program in Palangka Raya, Central Kalimantan.

Latex, crumb rubber, waste water, bark, and leaves samples were collected from PTPN 8 Cikumpay. Protein content of these samples was determined by Kjeldahl [25], using the Gerhardt Kjeldahltherm and Gerhardt Vadopest system.

Identification of Protein Fractions from Rubber Tree

Currently there are two material streams from rubber tree that are considered having (economical) importance, namely latex and wood. A small quantity of seeds with selected breed and quality are used for propagation. There is a growing interest in using the seeds for oil production. Another stream that has considerable amount of proteins, but is often overlooked, are the leaves of the rubber tree. The bark of the tree trunk is also discussed, due to its availability and ease of collection. The overview of these streams is presented in Fig. 2, and each stream is discussed separately as follows.

Latex

The latex of the rubber tree can be processed into a variety of rubber products, and currently is the main commercially applied fraction. Latex tapping usually starts when the tree is 5–7 years old. The maximum latex yield is reached for trees between 15 and 22 years old, after which the yield decreases. When the trees are 25–30 years old, latex yields only reach 50–67 % of their previous maximum [5, 33]. Latex tapping is performed by making an incision in the bark of the rubber tree to expose latex vessels in the bark to start the leaking of latex. The latex is collected in a cup that is attached to the tree. After 6–8 h, the latex in the collection cup is transferred into a larger container and brought to the processing plant. Ammonia is often added to prevent pre-coagulation of the latex (interview with farmers).

Latex Yield and Properties

The latex yield of the rubber tree is influenced by tree clone, tree age, seasons, climate, and soil conditions. Yields range from 24–32 g-fresh latex per tree/tapping in Nigeria [34] to 75–120 g-fresh latex per tree/tapping in Thailand [35]. Interviews with farmers and researchers indicate that latex yield in Indonesia varies between 25 and 110 g-fresh latex per tree/tapping, amounting to an annual yield of 4–6 tonnes-fresh latex per hectare for plantations and 3 tonnes-fresh latex per hectare for agroforests. Plantations can give higher yields because they use better clones and apply artificial fertiliser. Also, tree spacing in plantations is optimised for better yields while in agroforests the tree spacing is mostly arbitrary and sometimes too packed, making nutrition absorption not optimal. In agroforests, fertilising is rare to none, and sometimes old trees are still used as long as they still produce latex, albeit small.

Fresh latex can be separated by ultracentrifugation at 44,000–59,000*g*, and the resulting fractions are presented in a simplified form in Fig. 3. Fresh latex contains 1–2 % of protein that is distributed between rubber phase, serum, and bottom fraction; no protein is present in the phase containing Frey-Wyssling particles [15, 36].

The proteins in the rubber phase are mostly insoluble. They are attached to the rubber particles and stabilise their surface. Two proteins from the rubber phase with 14.6 and 23 kDa molecular mass are identified as allergenic proteins [9, 15].

Serum is the aqueous phase that makes up 40–50 % of the latex volume and contains a variety of proteins at different concentrations [15]. The most abundant protein is an acidic protein with an isoelectric point of 4.7 and a molecular weight of 40 kDa. This protein is important in preventing latex coagulation [14]. Free amino acids are present in the serum at total concentration of 16 mmol/l-latex, mostly consisting of alanine (26 %), and aspartic acid, glutamic acid, and glutamine (18–19 % each) [37].

The bottom fraction is viscous and has a yellowish colour; it contains 9 % rubber particles and 2 % protein [38]. The majority (50–70 %) of proteins in this fraction consists of hevein [12, 15], a 5 kDa protein that contains 18 % cysteine and is soluble in the presence of neutral salts [7, 12, 13]. The allergenic and antifungal properties of hevein are well identified [7, 8]. A previous study showed that most of the hevein from the latex is conserved after isolation from rubber factory effluent, obtaining a concentration of 0.7 g/l and suggesting that the effluent can be

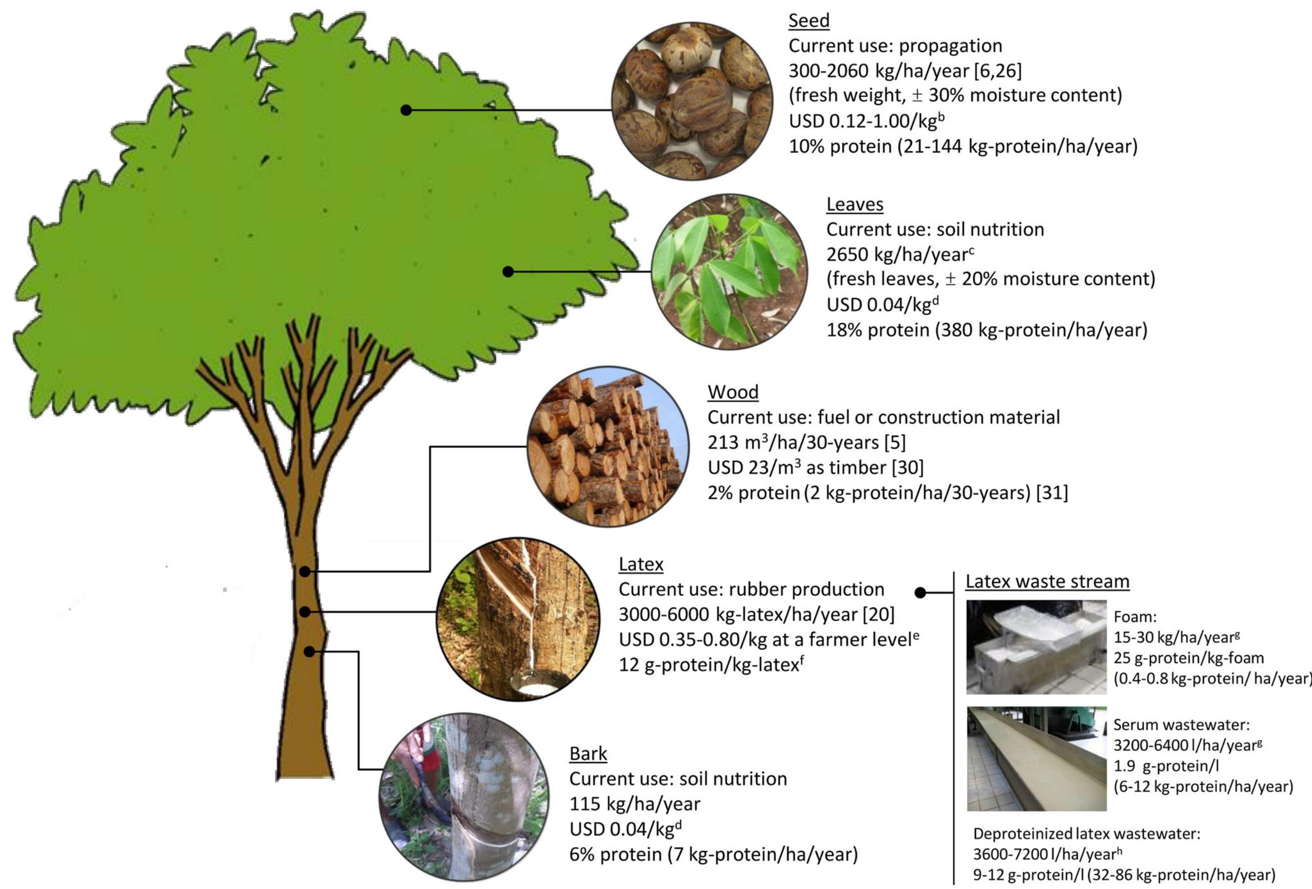
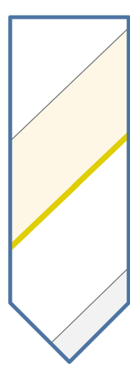


Fig. 2 Overview of mass streams from rubber tree, current use, and potential for protein[a]. [a]Data from interviews and own measurements, unless otherwise specified. [b]The price of USD 0.12/kg was obtained from interviewing the manager of pilot seed refinery. The price of USD 0.35–1.00/kg was estimated for rubber seed oil production in Malaysia [27], but might include the seeds for propagation purpose that comprise small quantities of selected breed and quality. [c]Assuming a leaf area index of 5, leaf mass area of 88 g/m^2, 80 % dry weight [28], and 60 % collection. [d]Price for organic fertiliser [29]. [e]Price of fresh latex based on interviews with farmers in West Java and Central Kalimantan. [f]Fresh latex with 30–35 % dry rubber content [32]. [g]Assuming all latex is processed into ribbed smoked sheet (RSS). [h]Assuming all latex is processes into deproteinised latex. Protein contents are in %-dry weight unless otherwise specified. USD 1 = IDR 13,000

Fig. 3 Fractionation of latex after ultracentrifugation



	Volume [32] (%)	Protein distribution [15,36] (%-w of total protein in latex)
Rubber phase	30-45	25-27
Frey-Wyssling particles	2-3	-
Serum (C-serum)	40-50	43-48
Bottom fraction	10-20	25-32

a source of proteins with antifungal properties [39]. Other proteins that are identified in the bottom fraction are 1,3-β-glucanase and hevamine; the latter shows high chitinase/lysozyme activity [11, 12].

A 43 kDa protein that is partially homologue to patatin, the main storage protein in potato, was also found in the bottom fraction and serum [10, 12]. The amount of this protein is 1 %-w of the bottom fraction [10].

Latex Processing

Rubber latex can be processed into various types of rubber products: crumb rubber, ribbed smoked sheet, concentrated latex, deproteinised rubber, air dried sheet, crepe, etc. Each of these products has different specifications and end-products. Most Indonesian smallholder farmers produce coagulated latex (lump), either by acid addition or natural coagulation at the plantation (interview with farmers). The coagulated lump is further processed into crumb rubber (CR) in rubber processing plants. Some of these plants also process liquid latex into ribbed smoked sheet (RSS) or concentrated latex. Simplified process of RSS and CR production is presented in Fig. 4. More than 80 % of Indonesian rubber products are in the form of CR because, unlike RSS processing, the lump is easier to produce and store by the farmers themselves [23].

Protein in latex is attached to rubber particles in the end-products and may cause allergenic reactions [15]. Therefore reduction of protein in the latex is beneficial, especially for latex used for products that come into contact with human skin e.g. gloves or mattress. Several processes have been designed and applied to produce deproteinised rubber (Table 1). The most common is centrifugation and washing (Fig. 5); the process can be combined with urea/surfactant or protease solubilisation [42, 43].

Based on current latex processing (Figs. 4, 5; Table 1), three potential streams were considered for protein extraction (Fig. 2): foam, serum wastewater, and the waste stream from deproteinised latex production. The other streams from current processes, e.g. RSS or CR wastewater (Fig. 4), were not of interest because their protein contents are too low.

- *Serum wastewater* Serum wastewater is obtained during slab formation in RSS production (Fig. 4). When rubber slabs are collected, serum wastewater is left in the vessels and then discarded into wastewater treatment, therefore it can be collected easily. When collected directly from the vessel, this wastewater contains 0.5 g-N/l. Only 50 % of the total nitrogen in the serum are proteins and amino acids [48], the rest is ammonia that is added to prevent pre-coagulation during collection. Based on this estimate, 1.9 g-protein/l is present in serum wastewater, the highest in all latex wastewater streams from RSS/CR production.
- *Foam* Foam is formed during the mixing of latex with acid to form slab in RSS production (Fig. 4). It is unwanted in the process because foam makes air columns in the slab, therefore the foam is removed from the mixing vessels, collected, and coagulated. The foam that is already coagulated has similar properties with dry latex and is usually used in CR line without any pre-treatment. Uncoagulated foam contains 5 %-dw protein. However, only less than 1 kg of foam with 49 % water content can be collected per 100 kg processed latex.
- *Waste streams from deproteinised rubber production* A combination of multiple centrifugation and washing steps is the most applied process to produce deproteinised rubber. The combined liquid streams from this process contain 9–12 g-protein/l (Fig. 5; [49]).

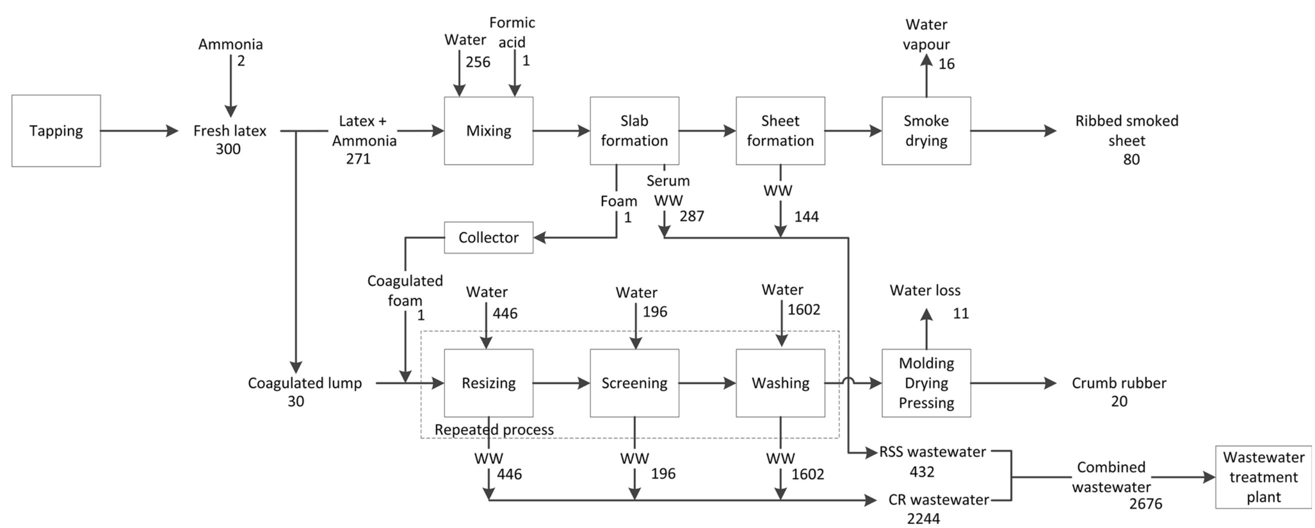


Fig. 4 Simplified process for producing crumb rubber (CR) and ribbed smoked sheet (RSS). *Numbers* indicate mass streams in tonne for producing 80 tonnes RSS and 20 tonnes CR. Numbers were calculated based on data from interview with plant manager at PTPN 8 Cikumpay, for the case of latex with 30 % dry rubber content. Water use was calculated from Leong et al. [40]

Table 1 Comparison of processes for deproteinised rubber production

Method	Current stage of application	Results	References
Multiple centrifugation steps and washing	Industrial	Rubber particles are concentrated. The separated proteins are present as native proteins in the liquid stream. The loss of rubber particles is ±10 % for every centrifugation step. Only 50–75 % protein is separated. Protein stream also contains rubber particles	[41]
Solubilisation with urea and/or surfactant	Industrial	Up to 100 % separation of protein is possible. (Denatured) proteins are present in the liquid stream, including water-insoluble proteins	[42]
Solubilisation and hydrolysis with protease	Industrial	Up to 98 % separation of protein; allergenicity can be totally removed. Hydrolysed proteins are present in the liquid stream	[43, 44]
Coagulation and precipitation	Industrial	Up to 98 % separation of protein. Proteins are precipitated together with Frey-Wyssling particles and components from bottom fraction	[45, 46]
Ion exchange	Patented process	Up to 98 % separation of protein is possible. Proteins are attached to resin and can be recovered by washing. Possible coagulation of rubber particles on resin	[47]

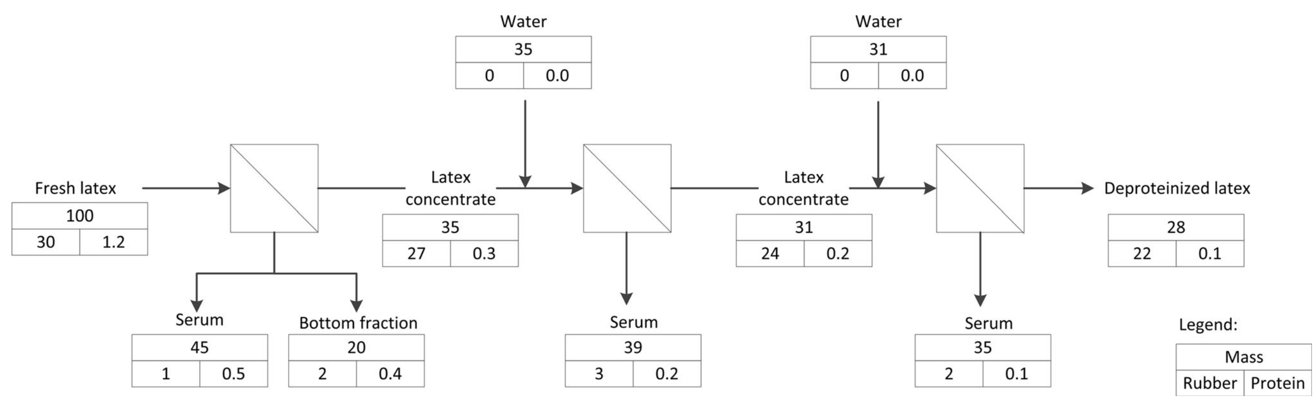


Fig. 5 Production of deproteinised latex by centrifugation and washing. *Numbers* indicate mass streams in tonne for processing 100 tonnes of latex (see *Legend*). Material balance calculations based on Yeang et al. [15] and Perrella and Gaspari [41]

Rubber Wood

In rubber plantations, regular replacement of old and unproductive trees is necessary to maintain latex production. The wood from the old trees is currently used as additional fuel, particularly in RSS production. However, there is a growing interest in using rubber wood as timber, particleboard, or fibreboard. Rubber wood has excellent physical properties, can be processed into various products, and is considered an eco-friendly source of timber because its production does not need a new land opening [22, 50]. At the end of a 30 years period, 213 m^3/ha rubber wood can be produced [5]. Rubber wood price at a farmer level is IDR 300,000 (USD 23) per cubic metre as logs [30], while the international market price is around USD 280/m^3 for hardwood logs and USD 500/m^3 for fibreboard [51, 52].

Rubber wood is a typical lignocellulosic material with protein content of only 2 %-dw [31]. These two properties present several challenges in protein extraction that render it not feasible. Furthermore, the recent use of rubber wood already presents a potential profit [5].

Seed

The flowering of rubber trees occurs 1 month after defoliation and coincides with the peak of solar radiation intensity. This is followed by fruit formation; each rubber fruit contains 3–4 seeds. After 4–5 months, the fruits will dehiscence and the seeds inside will fall to the ground and are available for collection [34, 53]. The annual yield of rubber seeds can vary between 300 and 2060 kg/ha [6, 26]. GT1, a clone of Indonesian origin and one of the most widely used varieties, produces 397,000 seeds/ha per year [54], corresponding to roughly 1900 kg of fresh material. In Indonesia the seeding season varies between regions but generally occurs between July and January. The seeding season coincides with the rainy season, therefore moisture content of the rubber seeds is relatively high (Table 2).

Table 2 Composition of rubber seed [19, 26]

Parameter	Unit	Range	Average
Whole seed			
Weight (fresh)	g	3.1–6.3	4.8
Hull fraction	%-w	32–53	40
Kernel fraction	%-w	47–64	60
Kernel			
Moisture (fresh)	%-w	28–50	36
Oil content	%-dw	40–50	49
Protein content	%-dw	17–20	18
Hull			
Moisture (fresh)	%-w		4
Oil content	%-dw		1
Protein content	%-dw		3
Crude fibre	%-dw		69

High moisture content makes the seeds prone to fungal contamination and deterioration, both in the plantation and during storage.

In most plantations, the seeds of rubber trees are currently left on the ground to become humus. A small amount of good quality seeds can be used for propagation. The oil, being one of the components that is present in the highest amount, is an interesting product that is currently getting more attention mainly as an alternative feedstock for biodiesel production [6, 18]. Valorisation of oil alone, however, may not be economically feasible [18]. Therefore, separation and use of all fractions to get a better value are envisaged. Pressing the kernel for the oil results in press cakes with 20–28 %-dw protein content. Oil pressing followed by protein extraction from the press cake is proposed as the optimal process to obtain both oil and protein from the rubber seed kernel [19]. A proposed biorefinery concept is presented in Fig. 6.

There is still limited information on proteins that are present in the rubber seed. Amino acid analysis of the proteins in the kernel showed high number of aspartic acid, glutamic acid, arginine, valine, and leucine [19], and overall 34 % essential amino acids that suggest the proteins can be used for feed applications. Direct application of the whole seeds or kernels as protein source, however, is not possible due to the presence of some anti-nutritional factors, most notably cyanide. Fresh rubber seed kernels contain the equivalent of 1640 mg-HCN/kg-dw, but the concentration is reduced to 42 mg/kg after 3 months of storage [26]. Application of high temperature, including during screw pressing, can reduce 61–93 % of the initial cyanide content [26, 55].

Rubber seed protein concentrate has a similar amino acid profile as the kernel, and is soluble in water at pH up and above 8.5, with isoelectric point between 4 and 5 [56].

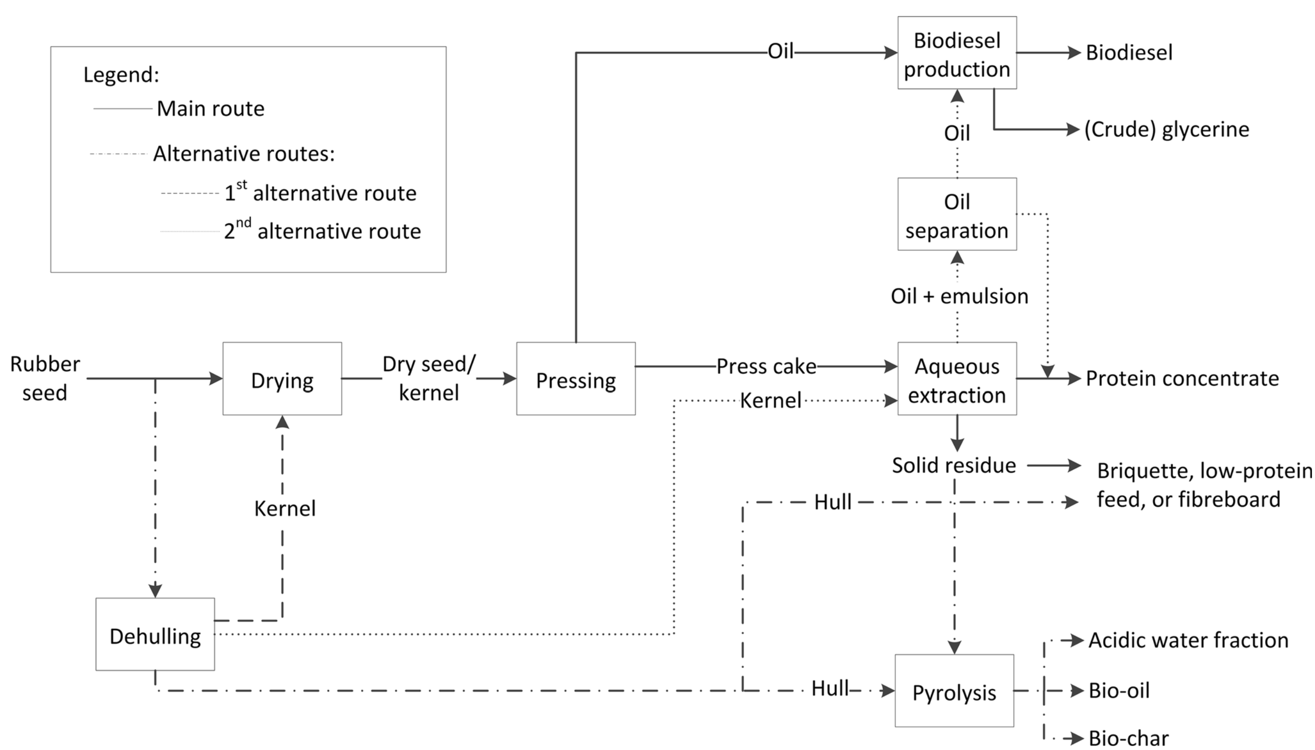


Fig. 6 Proposed concept for rubber seeds biorefinery

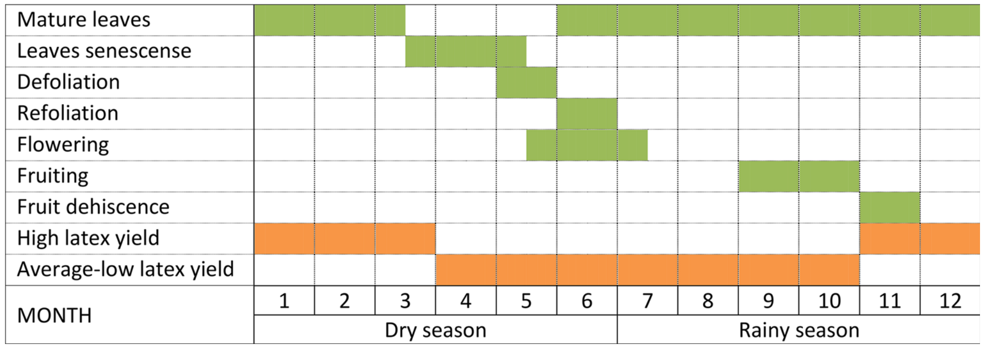


Fig. 7 Development phases of rubber tree [17, 34]. *Numbers* indicate months counted from the beginning of the dry season, which vary between regions, and do not correspond directly to months order in the Gregorian calendar

Leaves

During the dry season, mature rubber leaves enter a senescent phase for 2 months, which ends with one month of partial or complete defoliation. The tree can be leafless for 2–4 weeks, after which refoliation occurs during 1 month (Fig. 7) [17, 57, 58]. The amount of leaves varies between clones, age, and time of the year. In an 8-year old monoculture plantation, the leaf area index is 0.5 m^2/m^2 during the dry season and 5 m^2/m^2 during the rainy season [59]. In a mature plantation, a leaf area index of 7 m^2/m^2 was observed [60].

We measured the crude protein content of fallen fresh leaf as 18 %-dw. Similar values of 14–21 %-dw have also been reported [28]. Protein content changes with leaf age. The total protein content in the mature leaves increases during growth and reaches a peak right before the senescent phase, after which the protein content decreases significantly. Some of these proteins have been identified as antioxidative enzymes [17]. Proteins with molecular weights of 13 and 55 kDa were identified in the leaves [61], the latter being especially abundant. Both proteins may be RuBisCo small units [62]. Rubber leaves have been reported as part of the diets of proboscis monkeys and lesser short-nosed fruit bats [63, 64], and the leaf protein concentrate was used in rabbits diet without adverse effect [65]. Integration of sheep grazing with rubber plantation had been implemented [66], even though there is a concern that rubber leaves (and seeds) might cause metabolic problems due to the presence of anti-nutritional factors. Similar to the seeds, mature rubber leaves contain cyanide equivalent to 1300 mg-HCN/kg-dw [67]. The leaves also contain 7 %-dw tannins out of 11 % total phenols [64].

To harvest rubber leaves for their protein, it is important that leaf harvesting does not result in lower latex yield. Artificial defoliation using herbicide has been applied as a method to control leaf fall disease that is often found in rubber plantation [68]. Based on this finding, leaf harvesting might even present a benefit in plantation management. The optimum harvesting time still needs to be considered for influence on latex yield, the amount of available leaves, and the leaf protein content. In addition, rubber leaves cyanide content is influenced not only by leaf age, but also by latex tapping activities and sunlight exposure; young leaves harvested in the shade or during the night have the highest cyanide content [69]. Based on the development phases of rubber trees (Fig. 7), we propose to harvest the leaves before the mid of dry season; that is before the leaves enter the senescent phase. It is expected that protein content in this period is still high, while latex yield is not severely influenced. Assuming a leaf area index of 5 m^2/m^2, leaf mass area of 88 g/m^2, 80 % dry weight [28], and 60 % collection, 2650 kg fresh leaves/ha can be collected, which is equivalent to 2100 kg leaf-dry biomass or 380 kg crude protein (Fig. 2).

Bark

The bark of rubber trees is obtained during the latex tapping, but is not collected and left on the ground (interview with farmers). We estimated that for every 400 trees tapped (daily average number per worker), 1.5 kg of fresh bark can be collected easily. However, this will only amount to 115 kg of dry bark/ha/year (Fig. 2), which is very low considering it has to be collected and stored year-round. Furthermore, the protein content of the bark (6 %-dw) is too low and its high lignocellulosic content might pose difficulty in protein extraction. Protein recovery is therefore less feasible than from the other streams.

Isolation of Protein-Rich Products

Based on the protein contents and their availability, only latex residual streams, seeds, and leaves were considered interesting, and isolation of proteins from these streams is discussed as follows.

Latex

Three potential streams were considered for protein isolation from latex, namely foam, serum wastewater, and the waste stream from deproteinised latex production (Fig. 2). In general, at least two difficulties arise: dilute streams and attachment to rubber particles. The dilute streams mean that protein recovery from latex should be integrated into the current rubber production process instead of a stand-alone process, as processing outside the current plants will require transportation of large volumes of water. In practice, the most feasible process to obtain value from latex processing waste stream at present are coagulation-precipitation to recover rubber and anaerobic digestion to produce methane [49]. Considering the fungicidal properties of rubber latex proteins, it might be possible to use the wastewater directly as fungicide, e.g. in the nursery for rubber trees between 1 and 3 years old. Further investigation is needed to study the feasibility of this option. A possible drawback could be the remaining rubber particles in the wastewater, which might form a white-sticky layer in the spraying apparatus and on the leaf and soil.

As the proteins are present in dilute streams, the isolated proteins should have specific application and economic value to make the process feasible. According to our current knowledge, the protein with the most prospective application is hevein for antimicrobial or antifungal agents [7, 70]. The other protein with potential application is the 43 kDa patatin-homologue [10, 12], due to its similarity with patatin. Patatin is currently investigated for food application as emulsifier, gelling agent, and foaming agent [71–74], and synthesis of monoacylglycerols [75]. Proteomic and genomic studies [76–78] might reveal other proteins with potential high-value applications.

Once the target protein has been identified, a conceptual process design as illustrated in Fig. 8 is proposed to obtain the protein. A crucial step is separating the proteins from rubber particles, as the presence of rubber in the protein stream reduces its quality and may even attach to the separation equipment and create blockages. The use of additives, e.g. urea or SDS [42], is required to solubilise the proteins that may be attached to rubber particle surfaces, mostly in serum wastewater and foam. After solubilisation, the protein-containing fraction is separated from the rubber-containing stream via precipitation [42, 79]. Acetone was shown to be effective to separate protein from aqueous stream during deproteinised rubber processing [42]. To isolate the proteins and obtain the final product(s), chromatography and/or membrane filtration can be used. By using membrane with molecular weight cut-off of 30 kDa, the total solid of latex wastewater was concentrated from 39 to 154–275 g/l [80]. Ultra- or nano-filtration can also be used to separate hevein, which is relatively small (5 kDa), from the rest of the protein stream. Another alternative is using expanded bed adsorption chromatography, which is also used to isolate native potato proteins from potato juice, followed by ultrafiltration to concentrate the protein fractions and remove anti-nutritional factors [72]. The highest component cost is the purification via chromatography, with estimated processing cost of USD 184/kg-product [81]. Consequently this process is only feasible if the product has a high value application, e.g. pharmaceutical.

Seed

Alkaline extraction followed by isoelectric precipitation is commonly used to get protein from oilseed press cakes (Fig. 9). Alkaline conditions (0.1 M NaOH) can be used to extract protein from rubber seed kernel, press cake, and hexane-extracted meal, and 50–81 % protein from rubber seed kernel can be recovered in the extract [19]. The extracted proteins have 6–11 % degree of hydrolysis [19], indicating that some proteins are not in native forms. The process may also need to be adjusted to remove cyanide that is still present in the press cake. Using high(er) temperature for extraction and drying may aid in removing the cyanide. Higher extraction temperature, however, may result in lower protein purity because more non-protein compounds can also be extracted. The use of high temperature also increases energy consumption. An overall process optimisation is still needed by taking all these factors into account.

From the proposed process (Fig. 9), several products can be obtained. Starting with press cake containing 22–28 %-dw protein content, a protein concentrate with 48–63 %-dw protein can be obtained from this process (unpublished

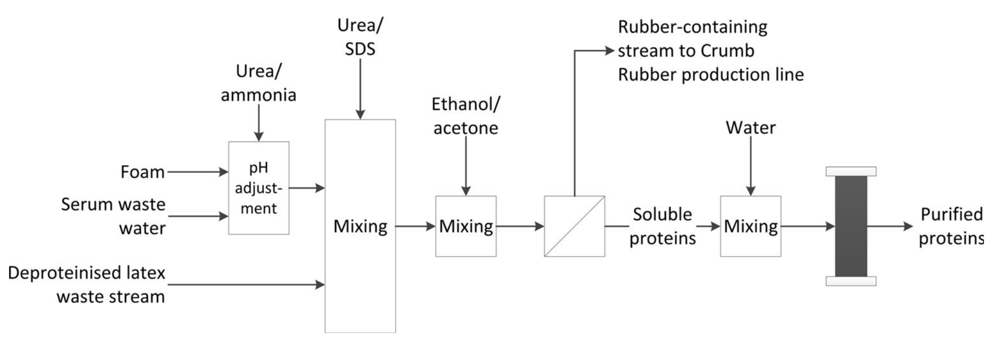


Fig. 8 Conceptual process design to isolate protein from latex processing waste stream

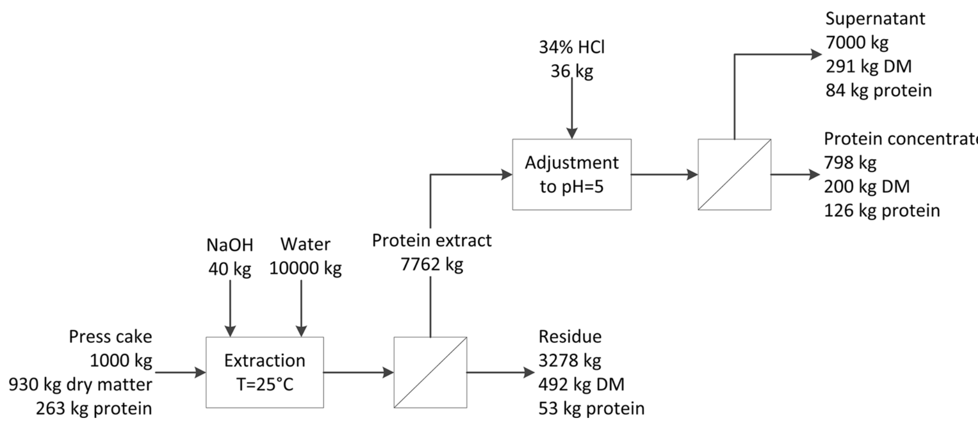


Fig. 9 Proposed process flow diagram to isolate proteins from rubber seed press cake

result). Protein concentrate price could be comparable to the price for soybean meal (44–48 %-dw protein) that is USD 0.32–0.35/kg or for cottonseed meal (41 %-dw protein) that is USD 0.28/kg [82].

Next to protein concentrate, briquettes can be produced by pressing the residue from protein extraction at elevated temperatures [83]. This process is low cost, can be operated by an untrained operator, and is almost without losses in dry weight. The residue can also be burned without oxygen to produce charcoal, however only 25–30 % of the original residue is then converted into product. The market for this product depends on local conditions. The briquette can be used for cooking or as an energy source in rubber production (Fig. 4). Alternatively, the residue can be used as low-protein ruminant feed [3].

The supernatant after precipitation, which still holds roughly 30 % of the press cake dry weight, can be used as liquid fertiliser for application in the rubber plantations. Fertiliser quality can be improved by selecting the appropriate alkaline and acid combination for the extraction and precipitation. In our experiments [19], sodium hydroxide (NaOH) was used as the alkali source because it is a strong alkaline, easy to obtain, and widely used in industries. Other alkali sources that can be used are calcium hydroxide, potassium hydroxide, and ammonia; the latter is already used by farmers to prevent latex coagulation on the field (interview with farmers). Instead of hydrochloric acid, sulphuric or phosphoric acid can be used for precipitation of protein.

Leaves

Isolation of protein from leafy materials can be done via mechanical pressing or alkaline extraction. The former has been extensively studied and implemented, from pilot to commercial plants [84–86]. The simplest mechanical pressing requires chopping and grinding leaf materials, pulping, and pressing to get protein-rich juice and press cake. Based on visual observation, rubber leaves are considered as soft biomass (unlike grass or alfalfa), therefore screw extrusion might not be suitable due to low friction coefficient [87]. However, leaf protein concentrate has been produced from cassava leaves, which are also soft leaves, both using screw extruder and hydraulic press [88, 89].

Protein-rich (press) juice can be processed into leaf protein concentrate via steam coagulation or isoelectric point precipitation, for use as animal feed or other protein applications. To improve protein quality and, consequently, increase the protein value, press juice can be treated with ultrafiltration or other means of purification. Activated carbon adsorption can remove the chlorophyll from the protein rich juice, results in a RuBisCo-rich fraction that can be used in food and beverage [90].

The other method to isolate protein from the leaves is using alkali. High temperature and high alkali amount are required to obtain high extraction yield [91, 92]. The advantage of alkaline extraction over mechanical pressing is the possibility to process dried material as well as fresh leaves. Alternatively, alkaline conditions can also be used to extract protein from the press cake that is left after press juice extrusion.

Ammonia pre-treatment, e.g. ammonia fibre explosion (AFEX), may increase extraction yield and allow the use of milder condition for alkaline extraction [93]. During AFEX, lignocellulosic material is treated with liquid ammonia under pressure followed by a rapid pressure release that breaks the fibres. AFEX pre-treatment followed by alkaline extraction is especially beneficial when leaf extraction for protein is combined with ethanol production [94].

Rubber leaves contain several anti-nutritional factors, particularly cyanide and tannins, and the influence of processing on these compounds should be taken into account. Alkaline conditions may hinder the formation of gaseous hydrogen cyanide that serves as a cyanide removal mechanism [95]. However, as shown in the processing of cassava leaves that also contain cyanide, chopping and drying the leaves before alkaline treatment and two-step drying after alkaline treatment can reduce the amount of cyanide [96]. Tannins and several other toxins and anti-nutritional factors, e.g. phorbol esters, phytate, and glucosinolate, can also be degraded or removed under alkaline conditions [96–98].

Bals and Dale [84] presented several scenarios for both mechanical pressing and alkaline extraction of leaves, in conjunction with the lignocellulosic biorefinery process. They concluded that compared to mechanical pressing, alkaline extraction gives less revenue due to lower protein recovery, but the overall process is less sensitive to changes in process conditions and biorefinery scale. Protein content in the final product is the determining factor in profitability [84], therefore an alkaline extraction process that can achieve up to 95 % protein recovery may be a feasible alternative [92]. On the other hand, protein degradation during storage and processing might reduce its profitability [84].

Combination of mechanical pressing and alkaline extraction to isolate proteins from rubber tree leaves is presented in Fig. 10. With this process, three protein products can be obtained: protein concentrate from press juice (40 %-dw protein), protein concentrate from press cake (52 %-dw protein), and a RuBisCo fraction (70 %-dw protein). For feed applications where protein degradation is not important, the price for protein concentrates could be comparable to the price for soybean meal (USD 0.32–0.35/kg) [82]. The process presented in Fig. 10 was able to maintain the RuBisCo functionality [90]. Based on estimated market price for cosmetic-grade proteins (90 %-dw protein, USD 1.10/kg) [86], the price of USD 0.80/kg for the RuBisCo fraction could be expected.

Discussion

Utilisation of protein fractions from rubber tree, particularly from rubber seeds and leaves, presents opportunities to increase revenue from rubber plantations. Even though previous studies have proposed potential uses of non-latex fractions of the rubber tree [4], biorefinery of these fractions is practically non-existent. To enable the use of protein fraction, a robust technology accompanied by a techno-economic assessment of the proposed process is required. Furthermore, especially for applications in rural areas, social aspect should be taken into consideration as this often becomes the determining factor.

The processes presented in this article (Figs. 6, 9, 10) can be applied either in local (small scale) or in centralised (large scale) biorefinery units. The application of certain equipment or technology is often only feasible at a large scale due to economy of scale. For instance when aiming for a large scale biodiesel production, seed collection from several plantations followed by processing at a centralised site is considered optimum since this approach will allow a continuous production [27]. For leaves processing (Fig. 10), protein refining to RuBisCo may be more beneficial at a large scale aiming for industrial markets.

Despite the benefits of large scale processing, local (plantation or village-based) processing may also present some benefits: processing can be adjusted to the farmers'

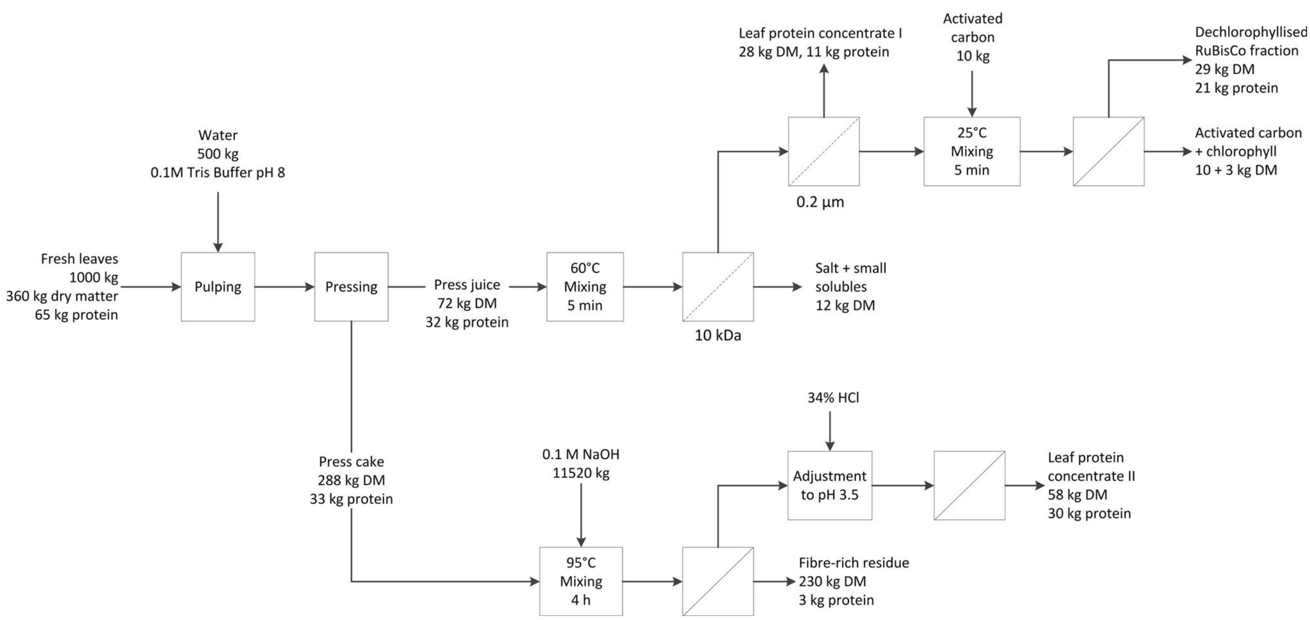


Fig. 10 Proposed process flow diagram to isolate proteins from rubber tree leaves. Material balance was calculated from Kamm et al. [86], Van de Velde et al. [90], and Zhang et al. [92]

daily activities, within a known community, and low energy input with local use of the undried products for feed. In the setting of Indonesian rural area, the use of protein concentrate for animal feed is the most straightforward and economically feasible. It can provide animal feed, especially for small scale/household farmers. Furthermore, using locally available agricultural residues also diverts the use of food harvest or imported feed ingredients, e.g. soybean meal. This approach also potentially reduces the negative environmental impact from agriculture and transportation.

In general, small scale (pre-)processing of biomass is more beneficial for processes with low capital and low energy use [24]. For the case of seed biorefinery (Fig. 6) and protein extraction from press cake (Fig. 9), the highest energy consumption is in the drying of the starting materials and product(s). When starting materials or products are not used directly, in situ drying is still preferred to prevent fungal growth and therefore alternatives to reduce energy consumption, e.g. sun drying, should be considered. For local processing, leaves processing (Fig. 10) can be modified for products that are suitable for local use.

Local processing also enables the recycle of nutrients and minerals to the soil. The seeds and leaves of rubber tree are currently not utilised, and only left on the plantation ground to become humus. Harvesting of the seeds and leaves, therefore, might reduce the soil organic carbons and nutrients in the plantation. One alternative for nutrients recycle is using the liquid fraction from the protein extraction as fertiliser.

Conclusions

Utilisation of protein fractions from rubber tree might increase the economics of rubber tree plantations. In Indonesia where most rubber plantations are owned by smallholder farmers, this can be a source of additional income for the farmers. Protein extraction from rubber seeds can be incorporated within a biorefinery plant that produces biodiesel as its main product. The protein extraction can be performed with the available knowledge and technology, and the product can be applied for animal feed. Protein extraction from rubber tree leaves can aim for animal feed proteins for local use or more polished products for food and industrial use. Utilisation of protein in the latex is not economically feasible at this moment, but may be feasible when specific use of the latex protein(s) can be identified.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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