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Biorefinery methods for separation of protein and oil fractions from rubber seed kernel

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ABSTRACT

Biorefinery of rubber seeds can generate additional income for farmers, who already grow rubber trees for latex production. The aim of this study was to find the best method for protein and oil production from rubber seed kernel, with focus on protein recovery. Different pre-treatments and oil separation methods were tested, and alkaline conditions were used to extract protein. Next to processes with subsequent oil and protein recovery, a one-step combined oil and protein extraction was tested. Our study showed that oil separation is not necessary to obtain high protein recovery, however most of the extracted oil is present as an emulsion. The origin of the seeds and their treatment on the plantation before processing were most important for high oil and protein recoveries, and in all cases tested had more influence on recoveries than its subsequent method of processing. Pressing the rubber seed kernel to separate the oil fraction followed by protein extraction from the press cake gives the highest protein recovery with satisfactory recovery for oil.

1. Introduction

The rubber tree (*Hevea brasiliensis*) is mainly cultivated for its latex, which can be processed to natural rubber and used in various products. Thailand, Indonesia and Malaysia are the largest natural rubber producing countries; their combined harvested area accounts for two-thirds of the world's harvested area (FAO, 2013). Even in these countries, the seed of the rubber tree is not widely collected for commercial use except for seeding, which accounts for less than 25% of seed with selected breed and quality (Suprayudi et al., 2014).

The annual production of rubber seed varies from 300 to 2060 kg/hectare (Jamieson and Baughman, 1930; Zhu et al., 2014). Plantation conditions often pose difficulties in collecting and rot preventing, therefore the realistic collectable yield without a dedicated collection method might be as low as 150–200 kg/hectare per year (Ramadhas et al., 2005; Ravindran and Ravindran, 1988). Seed weight in fresh condition is between 3 and 6 g (fresh weight), and consists of 42–51% shell and 49–58% kernel (Ravindran and Ravindran, 1988). On dry weight basis, the kernel of rubber seed contains 40–50% oil (Jamieson and Baughman, 1930; Ravindran and Ravindran, 1988) and 19–23% crude protein (Fetuga et al., 1977; Orok and Bowland, 1974; Ravindran and Ravindran, 1988). Based on a conservative estimation of 200 kg/ha with 30% moisture content, 38 kg-oil/hectare and 13 kg-protein/hectare are available annually.

Oil and protein production from rubber seeds can generate additional revenue to the latex production. The use of rubber seed oil as an alternative feedstock for biodiesel production has already been investigated (Morshed et al., 2011; Ramadhas et al., 2005). Other potential applications include corrosion inhibitor (Udiandeye et al., 2011), metal soap (Balköse et al., 2010), and precursor for resins and polymers (Aigbodion and Okieimen, 1996; Bakare et al., 2008; Joseph et al., 2004). Even though these potentials have been identified, studies on optimising oil separation from rubber seed are still limited. Ebewele et al. (2010) reported that the maximum 0.45 g-oil/g-kernel could be obtained by Soxhlet extraction using n-hexane as the solvent, while only 0.28 g-oil/g-kernel could be obtained using mechanical pressing at optimised condition. Higher results (0.21–0.34 g-oil/g-kernel) were obtained using supercritical carbon dioxide (Lee et al., 2013; Mohd-Setapar et al., 2013). The combination method using mechanical pressing with

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hexane addition gave as high as 0.49 g-oil/g-kernel (Morshed et al., 2011).

Early studies on full-fat and de-oiled rubber seed kernel suggested their potential use as food and feed materials because of their protein content (Achinewhu, 1986; Giok et al., 1967; Ukhun and Uwatse, 1988). Rubber seed kernel proteins contain 33–36% essential amino acid; lysine and methionine are the most limiting (Agunbiade et al., 1995; Fetuga et al., 1977; Ravindran and Ravindran, 1988). Heat and pressure treatment, soaking, and oil separation were observed to cause only limited changes in the amino acid composition (Agunbiade et al., 1995). Solvent oil extraction and soaking rubber seed kernel in 0.01 M HCl or NaOH decreased the protein quality as observed in experiments with rats (Fetuga et al., 1977), possibly due to protein denaturation. Soaking the full-fat kernel with water at 65 °C, however, showed slightly improved protein quality compared with the untreated kernel, possibly due to the leaching out of anti-nutritional factors. Other studies on the use of rubber seed kernel still give conflicting results. Biological assays on rats and chickens fed with diets containing de-oiled rubber seed kernel showed lower weight gain and reduction in food intake and fertility (Babatunde and Pond, 1990; Orok and Bowland, 1974; Ravindran et al., 1987). On the other hand, de-oiled rubber seed was used to replace 50% of protein in common carp diet without adverse effects (Suprayudi et al., 2014). To the authors' knowledge, no work on protein extraction from the rubber seed has been reported yet.

In oil containing biomass, oil is stored in the cell as oil bodies that are covered with proteins (Huang, 1992). Mechanical oil pressing or solvent extraction is used to separate oil, and oil recovery from these processes is influenced by several factors including temperature, pressure, and moisture content (Adeeko and Ajibola, 1990; Baümler et al., 2010). Application of high temperature aids in releasing the oil from the cells by means of breaking the cell structure, lowering oil viscosity, and adjusting moisture content. At high temperature, proteins that cover oil bodies also denature and coagulate, which helps releasing oil from the cell (Adeeko and Ajibola, 1990). Higher temperature, however, also influences proteins that are not associated with oil bodies and in general reduces the solubility of these proteins. High efficiency of oil pressing or extraction, therefore, might give reversed effect on protein extraction. Protein extraction from Jatropha seed showed that higher protein recovery was obtained from full-fat kernel instead of de-oiled kernel (Lestari et al., 2010). Protein extraction from microalgae with protease addition, however, shows that protein recovery from de-oiled microalgae was higher than full-fat microalgae (Sari et al., 2013).

Combined oil and protein extraction is an alternative method to separate protein and oil fractions, and has been used for oil and protein extraction from peanut, sesame, canola, soybean, and rapeseed (Jiang et al., 2010; Latif and Anwar, 2011; Latif et al., 2008; Rosenthal et al., 2001; Zhang et al., 2006). The method takes advantage of the insolubility of oil in water to create separate oil and aqueous protein phases. The recoveries of oil and protein are mainly influenced by pH and temperature (Rosenthal et al., 1998). The use of protease has been reported to increase both oil and protein recoveries (Latif and Anwar, 2011; Rosenthal et al., 2001; Zhang et al., 2006).

The aim of this study was to obtain high protein recovery from rubber seed kernel, without major losses in oil recovery. Different pre-treatments and oil separation methods were tested, and alkaline conditions were used to extract protein. Next to processes with subsequent oil and protein recovery, a one-step combined oil and protein extraction was tested. The influence of several processing parameters was examined, and results could be explained by interpreting differences and taking interactions between oil, protein, and other components into consideration. The envisaged process should have the highest protein recovery and a reasonable oil recovery, taking into accounts both protein and oil qualities. Energy and chemical uses were considered within the context of the intended use of the protein and oil fractions.

2. Materials and methods

2.1. Materials

Two types of rubber seeds were used in the experiments. The first batch was obtained from Subang, West Java, Indonesia, and the second batch was obtained from Bengkulu, Sumatera, Indonesia. The seeds were stored at room temperature in open containers until use.

The chemicals used for experiments and analysis were of analytical grade, unless otherwise specified. Protease enzyme (Protex 40XL) was obtained from Genencor International BV, the Netherlands. As specified by the manufacturer, the temperature range of the protease was 25–60 °C, pH range was 9–12, and activity was 52 MPU/g.

2.2. Pre-treatment

Before further treatment, the seeds were de-hulled manually to separate good condition kernels from the ones infected with fungi. The good kernels were cut into four parts to optimise drying. Some kernels were dried at 60 °C for three days and others were dried at 105 °C for 24 h.

2.3. Oil separation

Pre-dried Subang kernels were subjected to hydraulic pressing or hexane extraction.

2.3.1. Oil separation by hydraulic pressing

Before pressing, moisture content of the cut and pre-dried kernels was measured. To rule out the influence of different moisture content when measuring the influence of pre-drying temperature, dry kernels were exposed to ambient air to bring the moisture content to an equal value (3%). Pressing was performed using a laboratory scale hydraulic press that could operate from 30 to 120 °C with a maximum pressure of 25 MPa. For this experiment, the applied pressure was 25 MPa and temperature was 60 °C or 100 °C. At 60 °C, cell disruption and decrease in oil viscosity were expected, while protein coagulation could be avoided. At 100 °C, both effects were expected.

Pressing was performed in ten batches for each condition, using ±7 g of kernel per batch. Pressing time for each batch (including heating) was 30 min. De-oiled residues from the pressing (referred hereafter as press cake) were stored at 4 °C until further use.

2.3.2. Oil separation by hexane extraction

Pre-dried kernels (at 60 or 105 °C) were ground with a commercial coffee grinder, and stored in a desiccator until further use. Extraction was performed using technical grade n-hexane in a Soxhlet (70 °C) for 6 h. De-oiled residue from the hexane extraction (referred hereafter as meal) was dried at 60 °C to remove residual hexane, and stored at 4 °C until further use.

2.4. Protein extraction

Before protein extraction, press cakes were ground with a commercial coffee grinder. The meals from hexane extraction were already in powder form; therefore no other pre-treatment was applied.

To 4 g material, 40 g of 0.1 M NaOH (1:10 solid-to-solvent ratio) was added in a 100 ml Erlenmeyer flask. Flasks with the extraction