Non-food applications of Jatropha proteins

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Dianika Lestari

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For my supervisor

Wilhelmus J. Mulder

Researcher in Food & Biobased Research, Wageningen UR

Thank you for your guidance and supervision.

Untuk Ayah, Mamah, dan Adikku tercinta

Untuk belahan jiwaku

Untuk keluarga serta teman-teman yang aku sayangi

Dan untuk semua orang yang menyayangiku

TABLE OF CONTENTS

Chapter 1

General Introduction

1.1 BIOMASS, BIOENERGY, AND BIOFUEL

Currently, non-renewable fossil-based fuels supply about 85% of the primary energy resources. Until the year 2050, due to the increase of the world population, the global primary energy consumption is predicted to increase up to around 1500 EJ, or by as much as three times current usage [1, 2]. This may lead to the depletion of fossil feedstock and, as a consequence, an increase in oil prices. Moreover, combustion of fossil fuels also causes environmental problems, especially with regards to CO_2 emissions. The Intergovernmental Panel on Climate Change, IPCC, has proposed many different scenarios to predict emission, which covers a wide range of the main driving forces of future emissions, e.g. demographic, technological, and economic developments. One of the scenarios is the IPCC scenario A1F1, which is based on the global economy and intensive use of fossil fuels. According to this scenario, CO_2 emissions have doubled in the previous decades, and may still have a 5-fold increase to 821 Gt carbon in 2050 [2]. Therefore, in order to reduce CO_2 emission from fossil fuel combustion, many countries have started to use and develop alternative energy sources from biomass.

Biomass can be used as raw materials for food, feed, chemicals, materials, and energy. The energy from biomass is called bioenergy, while the crops that are used to produce bioenergy are called energy crops. Biofuels, such as bioethanol, biodiesel, and bio-oil, is the form of bioenergy that is specifically used for transportation. In 2009, Brazil produced about 25 GL of bioethanol from sugar cane, while the United States produced about 41 GL bioethanol from corn [3]. Together, Brazil and the United States contribute to around 88% of current bioethanol world production [4]. On the other hand, the EU was the world's largest producer of biodiesel, mainly from rapeseed and sunflower oil, with a total production of about 9 GL in 2009 [5]. Meanwhile, biodiesel production in Indonesia was still relatively low of about 0.23 GL in 2010 [6].

The Indonesian government has set a target to increase biofuel utilization in order to reduce fossil fuel consumption. Based on the Indonesian Presidential Regulation No. 5/ 2006 [7], biofuel is expected to supply up to 5% of the total energy source in 2025 (Figure 1.1). Biofuel utilization is expected to have a beneficial effect on the environment, economy, and society in Indonesia. Besides reducing the use of fossil fuels and the emission from their combustion, biofuel production should enable a village, or an area, to supply its own energy needs (and provide local energy security), while at the same time creating job opportunities for the villagers. In Indonesia, several food crops e.g. palm oil, sugar cane, and cassava, have already started to be partially used as energy crops. However, the use of food crops to produce biofuels can cause problems due to the possible competition between producing food and fuel. To overcome these problems, the potential of biofuel production from non-edible crops, e.g. *Jatropha curcas L.* or Jatropha should be investigated.

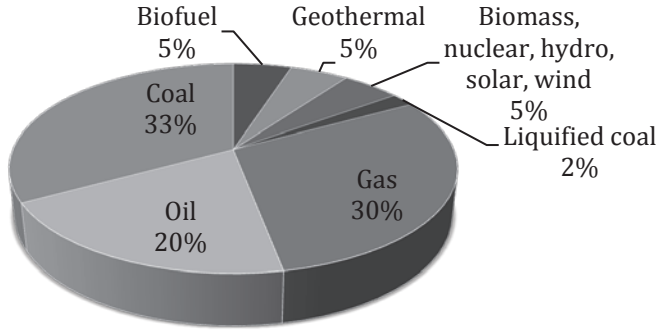

Figure 1.1 Indonesian energy mix target in 2025 (Based on the Presidential Decree No. 5/2006).

1.2 *Jatropha curcas*

Jatropha curcas L. or Jatropha or jarak pagar (in Indonesian) is a small oilseed tree belonging to the Euphorbiaceae family, which has strong pharmacological qualities and contains anti- nutritional and toxic components. Jatropha is one of the potential non-edible crops for biofuel production. Even though Jatropha is a native tropical plant from South America, it has already spread out in many regions in Asia and Africa. Jatropha can be grown in arid land. However, the productivity of Jatropha seed will be very low in such arid conditions and therefore, will not be feasible for biodiesel production [8]. Jatropha will result in a seed yield of less than 1 tonne dry seed/ ha with rainfall of 500 to 600 mm/ year [9]. On the other hand, the yield of seed fluctuates significantly, depending on the soil nutrition and plant density, from 0.6 to 4 ton dry seeds/ ha with rainfall of 1,200 to 1,500 mm/ year [10]. Francis [11] and Openshaw [8] estimated that Jatropha seed productivity may reach 5 tonnes seed/ ha in a good soil condition with sufficient water supply. Gunaselaan [12] reported that Jatropha seed yield was 4 tonnes/ ha per year and the pruned leaves yield was 1.1 tonnes/ ha per year from the rain fed dry land with a plant density of 4444 plant/ ha at 1.5 m x 1.5 m spacing.

Assuming the crop intercepted all incoming radiation, the dry matter distributions of Jatropha plant are about 25% in stems, 25% in leaves, and 50% in fruits. The fruit dry matter consists of about 30% fruit coat and 70% seed, while the seed dry matter consists of about 65% kernel and 35% shell (Figure 1.2) [8]. Traditionally, Jatropha is used to prevent and/or to control erosion, to reclaim land or to act as a living fence for excluding farm animals. Many plant parts have potential medicinal and commercial value, such as tannin from the bark for treating leather, honey from its flower, and Jatropha stem latex as a source of pharmaceutical components. Jatropha leaves can be used for silkworm feed, antiseptics, or remedy for dermatitis [8, 13]. Jatropha fruit coat is a sugar-rich material and can be used as a green fertilizer or for biogas production. Jatropha seed contains about 30-40% oil, which has similar fatty acids composition

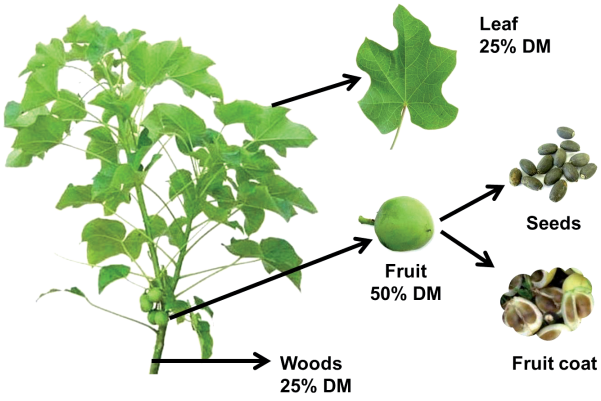

Figure 1.2 *Jatropha curcas* plant parts.

to sunflower or palm oil and can be converted into biodiesel. Jatropha oil is extracted from the seed by means of pressing, which results in about 30% oil and 70% press cake. Heller [14] reported the chemical composition of Jatropha seed and press cake, which are presented in Table 1.1.

Table 1.1 Jatropha seed and meal compositions [14].

	Compositions (% w/w DM)	
Components	**Seed**	**Press Cake**
Crude Fat	36	6
Crude fibre	28	39
Crude Protein	19	24
Carbohydrate	9	19
Ash	5	6
Minerals	3	6

1.3 CURRENT SITUATION OF JATROPHA IN INDONESIA

Currently, the growth of Jatropha plantations in Indonesia and the productivity of Jatropha seed per hectare are low. From 2007 to 2009, Jatropha plantations were increased from 68,200 ha to 69,315 ha [15]. This low growth may be due to the low yield and market value of Jatropha seed. The average Jatropha seed yield of 115 kg dry seed/ ha per year [15] is still much lower than the expected yield of around 0.6 to 4 ton dry seed/ ha per year [10, 12, 16]. East Kalimantan and West Nusa Tenggara are two of the provinces in Indonesia that are still growing Jatropha for biodiesel production. According to the Estate Agency of East Kalimantan, Jatropha plantations in East Kalimantan have reached 611 ha and produced around 85 tonnes of seed in 2010. This board is planning to develop another 10,000 ha of Jatropha plantations [17]. In West Nusa Tenggara, Jatropha plantations have reached 6000 ha and produced around 1000 tonnes of Jatropha seed [18]. Experimental Jatropha fields at Balittas in Pakuwon (Indonesia) has reached a seed yield of 5 tonnes/ ha per year. However, more efforts are needed to improve Jatropha seed yield in the actual fields.

Currently, Jatropha press cake is used as fertiliser or burned to produce heat [8, 19, 20]. Therefore, the press cake has a low value compare to the oil. However, press cake contains useful components, such as carbohydrates, minerals, fibre, and protein [14]. The total market value of Jatropha seed can be improved by converting these components into various products. As a rough estimation, the potential market value of Jatropha seed after total fractionation is about two times higher than the market value of the seed after partial fractionation (Table 1.2). Based on this, the total fractionation of Jatropha press cake should be conducted using a biorefinery to optimize the overall added value of Jatropha.

Table 1.2 the potential market value of Jatropha seed.

Scenario	Seed components	Compositions (% w/w DM)	Market value (EUR/ kg seed)
Partial fractionations	Oil	35	0.21
	Press cake	65	0.02
	Total market value		0.23
Total fractionations	Oil	35	0.21
	Crude protein	19	0.19
	Crude fibre	29	0.04
	Carbohydrates	9	0.02
	Ash/ minerals	8	0.02
	Total market value		0.48

1.4 BIOREFINERY: DEFINITIONS AND CLASSIFICATIONS

Biorefinery can be described as an integrated biomass processing to produce various added value products [19, 21, 22]. Another definition of biorefinery, which was defined within the framework of IEA Bioenergy Task 42 on Biorefineries, is the sustainable processing of biomass into a spectrum of marketable products and energy [23]. The use of biomass can be differentiated into the 5 F-cascades, which contain a list of priority products that can be manufactured from biomass as follows: 1) Food and feed; 2) Fine and bulk chemicals, and pharma; 3) Fibre and biomaterials; 4) Fuel and energy; and 5) Fertiliser and soil conditioners [24]. Sanders [1, 21] suggested that splitting the processing of biomass to produce several products, e.g. producing fuel, energy, and functionalized bulk chemicals, should give higher benefit or market value per hectare of cultivated biomass than producing only fuel and energy.

In a biorefinery, products can be manufactured from the feedstock using various types of conversion technologies: thermochemical conversion, e.g. pyrolysis or gasification; (bio-) chemical conversion, e.g. fermentation, reaction, and hydrolysis;

separations and purifications e.g. extraction [22]. Two examples of commercially operating biorefineries are the Novamont plant in Italy and Roquette at Lestrem in France. Novamont produces a range of chemicals, such as biodegradable polyesters, and starch-derived thermoplastics from corn starch, while Roquette produces many types of carbohydrate derivatives, sweeteners, polyols, and bioethanol from cereal grains [25, 26].

Cherubini et al. [23] proposed the latest classification approach of biorefinery, in which the four main features were used: 1) platforms; 2) products; 3) feedstock; and 4) processes. Of these, the platforms are the key intermediates between raw materials and final products that connect the different types of biorefineries. There are 10 proposed platforms for biomass processing: C5 sugars, C6 sugars, oils, biogas, syngas, hydrogen, liquid juice after wet biomass extraction e.g. grass juice, pyrolytic liquid, lignin, or electricity and heat. Among these proposed platforms, proteins and amino acids, despite their potential, are not yet included as a specific platform. Based on the assumption of the 10% substitution of fossil fuel for transportation, Sanders et al. [1] estimated additional annual production of 100 million tonnes of proteins as by-product of the biofuel productions, which is about four times protein requirement for the global human population. This over production of proteins may lead to the saturation of protein markets for food applications. Consequently, there will also be opportunities for protein markets for non-food/ technical applications [1, 27-29].

1.5 BIOREFINERY AS A TOOL TO OPTIMIZE JATROPHA UTILIZATION IN INDONESIA

In order to optimize the potential of Jatropha as an energy crop in Indonesia, biorefinery may be the best tool to optimize the overall market value of Jatropha seeds. The general scheme of Jatropha seed biorefinery is presented in Figure 1.3. The oil platform, where oil is extracted from the seed and converted to biodiesel, will be the main platform of Jatropha biorefinery. The press cake will be fractionated into its major components e.g. crude protein, crude fibre, carbohydrates, ash, and minerals. These components will undergo further processing to produce various marketable products. Among the components of Jatropha press cake, Jatropha protein has a high potential market value (Table 1.2). This research specifically investigated the potential of Jatropha protein to improve the market value of *Jatropha curcas*.

1.6 THE POTENTIAL OF PROTEINS IN BIOREFINERY

Protein serves significant roles in many biological processes e.g. enzymatic catalysis, transport, storage, and mechanical support in the skin and bones. Built-up by a sequence of 20 amino acids, protein can be used in various applications. Protein has not only nutritional values, but also many functional properties that are useful for food

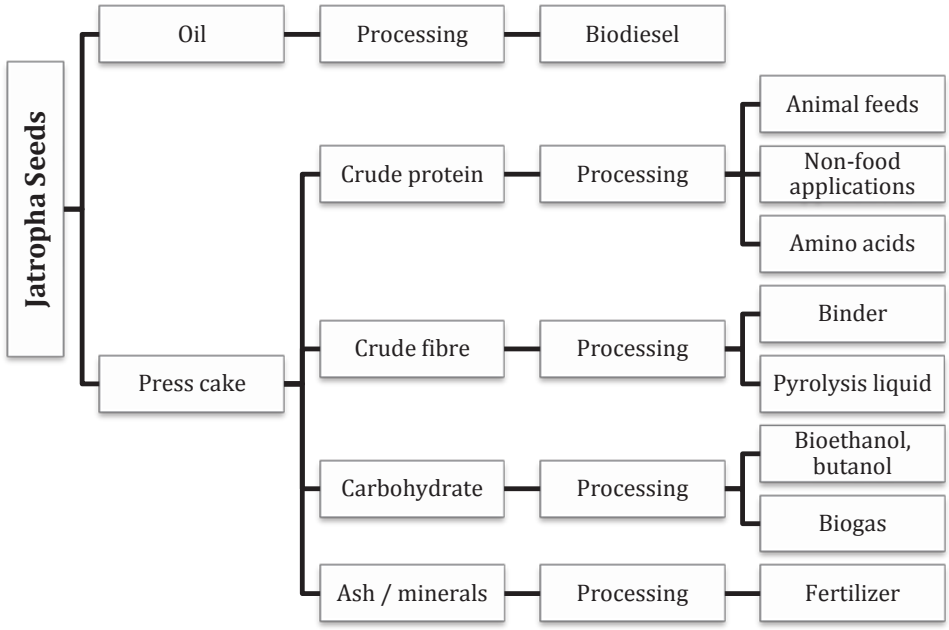

Figure 1.3 Jatropha seed biorefinery.

and non-food/ technical applications, for examples as emulsifier e.g. in mayonnaise, as foaming agent, or be used in drug encapsulation. Currently a number of proteins, such as soy protein, wheat gluten, and casein, have been produced commercially for both food and technical applications. Due to its ease of handling in high speed machines, casein is being used as adhesives in the bottle-labelling industry. Wheat gluten has applications as surfactants in adhesives, coatings, and cosmetics, while soy proteins has been used for a range of applications, including paper coatings, plywood adhesives formulations, and microencapsulation of insecticides [30, 31].

Soy protein is an example of industrial protein derived from oilseed crop. Soy protein products are sold in the market in the form of protein concentrate or protein isolate. There are three optional basic commercial processes to produce protein concentrate with 70% protein content: (i) washing defatted soy flour in 70-90% aqueous ethanol solutions, (ii) extraction of soluble by water acidification to pH 4.5 with food grade acid, or (iii) water extraction of toasted defatted soy flour [32-34]. Protein isolate is produced by solubilizing soy flour at pH between 6.8 -10 at 27 - 66°C using sodium hydroxide followed by precipitation at pH 4.2 - 4.5 using hydrochloric acid. After precipitation, the protein can be neutralized to pH 6.5 - 7.0 or spray dried at its isoelectric point, which results in protein content of about 90 - 95% [35]. Both soy proteins concentrate and soy protein isolate are widely used in food and industrial applications as emulsifier, as foaming agent, in adhesives formulations, as binder in ink and paint, or paper coating formulations.

Protein is also a potential source for amino acids, which can be used as food supplements, animal feed, and pharmaceutical applications. Amino acids contain carboxylic acid and amine groups, which gives them potential as alternative raw materials to produce functionalized N-containing chemicals [1, 27, 36]. Several lines of research have been conducted to convert amino acids into chemicals. Glutamic acid could be used as raw materials for chemicals such as 5-amino-1-butanol and glutaric acid [29]. Other studies report the decarboxylation of glutamic acid to γ-aminobutyric acid (GABA) [36, 37], which could be an intermediate for making N-methylpyrrolidone, an industrial solvent [38]. Glutamic acid can also be converted into acrylonitrile through two-step procedure of oxidative decarboxylation in water to 3-cyanopropanoic acid, followed by a decarbonylation-elimination reaction using a palladium catalyst [39]. Another example, L-aspartic acid, with the aid of Escherichia coli L-aspartate a-decarboxylase, can be converted into β-alanine (3-aminopropionic acid), a potential intermediate for the production of N-containing bulk chemicals e.g. acrylamide [40]. On the other hand, the hydrolysis of L-arginine produces L-ornithine, which can be decarboxylated to 1,4-diaminobutane, and urea, which could be directly applied as fertilizer [1].

Despite the outstanding performance and biodegradability of protein, protein-based technical applications are generally more expensive than petroleum-based technical applications. Among biopolymers, proteins could be as expensive as cellulose derivatives e.g. cellulose acetate or CMC, but commonly more expensive than starch and modified starch. For example, while the cost of industrial plant proteins are in the range of 0.5 to 4.0 US$/ kg and industrial animal proteins are more than 4 US$/ kg, the cost of starch derivatives or modified starch is only about 1 US$/ kg [31]. However, protein has specific functional properties, which make them better suited for some selected technical applications than other biopolymers, especially as adhesives formulations, emulsifiers, foaming agents, and coatings/films [30]. Some important functional properties for technical applications are solubility, emulsifying properties, foaming properties, film-forming properties, and adhesive properties.

1.7 THE POTENTIAL OF JATROPHA PROTEINS

Jatropha proteins could be produced as industrial proteins as the existing industrial proteins e.g. soy proteins, casein, or wheat gluten. Jatropha proteins can be obtained from the press cake and the leaves. Commonly, Jatropha leaves are only harvested during pruning to accommodate branching and to improve fruit production. Gunaselaan [12] reported that Jatropha seed yield was 4 tonnes/ ha per year and the pruned leaves yield was 1.1 tonnes/ ha per year, which were harvested from the rain fed dry land with a plant density of 4444 plant/ ha at 1.5 m x 1.5 m spacing. Based on this, the potential productivity of Jatropha press cake will be approx. 2.4 tonnes/ ha. Jatropha press cake contains around 25% w/w proteins, which leads to the total potential productivity of Jatropha press cake proteins of approx. 600 kg/ ha.

According to Chaudhary et al. [41], Jatropha leaf dry matter contained around 1.5 to 3.0% nitrogen or around 9.4% to 18.8% protein (based on N-factor of 6.25) of the dry matter. Assuming the dry matter content of 20%, the total potential availability of Jatropha leaf protein from the pruned leaves are approx. 220 kg/ ha per year.

In terms of functional properties and extraction conditions, Jatropha protein may have comparable properties to other well-known oilseed proteins, such as soy, canola, rapeseed, or sunflower protein. However, unlike the soy and sunflower, Jatropha seed contains toxic compounds such as curcin [42] and phorbol esters [43, 44]. The presence of these toxic compounds makes Jatropha protein unsuitable for food applications. After detoxification, Jatropha proteins are potential components for animal feed, while without detoxification, Jatropha protein applications will be limited to technical applications [45, 46].

Several studies on detoxification and protein extraction from Jatropha press cake have been conducted in the laboratory scale experiments. Protein extraction from Jatropha meal at pH 11 at 60°C and a solvent to solid ratio of 10 followed by precipitation at pH 5 resulted in protein recovery of 53% and a purity of 76-82% [46]. By using steam injection for protein precipitation, protein recovery is about 70-77% with a purity of 95-97% [45]. On the other hand, there is no method available yet to specifically extract proteins from Jatropha leaves. Detoxification of Jatropha press cake mainly consists of the removal of the toxic phorbol ester. Research have been conducted to reduce the phorbol ester content in Jatropha defatted kernel meal (without shell) using the combination of heat and chemical treatments. Aregheore [47] reduced around 95% phorbol ester content of Jatropha defatted kernel meal from 1780 ppm to 90 ppm using four cycles of extraction with 92% methanol. An alternative method developed by Martinez-Herrera et al. [43] reduced the phorbol ester content by 98% (from 3850 ppm to 80 ppm) in Jatropha defatted kernel meal using a 90% v/v ethanol-water extraction of 2 hour at room temperature, followed by a treatment using 0.07% $NaHCO_3$ and autoclaving at 121°C for 20 min. A recent patent from Makkar et al. [48] was able to reduce phorbol ester content in the protein isolate into undetectable amount at the HPLC detection limit of 10 ppm phorbol ester. In this patent, protein was extracted from defatted kernel meal using an aqueous solution of NaOH at pH 11 followed by protein precipitation by addition of ethanol. Another recent patent from the company D1 [49] has claimed that simultaneous oil extraction and detoxification of the flaked seed kernel using a combination of hydrophobic- hydrophilic solvent was able to reduce the phorbol ester content to an undetectable phorbol ester content. Currently, the industrial scale production of animal feed from Jatropha kernel meal or press cake is not yet available.

In order to extract the protein from Jatropha press cake or leaf at industrial scale, a more efficient protein extraction is required to obtain higher protein recovery at high protein concentration. Apart from these, an efficient protein extraction should also be able to extract protein with good functional properties e.g. solubility, foaming properties, film-forming properties, and emulsifying properties, which are essential for non-food/ technical applications. The functional properties of oilseed protein are

highly influenced by the extraction and preparation method [50, 51]. Several factors affecting protein functionality during extraction or applications are the process conditions—e.g. temperature, pH, ionic strength, and presence of other components such as surfactants or reactants. The extraction conditions may cause protein degradation such as S-S bridge reshuffling, denaturation, aggregation, chemical modification of side groups, proteolysis, and cross-linking reaction. Consequently, the degraded protein may lose their functional properties. This may limit their uses as technical applications [30, 52]. For these reasons, the extracted proteins should not be hydrolysed or denatured. Therefore, an extraction at ambient temperature is preferable as a starting point. Extraction at a higher pH and/or performing a multistage extraction, which consists of cross-flow extraction and counter current extraction, can be used to improve the protein recovery [53, 54].

1.8 RESEARCH OBJECTIVES AND THESIS OUTLINE

This research investigated whether Jatropha protein can be extracted with high recovery while still maintaining its functional properties. Furthermore, this protein will be used for many applications, such as animal feed and technical applications e.g. adhesives, foaming agent, coating, etc. After the general introduction of the research in Chapter 1, chapter 2 discusses the optimization of the toxic phorbol esters removal for industrial scale applications. Next, Chapter 3 presents the investigation on different multistage extraction configurations to improve protein recovery from press cake at room temperature. The extraction and characterization of Jatropha leaf protein are presented in Chapter 4. Chapter 5 discusses mainly about the functional properties of the extracted protein from press cake. The comparison of the total potential market value of Jatropha side products in five selected countries at different markets/ approach are presented in Chapter 6. Finally, Chapter 7 gives general discussions of the results and the perspectives of the future utilization of Jatropha, specifically in Indonesia.

REFERENCES

1. J.P.M. Sanders, E.L. Scott, R.A. Weusthuis, H. Mooibroek, Bio-Refinery as the Bio-Inspired Process to Bulk Chemicals, Macromolecular Bioscience, 7 (2007) 105-117.
2. IPCC, IPCC Special Report, Emission Scenarios. Summary for Polycymakers, in: N. Nakicenovic, R. Swart (Eds.), IPCC, Cambridge, 2000.
3. A.M.J. Kootstra, Processing Lignocellulosic By-products Streams Using Organic Acids, in: Valorisation of Plant Production Chains, Wageningen University, Wageningen, 2010, pp. 176.
4. RFA, Renewable Fuel Association; 2010 Ethanol Industry Outlook, (2010).
5. EBB, European Biodiesel Board Statistic; 2009 Production by country, in, 2009, pp. http://www.ebb-eu.org/stats.php.
6. R.K. Karindon, Pemanfaatan Biodiesel belum maksimal. Published date: 07-3-2011. Accessed date: 01-08-2011, in: http://www.mediaindonesia.com/read/2011/03/07/208439/21/2/-Pemanfaatan-Biodiesel-belum-Maksimal- 2011.
7. Indonesia, Indonesian Presidential Regulation No. 5/2006 on National Energy Policy, in, 2006.
8. K. Openshaw, A review of Jatropha curcas: an oil plant of unfulfilled promise, Biomass Bioenerg., 19 (2000) 1-15.
9. H. Euler, D. Gorriz, Case Study Jatropha curcas, in: Global Facilitation Unit for Unerutilized Species (GFU) and Deutsche Gessellschaft fur Technische Zusammenarbeit (GTZ), Frankfurt, Germany, 2004, pp. 63p. .
10. R.E.E. Jongschaap, W.J. Corre, P.S. Bindraban, W.A. Brandenburg, Claims and Facts on Jatropha curcas L. , in, Plant Research International B.V., Wageningen UR, Wageningen, 2007.
11. G. Francis, R. Edinger, K. Becker, A concept for simultaneous wasteland reclamation, fuel production, and socio-economic development in degraded areas in India: Need, potential and perspectives of Jatropha plantations, Natural Resources Forum, (2005) 12-24.
12. V.N. Gunaseelan, Biomass estimates, characteristics, biochemical methane potential, kinetics and energy flow from Jatropha curcus on dry lands, Biomass and Bioenergy, 33 (2009) 589-596.
13. E.T. Akintayo, Characteristics and composition of Parkia biglobbossa and Jatropha curcas oils and cakes, Bioresource Technology, 92 (2004) 307-310.
14. J. Heller, Untersuchungen über genotypische Eigenschaften und Vermehrungsund Anbauverfahren bei der Purgiernuß (Jatropha curcas L.) [Studies on genotypic characteristics and propagation and cultivation methods for physic nuts (Jatropha curcas L.)]. Dr. Kovac, Hamburg, (1992).
15. M. Syakir, Prospek dan Kendala Pengembangan Jarak Pagar Sebagai Bahan Bakar Nabati di Indonesia, Perspektif, 9 (2010) 55-65.
16. V. Nallathambi Gunaseelan, Biomass estimates, characteristics, biochemical methane potential, kinetics and energy flow from Jatropha curcus on dry lands, Biomass and Bioenergy, 33 (2009) 589-596.
17. Pada 2010, Produksi Tanaman Jarak di Kaltim Mencapai 85 Ton, in: http://m.korankaltim.co.id/read/m/4800 2011.
18. Disbun_NTB, Jarak Pagar. http://disbun.ntbprov.go.id/?p=119, in, 2010.
19. R. Manurung, D.A.Z. Wever, J. Wildschut, R.H. Venderbosch, H. Hidayat, J.E.G. van Dam, E.J. Leijenhorst, A.A. Broekhuis, H.J. Heeres, Valorisation of Jatropha curcas L. plant parts: Nut shell conversion to fast pyrolysis oil, Food and Bioproducts Processing, 87 (2009) 187-196.
20. R. Staubmann, G. Foidl, N. Foidl, G.M. Gubitz, R.M. Lafferty, V.M. Arbizu, W. Steiner, Biogas production from Jatropha curcas press-cake, Appl Biochem Biotechnol, 63-65 (1997) 457-467.
21. J.P.M. Sanders, Biorefinery, the bridge between agriculture and chemistry, in: The 14th European Biomass Conference and Exhibition : Biomass for Energy, Industry and Climate Protection, Paris, France, 2005.
22. F. Cherubini, The biorefinery concept: Using biomass instead of oil for producing energy and chemicals, Energy Conversion and Management, 51 (2010) 1412-1421.
23. F. Cherubini, G. Jungmeier, M. Wellisch, T. Willke, I. Skiadas, R. Van Ree, E. de Jong, Toward a common classification approach for biorefinery systems, Biofuels, Bioproducts and Biorefining, 3 (2009) 534-546.
24. E. Vandamme, T. Anthonis, S. Dobbelaere, Industrial Biomass: Source of Chemicals, Materials, and Energy! Implications and limitations of the use of biomass as a source for food, chemicals, materials and energy, in, Belgian Academy Council of Applied Science, 2011.
25. J.H. Clark, F.E.I. Deswarte, The Biorefinery Concept--An Integrated Approach, in: J.H. Clark, F.E.I. Deswarte (Eds.) Introduction to Chemicals from Biomass, John Wiley and Sons, Ltd., 2008.
26. B. Kamm, M. Kamm, Principles of biorefineries, Applied Microbiology and Biotechnology, 64 (2004) 137-145.
27. J. van Haveren, E.L. Scott, J.P.M. Sanders, Bulk chemicals from biomass, Biofuels, Bioproducts and Biorefining, 2 (2008) 41-57.
28. A.A. Koutinas, C. Du, R.H. Wang, C. Webb, Production of Chemicals from Biomass, in: J.H. Clark, F.E.I. Deswarte (Eds.) Introduction

to Chemicals from biomass, John Wiley and Sons, Ltd., 2008.

29. T. Werpy, G. Petersen, Top Value Added Chemicals From Biomass, Volume I: Results of Screening for Potential Candidates from Sugars and Synthesis Gas, in, the Pacific Northwest National Laboratory (PNNL), the National Renewable Energy Laboratory (NREL), 2004.
30. C.M. Vaz, L.A.d. Graaf, W.J. Mulder, Adhesives, coatings and bioplastics from protein sources, in: S.R. Fahnestock, A. Steinbuchel (Eds.) Polyamides and Complex Proteinaceous Materials II Wiley-VCH, New York 2003, pp. 383 - 404.
31. L.A. de Graaf, P. Kolster, Industrial proteins as a green alternative for 'petro' polymers: Potential and Limitations, Macromolecular symposia, (1998) 51-58.
32. E.W. Lusas, M.N. Riaz, Soy Protein Products: Processing and use, The Journal of Nutrition, 125 (1995).
33. J. Ohren, Process and product characteristics for soya concentrates and isolates, Journal of the American Oil Chemists' Society, 58 (1981) 333-335.
34. L. Sair, Proteinaceous soy composition and method of preparing. US Patent 2,881,076., (1959).
35. D.W. Johnson, S. Kikuchi, Processing for producing soy protein isolates, in: T.H. Applewhite (Ed.) Proceeding of the World Congress on Vegetable Protein Utilization in Human Foods and Animal Feedstuffs, American Oil Chemist's Society, Champaign, IL, 1989, pp. 66-67.
36. E.L. Scott, F. Peter, J.P.M. Sanders, Biomass in the manufacture of industrial products—the use of proteins and amino acids, Applied Microbiology and Biotechnology, 75 (2007) 751-762.
37. T.M. Lammens, D. De Biase, M.C.R. Franssen, E.L. Scott, J.P.M. Sanders, The application of glutamic acid [small alpha]-decarboxylase for the valorization of glutamic acid, Green Chemistry, 11 (2009) 1562-1567.
38. T.M. Lammens, M.C.R. Franssen, E.L. Scott, J.P.M. Sanders, Synthesis of biobased N-methylpyrrolidone by one-pot cyclization and methylation of [gamma]-aminobutyric acid, Green Chemistry, 12 (2010) 1430-1436.
39. J. Le Notre, E.L. Scott, M.C.R. Franssen, J.P.M. Sanders, Biobased synthesis of acrylonitrile from glutamic acid, Green Chemistry, 13 (2011) 807-809.
40. P.M. Konst, M.C.R. Franssen, E.L. Scott, J.P.M. Sanders, A study on the applicability of l-aspartate [small alpha]-decarboxylase in the biobased production of nitrogen containing chemicals, Green Chemistry, 11 (2009) 1646-1652.
41. D.R. Chaudhary, A. Ghosh, J. Chikara, J.S. Patolia, Soil Characteristics and Mineral Nutrient in Wild Jatropha Population of India, Communications in Soil Science and Plant Analysis, 39 (2008) 1476-1485.
42. J. Lin, F. Yan, L. Tang, F. Chen, Antitumor effects of curcin from seeds of Jatropha curcas, Acta Pharmacologica Sinica, 24 (2003) 241-246.
43. J. Martinez-Herrera, P. Siddhuraju, G. Francis, G. Davila-Ortiz, K. Becker, Chemical composition, toxic/antimetabolic constituents, and effects of different treatments on their levels, in four provenances of Jatropha curcas L. from Mexico, Food Chemistry, 96 (2006) 80-89.
44. H.P.S. Makkar, K. Becker, Jatropha curcas toxicity: identification of toxic principle(s), Toxic Plants Other Nat. Toxicants, [Proc. Int. Symp. Poisonous Plants], 5th, (1998) 554-558.
45. R.K. Devappa, B. Swamylingappa, Biochemical and nutritional evaluation of Jatropha protein isolate prepared by steam injection heating for reduction of toxic and antinutritional factors, J. Sci. Food Agric., 88 (2008) 911-919.
46. H.P.S. Makkar, G. Francis, K. Becker, Protein concentrate from Jatropha curcas screw-pressed seed cake and toxic and antinutritional factors in protein concentrate, J. Sci. Food Agric., 88 (2008) 1542-1548.
47. E.M. Aregheore, K. Becker, H.P.S. Makkar, Detoxification of a toxic variety of Jatropha curcas using heat and chemical treatments, and preliminary nutritional evaluation with rats, The South Pacific Journal of Natural Science, 21 (2003) 51-56.
48. H. Makkar, K. Becker, Patent number: WO 2010/092143 A1. Method for detoxifying plant constituents, in: European Patent Office, 2010.
49. J. Brooker, The methods for detoxifying oil seed crops. WO 2010/070264 A1, in: D1 (Ed.) PCT, 2010.
50. J. Kinsella, Functional properties of soy proteins, Journal of the American Oil Chemists' Society, 56 (1979) 242-258.
51. A. Moure, J. Sineiro, H. Dominguez, J.C. Parajo, Functionality of oilseed protein products: A review, Food Res. Int., 39 (2006) 945-963.
52. P. Walstra, Functional Properties of Industrial Proteins, in: Industrial Proteins, 2003, pp. 4-6.
53. A. Moure, D. Franco, J. Sineiro, H. Dominguez, M.J. Nunez, Simulation of multistage extraction of antioxidants from Chilean hazelnut (Gevuina avellana) hulls, Journal of the American Oil Chemists Society, 80 (2003) 389-396.
54. S.P. Adupeasah, L.L. Diosady, L.J. Rubin, A Multistage Hydrocyclone Stirred-Tank System for Countercurrent Extraction of Canola Oil Journal of the American Oil Chemists Society, 70 (1993) 755-762.

Chapter 2

The optimization of phorbol ester removal from Jatropha press cake by extraction using different types of organic solvents

This chapter is submitted as D. Lestari, M. Insanu, Widyarani, W. J. Mulder, J. P.M. Sanders, M. E. Bruins. The optimization of phorbol ester removal from Jatropha press cake by extraction using different types of organic solvents.

ABSTRACT

Jatropha press cake, the by-product after biodiesel production from Jatropha seed, will be used for animal feed. To be acceptable as animal feed, the toxic phorbol ester content should be reduced below the tolerance limit of 15 ppm. Some methods have been developed to remove phorbol ester from press cake, but further process optimization is needed for pilot plant scale operation. In our research, phorbol ester extractability in eight different organic solvents was investigated. Next, the extraction process conditions were optimized by varying solvent to water ratios, extraction times, or applying multi step extractions. Using the newly developed method for Ultra High Performance Liquid Chromatography (UHPLC), phorbol ester determinations were faster with a higher sensitivity. Petroleum ether extraction was able to remove up to 60% phorbol ester in the press cake. However, only 2% phorbol ester was present in the dried extract, which may indicate potential phorbol ester degradation. The highest phorbol ester extractability was obtained after 18-hour soxhlet extraction with 80% v/v ethanol-water mixture. This treatment reduced phorbol ester content of the press cake to 14 ppm. Moreover, the brine shrimp assay toxicity test of this treated press-cake gave the value of LC_{50} > 1000 μg/ mL, which indicated non-toxicity. After two-step consecutive soxhlet extractions by 50 % v/v ethanol-water mixture and petroleum ether 60/80 followed by protein extraction by 0.055 M NaOH, protein rich fractions with undetectable amount of phorbol ester was obtained.

Keywords:
Jatropha curcas; Jatropha press cake; animal feed; detoxification; phorbol esters; organic solvent extractions

2.1 INTRODUCTION

Jatropha curcas or Jatropha plants can be grown on a very dry soil, and therefore, can be potentially used for land recovery [1-5]. Jatropha seed contains around 30-40% oil, which can be converted into biodiesel. To be economically feasible, it is needed to valorise not only Jatropha oil, but also Jatropha press cake—the by-product after biodiesel production. Specifically, Jatropha press cake protein can be used for commercial purposes to improve the total value of the seed. Research has already been conducted to extract protein from Jatropha press cake [6-8]. It is also reported that autoclaving the press cake at 121 °C for 15 min prior to protein extraction at room temperature did not affect the molecular weight distributions of the extracted protein [9]. Due to the anti-nutritional and toxic components, protein applications will be limited to technical applications [10], or—after detoxification—for animal feed [6-8].

One of the major toxic components in Jatropha seeds is phorbol ester [11-17]. Haas et.al [18] has identified six phorbol esters, which are diterpene esters with tigliane skeletons. Phorbol esters are lipophilic components and mainly present in the oil with concentrations of about 200-400 ppm [19]. After oil pressing from the whole seed (kernel and shell), the phorbol ester content of the press cake was approx. 240-720 ppm. The phorbol esters that are bound in oil or press cake are heat stable [6, 8], but can be biodegraded in soil [20]. In order to use Jatropha press cake as animal feed, phorbol ester content should be reduced to a concentration of at least below 15 ppm, at which phorbol esters did not cause negative effect on carp [12].

The methods to remove phorbol esters from Jatropha press cake have been reported in several publications. The method from Aregheore [21] was able to reduce phorbol ester content of Jatropha defatted kernel meal to approx. 95% from 1780 ppm to 90 ppm, using four-stage extraction with 92% methanol. Martinez-Herrera et al. [17] has reported a method, where approx. 98% phorbol ester content of Jatropha defatted kernel meal was reduced from 3850 ppm to 80 ppm. This method used 90%v/v ethanol-water extraction for 2 hours at room temperature, followed by treatment with 0.07% $NaHCO_3$ and autoclaving at 121°C for 20 min. After protein extraction from seed cake with alkaline solutions at pH 9-11 followed by precipitation at pH 4-5, approx. 55-58% phorbol esters were distributed into the protein isolates [6]. In other reports, the phorbol ester content of the isolated protein from kernel meal was reduced to undetectable amounts by injecting 92°C steam into the supernatant of the alkaline protein extract followed by spray drying at 180°C. However, the phorbol ester content determination was conducted with different sample preparation method, using 90% v/v methanol and petroleum ether extraction followed by solubilisation in diethyl ether [8]. In a recent patent from Makkar et al. [22], protein was extracted from defatted kernel meal using an aqueous solution of NaOH at pH 11 and precipitated by adding ethanol solution into the extract. This method was able to reduce phorbol ester content in the protein isolate to undetectable amount at an HPLC detection limit

of 10 ppm phorbol ester. Another recent patent from the company D1 [23] has claimed that simultaneous oil extraction and detoxification of the flaked seed kernel using a combination of hydrophobic-hydrophilic solvents was able to reduce the phorbol ester content to an undetectable phorbol ester content. One of the examples of a hydrophobic-hydrophilic solvent combination was an ethyl acetate-methanol mixture at 40/60 ratio, which was used to simultaneously extract oil and to detoxify the seed kernel at 62°C and 1.2 bars. The research applied the detoxification procedure on Jatropha kernel (without shells). However, in this research, the detoxification was applied on the whole press cake (with shell). Consequently, the higher fibre content of the whole press cake may affect the mass diffusion of phorbol ester during extraction.

The objectives of this research were: 1) to determine the extractability of the phorbol ester in various organic solvents of different polarities, 2) to optimize the extraction conditions, and 3) to isolate proteins from the detoxified press cake for animal feed production at a pilot plant scale. To achieve these goals, the phorbol ester extractability was investigated in eight different organic solvents, which are allowed for feed applications. Next, phorbol ester removal was optimized by varying solvent to water ratios and extraction time and applying multi-step extraction. The Ultra High Performance Liquid Chromatography (UHPLC), which has lower retention times and lower detection limit than HPLC, was used to analyse the phorbol ester content. Consequently, this will give greater speed and accuracy on the phorbol ester content determination.

2.2 MATERIALS AND METHODS

2.2.1 Materials

Jatropha press cake was obtained from the Energy Technology Centre-Agency for the Assessment and Application of Technology (B2TE-BPPT), Serpong, West Java, Indonesia. Jatropha press cake, the by-product after oil pressing from the whole seeds (shell and kernels), was milled using a Retsch ZM 1000 milling machine into particle size of 2 mm and used for protein extraction without further oil removal. Protein content of the press cake is 23 ± 1% (n=6) and the oil content is 12%.

2.2.2 The effect of organic solvent extraction to reduce phorbol ester content of the press cake

The press cake was heated in an oven at 100°C for 3 hours to reduce the moisture content of press cake and to increase the efficiency of organic solvent extraction. Eight different organic solvents with different polarities and properties were used: petroleum ether 40/60, petroleum ether 60/80, dichloromethane, toluene, diethyl ether, ethyl acetate, ethanol, and methanol. About 10 gram of the pre-heated press cakes was weighted into pre-weighted thimble. Next, about 200 ml of different organic solvents was loaded into the pre-weighted round bottom flasks. Then, the press cake was extracted with the organic solvents using soxhlet for 18 hours. The temperatures

of the soxhlet extractions were the boiling temperature of each solvent to facilitate the reflux flow. After extraction, the solvent was evaporated by a rotary evaporator. The solvent-free extracts and the extracted press cakes were dried overnight at 100°C in the oven to a constant weight and then, the dry weight of each fraction was recorded. Afterwards, the phorbol ester content and the toxicity of the dried extracts and the extracted press cake were measured.

2.2.3 The effect of time and water concentration during ethanol extraction to reduce phorbol ester content of the press cake

Press cakes were extracted according to method described in section 2.2.2. To investigate the effect of ethanol concentration, about 10 gram of pre-heated press cake was extracted with 200 ml of ethanol-water mixture at different concentrations: 15; 30; 50; 75; 80; or 85 % v/v at constant extraction time of 3 hour using a soxhlet apparatus at the boiling temperature of each solution. To investigate the effect of extraction time, about 10 gram of the pre-heated press cake was extracted with 200 ml of ethanol 80% v/v at its boiling temperature at different extraction time: 1; 3; 6; or 18 hour.

2.2.4 The effect of number of extraction at room temperature to reduce phorbol ester content of the press cake

Multi-step extraction of press cake with a 80% v/v ethanol-water mixture were conducted at room temperature for 1 hour per extraction. The number of extractions (N) was varied from 1 to 4. For 1-time extraction, 80% v/v ethanol-water mixture was added into 4 gram of press cake to a total mass of 40 gram. The slurry was mixed with a rotary mixer at room temperature. After 1 hour, the treated press cake was separated from the extract by centrifugation at 4000 rpm for 20 minutes. For N (N=1, 2, 3, or 4) time extraction, the (N+1)-time extraction step was conducted by adding 80% v/v ethanol-water mixture directly to the N-time extracted press cake after centrifugation, into a total mass of 40 gram. Then, the slurry was mixed using a rotary mixer for 1 hour at room temperature. The treated press cake was dried overnight in the oven at 40°C (PC-N), while the extract from each stage was dried by evaporating the solvent by rotary evaporator (E-N).

2.2.5 Two-step organic solvent extractions followed by protein extraction

Press cakes were extracted according to the extraction method described in section 2.2.2, with two-step extractions using two organic solvents: petroleum ether 60/80 and 50% v/v ethanol-water mixture, following two different treatments: treatment A and treatment B. In treatment A, the press cake was extracted with petroleum ether 60/80 for 18 hours followed by extraction with 50% v/v ethanol-water mixture for 3 hours ; and, in treatment B, the press cake was extracted with ethanol 50% v/v for 3 hours followed by petroleum ether 60/80 extraction for 3 hours. Afterwards, around 2 gram of the treated press cake was solubilized into 20 gram of 0.055 M NaOH solutions for 30 minutes to extract the proteins followed by centrifugation at 4000 rpm for 20 minutes to separate the crude protein extract from the (de-proteinized)

residue. The pH of the crude protein extract was brought to pH 5 by adding 4 M HCl. Next, the mixture was left to settle overnight at 4°C to precipitate the proteins and then, the precipitated proteins were separated by centrifugation at 4000 rpm for 20 minutes. The residue and protein rich fractions were dried in the oven at 60°C for 24 hours to a constant weight. Afterwards, the dry weight, protein content and the phorbol ester content were measured for each fraction. All the treatment and protein extraction were conducted in duplicate.

2.2.6 Phorbol ester content quantification

To quantify the phorbol ester content, 40 ml of methanol was added to 2 gram of press cake or the total amount of dried extracts. These samples were mixed for 30 second using vortex and sonicated for 5 minutes, and were subsequently incubated for 24 hours at room temperature. After centrifugation at 4000 rpm for 20 minutes, the extracts were concentrated into a volume of approximately 2 to 5 ml by rotary evaporator at 40°C for approximately 10 to 15 minutes. The concentrated extract was filtered with minicart RC 15 (pore size 0.2 µm), and loaded to the Ultra High Performance Liquid Chromatogaphy (UHPLC). Phorbol esters were determined on a Kinetex 2.5u C18 100A (150 x 4.6 mm) column, protected by Krudkatcher Ultra HPLC in-line filter (0.5u x 0.004 in ID) guard-column from Phenomenex. Eluents consisted of liquid A (95% water + 5% methanol + 0.1% trifluoroacetic acid) and liquid B (100% methanol + 0.1% trifluoroacetic acid). Solvent wash and rear seal wash were performed using water-methanol solution (80/20). The system was run with a gradient from 30% A to 10% A at 0 to 5 minutes. The eluent composition was kept constant from 5-18 minutes with 10% A. The four phorbol esters peaks appeared between 8.0-9.5 min and were measured at 280 nm. The amount of phorbol esters is expressed as equivalents of a standard phorbol-12-myristate 13-acetate (Sigma Aldrich) (Figure 2.1). Using this method, the UHPLC can detect as low as 1 ppm of phorbol ester in the samples.

2.2.7 Toxicity test by brine shrimp assay

Brine shrimps (*Artemia salina*) were hatched using brine shrimp eggs in a conical shaped vessel (1L), filled with sterile artificial seawater (prepared using sea salt 38 g/L and adjusted to pH 8.5 using 1N NaOH) under constant aeration for 48 h. After hatching, active *nauplii*-free from eggshells were collected from brighter portion of the hatching chamber and used for the assay. Hundred microliters of suspension of *nauplii* containing about 9-12 larvae was added into 96-well microtiter plate. The brine shrimp assay was used to determine the residual toxicity of the press cake after extraction using different organic solvents. For sample preparation, about 40 ml of methanol was added to 4 gram extracted press cake, incubated for 24 hours, and filtered to obtain the methanol extracts. Methanol was evaporated from the extracts by a rotary evaporator. The weight of the dry extracts was then measured. The dry extracts were dissolved in the sea salt water with concentrations ranging between 31.25-1000 µl/ml. Dry extracts sample (50 mg) was added to each well and incubated for 24 h. The plates were examined under a microscope (12.5× magnification) and

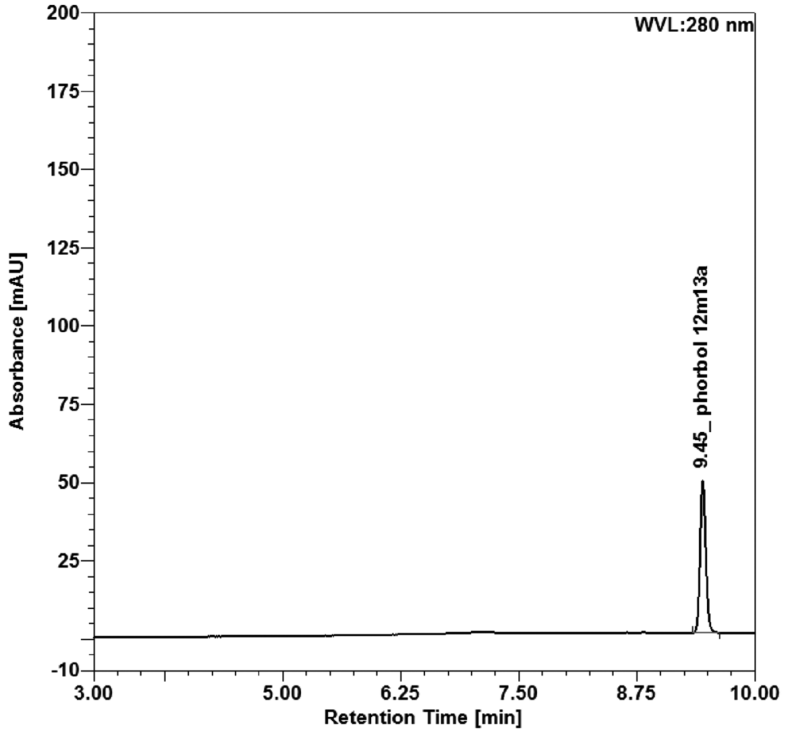

Figure 2.1 UHPLC profile of phorbol ester standard.

the number of dead *nauplii* in each well was counted. Next, about 100 µl of methanol was added. After 10 min, the total numbers of dead shrimp in each well were counted again. The value of lethal concentration 50 (LC_{50}) was calculated for each assay by taking the average of three experiments using a Finney Probit analysis [24-26].

2.3 RESULTS AND DISCUSSION

2.3.1 Dry matter and phorbol ester extractability in various organic solvents of different polarity

In general, the amount of extracted dry matter from press cake by different organic solvents was almost similar. About 13% dry matter was extracted from press cake by petroleum ether 40/60, petroleum ether 60/80, dichloromethane, toluene, ether, and ethyl acetate. Ethanol extracted around 14% dry matter, while methanol extracted around 17% dry matter from press cake after 18-hours of soxhlet extraction. Ethanol-water (80% v/v) mixture extracted more dry matter (19%) than the absolute ethanol. The amounts of extracted materials obtained with 80% v/v ethanol-water mixture were slightly increased by the increased of extraction time. About 13% material was extracted

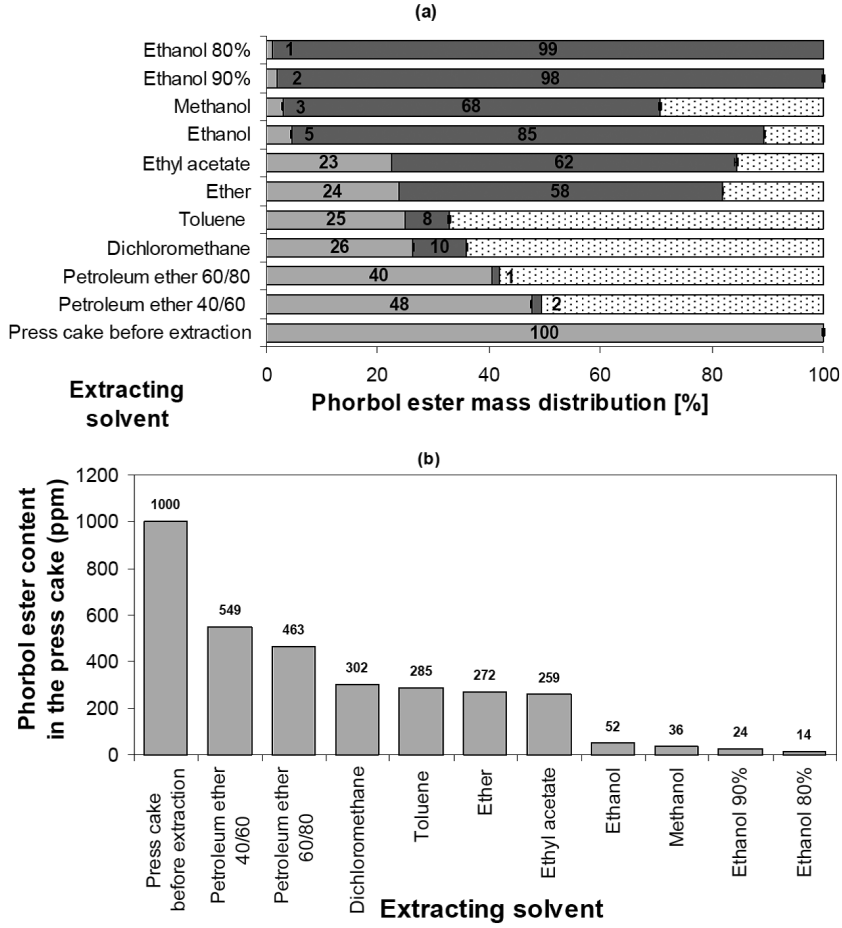

Figure 2.2 (a) Phorbol esters mass distribution and (b) phorbol ester content in the press cake after 18-hour extraction by different organic solvents. When the concentration is not mentioned, pure solvents were used. Legend: ■ Remained; ■ Extracted; ▒ Potential loss or degraded.

after 1 hour of soxhlet extraction with 80% v/v ethanol-water mixture. Extending the extraction time to 3 hours slightly increased the amount of extracted material from 13 to 18% dry matter. After 6 hour of extraction, around 20% dry matter was extracted and it remained constant until the extraction time of 18 hour. Based on visual observation, the extracted materials mainly contained residual oil, which remained in the press cake after oil pressing. There were colour differences in the extract, which indicates the presence of different components that extracted together with the residual oil. Most solvents extracted oil with clear yellow colour, while ethanol and methanol extracted oil with brown colour. The browning of the methanol and ethanol extract may have occurred due to the heating of the extracted carbohydrate.

Phorbol ester can be removed from press cake by organic solvent extraction. The effect of soxhlet extraction by different types of organic solvent (at their boiling points) on phorbol ester removal from press cake is shown in Figure 2.2. Phorbol ester extractability from press cake after 18 hours extraction was approximately 50% by petroleum ether; 75% by dichloromethane, toluene, ether, and ethyl acetate; and more than 95% by ethanol or methanol (Figure 2.2a). The residual phorbol ester content of the extracted press cake from the highest to the lowest was obtained after extraction with these following pure organic solvents: Petroleum ether 40/60 < Petroleum ether 60/80 < Dichloromethane < Toluene < Ether < Ethyl acetate < Ethanol < Methanol. Press cake had the lowest phorbol ester content after extraction with pure ethanol or methanol (52 and 36 ppm, respectively) suggesting that these were the best solvents to extract phorbol esters from press cake.

The use of ethanol-water mixture of 80 % v/v at 18-hour extraction further reduced the phorbol ester content of the treated press cake to 14 ppm (Figure 2.2b). The extractability of phorbol ester in different solvents may be influenced by the polarity of each solvent. In general, the increase of solvent polarity leads to an increase in phorbol esters extractability from press cake, except for dichloromethane, which has the polarity in between ether and ethyl acetate. In conclusion, petroleum ether (a non-polar solvent) gave the lowest phorbol ester extractability, dichloromethane, toluene, ether, and ethyl acetate (semi polar) gave medium phorbol ester extractability, while ethanol and methanol, the most polar protic solvents, gave the highest phorbol ester extractability. The UHPLC profile of press cake and extract after detoxification is shown in Figure 2.3.

2.3.2 The effect of ethanol concentration, and extraction time on residual phorbol ester content in the press cake

The effect of extraction time and ethanol concentration on the phorbol ester content in the press cake is shown in Figure 2.4. Phorbol ester content of the press cake decreased with an increase in the time of extraction. After 1 hour of soxhlet extraction with 80% v/v ethanol-water mixture at its boiling temperature, phorbol ester content was reduced from 1000 to 69 ppm. Extending the extraction time up to 6 hour reduced phorbol ester content to 16 ppm. Extending the extraction time up to 18 hours only reduced the phorbol ester content from 16 to 14 ppm (Figure 2.4a). Interestingly, phorbol ester content of the press cake was decreased by the decrease of ethanol concentration. After 3 hours of extraction, the lowest phorbol ester content in the press cake was obtained after extraction with 50% v/v ethanol-water mixture (26 ppm) (Figure 2.4b).

2.3.3 Phorbol ester removal by multi-step extraction

Multi-step ethanol extraction at room temperature

Phorbol ester removal from press cake was conducted by four extractions with 80% v/v ethanol-water mixture at room temperature for 1 hour per extraction. During the four extractions with 80% v/v ethanol-water mixture, approximately 72% mass of phorbol ester was extracted after the first extraction and was increased gradually up to 97% by the forth extraction. After four extractions at room temperature, phorbol ester content of the press cake was reduced from 1000 to 57 ppm (Figure 2.5).

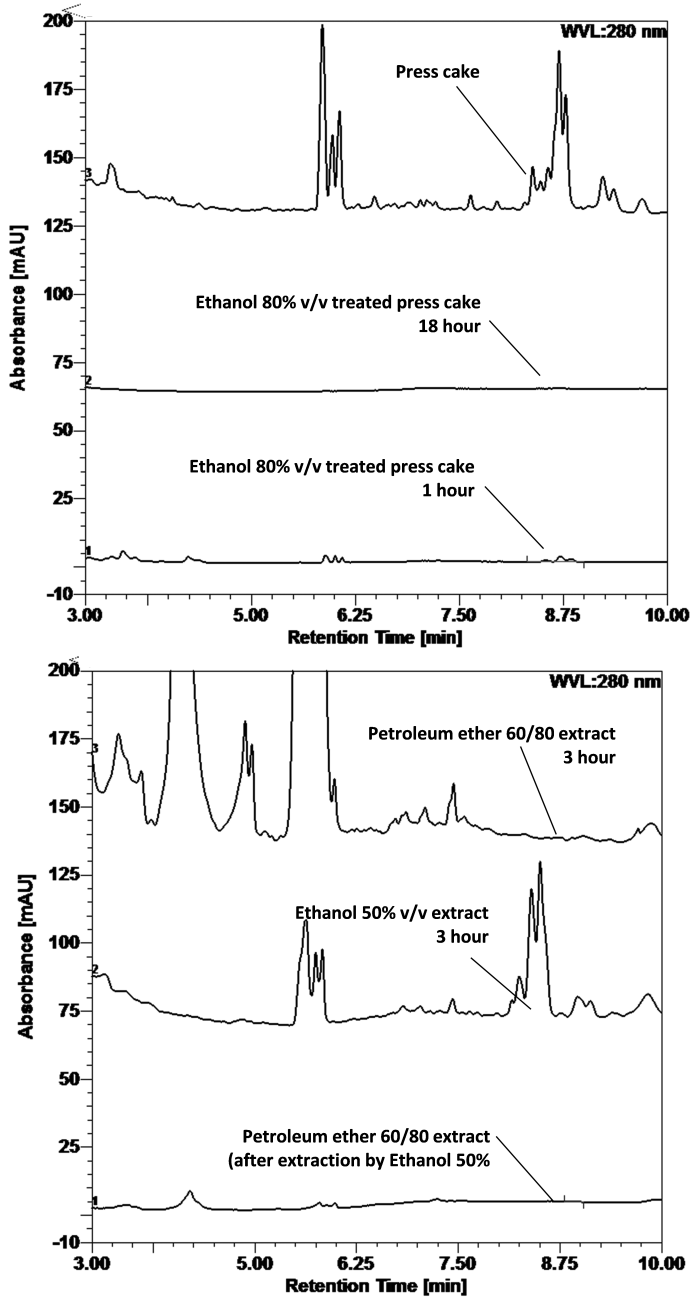

Figure 2.3 UHPLC profile of press cake and extract after detoxification. Phorbol ester retention time is between 7.5-9.0 min.

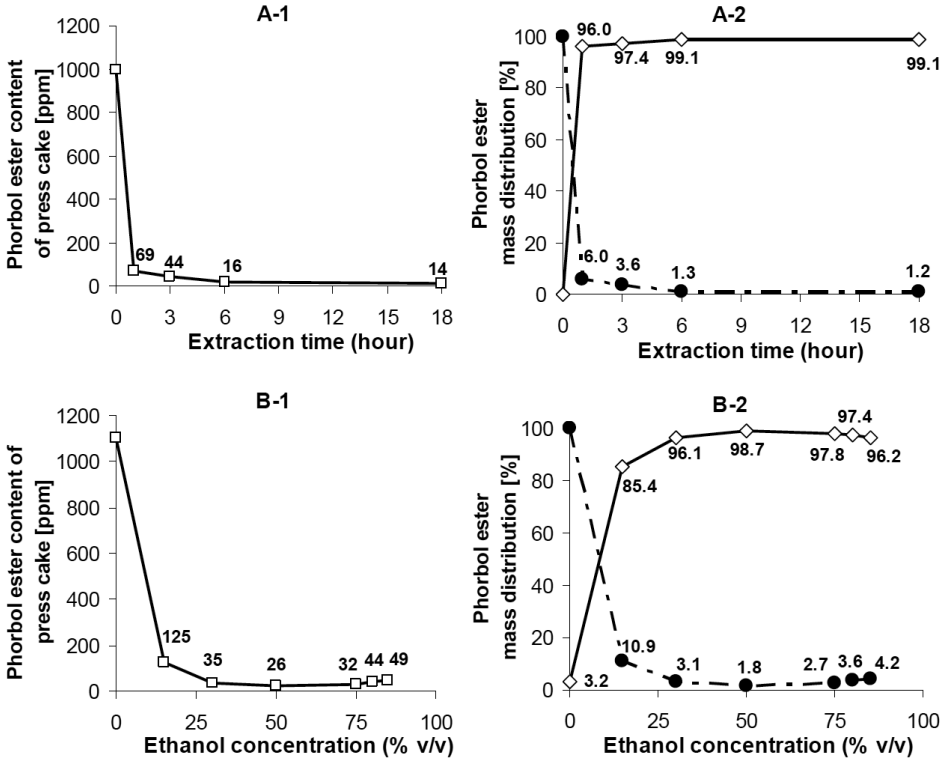

Figure 2.4 The effect of (A) extraction time after soxhlet extraction by 80% v/v ethanol at its boiling temperature and (B) ethanol concentration after 3 hour of soxhlet extraction at its boiling temperature on (1) the residual phorbol ester content of the press cake and (2) phorbol ester mass distribution. (Legend: □ ◊extracted; ● remained).

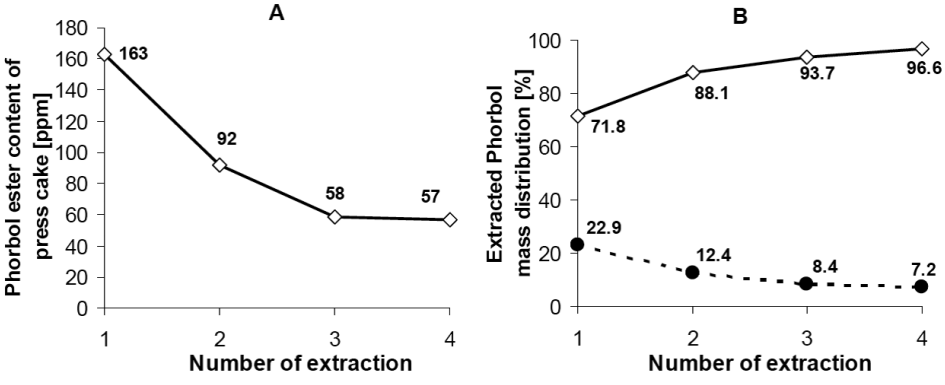

Figure 2.5 The effect of number of extraction on (A) phorbol ester content of press cake and (B) phorbol ester extractability after extraction at room temperature by Ethanol 80% v/v, each 1-hour per extraction (Legend: ◊ extracted; ● remained).

Two-step organic solvent extraction followed by protein extraction

A two-step soxhlet extraction followed by protein extraction with NaOH 0.055 M at room temperature for 30 minutes was conducted to optimize the phorbol ester removal and to produce phorbol ester free protein isolate for animal feed. Approximately 60% of the protein can be recovered after protein extraction from the untreated press cake, followed by precipitation at pH 5.5 [7]. Protein recovery was reduced after the two-step extraction by petroleum ether 60/80 and 50% v/v ethanol-water mixture. The recovery of the protein-rich fraction from the treated press cake was about two to four times lower than the recovery from the non-detoxified press cake. However, the protein-rich fraction had high(er) protein purity with undetectable amounts of phorbol ester (Table 2.1). In both treatments, the ethanol 50% v/v extraction step significantly reduced the amount of phorbol ester compared to the petroleum ether step. Phorbol ester content in the press cake was reduced to about 61 ppm after Treatment A (Figure 2.6a) and about 11 ppm after Treatment B (Figure 2.6b). The higher phorbol ester content of the press cake after Treatment A may be related to the fact that phorbol esters are oil soluble compounds. In the Treatment A, petroleum ether extraction removed the residual oil from the press cake, which may reduce the efficiency of the subsequent extraction by ethanol 50% v/v.

The protein extraction from press cake after Treatment A and Treatment B resulted in protein isolate and (protein free) residue fractions with low phorbol ester content. The phorbol ester content of the residue fractions after Treatment A (24 ppm) was higher than after Treatment B (11 ppm). Moreover, the phorbol ester was not detected in the protein isolate, produced after either Treatment A or Treatment B (Table 2.1). However, not only the phorbol ester content should be lower than the tolerance limit, but also the amount of protein to be consumed should fulfil the requirement of the animal, such as cattle or poultry. Therefore, we suggest the use of a phorbol esters to protein ratio, as a parameter to describe the potential applicability of Jatropha press cake as animal feed. Press cake or protein isolate with low phorbol ester to protein ratio is expected to fulfil the minimum protein intake for cattle or poultry, while limiting the phorbol ester intake below the tolerance limit per weight unit of cattle or poultry. The phorbol ester to protein ratio of the non-detoxified press cake was about 4.4 mg/ g proteins. After protein extraction from the non-detoxified press cake, the phorbol ester to protein ratio of the residue and protein isolate were about 1.5 and 0.6 mg/ g, respectively. After the two-step extraction, the phorbol ester to protein ratio of the press cake was reduced to around 0.26 mg/g after Treatment A and 0.05 mg/g after Treatment B (Table 2.1).

2.3.4 Toxicity test by brine shrimp assay

The residual methanol extracts after 18-hour soxhlet extraction of the press cake with different organic solvent were tested for toxicity. The results of toxicity test correlate to the amount of phorbol ester that can still be extracted from the treated press cake. These toxicity results against brine shrimp are shown in Table 2.2. High concentration of phorbol ester caused low LC_{50} values. Lagarto Parra [27] reported

Table 2.1 The protein recovery and phorbol ester content after protein extraction by using 0.055 M NaOH, followed by precipitation at pH 5.

	Dry matter recovery (%)	Protein content (%)	Protein recovery (%)	Phorbol ester content (ppm)	Phorbol esters to protein ratio[a] (mg/g)
Press cake after treatment A[b]					
Press cake	-	23.3 ± 0.3	-	60.8 ± 6.7	0.26 ± 0.03
Residue	73.6 ± 0.5	13.4 ± 0.8	42.9 ± 2.8	24.2 ± 2.2	0.18 ± 0.03
Protein isolate	9.6 ± 0.02	95.7 ± 4.3	40.1 ± 1.9	ND	ND
Press cake after treatment B[c]					
Press cake	-	22.2 ± 0.5	-	10.7 ± 0.1	0.05 ± 0.00
Residue	77.3 ± 0.8	17.4 ± 1.6	58.4 ± 5.9	10.8 ± 0.2	0.06 ± 0.00
Protein isolate	5.7 ± 1.0	93.8 ± 1.1	23.2 ± 4.4	ND	ND

[a]Phorbol ester to protein ratio is the ratio between phorbol ester amount (mg) and protein amount (g) in 1 gram of material i.e. press cake, residue, or protein isolate. [b]Treatment A: 18 hour soxhlet extraction by petroleum ether 60/80, followed by 3 hour extraction by 50% v/v ethanol. [c]Treatment B: 3 hour extraction by 50% v/v ethanol, followed by 3 hour extraction by petroleum ether 60/80

that there was also a good connectivity ($r=0.85$, $p<0.05$) between the LD_{50} of the oral toxicity in mice and LC_{50} of brine shrimp lethality assay. Nine different solvents were used for press cake extraction. The result from dichloromethane and ether extraction showed toxicity against the shrimp with LC_{50} values 31.6 and 33.3 µg/ml respectively. The methanol extract of press cake after 18-hour soxhlet extraction with 80% v/v ethanol-water mixture did not show toxicity (LC_{50} values higher than 1000 µg/ml).

2.3.5 Phorbol ester quantification by Ultra High Pressure Chromatography

With our newly developed UHPLC method, the phorbol ester standard peak has a retention time of 9.0 min (Figure 2.1). Based on this, the phorbol ester peaks of press cake and extract samples were expected to appear at retention times between 7.5 to 9.0 min, which was less than the retention time of HPLC (26 to 31 min). The sample preparation method to quantify the phorbol ester content of press cake from Makkar et.al [28] were also compared to the simplified method in section 2.5. By quantification using UHPLC, the phorbol ester content of press cake following the sample

Table 2.2 Toxicity test by brine shrimp assay on residual methanol extract of the press cake after extraction with different organic solvent.

Treatment	Extracting solvent	LC_{50} (µg/ml)
1	Ethanol 80%	>1000
2	Methanol	>1000
3	Ethyl acetate	92.6
4	Petroleum ether 40/60	43.3
5	Ether	33.3
6	Toluene	42.3
7	Dichloromethane	31.6
8	Ethanol 90%	55.3
9	Ethanol	56.2

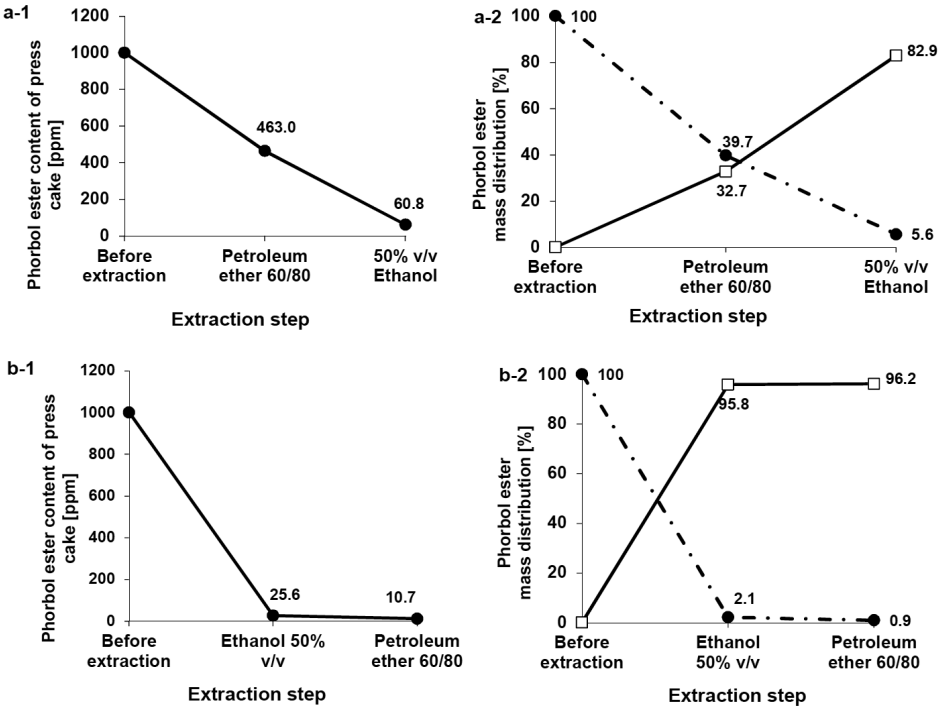

Figure 2.6 The effect of two-step extractions on (1) phorbol ester content of press cake and (2) phorbol ester extractability. (a) Treatment A, 18 hour Petroleum ether 60/80 soxhlet extraction, followed by 3 hour 50% v/v Ethanol soxhlet extraction, (b) Treatment B, 3 hour 50% v/v Ethanol soxhlet extraction, followed by 3 hour Petroleum ether 60/80 soxhlet extraction (Legend: □ extracted; ● remained).

preparation method from Makkar et.al [28] was 539 ± 26.9 ppm (n=2). This was slightly higher than the previously reported phorbol ester content in press cake of 460 ± 20 ppm [6]. By using the simplified method in section 2.5 and quantification by UHPLC, the phorbol ester content of press cake was 640 ± 85.7 ppm, which is also slightly higher than the phorbol ester content of press cake by using sample preparation method from Makkar [28]. This indicated that phorbol ester quantification by UHPLC not only has lower retention time but also a higher sensitivity and then HPLC, due to the higher-pressure column. In addition, the used of the simplified method will reduce the potential phorbol ester losses due to the multiple handling steps and reduce the sample preparation time, while maintaining accuracy.

2.4 CONCLUSION

Based on the results, we conclude that phorbol ester extractability in different types of organic solvents increased with the increase of solvent polarity. Phorbol ester extractability is highest with ethanol and methanol. By using 80% v/v ethanol-water,

the phorbol ester content was reduced from 1000 to 16 ppm after 6 hours of extraction. Extending the extraction time to 18 hours only slightly reduces the phorbol ester content to 14 ppm, which is lower than the minimum tolerance limit of 15 ppm. The methanol extract of press cake after 18 hours soxhlet extraction with 80% v/v ethanol-water mixture gave LC_{50} values higher than 1000 µg/ml, which indicates non-toxicity of the press cake for the shrimp larvae. After the optimization of ethanol-water concentration, 50% v/v ethanol gave the best results. After the two-step extraction by 50 % v/v ethanol and petroleum ether 60/80 followed by protein extraction by 0.055 M NaOH, the phorbol ester content of the residue fractions after Treatment A (24 ppm) was higher than after Treatment B (11 ppm). Moreover, the presence of phorbol ester was not detected in the protein isolate after protein extraction after Treatment A and Treatment B.

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REFERENCES

1. K. Openshaw, A review of Jatropha curcas: an oil plant of unfulfilled promise, Biomass Bioenerg., 19 (2000) 1-15.
2. E.T. Akintayo, Characteristics and composition of Parkia biglobbossa and Jatropha curcas oils and cakes, Bioresource Technology, 92 (2004) 307-310.
3. J. Heller, Physic nut : Jatropha curcas L. Promoting the conservation and use of underutilized and neglected crops.1 Institute of Plant Genetics and Crop Plant Research, Gatersleben/ International Plant Genetic Resources Institute, Rome Gaterslaben, 1996.
4. G.M. Gubitz, Exploitation of the tropical oil seed plant Jatropha curcas L, Bioresource technology, 67 (1999) 73.
5. R.E.E. Jongschaap, W.J. Corre, P.S. Bindraban, W.A. Brandenburg, Claims and Facts on Jatropha curcas L. , in, Plant Research International B.V., Wageningen UR, Wageningen, 2007.
6. H.P.S. Makkar, G. Francis, K. Becker, Protein concentrate from Jatropha curcas screw-pressed seed cake and toxic and antinutritional factors in protein concentrate, J. Sci. Food Agric., 88 (2008) 1542-1548.
7. D. Lestari, W.J. Mulder, J.P.M. Sanders, Improving Jatropha curcas seed protein recovery by using counter current multistage extraction, Biochemical Engineering Journal, 50 (2010) 16-23.
8. R.K. Devappa, B. Swamylingappa, Biochemical and nutritional evaluation of Jatropha protein isolate prepared by steam injection heating for reduction of toxic and antinutritional factors, J. Sci. Food Agric., 88 (2008) 911-919.
9. A. Marasabessy, M. Moeis, J.P.M. Sanders, R.A. Weusthuis, Enhancing Jatropha oil extraction yield from the kernels assisted by a xylan-degrading bacterium to preserve protein structure, Applied Microbiology and Biotechnology, 90 (2011) 2027-2036.
10. D. Lestari, W.J. Mulder, J.P.M. Sanders, Jatropha seed protein functional properties for technical applications, Biochemical Engineering Journal, 53 (2011) 297-304.
11. R.K. Devappa, H.P.S. Makkar, K. Becker, Jatropha Toxicity--A Review, Journal of Toxicology and Environmental Health, Part B: Critical Reviews, 13 (2010) 476 - 507.
12. G. Goel, H.P.S. Makkar, G. Francis, K. Becker, Phorbol esters: Structure, biological activity, and toxicity in animals, Int. J. Toxicol., 26 (2007) 279-288.
13. D. Huang, D. Huang, S. Guo, K. Pan, Z. Huang, J. Lin, Isolation and characterization of toxin

from Jatropha curcas, Shengwu Huaxue Yu Shengwu Wuli Jinzhan, 18 (1991) 149-151.

14. J. Lin, F. Yan, L. Tang, F. Chen, Antitumor effects of curcin from seeds of Jatropha curcas, Acta Pharmacologica Sinica, 24 (2003) 241-246.
15. H.P.S. Makkar, A.O. Aderibigbe, K. Becker, Comparative evaluation of non-toxic and toxic varieties of Jatropha curcas for chemical composition, digestibility, protein degradability and toxic factors, Food Chemistry, 62 (1998) 207-215.
16. H.P.S. Makkar, K. Becker, Jatropha curcas toxicity: identification of toxic principle(s), Toxic Plants Other Nat. Toxicants, [Proc. Int. Symp. Poisonous Plants], 5th, (1998) 554-558.
17. J. Martinez-Herrera, P. Siddhuraju, G. Francis, G. Davila-Ortiz, K. Becker, Chemical composition, toxic/antimetabolic constituents, and effects of different treatments on their levels, in four provenances of Jatropha curcas L. from Mexico, Food Chemistry, 96 (2006) 80-89.
18. W. Haas, H. Sterk, M. Mittelbach, Novel 12-Deoxy-16-hydroxyphorbol Diesters Isolated from the Seed Oil of Jatropha curcas, Journal of Natural Products, 65 (2002) 1434-1440.
19. H.P.S. Makkar, K. Becker, F. Sporer, M. Wink, Studies on Nutritive Potential and Toxic Constituents of Different Provenances of Jatropha curcas, Journal of Agricultural and Food Chemistry, 45 (1997) 3152-3157.
20. R.K. Devappa, H.P.S. Makkar, K. Becker, Biodegradation of Jatropha curcas phorbol esters in soil, J. Sci. Food Agric., 90 (2010) 2090-2097.
21. E.M. Aregheore, K. Becker, H.P.S. Makkar, Detoxification of a toxic variety of Jatropha curcas using heat and chemical treatments, and preliminary nutritional evaluation with rats, The South Pacific Journal of Natural Science, 21 (2003) 51-56.
22. H. Makkar, K. Becker, Patent number: WO 2010/092143 A1. Method for detoxifying plant constituents, in: European Patent Office, 2010.
23. J. Brooker, The methods for detoxifying oil seed crops. WO 2010/070264 A1, in: D1 (Ed.) PCT, 2010.
24. G.N. Wanyoike, S.C. Chhabra, C.C. Lang'at-Thoruwa, S.A. Omar, Brine shrimp toxicity and antiplasmodial activity of five Kenyan medicinal plants, Journal of Ethnopharmacology, 90 (2004) 129-133.
25. B.N. Meyer, N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols, J.L. McLaughlin, Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents, Planta Med, 45 (1982) 31,34.
26. A.V. Krishnaraju, T.V.N. Raoa, D. Sundararajua, M. Vanisreeb, H.-S. Tsayb, G.V. Subbarajua, Assessment of Bioactivity of Indian Medicinal Plants Using Brine Shrimp (Artemia salina) Lethality Assay, International Journal of Applied Science and Engineering, 3 (2005) 125-134.
27. A. Lagarto Parra, R. Silva Yhebra, I. Guerra Sardiñas, L. Iglesias Buela, Comparative study of the assay of Artemia salina L. and the estimate of the medium lethal dose (LD50 value) in mice, to determine oral acute toxicity of plant extracts, Phytomedicine, 8 (2001) 395-400.
28. H. Makkar, P. Siddhuraju, K. Becker, Plant Secondary Metabolites, Humana Press, Totowa, 2007.

Chapter 3

Improving *Jatropha curcas* seed protein recovery by using counter current multistage extraction

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ABSTRACT

Jatropha seed press cake contains 23 wt. % protein (dry basis). Due to the toxic compounds in Jatropha, we will use the protein for non-food applications. Related to non-food applications, an efficient protein extraction to obtain a high protein recovery and high protein concentration with good protein functional properties is required. To achieve this, we conducted protein extraction at room temperature by using different solvents at various solvent to solid ratios and performed multistage counter current extraction to improve protein recovery and protein concentration. We obtained the highest protein recovery of 82% by using 0.055 M NaOH at solvent to solid ratio of 10 g/g after four-stage counter current extraction. By using 0.055 M NaOH extraction at lower solvent to solid ratio of 4 g/g, protein recovery was 35% after one-stage extraction and improved up to 71% with a high protein concentration of 63mg/mL after four-stage counter current extraction. The highest amount of protein was precipitated from crude extracts at pH 4–5.5 and resulted on precipitate with protein content of 70%. The multistage counter current extraction did not influence Jatropha seed protein molecular weight distributions, which were within the range of 3–98 kDa.

Keywords:
Jatropha curcas; Counter current extraction; Protein recovery; Molecular weight distribution; Precipitation; Biorefinery

3.1 INTRODUCTION

Jatropha curcas (Jatropha) is an oilseed crop, which is grown mainly for oil production. Besides oil, Jatropha seed kernel contains approximately 25-30% protein [1,2]. After oil removal, proteins will remain in the Jatropha cake. Jatropha seed protein may have similarities with the other well-known oilseed protein such as soy, Canola, or sunflower protein. In contrast to soy and sunflower, Jatropha seed contains toxic compounds such as curcin [3] and phorbol esters [4,5], which makes Jatropha protein unsuitable for food applications. However, the use of Jatropha protein in non-food applications is a potential outlet. Possible non-food applications of proteins are in the field of adhesives, coatings, chemicals [6,7,8], fertilizer, such as seed press cake fertilizer [1] and amino acid chelated micronutrient fertilizer [9].

Indonesia has a high potential for growing Jatropha due to the large land availability. Based on the Indonesian Presidential Regulation No.5/2006 [10], Jatropha oil, together with palm oil, will be used as biofuel to provide 5% of the total energy supply in Indonesia by 2025. Considering this, there will be approximately 30 million ton/year of Jatropha seed to produce oil and that will result ca. 20 million ton/year of seed press cake as waste. This waste contains approximately ca. 5 million ton /year of Jatropha protein— a high amount that will be highly profitable to process further into a higher added value product.

Industrial proteins such as soy protein concentrate (48–70% protein) is produced by removal of non-protein constituents under neutral or acid medium. While, protein extraction, e.g. in alkaline medium followed by isoelectric precipitation, produces protein isolates—containing protein with more than 90% purity [11,12]. Either laboratory scale or large-scale extraction often uses water with sodium or potassium hydroxide, to modify pH, to extract oilseed protein e.g. soy, canola, corn [13,14]. Therefore, the same approach will be applied on *Jatropha* seed protein extraction. Makkar et al. [15] has performed Jatropha seed protein extraction in alkaline conditions followed by isoelectric precipitation. Protein concentrates obtained by Jatropha seed press cakes solubilisation at pH 11 for 1 h at 60°C followed by precipitation at pH 4 have protein recovery of over 53% of the total proteins presents in the seed press cake [15].

Higher protein recoveries can be obtained by extraction at a higher pH and/or performing a multistage extraction, which consists of cross-flow extraction and counter current extraction [16,17]. Besides high protein recovery, good functional properties— such as molecular weight distribution, solubility, foaming properties, film-forming properties, and emulsion properties— are required for proteins for technical / non-food application. For these reasons, the extracted proteins should not be hydrolysed or denatured and moderate extraction temperature is preferable. Therefore, the objectives of this research are both improving the recovery of the Jatropha seed protein and maintaining its functionality by optimizing protein extraction at room temperature by using multistage extraction. In relation to these

objectives, we conducted a series of experiments to answer several research questions. These questions are: 1) What is the most suitable solvent to extract protein from Jatropha seed; 2) How to improve protein recovery by using multistage extraction; 3) What is the protein recovery and its purity; and finally 4) Is there any effect of process on protein physical properties e.g. molecular weight distribution.

3.2 MATERIALS AND METHODS

3.2.1. Raw materials

Jatropha seeds were obtained from Subang plantation, West Java, Indonesia, while Jatropha whole seed press cakes were obtained from B2TE-BPPT, Serpong, West Java, Indonesia.

3.2.2. Non-defatted seed kernel

Jatropha seed kernels were manually separated from the husk. The seed kernels were milled by using a Retsch ZM 1000 milling machine into particle size of 2 mm. Protein content of the seed kernel is 24.42 ± 0.17 % (n=4) and the oil content is 56% (no oil removal prior to protein extraction).

3.2.3. Seed press cake

Jatropha seed press cake was produced after oil pressing from the whole seeds (husks and kernels) by using screw-press. The press cake was milled by using a Retsch ZM 1000 milling machine into particle size of 2 mm. Protein content of the press cake is 23.14 ± 0.92 % (n=4) and oil content is 12%.

3.2.4. One-stage extraction

Extraction of both seed kernels and whole seed press cakes were carried out by using 2 g of raw material and various solvents to solid ratio of 20 g/g for 30 minutes in 50 mL capped centrifuge tubes. The mixing was conducted at room temperature by using a rotary mixer. The extracting solvents were water, NaCl (0.1, 0.55, and 1.0 M), and NaOH (0.01 M, 0.055 M, and 0.1 M). Solid –liquid separation was conducted by HERMLE Z300 centrifuge at 4000 rpm.

3.2.5. Cross-flow extraction

Three stages of cross-flow extraction of both seed kernels and whole seed press cakes was carried out by using 2 g of raw material and 0.055 M NaOH at solvent to solid ratio of 20 g/g for 30 minutes in 50 mL capped centrifuge tubes as shown in Figure 3.1. The mixing was conducted at room temperature by using a rotary mixer. Solid –liquid separation was conducted by HERMLE Z300 centrifuge at 4000 rpm. Protein molecular weight distribution was analysed from the extract from each stage. Extract from each stage was freeze-dried and protein content was analysed.

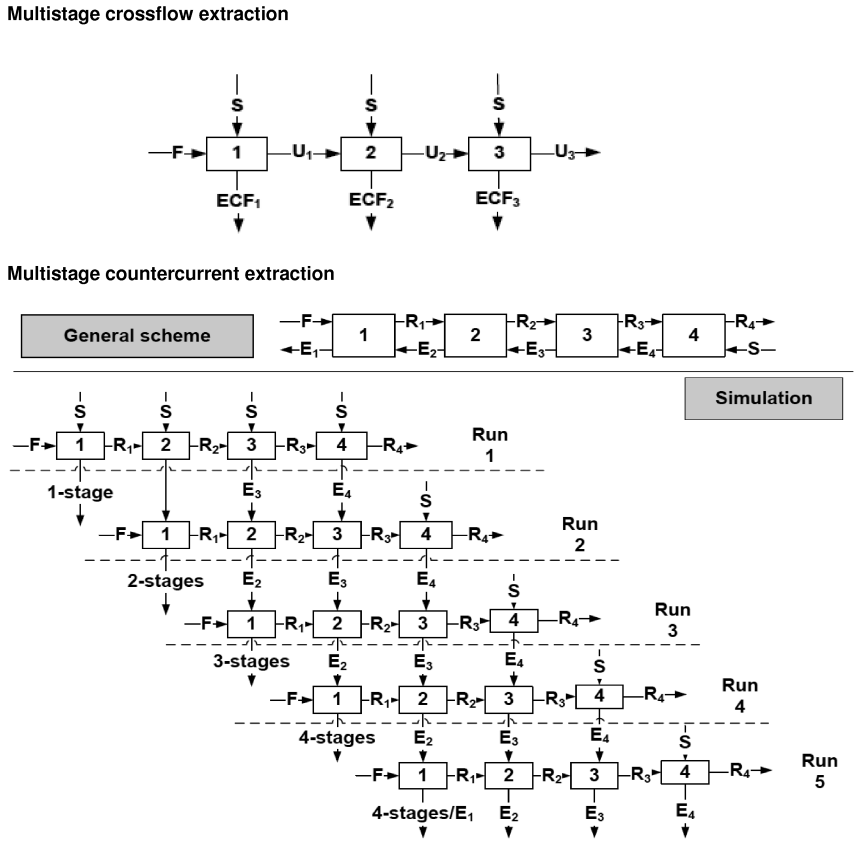

Figure 3.1 Cross-flow extraction in three stages (F = feed Jatropha seed kernel or press cake; S = fresh solvent; U= residue; ECF = extract cross-flow) and four stages counter current extraction of Jatropha seed kernel and press cake protein (F = feed Jatropha seed press cake; S = fresh solvent; R = residue; E = extract flow counter current).

3.2.6. Simulation of a multistage, continuous counter current extraction

Three stages of cross-flow extraction of both seed kernels and whole seed press cakes was carried out by using sequences of batch extractions modified from the four stages counter current extraction reported by Adu Peasah and Moure [16,17]. The scheme of four stages counter current extraction is shown in Figure 3.1. Each batch consisted of 2 g of raw material and 0.055 M NaOH at solvent to solid ratio of 20 g/g for 30 minutes in 50 mL centrifuge tubes. The mixing was conducted at room temperature by using a rotary mixer. The pH of the solution after a short time of mixing was re-adjusted between 12 and 12.7 (initial pH of 0.055 M NaOH) by using 1.0 and 2.0 M NaOH. Solid –liquid separation was conducted by HERMLE Z300 centrifuge at 4000 rpm. An aliquot of the extract from each stage was analysed for protein molecular weight distributions. Extract from each stage was freeze-dried and protein content was analysed.

3.2.7. Protein recovery from crude extracts

After protein extraction from the seed kernel, protein was recovered from the crude extracts by precipitation at pH 2; 3; 4; 5.5; and 7. The solutions were kept in a cold room of 4°C overnight and centrifuged (HERMLE Z300) at 4000 rpm for 20 minutes. The resulted precipitates were dialysed for 2 x 24 hour and freeze-dried.

3.2.8 Protein analysis and characterization

Protein molecular weight distribution (MWD) of crude extracts and protein precipitates was investigated by using SDS-PAGE (NuPage Electrophoresis System with NuPage Novex Bis-Tris Gels 10% from Invitrogen). We measured protein content for all samples—Jatropha seed kernel, flour, freeze-dried crude extract, and protein precipitate by using Kjeldahl method, which consist of destruction unit (Gerhardt Kjeldahlterm*)* and distillation unit (Gerhardt Vapodest). Calculation of the dry matter recovery and protein recovery from are shown in Equation (3.1) and Equation (3.2).

$$\text{Dry matter recovery [\% w/w]} = \frac{\textit{Dry matter weight in the extract or residue}}{\textit{Initial dry matter weight}} \times 100\% \quad (3.1)$$

$$\text{Protein recovery [\% w/w]} = \frac{\%\,\textit{protein content} \times \textit{Dry matter weight in the extract or residue}}{\%\,\textit{protein content} \times \textit{Initial dry matter weight}} \times 100\% \quad (3.2)$$

3.3 RESULTS AND DISCUSSION

3.3.1. One-stage protein extraction

Protein extraction from non-defatted seed kernel and seed press cake was performed at room temperature to prevent possible negative effects of heat treatment during extraction. Table 3.1 shows the effect of extraction parameters, particularly solvent types and molarities. In general, dry matter and protein recovery of the extracted material from seed press cake were lower than from kernel. Lower recovery from seed press cake may be due to the higher amount of non-extractable components, such as fibres from the husk. Because of that, selectivity of protein extraction from seed press cake was higher than from kernel. Therefore, protein content of the extracted material from seed press cake was also higher than from kernel.

In Table 3.1, it is shown that by using water as extracting solvent, the recovery of the total extracted material from seed press cake (7.6 %) was lower than from kernel (28.2%). Protein content of the extracted material from seed press cake (22.8 %), however, was higher than from kernel (13.5 %). In general, protein recovery from seed press cake (7.0 %) and kernel (14.6 %) obtained by water extraction was lower than by other extracting solvents. These results indicated that water was not a good extracting solvent to extract proteins from Jatropha kernel or seed press cake.

We also investigated the effect of salt on protein extraction (Table 3.1). Extraction by using NaCl 0.1 M gave a slightly higher protein recovery from kernel (16.8 %) and seed press cake (9.4 %) than extraction by using water. The increase of NaCl concentration from 0.1 M to 1.0 M improved protein recovery from both kernel and seed press cake up to about twofold. The use of NaCl solutions on protein extraction, however, gave disadvantage because salt was incorporated into the extract and decreased protein content. Therefore, further purification such as dialysis was needed. This will cause a significant increase in production cost and, therefore, Jatropha protein extraction by using NaCl solution seems less interesting for industrial scale.

The highest total extracted material and protein recovery, from Jatropha kernel and seed press cake, were obtained by using alkaline solutions (Table 3.1). Protein recoveries from kernel and seed press cake obtained by 0.01 M NaOH were up to three- and fivefold higher than protein recovery obtained by water extraction. Extraction by using higher concentrations of NaOH improved both total extracted material and protein recovery. Extraction by using 0.055 M NaOH improved both protein recoveries from kernel (69.6%) and seed press cake (64.9%) for about twofold higher than protein recovery obtained by 0.01M NaOH. We observed that an increase of NaOH concentration from 0.055 to 0.1 M resulted in a lower protein recovery. This may indicate that some proteins were denatured at NaOH concentration of 0.1 M NaOH that reduced total protein solubility in the crude extract. Therefore, the highest protein recovery, from kernel and seed press cake, was obtained from extraction by using 0.055M NaOH. In general, protein content of the extracted material from seed press cake after 0.055 M NaOH extraction was considerably high (53.3%). Based on these results, NaOH 0.055 M may be a good solution to extract Jatropha seed protein

Table 3.1 Dry matter recovery, protein content, and protein recovery of Jatropha seed kernel and press cake extracts after one-stage extraction by using different solvents and molarities at T=20◦C and extraction time of 30 min/stage.

	Dry matter recovery [%]		Protein content [%]		Protein recovery [%]	
Extracting Solvent	**Kernel**	**Seed Press Cake**	**Kernel**	**Seed Press Cake**	**Kernel**	**Seed Press Cake**
Water	28.2 ± 0.6	7.6 ± 0.9	13.5 ± 2.5	22.8 ± 0.7	14.6 ± 2.5	7.0 ± 0.9
NaCl[a]						
1.0 M[a]	144.8 ± 9.9	103.5 ± 0.8	5.9 ± 0.7	4.6 ± 0.4	33.9 ± 4.3	18.7 ± 1.4
0.55 M[a]	82.8 ± 3.7	64.8 ± 0.1	7.4 ± 0.1	6.9 ± 0.2	23.6 ± 1.1	14.6 ± 3.0
0.1 M[a]	36.8 ± 0.6	18.2 ± 0.2	11.9 ± 0.2	13.5 ± 0.6	16.8 ± 0.3	9.4 ± 0.4
NaOH						
0.1 M	57.0 ± 3.8	29.1 ± 0.8	30.2 ± 3.5	51.5 ± 1.2	65.9 ± 6.2	60.6 ± 1.1
0.055 M	50.7 ± 0.9	30.1 ± 2.2	35.8 ± 0.6	53.3 ± 2.0	69.6 ± 2.4	64.9 ± 5.8
0.01 M	32.9 ± 2.5	16.4 ± 0.4	34.0 ± 2.8	48.1 ± 0.6	42.7 ± 2.9	32.0 ± 0.7

[a] Extracts are dried without dialysis, extracted material and protein content include salt.

at a larger scale. Similar to other common oilseed proteins, such as soy and rapeseed protein, Jatropha seed protein had higher extractability in alkaline conditions. This result was comparable with the result on Jatropha seed protein extraction obtained by Makkar et al. [15] and Devappa et al. [18].

Jatropha seed proteins extractability was lower in water and NaCl solutions than in NaOH solutions, because NaOH solutions provided sufficient amount of alkaline needed to increase pH of extracting solution and promoted protein-solvent interaction. Protein-solvent interaction influences protein solubility and protein extractability in a specific solvent. Protein-solvent interaction can be improved by adjusting the pH of extracting solvent. As the pH of extracting solvent move further from the protein isoelectric point, protein net charge increases and leads to the increase of electrostatic repulsion and hydration of charged residue, which will promote protein solubilisation into the extracting solvent [19].

On the other hand, salts increases ionic strength of the solution and, as a result, protein solubility becomes higher. This explained why NaCl solutions extracted Jatropha seed cake proteins better than only plain water. However, the improvement was not as much as if we used NaOH solutions to extract protein from Jatropha seed cake.

Molecular weight distribution of the protein extracted by using NaOH and NaCl is shown in Figure 3.2. In general, at least 25 protein bands were present in Jatropha seed protein. The highest protein intensities were shown at molecular weights of 48, 40, 38, 37, 35, 36, 22, 23, 12, and 5 kDa. Increasing the NaOH concentration from 0.01 to 0.055 M significantly increased the amount of the extracted protein. Further increasing of the NaOH concentration to 0.1 M caused clear hydrolysis of the proteins. Increase of concentration of NaCl from 0.1 to 0.55 M gave only a slightly increase of protein intensity at molecular weight of 48 and 60 kDa. In addition, the intensity of protein with molecular weight of 29 kDa in NaCl crude extract was much higher than that in NaOH crude extract. This indicated that protein with molecular weight of 29 kDa might have converted into another compound under alkaline conditions.

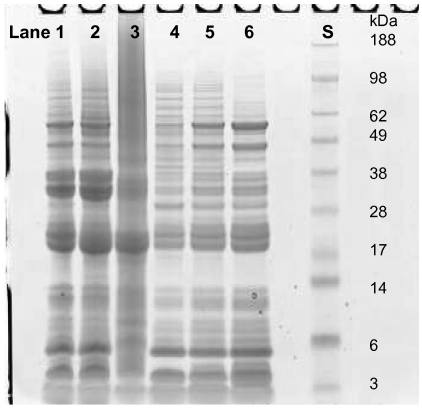

Figure 3.2 Protein molecular weight distributions of Jatropha seed kernel extracts after extraction at different concentrations of various solvents. Solvents:NaOH(1) 0.01M; (2) 0.055M; (3) 0.1M; and NaCl of (4) 0.1M; (5) 0.55M; (6) 1.0M; lane (S) protein marker

3.3.2. Three-stage cross-flow and counter current protein extraction

In order to improve protein recovery of a one-stage extraction, three-stage extractions were carried out for both non-defatted Jatropha kernel and seed press cake by using cross-flow extraction and counter current extraction (Figure 3.1). Compared to one-stage extraction, a three-stage cross-flow extraction at solvent to solid ratio of 20 g/g increased the protein recovery from both kernel (from 68.2 to 76.0%) and seed press cake (from 67.1 to 75.8%) (Table 3.2). By using a three-stage counter current extraction, the total protein recovery (E-1) of kernel crude extract (72.3%) was slightly lower than total protein recovery obtained by using cross-flow extraction. For Jatropha seed press cake extraction, the total protein recovery from counter current extraction (75.6%) gave almost similar results as the cross-flow protein recovery. Both three-stage of cross-flow extraction and counter current extraction gave a similar improvement of the total protein recovery from kernel and seed press cake extraction. These results may indicate that in both cases, extraction equilibrium has been reached and proteins have been extracted at its maximum value. The three-stage counter current extraction, however, used lower amount of solvent than in the cross-flow extraction. This reduce in solvent amount may lead to a higher process efficiency and lower operational cost.

Table 3.2 Dry matter recovery, protein content, and protein recovery of Jatropha seed kernel and press cake extracts during three-stage extraction with 0.055M NaOH at T=20◦C and extraction time of 30 min/stage.

	Dry matter recovery [%]		Protein content [%]		Protein recovery [%]	
Flow	**Kernel**	**Seed Press cake**	**Kernel**	**Seed Press cake**	**Kernel**	**Seed Press cake**
3 stages cross-flow (solvent to solid ratio 20 g/g)						
ECF-1	50.7 ± 0.1	30.1 ± 1.7	35.8 ± 1.6	53.3 ± 0.0	69.6 ± 3.3	64.9 ± 3.7
ECF-2	13.1 ± 0.6	10.2 ± 0.2	13.0 ± 3.9	18.8 ± 0.4	6.6 ± 2.3	7.8 ± 0.3
ECF-3	8.2 ± 0.3	6.8 ± 0.4	3.7 ± 0.3	3.1 ± 0.3	1.2 ± 0.1	0.9 ± 0.1
Σ ECF	72.0 ± 0.3	47.0 ± 0.8			77.3 ± 1.9	73.6 ± 1.4
U-3	30.2 ± 2.3	63.5 ± 1.2	10.8 ± 0.4	4.6 ± 0.2	12.5 ± 1.4	11.9 ± 0.4
Total	**102.2 ± 1.3**	**110.6 ± 1.0**			**89.9 ± 1.7**	**85.5 ± 0.9**
3 stages counter current (solvent to solid ratio 20 g/g)						
E-3	9.6 ± 0.5	7.0 ± 0.4	3.7 ± 1.7	4.1 ± 0.6	1.4± 0.7	1.2 ± 0.1
E-2	18.2 ± 0.6	13.9 ± 2.2	13.0 ± 3.0	22.2 ± 0.2	9.1± 2.0	12.5 ± 2.0
E-1	59.7 ± 1.7	34.4 ± 2.8	31.6 ± 0.5	54.1 ± 1.6	72.3± 1.0	75.6 ± 7.2
R-3	34.0 ± 4.3	64.2 ± 0.1	11.0 ± 1.5	5.6 ± 0.6	14.5± 3.8	14.5 ± 1.5
Total	**93.7 ± 3.0**	**98.6 ± 1.5**			**86.9 ± 2.4**	**90.1 ± 4.3**

3.3.3. The effect of solvent to solid ratio on one-stage protein extraction from press cake

One of the important aspects on protein extraction at a large scale is to use as low amount of solvent as possible, to obtain a high protein recovery at the highest protein concentration in the crude extracts. The use of low solvent amount reduces production cost and equipment cost, which finally increase the overall process efficiency. To achieve these goals, we performed experiments to extract protein from seed press cake at different solvent to solid ratio. The results are shown in Figure 3.3. We observe that total extracted material and protein recovery decreased by reducing solvent to solid ratio. By reducing solvent to solid ratio from 20 to 10 g/g, there was no effect on the total amount of extracted material, while protein recovery slightly decreased from 68.3 to 65.9%. However, a further reduction of solvent to solid ratio from 10 to 4 g/g decreased the total extracted material (from 27.4 to 14.6%) and protein recovery (from 68.3 to 35.3%) from seed press cake to about two times lower. However, protein concentration in the crude extract at solvent to solid ratio of 4 g/g (33 mg/mL) was about two times higher than protein concentration at solvent to solid ratio of 10 g/g (16 mg/mL) and about four times higher than protein concentration at solvent to solid ratio of 20 g/g (8 g/mL).

During solvent extraction, solvent to solid ratio affected the ease of protein diffusion from raw material into the solution and the saturation concentration. To achieve the optimum extraction rate, solvent amount should be high enough to facilitate better diffusion and to ensure that equilibrium concentration will be below the saturation concentration. Based on our observation, protein diffusion during Jatropha seed extraction became more limited at low solvent to solid ratio and finally reduced protein extractability. Related to equipment size, the lower solvent amount or the higher protein concentration, the smaller equipment size will be needed to extract the proteins. If we can find a way to improve the total protein recovery in the crude extract, the use of low solvent to solid ratio, e.g. 4 g/g may be a good option for Jatropha seed protein extraction at a larger scale.

3.3.4. The effects of solvent to solid ratio on four-stages counter current protein extraction

To improve protein recovery at lower solvent to solid ratio, we performed four-stage counter current extraction as shown in Figure 3.1. Due to counter current extraction, fresh seed press cake (F) entered the first stage and contacted with solvent from the second stage (E-2), which contained a high protein concentration from the previous extraction. In the meantime, the fresh solvent (S) entered from the opposite direction at the fourth stage and contacted with seed press cake residue from the third stage (R-3), which already had a low protein content after extraction at the previous stage. As a result, protein concentration of extracting solvent increased during extraction from the fourth stage and reached the highest at the outlet from the first stage (E-1).

From Table 3.3, protein recovery was increased gradually from E-4 to E-1 at solvent to solid ratio of 10 g/g. However, we also observed that the total extracted material

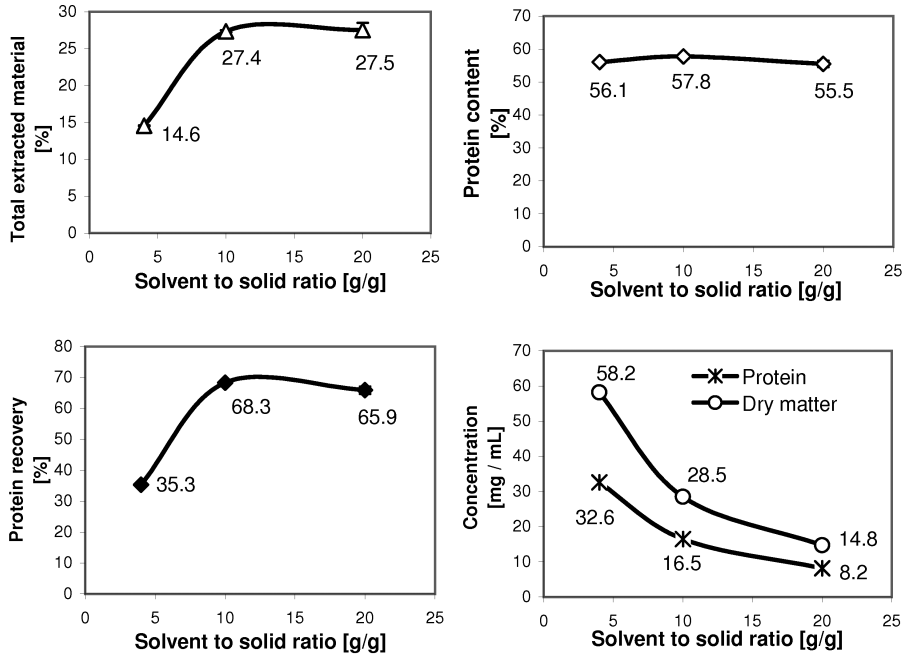

Figure 3.3 The effect of solvent to solid ratio on Jatropha seed press cake protein extraction by using 0.055M NaOH.

Table 3.3 Dry matter recovery, protein content, and protein recovery of Jatropha press cake extracts during four-stage counter current extraction with 0.055MNaOH at T=20◦C and extraction time of 30 min/stage.

Flow	Dry matter recovery [%]	Protein content [%]	Protein recovery [%]
4 stages counter current (solvent to solid ratio 10 g/g)			
E-4	5.0 ± 0.3	10.0 ± 3.5	2.1 ± 0.8
E-3	8.6 ± 0.1	35.9 ± 1.6	13.3 ± 0.5
E-2	19.8 ± 0.2	56.7 ± 0.8	48.6 ± 0.9
E-1	34.0 ± 0.1	55.8 ± 0.5	82.1 ± 0.7
R-4	65.5 ± 1.0	6.6 ± 1.7	18.7 ± 5.1
Total	**99.6 ± 0.56**		**100.7 ± 2.9**
4 stages counter current (solvent to solid ratio 4 g/g)			
E-4	5.8 ± 0.0	38.5 ± 2.7	9.6 ± 0.7
E-3	12.1 ±0.2	51.4 ± 1.7	26.9 ± 0.6
E-2	28.1 ± 0.5	58.6 ± 1.0	71.0 ± 0.5
E-1	28.4 ± 0.5	57.5 ± 2.0	70.6 ± 1.2
R-4	65.6 ± 0.1	10.4 ± 1.8	29.5 ± 5.1
Total	**94.0 ± 0.3**		**100.1 ± 3.1**

and protein recovery at flow E-2 were similar to flow E-1 (about 71 %) at solvent to solid ratio of 4 g/g. This may indicate that flow E-2 was already saturated with extracted material and equilibrium was reached faster at solvent to solid ratio of 4 g/g. By using a four-stage counter current extraction, protein content of residue (R-4) from extraction at solvent to solid ratio of 10 g/g was about 6.6% or about 40% lower than protein content of the residue (R-4) from extraction at solvent to solid ratio of 4 g/g (10.4%). These results corresponded to the higher protein recovery (82%) at solvent to solid ratio of 10 g/g.

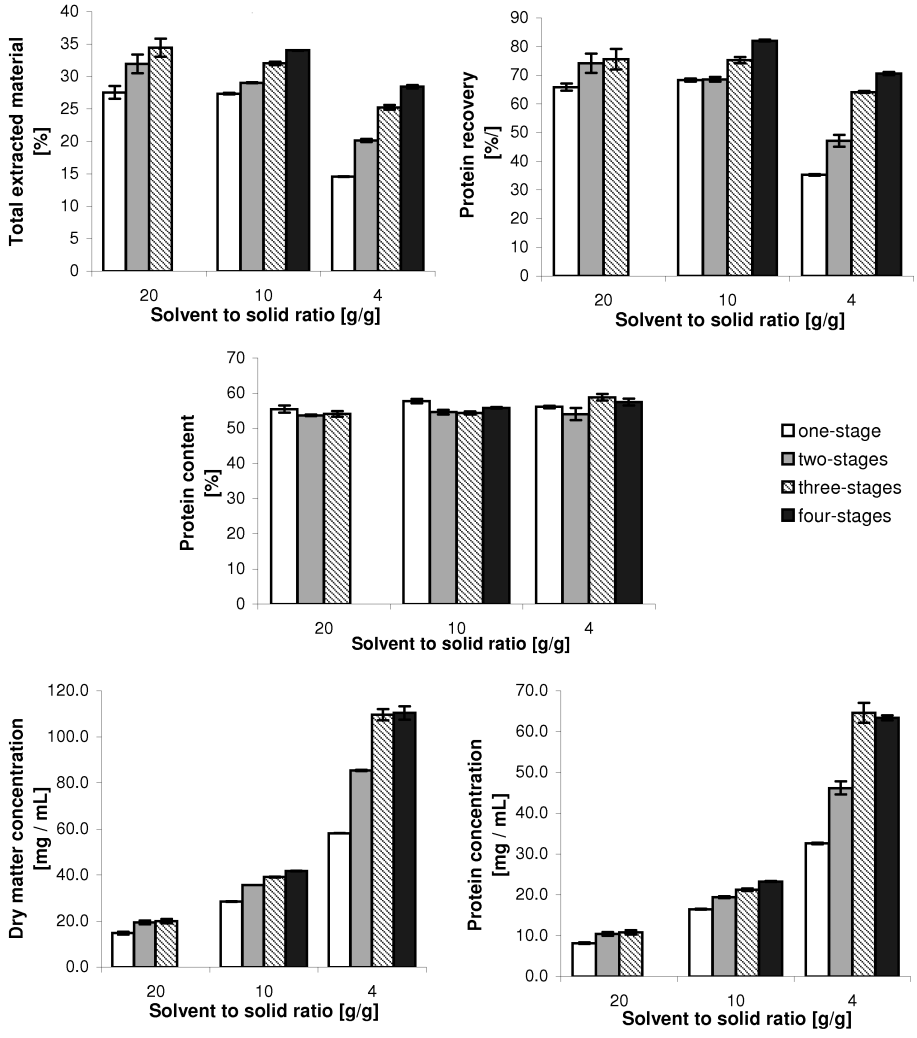

Figure 3.4 The effect of number of stages on the recovery, protein content, and concentration of the Jatropha seed press cake extracts after multistage countercurrent 0.055M NaOH extraction at different solvent to solid ratio.

3.3.5. The effect of number of stages on counter current protein extraction

Figure 3.4 shows the effect of number of stages on the recovery, protein content, and concentration of the Jatropha seed press cake outlet extracts (E-1) after multistage counter current 0.055 M NaOH at different solvent to solid ratio. From this figure, solvent to solid ratio and number of extraction stages had no effect on protein content in the dried crude extracts. On average, protein content of the extracted material was 55%; slightly lower than the protein content of commercial soy protein concentrate (65%). Lowering the solvent to solid ratio from 20 to 4 g/g increased the concentration of the total extracted material (from 2 to 11 mg/mL) and protein concentration in the outlet flow (from 15 to 63 mg/mL).

In addition, protein molecular weight distributions in the output flow (E-1) from both solvent to solid ratio 4 and 10 g/g at different number of stages showed similar pattern with the protein extracted by using NaOH 0.055 M in one-stage (Figure 3.2). Therefore, neither the number of extraction stages nor the solvent to solid ratio affected molecular weight distributions of the extracted protein. In terms of protein recovery, a four-stage counter current extraction at solvent to solid ratio of 10 g/g improved protein recovery from 69 to 82%. However, the increase of number of stages had the largest impact on extraction at solvent to solid ratio of 4 g/g. By using one-stage extraction, solvent to solid ratio of 4 g/g only gave protein recovery of 35%. Increasing the number of stages from one to four gradually improved protein recovery to about two folds higher (from 35 to 71%). The low recovery during one-stage extraction at solvent to solid ratio of 4 g/g indicated that solvent was saturated with protein before the equilibrium has been reached. Therefore, repeated contact with solvent during counter current extraction resulted in a large improvement on protein recovery. This improvement may give benefits, especially to lower production cost—by reducing amount of solvent, size of equipment, and further processing, especially drying and purification—and the possibility for direct application of the crude extract, due to a high protein concentration.

3.3.6. Protein recovery from crude extracts

After protein was extracted into solutions, a common method to recover proteins from the crude extracts and increase protein content is by isoelectric precipitation. To find the best conditions to recover protein by isoelectric precipitation, we conducted experiments by using one-stage extraction on press cake at solvent to solid ratio of 10 g/g.

Figure 3.5 shows the amount of protein concentrate, recovered from Jatropha seed press cake NaOH 0.055 M extracts by precipitation between pH 2 and 8. After precipitation at different pHs, all protein bands in the extracts were present in the precipitate as well (results not shown). From this result, we concluded that protein precipitation at different pH had no effect on the overall molecular weight distributions. Protein precipitation at the range of pH 4 - 5.5 resulted on the highest amount of protein precipitate. This result indicated that Jatropha seed protein had the lowest solubility between pH 4 and 5.5, comparable with the result from Devappa and Swamylingappa [18]. In this range of pH,

protein recovery and protein content was about 60% and 70%, respectively. This protein recovery was lower than protein recovery obtained by Devappa and Swamylingappa [18] by using steam injection heating (70-77%), but was slightly higher than protein recovery obtained by Makkar [15] after extraction at pH 11 for 1 hour at 60°C followed by precipitation at pH 4 (53%). This result indicated that extraction by using NaOH 0.055 M solution could result in a high protein recovery at room temperature. Extraction at room temperature will provided the mildest conditions to extract protein at alkaline conditions and maintained protein functional properties.

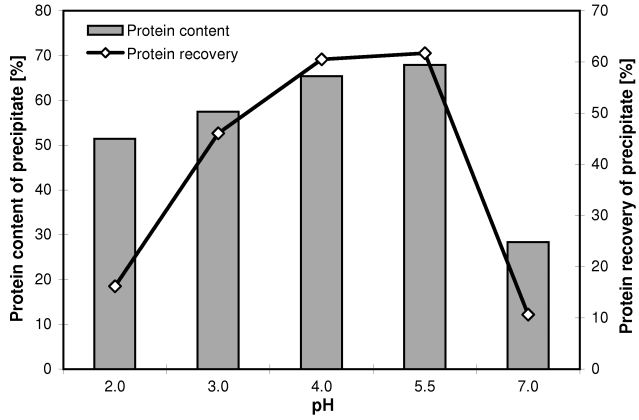

Figure 3.5 The recovery and protein content of the precipitate after NaOH 0.055M extraction followed by precipitation at various pH.

3.4 CONCLUSIONS

Among the tested extraction solvents, the use of 0.055 M NaOH resulted in highest protein recovery from both Jatroph kernel and seed press cake by using one-stage extraction. Protein recovery from kernel and seed press cake was 69.6 and 64.9%, respectively. The protein content of the extracted material from kernel and seed press cake are 35.8 and 53.3 %, respectively. Four-stages of counter current extraction successfully improved protein recovery from Jatropha seed press cake extraction at different solvent to solid ratio. Even though the highest protein recovery of 82% was obtained by four-stage counter current extraction at solvent to solid ratio of 10 g/g, extraction at solvent to solid ratio of 4 g/g may give more benefit—not only because of its high protein recovery of 71%, but also due to the higher protein concentration of 63 mg/mL. Furthermore, protein precipitation at pH 4 to 5.5 increased protein content to 70%, similar to commercial soy protein concentrates. SDS-PAGE shows the highest protein band intensities at molecular weight of 40, 48, 38, 37, 35, 36, 22, 23, 12, and 5 kDa. In addition, counter current extraction had no effect on protein molecular weight distributions.

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REFERENCES

1. K. Openshaw, A review of Jatropha curcas: an oil plant of unfulfilled promise, Biomass Bioenerg. 19 (2000) 1-15.
2. G.M. Gubitz, Exploitation of the tropical oil seed plant Jatropha curcas L, Bioresource technology 67 (1999) 73.
3. J. Lin, F. Yan, L. Tang, F. Chen, Antitumor effects of curcin from seeds of Jatropha curcas, Acta Pharmacologica Sinica 24 (2003) 241-246.
4. J. Martinez-Herrera, P. Siddhuraju, G. Francis, G. Davila-Ortiz, K. Becker, Chemical composition, toxic/antimetabolic constituents, and effects of different treatments on their levels, in four provenances of Jatropha curcas L. from Mexico, Food Chemistry 96 (2006) 80-89.
5. H.P.S. Makkar, K. Becker, Jatropha curcas toxicity: identification of toxic principle(s), Toxic Plants Other Nat. Toxicants, [Proc. Int. Symp. Poisonous Plants], 5th (1998) 554-558.
6. C.M. Vaz, L.A.d. Graaf, W.J. Mulder, Adhesives, coatings and bioplastics from protein sources, in: S.R. Fahnestock and A. Steinbuchel, (Eds.). Polyamides and Complex Proteinaceous Materials II Wiley-VCH, New York 2003, pp. 383 - 404.
7. E. Scott, F. Peter, J. Sanders, Biomass in the manufacture of industrial products—the use of proteins and amino acids, Applied Microbiology and Biotechnology 75 (2007) 751-762.
8. J.M. Krochta, Proteins as Raw Materials for Films and Coatings: Definitions, Current Status, and Opportunities, in: A. Gennadios, Editor. Protein-based films and coatings CRC Press, Boca Raton [etc.] 2002, pp. 1-32.
9. M. Jie, W. Raza, Y.C. Xu, Q.R. Shen, Preparation and optimization of amino acid chelated micronutrient fertilizer by hydrolyzation of chicken waste feathers and the effects on growth of rice, J. Plant Nutr. 31 (2008) 571-582.
10. President of Indonesia, Indonesian Presidential Regulation No. 5/2006 on National Energy Policy, 2006.
11. R. Kumar, V. Choudhary, S. Mishra, I.K. Varma, B. Mattiason, Adhesives and plastics based on soy protein products, Industrial Crops and Products 16 (2002) 155-172.
12. J. Kinsella, Functional properties of soy proteins, Journal of the American Oil Chemists' Society 56 (1979) 242-258.
13. D.S. Dijkstra, A.R. Linnemann, T.A.J.S. van Boekel, Towards Sustainable Production of Protein-Rich Foods: Appraisal of Eight Crops for Western Europe. PART II: Analysis of the Technological Aspects of the Production Chain, Critical Reviews in Food Science and Nutrition 43 (2003) 481 - 506.
14. A. Moure, J. Sineiro, H. Dominguez, J.C. Parajo, Functionality of oilseed protein products: A review, Food Res. Int. 39 (2006) 945-963.
15. H.P.S. Makkar, G. Francis, K. Becker, Protein concentrate from Jatropha curcas screw-pressed seed cake and toxic and antinutritional factors in protein concentrate, J. Sci. Food Agric. 88 (2008) 1542-1548.
16. A. Moure, D. Franco, J. Sineiro, H. Dominguez, M.J. Nunez, Simulation of multistage extraction of antioxidants from Chilean hazelnut (Gevuina avellana) hulls, Journal of the American Oil Chemists Society 80 (2003) 389-396.
17. S.P. Adupeasah, L.L. Diosady, L.J. Rubin, A Multistage Hydrocyclone Stirred-Tank System for Countercurrent Extraction of Canola Oil Journal of the American Oil Chemists Society 70 (1993) 755-762.
18. R.K. Devappa, B. Swamylingappa, Biochemical and nutritional evaluation of Jatropha protein isolate prepared by steam injection heating for reduction of toxic and antinutritional factors, J. Sci. Food Agric. 88 (2008) 911-919.
19. F. Vojdani, Solubility, in: G.M. Hall, Editor. Methods of Testing Protein Functionality, Blackie Academic & Professional, an imprint of Chapman & Hall, London, 1996, pp. 9-60.

Chapter 4

Jatropha leaf protein extraction

This chapter is to be submitted as D. Lestari, R. Oudman, W.J. Mulder, E. Ratnaningsih, J. P.M. Sanders; Jatropha leaf protein extraction

ABSTRACT

Jatropha curcas (Jatropha) is one of the oilseed crops that are grown mainly for biodiesel production. Besides the oil, Jatropha proteins can be extracted and utilized to obtain more value per hectare. Besides the press cake, Jatropha leaf is also a potential source of protein. However, the extraction and properties of Jatropha leaf protein have not been investigated so far. This research specifically investigated the chemical composition of Jatropha leaf and the properties of leaf protein, which include protein extractability, protein molecular weight distributions, and amino acid composition. Based on our results, Jatropha fresh leaf contains about 20% dry matter, which consists of fat (8%), crude protein (21%), carbohydrate (26%), lignin (26%), pectin (4%), and ash (12%). The molecular weight of Jatropha leaf proteins were distributed between 14.4 to 98 kDa, with the most prominent band presence at around 52 and 43 kDa. Because of the low extractability and low purity, utilization of native leaf protein may not be economically feasible. Nevertheless, Jatropha leaves are potential source of amino acids. Around 93% of the total amino acids can be recovered from Jatropha leaves by acid hydrolysis using 6 M HCl at 110°C for 24 h. The essential amino acids have potential as nutritional components for feed, while glutamine and glutamic acid (15 % of total nitrogen) have potential as intermediates to produce functionalized N-containing chemicals.

4.1. INTRODUCTION

Jatropha curcas (Jatropha) oilseed crop is one of the alternative energy sources to produce biodiesel. Currently, the economics of biodiesel production from Jatropha seeds may still be improved by optimizing the whole crop utilization to increase the overall crop value per hectare of Jatropha. To achieve this, researchers have investigated the potential of biorefinery as an integrated process to produce added value products from Jatropha. Biorefinery includes fractionation, conversion, and upgrading of various components in biomass [1-3]. Jatropha press cake, the by-product after oil pressing, contains valuable components e.g. proteins. Proteins, apart from their role in food and feed, also have potential in technical applications such as emulsifiers, foaming agents, coatings, bioplastics or adhesives [4, 5]. Due to the toxic components, Jatropha protein should be detoxified before using it as animal feed [6, 7]. Besides Jatropha seed or press cake, Jatropha leaves could be exploited as a protein source. In general, proteins extracted from other leaves, e.g. alfalfa or lucerne can be used as animal feed or for human nutrition [8]. In addition, leaf proteins are potential sources for amino acids, which can be converted into functionalized bulk chemicals [2, 9, 10]. Currently, the uses and characteristics of Jatropha leaf protein have not been explored so far.

According to Chaudhary *et al.* [11], Jatropha leaf dry matter contains around 1.5 to 3.0% nitrogen or around 9.4% to 18.8% protein (based on N-factor of 6.25). Jongschaap *et al.* [12] observed differences in nitrogen content between the mature and old leaves. According to Openshaw [13], about 35% of the dry matter is accumulated in the seed, while 25% of the dry matter is accumulated in the leaves. If Jatropha seed production is in the range between 0.6 to 3.9 ton dry seeds/ ha per year [12], the production of Jatropha leaf dry matter is approximately in the range between 0.4 to 2.9 ton/ ha per year, depending on the climate conditions, soil characteristics and plant density. Gunaselaan [27] has investigated the yield of Jatropha seeds and the amount of pruned leaves from a Jatropha plantation in rain fed dry land, with a plant density of 4444 plant/ ha (at 1.5 m x 1.5 m spacing). Under these conditions, the seed yield was approx. 4 tonnes dry seeds/ ha per year and the amount of pruned leaves was approx. 1.1 tonnes leaf dry matter/ ha per year, or 25% of the seed dry matter. Assuming the protein content of 20% of dry matter, the total potential availability of Jatropha leaf protein from the pruned leaves are about 220 kg/ ha per year. The leaf proteins availability could still be improved by harvesting more leaves, as long as it has no negative effects on seed productivity.

Literature has described methods to extract protein and produce leaf protein concentrate (LPC) from leaf e.g. alfalfa/ lucerne. A patent from the company France Luzerne [8] reported that protein can be obtained from alfalfa leaf by a certain pre-treatment followed by pressing. These pre-treatments were thermal, alkaline and a combination of these. Thermal pre-treatment was conducted before pressing by adding a liquid with pH between 5 and 8.5, at 50 or 60°C to fresh lucerne for 5 to 10 minutes, while alkaline pre-treatment was conducted by adding a strong alkaline liquid medium (e.g. sodium hydroxide) at pH 10-12 at 10-25°C for 10-20 minutes.

Currently, alkaline extraction and enzymatic treatment are the common methods to isolate protein from leaf [14]. In addition, some large-scale production of leaf protein concentrate carry out the removal of plant pigment e.g. chlorophyll prior to protein recovery using heat treatment [15, 16]. One of the methods to recover proteins from extract solutions is by precipitation at the isoelectric point. Protein from alfalfa leaf has minimum solubility at its isoelectric region between pH 3 to 5 as reported by Lu and Kinsella [17]. On the other hand, the isoelectric pH of soybean leaf protein is in the range between pH 3 and 7 as reported by Betschart and Kinsella [18].

In this paper, the objectives were to investigate the characteristics and extractability of Jatropha leaf protein, which included protein extractability in aqueous solution at different pH, the chemical compositions of leaf and leaf extracts, protein molecular weight distribution, and amino acid composition analysis.

4.2 MATERIALS AND METHODS

4.2.1 Raw materials

Jatropha leaves were obtained from the Subang plantation, West Java, Indonesia. Jatropha dried leaves for chemical composition analysis were prepared by air drying for overnight, followed by sun drying for 6 hour and oven drying at 40°C for 1 day. The dried leaves were milled into a green leaf powder.

4.2.2 Chemical compositions and sugar analysis

The chemical compositions of Jatropha leaf were investigated as described by TAPPI methods [19-25] with minor modifications. The milled samples were extracted using an accelerated solvent extraction (ASE200 Dionex) with ethanol: toluene 2:1 (v/v) and ethanol at 1500 psi and 100 °C, followed by a hot water extraction at boiling temperature. The extracted samples were dried using a rotary evaporator. The content of the neutral sugars and lignin of the extractive-free biomass was determined after hydrolysis with H_2SO_4 in two-steps: 1) H_2SO_4 12 M for 1 hour at 30 °C; 2) H_2SO_4 1 M for 3 hours at 100 °C. Neutral sugars were determined by a high-performance anion-exchange chromatography (HPAEC) with pulsed amperometric detection on a CarboPac PA1 column (Dionex) with a water-sodium hydroxide gradient and fructose as internal standard. The acid insoluble lignin (AIL) in the hydrolysate was measured by weight as Klason lignin, whereas the acid soluble lignin (ASL) content was determined by spectrophotometric determination at 205 nm [5,6]. Uronic acids in the sulfuric acid were determined by using a spectrophotometer at a wavelength of 520 nm. Ash content was determined on non-extracted milled samples by ignition at temperature of 525°C and 900°C. Protein content of materials were measured by Kjeldahl [26] using a semi-automatic Kjeldahl equipment from Gerhardt, which consist of destruction unit (Gerhardt Kjeldahlterm*)* and rapid distillation unit (Gerhardt Vapodest). Protein recovery was expressed as the percentage of total amount of protein extracted compared to total initial protein amount in leaf.

4.2.3 Protein extraction

Protein was extracted from Jatropha fresh leaf in three steps: 1) Pre-treatment by soaking with aqueous solvents: water, NaOH or HCl solutions; 2) Protein extraction and 3) Separation of the juice and press cake by mechanical pressing. About 40 gram of fresh leaves was used in each experiment. Before extraction, the fresh leaves were cut to reduce the size. Next, about 80 ml of extracting solvent, e.g. water, NaOH solution (0.05; 0.1; or 0.2 M) or HCl solution (0.2 M), was added to the cut leaves at room temperature. The leaf mixture was milled into leaf pulp with a wet mill designed for kitchen use for one minute. Afterwards, the leaf pulp was pressed for three minutes with a laboratory mechanical pressing unit at 3.2 bars, which resulted in leaf extract slurry and leaf press cake.

This laboratory mechanical pressing consists of two main parts: a basket with an inner diameter of 10.0 cm, made with a PVC tube drilled with holes to drain the water, and a closing lid having a hand-driven screw thread to apply the force. The bottom of the basket is fixed above the base. This also had holes drilled to drain the juice and a piece of metal grid on top to prevent the leaf pulp to pass the holes. To prevent leakage, the leaf pulp was packed in textile. After filling the basket with leaf pulp, a closing lid was put on top and the force was applied to the basket press with a hand-driven screw thread. The force was measured with two scales, indicating the weight applied. The force (2500 N) was kept at a constant level manually, giving a pressure of 3.2 bars inside the basket press for three minutes. During the experiments, the basket was placed in a plastic bin to collect the juice flowing out of the holes. The juice was then centrifuged at 8000 rpm for 15 min. For comparison, the leaf pulp after pre-treatment by 0.2 M NaOH was incubated at 8°C for 24 hours, followed by centrifugation (no pressing) to obtain the clarified extract. The clarified extract was decanted, freeze dried, and analysed for its protein content by Kjeldahl.

4.2.4 Determination of protein molecular weight distribution (SDS PAGE)

Samples were taken from the clarified water extract (prepared using the method at section 2.3). In addition, the molecular weight distribution was also analysed for the fractionated proteins, obtained by precipitating the proteins from the clarified water extract using ammonium sulphate at: 1) 20% saturation; 2) 20 to 60% saturation; and 3) 60 to 95% saturation. The analyses were conducted by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) following the method from Laemmli [27].

4.2.5 Acid hydrolysis and amino acid compositions analysis

The free amino acids and total amino acid composition of Jatropha leaf and dried leaf extract powder were investigated. To determine the content of free amino acids, samples were milled and extracted with acetone. Acetone extracts were then filtered and the amino acids were determined. To determine the content of total amino acids, the milled samples were hydrolysed with 6 N HCl solution containing 1% (w/v) phenol under vacuum after three alternating vacuum-nitrogen flushing steps and placed in an oven at 110° C for 24 h (or 20 h). The analysis was performed by using Ultra HPLC Dionex

RSLC instrument (Dionex Corporation, Sunnyvale, CA, USA), which consists of Ultimate 3000 RS (Rapid Separation) pump, Ultimate 3000 auto sampler, Ultimate 3000 column compartment with a thermo stable column area, and Ultimate 3000 variable wavelength detector. The system was running by using Dionex Chromeleon™ 6.8 software.

4.3 RESULTS AND DISCUSSION

4.3.1 Chemical composition of Jatropha leaf

The chemical composition of Jatropha leaf is shown in Table 4.1. Acid insoluble lignin (24% w/w dry matter) and protein (21% w/w dry matter) were the major components in Jatropha leaf. Carbohydrates in leaf were present in the form of soluble sugar (15% w/w dry matter) and polysaccharide (10% w/w dry matter). After strong acid hydrolysis, about 65% w/w of polysaccharide was converted to glucose. Jatropha leaf contained high contents of ash, soluble sugar and lignin (Table 4.1). Based on this, the method to extract and purify protein from Jatropha leaves should involve dialysis to remove salts and acid washing to remove acid insoluble lignin.

4.3.2 Jatropha leaf protein molecular weight distributions

The molecular weight distribution of Jatropha leaf protein is shown in Figure 4.1. The most prominent protein band of the clarified Jatropha leaf extract was observed at around 43 kDa. The other less abundant protein bands were observed between 14.4 and 20 kDa (4 bands), between 20 and 30 kDa (1 band), between 30 and 43 kDa (2 bands), between 43 and 67 kDa (2 bands), and between 67 and 98 kDa (2 bands). After the subsequent precipitation of the clarified Jatropha extract with ammonium sulfate ($(NH_4)_2SO_4$), the protein band at around 43 kDa was present at higher concentration after ammonium sulphate precipitation at 20-60% saturation and 60-95% saturation (Figure 4.1).

Rubisco, the major leaf protein component, has molecular weight of 550 kDa, consists of eight large and eight small subunits [28]. In the presence of SDS, the native structure of rubisco was dissociated completely into two subunits at around 12.5 and 52 kDa [29]. Lamsal *et.al* [30] reported that the most prominent protein band of the clarified alfalfa juice at around 49 kDa was identified as one of the dissociated subunit of rubisco. Jatropha leaf extract contained protein bands at around 14.4 and between 43 to 67 kDa, which could be indicated as the SDS-dissociated subunits of rubisco. However, the subunit that is located between 43 to 67 kDa was less prominent than the 43 kDa subunit, which may be due to the degradation of the the SDS-dissociated rubisco subunit to a smaller protein of 43 kDa.

4.3.3 Jatropha leaf protein extraction

Jatropha leaf protein extractability from fresh leaf was about 7% in either water or under acid conditions of 0.2 M HCl. The protein extractability was gradually improved by the increase of NaOH concentration. However, the highest protein extractability

(36%), which was obtained after extraction by NaOH 0.2 M, may not be high enough to ensure the economic feasibility of extraction at the industrial scale (Table 4.2). On the other hand, protein recovery from the pre-soaked dried leaf was about 10% after extraction using NaOH 0.2 M, which was much lower than from fresh leaf (unpublished results). In addition, the low protein concentration in the extract (1 to 5.5 mg/ ml) will increase the separation costs. The purity of the protein concentrate could not exceed 31-34%, even after dialysis and acid precipitation. This may be due to the high content of acid insoluble lignin, which precipitated along with the protein under the acidic conditions.

We found that it was difficult to separate acid insoluble lignin from protein fraction. This may indicate that the extraction of native protein from leaf extract powder may not be economically feasible. Surprisingly, protein extraction from fresh leaf after pre-treatment with NaOH 0.2 M, incubating for 24 hour at 8°C followed by centrifugation (no pressing) gave the highest soluble protein recovery of 48%. However, when this method was applied to extract proteins from larger amount of fresh leaf, i.e. 500 gram, the protein recovery was reduced to only about 20%, which may be due to the limited mass transfer from leaf cells into the extracting solvent during pulping in a small a kitchen mixer of 2 L (unpublished result).

Table 4.1 Chemical compositions of Jatropha leaf.

Components	Compositions (% weight dry basis)
Fat	8.2
Crude protein	20.6
Carbohydrate	
Soluble sugar	15.3
Polysaccharide	10.2
Arabinose	0.6
Xylose	1.0
Mannose	0.6
Galactose	1.5
Glucose	7.1
Pectin	3.9
Lignin	
Acid insoluble lignin	23.5
Acid soluble lignin	2.8
Ash	12.0
Total	**97.3**

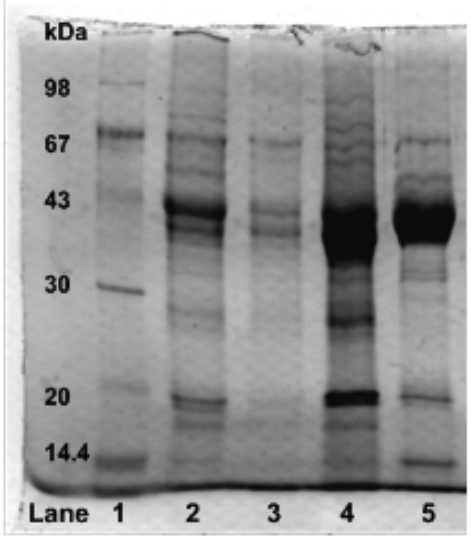

Figure 4.1 Jatropha leaf protein molecular weight distribution; Lane 1) standard protein marker; Lane 2) clarified water extract; Lane 3) Precipitated protein at 20% $(NH_4)_2SO_4$ saturation; Lane 4) Precipitated protein at 20-60% $(NH_4)_2SO_4$ saturation; Lane 5) Precipitated protein at 60-95% $(NH_4)_2SO_4$ saturation.

Table 4.2 Jatropha leaf extraction by adding extracting solvent, followed by pressing at 3.2 bars and centrifugation to clarify extract.

Extracting solvent	Clarified extract pH	Protein concentration (mg/ml)	Protein purity (% wt. DM)	Soluble protein (% initial protein)
Water	6.5	1.0	7	7
HCl 0.2 M	2	1.0	8	7
NaOH				
0.05 M	8.5	1.5	9	12
0.1 M	9.5	3.8	19	31
0.2 M	11	5.6	22	36
0.2 M, soaking 24h, no pressing	12	8.0	22	48

Table 4.3 The concentration and recovery of the free amino acids and total amino acids after Jatropha leaf proteins acid hydrolysis with 6 M HCl at 110° C for 24 h.

	Crude protein	Free amino acids	Total amino acids
Concentration (g/ kg DM of Jatropha leaf)	193.9	35.0	181.2
Amino acids recovery (% w/w crude protein)	-	18.1	93.5

4.3.4 Jatropha leaf protein as the source of amino acids

The low extractability of Jatropha native leaf protein in aqueous solutions may limit the use of this protein at industrial scale. However, the utilization of amino acids in Jatropha leaf may be a potential option to improve the value of Jatropha leaf. Jatropha leaf dry matter contained approx. 19% w/w crude protein, which consist of the free amino acids (~18%) and protein/ peptides (~82%). After hydrolysis with HCl 6 M at 110°C for 24 hour, about 93.5% of total protein was recovered in the form of amino acids (Table 4.3). The amino acid composition of Jatropha leaf protein was comparable with alfalfa protein and similar to the standard of FAO reference protein, except for methionine (Table 4.4). The amino acids from Jatropha leaf could have potential as building blocks for functionalized chemicals. Among the amino acids in Jatropha leaf, glutamine and glutamic acid were the biggest fractions with about 15% of the total nitrogen. Glutamic acid could be used as raw materials for commercial chemicals, such as 5-amino-1-butanol and glutaric acid [31]. Further decarboxylation of glutamic acid resulted in γ-aminobutyric acid (GABA) [10, 32], which could be a potential intermediate for making *N*-methylpyrrolidone, one of the industrial solvents [33].

Table 4.4 Total amino acid content of Jatropha leaf and leaf extract powder.

Amino acids (% w/w Crude protein)	*Jatropha* leaf		Alfalfa leaf	FAO ref.
	Free amino acid	Total amino acid		
Essential Amino Acids				
Isoleucine	0.20	5.00	5.5	4.20
Leucine	0.22	8.62	9.61	4.80
Lysine	0.02	4.30	6.56	4.20
Methionine		1.50	1.9	2.20
Phenylalanine	0.40	5.84	5.99	2.80
Threonine	0.91	5.09	5.14	2.80
Tryptophan			2.15	1.40
Valine	0.73	5.49	6.27	2.80
Non-Essential Amino Acids				
Alanine	0.51	6.20	6.2	
Arginine	0.12	5.40	6.6	
Asparagine + Aspartic acid	0.77	8.41	10.09	
Cysteine	0.12	1.06	1.03	
Glutamic Acid + Glutamine	11.51	15.31	11.5	
Glycine	0.04	5.33	5.46	
Histidine	1.43	1.87	2.32	
Proline_UV_263nm	0.31	5.26	4.63	
Serine	0.72	5.21	4.56	
Tyrosine	0.04	3.59	4.73	
Total (% w/w Crude protein)	**18.06**	**93.48**	**100**	

4.4 CONCLUSION

Jatropha leaf contains about 20% dry matter, which consists of fat (8%), crude protein (21%), carbohydrate (26%), lignin (26%), pectin (4%), and ash (12%). The molecular weight of Jatropha leaf proteins were distributed between 14.4 to 98 kDa, with the most prominent band presence at around 43 kDa, which may be the degradation product of the SDS-dissociated rubisco subunit. The low extractability and low purity of the protein concentrate suggest that the extraction and utilization of native leaf protein may not be economically feasible. Nevertheless, Jatropha leaf is a potential source of amino acids. By acid hydrolysis using 6 M HCl at 110°C for 24 h, about 93% of the total amino acids can be recovered. Jatropha leaf protein contain about 18% free amino acids. The total amino acids of Jatropha leaf contain mostly glutamine and glutamic acid of 15% of total nitrogen. The essential amino acids can have a potential as nutritional components for feed, while glutamine and glutamic acid can be converted into functionalized bulk chemicals.

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REFERENCES

1. J.P.M. Sanders, Biorefinery, the bridge between agriculture and chemistry, in: The 14th European Biomass Conference and Exhibition : Biomass for Energy, Industry and Climate Protection, Paris, France, 2005.
2. J.P.M. Sanders, E.L. Scott, R.A. Weusthuis, H. Mooibroek, Bio-Refinery as the Bio-Inspired Process to Bulk Chemicals, Macromolecular Bioscience, 7 (2007) 105-117.
3. F. Cherubini, The biorefinery concept: Using biomass instead of oil for producing energy and chemicals, Energy Conversion and Management, 51 (2010) 1412-1421.
4. D. Lestari, W.J. Mulder, J.P.M. Sanders, Jatropha seed protein functional properties for technical applications, Biochemical Engineering Journal, 53 (2011) 297-304.
5. D. Lestari, W.J. Mulder, J.P.M. Sanders, Improving Jatropha curcas seed protein recovery by using counter current multistage extraction, Biochemical Engineering Journal, 50 (2010) 16-23.
6. H.P.S. Makkar, G. Francis, K. Becker, Protein concentrate from Jatropha curcas screw-pressed seed cake and toxic and antinutritional factors in protein concentrate, J. Sci. Food Agric., 88 (2008) 1542-1548.
7. R.K. Devappa, B. Swamylingappa, Biochemical and nutritional evaluation of Jatropha protein isolate prepared by steam injection heating for reduction of toxic and antinutritional factors, J. Sci. Food Agric., 88 (2008) 911-919.
8. C. Gastineau, O.d. Mathan, J.-D. Dilly, Process for the treatment of green, leafy plants for the extraction of proteins in the pressing juices and for economical dehydration of cake, in: U.S. Patent No. 4,066,633, France Luzerne, 1978.
9. J. van Haveren, E.L. Scott, J.P.M. Sanders, Bulk chemicals from biomass, Biofuels, Bioproducts and Biorefining, 2 (2008) 41-57.
10. E.L. Scott, F. Peter, J.P.M. Sanders, Biomass in the manufacture of industrial products—the use of proteins and amino acids, Applied Microbiology and Biotechnology, 75 (2007) 751-762.
11. D.R. Chaudhary, A. Ghosh, J. Chikara, J.S. Patolia, Soil Characteristics and Mineral Nutrient in Wild Jatropha Population of India, Communications in Soil Science and Plant Analysis, 39 (2008) 1476-1485.
12. R.E.E. Jongschaap, W.J. Corre, P.S. Bindraban, W.A. Brandenburg, Claims and Facts on Jatropha curcas L. , in, Plant Research International B.V., Wageningen UR, Wageningen, 2007.
13. K. Openshaw, A review of Jatropha curcas: an oil plant of unfulfilled promise, Biomass Bioenerg., 19 (2000) 1-15.
14. L. Shen, X. Wang, Z. Wang, Y. Wu, J. Chen, Studies on tea protein extraction using alkaline and enzyme methods, Food Chemistry, 107 (2008) 929-938.
15. D.S. Dijkstra, A.R. Linnemann, T. van Boekel, Towards sustainable production of protein-rich foods: Appraisal of eight crops for Western Europe. PART II: Analysis of the technological aspects of the production chain, Crit. Rev. Food Sci. Nutr., 43 (2003) 481-506.
16. R.H. Edwards, R.E. Miller, D.D. Fremery, B.E. Knuckles, E.M. Bickoff, G.O. Kohler, Pilot-Plant Production of an Edible White Fraction Leaf Protein Concentrate from Alfalfa, Journal of Agricultural and Food Chemistry, 23 (1975) 620-626.
17. Pick-Seng Lu, J.E. Kinsella, EXTRACTABILITY AND PROPERTIES OF PROTEIN FROM ALFALFA LEAF MEAL, Journal of Food Science, 37 (1972) 94-99.
18. A.A. Betschart, J.E. Kinsella, Influence of storage on composition, amino acid content, and solubility of soybean leaf protein concentrate, Journal of Agricultural and Food Chemistry, 22 (1974) 116-123.
19. TAPPI, T 412 om-02, Moisture in pulp, paper and paperboard, in: TAPPI test methods 2004-2005, TMCD-04, 2004.
20. TAPPI, T 204 cm-97, Solvent extractives of wood and pulp, in: TAPPI test methods 2004-2005, TMCD-04, 2004.

21. TAPPI, T 249 cm-00, Carbohydrate composition of extractive-free wood and wood pulp by gas-liquid chromatography, in: TAPPI test methods 2004-2005, TMCD-04 2004.
22. TAPPI, T 222 om-02, Acid-insoluble lignin inwood and pulp, in: TAPPI testmethods 2004-2005, TMCD-04, 2004.
23. TAPPI, T 211 om-02, Ash in wood, pulp, paper and paperboard: combustion at 525 °C, in: TAPPI test methods 2004-2005, TMCD-04, 2004.
24. TAPPI, T 418 cm-97, Organic nitrogen in paper and paperboard, in: TAPPI testmethods 2004-2005, TMCD-04, 2004.
25. T.o.-. TAPPI, Moisture in pulp, paper and paperboard, in: TAPPI test methods 2004–2005, TMCD-04, 2004.
26. J. Kjeldahl, Nitrogen determination technique, Z. Anal. Chem., 22 (1883) 366.
27. U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature, 227 (1970) 680-685.
28. W.E. Barbeau, J.E. Kinsella, Ribulose bisphosphate carboxylase/oxygenase (rubisco) from green leaves-potential as a food protein, in, Taylor & Francis, 1988, pp. 93 - 127.
29. L.L. Hood, S.G. Cheng, U. Koch, J.R. Brunner, Alfalfa Proteins: Isolation and Partial Characterization of the Major Component — Fraction I Protein, Journal of food science, 46 (1981) 1843-1850.
30. B.P. Lamsal, R.G. Koegel, S. Gunasekaran, Some physicochemical and functional properties of alfalfa soluble leaf proteins, LWT - Food Science and Technology, 40 (2007) 1520-1526.
31. T. Werpy, G. Petersen, Top Value Added Chemicals From Biomass, Volume I: Results of Screening for Potential Candidates from Sugars and Synthesis Gas, in, the Pacific Northwest National Laboratory (PNNL), the National Renewable Energy Laboratory (NREL), 2004.
32. T.M. Lammens, D. De Biase, M.C.R. Franssen, E.L. Scott, J.P.M. Sanders, The application of glutamic acid [small alpha]-decarboxylase for the valorization of glutamic acid, Green Chemistry, 11 (2009) 1562-1567.
33. T.M. Lammens, M.C.R. Franssen, E.L. Scott, J.P.M. Sanders, Synthesis of biobased N-methylpyrrolidone by one-pot cyclization and methylation of [gamma]-aminobutyric acid, Green Chemistry, 12 (2010) 1430-1436.

Chapter 5

Jatropha seed protein functional properties for technical applications

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ABSTRACT

Jatropha press cake, by-product after oil expression from Jatropha seeds, contains 24-28% protein on dry basis. Objectives of this research were to investigate functional properties, such as solubility, emulsifying, foaming, film forming, and adhesive properties, of Jatropha press cake proteins and compared those with relevant industrial proteins. From our study, we found that protein extracted from press cake proteins had a solubility of about 90% above pH 9. Emulsifying properties of press cake protein were comparable to sodium caseinates and reached the highest value at pH 9-10. Jatropha proteins formed films with tensile strength of 0.4 – 1.8 MPa with 10-75% elongation, which were below soy protein or wheat-gluten. Further oil removal from press cake decreased emulsifying properties, while increased foaming and adhesive properties of the extracted proteins. Protein extracted from de-oiled press cake showed better foaming properties than sodium caseinate at pH 10, but lower than egg white protein at all pH. Furthermore, press cake protein showed better adhesive properties than casein adhesives at the same dry matter content. Based on these results, Jatropha press cake protein showed most promising results on adhesive and emulsifying properties, which indicate the potential of Jatropha press cake protein as emulsifier or paper adhesive.

Keywords: Jatropha seed protein; Protein extraction; Biorefinery; Protein functional properties; Solubility; Technical applications.

ABBREVIATIONS

PC = Press cake
DOPC = De-oiled press cake
EA = Emulsifying activity
ES = Emulsifying stability
$A_{500\ nm}$ = Absorbance at 500 nm
$A_{500\ nm,\ 30\ min}$ = Absorbance at 500 nm of the lower half emulsion after 30 min
FE = Foam expansion
FVS = Foam volume stability
FLS = Foam liquid stability
Vf_0 = Foam volume created
Vl_i = Initial liquid volume
$Vf_{30\ min}$ = Foam volume after 30 min
$Vl_{30\ min}$ = Liquid volume after 30 min
TS = Tensile strength
EM = Young's Modulus

5.1 INTRODUCTION

Jatropha curcas (Jatropha) is a potential energy crop, due to the high oil content in the seed and its drought tolerance. Jatropha has been grown widely for oil expression from its seed. After oil expression, by pressing and/or solvent extraction, about 60% of the mass of Jatropha meal is remained as waste stream [1, 2]. To increase the benefit of Jatropha seed, we need to valorise the side streams. Proteins are the major components of the Jatropha seed cake. A number of proteins, such as soy protein, gelatin and casein, have been commercially produced for decades for food and technical applications. However, Jatropha seed contains curcin [3] and phorbol esters [4, 5], which are toxic compounds. These compounds are also found in the seed cake, which limit the uses of Jatropha seed cake for food and feed applications, without further detoxification. For this study, we will focus on the technical applications of Jatropha seed protein.

Much research has been conducted to extract protein from Jatropha meal in combination with a detoxification process to produce protein concentrates or protein isolates for animal feed [6, 7]. Jatropha meal utilization for non-food applications, such as adhesives, coatings, and surfactants, has not been explored so far. The most common technical applications of the industrial proteins, e.g. adhesives, coatings, and surfactants have been reported by Vaz et al. [8]. Important functional properties that are related to technical applications are solubility, film forming properties, foaming properties, emulsifying properties, and adhesive properties. Several review papers reported that the functional properties of oilseeds protein are highly influenced by both the nature of the protein source and its preparation method [9, 10]. A study by L'Hocine et al. [11] reported that possible denaturation, due to heat treatment and residual lipid content of the soy flake after defatting procedures, affected protein functional properties of the soy protein isolate. Several studies on Jatropha seed protein extraction have been conducted. By using steam injection, protein recovery is about 70-77% with a purity of 95-97% [7]. Protein extraction from Jatropha meal at pH 11 at 60°C and a solvent to solid ratio of 10, followed by precipitation at pH 5 resulted in protein recovery of 53% and a purity of 76-82% [6]. Applying a four-stage counter current alkaline extraction at pH 12 increase protein recovery up to 70% at room temperature and solvent to solid ratio of 4 g/g [12].

In order to get an insight in the most promising technical applications for Jatropha protein, we investigated functional properties such as solubility, emulsifying, foaming, film forming, and adhesive properties of Jatropha press cake and Jatropha de-oiled press cake proteins.

5.2 MATERIALS AND METHODS

5.2.1 Raw materials and chemicals

Jatropha press cake was obtained from the Energy Technology Center-Agency for the Assessment and Application of Technology (B2TE-BPPT), Serpong, West Java, Indonesia. As reference proteins, we used milk protein EM-7 type sodium caseinate provided by DMV-International (85% protein content) and soy protein isolates Vicoprot-S provided by Fa. L. I. Frank (84% protein content).

Press cake

Jatropha seed press cake (PC) was produced after oil pressing from the whole seeds (husks and kernels) by using a screw-press. Jatropha seed PC was milled by using a Retsch ZM 1000 milling machine into particle size of 2 mm. Protein content of the PC was 23 ± 1% (n=6) and the oil content was 12%.

De-oiled press cake

Jatropha de-oiled press cake (DOPC) was produced after oil pressing from the whole seeds (husks and kernels) by using a screw-press followed by further oil extraction using hexane. Hexane extraction was conducted at Pilot Pflanzenöltechnologie Magdeburg e.V. (PPM), Germany by continuous hexane extraction. Protein content of DOPC was 27± 1% (n=6) and the oil content was less than <1%.

5.2.2 Protein preparation

We extracted protein from 1 kg of Jatropha DOPC or PC by using 10 L NaOH 0.055 M in a TERLET vessel with capacity of 12 L at 20°C for 1 hour. Subsequently, we separated the protein extract from the slurry by centrifugation using a Sorvall centrifuge with rotor SLA-3000 at 10816 x g for 15 min. Protein was precipitated from the extract at pH 5 and left overnight at 4°C. We measured protein content based on Kjeldahl method [13], by using Kjeldahl equipment from Gerhardt, which consist of digestion unit (Gerhardt Kjeldahlterm*)* and rapid distillation unit (Gerhardt Vapodest). Calculation of the dry matter recovery and protein recovery from are shown in equation 5.1 and 5.2.

$$\text{Dry matter recovery [\%]} = \frac{Dry\ matter\ \ weight\ \ protein\ \ precipitate}{Initial\ dry\ matter\ weight} \times 100\% \qquad (5.1)$$

$$\text{Protein recovery [\%]} = \frac{\%protein\ \ content \times Dry\ matter\ \ weight\ \ protein\ \ precipitate}{\%protein\ \ content \times Initial\ dry\ matter\ weight} \times 100\% \qquad (5.2)$$

5.2.3 Protein solubility

We performed solubility tests based on a method from Betschart [14]. We stirred 0.3 g protein (0.381 g PC or 0.307 g DOPC) into 25 ml demi water with a magnetic stirrer for 5 minutes, followed by pH adjustment to the desired pH with 1.0 M NaOH or HCl. Next, the protein solutions were shaken at 120 rpm at 20°C in a water bath for 1 hour. During this time, the pH was adjusted if necessary. After 1 hour, the total weight was

brought to 30 g and centrifuged for 30 min at 4000 rpm and 20°C. The experiments were conducted in triplicates. We measured protein content of the supernatant by using Kjeldahl method.

5.2.4 Emulsifying properties

We determined the emulsifying properties of Jatropha protein according to the procedure proposed by James and Patel [15]. First, a protein solution was prepared by stirring 0.1 g protein (0.128 g PC or 0.103 g DOPC) to 100 ml demi water, followed by pH adjustment to the desired pH. The emulsion was prepared by mixing 38 g of protein solution and 14 g triolein in a 60 ml glass container with lid (specially used for making emulsion), followed by homogenizing by using Ultra-Turrax (model T25) with shaft (S25N-18G) for 1 minute at 15000 rpm. To measure emulsifying activity, we immediately added 1 ml of the fresh emulsion into 250 ml 0.1% SDS solution, mixed it well, and measured the absorbance at 500 nm. To measure emulsifying stability, about 10 ml of emulsion was drawn up into a 10 ml syringe and let it stand vertically for 30 minutes. After these 30 minutes, the 5 ml of the lower part was transferred into a tube and mixed well by using vortex mixer. Then, we immediately added 1 ml of the *aged* emulsion into 250 ml 0.1% SDS solution, mixed it well, and measured the absorbance at 500 nm. The experiments were conducted in duplicates. We calculated emulsifying activity (EA) and emulsifying stability (ES) by using the following equations:

Emulsifying activity [EA] $$EA = A_{500\ nm} \tag{5.3}$$

Emulsifying stability [ES %] $$ES\%_{,30\ \min} = \frac{A_{500\,nm,30\,\min}}{A_{500\,nm}} \cdot 100\% \tag{5.4}$$

Where $A_{500\ nm}$ = absorbance of the fresh emulsion and $A_{500\ nm\ 30\ min}$ = absorbance of the emulsion after 30 min.

5.2.5 Foaming properties

Protein foaming properties were measured by whipping a 0.5% protein solution at room temperature according to the method from Patel et.al. [16]. About 0.5 g protein (0.640 g PC or 0.515 g DOPC) was solubilized into 100 ml distilled water, followed by pH adjustment to the desired pH. Next, the protein solution was whipped at maximum speed (scale 7) for 5 min by using a Kenwood Chef Excel mixer (Kenwood Electronic). Immediately, the foam was transferred to a measuring cylinder. We measured the foam and liquid volume at t = 0 and t = 30 minutes. The experiments were conducted in duplicates. From this data, we calculated foam expansion, foam volume stability and liquid volume stability by using equations as follows.

Foam expansion [FE %] $$FE\% = \frac{Vf_0}{Vl_i} \cdot 100\% \tag{5.5}$$

Foam volume stability [FVS %] $$FVS\,\%,_{30\,\min} = \frac{Vf_{30\,\min}}{Vf_0} \cdot 100\% \quad (5.6)$$

Foam liquid stability [FLS %] $$FLS\,\%,_{30\,\min} = \frac{Vl_i - Vl_{30\,\min}}{Vl_i - Vl_0} \cdot 100\% \quad (5.7)$$

Where Vf_0 = foam volume created; Vl_i = initial liquid volume; $Vf_{30\ min}$ = foam volume after 30 min; $Vl_{30\ min}$ = liquid volume after 30 min.

5.2.6 Film forming properties

Protein films were prepared from 10% protein solutions at the desired pH. In relation to the dry matter, 30, 40 or 50% glycerol as a plasticizer was added. Protein solutions and glycerol were mixed by using a propeller to provide good mixing. About 40 ml of the protein solution was transferred into Petri dishes by using a pipette to avoid incorporation of air bubbles, followed by oven drying at 40°C overnight. The thicknesses of the films were about 300 μm. The experiments were conducted in duplicates.

The common mechanical properties measurements to characterize films are: 1) tensile strength (TS) or the pulling force per film cross-sectional area required to break the film; 2) elongation (E) or a degree until a film can stretch before breaking; and 3) elastic modulus (EM) or film stiffness, which is determined by ratio of pulling force / area to degree-of-film-stretch [17]. The mechanical properties measurements, consist of tensile strength (TS), elongation at break (E), and the Young's Modulus (EM), were performed on samples based on standard ISO 527($l0$ = 20 mm, b = 4 mm, t = 0.3 mm) in quadruple with individually cast films at 50 % RH and 20 °C. The Zwick / Roell tensile testing machine (Zwick GmbH & Co. KG, Ulm, Germany) equipped with a 100N static load cell was used for measurement. The specimen grip (L0) was set to 55mm, with a crosshead speed of 10 mm/min, while the OptiXtens extensometer was used with a testing speed of 2 mm/min.

5.2.7 Adhesive properties

Adhesive preparation

For Jatropha seed protein adhesive preparation, 13 g PC or DOSC protein was dispersed in 77 g NaOH 0.055 M containing 7 g urea. After one hour stirring, 3 g borax was added to the solution and stirred for 1 h. As reference, we prepared a casein adhesive with the same composition as Jatropha seed protein adhesive, except that casein was dispersed in water. The experiments were conducted in duplicates

Viscosity

The viscosity of protein-based adhesives was measured at room temperature with a Brookfield DV-II Rheometer Model LVT (Brookfield Engineering Laboratory) by using spindle model LV2 or LV4 at 60 rpm.

Set-time

Adhesive was applied at a thin layer (~80 μm wet) by using a labcoater (RK Print-Coat Instrument Ltd.; motor Citenco speed scale 3) to a brown kraftliner paper (60 g / m^2),

purchased from Harolds Grafik , Rotterdam, the Netherlands,. Immediately, a second piece of paper (same type), which was first cut into pieces of 2.5 cm width, was put on the top of the adhesive layer. The setting time was determined as the minimum time after which paper tear occurred.

Open time

Adhesive was applied at a thin layer (~80 μm wet) by using a labcoater (RK Print-Coat Instrument Ltd.; motor Citenco speed scale 3) to a brown kraftliner paper (60 g / m^2), purchased from Harolds Grafik , Rotterdam, the Netherlands. A piece of paper (same type) of 10 cm x 2.5 cm was put on the top of the adhesive layer every 1-2 min. The open time is reached when the piece of paper does no longer stick to the substrate.

Adhesive properties

Adhesive was applied at a thin layer (~80 μm wet) by using a labcoater (RK Print-Coat Instrument Ltd.; motor Citenco speed scale 3) to a brown kraftliner paper (60 g / m^2), purchased from Harolds Grafik , Rotterdam, the Netherlands. The second paper (same type) was put immediately on the top of the adhesive layer. The sheets were dried in a gel dryer at 80°C for 5 minutes. After drying, paper was cut into paper strips with a width of 2.5 cm. The T-peel adhesion strength was manually tested at 60% RLV and after 10 minutes in water.

5.3 RESULT AND DISCUSSION

5.3.1 Protein extraction

The functionality of Jatropha protein may depend not only on the process conditions during protein extraction, but also on the process conditions during oil removal prior to protein extraction [10]. During oil removal, heat and the use of organic solvents (hexane) might negatively influence protein functional properties. De-oiled press cake (DOPC), which was produced after further oil removal from Jatropha press cake (PC) by hexane extraction, had lower oil content (<1%) than the initial PC (12%). The different oil contents and the changes on particle matrixes of these materials may influence the protein extraction parameters, e.g. protein recovery and protein content, and functional properties of the isolated protein, such as solubility, emulsifying,

Table 5.1 Protein content and protein recovery of Jatropha protein isolate (n=2).

	Protein content [% wt.]	Protein recovery from seed cake [% wt.]
Press cake	22.9 ± 0.03	100.0
Press cake protein	72.6 ± 0.54	56.4 ± 1.08
De-oiled press cake	27.2 ± 0.54	100.0
De-oiled press cake protein	97.7 ± 0.6	45.2 ± 0.29

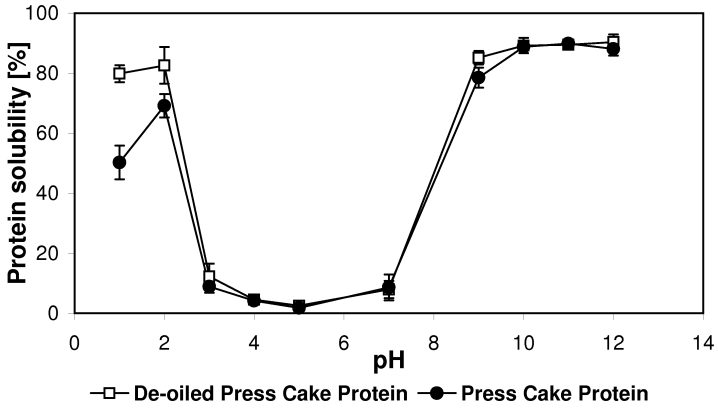

Figure 5.1 The solubility of Jatropha seed protein at different pH at 20°C (n=3).

foaming, film forming, and adhesive properties. To investigate the effect of oil removal by hexane extraction prior to protein extraction, we compared protein recovery and protein content of protein extracted from both PC and DOPC (Table 5.1).

We found that the protein recovery from DOPC (45%) was lower than from PC (56%) (Table 5.1). As we observed in section 5.3.2, hexane extraction prior to protein extraction did not affect the solubility of the extracted protein. Therefore, the lower extractability of protein from DOPC could be explained by the change of the matrix of press cake particle during hexane extraction, due to heat and contact with organic solvent. We observed that DOPC was dryer and its particles were sturdier than the PC. This might limit the diffusion of solvent into DOPC and finally reduced the rate of protein solubilization by the solvent. As consequence, the total extractability of the protein from DOPC was lower than from PC. Despite the lower extractability, protein extracted from DOPC had a higher protein content (98%) than protein extracted from PC.

5.3.2 Protein solubility

We investigated the effect of pH on the solubility of the protein isolated from DOPC and PC (Figure 5.1). We observed that DOPC protein and PC protein had similar protein solubility curves. Only between pH 1 and 2, the protein recovered from DOPC had a higher solubility. Despite the lower extractability of the protein from DOPC compare to that from PC (see section 5.3.1), the proteins extracted from both PC and DOPC showed similar solubility behaviors at most pH value. These results may indicate that hexane extraction prior to protein extraction had only little effect on the solubility of the extracted Jatropha proteins.

The minimum solubility of both PC and DOPC protein occurred at pH 5, which indicated the protein isoelectric point. This result is well corresponded to the estimation of Jatropha seed isoelectric point by using amino acid compositions data from Martinez-Herrera et al. [4], where the protein isoelectric point was calculated at pH 5.6. This result also confirmed the previous research on Jatropha seed protein precipitation,

which stated that the highest amount of Jatropha seed protein were precipitated at pH 4 to 5.5 [18].

In general, the protein solubility was low at neutral and slightly acid conditions (between pH 3 and 7). As consequence, this may limit Jatropha seed protein applications in neutral pH, especially, applications that need a high degree of solubilisation. The protein solubility increased sharply below pH 2 to 3 and above pH 8 to 9, which may be due to the increase of protein net charge as it went further from the protein isoelectric point. At acid conditions, protein solubility at pH 2 was high but then slightly decreased at pH 1. *Jatropha* seed proteins had the highest solubility (about 90%) above pH 9. If solubility is required, we may indicate that Jatropha seed protein is most suitable for applications in acid or alkaline environment, due to the high solubility under those circumstances.

5.3.3 Emulsifying properties

To form an emulsion, proteins should have good ability to assist the formation of oil into distinct small globules, which homogeneously distributed throughout the continuous aqueous phase [15]. According to Walstra [19], small oil globules can be obtained by increasing energy input and decreasing the interfacial tension. The role of proteins during emulsifications is to decrease the interfacial tension, which leads to the formation of small oil globules and the stabilization of the formed oil globules, by lowering the internal free energy of the system and adsorbed rapidly at the oil/water interfaces to form a new interfacial layer between oil/water interfaces [20]. According to Dickinson [21], the rate of protein unfolding at the surface is correlated

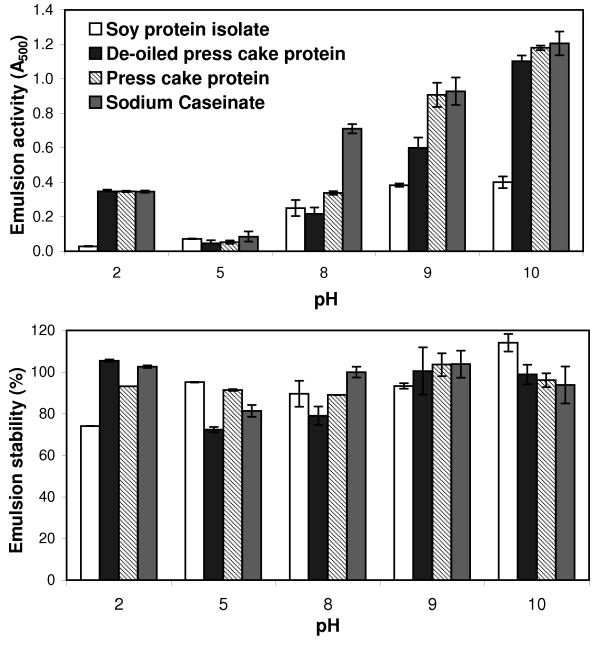

Figure 5.2 Emulsifying properties of Jatropha seed proteins at different pH at 20°C (n=2).

with the rate of adsorption and determine the maximum amount of protein adsorbed at the interface.

Based on the Mie theory for light scattering by dispersed spherical particles [22], absorbance value of diluted emulsion can give information about the average diameter and particle size distribution of the oil globules formed after emulsifications, which indicates protein emulsifying activity. Emulsifying activity of proteins is determined by protein film viscoelastic properties at the oil-water interface [21] and the amount protein adsorbed to the interface [23]. These two factors are affected by internal factors—such as protein structure and protein compositions—or external factors—such as pH or ionic strength, which influencing protein net charge or conformations.

We investigated the effect of pH on the emulsifying properties in terms of emulsifying activity (EA) and emulsifying stability (ES) of the DOPC protein and PC protein (Figure 5.2). For this study, we compared the emulsifying properties with sodium caseinate hydrolysate (EM-7), which has been used commercially as emulsifier, and soy protein, an industrial plant protein. At pH 2, the EA of all tested protein were considerably low. The EA of Jatropha proteins were similar to sodium caseinate (about 0.35) and higher than soy protein. At pH 5, the EA for all investigated proteins were very low (EA <0.1), which may due to the low protein solubility at the isoelectric pH. At pH 8, the EA of sodium caseinate (0.75) was higher than Jatropha DOPC proteins (0.2), Jatropha PC protein (0.35) and soy protein (0.25). The EA of Jatropha seed protein increased at alkaline pH and reached the highest value at pH 10, much higher than soy protein (0.4) and comparable to sodium caseinate (1.2). The higher absorbance value indicates a lower average diameter of the oil globules. Under identical emulsification conditions, the oil globules size varies for different proteins based on this order: soy proteins > whey proteins > sodium caseinate > soluble wheat proteins > blood proteins [24]. From our observations, the EA of soy protein and sodium caseinate were correlated with the order of oil globules size for different protein [24] while the EA of Jatropha seed proteins were between soy protein and sodium caseinates at almost all range of pH.

While the EA of both DOPC protein and PC protein were similar at acid pH, we observed that PC protein had higher emulsifying activities than DOPC protein at alkaline conditions. As discussed in section 5.3.1 and 5.3.2, protein extractability from DOPC was lower than from PC due to the changes of particle matrix of DOPC, which might affect the diffusion rate of several protein fractions. Related to this, the different of EA might be due to the possibility that the protein fractions extracted from PC had different structures or different compositions from the protein fractions extracted from DOPC. These different protein fractions may have different interfacial viscoelastic properties, which influenced the rate of unfolding during adsorption, and further influenced emulsifying activities

In relation to the protein solubility curve in Figure 5.1, the EA of Jatropha proteins were the highest value under alkaline conditions, low under neutral conditions, and reached the lowest at the protein isoelectric point, which corresponded to the trend in Jatropha protein solubility curve (Figure 5.1). At its isoelectric pH, Jatropha protein

solubility was very low (about 2%) and as a result, EA was the lowest. Jatropha protein solubility increased sharply from pH 8 to pH 9, which corresponded to the sharp increase of EA at the same pH range. At alkaline conditions, Jatropha protein was highly soluble (80-90%) and EA was also high. However, EA of Jatropha protein did not correspond to the protein solubility curve at acid pH. Although Jatropha protein solubility was high at pH 2 (about 80%), EA at pH 2 (0.4) was lower than EA at pH 9 (0.9), which had similar degree of solubility. This phenomenon was also occurring in protein isolated from Crambe oilseed [25]. This indicates that emulsifying activity is not only influenced by protein solubility, but also influenced by the rate of protein unfolding, which may be determined by protein structure or protein net charge in a certain pH. In general, EA of Jatropha protein was higher at alkaline pH. This might indicate that alkaline pH provided a higher rate of protein unfolding than acid or neutral pH. As a result, more protein was adsorbed to the oil-water interface and emulsifying activity became higher.

On the other hand, emulsion stabilization is determined by the ability of protein to retard the flocculation and coalescence of the oil globules. Flocculation is an initial step of coalescence and can be prevent by steric repulsion of the proteins in the oil-water interface, which is affected by pH, ionic strength, and temperature. Coalescence rate can be reduced if the protein, in addition to decreasing the interfacial tension, can form a film with good viscoelastic properties in the oil-water interface, to resist droplet-droplet collision [21, 26]. In general, Jatropha protein had a high emulsifying stability (above 70%), which indicated a good ability to stabilize emulsions. At neutral pH, PC protein had higher emulsifying stability than DOPC protein. On the other hand, PC protein had slightly lower emulsifying stability than DOPC protein in strong acidic environment (pH 2). Under alkaline conditions (pH 9 and 10), emulsifying stability of both proteins were similar and comparable to sodium caseinate (about 90%). Based on our results, we can indicate that Jatropha seed proteins can form films with sufficient steric repulsion and good viscoelastic properties at the interface, which further stabilize the emulsion. The role of proteins in emulsion industry may be differentiated into its use as a stabilizer or as emulsifier [20]. Based on these results, we can indicate that Jatropha seed protein, especially Jatropha PC protein might be an interesting alternative as emulsifier for oil/water mixture at alkaline pH, because of its high EA and ES at alkaline pH (pH>9), which is comparable to commercial emulsifier sodium caseinates. At neutral pH, Jatropha seed protein has a low EA but it has a good stability, which may be potential for an application as emulsion stabilizer.

5.3.4 Foaming properties

We investigated the effect of pH on foaming properties of Jatropha DOPC protein and PC protein, and compared it to sodium caseinate and soy protein isolates (Table 5.2). Foam expansion (FE) is the foam volume formed during a defined period of foaming generation relative to its initial volume, which shows the ability of the protein to form interfacial thin layer between gas bubbles.

Table 5.2 Foaming properties of Jatropha seed protein at different pH at 20°C (n=2; *n=1).

Protein	Foaming properties	pH				
		2	3	5	7	10
Protein PC	% FE	No Foam	No Foam	No Foam	No Foam	50*
	% FVS	No Foam	No Foam	No Foam	No Foam	9*
	% FLS	No Foam	No Foam	No Foam	No Foam	-
Protein DOPC	% FE	112.0 ± 11.3	95.0 ± 7.1	65.0 ± 7.1	90.0 ± 14.1	425.0 ± 35.4
	% FVS	48.2 ± 2.6	18.4 ± 3.7	4.9 ± 1.9	52.3 ± 1.7	85.0 ± 0.1
	% FLS	36.4 ± 1.6	45.8 ± 5.9	57.1 ± 20.2	48.0 ± 11.3	39.2 ± 3.5
Soy protein isolates	% FE	45.0 ± 7.1	70.0 ± 14.1	50.0 ± 0.0	35.0 ± 7.1	35.0 ± 7.1
	% FVS	23.2 ± 2.6	16.7 ± 1.5	21.7 ± 2.4	17.4 ± 0.7	22.8 ± 5.1
	% FLS	81.7 ± 2.4	58.3 ± 2.4	75.0 ± 0.0	80.0 ± 7.1	86.6 ± 2.3
Sodium caseinate	% FE	445.0 ± 7.1	430.0 ± 14.1	135.0 ± 7.1	335.0 ± 21.2	370.0 ± 21.2
	% FVS	20.2 ± 2.3	60.8 ± 4.4	23.3 ± 5.3	9.7 ± 3.6	20.1 ± 3.5
	% FLS	22.2 ± 0.0	36.2 ± 7.7	46.4 ± 5.1	22.5 ± 3.5	24.7 ± 6.7

PC protein showed no foaming properties in pH range of 3 to 9. At pH 10, Jatropha PC protein gave poor foaming properties with foam expansion of 50% and foam volume stability of 9% after 30 min (Table 5.2). These poor foaming properties may be due to the presence of oil in the PC protein, which lead to the emulsion formation instead of foam formation. Due to the higher surface tension of air-water interface, it was more likely that proteins were adsorbed more easily to the oil-water interface than to the air-water interface. As a result, this prevented the foam formation of the press-cake protein solutions. Table 5.2 shows that foaming expansion (FE) of Jatropha DOPC protein was low under acidic and neutral conditions (60 to 100%). The FE of the Jatropha DOPC protein solution was low, even at pH 2, where the protein solubility was rather high. The increase of pH from 2 to 3 slightly reduced FE, which may be due to the lower protein solubility. However, Jatropha de-oiled seed protein still showed foaming expansion at its isoelectric pH (pH 5), even if it was quite low (about 60%). FE of the Jatropha DOPC protein was low at pH 7 (about 80%), but an increase of pH to 10 improved the FE up to about 400%. Just like in emulsion formation, foaming expansion at different pH is more likely correlated to conformational state and rheological properties of the protein films. This means that pH will facilitate protein unfolding at the surface, which leads to protein adsorption to the air-water interfacial films.

According to Damodaran [26], there are two macroscopic processes that contribute to foam instability: liquid drainage and Ostwald ripening or gas disproportionation. The drainage of liquid from the interfacial air-water film leads coalescence of bubbles due to film thinning and rupture. On the other hand, gas disproportionation is a diffusion of gas from small bubbles to large bubbles, which leads to the shrinkage of small bubbles and expansion of large bubbles. As proteins are adsorbed to the interfacial

air-water film, the physicochemical and viscoelastic properties of the protein film can reduce the rate of liquid drainage and gas disproportionation, and, therefore, stabilize the foam [26]. The ability of proteins to stabilize foam will be expressed as foam liquid stability (FLS) and foam volume stability (FVS) [27]. FLS describes protein ability to prevent liquid drainage, while FVS describes protein ability to prevent bubbles coalescence.

We observed that FVS of the DOPC protein was well corresponded to the protein solubility curve (Figure 5.1). FVS of the DOPC protein was the lowest at its isoelectric pH, moderate at pH 2 and pH 7, and reached the highest at pH 10. On the other hand, FLS of the DOPC protein showed an opposite behaviour. FLS was the highest at the protein isoelectric pH, moderate at pH 3 and 7, and low at pH 2 and 10 (Table 5.2). However, even if FLS was low at pH 10 (~40%), foam volume stability at pH 10 was high (80%). Therefore, even though about 60% of the liquid in the foam was drained, about 80% foam volume can still be maintained. This may indicate that DOPC protein can form thin interfacial layers with a high viscoelasticity, which can prevent bubbles coalescence to some extent and stabilize the foam volume.

Compared to the reference proteins, DOPC protein had higher FE than soy protein isolate and lower FE than sodium caseinate at most of the tested pH, except in pH 10. At pH 10, Jatropha DOPC protein had higher FE than sodium caseinate. In terms of foam stability, Jatropha DOPC protein ability to retain liquid (FLS) were lower than soy protein isolate and higher than sodium caseinate at all tested pH. Despite that, Jatropha DOPC protein ability to maintain foam volume (FVS) was higher than both soy protein isolate and sodium caseinate at pH 2, 7, and 10.

However, Jatropha DOPC foaming properties were still inferior to egg white protein at all range of pH (FE 368-525%; FVS 71-91%; FLS 20-49%) [25]. DOPC protein foaming properties was comparable to egg white protein only at pH 10 (FE 425%; FVS 85%; FLS 39%). According to de Graaf [28], the foam expansion of the commercial foaming agent is about 750%, which is almost two-times higher than Jatropha protein foaming expansion. This indicates that some protein modifications—physical, chemical, or enzymatic— should be conducted to use Jatropha seed protein as foaming agent. However, we can indicate, based on our results, that Jatropha DOPC protein may be used as foaming agent at alkaline pH, for example as foaming agent in alkaline foaming detergent for industrial equipment cleaning, or alkaline foam for fire distinguisher [29].

5.3.5 Film forming properties

We investigated film-forming properties of the protein extracted from Jatropha PC and DOPC. The objectives are to investigate the effect of glycerol concentration on Jatropha protein film properties and to compare the film properties of film made from PC protein and DOPC protein. The film properties are analysed based on the mechanical properties tensile strength (TS), elongation, and Young's Modulus (E). We found that by increasing the glycerol concentration, TS was decreasing while elongation increased for both PC proteins film and DOPC protein film (Figure 5.3). PC protein film had TS of 0.5-1 MPa and elongation of 65-75%, while the DOPC protein

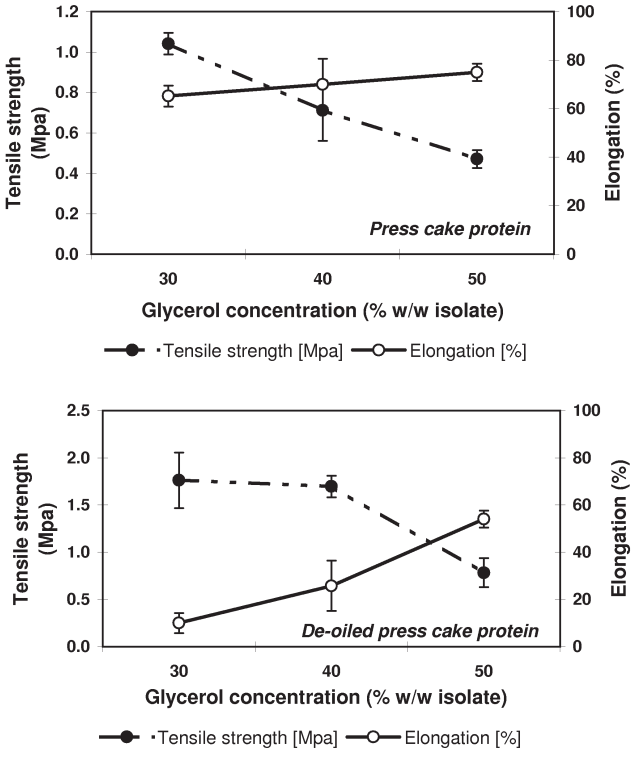

Figure 5.3 the effect of plasticizer concentration on tensile strength of the Jatropha seed protein film.

film had TS of 0.6-1.8 MPa and an elongation of 10-55%. Considering the film stiffness, expressed as E modulus, (Figure 5.4), films made by DOPC protein had higher Young's Modulus than films based on PC protein. This indicated that DOPC protein film was stiffer than PC protein film, which due to the higher oil content in PC protein.

Compared to some other plant proteins used for its film forming properties, such as soy protein and wheat-gluten, Jatropha seed protein films had a low tensile strength but a rather high elongation. Jatropha seed protein films have a lower tensile strength and comparable elongation to soy protein-glycerol films (TS 5-13 MPa; elongation 17-86%) [30, 31]; a comparable tensile strength and lower elongation than wheat gluten-glycerol films before modification (TS 0.5- 4 MPa; elongation

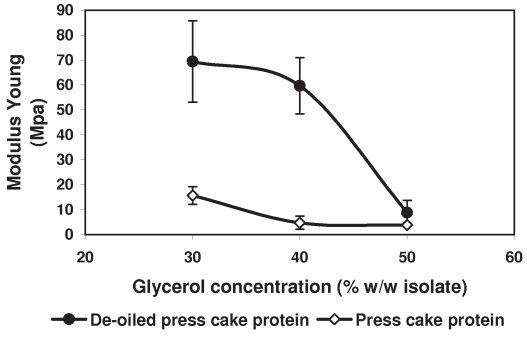

Figure 5.4 the effect of plasticizer concentration on Jatropha seed protein film stiffness.

170-208%) [32, 33]. Based on these mechanical properties, possible applications of Jatropha seed protein film may still be limited. We need to improve Jatropha seed protein films mechanical properties by protein modifications, such as protein heat denaturation or cross-linking [8, 28, 32, 33]. Other drawbacks of Jatropha seed protein is that it forms a brown colored film, which may not be suitable for coating or packaging that specifically required colourless film or transparency. However, Jatropha seed protein films may have potential uses for biodegradable trash bags, water-soluble bags or microcapsules for fertilizer and pesticides, or agricultural mulches, in which transparency may not be required [17].

5.3.6 Jatropha seed protein adhesive properties

We investigated adhesive properties of the protein extracted from Jatropha seed, PC and DOPC, and compared it with adhesive properties of casein, which commercially is being used as labelling adhesives. We found that Jatropha seed protein had comparable adhesive properties with casein adhesives (Table 5.3). Adhesives made from solubilising Jatropha press cake protein in 0.055 M NaOH solution had higher viscosity (4338 cP) than casein in water adhesive (888 cP) at the same dry matter content (Table 5.3). PC protein adhesive had higher viscosity than DOPC protein (931 cP). The set-time of PC protein adhesive (2.25 min) and DOPC (1.25 min) are faster than casein adhesive (2.5 min). For the open time, Jatropha seed protein adhesive had a longer open time (8 - 9.5 min) than casein adhesive (3.5 min) at the same dry matter content (13%). The commercial casein adhesive has about 23% casein in the formulation. However, when we applied this formulation at the lab, this formulation was too thick to be stirred and measured. For adhesive applications, viscosity is important to control the amount of adhesives applied onto a substrate, while ensuring the ease of applications.

From another source, the 23% casein adhesive has open time of 8 min and setting time of 2-3 min [34], which indicates that Jatropha seed protein adhesive formulation may have a slightly better performance than the commercial casein adhesive formulation.

Table 5.3 Jatropha seed protein adhesive properties (n=2).

Adhesive formulation	13% casein	13% DOPC protein	13% PC protein
Solvent	Water	0.055 M NaOH	0.055 M NaOH
Viscosity [cP] direct	888 ± 87	931 ± 115	4338 ± 442
Viscosity [cP] 2 days	116 ± 14	383 ± 6.2	170 ± 3.5
pH	7	8	8
Color	yellow	brown	brown
Set time [min]	2.5 ± 0.7	1.25 ± 0.4	2.25 ± 0.35
Open time [min]	3.5 ± 0.7	8 ± 2.83	9.5 ± 0.7
Adhesion (60% RH)	+++	+++	++
Adhesion (water)	-	++	+

This indicated that less Jatropha seed protein was needed than casein to make adhesive with the similar performance. In addition, Jatropha DOPC and PC protein adhesive had better adhesive properties in water than casein adhesive. Based on these results, Jatropha seed DOPC protein may have potential uses for paper adhesives. For other types of adhesives, e.g. bottle labelling adhesives or plywood adhesives, some other test and adjustment may still be needed to investigate its performance.

5.4 CONCLUSION

Based on our observations during protein extraction, protein recovery after extraction from PC was higher than after extraction from de-oiled PC. From this result, we concluded that hexane extraction reduced the protein extractability from the PC, due to the drier and more compact matrix of the de-oiled PC particles. However, the solubility test of the extracted proteins showed that the extracted proteins from either PC or de-oiled PC showed similar trends. This indicated that hexane extraction affected protein extractability from the raw material but not affected the solubility of the extracted proteins. Jatropha seed proteins had the highest solubility at alkaline conditions (above pH 9). Jatropha PC and DOPC protein had the best emulsifying properties at pH 9 and pH 10, in which they have comparable performance to sodium caseinates. For the foaming properties, DOPC protein had higher foam expansion and foam volume stability than sodium caseinate at pH 10. In general, Jatropha seed protein films had a low tensile strength but with rather high elongation. Jatropha seed protein, especially DOPC proteins, had adhesive properties comparable with casein adhesives. In addition, Jatropha press cake protein adhesive has a better set-time and adhesive properties in water than casein adhesive at the same dry matter content.

In general, Jatropha seed protein has the best performance in alkaline region, due to its high solubility, high emulsifying, and high foaming properties. Jatropha protein-based films and adhesives are best formed from alkaline solutions (NaOH 0.055 M), due to the high protein solubility. The other aspect of Jatroph*a* seed cake protein is its brown colour. Therefore, we should find some applications in alkaline environment where colour is not a big issue, such as emulsifier or stabilizer (emulsifying properties), foaming agent in alkaline detergent or fire fighter foam (foaming properties), biodegradable trash bags, water-soluble bags or microcapsules for fertilizer and pesticides, or agricultural mulches (film forming properties), and adhesives. For the better performance, we may still need to improve Jatropha seed protein functional properties, especially film-forming and foaming properties, by protein modifications, such as protein denaturation or cross-linking.

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REFERENCE

1. K. Openshaw, A review of Jatropha curcas: an oil plant of unfulfilled promise, Biomass Bioenerg., 19 (2000) 1-15.
2. G.M. Gubitz, Exploitation of the tropical oil seed plant Jatropha curcas L, Bioresource Technology, 67 (1999) 73.
3. J. Lin, F. Yan, L. Tang, F. Chen, Antitumor effects of curcin from seeds of Jatropha curcas, Acta Pharmacologica Sinica, 24 (2003) 241-246.
4. J. Martinez-Herrera, P. Siddhuraju, G. Francis, G. Davila-Ortiz, K. Becker, Chemical composition, toxic/antimetabolic constituents, and effects of different treatments on their levels, in four provenances of Jatropha curcas L. from Mexico, Food Chemistry, 96 (2006) 80-89.
5. H.P.S. Makkar, K. Becker, Jatropha curcas toxicity: identification of toxic principle(s), Toxic Plants Other Nat. Toxicants, [Proc. Int. Symp. Poisonous Plants], 5th, (1998) 554-558.
6. H.P.S. Makkar, G. Francis, K. Becker, Protein concentrate from Jatropha curcas screw-pressed seed cake and toxic and antinutritional factors in protein concentrate, J. Sci. Food Agric., 88 (2008) 1542-1548.
7. R.K. Devappa, B. Swamylingappa, Biochemical and nutritional evaluation of Jatropha protein isolate prepared by steam injection heating for reduction of toxic and antinutritional factors, J. Sci. Food Agric., 88 (2008) 911-919.
8. C.M. Vaz, L.A.d. Graaf, W.J. Mulder, Adhesives, coatings and bioplastics from protein sources, in: S.R. Fahnestock, A. Steinbuchel (Eds.) Polyamides and Complex Proteinaceous Materials II Wiley-VCH, New York 2003, pp. 383 - 404.
9. J. Kinsella, Functional properties of soy proteins, Journal of the American Oil Chemists' Society, 56 (1979) 242-258.
10. A. Moure, J. Sineiro, H. Dominguez, J.C. Parajo, Functionality of oilseed protein products: A review, Food Res. Int., 39 (2006) 945-963.
11. L. L'Hocine, J.I. Boye, Y. Arcand, Composition and functional properties of soy protein isolates prepared using alternative defatting and extraction procedures, Journal of Food Science, 71 (2006) C137-C145.
12. D. Lestari, W. Mulder, J. Sanders, Improving Jatropha curcas seed protein recovery by using counter current multistage extraction, Biochemical Engineering Journal, In Press, Corrected Proof.
13. J. Kjeldahl, Nitrogen determination technique, Z. Anal. Chem., 22 (1883) 366.
14. A.A. Betschart, Nitrogen solubility of alfalfa protein concentrate as influenced by various factors Journal of Food Science, 39 (1974) 1110-1115.
15. M.J. James, P.D. Patel, Development of a standard oil-in-water emulsification test for proteins, in: Research Report No. 631, Leatherhead Food R.A., 1988.
16. P.D. Patel, A.M. Stripp, J.C. Fry, Whipping test for the determination of foaming capacity of protein: a collaborative study, International Journal of Food Science & Technology, 23 (1988) 57-63.
17. J.M. Krochta, Proteins as Raw Materials for Films and Coatings: Definitions, Current Status, and Opportunities, in: A. Gennadios (Ed.) Protein-based films and coatings CRC Press, Boca Raton [etc.] 2002, pp. 1-32.
18. D. Lestari, W.J. Mulder, J.P.M. Sanders, Improving Jatropha curcas seed protein recovery by using counter current multistage extraction, Biochemical Engineering Journal, 50 (2010) 16-23.
19. R. Walstra, The role of proteins in stabilization of emulsions, in: G.O. Phillips, D.J. Wedlock, P.A. Williams (Eds.) Gums and stabilizers for the food industry, IRL Press, Oxford 1988, pp. p323–336.
20. S.E. Hill, Emulsions, in: G.M. Hall (Ed.) Methods of Testing Protein Functionality, Blackie Academic & Professional, an imprint of Chapman & Hall, London, 1996, pp. 153-185.
21. E. Dickinson, Adsorbed protein layers at fluid interfaces: interactions, structure and surface rheology, Colloids and Surfaces B: Biointerfaces, 15 (1999) 161-176.

22. M. Kerker, The Scattering of Light and Other Electromagnetic Radiation, Academic Press, London, 1969.
23. S. Tcholakova, N.D. Denkov, D. Sidzhakova, I.B. Ivanov, B. Campbell, Interrelation between Drop Size and Protein Adsorption at Various Emulsification Conditions, Langmuir, 19 (2003) 5640-5649.
24. P. Walstra, A.L. De Roos, Proteins at air-water and oil-water interfaces: Static and dynamic aspects, Food Reviews International, 9 (1993) 503 - 525.
25. E. Massoura, J.M. Vereijken, P. Kolster, J.T.P. Derksen, Proteins from *Crambe abyssinica* Oilseed. II. Biochemical and Functional Properties, JAOCS, 75 (1998) 329-335.
26. S. Damodaran, Protein stabilization of emulsions and foams, Journal of Food Science, 70 (2005).
27. P.J. Wilde, D.C. Clark, Foam formation and stability, in: G.M. Hall (Ed.) Methods of Testing Protein Functionality, Blackie Academie & Professional, an imprint of Chapman & Hall, London, 1996, pp. 110-152.
28. L.A. de Graaf, P. Kolster, Industrial proteins as a green alternative for 'petro' polymers: Potential and Limitations, Macromolecular symposia, (1998) 51-58.
29. J.L. Scheffey, R.L. Darwin, J.T. Leonard, Evaluating firefighting foams for aviation fire protection, Fire Technology, 31 (1995) 224-243.
30. A.H. Brandenburg, C.L. Weller, R.F. Testin, Edible Films and Coatings from Soy Protein, Journal of Food Science, 58 (1993) 1086-1089.
31. Y.M. Stuchell, J.M. Krochta, Enzymatic Treatments and Thermal Effects on Edible Soy Protein Films, Journal of Food Science, 59 (1994) 1332-1337.
32. L.A. de Graaf, Denaturation of proteins from a non-food perspective, J. Biotechnol., 79 (2000) 299-306.
33. A. Gennadios, A.H. Brandenburg, C.L. Weller, R.F. Testin, Effect of pH on properties of wheat gluten and soy protein isolate films Journal of agricultural and food chemistry 41 (1993) 1835-1839.
34. J. Gueguen, Green chemicals and biopolymers from rapeseed meal with enhanced end-uses performance. Final Report., in: Quality of life and management of living resources, ENHANCE, 1999.

Chapter 6

The method to calculate the potential economic value of potential products from Jatropha seed in five selected countries: Zimbabwe, Tanzania, Mali, Indonesia, and the Netherlands

This chapter is submitted as: D. Lestari and E. Zvinavashe, Johan P. M. Sanders, The method to calculate the potential economic value of potential products from Jatropha seeds in five selected countries: Zimbabwe, Tanzania, Mali, Indonesia, and the Netherlands.

ABSTRACT

Currently, Jatropha seeds are mainly used to produce biodiesel, and a resultant press cake is obtained as a by-product. Jatropha press cake fractionation and conversion into various potential products using biorefinery, could improve the economic value of Jatropha seed. The objectives of this study were to calculate the potential generated income and economic value of products from Jatropha. These were classified into two product groups: 1) Rural products, and 2) Industrial products. The potential generated income is the summation of the net profit and the labour costs. Using the national gross domestic product (GDP) per capita as a reference, we calculated and compared the potential economic value, which expresses the number of people that could obtain a certain income for every hectare of Jatropha plantation, in five selected countries. These countries consisted of four developing countries: Tanzania, Mali, Zimbabwe, and Indonesia, and a developed country, the Netherlands, as a reference. The study shows that the potential generated income from rural or industrial products per hectare of (imported) Jatropha was much lower than the income of one person in the Netherlands, which may be due to the high national GDP per capita. On the other hand, the potential economic value per hectare Jatropha in developing countries suggests that manufacturing the rural products can generate potential income for one person, while manufacturing the industrial products can generate a higher potential income for approx. 13 - 35 people.

Keywords:
Jatropha seed components, biorefinery, total revenue, production cost estimation, Jatropha potential economic value

6.1 INTRODUCTION

Jatropha curcas or Jatropha is a small tree with a smooth grey bark, which normally grows to a height of 3-5 m or of 8-10 m under favourable conditions [1]. Traditionally, Jatropha is used as a living fence around homesteads. Currently, Jatropha seeds are mainly used to produce biodiesel, and a resultant press cake is obtained as a by-product. Jatropha press cake fractionation and conversion into various potential products using biorefinery could improve the economic value of Jatropha seed [2, 3]. The potential economic value expresses the number of people that could obtain an income per hectare Jatropha plantation. In this study, the objectives were: 1) to calculate the total revenue and the net profit of (imported) Jatropha seeds after biorefinery using the estimated production costs, and 2) to calculate the potential generated income and the potential economic value from manufacturing products through biorefinery in five selected countries: Tanzania, Mali, Zimbabwe, Indonesia (developing countries) and the Netherlands (reference).

6.2 THE DEFINITIONS OF PRODUCT GROUPS

6.2.1 The potential of Jatropha side products

It is proposed that a farmers' interest to grow Jatropha is currently low due to the low yield and market value of Jatropha seeds compared to food crops. Francis et.al [4] reported that the yield of Jatropha seed varied between 0.2 to 2 kg dry seed per tree. Other study [5] reported a seed yield of 0.9 kg dry seed per tree, in a rain fed dry land. Assuming a plant density of 4444 plant/ ha (at 1.5 m x 1.5 m spacing), this results in a total seed yield of 4 tonnes/ha. However, the actual Jatropha seed yield in a commercial field is still lower than the expected yield of dry seeds / ha per annum [5-7]. Several experimental Jatropha fields are conducting research to improve the actual yield of Jatropha seeds.

The net profit of different crops was calculated by deducting the gross profit with the crop production cost from Purwono and Purnamawati [8]. This net profit comparison suggests that the net profit of Jatropha was much lower than that of selected food crops (Figure 6.1). To calculate the economic value, this study used Jatropha seed yield of 2 tonnes/ha (50% of the value reported Gunaselaan [5]), which is more realistic in relation to the current situation. Jatropha seed yield is dependent on soil conditions. However, here it is assumed that seed yield is similar in all the studied countries to be able to make a more direct comparison.

Table 6.1 Yield and price of Jatropha seed compare to other food crops in Indonesia.

Feedstock	Yield[1)] [kg/ha]	Market value[2)] [IDR/kg]	Market value[2)] [EUR/kg][3)]	Total revenue [EUR/ ha]
Rice	5000	4000	0.34	1694
Corn	4237	1500	0.21	898
Soybeans	1348	6000	0.51	686
Peanuts	1249	7800	0.66	826
Cassava	18764	1500	0.13	2383
Jatropha seed	2000	1200	0.10	200

Note: Data was accessed on October 2011 from:
[1)] Year 2009, http://www.bps.go.id/tnmn_pgn.php?eng=0 (Accessed on October 2011)
[2)] http://distanak.donggala.go.id/harga/hargaeceranjun2006.html, [3)] 1 EUR = IDR 12000

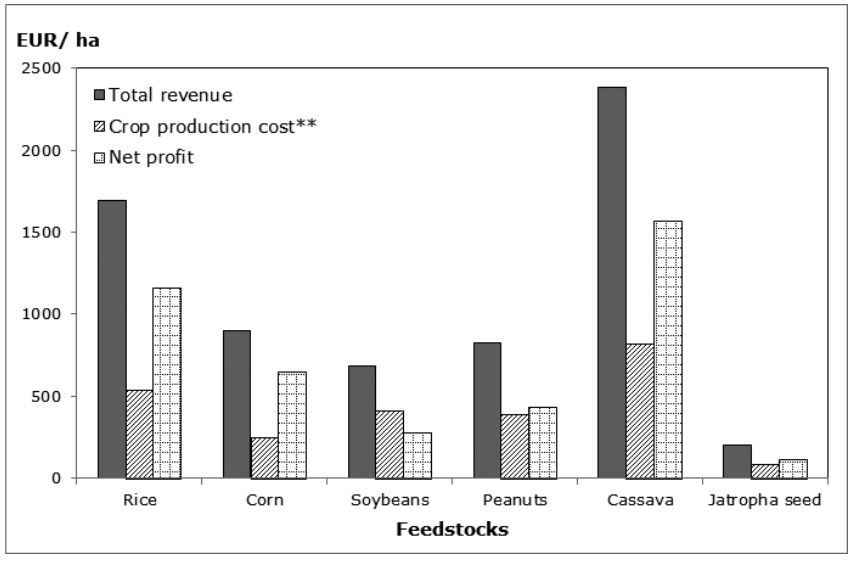

Figure 6.1 The total revenue and net profit from different food crops and Jatropha seeds in Indonesia at the farmer level. Note:**Crop production cost from Purwono and Purnamawati (2007).

The composition of Jatropha whole seed dry matter (before oil pressing) is approximately 36% fat, 28% crude fibre, 19% crude protein, 5% ash, 9% carbohydrate, and 3% minerals [9]. To calculate the yield of Jatropha components and their potential products, the following assumptions were used: 1) Jatropha seed yield was 2 tonnes/ ha; 2) biodiesel conversion from oil was 80% [10]; and 3) lignin-based binder formulation consist of about 20% w/w lignin as reported by Mathiasson [11]. Table 6.2 shows the potential yield of Jatropha seeds components after fractionation and their potential products.

Table 6.2 The potential yield of Jatropha seeds fractionated components and their potential products.

Fractionated components	Componentyield [kg/ha per year]	Potential selected products	Product yield from components [kg/kg]	Total product yield [kg/ ha per year]
Seeds	2000	-	-	-
Oil	720	Biodiesel	0.8[2)]	576
Proteins	380	Poultry feed	1.0	380
		Pig feed	1.0	380
		Coating/Adhesives	5.0[3)]	1900
Carbohydrates	180	Cattle feed	1.0	180
		Ethanol	0.5	90
Fibers/ lignin	560	Briquettes	1.0	560
		Binders	5.0[3)]	2800
Ash/ minerals	160	Fertilizer	1.0	160

Note: [1)] Assumption of Jatropha seeds yield; [2)] Based on the calculation from Syakir (2010); [3)] Based on lignin-based binder compositions from Mathiasson (1994).

Jatropha seed also contains anti-nutritional components e.g. trypsin inhibitors, curcin, saponins, phytate and toxic components e.g. lectins and phorbol esters, which limits its application in the food sector [12-14]. Several methods and patents to detoxify, or inactivate, the toxic or anti-nutritional compounds in Jatropha seeds have been developed [15, 16]. Jatropha press cake is rich in protein, fibres, lignin, carbohydrates, and some minor components (minerals and vitamins), where the use of biorefinery could generate different products and improve the total revenue and the net profit from Jatropha seed [17-20]. These components can be used not only as nutrients e.g. animal feed or vitamins, but also as technical products e.g. paper pulp, construction materials, adhesives, or coatings [21-25]. Jatropha press cake (the by-product after oil pressing) contains about 23-28% protein, which is potential for animal feed after detoxification [20, 26]. In addition, the functional properties of the protein could be suitable for non-food technical products, such as adhesives or coatings or paint formulations [27]. Several articles reported up to 60% recovery of the protein content from Jatropha press cake after extraction with alkaline solutions at pH 10 to 11 [20, 26, 28]. The press cake also contains carbohydrates that can be used for cattle feed or for second-generation (bio) ethanol. The remaining fraction will be rich in fibres and lignin, which has potential to produce briquettes or binders for fibreboard. In addition, the minerals such as nitrogen, potassium, and phosphorus can be used as soil fertilizer.

The market value of Jatropha seed components and their potential products in five selected countries (Table 6.3) were used to calculate the total market value of Jatropha products in those countries. The market value of the potential products were based on statistical data from 2005 to 2006 provided by FAO, UN Comtrade, and other sources, taken in January 2008 [29-34]. The price of jatropha protein was estimated using the

price of soy protein in the United States per January 2010. This was reported as soy functional concentrate (~70% proteins) at 1.3-1.9 EUR/ kg and soy protein isolates (>90% proteins) at 2.3-3.4 EUR/ kg [35].

Table 6.3 The cost of Jatropha seeds components and the market value of their potential products in five selected countries: Zimbabwe (Zim), Tanzania (Tan), Mali, Indonesia (Ind), the Netherlands (Ned).

Fractionated components	Component cost [EUR/ kg]	Selected potential products	Product market value in different countries [EUR/ kg]				
			Zim	Tan	Mali	Ind	Ned
Seeds	**0.10**[1)]	-	-	-	-	-	-
Oil	0.40[1)]	Biodiesel	0.48	0.62	0.64	0.76	0.54
Proteins[6)]	0.15	Poultry feed	0.20	0.20	0.20	0.20	0.80
	0.10	Pig feed	0.15	0.15	0.15	0.15	0.50
	1.50	Coating ingridient[2)]	1.84*	0.55	0.46**	1.52	2.03
	2.80	Adhesives ingridient[3)]	0.87*	1.40	1.00**	1.58	1.35
Carbohydrates	0.08	Cattle feed	0.10	0.10	0.10	0.10	0.20
	0.20	Ethanol[4)]	1.30	1.30	1.30	1.30	1.30
Fibers/ lignin	0.10	Briquettes	0.30	0.30	0.30	0.30	0.30
	0.40	Binders[2)]	0.61	1.22	3.83	1.26	1.09
Ash/ minerals	0.02	Fertilizer[5)]	0.02	0.02	0.02	0.02	0.02

Notes: Statistical data year 2006 except *2005 and **2004 from (all data were taken on January 2008)
[1)]Data from Syakir (2010) [10]
[2)]http://comtrade.un.org/db/mr/daCommoditiesResults.aspx?px=S3&cc=53341
[3)]http://comtrade.un.org/db/mr/daCommoditiesResults.aspx?px=H1&cc=390940 [4)]http://earthtrends.wri.org/searchable_db/index.php?action=select_variable&theme=9 [5)]http://faostat.fao.org/site/575/DesktopDefault.aspx?PageID=57;
[6)] the price of jatropha protein ~ soy protein in the United States per January 2010 as follows: soy functional concentrate (~70% proteins) of 1.3-1.9 EUR/ kg; soy protein isolates (>90% proteins) of 2.3-3.4 EUR/ kg [35].

6.2.2 The potential revenue of Jatropha seed components and their products

Jatropha seed products are produced using the two biorefinery steps:

a) Fractionation into different components e.g. oil, proteins, carbohydrates, fibre/ lignin, and ash/minerals. This fractionation step could produce either the non-functional components, e.g. low functionality proteins, or native cellulose, or the high-functional components, e.g. functional protein isolates, hydrolysed sugars, or purified lignin.

b) Conversion of the (non-functional and functional) components into various products. These are classified as rural products e.g. feed, briquettes, or fertilizer and produced using minor processing (group 1) and industrial products e.g. ethanol, coatings/ paint or adhesives, produced using more advanced processing (group 2). .

The revenue of each component or product can be calculated from the potential seed components yield (Table 6.2), the component cost and the product market value (Table 6.3), using the formula in equation 6.1 and 6.2. The total revenue of Jatropha seed components and their potential products in five selected countries are shown in Table 6.4.

Product revenue, = Yield, x Market value, (6.1)

With: *Product revenue,* = the revenue of product [EUR/ha per year)]
Yield, = Potential yield of seed components or product [kg/ha per year]
Market value, = Market value of product per unit [EUR/kg]

Total revenue, p = ∑ *Product revenue,* (6.2)

With: *Total revenue, p* = Total revenue of the package group [EUR/ha per year]
Product revenue, = Total potential value of product [EUR/ha per year]

6.2.3 The calculation of the net profit and potential economic value of Jatropha seed components and their potential products in five selected countries

The net profit of the product groups are calculated by deducting total revenue (Table 6.4) with production cost (Table 6.5) following Equation 6.3.

Net profit, p = Total revenue, p – Production cost, p (6.3)

With: *Net profit, p* = Net profit of the group [EUR/ha per year]
Production cost, p = Production cost of the group [EUR/ha per year]

Here the production costs are estimated from the cost of components as the raw material to manufacture the products. As references for simple processes, the cost of the oil is about 85% of the total biodiesel production cost and the cost of corn starch is about 80% of the total first generation ethanol production cost. As reference for the more advance process, the cost of wheat straw is about 30% of the total second generation ethanol production cost from lignocellulosic materials [36, 37]. These references suggest that the component cost comprises about of 30 to 85% of the total production cost, depending on the complexity of the process. A more straightforward process will have a higher component cost to total production cost ratio. Another part of the production costs is the labour cost. This will benefit the local community by providing jobs and income for workers. Labour costs in the biomass processing is about 20% of the total production cost [36, 37]. Although the costs are rough estimations, they give an indication for comparisons to be made between countries. Next, labour cost and net profit are used to calculate the potential generated income following Equation 6.4, which results are presented in Table 6.6. The potential economic value of each group is calculated by dividing the generated income from the selected group with GDP per capita following Equation 6.5. The results are presented in Figure 6.2.

Generated income, p = Net profit, p + Labour cost, p (6.4)

With: *Generated income, p* = generated income for workers [EUR/ha per year]
Labour cost, p = Approx. 20% of production cost [EUR/ha per year]

Table 6.4 the revenue of Jatropha seeds components and their potential products in five different countries: Zimbabwe (Zim), Tanzania (Tan), Mali, Indonesia (Ind), the Netherlands (Ned).

Jatropha seeds components	Potential products	Rural product revenue [EUR/ ha per year]				
		Zim	Tan	Mali	Ind	Ned
Oil	Biodiesel	276	357	369	438	311
Protein	Poultry feed	76	76	76	76	304
Carbohydrates	Cattle feed	18	18	18	18	36
Fiber/ lignin	Briquettes	168	168	168	168	168
Ash/ minerals	Fertilizer	3	3	3	3	3
Total	Total	542	622	634	703	822
Jatropha seed components	**Potential products**	**Industrial product revenue [EUR/ ha per year]**				
		Zim	**Tan**	**Mali**	**Ind**	**Ned**
Oil	Biodiesel	276	357	369	438	311
Protein	Coating/ paint	3496	-	-	-	3857
	Adhesives	-	2660	1900	3002	
Carbohydrates	Ethanol	117	117	117	117	117
Fiber/lignin	Binders	1708	3403	10712	3541	3052
Ash/ minerals	Fertilizer	3	3	3	3	3
Total	Total	5601	6541	13100	7101	7340

Economic value, p = Generated income, p ÷ GDP (6.5)

With: *Economic value, p* = Number of person income [number of persons/ ha]

GDP = Gross domestic product in each country [EUR/ person per year]

In Indonesia, manufacturing rural products from Jatropha seed components could generate net profit of 190 EUR/ ha per year (Table 6.6). This net profit is almost two-folds higher than the net profit from Jatropha seeds without processing (about 100 EUR/ ha per year), but it is still lower than that of food crops without further processing (Figure 6.1). The net profit from Jatropha seeds can be further improved by manufacturing industrial products, which opens up the possibility to generate more income for a number of people. The food crops may also result in higher net profit when they are processed further into various products e.g. oil and proteins from soybean, starch and proteins from corn or second-generation ethanol from cassava. Cultivation of Jatropha can give benefit and could be a good option for soil types that are unsuitable for these food crops.

Table 6.5 The cost estimation of Jatropha seeds fractionation and conversion into selected products.

Jatropha seeds components	Potential products	Component cost [EUR/ ha per year]	Component cost to total production cost ratio	Estimate total production cost [EUR/ ha per year]
Oil	Biodiesel	288	0.85	339
Proteins	Poultry feed	57	0.75	76
Carbohydrates	Cattle feed	38	0.75	19
Fiber/ lignin	Briquettes	56	0.75	75
Ash/ minerals	Fertilizer	3	0.75	4
Total	Total	419		513
Jatropha seeds components	**Potential products**	**Component cost [EUR/ ha per year]**	**Component cost to total production cost ratio**	**Estimate total production cost [EUR/ ha per year]**
Oil	Biodiesel	288	0.85	339
Proteins	Coating/ paint Adhesives	1064	0.70	1520
Carbohydrates	Ethanol	36	0.33	109
Fiber/lignin	Binders	224	0.70	320
Ash/ minerals	Fertilizer	3	0.75	4
	Total	1615		2292

Table 6.6 Estimated generated incomes from the net profits and labour cost from Jatropha product groups and the GDP per capita in 2006 in five different countries: Zimbabwe (Zim), Tanzania (Tan), Mali, Indonesia (Ind), the Netherlands (Ned).

	Zim	Tan	Mali	Ind	Ned
Rural products					
Estimate net profit [EUR/ ha per year]	29	109	121	190	309
Labour cost [EUR/ ha per year]	77	77	77	77	77
Potential generated income [EUR/ ha per year]	106	186	198	267	386
Industrial products					
Estimate net profit [EUR/ ha per year]	3308	4248	10808	4809	5048
Labour cost [EUR/ ha per year]	458	458	458	458	458
Potential generated income [EUR/ ha per year]	3767	4707	11267	5268	5506
Economic indicator					
National currency	US$	TZS	XOF	IDR	EUR
GDP per capita 2006 [National currency]	388	419000	214000	3338000	32700
GDP per capita 2006 [EUR/ person per year]	296	210	326	278	32700

6.3 THE POTENTIAL ECONOMIC VALUE OF JATROPHA SEED PRODUCTS

The potential economic value expresses the number of people that can obtain their income per hectare Jatropha plantation (Figure 6.2). Although this value is still an estimation, the comparison between countries is still relevant to show the potential of Jatropha biorefinery to generate income in different countries. In addition, the potential economic value in the rural area may be underestimated since the income in rural areas is often much lower than the average income per capita of the country. The calculation of the potential economic value suggests that one hectare of Jatropha plantation can generate the income for a number of people through manufacturing rural or industrial products from Jatropha seed components. Due to the high national GDP per capita in the Netherlands, the potential generated income from rural or industrial products per hectare of (imported) Jatropha was much lower than the income of 1 person. On the other hand, manufacturing rural products can generate the potential income for 1 person per hectare of Jatropha plantation in Tanzania, Mali, and Indonesia. Manufacturing industrial products from 1 hectare of Jatropha plantation can generate the potential income for approximately 13 people in Zimbabwe, 22 people in Tanzania, 35 people in Mali, and 18 people in Indonesia, which is higher than the potential economic value from some (unprocessed) food crops in Indonesia

6.3.1 Manufacturing rural product group

The results of potential economic value in Figure 6.2 suggests that approx. 1 person can already obtain income by manufacturing rural products per hectare Jatropha in

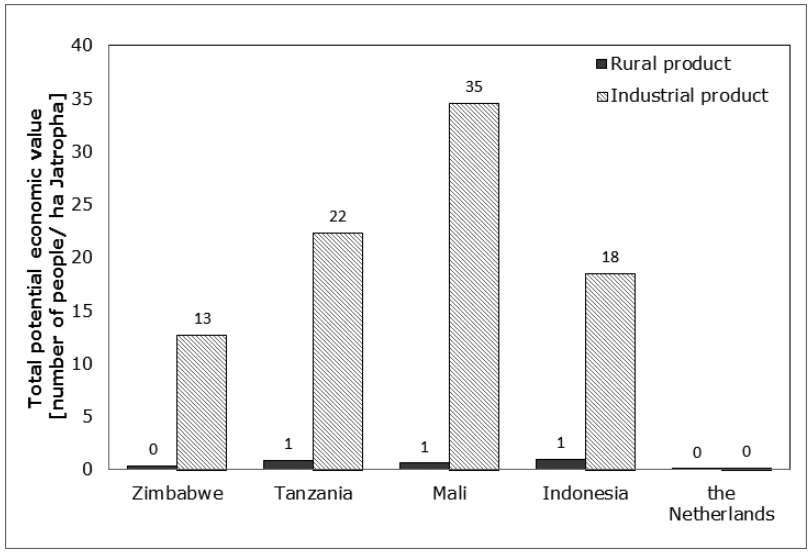

Figure 6.2 The potential economic values of Jatropha fractionated components and their potential products in different countries.

Tanzania, Mali, and Indonesia. The potential economic value from manufacturing rural products in Indonesia is still lower than the potential economic values from most of the food crops without biorefinery (Figure 6.1). The potential economic values of these food crops are similar to the income of about 5 people from rice, three people from corn, 1 people from soybeans, 2 people from peanuts, and 6 people from cassava.

Data of the market availability suggests that most of the studied countries imported rural products e.g. animal feed or fertilizer. For example in 2004, Tanzania and Mali were the net importer of animal feed, with the import amount of about 150,000 tonnes in Tanzania and 413,000 tonnes in Mali. On the other hand, Zimbabwe, Mali, and Indonesia were the net importer countries for fertilizer, with the import amount of about 30,000 tonnes in Zimbabwe, 30, 000 tonnes in Mali, and about 600,000 tonnes in Indonesia [33]. This may indicate that the market for rural products is still widely open, especially in these studied developing countries.

6.3.2 Manufacturing industrial product group

If manufacturing rural products has already given a basic value of economic benefit for the countries studied, then manufacturing industrial products could give much higher potential economic value per hectare plantation. In general, the potential economic value from manufacturing industrial products in the developing countries studied are similar to the income of approx. 13 to 35 people, which is much higher than the potential economic value from manufacturing rural product from the same feedstock. Among the other selected countries, Mali generated the highest potential economic value, which was equal to approx. 35 people per ha of Jatropha plantation (Figure 6.1). Although the potential economic value from manufacturing industrial products were higher than the unprocessed food crops in Indonesia, it is obvious that manufacturing industrial products from these food crops may also improve their potential economic value even higher. However, unlike the food crops, the utilization of the non-edible Jatropha seeds could be fully optimized for manufacturing the rural or industrial products.

Nowadays, there may not be large market volumes available yet in some countries for the industrial products. Nevertheless, exporting these products to other countries, where a larger market volume exists, could generate an additional income for the exporting country. For example, the market demand for adhesives was only about 900 kg in Zimbabwe, 2500 kg in Tanzania, and 6200 kg in Mali [29]. This means that by manufacturing Jatropha protein adhesives (~1900 kg/ ha Jatropha per year), these African countries can supply their national demand while creating a new export commodity to obtain additional income for the country.

6.3.3 Comparison between groups

Between the two groups, manufacturing industrial products from Jatropha seed components would give the highest benefit by generating the highest income for a number of people. Nevertheless, the access to the rural product market is much more realistic within the next few years. The manufacturing of rural products in the rural

area could generate a better social and environmental improvement due to the two main factors. Firstly, building Jatropha processing facilities in rural area could shorten the cycle for returning the minerals back to the soil. Secondly, manufacturing rural products could support the needs for the agriculture sector and the infrastructure of the rural area. Furthermore, valorisation of Jatropha seed components to manufacture rural products may support not only the people who work directly in the Jatropha plantations and/or processing facilities, but also the entire surrounding area where the processing facilities are located.

6.4 CONCLUSIONS

The potential economic value expresses the number of people that could get an income per hectare Jatropha plantation. This value was calculated using the potential generated income, which consists of the net profit and the labour cost. Using the national gross domestic product (GDP) per capita as reference, we calculated the potential economic value for both rural and industrial products in five selected countries. Based on the potential economic value, we conclude that one hectare of Jatropha plantation can generate the income for a number of people through manufacturing rural or industrial products from Jatropha seed components. It is obvious that manufacturing industrial products from food crops may also improve their potential economic value even higher. However, unlike the food crops, the utilization of the non-edible Jatropha seeds can be fully optimized for manufacturing the rural or industrial products. Although the potential economic value may not be too accurate, the comparison between countries is relevant to show the potential of Jatropha biorefinery to generate income in different countries. Our results suggest that the valorisation of Jatropha seed components through biorefinery will have the most impact in Mali, where the product prices are high. Even though the rural products have lower economic value, these products may give social benefits to the society by providing jobs for the rural community and reducing urbanization. Most African countries import industrial products, even those that are not petroleum-based products. By manufacturing industrial products from Jatropha seed components, these countries can supply their national demand while creating new export commodities to obtain additional income for the country.

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REFERENCE

1. J. Heller, Physic nut : Jatropha curcas L. Promoting the conservation and use of underutilized and neglected crops.1 Institute of Plant Genetics and Crop Plant Research, Gatersleben/ International Plant Genetic Resources Institute, Rome Gaterslaben, 1996.
2. E.T. Akintayo, Characteristics and composition of Parkia biglobbossa and Jatropha curcas oils and cakes, Bioresource Technology, 92 (2004) 307-310.
3. K. Openshaw, A review of Jatropha curcas: an oil plant of unfulfilled promise, Biomass Bioenerg., 19 (2000) 1-15.
4. G. Francis, R. Edinger, K. Becker, A concept for simultaneous wasteland reclamation, fuel production, and socio-economic development in degraded areas in India: Need, potential and perspectives of Jatropha plantations, Natural Resources Forum, (2005) 12-24.
5. V.N. Gunaseelan, Biomass estimates, characteristics, biochemical methane potential, kinetics and energy flow from Jatropha curcus on dry lands, Biomass and Bioenergy, 33 (2009) 589-596.
6. R.E.E. Jongschaap, W.J. Corre, P.S. Bindraban, W.A. Brandenburg, Claims and Facts on Jatropha curcas L. , in, Plant Research International B.V., Wageningen UR, Wageningen, 2007.
7. V. Nallathambi Gunaseelan, Biomass estimates, characteristics, biochemical methane potential, kinetics and energy flow from Jatropha curcus on dry lands, Biomass and Bioenergy, 33 (2009) 589-596.
8. Purwono, H. Purnamawati, Budidaya 8 jenis tanaman pangan unggul, Penebar Swadaya, Jakarta, 2007.
9. J. Heller, Untersuchungen über genotypische Eigenschaften und Vermehrungsund Anbauverfahren bei der Purgiernuß (Jatropha curcas L.) [Studies on genotypic characteristics and propagation and cultivation methods for physic nuts (Jatropha curcas L.)]. Dr. Kovac, Hamburg, (1992).
10. M. Syakir, Prospek dan Kendala Pengembangan Jarak Pagar Sebagai Bahan Bakar Nabati di Indonesia, Perspektif, 9 (2010) 55-65.
11. A. Mathiasson, D. Kubát, Lignin as binder in particle boards using high frequency heating, European Journal of Wood and Wood Products, 52 (1994) 9-18.
12. S.M.A. El Badwi, S.E.I. Adam, H.J. Hapke, Toxic effects of low levels of dietary Jatropha curcas seed on Brown Hisex chicks, Veterinary and Human Toxicology, 34 (1992) 112-115.
13. D. Huang, D. Huang, S. Guo, K. Pan, Z. Huang, J. Lin, Isolation and characterization of toxin from Jatropha curcas, Shengwu Huaxue Yu Shengwu Wuli Jinzhan, 18 (1991) 149-151.
14. H.P.S. Makkar, K. Becker, Jatropha curcas toxicity: identification of toxic principle(s), Toxic Plants Other Nat. Toxicants, [Proc. Int. Symp. Poisonous Plants], 5th, (1998) 554-558.
15. J. Brooker, The methods for detoxifying oil seed crops. WO 2010/070264 A1, in: D1 (Ed.) PCT, 2010.
16. H. Makkar, K. Becker, Patent number: WO 2010/092143 A1. Method for detoxifying plant constituents, in: European Patent Office, 2010.
17. J.P.M. Sanders, Biorefinery, the bridge between agriculture and chemistry, in: The 14th European Biomass Conference and Exhibition : Biomass for Energy, Industry and Climate Protection, Paris, France, 2005.
18. R. Manurung, D.A.Z. Wever, J. Wildschut, R.H. Venderbosch, H. Hidayat, J.E.G. van Dam, E.J. Leijenhorst, A.A. Broekhuis, H.J. Heeres, Valorisation of Jatropha curcas L. plant parts: Nut shell conversion to fast pyrolysis oil, Food and Bioproducts Processing, 87 (2009) 187-196.
19. F. Cherubini, The biorefinery concept: Using biomass instead of oil for producing energy and chemicals, Energy Conversion and Management, 51 (2010) 1412-1421.
20. D. Lestari, W.J. Mulder, J.P.M. Sanders, Improving Jatropha curcas seed protein recovery by using counter current multistage extraction, Biochemical Engineering Journal, 50 (2010) 16-23.
21. L.A. de Graaf, P. Kolster, Industrial proteins as a green alternative for 'petro' polymers: Potential and Limitations, Macromolecular symposia, (1998) 51-58.
22. J. van Haveren, E.L. Scott, J.P.M. Sanders, Bulk chemicals from biomass, Biofuels, Bioproducts and Biorefining, 2 (2008) 41-57.
23. J.P.M. Sanders, E.L. Scott, R.A. Weusthuis, H. Mooibroek, Bio-Refinery as the Bio-Inspired Process to Bulk Chemicals, Macromolecular Bioscience, 7 (2007) 105-117.
24. E.L. Scott, F. Peter, J.P.M. Sanders, Biomass in the manufacture of industrial products—the use of proteins and amino acids, Applied Microbiology and Biotechnology, 75 (2007) 751-762.
25. C.M. Vaz, L.A.d. Graaf, W.J. Mulder, Adhesives, coatings and bioplastics from protein sources, in: S.R. Fahnestock, A. Steinbuchel (Eds.) Polyamides and Complex Proteinaceous Materials II Wiley-VCH, New York 2003, pp. 383 - 404.
26. H.P.S. Makkar, G. Francis, K. Becker, Protein concentrate from Jatropha curcas screw-pressed seed cake and toxic and antinutritional factors in protein

concentrate, J. Sci. Food Agric., 88 (2008) 1542-1548.

27. D. Lestari, W.J. Mulder, J.P.M. Sanders, Jatropha seed protein functional properties for technical applications, Biochemical Engineering Journal, 53 (2011) 297-304.
28. R.K. Devappa, B. Swamylingappa, Biochemical and nutritional evaluation of Jatropha protein isolate prepared by steam injection heating for reduction of toxic and antinutritional factors, J. Sci. Food Agric., 88 (2008) 911-919.
29. UNcomtrade, Phenolic resins, in primary forms, Code: 390940 (HS1996), in, United Nations Statistics Division - Commodity Trade Statistics Database, 2006, pp. http://comtrade.un.org/db/mr/daCommoditiesResults.aspx?px=H1&cc=390940
30. UNcomtrade, Paints and varnishes based on synthetic polymers in an aqueous medium. Code: 53341, in, United Nations Statistics Division - Commodity Trade Statistics Database, 2006, pp. http://comtrade.un.org/db/mr/daCommoditiesResults.aspx?px=S3&cc=53341.
31. FAOSTAT, Fiber, in, Food and Agricultural Organization of the United Nations, 2004, pp. http://faostat.fao.org/site/514/DesktopDefault.aspx?PageID=514
32. EarthTrends, in, World Resouuces Institute, 2005, pp. http://earthtrends.wri.org/searchable_db/index.php?action=select_variable&theme=9
33. FAOSTAT, Fertilizer, in, Food and Agricultural Organization of the United Nations, 2004, pp. http://faostat.fao.org/site/575/DesktopDefault.aspx?PageID=557
34. FAOSTAT, FAO statistical yearbook 2010, in, Food and Agricultural Organization of the United Nations, 2010, pp. http://www.fao.org/economic/ess/ess-publications/ess-yearbook/ess-yearbook2010/yearbook2010-prices/en/.
35. OMNI, A survey of recent chemical price trends. The Potential Impact of Rising Petrochemical Prices on Soy Use for Industrial Applications. Prepared for the United Soybean Board, in, OMNI TECH International, Ltd., 2010.
36. A. McAloon, F. Taylor, W. Yee, K. Ibsen, R. Wooley, Determining the Cost of Producing Ethanol from Corn Starch and Lignocellulosic Feedstocks, in, National Renewable Energy Laboratory, Colorado, 2000.
37. N.D. Paulson, R.G. Ginder, The Growth and Direction of the Biodiesel Industry in the United States, in, Center for Agricultural and Rural Development, Iowa State University, Iowa, 2007.

Chapter 7

General Discussion

7.1 INTRODUCTION

The aim of this thesis is to gain more value per hectare *Jatropha curcas* by utilizing Jatropha proteins for various applications. Due to bioethanol and biodiesel production, large amount of proteins will be produced as by-product. Based on the assumption that 10% of current fossil transportation fuels will be replaced by biofuels [1], it is estimated that an additional annual production of around 100 million tonnes of proteins as by-products will be generated. This is more than the requirements for the global human population. This over production of proteins may lead to the saturation of protein markets for food applications. Consequently, there will also be opportunities for protein markets for non-food/ technical applications e.g. animal feed, adhesives, foaming agent, coating, etc. [1-4]. Specifically, this research investigated the extractability and functional properties of Jatropha proteins for non-food/ technical applications.

7.2 JATROPHA PRESS CAKE DETOXIFICATIONS

For animal feed applications, the phorbol ester content of Jatropha press cake should be reduced to at least below 15 ppm due to its toxicity. At this concentration, phorbol esters have been shown to not result in any negative effects in carp [5]. Although this value may not be the same for all animals, this value can be used as a reference to estimate the threshold of phorbol ester content. Jatropha non-de-oiled press cake contained phorbol ester at a level of 1000 ppm, which was higher than the results obtained by other research (240-720 ppm) [6, 7]. Our research investigated different extraction configurations and optimized the extraction conditions to remove phorbol ester from the press cake. Additionally, the phorbol ester mass balance during extraction was monitored. This can be useful to design a process for animal feed production at industrial scale.

We observed that phorbol ester extractability increased with the increase of the organic solvent polarity. The lowest phorbol ester extractability was obtained using petroleum ether. The highest phorbol ester extractability was obtained using alcohol solutions e.g. methanol and ethanol. Specifically, 50% v/v ethanol-water mixture extracted the highest amount of phorbol ester by means of soxhlet extractions (Chapter 2). The two-step consecutive extraction with 50% v/v ethanol-water mixture and petroleum ether 60/80 successfully reduced about 99% of phorbol ester content of the press cake, from 1000 to 11 ppm (Table 7.1). This two-step extraction using 50% v/v ethanol-water mixture and petroleum ether gave better phorbol ester removal than other reports. Aregheore [8] reduced around 95% phorbol ester content of Jatropha defatted kernel meal (from 1780 ppm to 90 ppm) using a four-stage extraction with 92% methanol, and Martinez-Herrera et al. [9], who reduced around 98% of the phorbol ester content (from 3850 ppm to 80 ppm) using extraction

with 90% v/v ethanol-water mixture for 2 hour at room temperature, followed by 0.07% $NaHCO_3$ treatment and autoclaving at 121°C for 20 min. A recent patent from Makkar et al. [10] was able to reduce phorbol ester content in the protein isolate into undetectable amount at the HPLC detection limit of 10 ppm phorbol ester. In this patent protein was extracted from defatted kernel meal using an aqueous solution of NaOH at pH 11 followed by precipitation of the protein by addition of ethanol. Another recent patent from the company D1 [11] has claimed that simultaneous oil extraction and detoxification of the flaked seed kernel using a combination of hydrophobic-hydrophilic solvent was able to reduce the phorbol ester content to an undetectable phorbol ester content. For example, the mixture ethyl acetate/ methanol at a 40/60 ratio at 62°C and 1.2 bars was used to simultaneously extract the oil and to detoxify the seed kernel. The difference between other reports and our research lies in the material that was subjected to detoxification. In the patent of Makkar et al. [10] and D1 [11], the detoxification was applied on the kernel meal and seed kernel (without shell); while here the detoxification procedure was applied to the whole press cake (with shell). Consequently, the higher fiber content of the whole press cake may affect the phorbol ester mass diffusion during extraction.

7.3 JATROPHA PROTEIN EXTRACTION

The production costs and the market value of the extracted protein are the most important parameters for Jatropha protein utilizations at industrial scale. The production costs include the cost of protein extraction, separation, purification, energy consumption, chemicals, and residue processing. Jatropha press cakes and leaves are the potential sources of protein. The extraction conditions will influence the structure and properties of the protein, which will also determine their potential application.

At ambient temperature, Jatropha press cake protein was successfully extracted with NaOH 0.055 M (pH ~ 12) followed by precipitation at pH 5 (protein recovery of 56%; protein content of 73%) (Table 7.1)[12]. This result was lower than the protein recovery obtained by Devappa and Swamylingappa [6] (70-77 %), where steam injection was used to heat the protein extract up to 92°C to precipitate the protein followed by spray drying up to 180°C. The protein recovery in this research was slightly higher than the protein recovery of 53 % obtained by Makkar [7] after extraction at pH 11 for 1 hour at 60°C followed by precipitation at pH 4. The use of a four-stage counter current extraction significantly improved protein recovery by up to two-fold after extraction using 0.055 M NaOH at a low solvent to solid ratio of 4 g/g at ambient temperature. This may become an interesting option for industrial scale protein productions, not only because of its high protein recovery (71 %) but also due to the higher protein concentration in the crude extract (Chapter 3) [12].

Our results showed that Jatropha press cake protein can be extracted with high recovery and the protein's native structure, indicated by the protein molecular weight

distribution, can still be maintained (Chapter 3). Similar to other well-known oil seed proteins such as soy [13-15], sunflower [16-18], or rapeseed [18], Jatropha press cake protein was extracted to a high degree under alkaline conditions. In addition, the extracted Jatropha press cake proteins still maintained several interesting functionalities that are suitable for many technical applications e.g. emulsifier or adhesives (Chapter 5) [12, 19]. The summary of protein recovery from detoxified press cake (chapter 2) and non-detoxified press cake (chapter 5) is shown in Table 7.1. Protein recovery from detoxified press cake was about two-times lower than protein recovery from non-detoxified press cake. Detoxification using ethanol seemed to denature the protein and reduce the extractability of Jatropha press cake protein.

Jatropha leaf protein consist of protein fractions with the most prominent protein molecular weight distribution at around 43, 20 and 14 kDa. Some attempts to extract the native Jatropha leaf protein, e.g. by extraction using 0.2 M NaOH solution at room temperature, resulted in a low protein recovery (36%) and low protein purity (22%). These results indicated that extraction and utilization of native leaf proteins might

Table 7.1 Jatropha press cake protein extracted from detoxified press cake (chapter 2), non-detoxified press cake (chapter 5), and leaves (chapter 4).

Products/ residues	DM recovery [kg product/ kg biomass]	Nitrogen recovery [%]	Protein content [%]	PE content [ppm]	PE to protein ratio [ppm/ kg]
Protein extractions by 0.055 M NaOH from non-detoxified press cake					
Non-de-oiled Press cake/ PC	1.00	100	23	1000	230
PC residue	0.82	36	10	155	16
PC protein isolates	0.18	56	73	400	292
De-oiled press cake/ DOPC	1.00	100	27	NM	NM
DOPC residue	0.85	38	12	NM	NM
DOPC protein isolates	0.15	45	98	NM	NM
Protein extractions by 0.055 M NaOH from detoxified press cake					
Detoxified press cake	1.00	100	22	11	2.4
DPC residue	0.94	73	17	11	1.9
DPC protein isolates	0.06	23	94	ND	-
Jatropha leaves protein hydrolysis					
Leaves dry matter	1.00	100	21	NM	NM
Total amino acids	0.20	95	NA	NA	NA
Essential amino acids	0.10	47	NA	NA	NA
Glutamine+glutamic acids	0.03	16	NA	NA	NA
Other amino acids	0.07	33	NA	NA	NA

Note: DPC=Detoxified press cake after detoxification using 50% v/v ethanol soxhlet extraction for 3 hours, followed by petroleum ether 60/80 soxhlet extraction for 3 hours; PE= phorbol ester; NM=not measured; ND=not detectable (by UHPLC with PE detection limit of 1 ppm); NA=not applicable.

not be feasible. Nevertheless, Jatropha leaf proteins are potential source for amino acids (Table 7.1). The essential amino acids are potential as nutritional components for feed, while glutamine and glutamic acid (15 % of total nitrogen) are potential as intermediates to produce functionalized N-containing chemicals [1, 2, 20].

7.4 PERSPECTIVE ON JATROPHA PROTEIN APPLICATIONS

To improve the value of Jatropha press cake, we proposed three scenarios to extract protein and detoxify press cake: 1) Protein extraction from the detoxified Jatropha press cake; 2) Protein extraction from non-detoxified Jatropha press cake; and 3) Protein extraction from non-detoxified de-oiled press cake. After detoxification, Jatropha protein is potential component for animal feed. While without detoxification, Jatropha protein applications will be limited to technical applications (Chapter 5) [19].

Based on the functionality tests, the film-forming properties of Jatropha press cake protein was rather low. This protein formed films with lower tensile strength than soy protein-glycerol films [21, 22] and lower elongation at break than native wheat gluten-glycerol films [23, 24]. Protein from Jatropha non-de-oiled press cake did not exhibit foaming properties. However, this protein showed better performance as emulsifier than de-oiled press cake protein. Therefore, non-de-oiled press cake protein has potential as emulsifiers (Chapter 5). Jatropha de-oiled press cake proteins had a high foaming expansion at pH 10, which was lower than that of egg white proteins and higher than that of soy protein isolates. Meanwhile, foaming stability of the de-oiled press cake protein was comparable to egg white protein and soy protein isolate. Jatropha de-oiled press cake protein showed the best performance as paper adhesives, with comparable performance to casein adhesive (Chapter 5).

7.4.1 The application of Jatropha protein from detoxified press cake

The process flow diagram of protein extraction from detoxified press cake (scenario 1) is shown in Figure 7.1. In scenario 1, Jatropha press cake is detoxified through a two-step consecutive extraction using 50% v/v ethanol and petroleum ether 60/80. Next, protein is extracted from the detoxified press cake using continues counter current extraction using NaOH 0.055 M followed by isoelectric precipitation at pH 5. After centrifugation and drying, the fibre-rich detoxified residues and the detoxified protein isolates are produced. The fibre-rich detoxified residue can be used as cattle feed, while the protein isolate can be used as poultry feed.

7.4.2 The application of Jatropha protein from non-detoxified press cake

In scenarios 2 and 3, protein is extracted from press cake before detoxification to prevent denaturation due to the contact with organic solvent. Ambient temperature is used to extract the press cake proteins to avoid any possible negative effect on the protein functional properties due to the strong alkaline extraction at high temperature. Extractions at ambient temperature were able to maintain protein

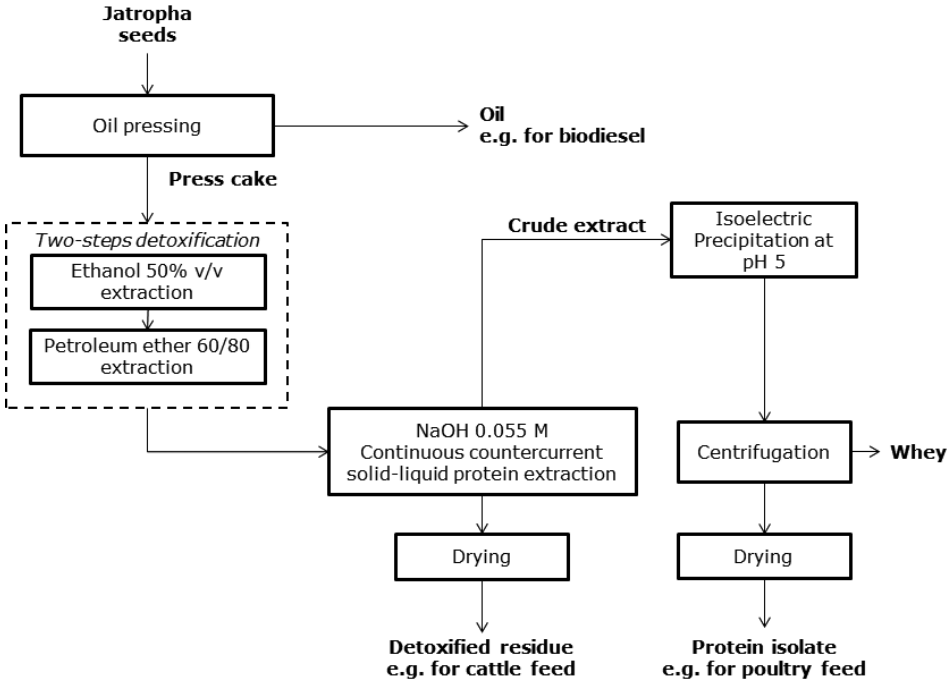

Figure 7.1 Process flow diagram for press cake valorizations scenario 1: Protein extractions from detoxified press cake.

functional properties during alkaline extractions. After protein extraction, a protein-rich fraction and fibre-rich residue fraction are produced. The fibre-rich residue is fed into the detoxification unit to reduce phorbol ester content. The protein-rich fraction has several functional properties that are suitable for technical applications. Jatropha protein can be extracted from non-de-oiled (scenario 2) or de-oiled press cake (scenario 3). As a result of the extra oil-extractions step, the proteins from de-oiled press cake have a higher protein content (98%) than the proteins from non-de-oiled press cake (73%) (Table 7.1).

The process flow diagram of protein extraction from non-detoxified press cake (scenario 2) is described in Figure 7.2. Compared to the process flow diagram of scenario 1, scenario 2 consists of the same unit operations, but the sequence between the detoxification and protein extraction unit is exchanged. The process flow diagram of protein extractions from non-detoxified de-oiled press cake (scenario 3) is described in Figure 7.3. Scenario 3 consists of the same unit operations with scenario 2, but with the additional unit of hexane extraction for further oil removal.

7.4.3 The potential revenues of Jatropha proteins products

To generate the maximum value per hectare *Jatropha curcas*, the potential total revenue from only oil pressing and with further press cake valorisation are compared

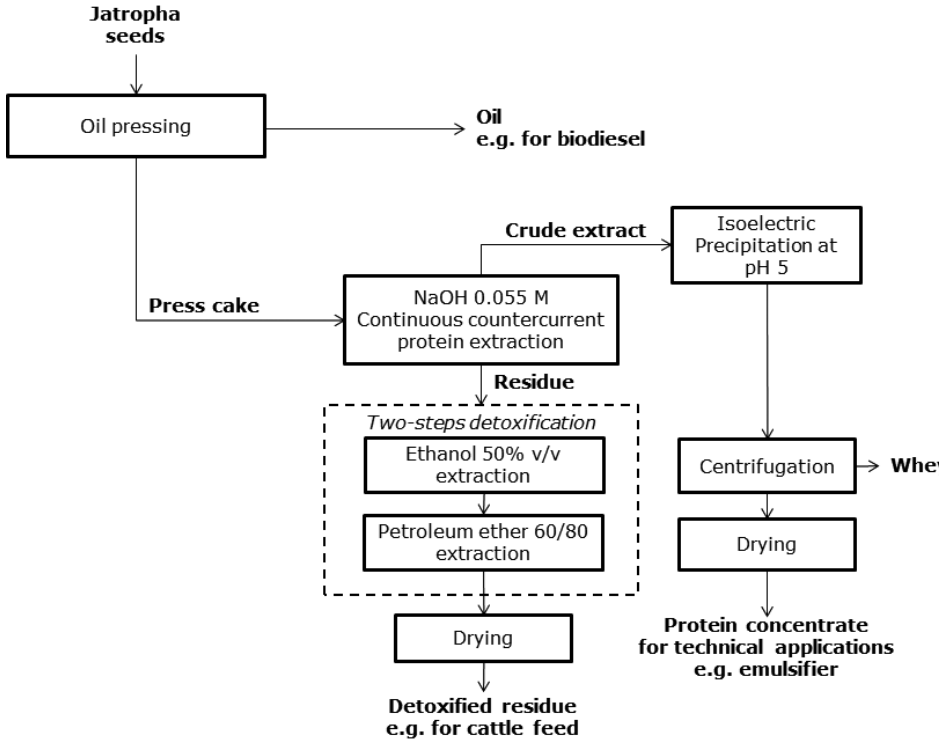

Figure 7.2 Process flow diagram for press cake valorisation scenario 2: Protein extraction from non-detoxified press cake and residue detoxification.

(Table 7.2). For the calculation, we estimated the market value of Jatropha protein concentrates/ isolates using the price of soy proteins in the United States per January 2010. These were reported to be soy functional concentrate (~70% proteins) at 1.3-1.9 EUR/ kg and soy protein isolates (>90% proteins) at 2.3-3.4 EUR/ kg [25]. As for the yield of Jatropha seeds, the reported value from Gunaselaan was used [26]. From this report, Jatropha seed yield was 4 tonnes/ ha per year and the pruned leaves yield was 1.1 tonnes/ ha per year, which were harvested from the rain fed dry land with a plant density of 4444 plant/ ha at 1.5 m x 1.5 m spacing. Currently in Indonesia, the Jatropha seed yield of 115 kg dry seed/ ha per year [27] is still much lower than the expected yield of around 0.6 to 4 ton dry seeds / ha per year [26, 28, 29]. Experimental Jatropha fields at Balittas in Pakuwon (Indonesia) has reached a seed yield of 5 tonnes/ ha per year. However, more efforts are needed to improve Jatropha seeds yield in the actual fields.

The utilization of protein can improve the total potential revenue of the seeds (Table 7.2). The market value of Jatropha seed is approached using the seed price in Indonesia [27]. Scenario 1 could improve the total potential revenue of the existing process up to 1.7 fold. The total potential revenue of scenario 2 could reach up to 1.5 fold higher

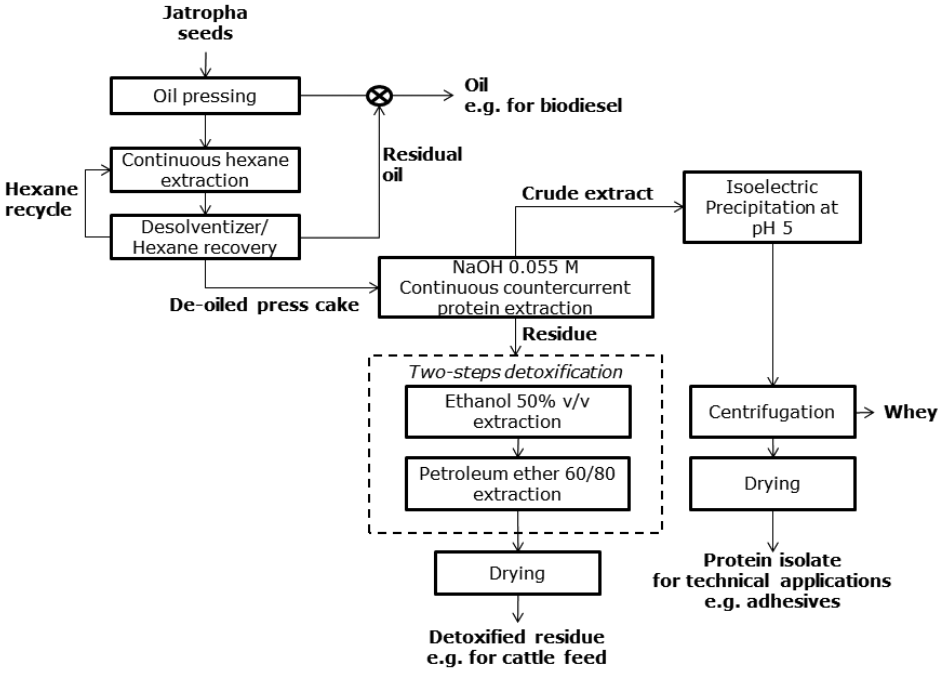

Figure 7.3 Process flow diagram for press cake valorization scenario 3: Protein extractions from non-detoxified de-oiled press cake and residue detoxification.

than scenario 1. By the addition of a hexane extraction unit, scenario 3 could generate slightly higher total potential revenue than the scenario 2. Although scenario 3 would have higher investment and operating costs, due to the additional hexane extraction unit, this scenario can produce protein isolates with higher functionality for more added value applications.

7.5 PERSPECTIVE ON JATROPHA BIOREFINERY PROCESS DESIGN

In Indonesia, ca. 611 ha of Jatropha plantations are available in East Kalimantan [30] and ca. 6000 ha of Jatropha plantations available in West Nusa Tenggara [31]. These low plantation areas indicated that the farmers currently have a low interest to grow Jatropha, which may be due the low total revenue per hectare of Jatropha compare to some food crops (Table 7.3).

To improve the potential total revenue, the valorisation of Jatropha press cake using biorefinery should be conducted (Figure 7.4). In general, the complete Jatropha biorefinery is the extended version of scenario 3 with an additional high temperature hydrolysis unit to process the de-proteinized residue. First, the protein is extracted from the press cake prior to the detoxification process to maintain the protein functional

Table 7.2 The comparison of the potential market value and gross profit margin of Jatropha protein products from different scenarios.

Products/residues	Productivity [kg product/ ha per year]	Potential applications	Market value [EUR/kg]	Revenue [EUR/ ha per year]
Current situation: Only pressing oil for biodiesel productions				
Seeds	4000		0.1	417
Oil	1400	Biodiesel	0.4	588
Press cake	2600	Biogas/ fertilizer	0.02	52
Total potential revenue [EUR/ha per year]				640
Potential gross profit [EUR/ha per year]				223
Scenario 1: Protein extractions from detoxified press cake				
Seeds	4000		0.1	417
Oil	1400	Biodiesel	0.4	588
Detoxified press cake (DPC)	2132			
DPC residues	2010	Cattle feed	0.2	402
DPCB protein isolates	115	Poultry feed	0.8	92
Total potential revenue [EUR/ha per year]				1082
Potential gross profit [EUR/ha per year]				665
Scenario 2: Protein extractions from non-detoxified press cake and residue detoxification				
Seeds	4000		0.1	417
Oil	1400	Biodiesel	0.4	588
Non-de-oiled Press cake (PC)	2600			
PC detoxified residues	2138	Cattle feed	0.2	428
PC protein concentrates [c)]	380	Emulsifier	1.5	570
Total potential revenue [EUR/ha per year]				1586
Potential gross profit [EUR/ha per year]				1169
Scenario 3: Protein extractions from non-detoxified de-oiled press cake and residue detoxification				
Seeds	4000		0.1	417
Oil	1400	Biodiesel	0.4	588
De-oiled press cake (DOPC)	2080			
DOPC detoxified residues	1772	Cattle feed	0.2	354
DOPC protein isolates [c)]	262	Adhesives	2.8	722
Total potential revenue [EUR/ha per year]				1664
Potential gross profit [EUR/ha per year]				1248

[a]Revenue [EUR/ ha per year] = Productivity x Market value; [b]Potential gross profit = Total potential revenue – Seeds revenue. All prices are the Indonesian prices, except for [c)] The price of jatropha protein concentrate ~ soy protein concentrate; the price of jatropha protein isolates ~ soy protein isolates in the United States per January 2010 as follows: soy functional concentrate (~70% proteins) of 1.3-1.9 EUR/ kg; soy protein isolates (>90% proteins) of 2.3-3.4 EUR/ kg [25].

Table 7.3 Yield and price of Jatropha seed compare to other food crops in Indonesia.

Feedstock	Yield[1)] [kg/ha]	Market value[2)] [IDR/kg]	Market value[2)] [EUR/kg][3)]	Total revenue [EUR/ ha]
Rice	5000	4000	0.34	1694
Corn	4237	1500	0.21	898
Soybeans	1348	6000	0.51	686
Peanuts	1249	7800	0.66	826
Cassava	18764	1500	0.13	2383
Jatropha seed	4000	1200	0.10	400

Note: data was accessed on October 2011 from:
[1)] Year 2009, http://www.bps.go.id/tnmn_pgn.php?eng=0 (Accessed on October 2011)
[2)] http://distanak.donggala.go.id/harga/hargaeceranjun2006.html, [3)] 1 EUR = IDR 12000

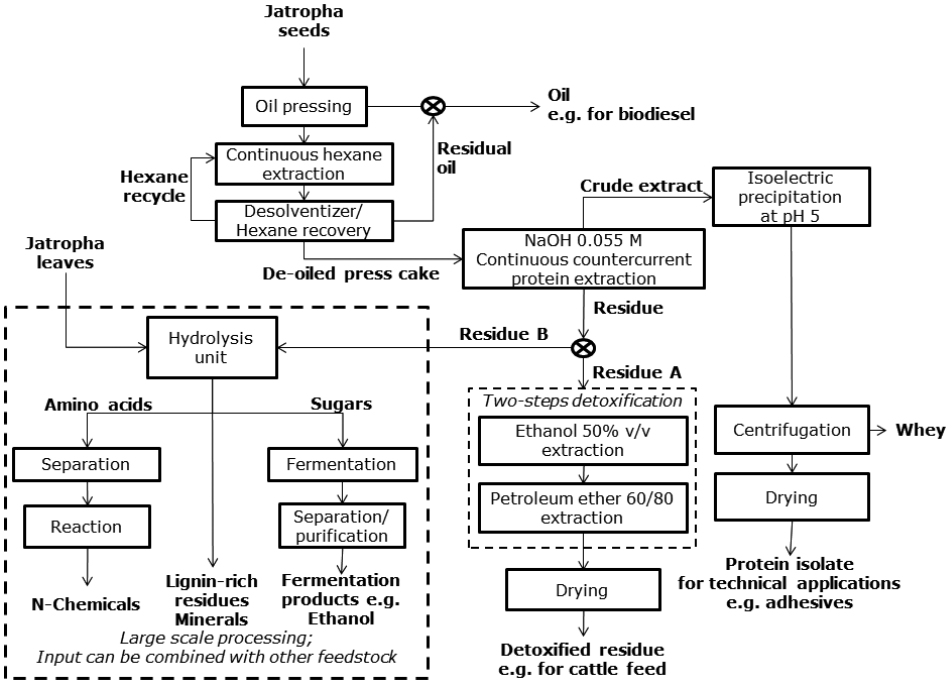

Figure 7.4 Process flow diagram of Jatropha biorefinery – combining the small and large scale processing.

properties and maximize the market value of the protein isolates. Second, the de-proteinized residue is hydrolysed, which results in the sugars and amino acids. These components can be used to produce fermentation products e.g. ethanol and chemical building blocks [1, 2, 20, 32, 33]. Third, the residues remaining after hydrolysis, which

Table 7.4 The estimated market value and gross profit margin of the overall products of Jatropha biorefinery.

Jatropha seed components	Yield [kg/ ha per year]	Component market value [EUR/ kg]	Revenue [EUR/ ha per year]	Potential applications
Oil	1400	0.4	588	Biodiesel
De-oiled press cake (DOPC)	2600			
DOPC protein isolates[a)]	262	2.8	722	Adhesives
DOPC residues A: detoxified	886	0.2	177	Cattle feed
DOPC residues B: hydrolized	886			
Amino acids	73	1.0	73	Animal feed
Sugars	222	0.2	44	Ethanol
Lignin rich-residues	439	0.4	175	Phenolic binders
Minerals	66	0.02	1	Fertilizer
Total potential revenue from seeds [EUR/ ha per year]			1086	
Pruned leaf dry matter	**1086**	**0.0**	**0.0**	
Total amino acids[b)]	217			
Essential amino acids	108	1.0	103	Animal feed
Glutamine + glutamic acids	36	0.4	14	Chemicals
Other amino acids	75	0.5	38	Chemicals
Sugars	319	0.2	64	Ethanol
Lignin rich-residues	375	0.4	150	Phenolic binders
Minerals	130	0.02	2.6	Fertilizer
Total potential revenue from leaves [EUR/ ha per year]			377	
Total potential revenue of Jatropha biorefinery [EUR/ ha per year]			2158	
Jatropha seed (feedstock) cost [EUR/ha per year]			400	
Estimate production cost [c)] [EUR/ha per year]			667	
Estimate net profit (EUR/ ha per year]			1093	

[a)]Gross profit margin/ ha (EUR/ ha per year) = ∑Total value of product– Total value of seeds. The price of jatropha protein concentrate ~ soy protein concentrate; the price of jatropha protein isolates ~ soy protein isolates. [b)]Market value of amino acids are taken from Sanders et al. [1]; [c)]Assume that the feedstock (seed) costs comprise 60% of the total production cost [37, 38]

are rich in lignin and minerals, will be processed further into products e.g. phenolic chemicals or binders [12, 34-36]. Finally, the flow of the de-proteinized residues can be split into two different unit operations: 1) detoxification and 2) a high temperature hydrolysis reactor, to optimize the total potential seed revenue.

The estimate of the potential total revenue and gross profit of the Jatropha biorefinery are presented in Table 7.4. Using a seed yield of 4000 kg/ ha per year, a total potential revenue can reached of around 2158 EUR/ ha per year. This value is higher than the

total revenue that is obtained by only pressing the oil (640 EUR/ ha per year). In addition to the seed and pruned leaves, Jatropha hulls can also contribute to improve the total revenue from Jatropha biorefinery. Using biorefinery processing, the net profit of Jatropha seed can be improved from approx. 300 EUR/ ha (before processing) into approx. 1093 EUR/ ha (after processing). After processing, the net profit of Jatropha seed would be higher than corn, soybean, or peanuts without biorefinery processing. Obviously, these food crops would have a higher net profit than Jatropha when they are processed further in a biorefinery. Moreover, the net profit from Jatropha seed after biorefinery is still lower than the net profit from rice and cassava without biorefinery (Figure 7.5). Still, cultivation of Jatropha can give benefit and could be a good option for soil types that are unsuitable for these food crops. As discussed in Chapter 6, manufacturing industrial products from Jatropha components could be a good option to improve the total revenue and the net profit from this crop to a higher value.

The market price of Jatropha seeds can still be improved by re-distributing the net profit after biorefinery (Table 7.3) to obtain the optimum value between the net profit for farmers and the net profit for the biorefinery. This optimum net profit could be obtained at the Jatropha seed price of approx. 0.2 EUR/ kg (IDR 2400/kg), which is twice the current price of 0.1 EUR/kg (Figure 7.6). Although this is still an estimate, this calculation shows that Jatropha seed market price can be improved by a total valorisation through biorefinery processing.

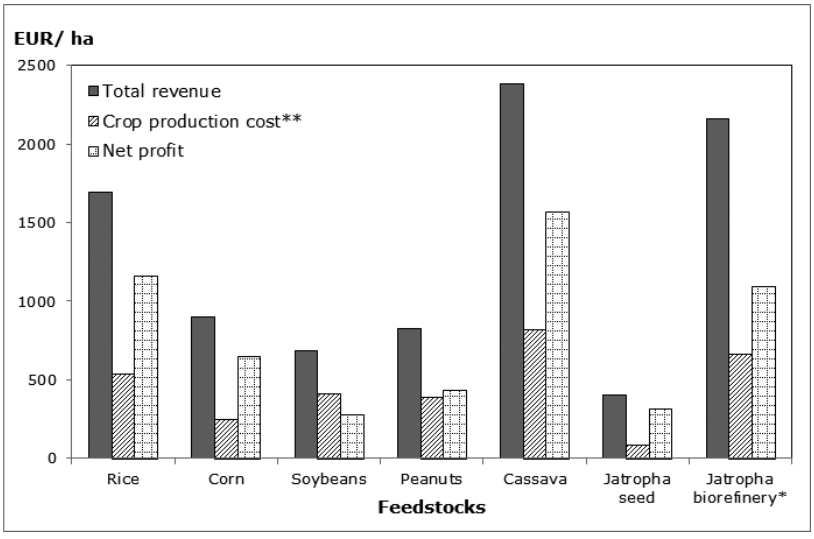

Figure 7.5 The total revenue and net profit for farmers from different food crops and Jatropha seeds in Indonesia. (Note:*Total revenue and net profit of Jatropha seeds after biorefinery processing from Table 7.4; **Crop production cost from Purwono and Purnamawati [39]).

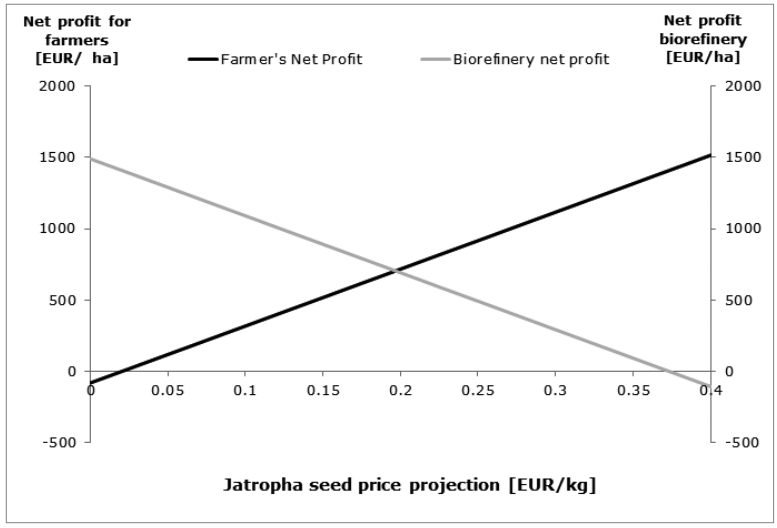

Figure 7.6 Jatropha seed price projection on the net profit for the farmer and the biorefinery processing company.

7.6 PERSPECTIVES ON THE SCALE OF OPERATIONS

Despite the claim that Jatropha can grow in marginal land and provide income for farmers through biodiesel production, there are many large scale Jatropha plantation projects (approx. 50,000 ha) in Africa that have failed due to the low seed yield and high cost for planting and harvesting the seed. In addition, Jatropha becomes more vulnerable to pests and diseases when it is planted at large scale in a monoculture. Consequently, growing Jatropha at a large-scale plantation for commercial biodiesel production is not yet economically viable. In addition, planting Jatropha in a monoculture may threaten biodiversity. On the other hand, some small scale Jatropha projects, which commonly involve local smallholders, have shown positive reports for their economic feasibility [40]. These small scale projects do not displace the land for food crops and can keep the profit within the region. These facts indicate that it may be better to plant Jatropha in a small to medium scale plantation. Jatropha plantation size could range from 0.4 to 4 ha for an individual farmer or up to 400 ha for a larger plantation company [41].

Jatropha biorefinery could be operated in a small to medium-scale, large-scale, or combination of both (Figure 7.7). The small to medium scale biorefinery should be located close to the field to reduce the transportation cost and shorten the mineral cycle back to the soil. Moreover, the small to medium-scale operation will provide job opportunities and products that are useful for daily life, while keeping the profit within the region. The small scale processing commonly has the capacity of 1000 kg biomass/ day [42], while the large scale processing is expected to have the capacity

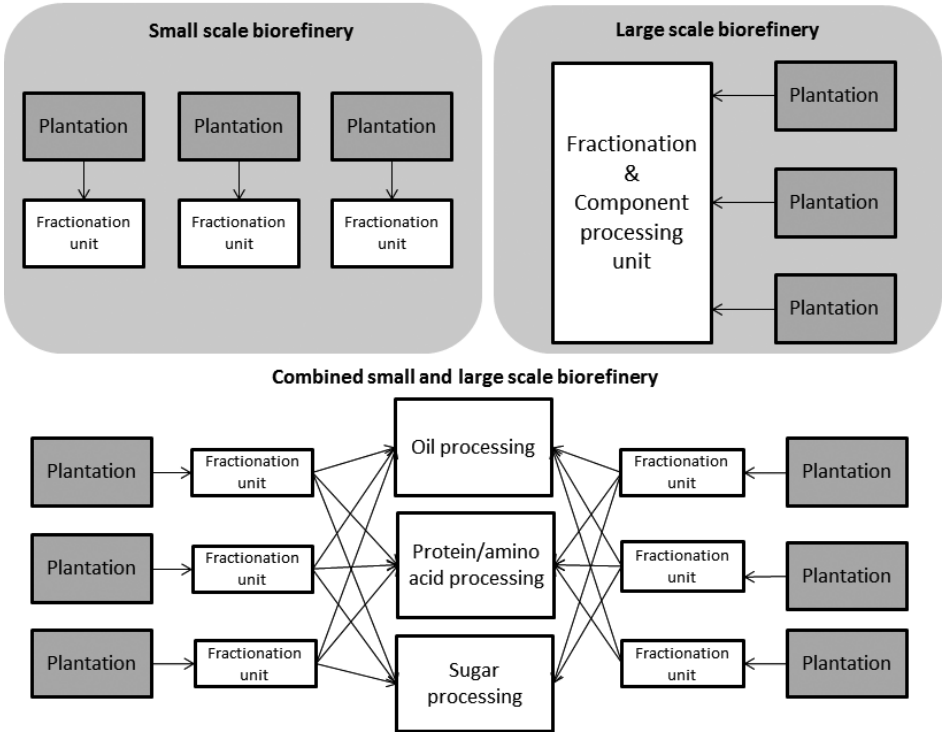

Figure 7.7 Illustration for Jatropha biorefinery operating scale.

of about three to four times larger than the small scale processing (about 3000 to 4000 kg biomass/ day). Using the seed yield of 4000 kg/ year per ha Jatropha, the seed availability could only reach approx. 13 kg dry seeds/ day per ha. Based on this availability, a pilot plant/ small scale Jatropha processing will need feedstock supply from a Jatropha plantation of at least 75 ha. On the other hand, a large scale processing will need feedstock supply from several Jatropha small plantations or a large plantation with the total areas of at least 300 ha. Despite the high capital cost, the large-scale biorefinery commonly has lower production costs per unit product.

Furthermore, the combination of small and large-scale operation may give the optimum benefit for Jatropha biorefinery. Parts of the biorefinery that consist of the primary fractionation and separation unit, e.g. leaf hydrolysis unit, press cake detoxification, protein extraction, solid-liquid separation, and drying unit, will be operated in a small to medium scale operation. The other parts of the biorefinery that consists of more advanced technologies, e.g. high temperature hydrolysis, amino acid separation, purification, and lignin processing, will be operated in a large-scale operation. The main objective of the large-scale operation parts is to convert amino acids, lignin and sugar streams into higher value products e.g. chemical building blocks, which may not be feasible in a small to medium scale operation (Figure 7.7).

7.7 RECOMMENDATION FOR FUTURE RESEARCH

To build a Jatropha protein platform at industrial scale, the future research should focus to obtain deeper knowledge on the extraction process, protein structure, protein functionality, and modifications to improve the protein performance. Regarding the extraction process, Marasabessy et.al reported that autoclaving Jatropha press cake at 121 °C for 15 min prior to protein extraction did not affect molecular weight distributions of the extracted protein [43]. This means that increasing the extraction temperature to some extent may improve the protein recovery without denaturing the protein. However, the effect of high temperature during Jatropha protein extraction under alkaline conditions is still unknown. Therefore, a deeper knowledge on the native protein structure of Jatropha proteins will be needed to explain this effect. Pots et al. have conducted several experiments to investigate the effect of pH and temperature on the denaturation of patatin, the major potato protein [44-46]. This investigation includes the observation on the protein structures and native conformation using FTIR, far UV circular dichroism, or fluorescence methods. These methods can also be used to study the effect of temperature on Jatropha protein denaturation, especially under strong alkaline extraction conditions.

In order to improve protein functional properties, alternative separation processes using chromatography, e.g. separation of patatin from the total native potato proteins, could be considered to produce specific protein with a better emulsifying or foaming properties [47]. At a commercial scale, the company AVEBE Solanic isolates native potato proteins that are heat unstable using large-scale chromatography. The process consists of potato-juice flocculation by divalent metal cation at pH of 7-9 followed by centrifugation to separate the supernatant. The supernatant is fed into the expanded bed adsorption chromatography, which uses specific adsorbent to bind potato protein. Finally, the potato protein isolate is separated from the adsorbent. This method successfully isolates native potato protein with high functionality and free of toxic/ anti nutritional compounds [48]. This process may be applied on Jatropha press cake protein to isolate native specific proteins with better emulsifying or foaming properties. Also, this process may be suitable to isolate native protein from Jatropha leaf juice.

To improve mechanical properties of Jatropha seed protein films, protein modifications could be done through enzymatic modification using transglutaminase or blending with other biopolymers [23, 24, 32, 33, 49]. Other alternatives to improve mechanical and permeability properties of protein films involve cross-linking with the aid of a cross-linking agent. Such approaches have been carried out with wheat gluten using a water-soluble carbodiimide [50] and by protein denaturation during processing [23]. Furthermore, the water/ vapour permeability of Jatropha protein films should be investigated to obtain more knowledge for coating applications. Another aspect of Jatropha press cake protein is its brown colour. Therefore, the use of proteins for applications where colour is not an issue, such as emulsifiers, foaming

agent, or wood adhesives, may be good options. Jatropha protein-based adhesives showed comparable performance with the commercial casein adhesives. However, it is necessary to investigate the ease of handling of this protein-based adhesive at high-speed machine.

7.8 GENERAL CONCLUSION

This research aimed to gain more value per hectare *Jatropha curcas* by utilizing Jatropha proteins for various applications, such as cattle or poultry feed, protein-based adhesives, or protein-based emulsifiers. Jatropha proteins can be extracted from seed/press cake or leaves. Our findings showed that Jatropha press cake protein had high performance as surfactants, e.g. emulsifier and foaming agent, and paper adhesives. More research is needed to improve the performance and mechanical strength of Jatropha press cake protein films. Jatropha leaf protein is a potential source of amino acids for either animal feed or as an intermediate for building blocks for functionalized N-containing chemicals. The valorisation of Jatropha proteins will improve the total potential revenue per hectare of Jatropha and increase the value of the seed. Consequently, this will attract the farmers' interest to grow Jatropha. Finally, even in areas where farmers can only grow Jatropha, there is an opportunity to build a biobased economy and create income for numbers of people through Jatropha biorefinery.

REFERENCES

1. J.P.M. Sanders, E.L. Scott, R.A. Weusthuis, H. Mooibroek, Bio-Refinery as the Bio-Inspired Process to Bulk Chemicals, Macromolecular Bioscience, 7 (2007) 105-117.
2. J. van Haveren, E.L. Scott, J.P.M. Sanders, Bulk chemicals from biomass, Biofuels, Bioproducts and Biorefining, 2 (2008) 41-57.
3. A.A. Koutinas, C. Du, R.H. Wang, C. Webb, Production of Chemicals from Biomass, in: J.H. Clark, F.E.I. Deswarte (Eds.) Introduction to Chemicals from biomass, John Wiley and Sons, Ltd., 2008.
4. T. Werpy, G. Petersen, Top Value Added Chemicals From Biomass, Volume I: Results of Screening for Potential Candidates from Sugars and Synthesis Gas, in, the Pacific Northwest National Laboratory (PNNL), the National Renewable Energy Laboratory (NREL), 2004.
5. G. Goel, H.P.S. Makkar, G. Francis, K. Becker, Phorbol esters: Structure, biological activity, and toxicity in animals, Int. J. Toxicol., 26 (2007) 279-288.
6. R.K. Devappa, B. Swamylingappa, Biochemical and nutritional evaluation of Jatropha protein isolate prepared by steam injection heating for reduction of toxic and antinutritional factors, J. Sci. Food Agric., 88 (2008) 911-919.
7. H.P.S. Makkar, G. Francis, K. Becker, Protein concentrate from Jatropha curcas screw-pressed seed cake and toxic and antinutritional factors in protein concentrate, J. Sci. Food Agric., 88 (2008) 1542-1548.
8. E.M. Aregheore, K. Becker, H.P.S. Makkar, Detoxification of a toxic variety of Jatropha curcas using heat and chemical treatments, and preliminary nutritional evaluation with rats, The South Pacific Journal of Natural Science, 21 (2003) 51-56.
9. J. Martinez-Herrera, P. Siddhuraju, G. Francis, G. Davila-Ortiz, K. Becker, Chemical composition, toxic/antimetabolic constituents, and effects of different treatments on their levels, in four provenances of Jatropha curcas L. from Mexico, Food Chemistry, 96 (2006) 80-89.
10. H. Makkar, K. Becker, Patent number: WO 2010/092143 A1. Method for detoxifying

plant constituents, in: European Patent Office, 2010.
11. J. Brooker, The methods for detoxifying oil seed crops. WO 2010/070264 A1, in: D1 (Ed.) PCT, 2010.
12. D. Lestari, W.J. Mulder, J.P.M. Sanders, Improving Jatropha curcas seed protein recovery by using counter current multistage extraction, Biochemical Engineering Journal, 50 (2010) 16-23.
13. E.W. Lusas, M.N. Riaz, Soy Protein Products: Processing and use, The Journal of Nutrition, 125 (1995).
14. J. Ohren, Process and product characteristics for soya concentrates and isolates, Journal of the American Oil Chemists' Society, 58 (1981) 333-335.
15. L. Sair, Proteinaceous soy composition and method of preparing. US Patent 2,881,076., (1959).
16. S. González-Pérez, J.M. Vereijken, K.B. Merck, G.A. van Koningsveld, H. Gruppen, A.G.J. Voragen, Conformational States of Sunflower (Helianthus annuus) Helianthinin: Effect of Heat and pH, Journal of Agricultural and Food Chemistry, 52 (2004) 6770-6778.
17. M. Saeed, M. Cheryan, Sunflower Protein Concentrates and Isolates' Low in Polyphenols and Phytate, Journal of Food Science, 53 (1988) 1127-1131.
18. L. Tranchino, R. Costantino, G. Sodini, Food grade oilseed protein processing: sunflower and rapeseed, Plant Foods for Human Nutrition (Formerly Qualitas Plantarum), 32 (1983) 305-334.
19. D. Lestari, W.J. Mulder, J.P.M. Sanders, Jatropha seed protein functional properties for technical applications, Biochemical Engineering Journal, 53 (2011) 297-304.
20. E.L. Scott, F. Peter, J.P.M. Sanders, Biomass in the manufacture of industrial products—the use of proteins and amino acids, Applied Microbiology and Biotechnology, 75 (2007) 751-762.
21. A.H. Brandenburg, C.L. Weller, R.F. Testin, Edible Films and Coatings from Soy Protein, Journal of Food Science, 58 (1993) 1086-1089.
22. Y.M. Stuchell, J.M. Krochta, Enzymatic Treatments and Thermal Effects on Edible Soy Protein Films, Journal of Food Science, 59 (1994) 1332-1337.
23. L.A. de Graaf, Denaturation of proteins from a non-food perspective, J. Biotechnol., 79 (2000) 299-306.
24. A. Gennadios, A.H. Brandenburg, C.L. Weller, R.F. Testin, Effect of pH on properties of wheat gluten and soy protein isolate films Journal of agricultural and food chemistry 41 (1993) 1835-1839.
25. OMNI, A survey of recent chemical price trends. The Potential Impact of Rising Petrochemical Prices on Soy Use for Industrial Applications. Prepared for the United Soybean Board, in, OMNI TECH International, Ltd., 2010.
26. V.N. Gunaseelan, Biomass estimates, characteristics, biochemical methane potential, kinetics and energy flow from Jatropha curcus on dry lands, Biomass and Bioenergy, 33 (2009) 589-596.
27. M. Syakir, Prospek dan Kendala Pengembangan Jarak Pagar Sebagai Bahan Bakar Nabati di Indonesia, Perspektif, 9 (2010) 55-65.
28. R.E.E. Jongschaap, W.J. Corre, P.S. Bindraban, W.A. Brandenburg, Claims and Facts on Jatropha curcas L. , in, Plant Research International B.V., Wageningen UR, Wageningen, 2007.
29. V. Nallathambi Gunaseelan, Biomass estimates, characteristics, biochemical methane potential, kinetics and energy flow from Jatropha curcus on dry lands, Biomass and Bioenergy, 33 (2009) 589-596.
30. Pada 2010, Produksi Tanaman Jarak di Kaltim Mencapai 85 Ton, in: http://m.korankaltim.co.id/read/m/4800 2011.
31. Disbun_NTB, Jarak Pagar. http://disbun.ntbprov.go.id/?p=119, in, 2010.
32. L.A. de Graaf, P. Kolster, Industrial proteins as a green alternative for 'petro' polymers: Potential and Limitations, Macromolecular symposia, (1998) 51-58.
33. C.M. Vaz, L.A.d. Graaf, W.J. Mulder, Adhesives, coatings and bioplastics from protein sources, in: S.R. Fahnestock, A. Steinbuchel (Eds.) Polyamides and Complex Proteinaceous Materials II Wiley-VCH, New York 2003, pp. 383 - 404.
34. J.P.M. Sanders, Biorefinery, the bridge between agriculture and chemistry, in: The 14th European Biomass Conference and Exhibition : Biomass for Energy, Industry and Climate Protection, Paris, France, 2005.
35. R. Manurung, D.A.Z. Wever, J. Wildschut, R.H. Venderbosch, H. Hidayat, J.E.G. van Dam, E.J. Leijenhorst, A.A. Broekhuis, H.J. Heeres, Valorisation of Jatropha curcas L. plant parts: Nut shell conversion to fast pyrolysis oil, Food and Bioproducts Processing, 87 (2009) 187-196.
36. F. Cherubini, The biorefinery concept: Using biomass instead of oil for producing energy and chemicals, Energy Conversion and Management, 51 (2010) 1412-1421.
37. A. McAloon, F. Taylor, W. Yee, K. Ibsen, R. Wooley, Determining the Cost of Producing Ethanol from Corn Starch and Lignocellulosic Feedstocks, in, National Renewable Energy Laboratory, Colorado, 2000.
38. N.D. Paulson, R.G. Ginder, The Growth and Direction of the Biodiesel Industry in the United States, in, Center for Agricultural and Rural Development, Iowa State University, Iowa, 2007.

39. Purwono, H. Purnamawati, Budidaya 8 jenis tanaman pangan unggul, Penebar Swadaya, Jakarta, 2007.
40. C. Pohl, Jatropha: money doesn't grow on trees. Ten reasons why jatropha is neither a profitable nor sustainable investment, in: H. Burley (Ed.), Friends of the Earth International, 2010.
41. N. Wahl, R. Jamnadass, H. Baur, C. Munster, M. Iiyama, Economic viability of Jatropha curcas L. plantations in Northern Tanzania-Assessing farmers' prospects via cost-benefit analysis.ICRAF. Working Paper no. 97, in, World Agroforestry Centre., Nairobi, 2009.
42. http://www.nrel.gov/biomass/integrated_biorefinery_research_facility.html, in.
43. A. Marasabessy, M. Moeis, J.P.M. Sanders, R.A. Weusthuis, Enhancing Jatropha oil extraction yield from the kernels assisted by a xylan-degrading bacterium to preserve protein structure, Applied Microbiology and Biotechnology, 90 (2011) 2027-2036.
44. A.M. Pots, Physico-chemical properties and thermal aggregation of patatin, the major potato tuber protein, in, Pots, [S.l.], 1999.
45. A.M. Pots, H.H.J. De Jongh, H. Gruppen, R.J. Hamer, A.G.J. Voragen, Heat-induced conformational changes of patatin, the major potato tuber protein, European Journal of Biochemistry, 252 (1998) 66-72.
46. A.M. Pots, H.H.J. de Jongh, H. Gruppen, M. Hessing, A.G.J. Voragen, The pH dependence of the structural stability of patatin, Journal of Agricultural and Food Chemistry, 46 (1998) 2546-2553.
47. A.M. Pots, H. Gruppen, M. Hessing, M.A.J.S. van Boekel, A.G.J. Voragen, Isolation and Characterization of Patatin Isoforms, Journal of agricultural and food chemistry, 47 (1999) 4587-4592.
48. M.L.F. Giuseppin, Native potato protein isolates. EP 1 920 662 A1, in: AVEBE (Ed.) European Patent Application, 2008.
49. J.-P. Lens, W.J. Mulder, P. Kolster, Modification of wheat gluten for nonfood applications, Cereal foods world, 44 (1999) 17-26.
50. V. Tropini, J.-P. Lens, W.J. Mulder, F. Silvestre, Cross-Linking of Wheat Gluten Using a Water-Soluble Carbodiimide, Cereal Chem., 77 (2000) 333-338.

SUMMARY

The aim of this thesis is to explore how to gain more value per hectare *Jatropha curcas* by utilizing Jatropha protein for various applications. Specifically, this research investigated the extractability and functional properties of Jatropha protein for non-food/technical applications. Jatropha press cake and leaves are the potential sources of protein. Jatropha proteins can be extracted from Jatropha seed press cake or leaves, with or without detoxification to remove the toxic phorbol esters. After detoxification, Jatropha proteins are potential components for animal feed. While without detoxification, Jatropha protein applications will be limited to technical applications such as bioplastics, surfactants, adhesives, or coatings.

Our research on press cake detoxification investigated different extraction configurations and optimized the extraction conditions to remove phorbol ester from the press cake. The research also monitored the phorbol ester mass balance during extraction, which can be useful to design the process for animal feed production at industrial scale. We observed that 50%v/v ethanol-water extracted the highest amount of phorbol ester by means of soxhlet extractions. Furthermore, a consecutive two-step soxhlet extraction using 50%v/v ethanol-water and petroleum ether 60/80, followed by a subsequent protein extraction using alkali (0.055 M NaOH) reduced the phorbol ester content in the protein isolate to undetectable amounts using UHPLC analysis (phorbol ester detection limit of 1 ppm) (Chapter 2).

The extraction conditions may influence the structure and properties of the proteins, which will then determine their potential application. In chapter 3, the aim was to find a more efficient method to extract protein from press cake. At ambient temperatures, Jatropha press cake protein was successfully extracted with NaOH (0.055 M) followed by precipitation at pH 5 with high protein recovery of 56% and protein content of 73%. The use of a four-stage counter current extraction significantly improved protein recovery by up to two-fold after extraction using NaOH (0.055 M) with a low solvent to solid ratio of 4 g/g at ambient temperature. This may become an interesting option for industrial scale protein productions, not only because of its high protein recovery (71 %), but also due to the higher protein concentration in the crude extract.

The potential of Jatropha leaves as the potential sources of proteins was also investigated in Chapter 4. The molecular weight of Jatropha leaf proteins were distributed between 14.4 to 98 kDa, with the most prominent band presence at around 52 and 43 kDa. Some attempts to extract the native Jatropha leaf proteins using NaOH solution (0.2 M) at room temperature, resulted in a low protein recovery (36%) and low protein purity (22%). These results indicated that extraction and utilization of native leaf proteins might not be economically feasible. Nevertheless, Jatropha leaf proteins are a potential source for amino acids. The essential amino acids can contribute as nutritional components for feed, while glutamine and glutamic acid (15 % of total nitrogen) can be used as intermediate to produce functionalized N-containing chemicals

In Chapter 5, the research investigated and compared the functional properties of Jatropha press cake proteins with the relevant industrial proteins. Jatropha press cake protein films had a low tensile strength but with rather high elongation at break. De-oiled press cake protein had higher foam expansion and foam volume stability than sodium caseinate at pH 10. Jatropha press cake protein showed most promising results as emulsifier and paper adhesive. Emulsifying properties of press cake protein were particularly the highest at pH 9-10. Jatropha press cake proteins, especially de-oiled press cake proteins, had adhesive properties with comparable performance to casein adhesives.

The objectives of the study in Chapter 6 were to calculate the potential generated income and potential economic value from Jatropha products, which were classified into two product groups: 1) Rural products, and 2) Industrial products. The potential generated income consists of the net profit and labour costs. Using the national gross domestic product (GDP) per capita as reference, we calculated and compared the potential economic value, which expresses the number of people that can obtain a certain income for every hectare of Jatropha plantation, in five selected countries: Tanzania, Mali, Zimbabwe, Indonesia, and the Netherlands (reference). The potential economic value per hectare Jatropha in the developing country suggests that manufacturing the rural products can generate a potential income for 1 person, while manufacturing the industrial products can generate a higher potential income for approx. 13 - 35 people.

Chapter 7, the general discussion, describes and compares the results from the previous chapters and their contributions to current knowledge. Perspective for a Jatropha biorefinery process design is described, which correlates the results from the previous chapters. Using a seed yield of 4000 kg/ ha per year, the total potential revenue can reach around 2158 EUR/ ha per year. This is a major additional income compared to the potential revenue that is obtained only from Jatropha oil of 640 EUR/ ha per year. From the total revenue, approx. 75% revenue was generated from seeds and approx. 25% revenue was generated from the pruned leaves. Through biorefinery processing, the net profit of Jatropha seed can be improved from approx. 300 EUR/ ha (before processing) into approx. 1093 EUR/ ha (after processing).

In conclusion, Jatropha press cake proteins have the most promising results as emulsifier and paper adhesive. On the other hand, Jatropha leaf proteins are a potential source of amino acids, which can be applied as animal feed or intermediate building blocks for functionalized N-containing chemicals. The valorisation of Jatropha proteins will improve the total potential revenue per hectare of Jatropha and increase the seed market value, to attract the farmers' interest to grow Jatropha. Finally, even in areas where farmers can only grow Jatropha, there is an opportunity to build a biobased economy and create income for numbers of people through Jatropha biorefinery.

SAMENVATTING

Het doel van dit proefschrift is om te onderzoeken hoe meer waarde per hectare *Jatropha curcas* te verkrijgen door gebruik te maken van Jatropha eiwit voor diverse toepassingen. In het bijzonder, werd de winbaarheid en de functionele eigenschappen van de Jatropha eiwit onderzocht voor non-food/technische toepassingen. Jatropha perskoek en bladeren zijn de potentiële bronnen van eiwit. Jatropha eiwitten kunnen worden gewonnen uit Jatropha zaad perskoek en bladeren, met of zonder ontgiftiging om de giftige phorbol esters te verwijderen. Na de ontgiftiging, zijn Jatropha eiwitten potentiële componenten voor veevoer. Terwijl zonder ontgiftiging, Jatropha eiwit beperkt zal blijven tot technische toepassingen, zoals bioplastics, oppervlakte actieve stoffen, lijmen, of coatings.

Ons onderzoek om de perskoek te ontgiftigen verkende verschillende extractie-configuraties om de phorbolester te verwijderen uit de perskoek. Er werd een phorbolester massabalans tijdens de winning opgesteld, welke nuttig kan zijn om het proces te ontwerpen voor de productie van diervoeders op industriële schaal. We zagen dat 50% v/v ethanol-water het meeste van de phorbolester kon verwijderen door middel van soxhlet extracties. Bovendien verlaagde een aaneengesloten tweestaps soxhlet-extractie met 50%v/v ethanol-water en petroleumether 60/80, gevolgd door een volgende eiwit extractie met behulp van alkali (0,055 M NaOH) de phorbolester inhoud in het eiwit isolaat naar niet-detecteerbare hoeveelheden met behulp van UHPLC analyse (Hoofdstuk 2).

De extractie omstandigheden kunnen invloed hebben op de structuur en eigenschappen van de eiwitten, welke bepalend is voor hun mogelijke toepassing. In hoofdstuk 3, was het doel om een meer efficiënte methode om eiwitten te winnen uit perskoek te ontwikkelen. Bij kamertemperatuur werd Jatropha perskoek eiwit succesvol geëxtraheerd met NaOH (0,055 M), gevolgd door precipitatie bij pH 5 met een hoge recovery van 56% en een eiwitgehalte van 73%. Het gebruik van een vier-traps tegenstroom extractie verbeterde de eiwit extractie twee-voudig met NaOH (0,055 M) als oplosmiddel en een verhouding van 4 g oplosmiddel per gram perskoek. Dit kan een interessante optie zijn voor industriële schaal eiwitten productie, niet alleen vanwege de hoge eiwit-recovery (71%), maar ook vanwege de hogere eiwit concentratie in het ruwe extract.

Het potentieel van Jatropha bladeren als de potentiële bronnen van eiwit werd onderzocht in hoofdstuk 4. Het molecuulgewicht van de meeste Jatropha blad eiwitten liggen tussen de 14 en 98 kDa, met de meest prominente eiwitbanden rond 52 en 43 kDa. Een aantal pogingen om de inheemse Jatropha blad eiwitten met behulp van NaOH-oplossing (0,2 M) extractie bij kamertemperatuur in handen te krijgen, resulteerde in een lage eiwit recovery (36%) en een lage eiwit zuiverheid (22%). Deze resultaten gaven aan dat winning en gebruik van inheemse blad eiwitten op deze wijze niet economisch haalbaar. Toch kunnen Jatropha blad eiwitten een potentiële bron voor aminozuren zijn. De essentiële aminozuren kunnen een bijdrage leveren als nutritionele componenten voor voeding, terwijl glutamine en glutaminezuur (15%

van de aminozuren) kunnen worden gebruikt als intermediair om gefunctionaliseerde N-bevattende chemicaliën te produceren

In Hoofdstuk 5, werden de functionele eigenschappen van Jatropha perskoek eiwitten onderzocht in vergelijking met relevante industriële eiwitten. Jatropha perskoek eiwit films hadden een lage treksterkte maar met een vrij hoge rek bij breuk. De eiwitfractie geisoleerd uit perskoek waarbij hexaan extractie heeft plaatsgevonden, had een hogere schuim expansie en schuim volume stabiliteit dan natriumcaseïnaat bij pH 10. Jatropha perskoek eiwit toonde veelbelovende resultaten als emulgator en papierlijm. Emulgerende eigenschappen van perskoek eiwit waren vooral het beste bij pH 9-10. Jatropha perskoek eiwitten, in het bijzonder de eiwitfractie geisoleerd uit perskoek waarbij hexaan extractie plaatsvond, hebben goede kleef eigenschappen met vergelijkbare prestaties met caseïne lijmen.

De doelstelling van de studie in Hoofdstuk 6 was om het potentieel gegenereerde inkomen en de potentiële economische waarde te berekenen van de Jatropha-producten, die werden ingedeeld in twee productgroepen: 1) plattelandsproducten, en 2) Industriële producten. Het potentiële gegenereerde inkomen bestaat uit de nettowinst en de arbeidskosten. Met behulp van het nationale bruto binnenlands product (BBP) per hoofd van de bevolking als referentie, hebben we de potentiële economische waarde, die het aantal mensen dat een bepaald inkomen van elke hectare Jatropha plantage kan verkrijgen, berekend en vergeleken in vijf geselecteerde landen: Tanzania, Mali , Zimbabwe, Indonesië en Nederland. De potentiële economische waarde per hectare Jatropha in een ontwikkelingsland laat zien dat de productie van de landelijke producten van één hectare het potentiële inkomen voor 1 persoon kan genereren, terwijl de productie van de industriële producten het inkomen kan genereren voor 13 tot 35 mensen.

Hoofdstuk 7, de algemene discussie, beschrijft en vergelijkt de resultaten uit de vorige hoofdstukken en hun bijdragen aan de huidige wetenschappelijke kennis. Het perspectief voor een Jatropha bioraffinage procesontwerp is beschreven, afgeleid uit de resultaten van de voorgaande hoofdstukken. Met behulp van een zaadopbrengst van 4000 kg/ ha per jaar, kan de totale potentiële opbrengst oplopen tot circa 2.158 euro/ ha per jaar. Dit is een belangrijke extra inkomsten ten opzichte van de potentiële inkomsten welke alleen uit Jatropha-olie van 640 EUR/ ha per jaar kan worden verkregen. Van de totale omzet wordt ongeveer75% van de omzet gegenereerd uit zaden en ongeveer 25% de omzet uit de gesnoeide bladeren. Door middel van bioraffinage verwerking, kan de netto winst van Jatropha zaden worden verbeterd van ongeveer 300 EUR / ha (zonder verwerking) tot ongeveer 1.093 EUR / ha (na verwerking).

Jatropha perskoek eiwitten hebben veelbelovende eigenschappen als emulgator en papierlijm. Jatropha blad eiwitten zijn een potentiële bron van aminozuren, die kunnen worden toegepast als veevoer of als intermediaire bouwstenen voor gefunctionaliseerde N-bevattende chemicaliën. De valorisatie van Jatropha eiwitten zal de totale potentiële opbrengst per hectare van Jatropha teelt verhogen. Tot slot, zelfs in gebieden waar boeren slechts Jatropha kunnen verbouwen, is er een kans om een biobased economie op te bouwen en het inkomen voor grote aantallen mensen door middel van Jatropha bioraffinage te genereren.

RINGKASAN

Tujuan penelitian ini adalah untuk mendapatkan nilai tambah per hektar dari *Jatropha curcas* (Jatropha/jarak pagar) dengan cara mengekstraksi protein untuk aplikasi nonpangan/teknis. Protein jarak pagar dapat diekstrak baik dari *press cake* (ampas yang diperoleh setelah ekstraksi minyak dari biji jarak pagar) maupun dari daun, dengan atau tanpa proses detoksifikasi untuk menghilangkan komponen toksik phorbol ester. Protein jarak pagar yang diekstrak dari *press cake* yang telah didetoksifikasi dapat digunakan sebagai pakan ternak. Protein jarak pagar yang diekstrak dari *press cake* tanpa detoksifikasi terbatas penggunaannya untuk aplikasi nonpangan/teknis seperti bioplastik surfaktan, perekat, atau bahan pelapis (cat), yang mempunyai nilai jual yang lebih tinggi daripada pakan ternak.

Selanjutnya, proses detoksifikasi atau penghilangan komponen toksik phorbol ester dari *press cake* jarak pagar dioptimalkan untuk aplikasi di *pilot plant* (Bab 2). Ekstraksi *soxhlet* dengan pelarut 50% v/v etanol memberikan kinerja terbaik untuk mengurangi kadar phorbol ester dari *press cake*. Untuk hasil yang lebih optimal, dilakukan ekstraksi *soxhlet* dua-tahap dengan pelarut etanol 50% v/v dan petroleum eter 60/80, secara berurutan, yang kemudian diikuti dengan ekstraksi protein dengan NaOH 0,055 M. Dengan menggunakan metode tersebut, kadar phorbol ester dalam isolat protein dapat dikurangi hingga tidak terdeteksi oleh pengukuran UHPLC.

Kondisi ekstraksi akan mempengaruhi struktur dan sifat fungsional dari protein, yang akan menentukan aplikasi potensial dari protein tersebut. Tujuan dari penelitian pada Bab 3 adalah untuk menemukan metode yang lebih efisien untuk mengekstraksi protein dari *press cake* jarak pagar. Menggunakan metode ekstraksi protein satu-tahap pada temperatur ruang dengan pelarut NaOH 0,055 M yang dilanjutkan dengan presipitasi pada pH 5, perolehan protein dari *press cake* adalah 56% dengan kemurnian protein mencapai 73%. Penggunaan metode ekstraksi protein empat-tahap secara *countercurrent* berhasil meningkatkan perolehan protein dari *press cake* pada perbandingan pelarut/padatan yang rendah (4 g/g) hingga dua kali lipat, dari 35% menjadi 70%. Dalam aplikasi industri, ekstraksi protein dengan perbandingan pelarut/padatan yang rendah akan memberikan keuntungan karena perolehan protein yang tinggi dan konsentrasi protein yang tinggi dalam ekstrak, sehingga mengurangi biaya pemurnian protein.

Potensi daun jarak pagar sebagai sumber protein potensial diselidiki secara lebih saksama pada Bab 4. Daun jarak pagar mengandung sekitar 20% bahan kering, yang terdiri dari 8% lemak, 21% protein, 26% karbohidrat, 26% lignin, 4% pektin, dan 12% abu. Berat molekul protein daun jarak pagar terdistribusi antara 14,4-98 kDa, di mana protein utama terdistribusi di sekitar 52 dan 43 kDa. Ekstraksi protein natif dari daun jarak pagar belum dapat disebut layak secara ekonomi karena perolehannya yang rendah, walaupun telah diekstrak dalam kondisi basa kuat pada suhu ambien. Walaupun begitu, daun jarak pagar mempunyai potensi yang besar sebagai sumber asam amino untuk penggunaan sebagai pakan hewan. Selain itu, glutamin dan asam

glutamat, dengan kadar sekitar 15% dari asam amino total, dapat dikonversikan menjadi bahan kimia berbasis nitrogen.

Sifat-sifat fungsional protein jarak pagar yang diekstrak dari *press cake* diobservasi secara lebih terperinci dan dibandingkan dengan protein industrial (Bab 5). Lapisan film yang terbuat dari protein *press cake* mempunyai *tensile strength* yang rendah dan *elongation at break* yang tinggi. Pada pH 10, protein yang diekstrak dari *de-oiled press cake* mempunyai sifat ekspansi dan kestabilan pembusaan yang lebih tinggi dari natrium kaseinat (salah satu protein industrial). Protein dari *press cake* memperlihatkan hasil yang paling menjanjikan sebagai bahan pengemulsi dan bahan lem/perekat kertas. Sifat pengemulsi tertinggi dari protein *press cake* diperoleh pada pH 9-10. Protein dari *press cake* jarak pagar mempunyai sifat perekat dengan kinerja yang sebanding dengan kasein, protein komersial untuk bahan perekat.

Selanjutnya, tujuan dari studi pada Bab 6 adalah untuk menghitung pendapatan potensial total dan nilai potensi ekonomi yang dihasilkan dari produk jarak pagar yang dikelompokkan menjadi: 1) produk pendukung pertanian, dan 2) produk industrial. Pendapatan potensial total terdiri dari keuntungan bersih produksi dan ongkos buruh. Pendapatan potensial total dan produk domestik bruto per kapita (GDP) adalah dua parameter ekonomi yang digunakan untuk perhitungan nilai potensi ekonomi. Nilai ini mengindikasikan jumlah orang yang dapat memperoleh sejumlah pendapatan per hektar jarak pagar. Perbandingan dilakukan pada 5 negara terpilih, Tanzania, Mali, Zimbabwe, Indonesia, dan Belanda, dengan basis perolehan biji jarak pagar sebesar 2000 kg/ha per tahun. Hasil perhitungan nilai potensi ekonomi per hektar jarak pagar di negara berkembang mengindikasikan bahwa konversi komponen biji jarak menjadi produk pendukung pertanian dapat memberikan pendapatan potensial untuk 1 orang, sedangkan konversi komponen biji jarak menjadi produk industrial dapat memberikan pendapatan potensial untuk 15 hingga 35 orang.

Hasil-hasil penelitian dari bab-bab sebelumnya dan perspektif dalam perancangan proses biorafinasi jarak pagar dibahas secara lebih umum pada Bab 7. Dengan menggunakan perolehan biji jarak pagar sebesar 4000 kg/ha per tahun, pendapatan potensial total dapat mencapai 2158 EUR/ha per tahun (sekitar Rp 26 juta/ha per tahun), jauh lebih besar daripada pendapatan total 640 EUR/ha per tahun (sekitar Rp 8 juta/ha per tahun) dari produksi minyak jarak pagar. Dari total pendapatan, sekitar 75% dihasilkan dari biji dan sekitar 25% dihasilkan dari daun jarak pagar hasil *pruning*. Melalui proses biorafinasi, estimasi keuntungan bersih dari pengolahan biji jarak pagar dapat ditingkatkan dari sekitar 300 EUR/ha (sekitar Rp 3,6 juta/ha) (sebelum pengolahan) hingga mencapai sekitar 1093 EUR/ha (sekitar Rp 13 juta/ha).

Sebagai kesimpulan umum, protein dari *press cake* jarak pagar mempunyai potensi sebagai bahan pengemulsi dan bahan perekat kertas, sementara protein dari daun jarak pagar adalah sumber asam amino yang berpotensi sebagai pakan ternak atau bahan kimia berbasis nitrogen. Pemanfaatan protein jarak pagar dapat meningkatkan pendapatan potensial total per hektar jarak pagar dan nilai pasar dari biji jarak pagar. Hal tersebut diharapkan dapat meningkatkan kembali ketertarikan para petani untuk

menanam jarak pagar. Bahkan di suatu daerah di mana petani hanya dapat menanam jarak pagar, terdapat kesempatan untuk mengembangkan *biobased economy* dan menghasilkan pendapatan bagi masyarakat sekitar melalui biorafinasi jarak pagar.

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CURRICULUM VITAE

Dianika Lestari was born on February 10th, 1982 in Bandung, Indonesia, where she attended the local primary and secondary school. From 1992 to 2004, she also followed the electone (electronic organ) study course in the Yamaha Music Foundation. From 2000 to 2004, she continued her education and graduated with honour in the Chemical Engineering Department of Institut Teknologi Bandung (ITB), Indonesia. She directly continued her master programme in the same university and graduated with honour in 2006. While pursuing her Master degree, she was working as academic and project assistant in the university. In addition, from 2005 to 2007, she was also working as a part-time music teacher in Yamaha Music Foundation, where she taught keyboard and popular piano. In 2007, she came to Wageningen, the Netherlands, to start her PhD in the Department Valorisation of Plant Production Chains at Wageningen UR, under the supervision of Prof. Dr. Johan P.M. Sanders. The aim of her PhD research 'Non-food applications of Jatropha proteins' is to gain more value per hectare Jatropha curcas by extracting Jatropha proteins and use them for various applications, which results are described in this thesis. Currently, she is starting her career as a lecturer and researcher in the Chemical Engineering Department of ITB, Indonesia.

LIST OF PUBLICATIONS

Peer reviewed

D. Lestari, W.J. Mulder, J.P.M. Sanders, Jatropha seed protein functional properties for technical applications, Biochemical Engineering Journal, 53 (2011) 297-304.

D. Lestari, W.J. Mulder, J.P.M. Sanders, Improving Jatropha curcas seed protein recovery by using counter current multistage extraction, Biochemical Engineering Journal, 50 (2010) 16-23.

D. Lestari, M. Insanu, Widyarani, W. J. Mulder, J. P.M. Sanders, M. E. Bruins. The optimization of phorbol ester removal from Jatropha press cake by extraction using different types of organic solvents. 2011. Submitted.

D. Lestari, R. Oudman, W.J. Mulder, E. Ratnaningsih, J. P.M. Sanders; Jatropha leaf protein extraction, 2011. To be submitted.

D. Lestari and E. Zvinavashe, J. P.M. Sanders, The method to calculate the potential economic value of potential products from Jatropha seed in five selected countries: Zimbabwe, Tanzania, Mali, Indonesia, and the Netherlands, 2011. Submitted.

Oral/ poster presentations

D. Lestari, W.J. Mulder, J.P.M. Sanders, Non-food applications of Jatropha proteins. International Conference on Jatropha curcas, 2010. Groningen, the Netherlands.

D. Lestari, W.J. Mulder, R.A. Weusthuis, J.P.M. Sanders, Non-food applications of Jatropha proteins: Extraction and functional properties. The Netherlands Process Technology Symposium 9, 2009, Veldhoven, the Netherlands

D. Lestari, W.J. Mulder, R.A. Weusthuis, J.P.M. Sanders, Jatropha protein for technical applications. The 2nd Jatropha World Africa, 2009, Brussel, Belgium

D. Lestari, A. Marasabessy, R.A. Weusthuis, J.P.M. Sanders, Jatropha curcas: Applying the biorefinery concepts to a new biofuel crop. Indonesian Student's Scientific Meeting, 13-15 May 2008, Delft, the Netherlands

OVERVIEW OF COMPLETED TRAINING ACTIVITIES

Discipline specific activities

Sustainable Process, Product, and System Design, organized by OSPT, Groningen University, the Netherlands, 2010

Intensive Program Biorenewable Material, organized by Bioscience Engineering, Ghent University and INP ENSIACET, Touluose and Tarbes, France, 2007

International Conference of Jatropha curcas, University of Groningen, the Netherlands, 2010

Netherlands Process Technology Symposium (NPS9), Veldhoven, the Netherlands, 2009

The 2nd Jatropha World Africa, Brussel, Belgium, 2009

The 5th International Conference on Renewable Resources and Biorefineries (RRB5), Ghent, Belgium, 2009

Expert Seminar on Jatropha curcas, agronomy and genetics, FACT, Wageningen, The Netherlands, 2007

General courses

Presentation Skill, Wageningen Graduate School, 2010

Career Orientation, Wageningen Graduate School, 2010

Techniques for Writing & Presenting a Scientific Paper, Wageningen Graduate School, 2008

Information Literacy, Wageningen Graduate School, 2008

The Art of Writing, Wageningen Graduate School, 2007

Optional courses/ activities

Presentation in the meeting of the Netherlands Group of Users of Technologies for Separation (NL GUTS), Son, the Netherlands, 2010

Advanced Crop Physiology, Wageningen UR, the Netherlands, 2007

Biorenewable resources for bulkchemical industries, Wageningen UR, 2009

Food Chemistry, Wageningen UR, the Netherlands, 2007

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