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Recent Bio-utilization of *Jatropha Curcas* Seed

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Abstract. *Jatropha curcas* plant is widely cultivated in many areas in Thailand as it has seeds with high quality of oil. Its seed oil has been extracted and used as an alternative fuel to solve an energy shortage in the country. After oil extraction, the seed cake is a low-cost and under-utilized by-product with a high amount of protein. Unfortunately, protein-rich seed cake cannot be applied to human food or animal feed since it has toxic compounds, phorbol esters, and anti-nutritional factors such as phytate, trypsin inhibitor, lectin and saponin. Therefore, the detoxification of these toxins is necessary for the seed cake utilization. Recently, researches on the utilization of the under-utilized seed cake are under progress. This paper aims to review the recent research works on the utilization of the seed cake including removal of toxins in the seed cake by microbial fermentation and production of bioactive compounds expressing anti-oxidative, anti-hypertensive and plant growth promoting activities.

Keywords: *jatropha curcas*, detoxification, protein hydrolysis, anti-oxidation, anti-hypertension, plant growth promotion.

1. Introduction

At present, the insufficiency of energy resources due to the high demand for transportation and industrial uses leads to global energy crisis. The supplementary and alternative energy sources are urgent needs. In Thailand, the fuel consumption rate continuously increases by approximately 4.5% each year [1]. Currently, *Jatropha curcas* is an interesting energy source for biofuel production. It has been cultivated around the country with the supports of Thai government. It is also easily cultivated under climatic conditions of Thailand. Its seeds are extracted for oil and processed to become the biofuel. After oil extraction, seed cake is a non-valuable by-product which is normally used as a green manure or a fertilizer [1].

J. curcas is a tropical short-lived plant in the Euphorbiaceae family, which can be cultured in Central and South America, South-East Asia, India and Africa [2]. It is a multipurpose small tree because of industrial and medicinal uses [3]. It is well adapted to arid and semi-arid conditions and often used for prevention of soil erosion [2]. Within 3-4 months after flowering, *J. curcas* mature seeds can be harvested from the ripen fruits [3]. A fruit contains 3 triangular-convex ellipsoid seeds which have 300-350 g/kg oil [3], [4]. Its seed oils are widely used as a fuel substituent, after trans-esterification. After oil extraction by a screw press, there are 500-600 g/kg indigestible seed cakes left which contain a high amount of proteins, approximately 190-270 g/kg [5]. The seed cake has been utilized as a fertilizer or a green manure. Proteins in the seed cake consist of all essential amino acids, except for lysine, which are higher than FAO and WHO reference proteins for 2-5 year old children as shown in Table 1 [2]. However, the application of its seed cake protein in animal feed industry is restricted since there are toxic compounds; phorbol esters, and anti-nutritional factors such as trypsin inhibitor, phytic acid, lectin and saponin, which cause the negatively effects to humans and animals [6]-[11]. The detoxification of those toxic compounds is important for seed cake protein application either as food or feed ingredients and it is a good way to add the value to this under-utilized by-product.

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Isolated protein from the seed cake would be an important protein source of bioactive peptides or compounds expressing anti-microbial, anti-oxidative, anti-angiotensin I converting enzyme (ACE) and plant growth promoting activities as reported on whey and chickpea proteins [12]-[15]. This paper reports the recent works on bio-utilization of *J. curcas* seed cake.

2. Bio-utilization of *Jatropha Curcas* Seed Cake

2.1. Detoxification of *jatropha curcas* seed cake

Toxin, phorbol esters, and anti-nutritional factors in the seed cake were detoxified by *Bacillus* sp. reported by Phengnuam and Suntronsuk [16]. Submerged fermentation of the seed cake by *Bacillus licheniformis* incubated on rotary shaker (150 rpm) at 37°C for 5 days could reduce phorbol esters by 62%, phytic acid by 42% and trypsin inhibitor by 75%.

Table 1. Amino acid compositions of *J. curcas* defatted seed cake and essential amino acid pattern suggested by FAO/WHO for 2-5 year old children

Amino acid	g/16 g Nitrogen [2, 4]	
	J. curcas defatted seed cake [a]	FAO/WHO reference
Essential		
Cystine	1.34-1.74	2.5 [b]
Methionine	1.49-1.66	
Valine	4.10-5.18	3.5
Isoleucine	3.51-4.47	2.8
Leucine	6.13-7.08	6.6
Tyrosine	2.77-3.20	6.3 [c]
Phenylalanine	4.00-5.42	
Histidine	2.76-3.51	1.9
Lysine	3.00-3.55	5.8
Threonine	3.25-3.56	3.4
Trypthophan	1.23	1.1
Non-essential		
Aspartic acid	11.7-12.50	
Proline	4.03-5.45	
Serine	4.71-5.23	
Glutamic acid	16.10	
Glycine	4.24-5.10	
Alanine	4.36-5.47	
Arginine	11.13-14.16	

[a] The average value of *J. curcas* defatted seed cake form four regions of Mexico; Castillo de Teayo, Veracruz state, Pueblillo, Papantla, Veracruz state, Coatzacoalcos, Veracruz state and Yautepec, Morelos state.

[b] Cystein + methionine. [c] Tyrosine + phenylalanine.

ND, not determined.

The fermented seed cake has a good nutritional value that might be applied in animal feeds. Phengnuam and Suntornsuk [16] also found that the submerged fermentation better degraded toxin and anti-nutrients compared to the solid state fermentation. The reductions of those toxic compounds were well related to Bacillus enzymes; protease, phytase and esterase, produced during the fermentation. The bio-degradations

of toxin and anti-nutritional factors in the seed cake were also found in fungal fermentations by *Rhizopus oligosporus*, a novel Streptomyces fimicarius YUCM 310038 and Aspergillus versicolor CJS-98 reported by Sawang-arom and Suntornsuk [17], Wang et al. [18] and Veerabhadrappa et al. [19], respectively. *R. oligosporus* under submerged fermentation significantly eliminated phorbol esters by approximately 36% within 7 days of fermentation [17] while a novel *S. fimicarius* YUCM 310038 could degrade total toxins in the seed cake by more than 97% under solid state fermentation within 9 days [18]. The fermented seed cake by *S. fimicarius* was fed to carp fingerlings and used as fertilizer for tobacco plant and found that it was non-toxic to the carp and significantly promoted the growth of the plant (Wang et al., 2013) [18]. Toxic compounds in the seed cake were rapidly detoxified by A. versicolor CJS-98 under solid state fermentation [19]. The seed cake was supplemented with 2% (w/w) peptone, adjusted pH to 7.0, added water for moisture content to 40%, inoculated with 1x107 spores/5 g seed cakes and incubated at 25oC. The toxin; phorbol esters, and anti-nutritional factors; phytate, trypsin inhibitor and lectin, in the seed cake were remarkably reduced by approximately 82%, 72%, 98% and 89%, respectively within 96 h. The difference bio-degradation rates of toxic and anti-nutrient compounds in the seed cake possibly come from the different microorganisms used, amounts of inoculation and additional supplements.

2.2. Anti-microbial activity

The effective bacteriostatic peptides should contain 12-50 amino acids which include two or more positive charges and high proportion of hydrophobic residue (more than 50%) to make them an amphipathicity property. The amphipathicity property enhances the ability of the peptides to associate and permeate through the membrane of bacteria which are the typical mechanisms of anti-microbial peptides [20], [21].

Xiao et al. [22] also successfully used a novel method named cell membrane affinity chromatography to isolate an anti-microbial peptide from *J. curcas* meal protein. The peptide was identified as a cationic peptide, Lys-Val-Phe-Leu-Gly-Leu-Lys (JCpep7) which inhibited the microbial growth against *Salmonella* Typhimurium ATCC50013, *Shigella dysenteriae* ATCC51302, *Pseudomonas aeruginosa* ATCC27553, *Staphylococcus aureus* ATCC25923, *Bacillus subtilis* ATCC23631 and *Streptococcus pneumonia* ATCC49619. The anti-microbial mechanisms based on Fourier transform infrared (FTIR) spectroscopy and transmission electron microscopy (TEM) techniques showed that JCpep7 killed microbes principally via disrupting of their cell walls and membranes, followed by cell lysis.

2.3. Anti-oxidative activity

The oxidation reaction produces free radicals which grab electrons from surrounding molecules and starts chain reaction. This reaction causes a damage or death of cell and DNA mutation leading to an aging or a cancer. Anti-oxidants are molecules that terminate oxidation reactions by transferring electrons or hydrogens to the radicals. Effective anti-oxidants are basically reducing agents.

Several studies have revealed the anti-oxidative activities found in protein hydrolysates obtained from *J. curcas* seed protein hydrolysis. Marrufo-Estrada et al. [23] produced the anti-oxidant hydrolysate from *J. curcas* seed protein isolate using pepsin-pancreatin sequential hydrolysis system. The hydrolysate had the anti-oxidant activity against 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS+) radical by approximately 15 mM/mg protein equivalent to standard anti-oxidant Trolox (TEAC value) while Gallegos-Tintoré et al. [24] could produce anti-2,2-diphenyl-1-picrylhydrazyl ($DPPH^-$) radical and chelating peptides form Alcalase hydrolysis of the seed protein isolate. They also found that the hydrolysate could chelate Fe^{3+} and turn into Fe^{2+} and scavenge $DPPH^-$ correlated to the hydrolysis time. They concluded that those activities were related to the peptide molecular weight by which the lower molecular weight had higher activity. The results were supported by the finding of Phengnuam et al. [25]. They produced the anti-$DPPH^-$ hydrolysate from the seed cake protein isolate by using Neutrase, a commercial enzyme. Moreover, after series of liquid chromatography purification the compounds responsible for the anti-oxidant were characterized as a mixture of fatty acids, fatty acid derivatives and a small amount of peptides by mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy. Additionally, the purification of the anti-$DPPH^-$ hydrolysate obtained from Neutrase hydrolysis of the seed cake protein for 1 h by anion exchange chromatography

(Hiprep DEAE FF 16/10) and strong cation exchange cartridge, the active fraction was then characterized its amino acids constituent as arginine rich compounds by approximately 25 mg/g [25].

2.4. Anti-hypertensive activity

The angiotensin I converting enzyme (ACE) is an important enzyme causing a hypertension, the most common worldwide disease in human. The ACE inhibitory peptides are reported as small peptides containing 2-12 amino acids. The most effective ACE inhibitory peptides are tripeptide residues, normally hydrophobic amino acid (aromatic or branched side chains) or positively charged by lysine and arginine at C-terminal. The tripeptide residues inhibit ACE by competitive binding at the active site of enzyme [26]. Many studies showed that pepsin (preferential cleavage between hydrophobic residues) or trypsin (preferential cleavage in arginine and lysine) hydrolysis could be successfully used in antihypertensive peptide production [12].

Protein hydrolysate obtained from protein isolated from *J. curcas* seed cake hydrolysed by pepsin at 2 h with degree of hydrolysis at 15% showed remarkably ACE inhibitory activity at an IC50 of 0.76 mg/ml as shown in Fig. 1. ACE inhibitory activity of pepsin hydrolysate in this study was much higher than that of Alcalase hydrolysate of defatted *J. curcas* flour protein with an IC50 value of approximately 3 mg/ml [23]. The difference of ACE inhibitory activity would be because of different enzyme and hydrolysis condition used. Furthermore, Segura-Campos et al. [27] reported that the hydrolysate of the *J. curcas* kernel meal hydrolysed by Alcalase had ACE inhibitory activity and its purified fraction with a molecular weight lower than 1 kDa obtained by ultrafiltration and gel permeation chromatography possessed the highest ACE inhibitory activity at an IC50 value of 4.78 μg/ml.

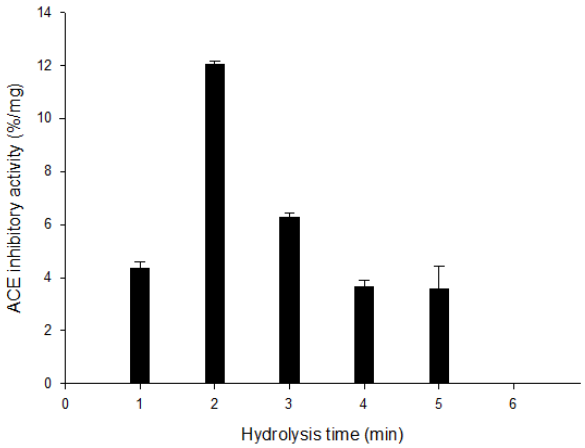

Fig. 1: ACE inhibitory activity of *J. curcas* protein hydrolysate by pepsin

2.5. Plant growth promotion

Protein hydrolysate obtained from *J. curcas* seed cake protein hydrolyzed by Neutrase at 2 h gave the highest plant growth indices of chili pepper, *Capsicum annuum* L. It gave germination percentage of 75%, radical emergence percentage of 95%, seedling growth rate of 4.33 and germination index of 7.4 which exhibited higher growth than a commercial plant growth promoter and other controls [15]. In addition, Sawang-arom and Suntornsuk [17] found that fermented liquid obtained fungal fermentation of the seed cake by *Rhizopus oligosporus* under submerged cultivation for 7 days showed plant growth promoting effects on chili pepper and Chinese kale.

3. Conclusions

It is clearly demonstrated that the toxic compound and anti-nutritional factors in *J. curcas* seed cake could be eliminated by bacterial and fungal fermentations. The fermented seed cake would then retain high protein content and other nutritional values applicable to the animal feed industry. The seed cake protein could be hydrolyzed and be further utilized as anti-oxidant, anti-microbial agent, anti-ACE agent and plant

growth promoter. The hydrolysate would be applied for the functional food, medicine and cosmetic industries.

The summary of bio-utilization of *J. curcas* seed cake is shown in Fig. 2.

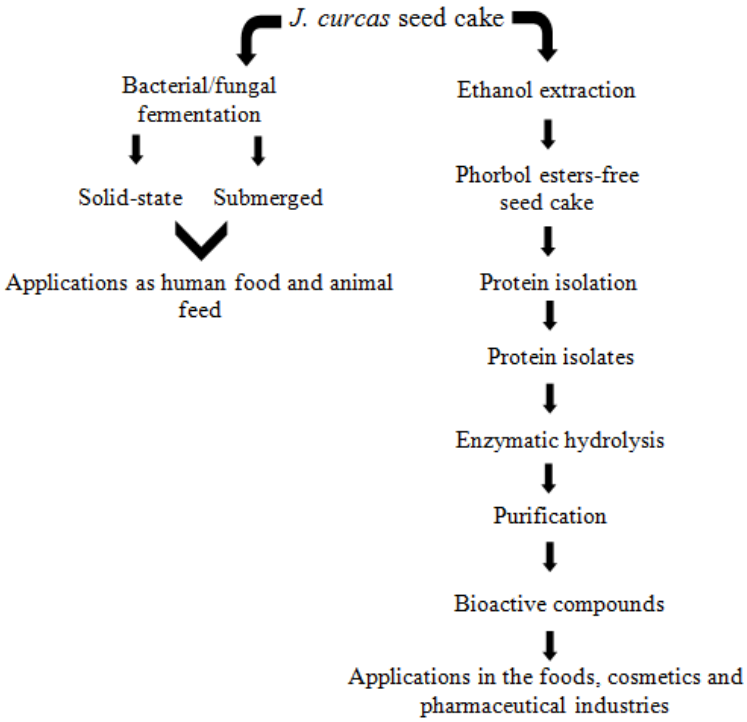

Fig. 2: Summary of bio-utilization of *J. curcas* seed cake

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5. References

[1] L. Na Ayudhaya, and S. Garivait. Potential of *Jatropha curcas* derived biodiesel for rice farmers in Thailand. *The 9th Eco-Energy and Materials Science and Engineering Symposium, Energy Procedia*. 2011, **9**: 252-263.

[2] J. Martinez-Herrera, P. Siddhuraju, G. Francis, G. Dávila-Ortíz, and 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 Chem.*2006, **96**: 80-89.

[3] A.N. Blessing, O.A. Ikechukwu and E.O. Bosa. Vegetative and floral morphology of *Jatropha* species in the Niger Delta. *J. Plant Sci.* 2012, **5**: 163-175.

[4] H.P.S. Makkar, G. Francis and K. Becker. Protein concentrate from *Jatropha curcas* screw-pressed seed cake and toxic and antinutritional factors in protein concentrate. *J. Sci. Food Agric.* 2008, **88**: 1542-1548.

[5] H.P.S. Makkar and Becker, K. Potential of *Jatropha* seed meal as a protein supplement to livestock feed and constraints to its utilization. In *Proc. International Symposium on Biofuel and Industrial Products from Jatropha curcas and other Tropical Oil Seed Plants*, February 23-27, Managua, Nicaragua, Mexico, 1997.

[6] S.M. Dimitrijevic, U. Humer, M. Shehadeh, W.J. Ryves, N.M. Hassan, and F. J. Evans. Analysis and purification of phorbol ester using normal phase HPLC and photodiode-array detection. *J. Pharm. Biomed. Anal.* 1996, **15**: 393-401.

[7] W. Haas and M. Mittelbach. Detoxification experiments with the seed oil from *Jatropha curcas* L. *Ind. Crop. Prod.* 2000, **12**: 111-118.

[8] J.T. Lin and D.J. Yang. Determination of steroidal saponin in different organs of Yam (*Dioscorea pseudojaponica* Yamamoto). *Food Chem.*2008, **108**: 1068-1074.

[9] H.R. Park, H.J. Ahn, S.H. Kim, C.H. Lee, M.W. Byun, and G.W. Lee. Determination of the phytic acid levels in infant foods using different analytical methods. *Food Control*. 2006, **17**: 727-732.

[10] C. Rizzi, L. Galeoto, G. Zoccatelli, S. Vincenzi, R. Chignola and A.D.B. Peruffo. Active soybean lectin in foods: quantitative determination by ELISA using immobilised asialofetuin. *Food Res. Int.* 2003, **36**: 815-821.

[11] L. Yoshizaki, M.F. Troncoso, L.S.L. Jose, U. Hellman, L.M. Beltramini and C. Wolfenstein-Todel. *Calliandra selloi* macbride trypsin inhibitor: isolation, characterization, stability, spectroscopic analyses. *Phytochem.* 2007, **68**: 2625-2634.

[12] M.M. Mullally, H. Meisel, and R.J. FitzGerald. Angiotensin-I-converting enzyme inhibitory activities of gastric and pancreatic proteinase digests of whey proteins. *Int. Dairy J.* 1997, **7**: 299-303.

[13] S. Medina-Godoy, D.L. Ambriz-Pérez, C.I. Fuentes-Gutiérrez, L.J. Germán-Báez, R. Gutiérrez-Dorado, C. Reyes-Moreno and A. Valdez-Ortiz. Angiotensin-converting enzyme inhibitory and antioxidative activities and functional characterization of protein hydrolysates of hard-to-cook chickpeas. *J. Sci. Food Agric*. 2012, **92**: 1974-1981.

[14] I.M.P.L.V.O. Ferreira, O. Pinho, M.V. Mota, P. Tavares, A. Pereira, M. P. Goncalves, D. Torres, C. Rocha, and J.A. Teixeira. Preparation of ingredients containing an ACE-inhibitory peptide by tryptic hydrolysis of whey protein concentrates. *Int. Dairy J*. 2007, **17**: 481-487.

[15] O. Selanon, P. Jitareerat, and W. Suntornsuk. Plant growth promotion by protein hydrolysate obtained from enzymatic digestion of protein isolated from *Jatropha curcas* seed cake. *Agric. Sci. J*. 2009, **40**: 345-348.

[16] T. Phengnuam and W. Suntornsuk. Detoxification and anti-nutrient reductions of *Jatropha curcas* seed cake by *Bacillus* fermentation. *J. Biosci. Bioeng*. 2013, **115**: 168-172.

[17] P. Sawang-arom, and W. Suntornsuk. Fungal submerged fermentation on *Jatropha curcas* seedcake for its detoxification and production of plant growth promoter. In *Proc. 49th Kasetsart University Annual Conference, Bangkok, Thailand*, 2011, pp. 105-112.

[18] X.H. Wang, L. Ou, L.L. Fu, S. Zheng, J.D. Lou, J. Gomes-Laranjo, J. Li and C. Zhang. Detoxification of *Jatropha curcas* kernel cake by a novel *Streptomyces fimicarius* strain. *J. Hazard. Mater*. 2013, **260**: 238-246.

[19] M.B.Veerabhadrappa, S.B. Shivakumar and S. Devappa. Solid-state fermentation of *Jatropha curcas* seed cake for optimization of lipase, protease and detoxification of anti-nutrients in *Jatropha* seed cake using *Aspergillus versicolor* CJS-98. *J. Biosci. Bioeng*. 2014, **117**: 208-214.

[20] M. Charnley, A.J.G. Moir, A.W.I. Douglas and J.W. Haycock. Anti-microbial action of melanocortin peptides and identification of a novel X-Pro-D/L-Val sequence in Gram-positive and Gram-negative bacteria. *Peptides*. 2008, **29**: 1004-1009.

[21] J. Herbiniere, C. Braquart-Varnier, P. Greve, J.M. Strub, J. Frere, A.V. Dorsselaer and G. Martin. Armadillidin: a novel glycine-rich antibacterial peptide directed against Gram-positive bacteria in the woodlouse *Armadillidium vulgare* (Terrestrial Isopod, Crustacean). *Dev. Comp. Immunol*. 2005, **29**: 489-499.

[22] J. Xiao, H. Zhang, L. Niu and X. Wang. Efficient screening of a novel antimicrobial peptide from *Jatropha curcas* by cell membrane affinity chromatography. *J. Agric. Food Chem*. 2011, **59**: 1145-1151.

[23] D.M. Marrufo-Estrada, M.R. Segura-Campos, L.A. Chel-Guerrero and D.A. Betancur-Ancona. Defatted *Jatropha curcas* flour and protein isolate as material for protein hydrolysates with biological activity. *Food Chem*. 2013, **138**: 77-83.

[24] S. Gallegos-Tintoré, C. Torres-Fuentes, A.L. Martínez-Ayala, J. Solorza-Feria, M. Alaiz, J. Girón-Calle and J. Vioque. Antioxidant and chelating activity of *Jatropha curcas* L. protein hydrolysates. *J. Sci. Food Agric*. 2011, **91**: 1618-1624.

[25] T. Phengnuam, A.K. Goroncy, S.M. Rutherfurd, P.J. Moughan and W. Suntornsuk. DPPH radical scavenging activity of a mixture of fatty acids and peptide-containing compounds in a protein hydrolysate of *Jatropha curcas* seed cake. *J. Agric. Food Chem*. 2013, **61**: 11808-11816.

[26] B. Hernández-Ledesma, M.M. Contreras and I. Recio. Antihypertensive peptides: production, bioavailability and incorporation into foods. *Adv. Colloid Interface Sci*. 2011, **165**: 23-35.

[27] M.R. Segura-Campos, F. Peralta-González, A. Castellanos-Ruelas, L.A. Chel-Guerrero and D.A. Betancur-Ancona. Effect of *Jatropha curcas* peptide fractions on the angiotensin I-converting enzyme inhibitory activity. *Biomed. Res. Int*. 2013, **2013**: Article ID 541947.