ORIGINAL ARTICLE

Enzymatic saccharification of hemicellulose extracted from palm oil mill wastes

Poonsuk Prasertsan[1] and Susumu Oi[2]

Abstract

Prasertsan, P.[1] and Oi, S.[2]
Enzymatic saccharification of hemicellulose extracted from palm oil mill wastes
Songklanakarin J. Sci. Technol., 2001, 23(Suppl.): 789-795

Various parameters affecting the extraction of hemicellulose from palm cake by alkali method and sterilizer condensate by solvent method were investigated. For extraction of hemicellulose from palm cake, the optimal ratio of palm cake to sodium hydroxide (NaOH) (1.5% conc.) was 1:10. However, potassium hydroxide (KOH) was a better source of alkali than NaOH and the optimum ratio of palm cake to 12% KOH was 1:50 (w/v). Temperature over 100ºC (100 and 121ºC) extracted significantly higher hemicellulose than at 80ºC after 20 min treatment. The addition of ethanol to the extracted solution in the ratio of 1:1 (v/v) gave the highest hemicellulose yield of 8.67 g/100 g palm cake. For extraction of hemicellulose from sterilizer condensate, the optimum ratio of ethanol to the condensate was 2:1 (v/v), which gave a hemicellulose yield of 6.42 g/100 ml. The enzymatic saccharification of the hemicelllulose extracted from palm cake (HEPC) and from sterilizer condensate (HESC) was 3-10 times lower than that of xylan. The enzyme from *Aspergillus niger* ATCC 6275 and Meicellase gave higher saccharification rates than that of Sumyzyme. The contents of reducing sugars in xylan, HEPC and HESC were 96.4, 36.2 and 20.6%, respectively and 75.3, 67.9 and 97.6% of these values could be hydrolysed by the enzymes. Hence, palm cake was a better source of substrate for extraction of hemicellulose while hemicellulose extracted from sterilizer condensate gave higher percentage of enzymatic saccharification.

Key words : enzyme, saccharification, hemicellulose, extraction, palm oil mill waste

[1]Ph.D.(Biotechnology), Assoc. Prof., Department of Industrial Biotechnology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla 90110 Thailand [2]Ph.D.(Biology), Prof., Department of Biology, Faculty of Science, Osaka City University, Sumimoto-cho, Sumiyoshi-ku, Osaka 558, Japan
Corresponding e-mail: ppoonsuk@ratree.psu.ac.th

บทคัดย่อ

พูนสุข ประเสริฐสรรพ์ [1] และ Susumu Oi [2]

การย่อยสลายเฮมิเซลลูโลสที่สกัดจากวัสดุเศษเหลือของโรงงานสกัดน้ำมันปาล์มด้วยเอนไซม์

ว. สงขลานครินทร์ ฉบับวทท. 2544 23(ฉบับพิเศษ): 789-795

ศึกษาพารามิเตอร์ต่าง ๆ ที่มีผลต่อการสกัดเฮมิเซลลูโลสจากกากปาล์มด้วยวิธีการใช้ด่างและน้ำนึ่งปาล์มด้วยวิธีการใช้สารทำละลาย ผลการสกัดเฮมิเซลลูโลสจากกากปาล์ม พบว่าอัตราส่วนที่เหมาะสมของกากปาล์มต่อโซเดียมไฮดรอกไซด์ (เข้มข้น 1.5%) เท่ากับ 1:10 อย่างไรก็ตามโปแทสเซียมไฮดรอกไซด์เป็นด่างที่ดีกว่าโซเดียมไฮดรอกไซด์ และอัตราส่วนที่เหมาะสมของกากปาล์มต่อโปแทสเซียมไฮดรอกไซด์ (เข้มข้น 12%) เท่ากับ 1 : 50 (นน./ปริมาตร) การสกัดที่อุณหภูมิ 80°C เป็นเวลา 20 นาที ทำให้ได้ปริมาณเฮมิเซลลูโลสเพิ่มขึ้นอย่างมีนัยสำคัญ การเติมเอทธานอลในสารละลายสกัดในอัตราส่วน 1:1 ให้ผลผลิตของเฮมิเซลลูโลสสูงสุดเท่ากับ 8.67 กรัม/กากปาล์ม 100 กรัม สำหรับการสกัดเฮมิเซลลูโลสจากน้ำนึ่งปาล์ม พบว่าอัตราส่วนที่เหมาะสมของเอทธานอลต่อน้ำนึ่งปาล์มคือ 2:1 (ปริมาตร/ปริมาตร) ซึ่งให้ค่าเฮมิเซลลูโลส 6.42 กรัม/100 มล. การใช้เอนไซม์ในการทำแซคคาริฟิเคชั่นต่อเฮมิเซลลูโลสที่สกัดจากกากปาล์มและที่สกัดจากน้ำนึ่งปาล์มมีค่าต่ำกว่าค่าที่ได้จากการทำแซคคาริฟิเคชั่นต่อไซแลน 3-10 เท่า ส่วนเอนไซม์จาก *Aspergillus niger* ATCC 6275 และเอนไซม์ Meicellase ให้ค่าอัตราแซคคาริฟิเคชั่นสูงกว่าค่าที่ได้จากการใช้เอนไซม์ Sumizyme ปริมาณน้ำตาลรีดิวซ์ในไซแลน เฮมิเซลลูโลสที่สกัดจากกากปาล์มและที่สกัดจากน้ำนึ่งปาล์ม มีค่าเท่ากับ 96.4%, 36.2% และ 20.6% ตามลำดับ และเอนไซม์สามารถไฮโดรไลซ์ค่าเหล่านี้ได้ 75.3%, 67.9% และ 97.6% ตามลำดับ ดังนั้นกากปาล์มจึงเป็นสับสเตรทที่ดีกว่าในการสกัดเฮมิเซลลูโลส ในขณะที่เฮมิเซลลูโลสจากน้ำนึ่งปาล์มสามารถถูกย่อยสลายเป็นน้ำตาลได้ดีกว่าจากการย่อยด้วยเอนไซม์

[1]ภาควิชาเทคโนโลยีชีวภาพอุตสาหกรรม คณะอุตสาหกรรมเกษตร มหาวิทยาลัยสงขลานครินทร์ อำเภอหาดใหญ่ จังหวัดสงขลา 90112 [2]Department of Biology, Faculty of Science, Osaka City University, Sumimoto-cho, Sumiyoshi-ku, Osaka 558, Japan

Oil palm fruit consists of 68.3% pericarp and 31.7% nuts. The pericarp itself contains 23.3% pericarp fibre which has 6.4% (dry weight) holocellulose (Kirdaldy and Sutanto, 1976). Holocellulose consists of cellulose and hemicellulose. It is known that palm oil mill wastes also contain this component in different quantities. There are two major types of palm oil milling processes; wet process and dry process. While palm cake is the only waste from dry milling process, sterilizer condensate, empty fruit bunch (EFB), shell and palm pressed fibre (PPF) are the wastes generated from wet process (Prasertsan *et al*., 1990). Palm cake posseses the total amount of hemicellulose since only oil and water are extracted during the process. Sterilizer condensate contains soluble matters including hemicellulose as they are extracted during steaming at 40 lb/in^2. They are therefore the two sources of interest for extraction of hemicellulose.

Hemicellulose consists of a heterogenous group of polymers of mannose, galactose, certain beta-glucanases, the heteropolymers from uronic acid and methyl or acetyl-substituted hexoses, including pentosans that are polymers of xylose and arabinose (Linsmaier-Bednar, 1998). Enzymatic saccharification of hemicellulose would result in the production of various sugars. Xylobiose, one of the enzymatic products from xylan, is available commercially as a low calorie sweetener for dietetics (Nobuzo, 1988).

The aim of this work is to investigate the effects of various parameters on the extraction of hemicellulose from palm cake using alkali method and sterilizer condensate using solvent method, then to use the extracted hemicellulose for the production of sugars by enzymatic saccharification.

Materials and Methods

Palm oil mill wastes

Palm cake and sterilizer condensate were kindly provided by Rungruangkit Co., Ltd. and Pure Oil Co., Ltd., respectively, at Songkhla Province.

Enzyme

Crude enzyme from *Aspergillus niger* ATCC 6275 was prepared as described earlier (Prasertsan *et al*., 1997). The commercial enzymes; Meicelase (CEP 4510) and Sumyzyme were purchased from Meiji Co., Ltd. and Shin Nikon Chemical Co., Ltd., respectively. They were prepared to reach the same level of xylanase activity as the crude enzyme from *Aspergillus niger.*

Factors affecting the extraction of hemicellulose from palm cake and sterilizer condensate

Hemicellulose was first extracted from palm cake by alkali treatment in which 1.5% (w/v) NaOH was added. The mixture (in the ratios of palm cake to alkali, 1:5 and 1:10) was autoclaved at 121ºC for 20 min. After cooling and separation of solid particles, the filtrate was adjusted to pH 4.5 using conc. acetic acid. Hemicellulose was precipitated from the solution by adding 2 volumes of 95% alcohol. After leaving overnight at room temperature, the precipitate was separated by filtration and washed 2-3 times with absolute alcohol. The precipitate was dried at 45ºC for 10 h. Extraction of hemicellulose from sterilizer condensate followed the above procedure but did not include alkali treatment.

The effects of various parameters on the extraction of hemicellulose from palm cake and sterilizer condensate were studied. For palm cake, the involved parameters were type (NaOH and KOH) and concentration (1.5, 3.0, 6.0, 12.0) of alkali, the ratio of palm cake to alkali (1:10, 1:25 and 1:50 v/v), temperature and time of extraction (80ºC at 20 min, 4 h and 8 h; 110ºC/20 min; and 121ºC/20 min) and the ratios of extracted solution to ethanol (1:1, 1:2 and 1:3 v/v). For extraction of hemicellulose from sterilizer condensate, only the effect of ethanol was studied using the same ratios (1:1, 1:2 and 1:3).

Effect of enzyme source on the saccharification of the extracted hemicellulose

The extracted hemicellulose from palm cake and xylan (Oat Spelt) were used as substrates for saccharification by three different enzymes: crude enzyme from *A. niger*, Meicelase and Sumyzyme. The reaction mixture contained 10 ml of each enzyme solution and 10 ml of 5% extracted hemicellulose dissolved in 0.05 M citrate buffer, pH 4.8. Chloramphenicol was added to bring the final concentration to 100 *u*g/ml for microbial growth inhibition. The mixed solution was incubated at 40ºC with shaking. Samples were taken at 0, 4, 8, 16 and 24 h, and their filtrates were used for reducing sugars determination (Miller, 1959) using glucose as a standard. The percentage saccharification was taken as mg glucose/ml x 1.8 (Prasertsan and Oi, 1992).

Comparison between acid and enzyme hydrolysis on the extracted hemicelluloses and xylan

The commercial xylan represents the hemicellulose with xylose backbone and is normally used as a substrate for determination of enzyme (xylanase) activity. Substituted xylans are components of the plant cell wall. Xylan itself has chains of 1,4-linked beta-D-xylopyranose residues (Linsmaier-Bednar, 1998).

The hemicellulose extracted from palm cake (HEPC) and the hemicellulose from sterilizer condensate (HESC) and xylan (50 mg each) were mixed with 5 ml each of 5% H_2SO_4 and 2% Meicelase (prepared in 0.05 M citrate buffer, pH 4.8). The acid hydrolysis was carried out at 100ºC for 2 h, while enzymatic saccharification took place at 40ºC overnight.

Results and Discussion

Factors affecting the extraction of hemicellulose from palm cake and sterilizer condensate

The effect of the ratio of palm cake to NaOH (1.5% conc.) at 1:5 and 1:10 was studied. The result showed higher hemicellulose at higher ratio, 4.52± 1.01% at 1:10 ratio compared to 3.87±0.07% (w/w) at 1:5 ratio (data not shown). The ratio of 1:10 was then used for subsequent studies. The effects of NaOH and KOH at different concentrations (1.5, 3, 6 and 12%) on the extraction of hemicellulose are illustrated in Figure 1. The extracted hemicellulose yields were 6.80, 7.21, 7.35 and 8.37 g per 100 g of palm cake using 1.5%, 3%, 6% and 12% KOH, respectively. The corresponding values at the respective NaOH concentrations were 4.50, 5.29, 5.54 and 6.92 g hemicellulose per 100g of palm cake. KOH therefore gave a higher percentage of extracted hemicellulose than NaOH at every concentration tested and the higher the concentration the higher the yield. Potassium hydroxide was also used to extract xylan from pine kraft pulp (Viikari *et al*., 1994). Hence, 12% KOH was selected and used in the following experiment.

The effect of palm cake to 12% KOH ratio (1:10, 1:25 and 1:50) on the extraction of hemicellulose was investigated. The optimum ratio was found to be 1:50 (w/v) giving 9.44% (w/w) of extracted hemicellulose, compared to 5.91% and 5.66% at the ratio of 1:25 and 1:10, respectively (Figure 2).

Temperature had an influence on the extraction of hemicellulose from palm cake. The temperature over 100ºC gave significantly higher hemicellulose yield than at 80ºC, although there was no difference using 121ºC or 110ºC for 20 min giving the values of 8.55% and 8.45% hemicellulose, respectively (Figure 3). The reaction time, however, had no effect on the concentration of extracted hemicellulose obtained (6.04-6.51%) at the temperature tested (80ºC).

For precipitation, the optimal ratio of ethanol to the extracted solution was 1:1 in which 8.67 g hemicellulose per 100 g palm cake was obtained (Figure 4). This yield (8.67%, w/w) was lower than the expected hemicellulose (20%) in plant cell wall (Linsmaier-Bednar, 1998). The optimum ratio of ethanol to sterilizer condensate, on the other hand, was found to be at 2:1, giving the yield of 6.42 g hemicellulose per 100 ml of sterilizer condensate. The yields at 1:1 and 1:3 ratio were 4.50% and 2.91%, respectively.

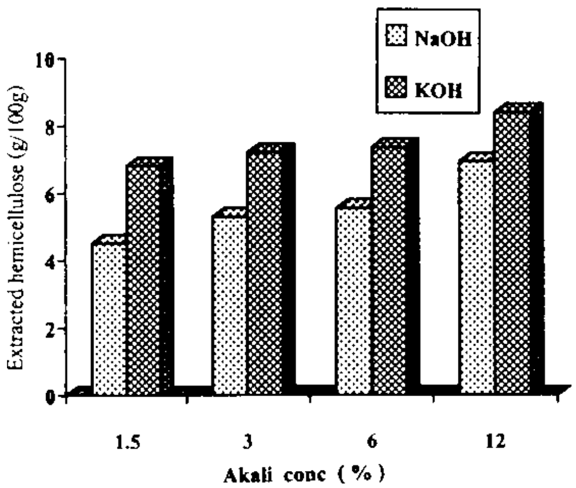

Figure 1 Effect of NaOH at various concentrations on the extraction of hemicellulose from palm cake.

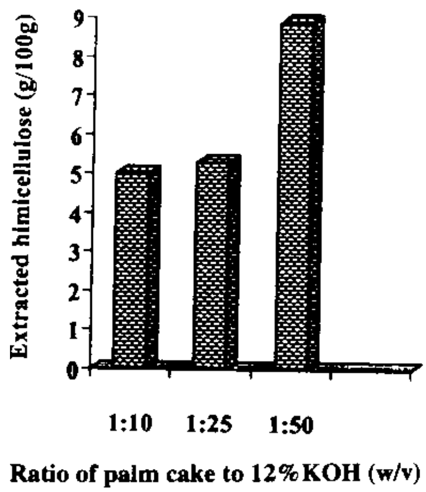

Figure 2 Effect of the ratio of palm cake to 12% KOH on the extraction of hemicellulose from palm cake

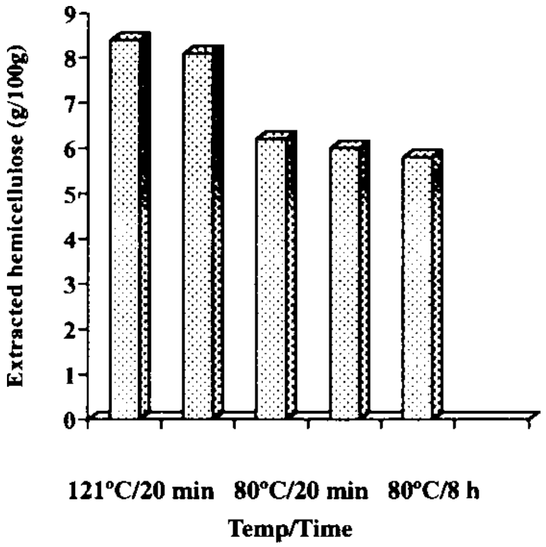

Figure 3 Effect of temperature and time on the extraction of hemicellulose from cake.

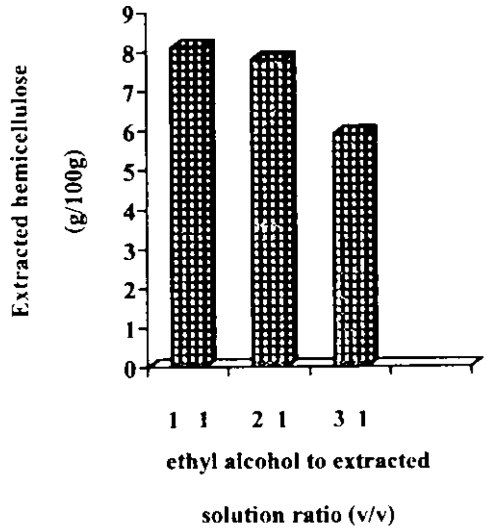

Figure 4 Effect of ethyl alcohol to extracted solution ratio on the extraction of hemicellulose from palm cake.

Effect of enzyme source on the saccharification of the extracted hemicellulose

The hydrolysis of hemicellulose extracted from palm cake and sterilizer condensate, so called HEPC and HESC, respectively, using the enzyme from *A. niger* and the two commercial enzymes is presented in Table 1. It could be demonstrated that the hydrolysis rate on xylan was much higher (3-10 times depending on the enzyme source) than that on the extracted hemicellulose, due to the purity of the substrate. Xylan is a straight chain of xylose residues while extracted hemicellulose consists of a xylose backbone with branching chains of other sugars such as arabinose, mannose, etc. Comparison of the two substrates showed that hemicellulose from palm cake seems to be hydrolysed slightly better than that from sterilizer condensate. Both percentage and rate of saccharification on the hemicellulose from palm cake was about 3 times higher than those on native and ball-milled palm cake using the enzyme from *A. niger* ATCC 6275 (Prasertsan and Oi, 1992). Among the different sources of enzymes, Meicelase and enzyme from *A. niger* exhibited similar saccharification, while Sumyzyme showed lower saccharification activity. Enzymatic additive was reported to enhance the animals to utilize fodder rapidly and efficiently. In addition, the enzyme preparation should contain xylanase activity, in addition to the beta-glucanases and cellulases, in order to have a strong binding of water to the feed component (Linsmaier-Bednar, 1998).

Comparison between acid and enzyme hydrolysis on the extracted hemicelluloses and xylan

Results from acid and enzymatic hydrolysis on hemicelluloses, HEPC and HESC, and xylan are given in Table 2. Reducing sugars obtained from acid hydrolysis of xylan were higher than those from extracted hemicelluloses; 9.64 g/l compared to 3.62 and 2.06 g/l from HEPC and HESC, respectively. This, on the other hand, also indicated a higher content of hemicellulose in palm cake than in the sterilizer condensate. It also correlated with the results achieved from enzymatic hydrolysis, but at lower values: 7.26, 2.46 and 2.01 g/l, res-

Table 1 Hydrolysis of hemicellulose extracted from palm cake and sterilizer condensate by enzyme from *Aspergillus niger* ATCC 6275 and two commercial enzymes

Source of enzyme	Incubation Time (h)	% Saccharification		
		Extracted hemicellulose		Xylan
		Palm cake	Sterilizer condensate	
Enzyme	4	5.71	1.41	16.16
from *A. niger*	8	13.04	10.61	49.39
ATCC 6275	16	12.17	10.73	55.71
	24	11.56	13.13	61.16
Meicellase	4	4.16	3.58	15.97
	8	14.86	11.92	43.67
	16	11.84	9.13	50.88
	24	8.78	8.46	55.28
Sumyzyme	4	3.29	3.18	15.97
	8	8.01	4.07	39.38
	16	4.68	3.83	45.16
	24	4.36	3.80	58.10

Table 2 Reducing sugars from acid hydrolysis and enzymatic hydrolysis on hemicellulose extracted palm cake (HEPC) and hemicellulose sterilizer condensate (HESC)

Substrate	Total reducing sugars (mg/ml)		Yield
	Acid hydrolysis	Enzyme hydrolysis	(%)
Xylan (oat spelt)	9.64	7.26	75.3
HEPC	3.62	2.46	68.0
HESC	2.06	2.01	97.4

pectively. The higher values of reducing sugar from acid hydrolysis were due to the complete hydrolysis on the substrate. Enzymatic hydrolysis, on the other hand, is a more specific reaction and may be limited by various factors including the inhibition by soluble products (Sinitsyn *et al.*, 1981). If based on the amount of substrate used (50 mg substrate in 5 ml of acid or enzyme), the content of reducing sugars in the xylan was 96.4% in which 75.3% was obtained after enzymatic hydrolysis for 19 h at 40ºC. For HEPC and HESC, the quantities of hemicellulose obtained were 36.2 and 20.6% in which 67.9 and 97.6% of them could be hydrolysed by the xylanase. The higher value from HESC was due to the higher accessibility of the hemicellulose extracted from sterilizer condensate.

Conclusion

Palm oil mill wastes; palm cake and sterilizer condensate, contain hemicellulose that could be extracted by alkali and solvent methods, respectively. The extracted hemicelluloses obtained could be saccharified using either the crude enzyme from *Aspergillus niger* ATCC 6275 or the commercial enzyme Meicellase. The yields of total reducing sugars from hemicellulose of palm cake and sterilizer condensate were 68% and 97.4%, respectively, compared to the products from the acid hydrolysis.

Acknowledgement

The authors would like to thank the Monbusho and the JSPS for supporting this collaborative research work.

References

Kirdaldy, and Sutanto, J.B. 1976. Possible utilization of by-products from palm oil industry. Planter, Kuala Lumpur. 52: 118-126.

Linsmaier-Bednar, E.M. 1998. Industrial Enzymes and Their Applications. John Wiley & Sons, Inc. NY.

Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry. 31: 426-430.

Nabuzo, T. 1988. Recent topics on enzyme utilization for food processing in Japan. Food Conference' 88. P. 205-209..

Prasertsan, P., H-Kittikul, Kunghae, A., Maneesri, J. and Oi, S. 1997. Optimization for xylanase and cellulase production from *Aspergillus niger* ATCC 6275 in palm oil mill wastes and its application. World J. Microbiol. Biotechnol. 13: 555-559.

Prasertsan, P., Jitbunjerdkul, S. and H-Kittikul, A. 1990. Process, waste utilization and characteristics of palm oil mill effluent. Songklanakarin J. Sci. & Technol. 12(2): 169-176.

Prasertsan, P. and Oi, S. 1992. Production of cellulolytic enzymes from fungi and use in the saccharification of palm cake and palm fibre. World J. Microbiol. Biotechnol. 8: 536-538.

Sinitsyn, A.P., Nadzhemi, B. and Klisov, A.A. 1981. Enzymatic conversion of cellulose to glucose: influence of product inhibition and the alteration in the reacting of the substrate on the rate of enzymatic hydrolysis. Appl. Biochem. Microbiol. 17(3): 225-235.

Viikari, L., Kantelinen, A., Buchert, J. and Puls, J. 1994. Enzymatic accessibility of xylans lignocellulose materials. Appl. Microbiol. Biotechnol. 41(1): 124-129.