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Enzyme-Supported Oil Extraction from *Jatropha curcas* Seeds

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ABSTRACT

Jatropha curcas is a tropical plant widely distributed in arid areas. The seeds contain about 55% of oil, which is mainly used for the production of soap as a fuel and after transesterification as biodiesel. Various methods for recovering of oil from the seeds, including extraction with organic solvents and water, have been investigated. Compared to hexane extraction (98%) the oil extraction using water only yielded 38% of the total oil content of the seeds. Using several cell wall degrading enzymes during aqueous extraction a maximum yield of 86% was obtained. The influence of cellulolytic, hemicellulolytic enzymes, as well as proteases was studied. The experiments were carried out at different pH-values and temperatures to find out the optimum for oil recovering using enzymes. Surprisingly, the best results (86%) were obtained using an alkaline protease. Combinations of proteases with hemicellulases and/or cellulases did not further increase the extraction yield. The enzyme-supported aqueous extraction offers a nontoxic alternative to common extraction methods using organic solvents with reasonable yields.

Index Entries: Enzyme-supported oil extraction; *Jatropha curcas*; oil seeds; aqueous oil extraction.

INTRODUCTION

Jatropha curcas Linn is a plant widely distributed in the arid regions of the hemispheres, mainly Central and South America. The high share of oil, but also the resistance to dryness and sterile soil make *Jatropha curcas* inter-

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Table 1
Composition of *Jatropha curcas* Seeds

compounds	seeds with shells [%]	seeds without shells [%]
dry substance	94.23	92.00
rash	3.17	3.96
organic dry substance	91.06	88.16
protein	17.08	22.24
raw fat	34.38	54.38
raw fibre	22.96	2.21
starch	0.04	0.15
sugar	2.67	3.30
hemicellulose	3.22	0.18
raw cellulose	13.98	2.91
raw lignin	14.25	0.17

esting for afforestation and desirable as a source of alternative energy. Using the seeds that are rich in oil (55%) and proteins (22%) (Table 1) as a feed staff is not taken into account, because of contained phorbol ester and lectine (curcine). Therefore, the seeds are mainly used for the production of soap as a fuel and after transesterification as biodiesel.

Jatropha curcas fruits are as big as walnuts, divided in three parts containing three seeds formed like kidneys in a hard brown shell with a length of about 2 cm. Plant cell walls are unlignified and composed of cellulose fibers to which strands of hemicellulose are attached. The fibers are often embedded in a matrix of pectic substances linked to structural protein *(1)*. The different possibilities of oil extraction are based upon the fact that the oil inside the cells is partly bounded to proteins and complex carbohydrates, like starch, pectin, cellulose, and xylan. During extraction with an organic solvent, the cell wall of cells not mechanically opened is distributed by osmotic pressure. The use of aqueous solutions has not played a part until now, because of the very bad solution quality of oil in water. This changed with the use of cell-degrading enzymes, mainly fungal preparations rich in xylanases, cellulases, and proteases, which have been interesting for the food processing industry to aid the isolation of proteinaceous rapeseed and other materials from plants *(2,3)*. The degree of mechanical crushing is insofar important as a high crushing rate implies an emulsion of oil drops, cell remnants, proteins, and lipids *(4)*. If the use of emulsion-

breaking substances as detergents is not wanted, an optimal mechanical crushing degree must be determined. It should be remarked that a centrifugation can separate the emulsion partly. Since the composition of plant cell walls varies, individual enzyme combinations comprising xylanases, cellulases, pectinases, and proteinases are required for enzyme-supported oil extraction *(5,6)*.

The aim of this work was to compare commercial available enzymes and to find the optimal enzyme combination, concentration, and conditions for enzyme-supported oil extraction of *Jatropha curcas* seeds.

MATERIAL AND METHODS

Substrate

Table 1 shows the composition of seeds with and without shells *(7)*. For our experiments we used *Jatropha curcas* seeds, obtained from UNI Managua/Nicaragua with an average oil content of 59.8%. The pretreatment of the seeds included the removal of the external fruit flesh and the drying to an average water content of 8 to 10%. Before treatment with enzymes, the seeds were shelled and grinded, to a size of about 0.2 mm in a coffee mill.

Enzymes

Two protease preparations (Alcalase and Neutrase) and a hemicellulase/cellulase preparation (Viscozyme) were kindly provided from Novo Nordisk (Bagsvaerd, Denmark). The protease preparations BLAP and Corolase were obtained from COGNIS (Düsseldorf, Germany) and Röhm (Darmstadt, Germany), the hemicellulase LYX and the cellulase Cytolase CL from Gist-Brocades (Seclin, France), respectively.

Enzyme Activity Assay

Enzyme activities of the commercial preparations was measured at optimal pH and temperature as specified in the manufacturer's instructions. Protease activity was determined using the Anson/Kunitz method *(8)*, xylanase activity using the DNS method *(9)*, and cellulase activity (FPU) according to IUPAC recommendations *(10)*.

Other Analytical Methods

The calculation of extraction yield is based on the initial oil content determined with soxhlet extraction and direct measurements of the weight of the oil after the extraction process. Free fatty acids were determined using the titration method with ethanol/diethyl ether and phenolphthalein as indicator to qualify the oil *(11)*. The water content of the oil was quantified using the Karl Fischer method.

Table 2
Optimal pH and Temperature for Enzymatic Activity with Casein (Proteases), Filter Paper (FPU), and Xylane (XU) as Standard Substrates and with *J. curcas* Seeds for Oil Extraction*

Enzyme	pH standard	T [°C] standard	Anson [U/g]	FPU filterpaper [U/g]	XU xylan [U/g]	pH *J. curcas*	*T [°C] J. curcas*	*oil yield [%]*
alcalase	8	50	1027	-------	-------	7	60	78
neutrase	6	45	852	-------	-------	6	45	61
corolase	9	45	99	-------	-------	9	45	59
BLAP	11	65	623	-------	-------	11,5	60	60
cytolase cl	5	45	-------	175	37	4,5	45	40
LYX	5	40	-------	23	165	5	45	42
viscozyme	4	45	-------	25	225	4,5	45	68

*Incubation system in case of *Jatropha curcas* as substrate: 0.5*M* citrat or phosphate buffer (depends on optimal pH), 5 Anson U/g seed or 3 XU/g seed for 2 h.

Enzyme Treatment

A suspension of *Jatropha curcas* seeds (460 g/L, dry weight) was incubated with different enzymes for 2 h at different pH (0.05*M* citrate buffer [pH ≤ 7] 0.05*M* phosphate buffer [pH > 7]) and temperatures as indicated in Table 2. Thereafter, the suspension was centrifuged at 10,000 rpm for 10 min yielding a four-phase system (Fig. 1). The oil phase was separated after dilution with n-hexane (100% hexane v/v), which was removed by distillation afterwards. The oil content was quantified gravimetrically after drying at 105°C for 1 h.

RESULTS AND DISCUSSION

First, the correlation between substrate concentration and oil extraction yield was studied. A suspension of the seeds was incubated with 3 XU/g seed for Viscocyme activity or 5 Anson U/g seed for protease activity (Neutrase, Alcalase, Corolase, and BLAP) and the extraction yield was determined. For all enzyme preparations, the best results were obtained at a substrate concentration around 46% w/w, dryweight, which is shown in Fig. 2 for Alcalase and Viscozyme. These findings are in contrast to observations from Barrios et al. *(12)* for coconuts of 25% w/w and from

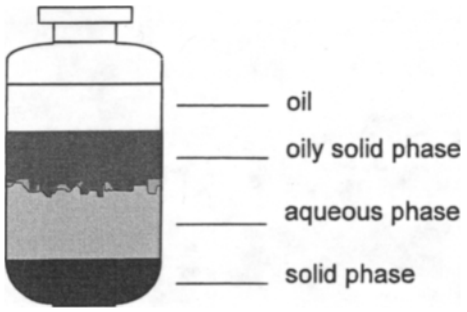

Fig. 1. Four-phase system after centrifugation of the enzyme-treated seed-water solution.

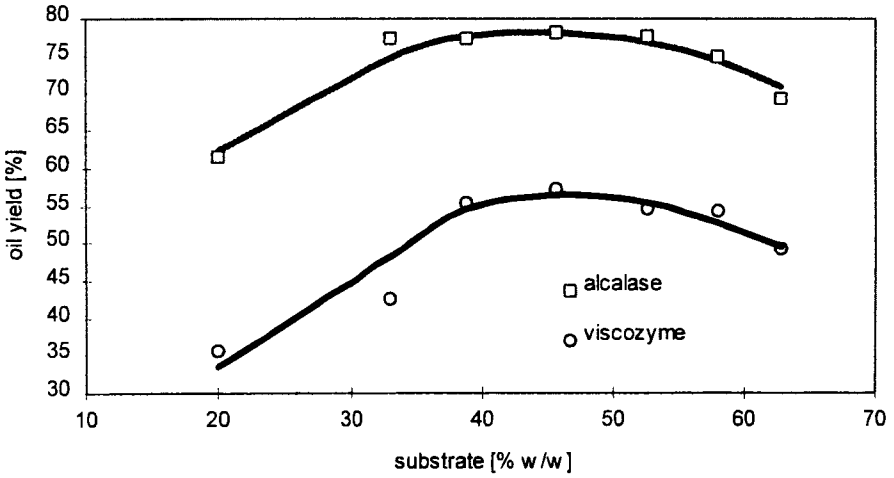

Fig. 2. Effect of substrate concentration (w/w) on *J. curcas* seed extraction yield with Alcalase (5 Anson U/g seed, pH: 7) and Viscozyme (3 XU/g seed, pH: 4.5); incubation time: 2 h, temperature: 60°C (Alcalase), 45°C (Neutrase).

Buenrostro *(13)* for avocados of 20% w/w. All further experiments were carried out at a substrate concentration of 460 g/L.

In a second step, the optimal pH and temperature for the enzyme treatment of *J. curcas* seeds were determined. The cellulase-hemicellulase gave the best results at pH around 5.0, whereas proteases preferred higher pH between 6.0 and 11.5 (Table 2). All preparations showed different optimum temperature ranging from 45 to 60°C. Optimal pH and temperature measured on *J. curcas* seeds did not vary significantly from those reported in the manufacturer's instructions for standard substrates. The oil extraction yields obtained with the individual enzyme preparations under these conditions are also listed in Table 2.

Enzyme treatment was performed with different enzyme concentrations varying from 0.4 to 4 XU/g (Viscozyme) and from 0.3 to 30 Anson U/g for proteases (Neutrase, Alcalase, Corolase, and BLAP). The maximum

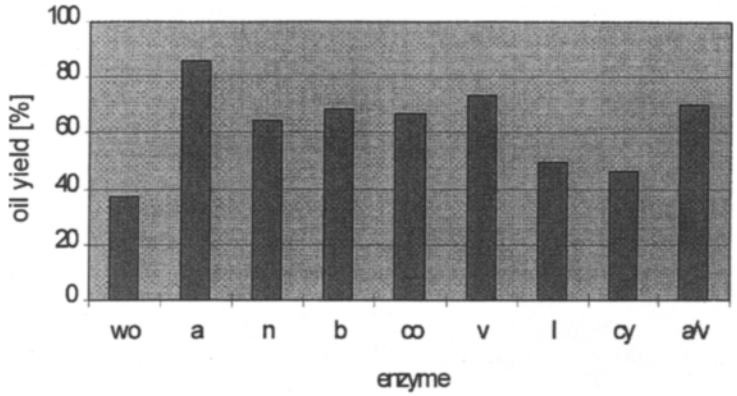

Fig. 3. Oil yields after enzymatic aqueous extraction with different enzymes at optimal conditions: 15 Anson units for proteases, 2 XU for hemicellulases/cellulases. (wo = without enzyme, a = Alcalase, n = Neutrase, b = BLAP, co = Corolase, v = Viscozyme, l = LYX, cy = cytolase and a/v = Alcalase/Viscozyme).

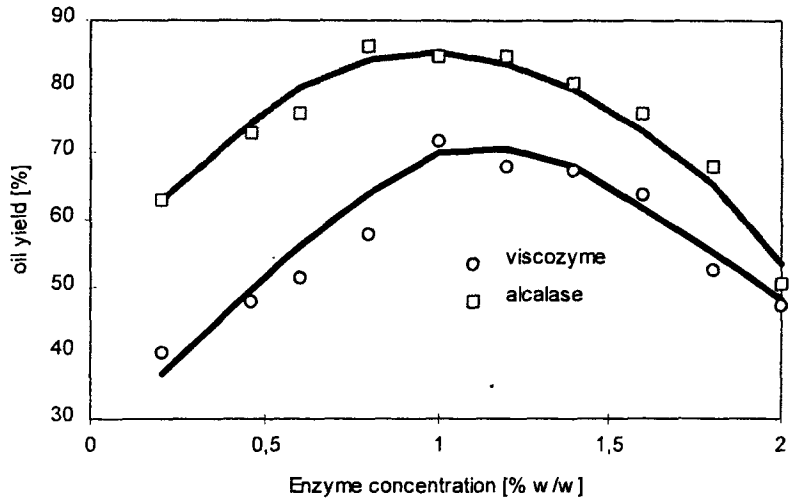

Fig. 4. Effect of enzyme concentration (w/w) on *J. curcas* seed extraction yield with Alcalase (1% w/w = 15 Anson U/g seed, pH: 7) and Viscozyme (1% w/w = 2 XU/g seed, pH, 4.5); incubation time: 2 h, temperature: 60°C (Alkalase), 45°C (Viscozyme).

extraction yield was reached with different activities of the individual enzymes as shown in Fig. 3. The highest extraction yield of 85.6% was obtained with the protease Alcalase. This is in a good agreement with data reported by Barrios *(12)* for avocados (80%), Buenrostro et al. *(13)* for coconuts (78%), Olsen *(2)* for rapeseed (95%), coconuts (95%), and flax seed (72%). In this case proteases seem to be more effective than cellulases or hemicellulases preparations that gave a maximum extraction yield of 73% in case of Viscozyme. Figure 4 shows the extraction yields obtained with different

Table 3
Content of Free Fatty Acids and Water in the Oil after Enzymatic Treatment

Enzyme	free fatty acids [%]	water [%]
Alcalase	7.3	0.1621
BLAP	9.07	0.1690
Neutrase	11.18	0.1688
Viscocyme	7.5	0.1705
blank	2.5	0.1993
pressed oil	5.6	0.1677

enzyme concentrations using Viscozyme and Alcalase (Fig. 4). Interestingly, high enzyme concentrations resulted in a decrease of the extraction yield because of worse separation properties of the resulting suspension.

In order to study the effect of combinations of proteases with hemicellulases/cellulase, a common oil seed suspension (460 g/L) was incubated with Alcalase (15 U/g seed, dry weight, activity Alcalase: 1027 U/mL) and Viscozyme (2 XU/g seed, activity Viscozyme: 25 FPU/mL, 225 XU/mL). The optimal pH- and the temperature for combined enzyme treatment was determined, but an increase of the yield could not be obtained compared to separate treatment with Alcalase and Viscozyme. Under optimal conditions, an oil yield of 70% could be measured that is about the same as with Viscozyme alone. This is in contrast to results reported in the literature *(6,11,12,14)* where combinations of proteases, hemicellulases, and cellulases gave the best result.

Incubation of *J. curcas* seeds after milling for 1 h at 105°C before enzyme treatment decreased the yield about 10 to 15% in contrast to the results from Jensen et al. *(15)* for rapeseeds.

To characterize the oil quality with regard to followed esterification, the content of water and free fatty acids in the oil was determined (Table 3). The results were compared to data measured for oil extracted by the Soxhlet method with n-hexane. The content of free fatty acids was in all cases above the values of blank, and the contents of water were very similar. An aftertreatment of the oil seems to be necessary since fatty acids and water cause disturbing coreactions during esterification *(16)*.

CONCLUSIONS

When applying the enzyme-supported oil extraction of *Jatropha curcas* seeds described here, an oil yield of 86% is achieved, that is signifi-

cant higher than aqueous extraction (38%). In general, the enzymatic process requires a considerably lower capital investment and consumes less energy, that makes this process interesting for developing countries, specially in case of *Jatropha curcas*, which is used for afforestation of arid areas.

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