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PRELIMINARY STUDIES ON THE ENZYMATIC TREATMENT OF COTTONSEED FOR HIGHER OIL YIELD

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ABSTRACT

Enzymatic pretreatment of oilseeds prior to oil extraction is an alternative to the thermal / hydrothermal treatment carried out in the industry to degrade the cell walls. In this work cottonseed flakes were enzymatically treated with cellulase, hemicellulase, and pectinase. The enzyme concentrations investigated were 1, 2, and 3%, at moisture : cottonseed flakes ratio of 5.5:1, 7:1and 10.5:1 (w/w) for 3 and 6 hours.The pH and temperature of the reactions were those stated by the manufacturers. Pectinase proved the most efficient of the three enzymes followed by cellulase, extracting ca. 45% and 40% oil, respectively, from the treated flakes, compared to 37% extracted oil from nontreated cottonseed flakes. All treatments resulted in highly significant differences ($P<0.001$) compared to nontreated flakes. Enzyme mixtures were formulated between pectinase : Cellulase (1:1,w/w) and pectinase : cellulase : hemicellulase (0.66 : 0.66 : 0.66, w/w). Percentage increase in oil extractability was in the following order pectinase : cellulase > pectinase > pectinase : cellulase : hemicellulase > cellulase > hemicellulase yielding 28% , 22% , 22 % and 10.5%, respectively. Iodine value, acid value and total gossypol of all the resulting oils were examined. The fatty acid composition of the oils resulting from the treatments together with the iodine value show that the ratio of saturated to unsaturated fatty acids were either the same as the untreated oil or the saturation increased slightly.

Keywords: Cottonseed oil, pectinase, cellulase, hemicellulase, oil extractability

INTRODUCTION

The global demand for edible oils is increasing with the increasing world population, especially in developing countries. People are becoming more apprehensive about their health, eating habits, and the quality of the food they eat. These issues promoted the search for new oil sources, as well as the improvement of existing oil seed processing technologies, to improve oil yield and reduce oil loss. Genetic engineering together with research and development should work hand in hand to achieve high oil yields of premium health quality.

Oilseeds need to undergo some treatments before the mechanical expelling or solvent extraction to break the cell walls and facilitate the flow of oil. Oil is usually found inside the vegetable cells linked with other macromolecules such as proteins. Recent cell wall model studies envision a cellulose-hemicellulose structural domain embedded in a secondary domain consisting of pectic substances, while a third domain consists of covalently cross linked proteins Carpita and Gibeaut (1993).

Among the conventional pretreatment carried out in the oilseed industry to break the cell walls to release the oil, are thermal / hydrothermal treatments. This heat treatment causes damage to oil and protein. An alternative to this treatment is the enzymatic treatment, which results in degrading the cell walls but in the mean time will preserve the oil and protein in their native form. Specific enzymes are needed to be used, such as

cellulase and hemicellulase to break the cellulose and hemicellulose structure, pectinase to hydrolyse the pectins and proteases to break the lipid-protein complexes, and others.

Hitze *et al.* (1972) and Bocevska *et al.* (1993), reported on the application of enzymatic extraction of oil from corn germ and the resulting oil was of good quality. The extraction of olive oil using enzymes was carried out by Montedoro *et al.* (1973). Lanzani *et al.* (1975) followed the same technique for the extraction of rapeseed oil. Fullbrook (1983) investigated the treatment of ground dehulled oilseeds with enzymes in the presence of hexane and reported that the oil yield could be improved under such conditions. McGlone *et al.* (1986), Che Man *et al.* (1996 and 1997) developed an aqueous enzymatic extraction method for coconut oil. Among the enzymes investigated were polygalacturonases, amylase, cellulase, protease and *Lactobacillus plantarum* 1041 1AM. They reported a significant improvement in oil yield and quality over the conventional wet process. The use of microbial enzymes for the treatment of cottonseed, sunflower seed, Soybean and castor bean before oil extraction was reported by Bhatnagar and Johari, (1987). They confirmed better oil yield.

Sitohy *et al.* (1993) characterized the enzymatically extracted sunflower oil as well as the protein residues and reported good results with cellulase and hemicellulase but not with pectinase and protease for both oil and protein. Dominguez *et al.* (1995) also applied the aqueous processing of sunflower with the enzymatic technology and their results showed a 30% improvement in oil extraction yield. The application of the enzyme pretreatment of soybeans before oil extraction was reported by (Smith *et al.*, 1993). They followed the enzymatic treatment with mechanical expelling. Shankar *et al.* (1997) applied enzymatic treatment in conjunction with conventional pretreatment of soybeans. While Rosenthal *et al.* (2001), investigated the effect of operational variables and enzyme activity on aqueous enzymatic extraction of oil and protein from soybean.

Sengupta and Bhattacharyya (1996) reported that the quality of enzyme extracted mustard seed oil was better with regard to color and odor than commercial expeller and solvent extracted oils. They also reported that the characterization of enzyme extracted rice bran oil was the same as the commercial oils. Hernandez *et al.* (2000) proposed a modification of the process of oil extraction from rice bran, introducing one or two enzymatic reactions previous to solvent extraction. Results showed 5% higher oil yields when treated with amylase.

Until recently the oilseed industry in Egypt was based solely on the cottonseed crop. Soybeans have been introduced in the 1970's. The present work is just a preliminary study to investigate the enzymes that will result in optimum oil yield from cottonseed. This will be followed by coming work to investigate the extraction of the enzymatically treated cottonseed hulls by hydraulic pressing instead of solvent extraction to obtain high yield and quality. So the aim of the present study is to investigate the enzymatic pretreatment of cottonseed flakes, using cellulase, hemicellulase and pectinase.

MATERIALS AND METHODS

Materials

Cottonseed (*Gossypium barbadence*) flakes were supplied by El-Minya Ginning Company.

Enzymes

Cellulase and hemicellulase are products of Sigma. Pectinase is a product of Novo-Nordisk.

Methods

- **Enzymatic treatment of cottonseed flakes**

A calculated amount of water was added to the flakes to reach the desired moisture:flakes ratio, the enzyme (calculated as % of sample weight) was added, then the pH was adjusted while stirring on a magnetic stirrer continuously for 15-20 minutes to ensure stability of pH. The mixture was transferred to a glass stoppered Erlenmeyer flask then placed on a shaking thermostatic water bath adjusted to the optimum temperature of activity of the used enzyme and shaking was continued for a predetermined time. At the end of the experiment the temperature was raised to 104°C for 5 minutes to inactivate the enzyme. The hydrolysed flakes were then placed in an air draft oven at 60°C and dehydrated to a moisture level of 5%. The dried flakes were ground then subjected to oil extraction. A control sample was carried together with the experiments without enzyme. Optimum conditions for enzymes as stated by the manufactures, for cellulase, pH 5.0 and 37°C, hemicellulase, pH 5.7 and 37°C, and pectinase pH 4.0 and 25°C .

The investigated variables were : Enzyme concentration (1, 2 and 3%) ; time 3 and 6 hrs, and moisture : cottonseed flakes ratio of 5.5 :1 , 7:1 and 10.5 :1 (w/w).

2- Extraction of oil from enzyme treated flakes

The oil was extracted from both control and enzyme treated samples with petroleum ether (40°C – 60°C) in a soxhlet apparatus for 12 hours. The flakes were dried at 60°C, reground and re-extracted with the same solvent for further 12 hours. The extract was dried over anhydrous sodium sulphate, filtered and evaporated in a rotary evaporator till near dryness, then completely dried in a vacuum oven at 60°C overnight and the extracted oil then weighed and the oil percentage was calculated.

Chemical analyses:

Moisture of cottonseed flakes, acid value and iodine value were determined according to standard methods of AOCS (1994), and total gossypol as described by Pons *et al.* (1956).

Fatty acid composition :

The component fatty acids of the oil sample extracted from cottonseed flakes was converted to its methyl ester by esterification according to Luddy *et al.* (1960). The fatty acid esters were subjected to gas liquid

chromatographic (GLC) analysis. The column was 4 feet long, and 0.3 mm i.d., packed with polyethylene glycol adipate on celite. The inlet temperature was 325°C. Nitrogen was used as a carrier gas at a flow rate of 30 ml/min. The detector current was maintained at 1000V. The identity of the peaks was achieved through comparison of the retention times with those of authentic standards.

The enzymatic hydrolysis experiments were replicated three times for each enzyme. Analysis of the resulting oil was determined in triplicates.

Statistical analysis :

Statistical analysis of the results were computed with compatible IBM personal computer and performed using the arithmetic mean, standard deviation, standard error and hypothesis "T" test as described by Strike (1966). Non-significant difference (NS) if $P > 0.05$; highly significant difference if $P < 0.01$; very highly significant difference if $P < 0.001$.

RESULTS AND DISCUSSION

1- Preliminary experiments on the enzymatic treatment

The first investigation was carried out on the treatment of cottonseed flakes with the three investigated enzymes, namely, cellulase, hemicellulase and pectinase. This preliminary treatment with the three enzymes was carried out to compare their effects on the extracted oil yield.

Results in Table (1) indicates the results of treating cottonseed flakes with 1% of each enzyme, separately, for a period of 3 hrs at different moisture ratios. Data revealed that treating cottonseed flakes with pectinase resulted in the highest percentage of extracted oil. At moisture : flakes ratio of 5.5 : 1 , 7 : 1 and 10.5 : 1 (w/w) the extracted oils were 41.4, 39.9 and 45.9%, respectively. Pectinase was followed by cellulase which extracted 38.7, 37.6 and 38.8% oil at the same ratios mentioned before, respectively.

Performance of hemicellulase was the least of the three enzymes, yet statistical analysis of the data showed a highly significant difference ($P < 0.01$) between the control (non-treated flakes) and all the enzymatically treated flakes.

2- Effect of pectinase on the oil extractability

The treatment of cottonseed flakes with 1, 2, and 3% concentration of pectinase at moisture : cottonseed flakes ratios of 5.5 : 1 , 7 : 1 and 10.5 : 1 (w/w) for 3 and 6 hours at pH 4.0 and temperature 25°C is represented in Table (2). The used Temperature 25°C and pH 4.0 were recommended by the manufacturer. Results in the table clearly show that hydrolysis of cottonseed flakes under different conditions using pectinase resulted in a general increase in the amount of the extracted oil, ranging from 10.0 to 23.0

Table (1): Effect of enzyme type on the oil extractability from cottonseed flakes.

Enzyme type	Moisture : cotton Seed flakes (w/w)	Extracted oil (%)
	(5. 5 :1)	38.696±0.197
Cellulase	(7 :1)	37.586±0.205
	(10. 5 :1)	38.836±0.0796
	(5. 5 :1)	34.373±0.254
Hemicellulase	(7 :1)	36.863±0.055
	(10. 5 :1)	36.6±0.200
	(5. 5 :1)	41.433±0.260
Pectinase	(7 :1)	39.883±0.181
	(10. 5 :1)	45.89±2.585
Control (Non-treated cottonseed flakes)		36.75±0.595

- Experiments were carried out as 1% enzyme concentration and for a duration of 3 hrs.
- Values are given on wet basis.
- Results are expressed as mean ± SE.

% when compared with the control. Highest yield was achieved with 2% pectinase at 6hrs hydrolysis with 5.5 : 1 and 7: 1 (w/w) moisture ratios reaching 45.82 and 45.09%, respectively. The Least amount of extracted oil resulted from hydrolysis with 1% pectinase for 3hrs at 7: 1 moisture ratio , yielding 39.64% oil. All treatments resulted in a highly significant difference ($P < 0.01$) over the control.

Table (2) : Effect of pectinase treatment of cottonseed Flakes on oil extractability.

Enzyme Conc. (%)	Time (hr.)	Moisture : cotton Seed flakes (w/w)	Extracted Oil (%)	Increase in oil extractability (%)
		(5. 5 :1)	42.82±0.092	16.5
1	3	(7 :1)	39.646±0.163	7.86
		(10. 5 :1)	44.58±0.2055	21.31
		(5. 5 :1)	43.643±0.211	18.74
1	6	(7 :1)	41.176±0.312	12.02
		(10. 5 :1)	43.513±0.153	18.39
		(5. 5 :1)	42.333±0.189	15.10
2	3	(7 :1)	41.1066±0.115	11.83
		(10. 5 :1)	43.65±0.101	18.78
		(5. 5 :1)	45.096±0.118	22.74
2	6	(7 :1)	45.816±0.113	23.0
		(10. 5 :1)	41.01±0.143	11.59
		(5. 5 :1)	42.293±0.289	15.07
3	3	(7 :1)	42.333±0.240	15.18
		(10. 5 :1)	42.466±0.218	15.56
		(5. 5 :1)	43.313±0.103	17.85
3	6	(7 :1)	43.783±0.072	19.12
		(10. 5 :1)	40.4±0.264	9.93
Control (Non-treated cottonseed flakes)			36.75±0.595	

- Values are given on wet basis. Results are expressed as mean ± SE.

3- Effect of cellulase on the oil extractability

The investigated conditions 1, 2 and 3% cellulase, time of hydrolysis 3 and 6 hours, and moisture : cottonseed flakes ratios of 5.5: 1, 7: 1 and 10.5: 1 (w/w) . Results are represented in Table (3). Statistical analysis of the data show a high significant difference ($P< 0.01$) represented by the increase in the extracted oil resulting from the treated flakes compared to the non-treated flakes (control) . The highest oil yield was achieved under hydrolysis condition of 3% cellulase, 6 hours, and moisture:flakes ratio of 10.5: 1 (w/w) amounting to 40.61% oil compared to 36.75% oil extracted from non treated flakes (control). About 39 % oil was obtained under the following condition, 1% enzyme concentration, 6 hours, and 5..5: 1 moisture : flakes ratio and at 2% enzyme concentration, 6 hours, and 10.5 :1 moisture : flakes ratio .The increase in oil extractability over the control for all the treatments ranged between 2.2 and 10.5 % .

Bhatngar and Johari (1987) reported an increase between 8 and 23% in the extractable oil from cottonseed when using cellulase and hemicellulase. They also used several microbial enzymes and obtained close results to the former enzymes. When treating sunflower with the same enzymes maximum increase in the extracted oil reached only 12%, soybean reached 15%increase in extracted oil over the untreated seeds.

Lanzani *et al.* (1975) working with rapeseed, peanut, sesame, sunflower and soybean investigated the action of several enzymes to increase oil extractability from these seeds. They reported oil obtained from total available oil in the seeds to reach ca. 72-78% for peanut, 50-78% for rapeseed and 30-44%for sesame. Sengupta and Bhattacharyya (1996) reported % oil recovery from mustard seed treated with a mixture of cellulase and pectinase in the presence of hexane to reach a maximum of 100% oil recovery. They also reported 90% oil recovery from rice bran with the same mixture of enzymes.

Sosulski *et al.* (1988) found that treatment of different canola cultivars with Cellulast, Finizym, Pectinex, Enzeco, Novozym resulted in % oil extraction of 34-41% compared to 22.3% for untreated canola.

4- Effect of mixed enzymes on the oil extractability

Dominquez *et al.* (1994) in a review on the enzymatic pretreatment to enhance oil extraction from fruits and oilseeds, stated that multi-activity enzymatic formulations are the most favorable during the pretreatment of the seeds when trying to improve the oil yield.

Thus two formulations from the previously investigated enzymes were prepared and their effect on the oil yield from cottonseed flakes was investigated. The two enzyme formulations included : 1) cellulase : pectinase (1:1, w/w), hydrolysis conditions : 2% enzyme mixture concentration, 7:1 moisture : flakes ratio, 6 hours, pH 4.5 and temperature 30°C. 2) cellulase : hemicellulase : pectinase (0.66 : 0.66 : 0.66 w/w), hydrolysis conditions : 2% enzyme mixture concentration, 7 : 1 moisture : flakes ratio, 6 hours, pH 5.0, temperature 30°C.

Table (3): Effect of cellulase treatment of cottonseed flakes on oil extractability.

Enzyme Conc. (%)	Time (hr.)	Moisture: cotton Seed flakes (w/w)	Extracted Oil (%)	Increase in oil extractability (%)
		(5. 5 :1)	38.56±1.505	4.9
1	3	(7 :1)	37.586±0.205	2.2
		(10. 5:1)	38.823±0.072	5.6
		(5. 5 :1)	39.786±0.099	8.24
1	6	(7 :1)	35.95±0.223	-
		(10. 5 :1)	38.08±0.066	6.34
		(5. 5 :1)	37.3833±0.291	1.71
2	3	(7 :1)	37.266±0.193	1.41
		(10. 5 :1)	38.213±0.194	3.97
		(5. 5 :1)	38.323±0.178	4.28
2	6	(7 :1)	37.943±0.119	3.23
		(10. 5 :1)	39.37±0.075	7.12
		(5. 5 :1)	38.51±0.274	4.78
3	3	(7 :1)	38.063±0.073	3.57
		(10. 5 :1)	38.59±0.202	5.00
		(5. 5 :1)	37.893±0.061	3.10
3	6	(7 :1)	38.556±0.248	4.91
		(10. 5 :1)	40.616±0.136	10.52
Control (Non-treated cottonseed flakes)			36.75±0.595	

- Values are given on wet basis.
- Results are expressed as mean ± SE.

Results in Table (4) clearly show that mixed enzymes or multiactivity enzyme mixtures are superior to single enzymes. The mixture of celluiase : pectinase extracted 47.09% oil compared to 36.75% extracted oil from the untreated flakes (control) resulting in 28.15% increase in oil extractability and a highly significant difference ($P < 0.01$) over control. While cellulase alone resulted in 10.52% and pectinase alone in 23.0% increase in oil extractability. The cellulase : hemicellulase : pectinase mixture resulted in 22.38% increase in oil extraction over the control. This value is similar to % increase in oil extraction when using pectinase, but higher than that when using cellulase alone.

Table (4) : Effect of enzyme mixture treatment of cottonseed flakes on the oil extractability.

Enzyme mixture	Extracted oil (%)	%Increase in oil extractability
Cellulase : Pectinase (1 : 1)	47.096±0.097	28.15
Cellulase : Hemicellulase : Pectinase (0.66 : 0.66 : 0.66)	44.976±0.156	22.38
Control (Non-treated cottonseed flakes)	36.75±0.595	

Sosulski *et al.* (1988) extracted oil from canola by hydrolysis of canola with carbohydrase enzymes. They reported higher oil yields when using a mixed activity enzymes. *Lanzani et al., (1975)*, reported higher oil yields from peanut, rapeseed and sunflower when using a mixture of enzymes. The pretreatment of soybean with a mixture of pectinase and cellulase was reported by *Fullbrook (1983)* to yield more oil than the individual enzymes.

5- Characteristics of enzymatically treated extracted oils

Table (5) shows the effect of the enzymatic treatment of cottonseed flakes prior to extraction of oil with petroleum ether on the iodine value, acid value and gossypol content of the extracted oils, compared to oil extracted from nontreated flakes. Results in the table show that the effect of treatment of flakes with a single or a mixture of enzymes results in a significant difference ($P < 0.001$) on the iodine value, acid value, and % gossypol content. Acid value ranged between 4.6 to 5.3 compared to 5.4 for control oil. Iodine value ranged between 103 to 101 compared to 103 of control oil. The gossypol content was 0.37 % for untreated oil (control) whereas it ranged between 0.30 to 0.40% for enzyme treated oils.

Table (5) : Effect of enzyme treatment of cottonseed flakes on the iodine value, acid value and gossypol content of the oil.

Enzyme treatment	Iodine value	Acid value	%Gossypol
Control	103.4±0.0509	5.383±0.021	0.37±0.004
Pectinase treated	103±0.063	5.291±0.032	0.303±0.004
Cellulase treated	100.69±0.011	4.856±0.022	0.301±0.004
Cellulase : Pectinase	101.12±0.062	4.55±0.042	0.398±0.005
Cellulase : Hemicellulase : Pectinase	102.82±0.005	4.633±0.049	0.4±0.005

6- Fatty acid composition of extracted oils

Table (6) gives the fatty acid composition of the oils extracted from enzymatically treated cottonseed flakes, together with the oil extracted from nontreated flakes. Results of Gas-Liquid Chromatographic analysis of the oils revealed slight differences between those from enzymatically treated flakes and the nontreated flakes. If we look to the ratio between the total saturation : total unsaturation we find that it is in agreement with the results of the iodine value in table 5. The ratio of saturation : unsaturation is 1 : 2.3 for control, pectinase treated oil and mixture of cellulase : hemicellulase : pectinase treated oils, but is 1 : 2.2 for cellulase and cellulase : pectinase treated oils.

In conclusion this technique proved to be promising as a tool to increase the yield of extracted oil without damaging the oil or meal protein. Yet further work on individual enzymes and mixtures of enzymes including proteases and carbohydrases have to be carried out.

Table (6) : Fatty acid composition of cottonseed oil extracted from enzymatically treated cottonseed flakes.

Fatty acid (%)	Non-treated cottonseed oil	Pectinase treated cottonseed oil	Cellulase treated cottonseed oil	Pectinase : cellulase treated cottonseed oil	Cellulase : Hemicellulase : Pectinase treated cottonseed oil
$C_{14:0}$	0.5	0.5	0.74	0.85	0.76
$C_{16:0}$	29.5	28.7	28.43	27.46	26.99
$C_{16:1}$	-	-	0.8	1.06	0.8
$C_{18:0}$	0.6	0.5	2.36	2.85	2.39
$C_{18:1}$	22.5	23.0	20.0	20.86	21.62
$C_{18:2}$	46.5	46.0	47.84	47.25	46.69
$C_{18:3}$	0.4	0.3	0.1	0.67	0.78
Saturation : Unsaturation ratio	1:2.26	1:2.33	1:2.18	1:21	1:232

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دراسة أولية على المعاملة الإنزيمية لبذور القطن لإنتاج محصول عالى من الزيت

فخرية طه ، إلهام يوسف ، سلامه عمر

قسم الدهون والزيوت ، المركز القومى للبحوث ، القاهرة

المعاملة الإنزيمية للبذور الزيتية قبل إستخلاص الزيت يمكن أن تعتبر بديل للمعاملات الحرارية التى تجرى فى المصانع بغرض تكسير جدران الخلايا لتسهيل إستخراج الزيت. تمت معاملة قشور بذرة القطن بالإنزيمات التالية : بكتينيز ، سليوليز والهيميسليوليز. تركيز الإنزيمات المختبرة كان ١ ، ٢ ، ٣% ونسبة الرطوبة : قشور بذرة القطن كانت ١:٥,٥ و ١:٧ و ١:١٠,٥(وزن /وزن) ومدة التجربة ٣ و ٦ ساعات . درجة الحرارة وال pH هما الموص بهما من قبل شركة التصنيع. أثبتت التجارب أن البكتينيز أعطى أعلى نسبة إستخلاص للزيت (حوالى ٤٥%) يليه إنزيم السليوليز (٤٠ %) بالمقارنة بالقشور الغير معاملة (٣٧%) . تم بعد ذلك تكوين خلطات إنزيمية وقد أوضحت النتائج أن نسبة الزيادة فى الزيت المستخلص كان بالترتيب التالى : بكتينيز : سليوليز ،بكتينيز :بكتينيز : سليوليز : هيميسليوليز ، سليوليز، هيميسليوليز بنسب ٢٨% ،٢٢% ، ٢٢% > ١٠,٥% على التوالى . وقد تم تقدير الرقم اليودى والرقم الحامض و نسبة الجوسيبول فى جميع الزيوت الناتجة من المعاملات الإنزيمية وقد أوضحت نتائج التحليل الكروماتوجرافى الغازى للأحماض الدهنية تناسب نتائجها مع نتائج الرقم اليودى كما أن المعاملات الإنزيمية اوضحت ان نسبة الأحماض المشبعة للغير المشبعة هى نفس النسبة الموجودة للزيت الغير معامل أ و قد تزيد نسبة الأحماض المشبعة قليلا