Optimizing conditions for enzymatic extraction of sunflower oil.

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RESUMEN

Condiciones de optimización para la extracción enzimática de aceite de girasol.

Aceite de semilla de girasol fue extraído mediante un proceso enzimático usando diferentes enzimas hidrolíticos: celulasa, hemicelulasa, proteinasa animal, proteinasa ácida, pectinasa y pectinex, comparando con la extracción acuosa libre de enzima.

Todos los enzimas hidrolíticos incrementan la extracción de aceites de semilla de girasol. Las condiciones óptimas para la extracción de aceite a partir de semillas de girasol fueron: 2% de concentración de enzima, 30% de concentración de sustrato y un período de 3 horas.

La ecuación de Boganov y Buchkov mostró que el tiempo debe ser prolongado para alcanzar altos rendimientos. El máximo rendimiento durante tres horas de extracción con proceso enzimático osciló entre el 44,5%-57,1% del aceite extraído con soxhlet.

La potencia de los enzimas investigados en la extracción de aceite siguió el orden: proteinasa ácida > celulasa > hemicelulasa > proteinasa animal > pectinex > pectinasa cuando fue previamente comparado con las condiciones óptimas.

PALABRAS-CLAVE: Aceite de girasol - Enzima hidrolítico - Extracción - Optimización.

SUMMARY

Optimizing conditions for enzymatic extraction of sunflower oil.

Sunflower seed oil was extracted with an enzymatic processes using different hydrolytic enzymes: cellulase, hemicellulase, animal proteinase, acid proteinase, pectinase and pectinex, as compared to enzyme - free aqueous extraction.

All the hydrolytic enzymes enhanced oil extraction from sunflower seeds. The most optimal conditions for oil extraction from sunflower seeds were: 2% enzyme concentration, 30% substrate concentration and 3 hrs period.

Using Boganov and Buchkov equation showed that time must be prolonged to get higher yields. The maximum yield during 3 hrs extraction with enzymatic process ranged between 44,5%-57,1% of the soxhlet extractable oil.

The potency of the investigated enzymes in extracting oil was in the following order: acid proteinase > cellulase > hemicellulase > animal proteinase > pectinex > pectinase when compared at the previous optimal conditions.

KEY-WORDS: Extraction - Hydrolytic enzyme - Optimizing - Sunflower oil.

1. INTRODUCTION

Vegetable oil exists in the vegetable cells linked with other macromolecules (Gunetileke and Laurentius, 1974).

Neutral triglycerides bind to proteins via hydrophobic interactions while phospholipids bind more tightly through polar linkages. Covalent bonds were also found between the oxidation products of unsaturated lipids constituent and proteins (Cheftel et al., 1984).

Hence, separation of oil with apolar organic solvents, e.g. hexane, might be only effective in case of neutral glycerides linked to protein through apolar linkages, but lipid constituents bound through polar linkages require polar solvent. Solvent mixtures consisting of polar and apolar solvents such as ethyl: chloroform (1:1) were developed for more efficient oil extraction (Steinkraus, 1973).

Using organic solvents in oil extraction may pose some hygienic hazarads to those working with them or the food if contaminated with traces of them. They also may affect quality of the separated protein. So, the use of enzymatic extraction of vegetable oil may be safe and more efficient as the enzymes may be capable of dissociating most of the linkages between the lipid and other constituents as indicated by Mac-Glone et al., (1986) who developed an enzymatic process for the extraction of coconut oil.

Therefore, this work was designed to identify the optimal conditions for an enzymatic extraction of sunflower oil using different enzymes such as proteases, cellulases and pectinases.

2. MATERIALS AND METHODS

Sunflower seeds obtained from Zagazig local market were dehulled and thoroughly chopped. The ground dehulled seeds were used as the substrate for oil extraction.

Animal and acid proteinase were obtained from Institute of Microbiology, Sofia, Bulgaria, Cellulase 9108, Hemicellulase AS and pectinase were obtained from Rapidase Seclin, France. Pectinex ultra SP was purchased from Novo Industri A/S Bagsvaerd, Denmark.

2.1. Substrate preparation

An amount of 50 g ground dehulled seeds were combined with a suitable aliquot of phosphate buffer (pH 5) to give either 30 or 50% concentration, then mixed thoroughly with mechanical stirrer.

2.2. Enzyme preparation

Proper amounts of enzymes were dissolved in 10 ml phosphate buffer (pH 5) and added to the substrate emulsion in the ratios of 0,5 or 2% (g E/100 g substrate).

2.3. Extraction procedure

Proper aliquots of enzymes were combined with substrate emulsions at a ratio of 0,5 or 2% (E/S) and adjusted to pH 5 before stirring at 50°C for 1 or 3 hrs. Then 10 ml of benzene were added at the end of extraction time to inactivate enzymes. The emulsions were centrifuged at 3.000 rpm for 15 minutes then the oil - solvent layer was decanted and filtered through anhydrous sodium sulfate to get rid of moisture. Benzene was evaporated in Soxhelt apparatus and the remaining oil was weighed. Respective controls were conducted under the aforementioned conditions without the addition of enzymes. Different combinations of these conditions were designed in 8 different treatments as well be shown in the results.

The equation of Boganov and Buchkov (1973) was used to compare the effectiveness of the reaction conditions on the oil yield. This equation is;

$$y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3$$

were y = Oil yield.

b_0 = factor which indicates the effectiveness of the enzyme and yield magnitude.

x_1 = enzyme concentration.

x_2 = substrate concentration.

x_3 = time in hrs.

b_1 = factor indicates the effectiveness of x_1.

b_2 = factor indicates the effectiveness of x_2.

b_3 = factor indicates the effectiveness of x_3.

3. RESULTS AND DISCUSSIONS

The approximate chemical composition of sunflower seeds was as follows: 28% hulls, 9,4% moisture and 45,9% oil of the dry seeds.

Sunflower seed oil extractability using cellulase and hemicellulase are presented in Table I. Data show that oil yield during aqueous processing reached to a maximum of 41,6% (calculated as % of Soxhelt extracted oil) at 30% substrate concentration after 3 hrs while it reached 10,3% at 50% substrate concentration and after 1 hr of extraction. The aqueous extraction of oil from sunflower seeds in the absence of any external addition of enzymes might be due to the action of the internal natural degrading enzymes. These results are in line to those obtained by Lorenz (1980) who detected low cellulase activity and high hemicellulase and protease activity in the Kernels of sunflower seeds.

Addition of cellulase or hemicellulase to the aqueous extraction medium enhanced oil extraction at the different studied conditions which could be attributed to their degrading action upon the cell walls composed of cellulase fibers to which strands of hemicellulase are attached. Olsen (1988) indicated that vegetable oils inside plant cells are linked with starch, cellulose, hemicellulose, protein and pectin. So it could be concluded that cellulase and hemicellulase helped to break the links between oil molecules and both of cellulose and hemicellulose.

Table I
Sunflower seed oil extractibility with using cellulase and Hemicellulase (% of the soxhlet extractable oil)

Treatment No	Extraction conditions: Enzyme concentration%	Substrate concentration%	Time hr	Oil Extractibility: Control (water extraction)	Cellulase	Hemicellulase
1	2,0	30	3	41,6	55,77	54,07
2	0,5	30	3	41,6	50,89	42,00
3	2,0	50	3	20,8	41,10	36,78
4	0,5	50	3	20,8	30,37	28,50
5	2,0	30	1	20,9	36,38	31,98
6	0,5	30	1	20,9	34,14	18,28
7	2,0	50	1	10,3	26,14	16,67
8	0,5	50	1	10,3	23,31	15,40

Equation (1) cellulase $y = 15,41 + 0,9x_1 + 2,76 x_2 + 4,66 x_3 + 1,86 x_2x_3 + 1,05x_1x_3$

Equation (2) Hemicellulase $y = 12,62 + 1,2x_1 + 2,36x_2 + 3,70x_3 - 2,34x_1x_3 - 2,41x_1x_2x_3$

Results obtained in Table I revealed that cellulase was more powerful in enhancing oil extraction from sunflower seeds at most extraction conditions than hemicellulase which could be indicated by Boganov and Buchkov equations 1 and 2, respectively. As the factor bo in equation 1 (15,04) is higher than the respective one in equation 2 (12,26). Moreover, it could be observed that the most effective conditions were: 2% enzyme concentration, 30% substrate concentration and 3 hours extraction period which increased the oil yield by about 34 and 30% in case of cellulase and hemicellulase compared to respective controls. These findings indicated that low substrate concentration would allow more water and enzyme to penetrate cell structures and act upon them releasing oil. So, high concentration of solvent (water) plays a major role in disintegrating cell structures and complexes and helping in the mobilization and action of the hydrolytic enzymes.

Applying Boganov and Buchkov equation to the data presented in Table I it could be observed that the time factor is most effective upon the final yield followed by substrate concentration. Besides, the interaction between time and substrate concentration factors is more effective upon oil yield than the interaction between time and enzyme concentration. Therefore, it is expected that increasing the time of extraction and reducing the substrat concentration may effectively increase oil extraction yield from sunflower seeds.

Concerning proteolytic enzymes, it could be observed, as shown in Table II, that addition of these enzymes to aqueous extraction medium lead to enhanced oil extraction from sunflower seeds especially at the high enzyme concentration (2%). Low enzyme concentration (0,5%) either slightly enhanced oil extraction or did not affect it at

all. Generally, acid proteinase was more effective than animal proteinase as proved from the factors of equations 3 and 4. The most optimal conditions for oil extraction from sunflower seeds with the two proteolytic enzymes were; 2% enzyme concentration, 30% substrate concentration and 3 hrs period. Under these conditions, oil extraction was increased by 23,4 and 37,2%, compared to respective controls, in case of animal and acid proteinase.

Table II
Extractibility of sunflower seed oil using animal proteinase (% of the soxhlet extractable oil)

Treatment No	Extraction conditions: Enzyme concentration%	Substrate concentration%	Time hr	Oil Extractibility: Control (water extraction)	Animal proteinase	Acid proteinase
1	2,0	30	3	41,6	51,33	57,08
2	0,5	30	3	41,6	41,18	41,83
3	2,0	50	3	20,8	36,01	40,52
4	0,5	50	3	20,8	23,12	25,71
5	2,0	30	1	20,9	25,86	36,60
6	0,5	30	1	20,9	19,72	22,88
7	2,0	50	1	10,3	25,23	26,80
8	0,5	50	1	10,3	22,59	13,94

Equation (3) Animal proteinase $y = 12{,}02 + 2{,}09x_1 + 2{,}91x_3 + 1{,}49x_2x_3$.
Equation (4) Acid proteinase $y = 13{,}98 + 0{,}79x_1 + 2{,}19x_2 + 2{,}28x_3$

The abovementioned findings could be ascribed to the degrading action of proteolytic enzymes upon the lipid protein complexes in accordance with that obtained by Gunetilekeand Laurentius (1974) and Cheftel et al., (1984).

Regarding Boganov and Buchkov (1973) equation, it could be observed that time is the most effective factor followed by substrate concentration in case of animal and acid proteinase.

Data presented in Table III indicate that both pectinase and pectinex could slightly enhance oil extraction compared to the aqueous free enzyme extraction (control) when the extraction conditions were; 2% enzyme concentration; 30% substrate concentration and 3 hrs extraction time. The increase in oil yield under these conditions were 6,9% and 12,5% for pectinase and pectinex over respective controls. Hence, pectinex was more powerful than pectinase as supported by bo values in equation 5, 6 respectively.

From the same equations, it can also be noticed that the time is still the most effective factor as with the other previously studied enzymes.

The oil yield enhancing effect of pectinase and pectinex might be due to their degrading action upon pectins found in sunflower seeds material and hence help release the oil bound to pectin molecules. However, the action of these two enzymes was inferior to the action of either proteases or cellulases. This may be due to the absence of an appreciable content of pectin in sunflower seed

Table III
Sunflower seed oil extractibility with using pectinase and pectinex (% of the Soxhlet extractable oil)

Treatment No	Extraction conditions: Enzyme concentration%	Substrate concentration%	Time hr	Oil Extractibility: Control (water extraction)	Pectinase	Pectinex
1	2,0	30	3	41,6	44,47	46,80
2	0,5	30	3	41,6	43,51	42,70
3	2,0	50	3	20,8	29,37	40,72
4	0,5	50	3	20,8	22,61	34,42
5	2,0	30	1	20,9	25,42	25,86
6	0,5	30	1	20,9	22,83	19,26
7	2,0	50	1	10,3	16,78	32,90
8	0,5	50	1	10,3	12,83	18,98

Equation (5) Pectinase $y = 11{,}85 + 0{,}49x_1 + 2{,}61x_2 + 3{,}10x_1 - 0{,}89x_1x_2 + 0{,}61x_1x_3$.
Equation (6) pectinex $y = 13{,}98 + 2{,}01x_1 + 3{,}49x_3 - 0{,}81x_1x_2 + 1{,}31x_2x_3$

material (Sabir et al., 1975; Earle et al., 1968). So, the role of pectin in binding oil was relatively trivial and hence using pectin degrading enzyme may not be effective tools for oil extraction from sunflower seeds.

Finally, it is worthy to note that proteases and cellulases enzymes were of meanly equal potencey in oil extraction from sunflower seeds while pectinases showed only a limited action as well as prolonging the time of extraction and manipulating substrate concentration might further maximize the oil extraction yield.

These findings are in agreement with the results obtained by McGlone et al., (1986) and Olsen (1988) who used polygalactourenase in coconut oil extraction and cell proteases in the extraction of different vegetable oil from rapeseed, flak seed, coconut and corn germ.

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