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Enzyme-assisted hexane extraction of soya bean oil

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An enzymatic treatment with carbohydrases was performed either simultaneously with or prior to the hexane extraction of oil from soya grits. The enzymatic treatment increased the oil extractability by 5% of the extractable oil when it was carried out simultaneously with the oil extraction and 8–10% if the treatment was carried out prior to the solvent extraction. For this latter case, the fraction 'easily' extractable increased up to 7·5%. With regard to the products, the in-vitro apparent digestibility of the meal was slightly improved by 3%, and the enzyme assisted extracted oil contained higher free fatty acids and phosphorus contents than the oil from untreated samples ($P < 0·05$).

INTRODUCTION

The solvent extraction of oil from seeds is the most usual and efficient extraction technique, both from seeds with an oil content lower than 20% and from those with high oil content (Bernardini, 1983). Oil from fruits and seeds provides 70% of the total world oil production, soya bean oil accounting for 30% of this production. The recognised quality of its oil and the nutritive value of the meal protein make soya bean a primary oilseed.

Before extraction, soya beans must be cleaned, cracked and flaked as efficient solvent extraction of oil requires that every oil cell is broken (García Serrato, 1981), so improving the mass transfer (Fan *et al.*, 1948; Smith, 1952). Cell walls (mainly composed of cellulose, associated with hemicelluloses, pectic substances and lignin), could also be broken by means of enzymes, to achieve a significant improvement in extraction yields and rates (Olsen, 1988; Domínguez *et al.*, 1994*a*).

Oil extraction with enzymes as processing aids has been extensively reported for fruits (Buenrostro & López-Munguía, 1986; Cintra *et al.*, 1986; Alba *et al.*, 1987; Christenson & Olsen, 1988). The enzymes can be incorporated during the mixing of the paste. Oil extraction yields from seeds became greatly enhanced when the enzymatic treatment was performed during the aqueous oil extraction (Olsen 1988; Laiho *et al.*, 1990; Marek *et al.*, 1990; Badr & Sithohy, 1992), since water is not a specific oil solvent, and the extraction efficiency is considerably lower. Furthermore, a reduction in the operation time, a good quality of the obtained oil and higher protein yields were also observed.

In established processes for olive oil extraction, the oil increase as a result of the enzymatic treatment could account for 1–2 kg/100 kg olive while, for coconut and avocado, higher yields could be attained. For seeds, 15–30% more of the total oil can be extracted from aqueously processed soya bean and rapeseed, and up to 60% in the presence of an organic solvent (Fullbrook, 1983).

Nowadays, the aim is to incorporate enzymatic treatment in the industry without significant alteration of the conventional process. However, a number of difficulties arise, because (i) the enzyme efficiency is lower at the reduced moisture common in the industry, (ii) particle size is optimised for industrial operation but not for enzyme attack, and (iii), being the basic process maximised for oil extraction, the attainable increases in the oil extraction yield will be lower. Among these three, the moisture value is decisive since, for cellulases, water activity plays an important role in swelling, expanding the structure of fibre, increasing the surface area accessible to cellulolytic enzymes, and also facilitating the diffusion of enzymes and the inhibiting products formed (Fan *et al.*, 1987).

The incorporation of the enzymatic treatment (at 20–40% moisture during the incubation with enzymes) prior to the conventional extraction process, has been studied for canola seeds (Sosulski *et al.*, 1988; Sosulski & Sosulski, 1990). A similar enzymatic pretreatment

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was applied before the mechanical expelling of soya beans (Smith *et al.*, 1993) and sunflower (Domínguez *et al.*, 1993).

In the present study, the ability of the enzymatic pretreatment performed at low moisture conditions to enhance soya bean oil extractability was evaluated. For this purpose the treatment was carried out either prior to (at 15–20% moisture) or simultaneously with the hexane extraction (under 12% moisture). Under-optimal conditions (particle size, temperature and solvent to seed ratio) were stated for better-observing the effect on the oil extraction. Product quality and extraction rate are also examined.

MATERIALS AND METHODS

Seeds

Soya beans (Amsoy N.2 cultivar) kindly supplied by Cereol Ibérica (La Coruña) were stored at 4°C until used. The seeds were cracked, dehulled and screened to obtain the desired particle size distribution of 1–0·75 mm, for certain experiments, and 0·75–0·4 mm for other ones.

Solvent

Industrial hexane, kindly supplied by the above-mentioned local factory, was rectified before use.

Enzymes

A commercial cellulolytic formulation, Celluclast 1.5 L (Novo Nordisk A/S) and Multifect (Finnish Sugars Co. Ltd), a mixed activity consisting of cellulase, hemicellulase and other side-degrading activities were used for these experiments, both with similar activity per unit volume.

Adsorption assays

The enzymatic solution was added to the cracked seeds (0·05 v/w, db) and after periods of less than 1 h at room temperature (where virtually no hydrolysis occurs) 2·5 ml citric buffer/g seed db were added, mixed and then centrifuged at 5000 rpm (3696 × *g*). The supernatant was assayed for cellulolytic activity, and the adsorbed activity was calculated as the difference between the initially added (100% activity) and that removed in the supernatant.

Enzymatic treatment

Simultaneous enzymatic treatment–solvent extraction

Under-optimal conditions for extraction were stated to discern the effect of the enzymatic treatment, because low improvements in the oil extractability were expected at reduced moisture conditions. For this reason, a particle size in the range 0·75–1 mm was chosen; this size is small enough both to favour the enzymatic action (as in small sizes) and to slow down the extraction kinetics, since after periods of 2 h (necessary to allow the enzymatic action) only 80% of oil potentially extractable with Soxhlet from the ground seeds was extracted.

Moisture values should be kept below 11·5%, to avoid negative effects on the extraction rate; also, during industrial operation, moisture must be kept below this value in order for the extractor to function efficiently, without irregular draining and plugging. Before the addition of the enzyme, the seeds were dried at moderate temperatures (40°C) in order to minimise the effect on both the quality of the end-products and the porosity of the material. The enzyme was added in water, so the pH during the treatment was that of the unmodified natural seeds; the enzymatic solution was sprayed onto the solid sample so the moisture content was adjusted to the desired level for hydrolysis. After equilibration to stabilise and homogenise the moisture, the solvent was added in a dry weight to volume ratio of 1:17, since the oil yield is not significantly increased if higher ratios are used (Bernardini, 1983). Both the enzymatic treatment and solvent extraction were simultaneously performed in an orbital incubator at 50°C; this is the usual value in commercial solvent extraction practice and the optimum for cellulolytic activity. To keep a good contact between seeds and solvent, 150 rpm agitation was maintained in 250 ml Erlenmeyer flasks. After extraction, samples were cooled at room temperature and the separation of seeds and oil was accomplished by means of vacuum filtration, measuring the oil extracted by weight difference once the solvent was removed.

Enzymatic treatment previous to solvent extraction

The enzymatic solution was sprayed onto the grits, reaching 15–20% moisture. The enzymatic treatment was carried out at 50°C for 3 h and, before extraction, the moisture content was reduced to 10% under carefully controlled drying conditions in half-opened flasks, to avoid affecting the structure during drying, in which hydrolysis could occur in some extent. To follow the extraction kinetics, samples of miscella were taken, filtered through 0·5 μm filters and the oil concentration was spectrophotometrically measured.

This procedure was used with the following purposes:

(i) to analyse the effect of the enzymatic treatment on the kinetics in a system where the concentration of oil in the solvent was kept practically constant; and

(ii) to evaluate the reduction of the operation time in equipment where the solvent becomes progressively enriched in oil.

Depending on the objective, the extraction was performed:

(i) in Erlenmeyer flasks at a sub-optimal temperature for hexane extraction (30°C), with a solvent to seed ratio of 20 (v/w).

(ii) in a vertical extractor heated at 50°C by a thermostatted external water bath. To simulate the use of a miscella progressively enriched in oil, the solvent to seeds ratio was fixed as a function of the shape and size of the equipment; so the available volume was occupied with the seeds (40 g grits), and 115 ml hexane were used.

The vertical extractor was capable of operating with ascendant or descendant flow. A condenser was used to avoid solvent losses, since the system was not hermetically closed and, even with condenser, the evaporation losses accounted for 3 ml/h. Hexane was pumped through the bed of seeds with a peristaltic pump at a flow suitable to allow a good circulation of solvent (65–70 ml/min). During extraction by percolation, the solvent was sprayed from the upper part, where a filter filled with glass spheres (6 mm diameter) continuously shared the flow over the bed of seeds without completely filling up the empty spaces. For extraction by immersion, the solvent was pumped from the bottom, also filled with glass spheres, before reaching the seeds that were kept immersed in the solvent. In both operation schemes, hexane was continuously recirculated. Since no pure solvent was used in further extraction stages, complete oil extraction was not possible, but the oil concentration in the miscella was far from that affecting the extraction rates (20% w/w) (Othmer & Jaatinen, 1959).

Either when the enzymatic treatment was done previously or simultaneously with the hexane extraction, a control sample (with water instead of enzyme) was examined for oil extractability in order to assure that the drying conditions did not affect the oil extractability.

Analytical methods

Total oil content was determined in a Soxhlet apparatus with hexane. Experiments on the simultaneous enzymatic treatment–solvent extraction were carried out in Erlenmeyer flasks and the extracted oil was calculated by weight differences in previously tared flasks, after separating the miscella from the solid by filtration and evaporating the solvent. The extraction kinetics when the enzymatic treatment was carried out previous to solvent extraction were followed by measuring the oil concentration in the extracting miscella. Samples were conveniently diluted in hexane, and analysed by UV spectrophotometry (Shimadzu UV-160A), at 225 or 300 nm, depending on the required sensitivity. Hexane was used as reference. The standard calibration curve at each wavelength was obtained with known concentrations of crude soya bean oil in hexane (Wiese & Snyder, 1987). Oil concentration in the miscella (%, vol), was calculated as a function of the absorbance values, 0·083 · Abs [225 nm] and 1·96 · Abs [300 nm].

Ash and moisture were determined according to the methodology of AOAC (1990). The crude protein content was calculated from Kjeldahl nitrogen values (N6.25). The in-vitro apparent digestibility coefficient (ADC) of the meal was estimated by a multi-enzymatic digestion with trypsin, chymotrypsin and peptidase (Hsu *et al.*, 1977). Available lysine was determined by the TNBS method (James & Ryley, 1986).

Reducing sugars were determined by the DNS method (Miller, 1959), once extracted from the defatted meal with 80% ethanol.

The free fatty acid (FFA) content in the oil, expressed as percent of oleic acid, was determined by the colorimetric method of Lowry and Tinsley (1976). The phosphorus content, measured as phospholipids in the oil, was measured by the method of Raheja *et al.* (1973). Peroxide value was measured as the cadmium iodate complex formed from the cadmium-iodide oxidised by the peroxides in the oil (AOCS, 1985).

Cellulolytic activity was measured as endo-ß-glucanase on 1% CMC (Panreac), cellobiohydrolase on 5% Avicel (Merck), and total activity on 50 mg Filter Paper (Whatman N.1). Assays were carried out in 0·1 M citric buffer at pH 4·8 at 50°C during 1 h for the latter activities and during 0·5 h for endoglucanase.

RESULTS AND DISCUSSION

Adsorption studies

The adsorption of endoglucanase and total celullolytic activity (measured in filter paper) of the enzymes used during this work revealed that the maximum values of adsorbed cellulolytic activity occurred 15–25 min after the addition of enzymes, with average values of 90% for FPase and 70–80% for CMCase. The adsorption of enzymes in concentrated solutions (as those used during this treatment) becomes difficult (Fan *et al.*, 1987); also the particle size and the lack of water-swelling to increase the available surface area could in some measure hinder adsorption during this treatment.

Selection of the operational conditions during the simultaneous enzymatic treatment–solvent extraction

For studying this process, a central composite design of experiments with six replicates in the central point, to evaluate the experimental error and the reproducibility of the experiment, was used. An additional experiment consisting in extracting untreated soya bean grits was performed and considered as reference. Two operational variables were considered: the moisture content during the enzymatic treatment, m (g water/100 g soya grits) and the enzyme to kernel ratio, r (g enzyme/100 g dry soya grits). Likewise, their effects on the oil extraction yield and the cell wall degradation were studied.

The appropriate range for each operational variable was selected by previous experience (Domínguez *et al.*, 1994*b*). Moisture was varied in a very limited range, since it was determined by the operational conditions compatible with solvent extraction (8–12%). The enzyme to seed ratio was chosen in the range of the lower values, showing significant action on the oil

Table 1. Operational conditions and experimental results obtained in the study of the enzymatic treatment of soya bean grits simultaneous with solvent extraction following a central composite design for two factors

Experiment	M	R	Moisture	E/S	F_1 (% total oil)		F_2 (OD/g meal)	
					Experimental	Calculated	Experimental	Calculated
1	−1	−1	8·5	0·25	81·96	82·39	0·03	0·13
2	−1	+1	8·5	1·25	82·34	82·99	0·04	0·24
3	+1	−1	11·5	0·25	82·06	82·20	0·47	0·45
4	+1	+1	11·5	1·25	84·33	84·69	0·95	1·04
5	0	0	10	0·75	84·20	83·97	0·55	0·54
6	0	0	10	0·75	83·86	83·97	0·44	0·54
7	0	0	10	0·75	82·77	83·97	0·35	0·54
8	1·41	0	12·1	0·75	84·19	83·99	0·95	0·94
9	−1·41	0	7·8	0·75	83·53	82·93	0·33	0·15
10	0	1·41	10	1·46	84·33	83·77	0·81	0·64
11	0	−1·41	10	0·04	81·82	81·58	0·16	0·14
12	0	0	10	0·75	83·91	83·97	0·50	0·54
13	0	0	10	0·75	84·85	83·97	0·74	0·54
14	0	0	10	0·75	84·22	83·97	0·67	0·54
Untreated[a]			—	—	79·50		0·00	

[a]Untreated: % oil extraction from soya bean grits not treated with enzymes, 79·50 ± 0·52.

extractability (0·05–1·5 g/100 g dry seeds). The coded variables were the moisture percentage during the treatment, $M = (m–10)/1·5$, and the enzyme to seed ratio, $R = (r–0·75)/0·5$.

The response for each objective function F can be described as a function of the selected factors, M and R. The empirical relation shows individual and crossed effects of each variable, in a second-order model with two first-order effects (B_M, B_R), two second order effects (B_{MM}, B_{RR}) and the interaction term (B_{MR}):

$$F_i(M, R) = B_0 + B_M \cdot M + B_R \cdot R + B_{MM} \cdot M^2 + B_{RR} \cdot R^2 + B_{MR} \cdot M \cdot R \quad (1)$$

To measure both the extent and efficiency of the enzymatic reaction, two objective functions were taken into consideration. The oil extractability at 3 h (to observe the effect of the enzymatic action), F_1 (measured as % of Soxhlet extractable oil from the ground untreated grits) and the cell wall degradation of the defatted meal, F_2 (measured as OD by the DNS method).

Previous to the experiments, we verified that the activity and thermostability of the different enzymatic activities were not affected by hexane. Also, thermal stability was considerably enhanced with respect to that in hydrated media (Sineiro *et al.*, 1994).

Table 1 lists the set of experiments expressed as dimensionless and dimensional variables corresponding with the experimental design. The values reached for the objective functions are also shown in this table. The values named as calculated were determined with coefficients in Table 2.

Table 2 shows the set of coefficients calculated by multiple regression of data and their statistical significance (based on the values determined by a t-test), as well as the mathematical parameters measuring both the correlation and the significance of the empirical models.

Table 1 shows that the oil extraction yield (F_1) varied in a very limited range. It can be inferred from the coefficients in Table 2 that the oil extractability was mainly affected by the enzyme to kernels ratio (includ-

Table 2. Regression coefficients and statistical parameters obtained for the empirical models[a]

Coefficient	F_1	F_{exp}	P-value	F_2	F_{exp}	P-value
(a) Regression coefficients						
B_0	83·97	—	—	0·54	—	—
B_M	0·38	2·36	0·16	0·28	21·55	<0·01
B_R	0·77	9·92	0·01	0·17	8·68	0·02
B_{MM}	−0·25	0·63	0·45	0·00	0·01	0·92
B_{RR}	−0·64	6·35	0·03	−0·08	1·53	0·25
B_{MR}	0·47	1·84	0·21	0·12	1·91	0·20

(b) Statistical parameters				
Objective function	R^2	Corr R^2	F_{exp}	Prob. ($F_{exp} > F_{st}$)
F_1	0·7251	0·5533	4·22	<0·04
F_2	0·8081	0·6881	6·74	<0·01

[a]F_{exp} defined as the ratio between the mean squares of model and error. F_{st} defined as the statistical value of F for the degrees of freedom of model and error.

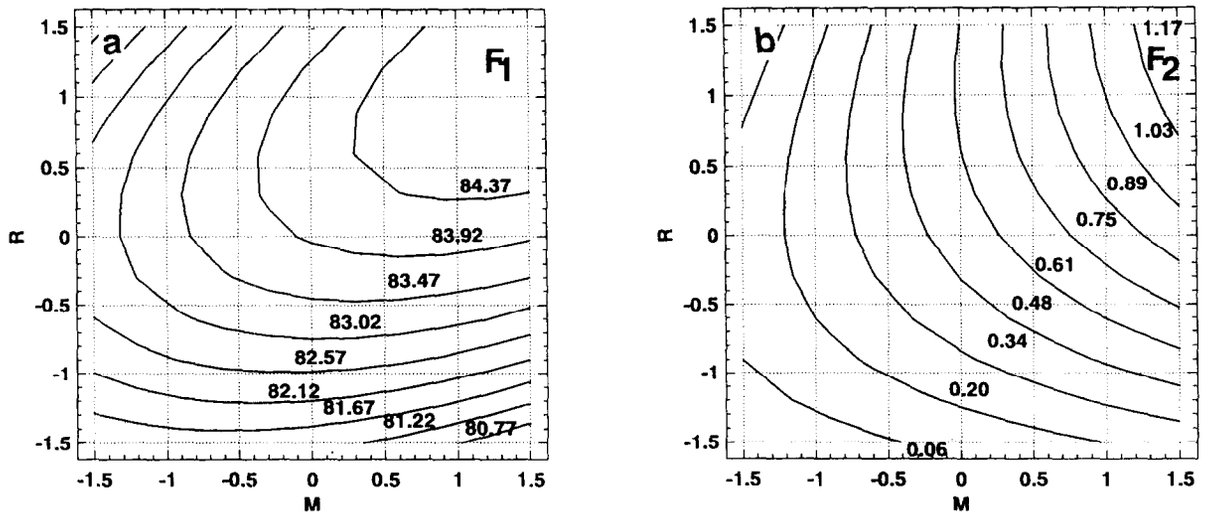

Fig. 1. Predicted dependence of (a) F_1, oil extraction yield, and (b) F_2, cell wall degradation of the defatted meal on the moisture content and the enzyme to seed ratio during the simultaneous enzymatic treatment with solvent extraction of soya bean grits.

ing linear and second-order terms, both significant at 99% and 97% confidence level, respectively), being the favourable influence of the moisture content, in the range studied, statistically non-significant.

Figure 1(a) shows the predicted dependence of the oil extraction yield on the enzyme to seed ratio and moisture content. When no enzyme or low enzyme to seed ratios were used, the oil extraction was slightly favoured operating at reduced moisture percentages, as this variable has a crucial effect on the solvent extraction (Karlovic *et al.*, 1992). The oil extractability was progressively enhanced as the enzyme to seed ratio increased, regardless of the moisture during the treatment. With lower moisture percentages, an increase in the enzyme to seed ratio does not have a favourable repercussion on the oil extraction yield. This fact could be explained by the unfavourable conditions for the enzymatic action, where the extension of the reaction after relatively short periods of hydrolysis and the consequent effect on the extractability are actually low.

The experimental values (Table 1) for the cell wall degradation (F_2) indicate that this function was strongly dependent on the operational conditions. The extreme values were obtained in experiments 1 and 4, performed with the minimum and the maximum values of both assayed variables, respectively. This function is significantly affected by M and R as can be deduced from the respective coefficients. The moisture content presents a more marked influence. The terms involving both the interaction and second-order terms are not significant and only slightly influence the objective functions. Figure 1(b) shows the predictions of the empirical model for the dependence of the cell wall degradation on the moisture content and the enzyme to seed ratio.

From these results, the more suitable values for both variables during the enzymatic treatment simultaneous with hexane extraction were the higher enzyme to seed ratio and the higher moisture studied in the interval (1·25–1·5 g enzyme/100 g dry seed and 11·5–12% moisture), always in order to attain higher enzyme efficiency.

Product characteristics

Under the above optimum conditions, the enzymatic treatment was prolonged during 6 h in the simultaneous hydrolysis–extraction process. The properties of oil and meal obtained from untreated samples (directly solvent-extracted), treated samples (the enzyme solution was added after a previous drying) and control samples (a volume of water equivalent to the enzymatic solution was added and incubated as those treated with enzymes) were evaluated.

Oil

The hexane–oil miscella was vacuum evaporated at 40–50°C to remove the solvent until no characteristic odour was detected. The crude oil obtained was analysed for some quality indices to compare the effect of the enzymatic treatment (Table 3). FFA values overpass the maximum value of 0·75 proposed for physical

Table 3. Characteristics of the crude oil from soya beans treated and untreated with enzymes during extraction with hexane[a]

Characteristics	Untreated	Treated	
		Control	Enzyme
Oil			
FFA (% oleic)	0·59a	0·78b	0·82b
Phosphorus (ppm P/g oil)	44a	124b	151a
Peroxide value (meq/kg)	3·89a	3·77a	3·43a
Colour			
420 nm	0·43a	0·60b	0·40a
453 nm	0·53a	0·63b	0·47a
Meal			
Ash (%, db)	6·35a	6·66a	6·45a
Reducing sugars (%, db)	0·40a	0·49a	0·71b
In-vitro ADC (%)	75·6a	75·0a	77·6b
Available lysine (mg/16 N)	4·99a	—	4·92a

[a]Means within rows with the same following letter are not significantly different (5% level).

refining. However, values are in the range of those of crude commercial soya bean oils, from 0·26 to 1·33%.

The low phosphorus values, as compared to the 500–700 ppm of the crude commercial oils, may be attributed to the 0·75–1 mm grits which do not allow all the fat fractions to be extracted in the time interval studied, especially the last ones where the phospholipid content is higher. Likewise, the absence of a thermal treatment before extraction could explain these low values. An increase in the phosphorus content in enzyme-treated samples with respect to untreated was registered; perhaps this increase was produced by the moist thermal treatment prior to oil extraction to inactivate endogenous enzymes such as lipoxygenase or lipases. However, this fact was also found in the control samples, probably caused by the addition of water, although the temperatures are lower than those used during the thermal conditioning (80–90°C) (Koch, 1981; Ong, 1981).

Peroxide value was not significantly different for the treated samples, remaining under 10 meq/kg, the highest value allowed to remain within the standard limits. The colour of the crude oil, measured in an 1:1 oil to hexane solution at 420 and 453 nm with hexane as reference, did not differ significantly in samples enzymatically treated or untreated.

Meal

The increase in the reducing sugars in the meal (0·31%, equivalent to 4 mg/g dry defatted basis) was lower than that obtained at higher water contents (40–60%) (12 mg/g ddb) (Domínguez *et al.*, 1993). This fact was also observed with artificial substrates, endoglucanase activity at low moisture being 11·5% of that obtained in standard conditions, Avicelase slightly over 7% and FPase hardly 4%. As the mechanism of cellulose hydrolysis takes place with a depolymerisation in the surface and a posterior rupture of the cellobiose units in glucose in the liquid phase, the low reducing sugars production can be anticipated. This reduced hydrolysis rate could be explained by the inhibition by-product to which enzyme adsorbs, thus precluding its diffusion towards other sites where the reaction could take place.

A slight but statistically significant increase (3%) in the in-vitro digestibility of the meal from treated samples over that from untreated meal was observed. Although it has not been checked, this increase could be ascribed to the use of cellulolytic and related activities, that frequently result in freeing of the proteins from the polysaccharide matrix in which they may be entrapped, especially in the protein bodies of the aleurone cells of seeds (Schwimmer, 1980). Walsh *et al.* (1993) reported that the incorporation of cellulolytic activities in monogastric feed can increase the availability of the simple sugars released from cellulolytic substrates.

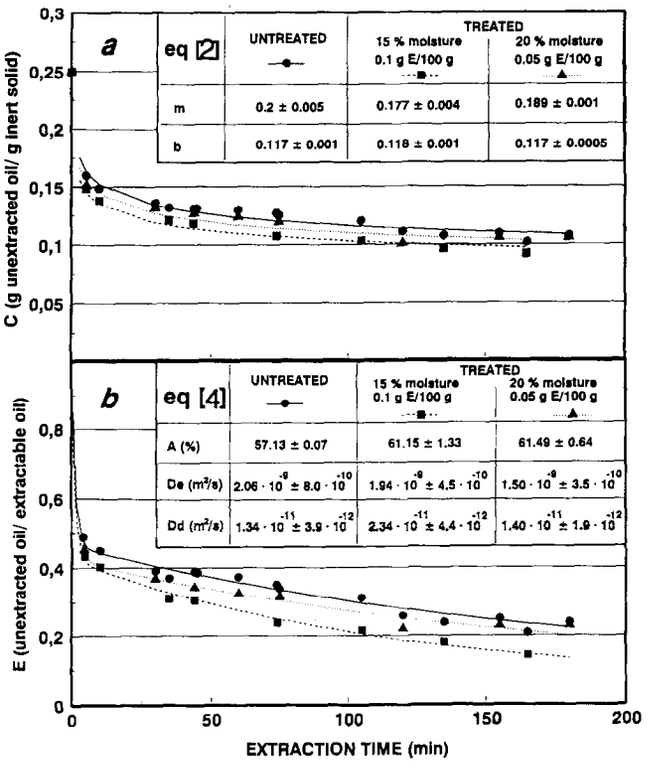

Fig. 2. Fraction of unextracted oil as a function of time for soya bean grits. Curves are fitted to (a) eqns (2), and (b) eqn (4).

Enzymatic treatment prior to the solvent extraction: effect on the kinetics

The application of the enzymatic treatment prior to the solvent extraction gave extraction times more akin to industrial extraction times. Two kinds of experiments were carried out. In the first series (i) where the oil concentration in the solvent was constant and almost zero (in fact the maximum attainable oil concentration in the miscella is 1% w/w), the objective was analysing the effect of the enzyme on the wall degradation, which, in turn, influences extraction rate. In the second series of experiments (ii), the possible reduction of operation time was evaluated.

Effect of the enzymatic treatment on the oil removal

Extraction data from untreated grits were compared to those from samples treated at 15% moisture with 0·1 g enzyme/100 g grits and at 20% moisture with 0·05 g enzyme/100 g grits during 3 h, and dried before extraction in Erlenmeyer flasks at 30°C.

C (g unextracted oil/g inert solid) was plotted versus t (min), according to eqn (2) (Othmer & Jaatinen, 1959):

$$C = m \cdot t^{-b} \qquad (2)$$

The constant m, which depends on the particle size, was slightly lower for the treated samples (Fig. 2(a)), indicating an enhanced oil extractability in those samples.

The mechanism of vegetable oil extraction depends largely on diffusion (Nieh & Snyder, 1991). Assuming that extraction takes place purely by diffusion, since the resistance of the external liquid film is negligible compared to the internal resistance in the pores and applying the Fick's law, if spherical geometry is supposed for soya grits, the mathematical problem can be solved as

for heat conduction in a sphere of radius R. The fraction of extractable oil left unextracted at time t (E) can be expressed as a function of a series of terms depending on the diffusivities, D (Bird *et al.*, 1975); E can be calculated as

$$E = \frac{C-C_e}{C_i-C_e} = \frac{C_{M\infty}-C_M}{C_{M\infty}} \quad (3)$$

where C is the residual oil in the solid at time t, C_e is the equilibrium oil (value at 15 h); C_i is the initial oil content, C_M is the oil content in the miscella at time t and $C_{M\infty}$ is the equilibrium oil content in the miscella (value at 15 h: 0·62–0·7 g oil/100 ml miscella). Since the series converges rapidly the first terms are sufficient for practical calculations:

It is possible to resolve the curve for E into two theoretical curves, each corresponding to a constant diffusion coefficient on the assumption that there are two fractions in the material being extracted, with different extractabilities:

$$E = A * E_{\text{easy}} + (1-A) * E_{\text{difficult}} \quad (4)$$

where A is the fraction of easily extractable oil, and E is a function of the diffusivity, D.

Osburn and Katz (1944) found that the extraction curves for the oil from soya bean flakes might be explained on the basis of the existence of an oil fraction easily extractable and a small amount that extracts more slowly. Although flaking facilitates the extraction of oil which in larger grits is unextractable, the curves for grits (higher than 0·24 mm) and flakes have the same character (Karnofsky, 1949).

The mechanical treatment leads to a fraction with easily accessible oil and another more difficult to extract, which could be modelled with the two diffusion coefficients, D_e and D_d. The curves obtained for soya bean grits were fitted in order to evaluate the effect of the enzymatic treatment on the relative importance of these fractions. The 'easy' fraction was hardly 60%, the diffusion coefficient for this fraction being about 100 times higher than the coefficient of the 'difficult' fraction (Fig. 2(b)). The application of the enzymatic treatment led to a slightly higher fraction of easily extractable oil, because enzymes cause additional breaking of the cell wall structure, enhancing the liberation of oil. Osburn and Katz (1944), working with flakes instead of grits, found that the coefficient of the 'easy' fraction was only 10 times higher than that of the 'difficult' fraction. Moreover, in flakes the fraction of unextractable oil is lower than in grits due to the intense disruption caused by the flaking process. The 'difficult' fraction is considerably smaller than the 'easy' fraction, the difference between the diffusion coefficients being also lower than the values found for grits, probably due to the more homogeneous cell wall distortion.

Reduction in the operation time

To evaluate this reduction, the extraction of grits previously treated with enzyme at 15% moisture was performed at 50°C in the vertical extractor. To simulate the use of an oil-enriched miscella, the equipment was initially operated by percolation and then by immersion. In the previous section an enhanced oil-extractability was

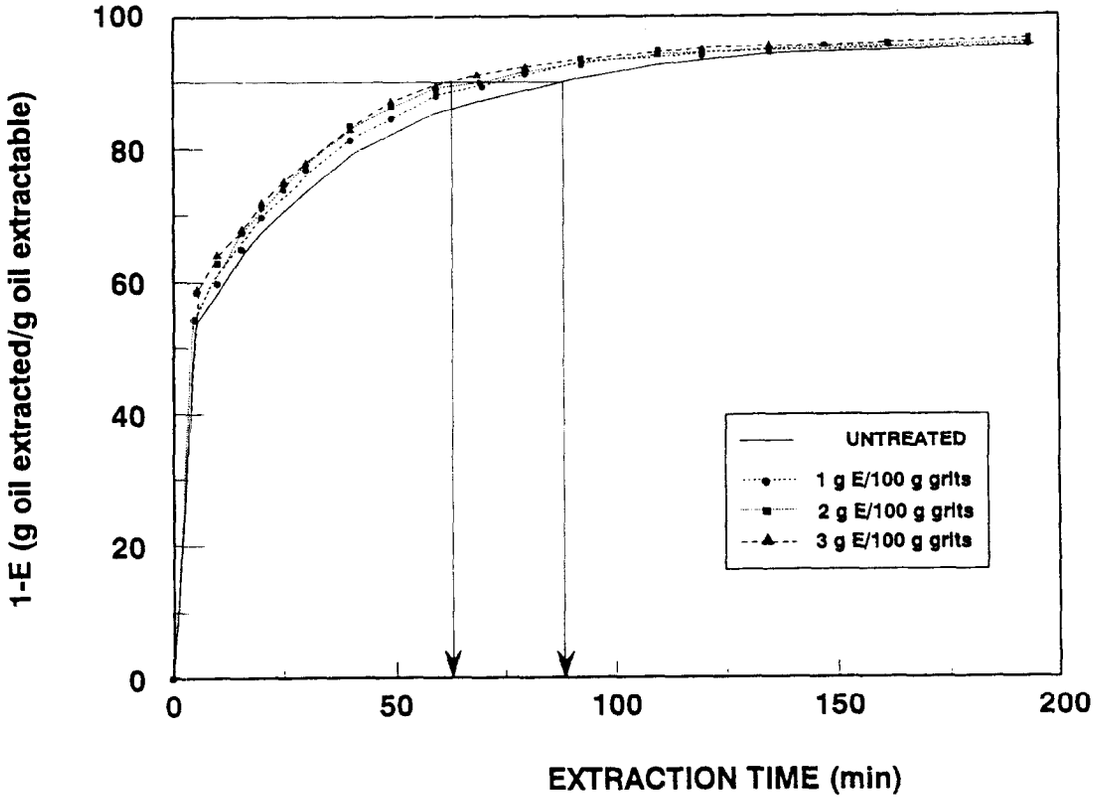

Fig. 3. Fraction of extractable oil extracted at 50°C in the vertical extractor as a function of time for soya bean enzymatically treated with 1–3 g enzyme/100 g seeds as compared to untreated samples.

observed if the enzymatic treatment was performed with 0·05–0·1 g enzyme/100 g seeds, at 15 and at 20% moisture when the extraction was done at under-optimal temperature conditions (30°C). However, higher enzyme to seed ratios (1–3 g/100 g seeds) had to be used during the enzymatic treatment of smaller grits (0·4–0·75 mm) to notice the effect at 50°C.

The extraction times to attain 90% extraction of the total extractable oil under these operational conditions were compared; whereas for untreated samples 90 min were required, in treated samples this was reduced to 60–70 min (Fig. 3). From the operational point of view this reduction in the extraction time would yield important benefits in either higher processing capacity or reduced extractor volume.

An effect of the enzyme concentration on the extraction rate was noted, enzyme to seed ratios of 2 and 3 g enzyme/100 g seeds being superior to 1 g/100 g. No significant improvement was observed with higher concentrations.

In conclusion, the proposed scheme is adequate for the application of an enzymatic treatment under low moisture conditions to enhance oil extractability, and is compatible with conventional solvent extraction. During work, the operational conditions (particle size, extraction temperature, solvent to seed ratio) were fixed at under-optimal values to observe the effect of the enzymatic treatment, but additional studies are required to see whether significant benefits could be attained during a continuous countercurrent process.

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REFERENCES

Alba, M. J., Ruiz, G. A., Prieto, G. C. & Gutiérrez, R. F. (1987). Eficacia de la formulación enzimática en la tecnología del aceite de oliva. Composición y valoración organoléptica de los aceites obtenidos. *Grasas y Aceites*, **38**, 271–7.

AOAC (1990). *Official Methods of Analysis of the Association of Official Analytical Chemists* (15th edn), ed. K. Helrich. AOAC, Arlington, VA, USA.

AOCS (1985). *Official Tentative Methods of the American Oil Chemists' Society*, ed. R. G. Walker. American Oil Chemists' Society, Champaign, IL, USA, Method Cd 8-53.

Badr, F. H. & Sithohy, M. Z. (1992). Optimizing conditions for enzymatic extraction of sunflower oil. *Grasas y Aceites*, **43**, 281–3.

Bernardini, E. (1983). *Oilseeds, Oils and Fats*. B. E. Oil, Publishing House, Rome, Italy.

Bird, R. B., Stewart, W. E. & Lightfoot, E. N. (1975). *Fenómenos de Transporte*. Reverté, Barcelona, Spain.

Buenrostro, M. & López-Munguía, C. A. (1986). Enzymatic extraction of avocado oil. *Biotechnol. Lett.*, **8**, 505–6.

Christenson, F. M. & Olsen, H. A. S. (1988). Method for production of an upgraded coconut product. UK Pat. Appl. GB-A-2115820 (Novo Industri A/S).

Cintra, M. O., López-Munguía, C. A. & Vernon, C. J. (1986). Coconut oil extraction by a new enzymatic process. *J. Food Sci.*, **51**, 695–7.

Domínguez, H., Núñez, M. J. & Lema, J. M. (1993). Oil extractability from enzymatically treated soya bean and sunflower: range of operational variables. *Food Chem.*, **46**, 277–84.

Domínguez, H., Núñez, M. J. & Lema, J. M. (1994*a*). Enzymatic pretreatment to enhance oil extraction from fruits and oilseeds: a review. *Food Chem.*, **49**, 271–86.

Domínguez, H., Núñez, M. J. & Lema, J. M. (1994*b*). New strategies for the oil extraction process of soya beans coupled with enzymatic treatment. *Afinidad*, **51**, 17–23.

Fan, H. P., Morris, J. C. & Wakeman, H. (1948). Diffusion phenomena in solvent extraction of peanut oil. *Ind. Engng Chem.*, **40**, 195–9.

Fan, L. T., Gharpuray, M. M. & Lee, Y.-H. (1987). *Cellulose Hydrolysis*. Springer-Verlag, Berlin, Germany.

Fullbrook, P. D. (1983). The use of enzymes in the processing of oilseeds. *J. Am. Oil Chem. Soc.*, **60**, 476–8.

García Serrato, A. (1981). Extraction of oil from soybeans. *J. Am. Oil Chem. Soc.*, **58**, 157–9.

Hsu, H. W., Vavak, D., Satterlee, L. D. & Miller G. A. (1977). A multienzyme technique for estimating protein digestibility. *J. Food Sci.*, **42**, 1269–73.

James, N. A. & Ryley, J. (1986). The rapid determination of chemically reactive lysine in the presence of carbohidrates by modified trinitrobenzensulphonic acid procedure. *J. Sci. Food Agric.*, **37**, 151–6.

Karlovic, D., Sovilj, M. & Turkulov, J. (1992). Kinetics of oil extraction from corn germ. *J. Am. Oil Chem. Soc.*, **69**, 471–6.

Karnofsky, G. (1949). The theory of solvent extraction. *J. Am. Oil Chem. Soc.*, **26**, 564–9.

Koch, M. (1981). Production of an edible oil from crude soy oil. US Patent 4,255,346.

Laiho, S., Tulisao, U., Oksanen, H. & Nyström, R. (1990). A process for the production of a vegetable-oil product. WO-A-91/13956 (Öljynpuristamo OY).

Lowry, R. R. & Tinsley, I. J. (1976). Rapid colorimetric determination of free fatty acids. *J. Am. Oil Chem. Soc.*, **53**, 470–2.

Marek, E., Schalinatus, E., Weigelt, E., Mieth, G., Kerns, G. & Kude, J. (1990). On the application of enzymes in the production of vegetable oil. *Prog. Biotechnol.*, **6**, 471–4.

Miller, G. L. (1959). Use of DNS reagent for determination of reducing sugars. *Anal. Chem.*, **31**, 426–8.

Nieh, C. D. & Snyder, H. E. (1991). Solvent extraction of oil from soybean flour I. Extraction rate, a countercurrent extraction system, and oil quality. *J. Am. Oil Chem. Soc.*, **68**, 246–9.

Olsen, H. S. (1988). Aqueous enzymatic extraction of oil from seeds. Presented at Asean Food Conference '88. 24–26 October, Bangkok, Thailand. (Novo A-06041a/HSO).

Ong, J. T. L. (1981). The impact of the preparation conditions of soybeans on the oil quality and yield. Proc. of the 2nd Symp. of ASA on Soybean Processing (Antwerpen, Netherlands).

Osburn, J. O. & Katz, D. L. (1944). Structure as a variable in the application of diffusion theory to extraction. *Trans. AIChE*, **40**, 511–31.

Othmer, D. F. & Jaatinen, W. A. (1959). Extraction of soybeans. *Ind. Engng Chem.*, **51**, 543–6.

Raheja, R. K., Kaur, C., Singh, A. & Bathia, I. S. (1973). New colorimetric method for quantitative estimation of phospholipids without acid digestion. *J. Lipid Res.*, **14**, 695–7.

Schwimmer, S. (1980). Enzyme action in protein modification and health-associated aspects of food-quality. In *Source Book of Food Enzymology*. The AVI Publishing Company Inc., Wesport, CT, USA.

Sineiro, J., Domínguez, H., Núñez, M. J. & Lema, J. M. (1994). Hydrolysis of microcrystaline cellulose by cellu-

lolytic complex of *Trichoderma reesei* in low moisture media. *Enzyme Microb. Technol.* (in press).

Smith, A. S. (1952). Evaluation of extraction rate measurements. *J. Am. Oil Chem. Soc.*, **29**, 421–5.

Smith, D. D., Agrawal, Y. C., Sarkar, B. C. & Singh, B. P. N. (1993). Enzymatic hydrolysis pretreatment for mechanical expelling of soybeans. *J. Am. Oil Chem. Soc.*, **70**, 885–90.

Sosulski, K. & Sosulski, F. W. (1990). Quality of oil and meal from enzyme treated canola seeds. *Proc. Can. Inst. Food Sci. Technol.*, **3**, 656.

Sosulski, K., Sosulski, F. W. & Coxworth, E. (1988). Carbohydrase hydrolysis of canola to enhance oil extraction with hexane. *J. Am. Oil Chem. Soc.*, **65**, 357–61.

Walsh, G. A., Power, R. F. & Headon, D. R. (1993). Enzymes in the animal-feed industry. *Tibtech*, **11**, 424–30.

Wiese, K. L. & Snyder, H. E. (1987). Analysis of the oil extraction process in soybeans: A new continuous procedure. *J. Am. Oil Chem. Soc.*, **64**, 402–6.