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Oil extractability from enzymatically treated soybean and sunflower: range of operational variables

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An evaluation of the feasibility of enzymatically treating oil seeds (soya beans and sunflower kernels) to improve the extractability of oil is presented. The effects of each variable affecting the process (mechanical treatment, moisture percentage, enzyme concentration and time of hydrolysis) were studied to define the optimum range of operation for each of them as a function of the extractability of samples treated with different enzyme formulations.
Size reduction of the seeds favours the efficiency of the enzymatic treatment related to oil extractability. For a fixed particle size, moisture contents of 60 and 40% selected for soybean and sunflower respectively, regardless of the kind of enzyme, are suitable to efficiently increase the extractability of the seed. The enzyme/seed ratio is dependent on the formulation. Time of hydrolysis must also be optimized to avoid long reaction times which do not result in notable increases in the amount of recovered oil.

INTRODUCTION

Vegetable cells of some fruits and seeds accumulate oil in intracellular vacuoles, so the extraction of oil could be enhanced by the hydrolytic action of carbohydrates, which acting on glucans of the cell wall, would favour liberation of the oil. Depending on the nature of the seeds or fruits the characteristics and definition of the process are different. Application of an enzymatic treatment requires a specific strategy for each case and the values of implied variables will depend on the process under study.

Oil extraction processes from oilseeds (rapeseed, sunflower and soybean) are done through different stages, with a primary thermal and mechanical treatment followed by an extraction step with organic solvents. Solvent extraction can sometimes be avoided due to the efficiency reached during the pressing stage (Sosulski & Sosulski, 1990). Together with these conventional processes there is an aqueous process, safe and ecological, already applied to soybean and rapeseed oil extraction (Fullbrook, 1983).

Improvement of the extractability of vegetable oils from fruits and seeds enzymatically treated has been confirmed (Soroa, 1967), although complete studies on the subject are scarce. Optimum conditions for hydrolysis and characterization of the resulting products have been reported for some seeds such as rapeseed (Sosulski *et al.*, 1988) and fruits such as coconut (Cintra *et al.*, 1986) and olives (Montedoro & Petruccioli, 1974; Santos Antunes, 1978; Alba *et al.*, 1990), these assays being performed both on lab and pilot or industrial scale.

Some previous studies dealt with the application of the enzymatic treatment for oil extraction from canola (Sosulski *et al.*, 1988), peanuts, sunflower (Lanzani *et al.*, 1975) and soybean (Fullbrook, 1983). Enzymes to enhance extractability of the oil seeds vary in efficiency, but formulations with mixed activities have proved to be very effective (Sosulski *et al.*, 1988; Domínguez *et al.*, 1991).

The main variables affecting the hydrolytic process should be particle size, moisture, hydrolysis time and enzyme/seed ratio. The nature of enzymatic formulation and the enzyme/seed ratio determine the optimum hydrolysis time. The effects of these variables on the extractability of oil from soya and sunflower were studied in our work. For this purpose an experimental procedure, allowing us to discern the action of the enzymes on the degree of extractability of the samples was defined and performed.

MATERIALS AND METHODS

Materials

Seeds

Soybean seeds, Amsoy No2 cultivar, and sunflower kernels KE-474 employed for this research were kindly

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Table 1. Enzymatic formulations selected for the enzymatic treatment of soya beans and sunflower kernels

Commercial enzyme	Manufacturer	Source	Main activity
*Enzeco hemicellulase	Enzyme Development Corporation	*Aspergillus niger*	Hemicellulase
* o Celluclast 1·5L	Novo Nordisk A/S	*Trichoderma reesei*	Cellulase
* o Multifect™	Finnish Sugar Co. Ltd	*Trichoderma longibrachiantum*	Cellulase, hemicellulase
o Olease	Biocon (US) Inc..		Multiactivity
*o Pectinex	Novo Nordisk A/S	*Aspergillus niger*	Polygalacturonase Pectinesterase pectintranseliminase
o Röhament P	Röhn Enzymes		Pectinase
* Röhapect B	Röhn Enzymes		Pectinase

supplied by Cereol Ibérica. Samples of these seeds, cut in grits (Soybean) and as the whole kernel or cut in halves (sunflower), were treated with commercial enzymes.

Enzymes

Enzymatic formulations were selected after several runs of comparative assays among enzymes of different activities: amylases, glucanases, cellulases, hemicellulases, pectinases, proteases and multiactivity complexes, all of them commercially available. These experiments were carried out at the Saskatchewan Research Council (Domínguez *et al.*, 1991). Table 1 summarizes the selected enzymes for the treatment of soya beans (*) and sunflower kernels (o).

Commercial enzymes can be applied as such or blended with others. So, for our experiments all the formulations, as well as a combination Cellulast 1·5 L + Pectinex ULTRA SP were used.

Methods

The experimental procedures are slightly dependent on the seed (Fig. 1). Commercially, soybean is solvent-extracted after mechanical and thermal conditioning; hulls can be either eliminated or mixed back with the high protein content meal to produce a meal with lower protein content. In our case, soya beans were mechanically conditioned (cracked, dehulled and sieved) and mixed with the diluted enzyme solution, which was applied as homogeneously as possible to the seeds. The dilution employed depends on the final moisture desired.

Dehulling of sunflower permits an increase in the capacity of the industrial plant (about 20%) resulting in considerable sparing in energy, and low wear of the equipment, especially the press. Furthermore, it allows higher oil yields and lower solvent wastes, leaving a higher protein content in the meal. The high oil cultivars are more difficult to dehull efficiently. Additional problems are related with the low density of hulls (about 127·9 kg/m^3), which makes storage more expensive, and with low commercial value; usually hull is used as fuel in the same factory, its heat of combustion being about 18·8 × 10^6 J/kg) (Ward, 1984; Signoret & Evrard, 1987). After dehulling, the whole or transversally cut kernels were enzymatically pretreated. That operational scheme does not alter the conventional process significantly; in this latter the kernels are not only whole, but also in pieces, as a result of the dehulling process.

The hydrolytic process takes place at a temperature in the range of 45–55°C for a period of 3–12 h. This

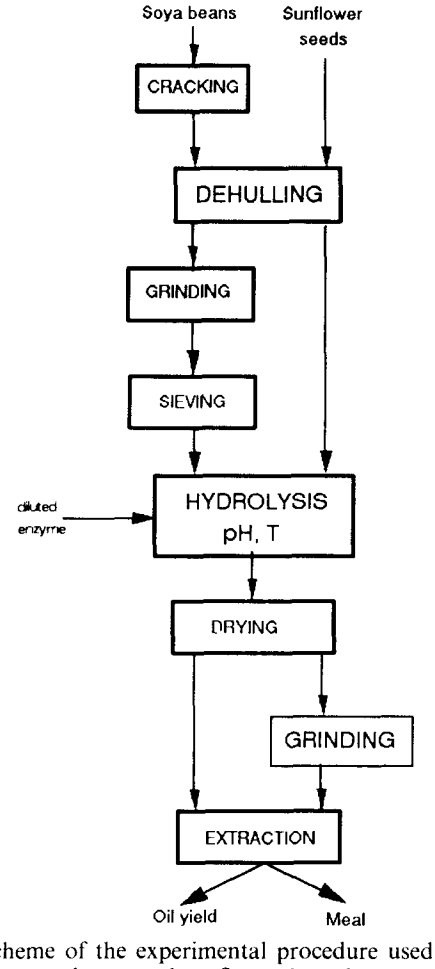

Fig. 1. Scheme of the experimental procedure used for soya beans and sunflower kernels.

range was chosen as a function of the enzyme activity (see enzyme activity below), the control samples (without enzyme) being incubated always at 50°C.

To compare the extractability of the different samples they were extracted (in Soxhlet) for 6 h for soybean samples and 8 h for sunflower, with commercial hexane, the usual solvent in industrial processes. Finally the seeds are dried in an air oven (60–70°C) up to their desired final moisture (8–10% for soya bean and 4–6% for sunflower). The percentage of recovered oil determined by weight after removing the solvent from the extract, allows us to compare the efficiency of the enzymatic treatment at the several operating conditions.

Analyses

Seed samples were analysed by the Standard AOAC (Helrich, 1990) procedures for moisture (air-oven method) and oil content (Soxhlet extraction with hexane as solvent) (AOAC Methods). Enzyme activity assays in formulations of cellulolytic activity were conducted on Filter Paper (Whatman No. 1) (Mandels *et al.*, 1976). Reducing sugars from the ground defatted samples were measured by DNS method after extraction with hot water (50°C) in a suspension 1/100 (w/v); then liquid samples were centrifuged until a clear supernatant was obtained.

RESULTS AND DISCUSSION

Experiments were carried out mainly to pre-evaluate the feasibility and interest of the enzymatic treatment of oilseeds and select the operational range of the most important variables. Several assays using the more adequate formulations found in previous experiments were carried out. The effects of moisture, enzyme : seed ratio and time of hydrolysis for a fixed particle size will allow us to define the operational characteristics of the process.

Enzymatic activity

The selected pH was 6·6 corresponding to the natural value of the seeds suspension. The activity of the enzymes and mixtures of enzymes strongly depends on the temperature as shown in Fig. 2.

For cellulolytic activity of the formulations (measured on filter paper) the most suitable temperatures are 50°C for a mixed activity complex like Multifect™ and Olease, 55°C for a cellulolytic enzyme, Celluclast and 40–45°C for the Enzeco Hemicellulase. Pectinex U. SP and Röhapect B present low cellulasic activity, their maximum pectinase activity being 40–50°C. The operating temperature in the experiments was that corresponding to the maximum activity of each enzymatic complex. For the mixture (2 : 1) of Celluclast and Pectinex U. SP, an intermediate temperature between the two individual optima, 50°C, was maintained. Control samples were incubated at 50°C.

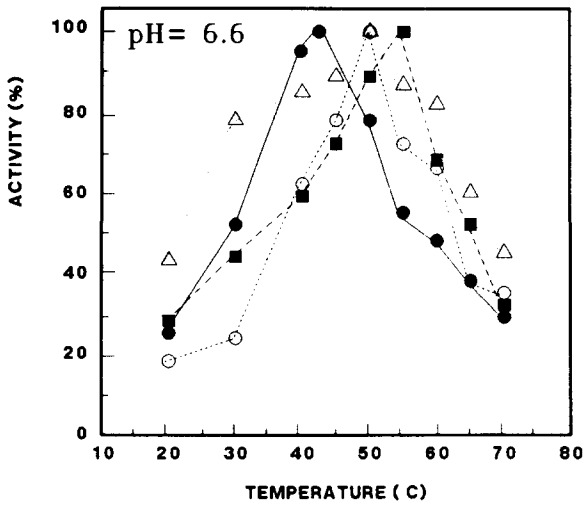

Fig. 2. Effect of temperature of hydrolysis on the cellulolytic activity of the formulations at the natural pH of the seeds: (Δ) Multifect™; (○) Olease; (■) Celluclast 1·5 L; (●) Enzeco Hemicellulase.

Soybean

In this section we separately determine the effect of each considered variable, keeping the others constant at the most favourable value found in the previous experiments.

Grit size

The effect of particle size on extractability, determined as the percentage recovered oil by Soxhlet over the total extractable oil, was studied. Soya beans, cut in grits, were treated with one of the selected enzymatic preparations (Multifect™). The favourable effect of a size reduction of the seeds on the amount of obtained oil can be observed in Fig. 3, where extractability increases more than twice when size becomes reduced to one quarter.

Furthermore, the effect of the enzyme is more evident at the lower sizes, indicating that a better accessibility of enzyme to the cell wall is achieved. The remaining experiments were performed using grits of 0·75–1 mm, as this

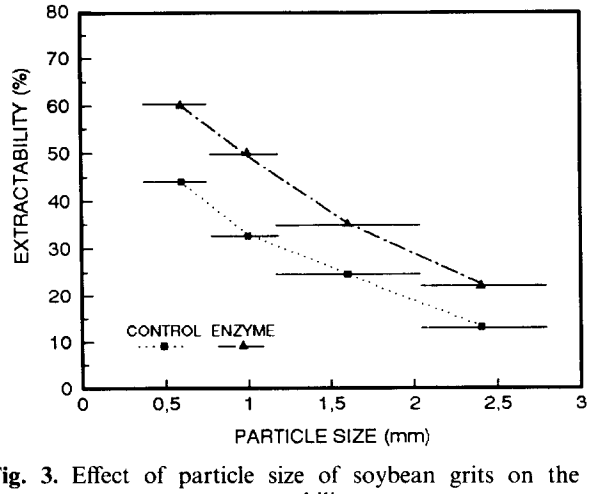

Fig. 3. Effect of particle size of soybean grits on the oil extractability.

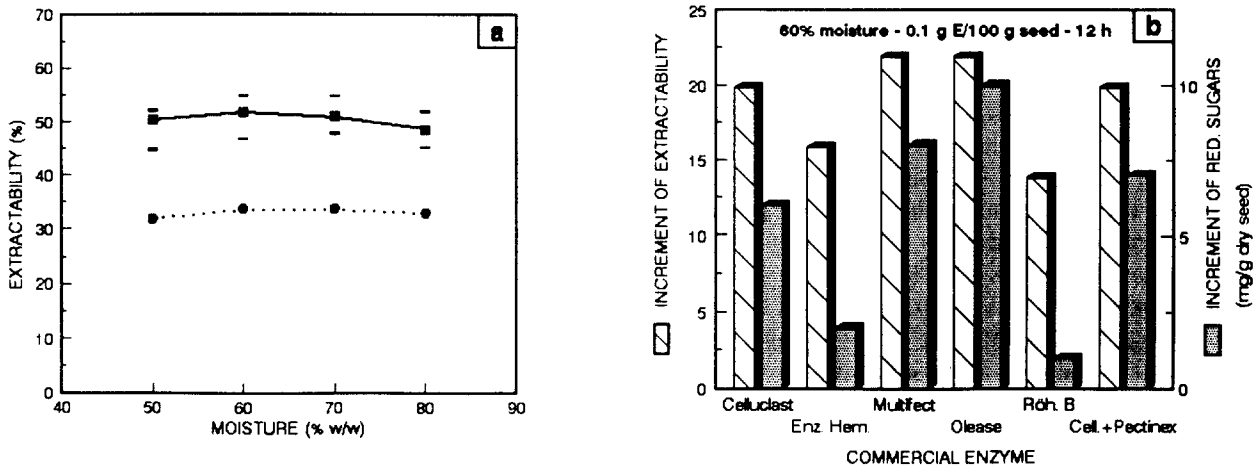

Fig. 4(a). Effect of moisture on the extractability of oil from soybean grits treated with enzymes. The dotted line represents the control samples; squares are the standards average of all the assayed enzymes; hyphens correspond with the highest and lowest extractability values. (b) The effect of the different commercial enzymes on oil extractability and reducing sugar production during the enzymatic treatment of semi-dehulled soybean pieces of 0·75–1 mm (16–22 mesh).

range of sizes presents a better mechanical resistance than smaller ones, which tend to stick together and lose their identity during the following drying process.

Moisture (% w/w)

In order to determine the effect of the water percentage on the enzymatic activity, several experiments at moistures ranging from 50 to 80% were conducted. An enzyme : seed ratio of 0·1 g of enzyme/100 g seed (dry weight) and a treatment period of 12 h were employed in these experiments.

All formulations show a similar behaviour as regards the influence of moisture on extractability, the apparent maximum being in the range of 50–70% (Fig. 4(a)), so the effect of moisture during treatment in the process affects more than the kind of used formulations. The selected moisture value was 60%, which was employed in the following experiments. Fifty percent would be the minimum value needed to moisten the whole sample. Higher values than 70% make the soybean pieces lose consistency, mainly during drying, causing disaggregation of the particles, as well as undesirable odours after prolonged period of treatment.

In Fig. 4(b) increments (over the control) of percentage of extractability are plotted for the different enzyme formulations. Mixed activity enzymes or those with high cellulolytic activity appear to exert a more favourable action on the extractability of oil. The same figure also shows the net production of reducing sugars, due to the action of the different enzymes. There seems to exist a relationship between the increment of the extractability and the partial breakdown of the cellular wall, measured as reducing sugars in the defatted meal. So pectinase and hemicellulase activities give the lower increase on extractability as well as on reducing sugar production.

Enzyme : seed ratio (w/w)

It is important to determine whether a higher enzyme concentration could exert a more efficient action and in this case, to define the range in which maximum activity is observed. For this purpose, seeds were

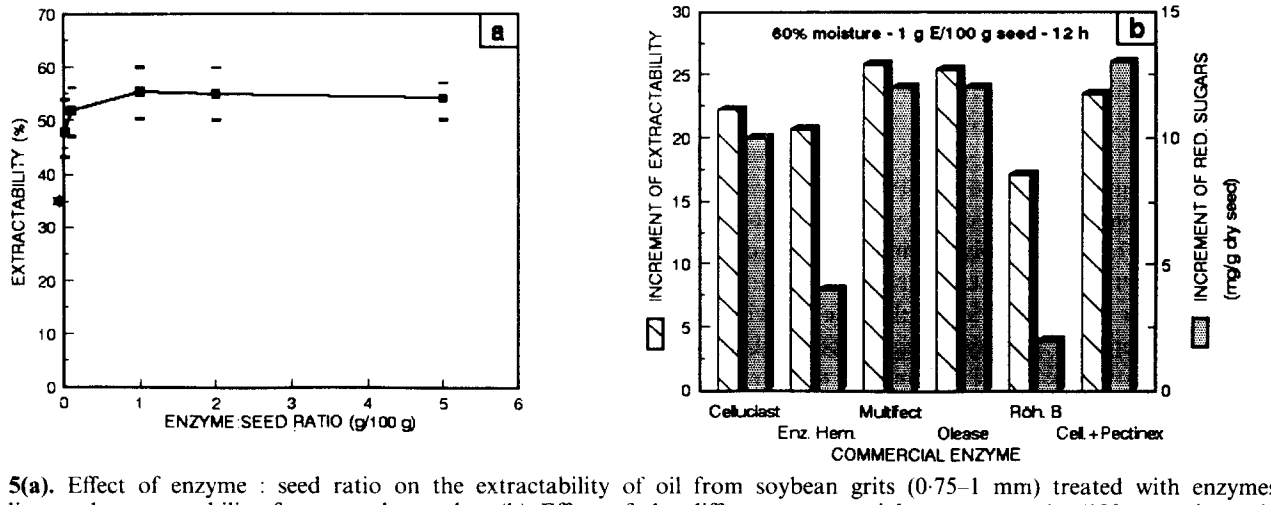

Fig. 5(a). Effect of enzyme : seed ratio on the extractability of oil from soybean grits (0·75–1 mm) treated with enzymes. * indicates the extractability for control samples. (b) Effect of the different commercial enzymes at 1 g/100 g seed on the increment of oil extractability and reducing sugars.

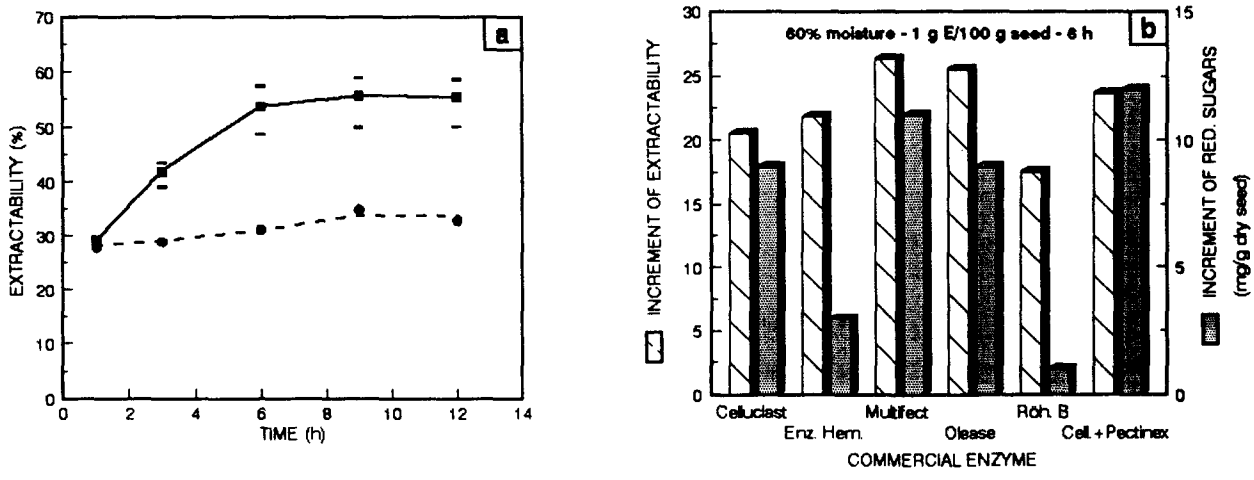

Fig. 6(a). Effect of the length of treatment on the extractability of oil from soybean grits treated with enzymes. (b) Effect of the different commercial enzymes on the increment of oil extractability and reducing sugars production after 6 h of enzymatic treatment.

treated for a period of 12 h with the enzymatic preparations properly diluted to keep a final moisture of 60% at five enzyme : seed ratios 0·01, 0·1, 1, 2 and 5 g/100 g seed.

Figure 5(a) shows the effect of this variable. The enzymatic formulation sensitively affects the extractability of the seeds, giving a range of obtained values wider than in moisture assays; this is due to the different enzymatic activities of the enzymes. Even the lower enzyme : seed ratios have a marked effect on the extractability of oil, only slightly improved by higher concentrations of enzyme up to 1 g/100 g seeds. The global effect on the oil extractability remains almost unaffected when this ratio is raised from 1 to 5.

By comparing Figs 5(b) and 4(b), the change in the efficiency of the extractability, when the enzyme : seed ratio is increased from 0·1 to 1 g/100 g seed, can be observed. Improvements in the order of 2·5–3·5 are obtained, being even higher in the treatment with the hemicellulase. Two reasons can be adduced to explain this latter effect: the percentage of hemicelluloses existing in the cell wall polysaccharides and the cellulolytic effect so that, when the overall concentration increases, the cellulolytic effect becomes more important. This is also observed by comparing the higher production of reducing sugars (more than two fold) for higher enzyme : seed ratios.

The analysis of these results indicates that the enzyme : seed ratio should be a compromise between the improvement of the extractability and the cost of enzyme. In any case the optimum should be in the range between 0·1 and 1 g/100 g seeds.

Time of hydrolysis

The hydrolysis time would affect the size and consequently the economy of the enzymatic pretreatment stage, so that an evaluation of the influence of the duration of this step is of interest. For these assays, moisture values were kept at 60%, enzyme seed ratio at 1 g/100 g and hydrolysis was performed in a period ranging from 1 to 12 h.

Long periods (12 h) do not favour extractability of seeds more than intermediate values (around 6 h), and produce undesirable effects on odour due to the amount of water present.

By comparing Figs 5(b) and 6(b) the effect of a more prolonged treatment can be observed; although reducing sugar production is more affected by the length of the hydrolysis time, there is not any favourable action with respect to the extractability of the oil. Because of that, a period around 6 h should be chosen for the treatment.

Sunflower

Sunflower is a seed with an oil content on a dry weight basis of about 50–60% of the whole kernel, two different extraction processes are commercially employed: prepressing and solvent extraction of the cake, or direct solvent extraction. Although enzymatic pretreatment is expected to improve both steps (pressing and extraction) in this work only experiments based on the oil extracted by a solvent were carried out.

A preliminary experiment aiming to determine the analysis time necessary to study the kinetics of the extraction process from untreated whole, transversally cut in halves or ground kernels was done. The experiments were performed using a lower extraction rate (50–70 drops/min) than that recommended for standard analysis (175 drops/min) and the results are shown in Fig. 7.

The amount of extracted oil obviously depends on particle size, and after 5 h of extraction of ground samples (in Soxhlet) nearly all of the oil present was extracted. On the whole kernels only 5% of the total oil was extracted after 12 h, and from kernels transversally cut in halves about 15% total oil can be extracted in this period. Ground kernels allow the reduction of the time of extraction to 3 h, with a significant removal of the oil from samples.

For analysis purposes a period of extraction of 6 h was selected for whole and half kernels, while for ground kernels 3 h at a reduced rate was chosen. In

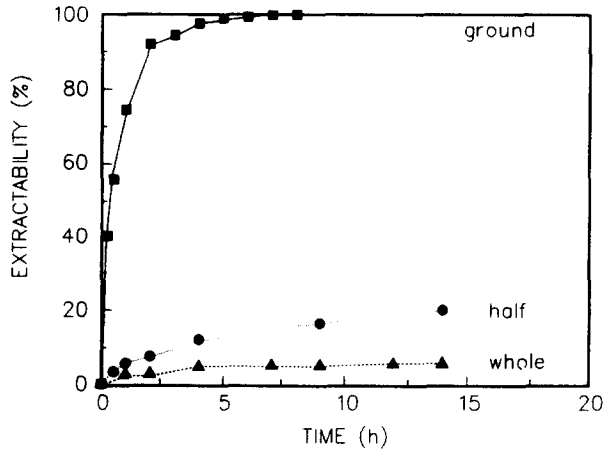

Fig. 7. Kinetics of the extraction process in Soxhlet of untreated sunflower kernels: whole, halves and ground.

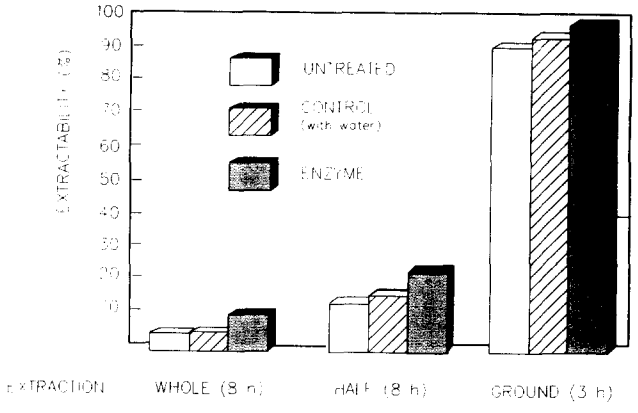

Fig. 8. Effect of particle size on the efficiency of the enzymatic treatment during 12 h (first two groups of columns), and on the extracted fraction after grinding.

this last case, extraction periods higher than 3 h would not allow us to see the possible effect of the enzymatic treatment.

The effects of the main parameters affecting the efficiency of the enzymatic pretreatment have been studied (kernel size, moisture percentage (20–60%), enzyme : kernels ratio (0·01–5% w/w dry basis) and hydrolysis time (1–12 h) at the most adequate temperature for each formulation.

We proceed by changing the variables one by one, keeping the others constant at the most favourable value found in previous experiments. The criteria for selecting the operating conditions is, as previously, the oil extractability from seeds.

Size

Figure 8 shows a comparison of extractability for whole and half kernels enzymatically treated, dried and extracted as such in the first two groups of columns and after grinding treated whole kernels in the third one. Only one enzyme, Multifect™, was employed at 0·1 g/10 g dry seed. The enzymatic treatment was realized during 12 h and the extraction time was 8 h, for whole and half and 3 h for ground kernels.

Treated samples always present higher oil extractability. The influence of the size on the efficiency of the enzymatic treatment was determined for the two particle sizes from the difference between the percentage of extracted oil from treated and untreated samples. For whole seeds enzyme treatment provides an increase of a 5%, while in half seeds a 7–8% of difference can be appreciated. As expected, reduction in particle size means more available surface for the action of enzymes. The addition of water has a slight effect on the extractability (2%) in the three cases.

The effect of particle size during extraction can be determined from the difference in the percentage of extracted oil from untreated whole and half kernels, accounting for 10%. But this improvement can be passed by grinding seeds (85% more oil in a period three times lower).

Further experiments were conducted applying the enzyme treatment on whole kernels, that after drying

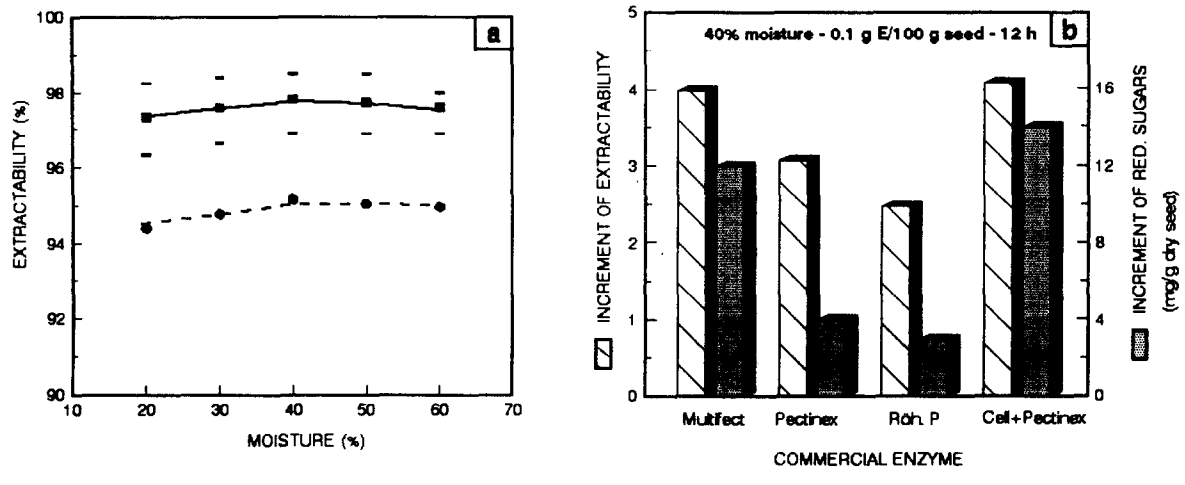

Fig. 9(a). Influence of moisture on extractability of oil from whole treated sunflower kernels. (b) Effect of the different enzymes on the extractability and reducing sugars production with respect to control samples at the selected moisture conditions.

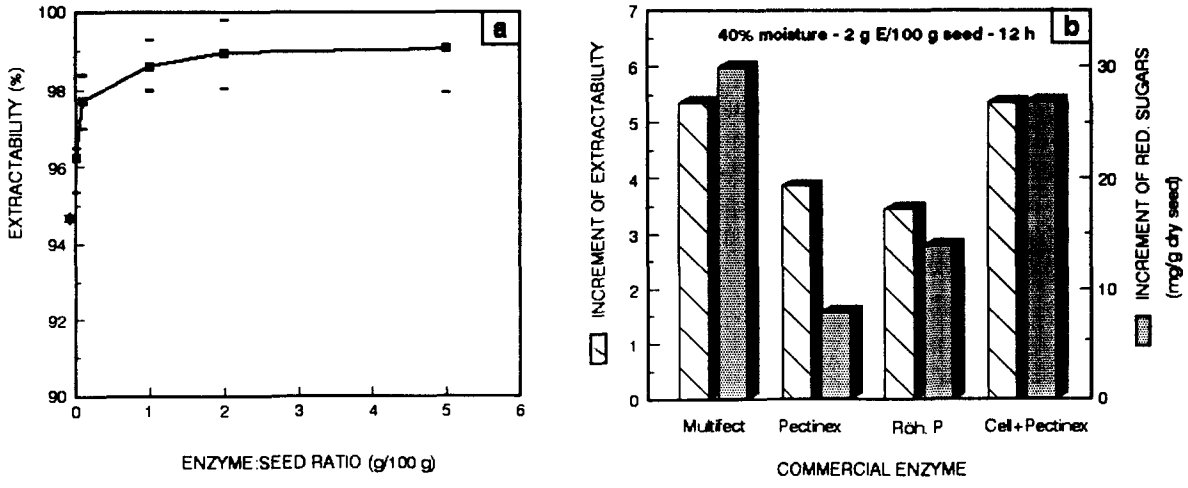

Fig. 10(a). Influence of enzyme : seed ratio on extractability of oil from whole treated sunflower kernels. * indicates the extractability for control samples. (b) Effect of the different enzymes at 2 g/100 g seeds on the extractability and reducing sugar production with respect to control samples.

were ground and extracted, allowing this method to keep a difference between enzymatically treated and untreated samples of 5%.

Moisture

Water is required to moisten the seeds and make effective the action of the enzymatic formulation. Three enzymes and a (2 : 1) Celluclast-Pectinex mixture were employed at a 0·1% (w/w) enzyme : kernels ratio. Tested moisture contents ranged from 20 to 60%. Figure 9(a) shows the effect of moisture percentage on the extractability of the kernels. Maximum values of the percentage of total extracted oil are achieved for a wide range of moisture percentages, the global optimum being not too marked. Due to the nature and shape of the seeds, even low values allow dispersion of the enzyme solution on the kernels. Higher moisture values require more drying energy after treatment (to a residual moisture of 5%). The performance of some preparations is greatly affected by moisture content during treatment, and a 40% level was chosen to carry out the following experiments. Control samples, treated only with water, are slightly affected by the moisture, the extractability being practically the same when enzymes are not present (94·5%).

The most efficient enzymes are cellulolytic and mixtures of cellulase with pectinase, the increase in oil yield being parallel to the cell wall degradation, measured as reducing sugars (Fig. 9(b)).

Enzyme : kernels ratio (w/w)

For a favourable moisture value (40%), the effect of enzyme : substrate ratio on the extractability of sunflower kernels was studied; the beneficial effect is more marked for the range between 0·01 and 2 g/100 g kernels, than when it is raised from 2 to 5% (Fig. 10(a)). An additional rise of this variable does not imply a parallel improvement in oil yield, but an increase of the costs, not advantageous from the economic point of view. As the oil extractability is enhanced by raising the enzyme : seed ratio, the net reducing sugar production is also increased.

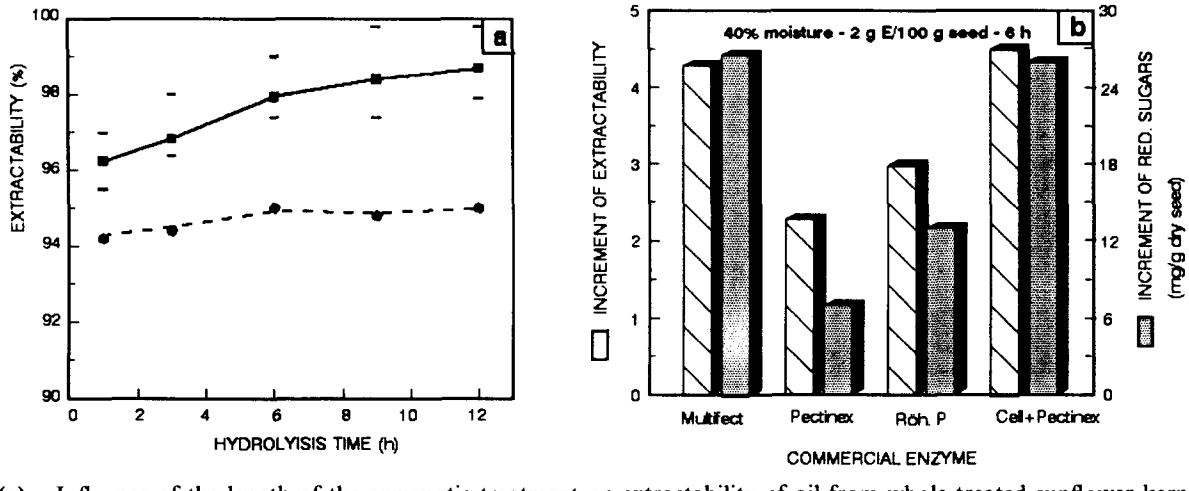

Fig. 11(a). Influence of the length of the enzymatic treatment on extractability of oil from whole treated sunflower kernels. (b) Effect of the different enzymes on the extractability and reducing sugars production with respect to control samples at the selected moisture conditions.

Time of hydrolysis

The effect of this variable on the oil extractability of sunflower was studied keeping 40% moisture and 2 g enzyme/100 g kernels. Although previous studies were carried out for more prolonged times, about 6 or 8 h is long enough to carry out enzymatic hydrolysis more efficiently (Fig. 11(a)). The effects of water do not change with the exposure time, keeping the extractability of control samples fairly constant.

CONCLUSIONS

Several operating variables (size, moisture, enzyme : seed ratio and length of the treatment), as well as the kind of enzymatic complex, affect the efficiency of the enzymatic pretreatment of soybean and sunflower before the extraction process.

Size reduction is favourable during enzymatic treatment because a better accessibility of the enzyme to the cellular wall is achieved. So when soya beans are reduced from 2 mm to 1 mm, the increment in the efficiency is more than 35%, and for sunflower an increase of 50% is obtained when the enzyme is applied on kernels transversally cut in halves instead of whole ones.

The minimal moisture required during the enzyme treatment of soybean is 50%, and 20–30% for sunflower; a further increase in moisture affects the extractability slightly (Figs 4(a) and 9(a)). Because of that, and also for operational reasons (loss of consistency during drying, undesirable odours, sticking), as well as the need for more energy during the drying process, very high values are not recommended. Water treatment (without enzyme), for prolonged periods of treatment also exerts a very slight beneficial effect on extractability.

Hydrolytic enzymes are more effective for the extractability of both seeds, because they provoke a partial breakdown of the cell wall; this explanation is based on observations that the higher increments of extractability are always accompanied by greater production of soluble reducing sugars.

Even when a very low enzyme : seed ratio (0·01 g/100 g seeds) is applied, a beneficial effect of extractability is clearly observed (Figs 5(a), 10(a)), although increases in efficiency are obtained for enzyme : seed ratios of up to 1 for soybean and 2 g/100 g seed for sunflower. Higher values do not result in further improvements. The final decision about the value to be employed must be based on an economical balance between the increase of efficiency and the cost of enzyme which is lost in the process.

The effects of the enzymatic pretreatment are also completed in a period of 6 h in both cases (Figs 6(a), 11(a), although slight increments can be obtained in more extended periods.

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