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Enzymatic pretreatment to enhance oil extraction from fruits and oilseeds: a review

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Enzymatic treatment to enhance oil recovery from olive, avocado or coconut pastes has been used with excellent results both on a laboratory and industrial scale (olive) obtaining the oil in shorter times and increasing the capacity of the equipment. This treatment was tried for the extraction of oil and protein from oilseeds on a laboratory scale (peanut, rapeseed — also in a pilot plant — sunflower and soybean). Considering that two thirds of the total fat and oil production is supplied by oilseeds (soybean, sunflower, rape and palm accounting for more than 70% of vegetable oils) this is a promising field for biotechnological applications. In the present work the different processes, as well as the factors affecting their efficiency, are discussed.

INTRODUCTION

The profitable effects of utilizing enzymes in the alimentary field have long been recognized, with regard to the increasing yields of the main products by reducing side products, and to the low waste treatment costs (Voragen & Pilnik, 1989). Another advantage is the tailoring of enzyme complexes to fit the processing requirements because of the mild conditions that avoid drastic operational conditions. An important deterrent of their use has been the high enzyme cost, although advances in aspects like microbial genetics, thermal stability enhancement and purity have improved the activity as well as the economic balances, which is decisive when substituting old processes by enzymatic ones (Spradling, 1989).

Several applications of enzymes in fats and oils processing have been reviewed: oil extraction, mono and diglyceride production, steroid and fatty acid transformation (Caragay, 1983; Posorske, 1984; Ratledge, 1984; Holló, 1987; Graille *et al.*, 1988; Friedt, 1988). In the case of oil extraction processes two advantages are offered by this treatment: higher yields of oil and higher quality of the meal, this field being considered as a new perspective of development in this kind of industry. The favourable effect on the oil yield after enzymatic pretreatment was already observed in the 1950s, when economic aspects did not allow its industrial application. In the 1970s this subject again attracted the attention of several researchers and the existing interest in this technology has led to the development of pilot and industrial processes with olive (Montedoro & Petruccioli, 1973; Santos, 1978; Alba *et al.*, 1987), pilot processes with rapeseed (Olsen, 1987) and semipilot with coconut (Cintra *et al.*, 1986).

Enzymatic treatment is different depending on its action on seeds or fruits:

(a) Oilseeds

Enzymatic action, as well as mechanical and thermal treatment, damages cell walls, favouring the permeability for oil. A number of enzymes from vegetable cell degrading microorganisms have been used to enhance the extractability of oil from oilseeds: amylase, glucanase, protease, pectinase, as well as cellulolytic and hemicellulolytic enzymes (Fullbrook, 1984).

The cell walls have to be degraded to make possible the extraction of oil from oilseeds. Degradation affects carbohydrates, but the resulting components must not interact with the products to be purified. Enzymatic treatment offers a high yield and a preservation of valuable extracted components, because of the mild conditions employed (Olsen, 1988). All the analyses agree in showing favourable effects for enzyme mixtures, as well as for multiactivity formulations which produce a breakdown of the cellular structures to obtain a total degradation of the cell walls, causing the release of oil (Olsen, 1986). The removal of cell walls was sometimes observed: in Fig. 1 shows the coat of soybean before and after treatment with cellulase at 28°C and 200 rev/min during 4 h (Toyama, 1969).

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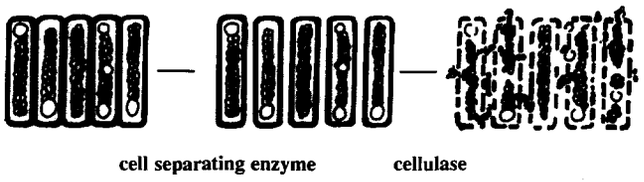
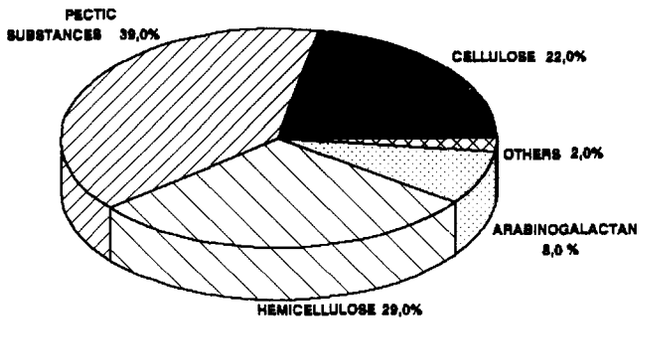


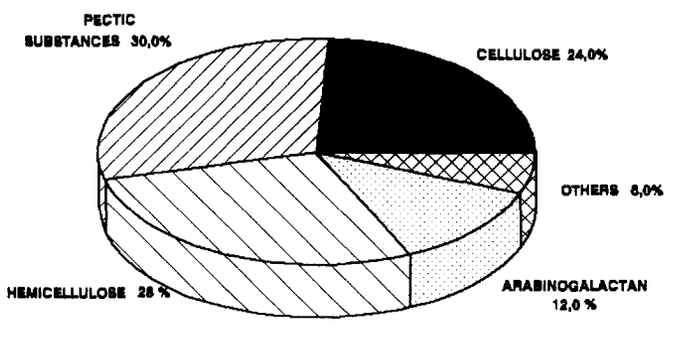
Fig. 1. Enzymatic degradation of soybean cell walls (Toyama, 1969).

Enzymatic formulations with cellulase and hemicellulase activities are the most favourable for the purpose of removing cell walls, because of the composition of these latter in polysaccharides. Figures 2 (a) and (b) show the cell wall composition of soybean and rapeseed, respectively. Cellulose and hemicellulose account for 52% of cell wall polysaccharides in soybean and 51% in rapeseed.

Pectinases are also effective because pectic substances are structural components of the cell walls of fruits and vegetables. A double membrane exists in these compounds; the primary wall is a matrix of pectins, hemicellulose and protein with cellulose microfibrils. In the secondary wall, cellulose and hemicellulose prevail. In seeds, oil is in intracellular vacuoles linked to other macromolecules, and its extraction is enhanced by the hydrolytic action of carbohydrases. So, exogenous enzymes are employed to increase the oil recovered. Bhatnagar & Johari (1987) assayed several thermophilus moulds, superior to mesophilus in productivity and industrial performance, for degradation of oilseeds and fruits (castor, sunflower, soybean, cotton), finding that their secreted enzymes improved oil recovery to a greater degree than purified cellulase or hemicellulase.



(a)



(b)

Fig. 2. Composition of the cell wall polysaccharides of: (a) rapeseed; (b) soybean.

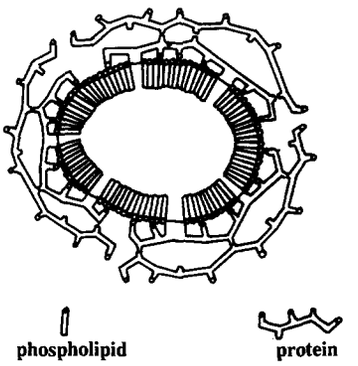


Fig. 3. Structure of the lipoproteic membranes of the emulsion (Santos, 1979).

(b) Fruits

The extraction of oil from oil fruits is accomplished by the addition of hot water to the ground fruit, mixing of the paste and separation of the three phases, the solid, the aqueous and the oily. When enzymatically treated oilseeds are processed either by pressing or solvent extraction, the registered increase in oil is due to cell wall rupture. In oil fruits, the higher amount of oil recovered is due to this same reason, as well as to an additional effect: the rupture of the interphase of lipoproteic membranes, thus allowing the dispersion of the colloidal system formed during grinding. This colloidal system presents a lipophilic phase in contact with oil and a hydrophilic phase in contact with water (Fig. 3) (Santos, 1979). The distribution can be justified by the particular structure of the oil drops surrounded by a membrane of dispersed protein in the aqueous phase forming lipoproteic compounds, so the enzymatic treatment is specially useful for 'difficult' pastes, which present operational difficulties due mainly to the emulsion formed.

FACTORS AFFECTING THE ENZYMATIC TREATMENT OF OIL SEEDS AND FRUITS

Several investigations have been carried out to analyse the effects of the pretreatment on the yield and quality of oil from fruits and seeds with different microbial enzymes. Taking into account the different compositions (Table 1), the mode of operation as well as the effectiveness of the treatment has to be appropriate for the oil-bearing materials. The conditions affecting enzymatic hydrolysis of oil fruits and oilseeds are reviewed below. However, the pH and temperature condition for hydrolysis are similar for each enzyme.

(a) Temperature

The values of this variable should be in the range of maximum activity of the enzyme, and the quality of the product should not be affected. Table 2 summarizes the temperature ranges used for different fruits and oilseeds. Fullbrook (1983) used a temperature programme for rapeseed and soybean consisting of 60 min at 50°C,

Table 1. Average composition of three oil bearing fruits and three oilseeds (dry basis, %)

	Olive pulp	Avocado	Coconut	Rapeseed	Sunflower kernel	Soybean
Water	50·0	—	48·1	5·0	6·7	9·0
Oil	22·0	70·0	27·6	41·6	53·3	20·0
Protein	1·6	8·1	3·8	26·2	22·9	40·0
Carbohydrate + fibre	24·9	17·7	19·6	23·4	14·0	26·0
Ash	1·5	4·2	0·9	3·8	3·1	5·0

120 min at 63°C and a short period (13 min) at 80°C to inactivate enzymes. Lanzani *et al.*, (1975) treated sunflower, rapeseed and peanut using a sequence of increasing temperatures (40°C, 50°C and 65°C) during 3 h.

(b) pH

This variable, as well as temperature, can be adjusted during the course of the reaction and it is also strongly dependent upon the enzyme. The pH range of 4·5–5·5 is generally suitable, 3–8 is the range of maximum activity.

OIL FRUITS

The extraction of oil from fruits can be enhanced with partial enzymatic hydrolysis to accelerate the natural enzymatic process of the paste, so favouring the separation of oil from other macromolecules to which oil is linked. Enzymatic treatment must inhibit the formation of interphases, or destroy them, because the yield in oil can be reduced by the production of emulsions surrounded by lipoproteic membranes or by the lipophilic solids of the paste, which can absorb part of the oil. The rheologic modification of the pastes favours the flow of liquids reducing either the pressing or the centrifugation time (depending on the process) as well as the quantity of residual oil in treated pastes. The enzyme is added with water, modifying the conventional process (Fig.4).

Enzymatic formulations are added to the paste, maintaining pH and temperature in the desired range. After this treatment, aqueous and solid phases are separated by centrifugation or pressing (single or double pressing) of the paste. Oil has to be recovered from the emulsion by further centrifugation.

Table 2. Temperature ranges for the enzymatic treatment of fruit and oilseeds

Fruit/Seed	*T* (°C)	Time (h)	Enzyme	Reference
Avocado	65	1	α-Amylase	Buenrostro & López-M. (1986)
	65	1	Mixture of protease and cellulase	Buenrostro & López-M. (1986)
Coconut	40	0·33	Mixture of α-amylase and protease	Cintra *et al.* (1986)
	40	0·33	Polygalacturonase	Cintra *et al.* (1986)
Olive	50	1 to 4	α-Amylase	Montedoro & Petruccioli (1973)
	35	1·5 to 2	Cellulase	Montedoro & Petruccioli (1973)
	45	1·5 to 2	Pectinase	Montedoro & Petruccioli (1973)
	35	1·5 to 2	Cellulase	Montedoro & Petruccioli (1974)
	30	1	Pectinase	Siniscalco & Montedoro (1988)
	30–37	0·75	Pectinglycosidase–cellulase–hemicellulase	Alba *et al.* (1987)
	35–40	0·5–0·67	Pectinase–hemicellulase–polysaccharidase	Alba *et al.* (1990)
	18–20	0·33–0·5	Pectinase-cellulase	Leone *et al.* (1977)
Peanut	40–50–65	3	Mixture of protease, cellulase and pectinase	Lanzani *et al.* (1975)
Rapeseed	40–50–65	3	Protease	Lanzani *et al.* (1975)
	40–50–60	3	Mixture of protease and pectinase	Lanzani *et al.* (1975)
	50–63	3	Mixture of α-amylase, β-glucanase and *n*-protease	Fullbrook (1984)
	50–63	3	Hemicellulase	Fullbrook (1984)
	50	6	Multi-activity	Sosulski *et al.* (1988)
	50	6	Pectinase	Sosulski *et al.* (1988)
	45–50	6	Multi-activity	Sosulski & Sosulski (1990)
	50	4	Multi-activity	Olsen (1987)
Soybean	50–63	3	Mixture of pectinase and cellulase	Fullbrook (1984)
	50–63	3	Hemicellulase	Fullbrook (1984)
	45	8	Cellulase	Fullbrook (1984)
	45	8	Hemicellulase	Bhatnagar & Johari (1987)
Sunflower	40–50–65	3	Mixture of cellulase and pectinase	Lanzani *et al.* (1975)
	45	8	Cellulase	Bhatnagar & Johari (1987)
	45	8	Hemicellulase	Bhatnagar & Johari (1987)
	50	8	Multiactivity	Domínguez *et al.* (1991)

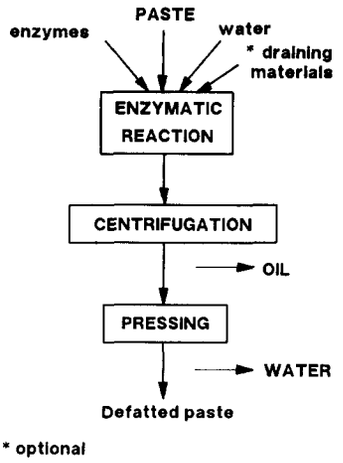


Fig. 4. Enzymatic process for oil extraction from pastes of oil fruits (Buenrostro & López-Munguía, 1986).

Olives are the most studied of the oil fruits; experiments on a laboratory, pilot or industrial scale have shown that enzymatically treated olive pastes offer higher extraction yields than untreated ones. Studies on laboratory scale (avocado) and semipilot plant (coconut) have corroborated this improvement. The beneficial effects are summarized in Table 3, representing the increment in the percentage of extracted oil from enzymatically treated pastes versus pastes to which only water was added. Mixtures of enzymes are more favourable in oil extraction from avocado, coconut and olive. For the first two fruits, the increase reached with the enzymatic treatment as a percentage of the total extractable is 4–5 times superior to that obtained for olive oil. The results from pilot or industrial plant operations differ from those ones obtained in laboratory experiments.

A number of variables and their effect on the oil extraction yield were studied by several authors when the enzymatic treatment was applied to olive, avocado and coconut.

Dilution ratio

The amount of water added during the treatment affects the recovered oil yield. The conclusions are different depending on the fruits. Buenrostro & López-Munguía (1986) have found that the maximum yield of avocado oil after pressing is reached with dilution ratios of 1/5, while with coconut the best yields are obtained at a 1/4 dilution ratio (Cintra *et al.*, 1986) (Fig. 5). This effect has also been studied for the extraction from olive pastes, after drying the fruit to a final

Table 3. Increment of the oil yields obtained with enzymatically treated samples of avocado, coconut and olive

Fruit	Main enzyme activities (commercial names)	Oil yield increase[a]	Reference
Avocado	α-Amylase (TANASE)	68·0	Buenrostro & López-M. (1986)
	Protease (PAPAIN)	49·0	Buenrostro & López-M. (1986)
	Cellulase (CELLUFERM)	40·0	Buenrostro & López-M. (1986)
	Mixture of α-Amylase and Protease	65·0	Buenrostro & López-M. (1986)
Coconut	Pectinase (CLAREX)	28·1	Cintra *et al.* (1986)
	α-Amylase (TANASE)	19·2	Cintra *et al.* (1986)
	Protease (HT PROTEOLITIC)	7·0	Cintra *et al.* (1986)
	Mixture of pectinase and α-amylase	46·9	Cintra *et al.* (1986)
	Mixture of pectinase and protease	37·0	Cintra *et al.* (1986)
	Mixture of pectinase, α-amylase and protease	68·0	Cintra *et al.* (1986)
		62·7[b]	Cintra *et al.* (1986)
Olive	Pectinase (PECTINEX)	1·8 7·4[b]	Siniscalco & Montedoro (1988)
	Pectinase (PECTINEX)	0·9 3·8[c]	Siniscalco & Montedoro (1988)
	Pectinase–cellulase	1·8 7·0	Leone *et al.* (1977)
	Pectinglycosidase–cellulase–hemicellulase (RÖHAMENT O)	1·1 3·7	Alba (1987)
	Pectinglycosidase–cellulase–hemicellulase (RÖHAMENT O)	1·1 4·2[c]	Alba (1987)
	Pectinglycosidase–cellulase–hemicellulase (RÖHAMENT O)	0·5 1·8[c]	Alba (1987)
	Pectinglycosidase–cellulase–hemicellulase (RÖHAMENT O)	1·7 8·3	Alba *et al.* (1987)
	Pectinase–hemicellulase–polysaccharidase (OLIVEX)	1·3 4·4	Alba et al. (1987)
	Pectinase–hemicellulase–polysaccharidase.(OLIVEX)	2·0 9·9[c]	Alba *et al.* (1990)
	Pectinase-cellulase (ULTRAZYM SE604)	3·8 15·0	Santos (1978)
	Cellulase (CCG 20385) + pectinase (CGA 20408)	2·1 8·0	Santos (1978)
	Fungal cellulase (CCG 20385)	2·6 10·1	Santos (1978)
	Pectinase (ULTRAZYM 100) + pectinmethyl esterase + cellulase + papain (MERCK)	3·1 11·7[b]	Montedoro & Petruccioli (1973)
	Cellulase (CGA 20385) + pectinase (CGA 20408)	2·1 8·0[c]	Montedoro & Petruccioli (1974)
	Cellulase (CGA 20385)	1·8 7·1	Montedoro & Petruccioli (1974)
	Hemicellulase (CGA 20393)	1·4 5·6	Montedoro & Petruccioli (1974)
	Mixture of cellulase (CGA 20385) and pectinase (CGA 20408)	2·1 8·0	Montedoro & Petruccioli (1974)
	Mixture of cellulase (CGA 20385) and acid protease (CG 23352)	2·0 7·7	Montedoro *et al.* (1975)

[a] Oil yield (extracted oil measured as difference in percentage of total oil) from enzyme treated pastes minus oil yield of control pastes; olive first column: oil yield increase (kg oil/100 kg fruit), second column: oil yield increase (% of total oil).
[b] Pilot or semipilot.
[c] Industrial.

moisture content of 10 % (weight) and addition of the enzymatic solution in buffered media. Enzymes showed their maximum activity in media with a water content of 35% or more (Montedoro & Petruccioli, 1973).

Enzyme concentration

It is known that an increase in the enzyme concentration increases the rate at which the oil is separated, but the optimum must be established. Figure 6 illustrates the results from olive pastes treated with different commercial enzymes. It was observed that cellulase and acid protease yielded the maximum amount of oil, their optimum concentrations depending on the enzyme. Cellulase enhances the oil extraction especially at 25–30 g/100 g olive, its efficiency being considerably reduced when working at higher or lower concentrations. Acid protease yields the maximum at 50 g/100 g. Hemicellulase does not present a marked optimum and the better values range between 50 and 80 g of enzyme/100 g of paste. These activities present a great affinity for this substrate which is composed mainly of cellulosic and proteic substances. Pectinase shows a maximum at 4 g/100 kg but the observed increment is lower. As expected, mixtures of enzymes yield more oil due to their combined effect on colloidal and lipoproteic structures. Several authors demonstrated that the addition of pectinase and protease to cellulolytic enzymes reduced by half the optimum concentration of this latter enzyme, obtaining the same oil yield.

Several works indicate that the mixture of enzyme activities provides higher extraction oil yields (Montedoro & Petruccioli, 1974). The influence of different formulations on the olive oil yield obtained by single pressing after enzymatic treatment was examined, showing that mixed cellulase associated with pectinase, or cellulase with acid protease was more efficient in increasing the yield than cellulase alone although added in double concentration.

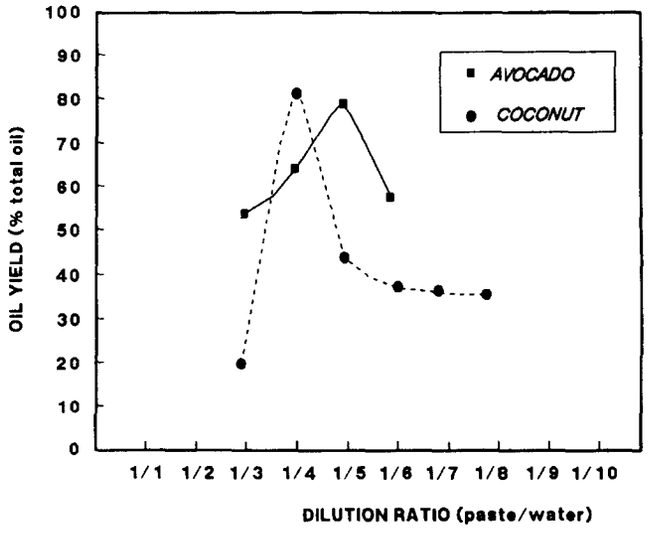


Fig. 5. Effect of dilution ratio on the extraction yield of avocado (Buenrostro & López-Munguía, 1986) and coconut (Cintra *et al.*, 1986).

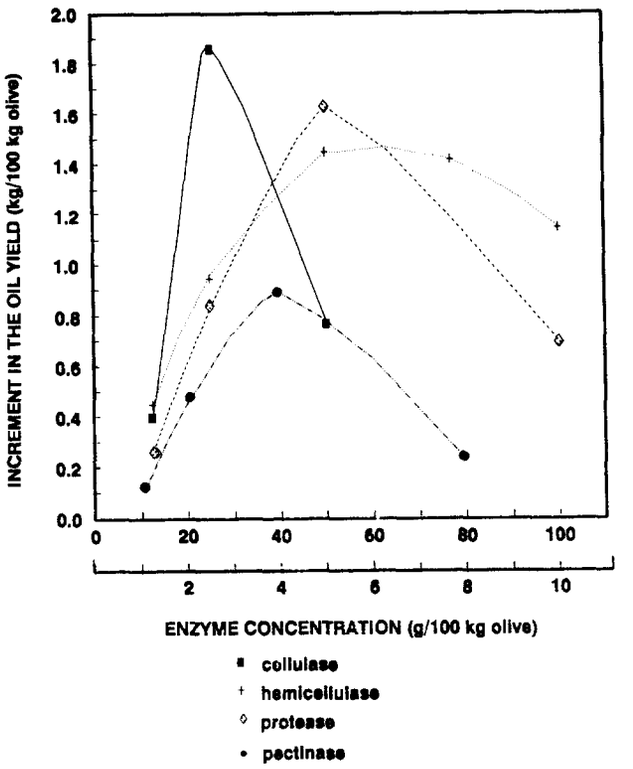


Fig. 6. Effect of enzyme concentration on the increment of oil yield from oil pastes treated with cellulase, hemicellulase, acid protease and pectinase (Montedoro & Petruccioli, 1974).

An increase in the enzyme concentration makes the emulsion more unstable. Figure 7 shows this effect on the number of particles per unit volume, N. At high enzyme concentrations N decreases, making more rapid the separation of oil (Cintra *et al.*, 1986). The number of particles N was determined by means of the equation

$$N = \frac{6 \cdot \Theta \cdot 10^{12}}{\pi \cdot D_a^3}$$

Θ being the volume fraction of the disperse phase and D_a the average diameter of the oil droplets. The

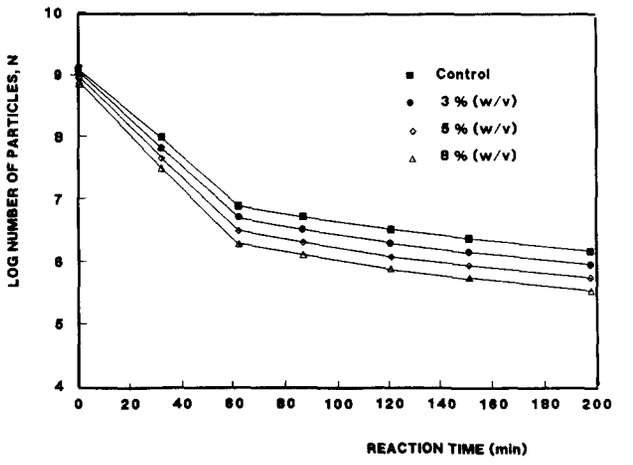


Fig. 7. Emulsion stability measured in numbers of particles per unit of volume (N) during the enzymatic reaction at 40°C (Cintra *et al.*, 1986).

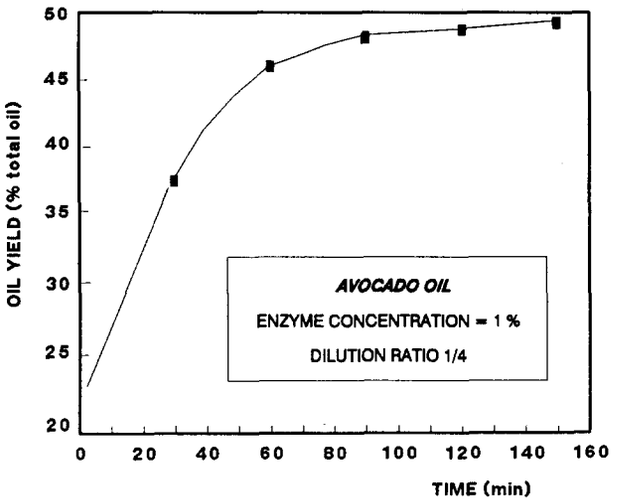


Fig. 8. Effect of reaction time in aqueous treatment of avocado (Buenrostro & López-Munguía, 1986).

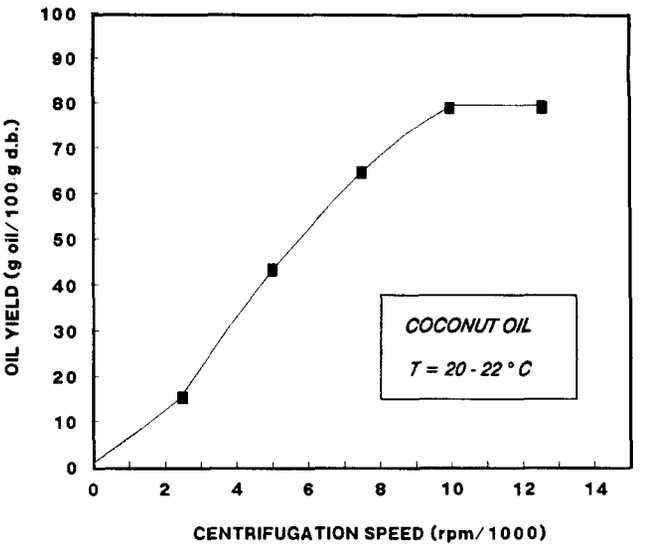


Fig. 9. Effect of the time of centrifugation on the oil yield of coconut pastes (Cintra *et al.*, 1986).

slope of the line represents the coalescence rate, inversely proportional to the emulsion stability and slightly more pronounced in treated pastes with increasing concentration as compared to control. However, after 60 min of reaction the difference is not significant, the coalescence rate being the same for treated and untreated samples.

Reaction time

Enzyme incubation times reported in the literature (0·33–2 h) are enough to instigate significant increase of the recovered oil yield. The enzymatic incubation is maintained during the mixing stage substituting the addition of hot water in the conventional process. Figure 8 shows the effect of reaction time on the extraction yield of avocado oil at an enzyme concentration of 1% and T = 65°C. Incubation time of 90 min is significantly more favourable than shorter ones and the amount of oil obtained is not surpassed after longer times of contact with enzymes.

Centrifugation speed and time of pressing

These two factors, although independent of the enzymatic reaction have been studied after the treatment of fruit pastes in order to optimize the yield of oil. Two cases are presented, corresponding to different fruits, illustrating the importance of these operational variables during the extraction process.

Cintra *et al.*, (1986) optimized the centrifugation conditions of coconut oil extraction after enzymatic treatment, in the range of 2500–12500 rev/min (Fig. 9). A maximum yield was obtained at 10 000 rev/min at room temperature at 20–22°C for a centrifugation time of 10 min.

Figure 10(a) shows the effect of a pectolytic-cellulolytic

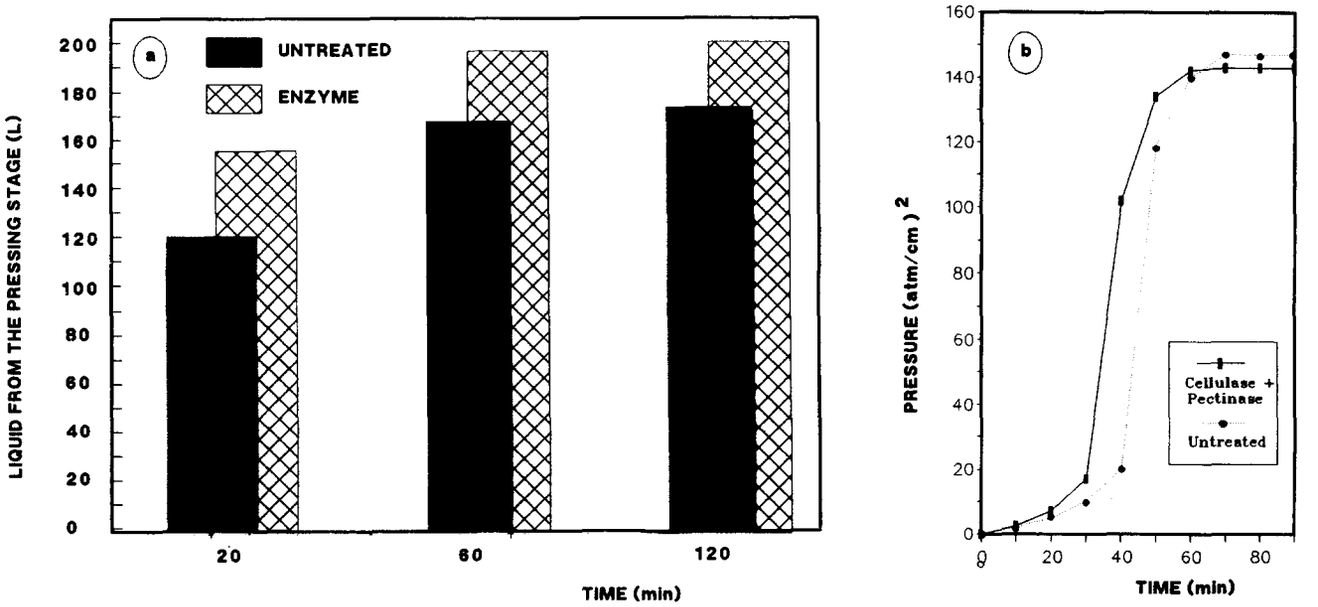


Fig. 10. (a) Volumes of pressed liquid from the pressing stage (Santos, 1978); (b) pressure increment during the first 45 min (Montedoro & Petruccioli, 1974) for untreated and treated olive pastes.

Table 4. Effect of the addition of draining material on the oil yield (kg/100 kg olive) (industrial olive oil extraction by single pressing) (Montedoro & Petruccioli, 1974)

	Drainer concentration (g/100 kg olive)	Without enzyme		Cellulase + pectinase	
		(kg oil/100 kg olive)	Increment (% total oil)	(kg oil/100 kg olive)	Increment (% total oil)
Without treatment		24·35	—	28·30	16·2
Polyclar AT	25	26·08	7·1	—	—
	50	26·70	9·6	28·40	16·6
	100	—	—	28·45	16·8
	150	27·20	11·7	—	—
	200	26·95	10·8	28·40	16·6
Methylcellulose	25	27·00	10·9	28·30	16·2
	50	27·20	11·7	28·33	16·8
	100	—	—	28·50	17·0
Albumin	15	25·91	6·4	—	—

enzyme during industrial assays with olive at 10 g/100 kg after 10 min of treatment during the mixing stage and 2 h of pressing, resulting in a higher extraction rate and oil yields in treated pastes, as well as better drainage. This effect is caused by a better dispersion of the lipoproteic structures. The volume obtained during the pressing process is measured every 5 min for an enzyme treated sample which is compared to a control one; the recovered oil is also higher in the treated sample (Santos, 1978). Pressure values, for treated and untreated pastes (Fig. 10(b)) are higher during the initial stages of the process for enzymatically treated pastes, but once stabilized the final value (after 60 min) is maintained in both samples till the end of the pressing.

Draining materials

Additives can be used to destroy the colloidal structures that limit the mechanical extraction and also to absorb tannin phenolic compounds with an inhibitory action on the enzymes used (Montedoro *et al.*, 1975), making easier the fluidity and recovery of the oil. On the other hand, the association of these absorbent agents with the carbohydrases enhances the enzymatic activity by eliminating the polyphenols. Several materials have been tested with different mechanical extraction methods with olive pastes either alone or with enzymes, mineral talc (Giovacchino, 1988; Siniscalco & Montedoro, 1988; Siniscalco *et al.*, 1989), highly substituted methylcellulose, egg albumin, insoluble polyvinyl pyrrolidone (Polyclar AT), as well as a fibrous product from wood, Silvacel (Giovacchino, 1990). These materials were employed on both a laboratory and industrial scale with different techniques, the yield of oil being favourably influenced by all of them. The oils obtained are clear and transparent to such a degree that no further filtration is required. The combined effect of draining materials and enzymes is summarized in Table 4, being more favourable when they are acting together. Oil is extracted by percolation and centrifugation; this latter stage improved considerably with the addition of drainers.

Mono and divalent cations

The presence of mono and divalent cations activates enzymes with pectinase activity, and given that under certain concentrations cellulolytic and proteolytic activities are unaffected, the addition of salts is favourable to the extraction of difficult olive pastes. An optimum value in the range 2–4% of NaCl and 0·5–1·5% of $CaCl_2$ was found (Montedoro & Petruccioli, 1973).

OIL SEEDS

For the extraction of oil and protein from oilseeds the enzymatic pretreatment was tried on a laboratory and pilot scale; different strategies were employed depending on the seed, the industrial process used to separate both the oil and the meal and the aspects of the process that could be improved.

(a) Aqueous process

A high percentage of moisture is used during the enzymatic treatment as water is the solvent used to recover oil and protein.

(b) Solvent assisted oil separation in aqueous processes

Water-inmiscible solvents, easily recovered are added either after the enzymatic treatment to separate the product or simultaneously with the enzymes. Solvents, whose function is to recover the released oil are used in small quantities.

(c) Conventional process, enzymatic treatment in the presence of reduced moisture

This alternative requires optimized moisture during the enzymatic incubation because it has to be removed before extraction by drying. While the previous options are suitable for both oil fruits and seeds, this latter is especially adequate for oilseeds.

Table 5. Comparison between enzymatic and conventional rapeseed oil and meal extraction (Olsen, 1987)

	Conventional	Enzymatic process (aqueous)
Pretreatment	Rolling Toasting	Milling Boiling in water
Release of oil	Pressing Solvent extraction	Enzyme reaction (Degradation of fibre)
Products	Raw oil (with lecithin) Cakes Flakes (Extraction residue)	Raw oil (with lecithin) Protein Molasses Hulls

AQUEOUS PROCESS

The main drawbacks with most extractive processes (mechanical pressing and solvent extraction) are economic, environmental and safety aspects (Johnson & Lusas, 1983). The high temperatures reached at certain stages are the negative points of the conventional oilseed processing plants, because of their undesirable side effects on the quality of the finished products; the protein that results is denatured, limiting its use for food and feed products. These problems could be overcome by the aqueous alternative. The previous enzymatic action and the aqueous process can favour the extraction of seeds in environmental safety and economic aspects, yielding a detoxified protein product. Lower operation temperature can be used, with consequent lower energy requirements, and the oil extracted meets the required quality specifications.

In the case of seeds this process is an alternative to the pressing and/or solvent extraction processes of oil and protein from oil fruits and seeds (Hron *et al.*, 1982) such as peanuts (Rhee *et al.*, 1972; Hagenmaier, 1974; Lawhon *et al.*, 1981) sunflowers, coconut (Hagenmaier *et al.*, 1973), soybeans, cottonseed. It consists of grinding,

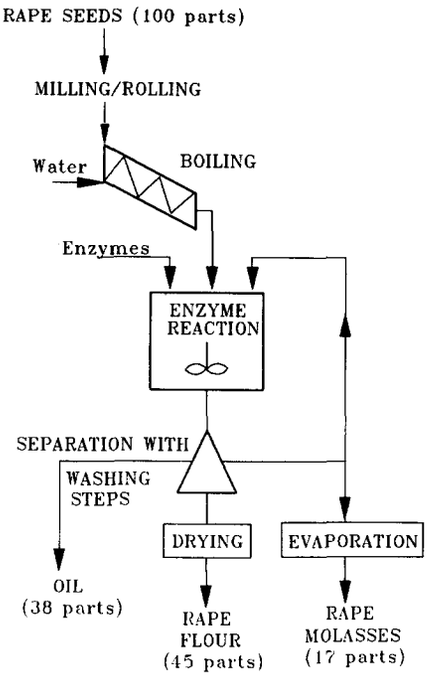


Fig. 11. Experimental enzymatic aqueous process for oil recovery from rapeseed (Olsen, 1987).

mixing and extraction followed by solid-liquid, liquid-liquid separations and drying of the solid product.

With the enzymatic attack on the cell walls of oilseeds the oil can be separated more efficiently from both protein (solid phase) and water (liquid phase) (Lanzani *et al.*, 1975). Either centrifugation or filtration are suitable to separate solid and liquid phases by mechanical or enzymatic means (Hagenmaier *et al.*, 1973; Gresch, 1989). This process can be used on a large scale, gives no harmful or polluting waste, and the aqueous phase can be used as a feedstuff. Over 90% oil extractable can be recovered from a number of seeds or fruits: olive, rapeseed, soybean, flax, palm kernel, castor, jojoba, cotton... (Marek *et al.*, 1990).

A comparison between enzymatic and conventional

Table 6. Increase in the percentage of oil extracted from enzymatically treated seeds compared with untreated in aqueous process

Seed	Enzyme activities	Increment	Reference
Peanut	Cellulase (CGA 20394)	3	Lanzani *et al.* (1975)
	Pectinase (ULTRAZYM)	2	Lanzani *et al.* (1975)
	Protease (MERCK)	6	Lanzani *et al.* (1975)
	Mixture of pepsin (MERCK) and cellulase (CGA 20394)	6	Lanzani *et al.* (1975)
	Mixture of pectinase, protease and cellulase	6	Lanzani *et al.* (1975)
Rapeseed	Cellulase (CGA 20394)	6	Lanzani *et al.* (1975)
	Pectinase (ULTRAZYM)	1	Lanzani *et al.* (1975)
	Protease (CGA 20391)	33	Lanzani *et al.* (1975)
	Mixture of pectinase and protease	35	Lanzani *et al.* (1975)
	α-Amylase, β-glucanase and *n*-protease (CEREMIX)	31	Fullbrook (1984)
	Cellulases from *A. terreus*	9	Marek *et al.* (1990)
Soybean	α-Amylase, β-glucanase and *n*-protease (CEREMIX)	33	Fullbrook (1984)
	Cellulases from *P. verruculosum*	10	Marek *et al.* (1990)
Sunflower	Cellulase (CGA 20394)	14	Lanzani *et al.* (1975)
	Pectinase (ULTRAZYM)	14	Lanzani *et al.* (1975)
	Protease (MERCK)	8	Lanzani *et al.* (1975)
	Mixture of pectinase and cellulase	22	Lanzani *et al.* (1975)
	Mixture of pectinase and protease	13	Lanzani *et al.* (1975)
	Mixture of cellulase and protease	16	Lanzani *et al.* (1975)

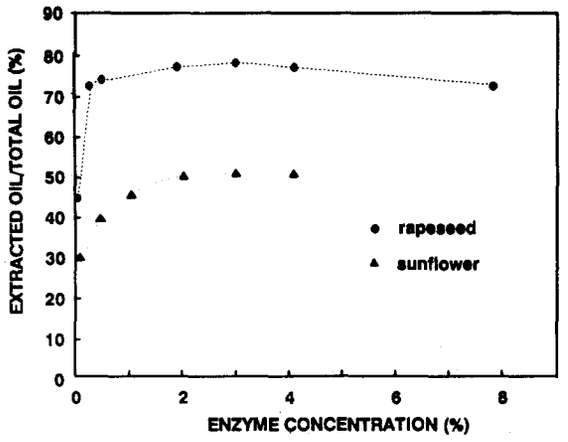


Fig. 12. Effect of enzyme concentration in the percentage of total oil extracted from rapeseed and sunflower (Lanzani *et al.*, 1975), with aqueous processes.

processes of rapeseed oil and protein extraction according to the products released is shown in Table 5 (Olsen, 1987). Figure 11 shows a scheme for the aqueous process where the seed is fractionated, improving the economic value of the products compared to the conventional process. Pilot plant studies with rapeseed have been carried out following this scheme. First the selected fruits are ground, prior to the enzymic treatment; the inactivation of rapeseed antinutritional factors is required. The temperature is kept at 50°C, pH at 4·5 during 4 h with an enzyme dosage of 0·5 % w/w. The recycled aqueous phase from the separation step is added at a ratio of 2:1 to the milled seeds. Three products are obtained, oil, protein and a fibrous residue. Once the optimum amount of oil has been released, after successive washing steps, most of the fibrous material is removed with a decanter centrifuge, or a vibrating sieve, where most of the insoluble proteins are recovered. Most of the oil is recovered from the centrifuge as clear oil. The aqueous phase may be concentrated by evaporation and separated at 90–95°C to recover more oil.

The improvement reported in the increase of extraction yield depends mainly on the seed; while considerable enhancement of oil recovery is achieved with sunflower and rapeseed, the aqueous process for peanut does not allow such a significant improvement. Table 6 shows the increase in oil yield for treated samples versus untreated ones during aqueous processing to recover the oil from different oilseeds.

Enzyme concentration

Lanzani *et al.*, (1975) studied the effect of enzyme concentration on rapeseed and sunflower oil recoveries with a mixture of enzymes: protease (CGA 20391) and α1–4 galacturonidase (Ultrazym) for rapeseed, and a mixture of cellulase (CGA 20394) and Ultrazym for sunflower; both mixtures appeared to be the best in the previous experiments. For rapeseed the maximum oil recovery can be reached with 3% enzyme concentration. By increasing the enzyme concentration over 2%, the yield of sunflower oil can not be increased (Fig. 12).

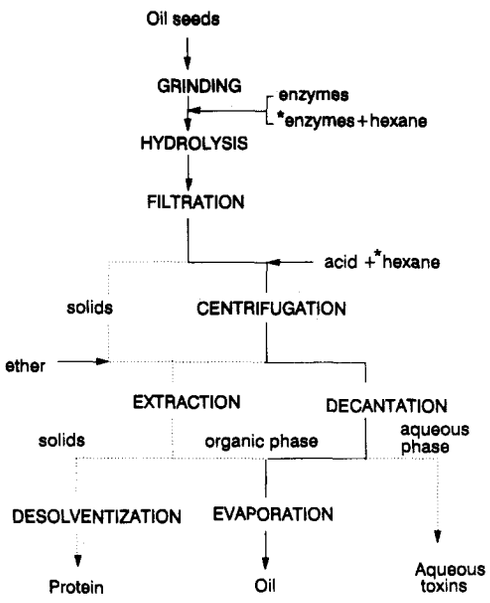


Fig. 13. Solvent assisted hydrolysis/extraction process (Fullbrook, 1983).

SOLVENT ASSISTED PROCESS

A slight difference exists between the solvent-assisted aqueous process and the previous process; it lies in the simultaneous presence of an organic solvent during the enzymatic incubation or later, during the separation of the phases. Fullbrook (1984) used, for rapeseed and soybean, a strategy consisting of the stages indicated in Fig. 13. It was observed that the addition of hexane to the aqueous slurry increased the amount of oil from the finely ground soybean, rapeseed and melon seeds. There are two alternatives for carrying out this process, the addition of solvent (a) during or (b) after the enzymatic hydrolysis.

(a) Diluted enzymes are incubated with the ground seeds at a pH of 6 for a period of 1–6 h at two different temperatures and at a third stage, 80°C is used to

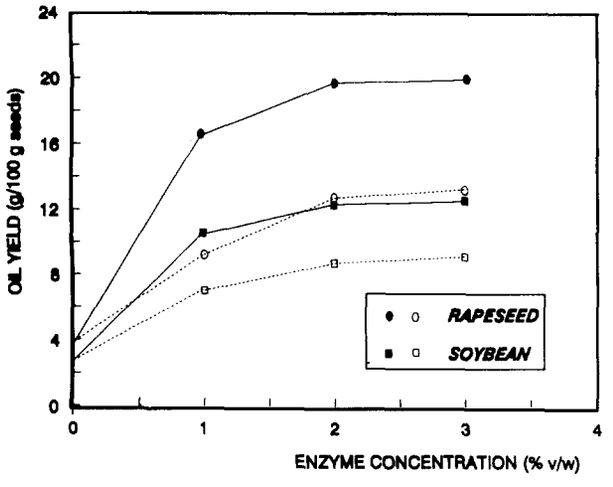


Fig. 14. Effect of the presence of hexane in the yield of soybean and rapeseed incubated with a mixture of α-amylase, β-glucanase and *n*-proteinase from *Bacillus subtilis* (Ceremix, Novo Ind. A/S)). Void symbols correspond with absence of solvent (Fullbrook, 1984).

inactivate the enzymes, finishing the hydrolysis. In the filtration step the organic solvent is added to the incubated slurry to separate the oil. After the filtration of this mixture, two phases are obtained. In the filtrate, water, soluble protein, oil and solvent are recovered. Modifying the pH into the acidic range 3–5, the protein can be easily precipitated, and after the removal of solvent, a protein concentrate (solid phase) can be obtained, as well as a water phase and an organic one containing oil and solvent. The latter two are separated by decantation, while the insolubles are added to the solids from the filtration step, constituting the protein concentrate.

(b) Enzymes, water and water-immiscible solvent are added to the ground oil seeds. Once the hydrolysis has proceeded as in the previous alternative, the mixture is filtered and the process presents no significant operational differences from that described above, but the yield of recovered oil is higher than with the alternative (a), as Fig. 14 shows for soybeans and rapeseed.

Enzyme concentration

Figure 15 shows the effect of different enzyme concentrations on the extracted oil in the simultaneous

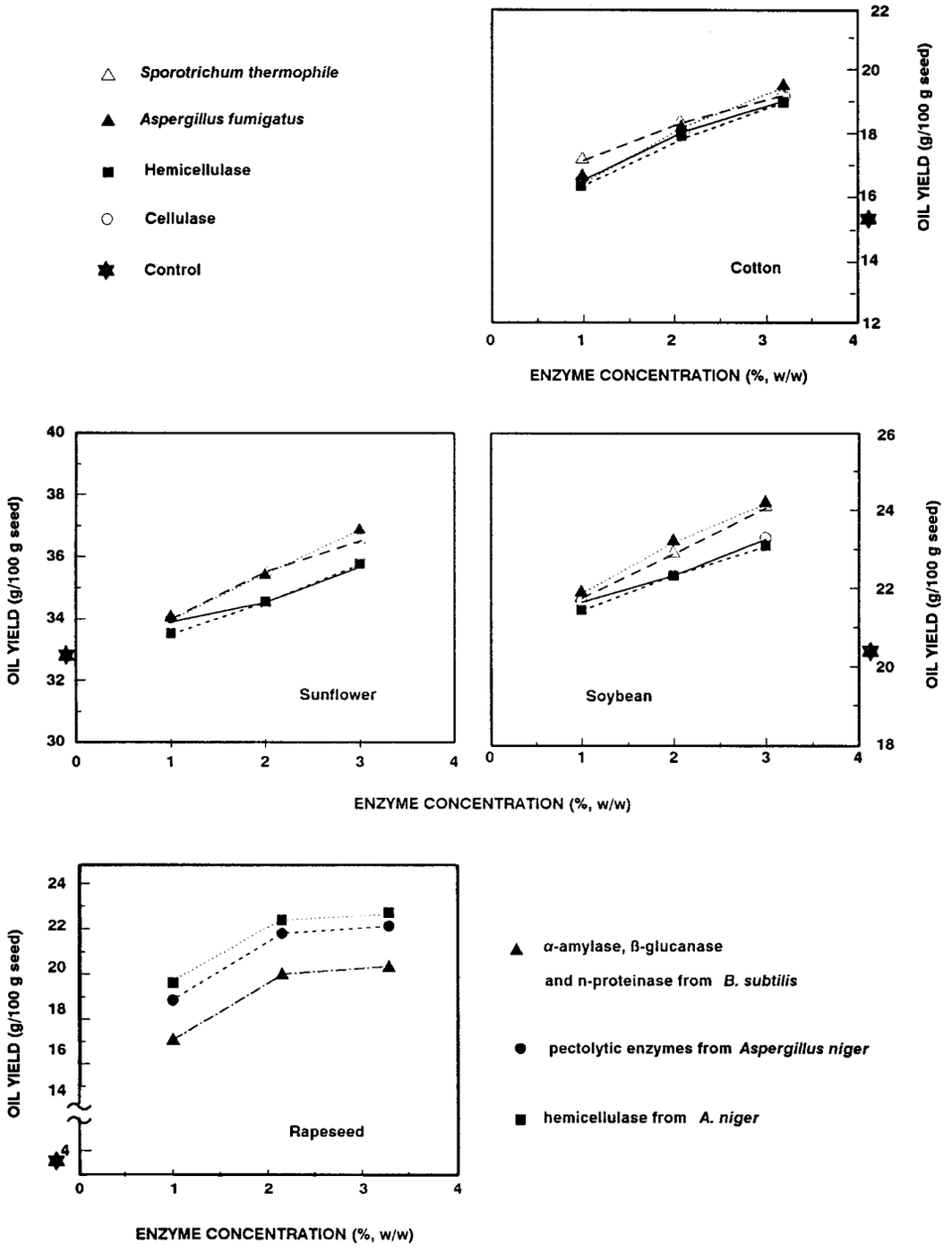


Fig. 15. Effect of the enzyme concentration on the yield of oil when the hydrolysis is carried out in the presence of solvent during incubation of rapeseed (Fullbrook, 1984), cotton, sunflower and soybean (Bhatnagar & Johari, 1987).

Table 7. Increment of the percentage of extracted oil from ground seeds, enzymatically treated in the presence of hexane

Seed	Enzyme activities (commercial name)	Increment (g/100 g seed)	Reference
Castor	Cellulase (SIGMA)	3·11	Bhatnagar & Johari (1987)
	Hemicellulase (SIGMA)	2·92	Bhatnagar & Johari (1987)
Cotton	Cellulase (SIGMA)	3·62	Bhatnagar & Johari (1987)
	Hemicellulase (SIGMA)	3·57	Bhatnagar & Johari (1987)
Sunflower	Cellulase (SIGMA)	3·21	Bhatnagar & Johari (1987)
	Hemicellulase (SIGMA)	3·10	Bhatnagar & Johari (1987)
Soybean	Cellulase (SIGMA)	2·99	Bhatnagar & Johari (1987)
	Hemicellulase (SIGMA)	2·79	Bhatnagar & Johari (1987)
	α-Amylase, β-glucanase, *n*-protease (CEREMIX)	9·90	Fullbrook (1984)
	Pectinase–cellulase (PECTINEX Swiss F. Co)	13·90	Fullbrook (1984)
	Hemicellulase (GAMANASE, Novo)	15·10	Fullbrook (1984)
Rapeseed	α-Amylase, β-glucanase, *n*-protease (CEREMIX)	16·20	Fullbrook (1984)
	Pectinase–cellulase (PECTINEX Swiss F. Co)	17·70	Fullbrook (1984)
	Hemicellulase (GAMANASE, Novo)	18·30	Fullbrook (1984)

presence of hexane (1:2 hexane:water ratio) during the enzymatic treatment. Enzymes were used purified, i.e. hemicellulase, cellulase, or raw from moulds, accompanied by other products. Enzymes from unpurified mould cultures are more efficient than those from pure enzymes in the removal of oil from seeds as the higher yields obtained with cultures from *Aspergillus fumigatus* and *Sporoctrichum thermophile* indicate. The reason lies in the nature of the cell wall of oil seeds, which is a complex substrate, more easily degraded by a mixture of enzyme activities than by pure enzymes. The results are congruent for different authors as long as the enzymes used with rapeseed are other than those used for cotton, soybean and sunflower. Extracted oil increases with enzyme concentration, but not continuously. Therefore an optimum concentration value can be deduced from the behaviour of the different seeds. The increase in the enzyme seed ratio has a different effect depending on the seed, even in the same range of 1–3 g /100 g seed; for sunflower and soybean the tendency is to increase continuously, while for rapeseed 2 g/100 g appears to be the optimum enzyme/seed ratio.

For different seeds, the increment in the yield of recovered oil is higher if an organic solvent is used. Table 7 summarizes the increment of the oil yield from several seeds. Depending on the enzyme and the experimental strategies, different yields are obtained, as the data from different authors reflect. Bhatnagar & Johari (1987) obtained the greatest improvement in the extraction yield for cotton (3.62%, representing more than 20 % of the total oil), while for castor and sunflower the increment was less than 10 %. Fullbrook (1984) reported increments over 100% with respect to untreated samples.

ENZYMATIC TREATMENT IN THE PRESENCE OF REDUCED MOISTURE

Depending on the oil content of the seed, the extraction process is different; for high oil content seeds, a prepressing stage is used and the presscake is solvent extracted (sunflower, rapeseed; soybean with low oil content is directly extracted). Solvent extraction allows the recovery of almost all the oil, whereas in aqueous processes oil yields greater than 90% are difficult to obtain: 90% of soybean oil, but only 70–72% of the total oil from rapeseed, a different technique being required for this seed. Sosulski & Sosulski (1990) developed the optimum conditions for enzymatic treatment of canola in order to enhance oil recovery from several canola cultivars. The experimental process is represented in Fig. 16.

Assays to study the effect of the variables that most influence the process were carried out in smaller samples, whose extractability was analysed by extraction (Soxhlet) with hexane. Seeds are flaked to a 99% seed coat rupture and endogenous enzymes are inactivated by heat treatment; after the incubation with enzymes, the samples are dried and solvent-extracted. The percentage of extracted oil from treated samples was compared with that from untreated ones of canola (average values of three cultivars: Regent, Westar and Tobin), soybean and sunflower (Table 8). The conditions

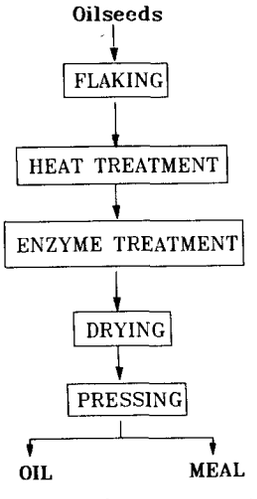


Fig. 16. Enzymatic process for oil extraction from oil seeds (Sosulski & Sosulski, 1990).

Table 8. Enhanced extractability of the oil from enzymatically-treated intact Westar canola seeds incubated 12 h at 30% moisture, and ground before extraction. Soybean grits of 1·2–0·8 mm and half sunflower kernels were treated at 40 and 60% moisture respectively for 16 h

Seed	Main enzyme activities	Increment		Reference
		% d.b.	% total oil	
Canola	Cellulase–β-glucanase–arabinase–hemicellulase–xylanase–pectinase–α-galactosidase (SP 249)	5·4	12·6	Sosulski *et al.* (1988)
	Hemicellulase (ENZECO HEM.)	2·2	5·1	Sosulski *et al.* (1988)
	Pectinase (PECTINEX U. S.P.)	3·2	7·4	Sosulski *et al.* (1988)
	Cellulase (CELLUCLAST 1.5 L)	0·9	2·1	Sosulski *et al.* (1988)
	β-Glucanase (FINIZYM)	0·5	1·2	Sosulski *et al.* (1988)
Soybean	Cellulase (CELLUCLAST 1.5 L)	1·7	8·3	Domínguez *et al.* (1991)
	Hemicellulase (ENZECO)	0·7	3·7	Domínguez *et al.* (1991)
	Multiactivity (MULTIFECT™)	2·5	12·1	Domínguez *et al.* (1991)
	Multiactivity (OLEASE)	2·4	11·4	Domínguez *et al.* (1991)
Sunflower	Pectinase (RÖHAMENT P)	1·2	2·2	Domínguez *et al.* (1991)
	Multiactivity (MULTIFECT™)	2·0	3·5	Domínguez *et al.* (1991)
	Pectinase (PECTINEX ULTRA SP)	2·3	4·0	Domínguez *et al.* (1991)
	Cellulase (CELLUCLAST) + Pectinase (PECTINEX)	1·7	3·1	Domínguez *et al.* (1991)

[a] With respect to control samples.

were maintained at the optimum recommended temperature for each enzyme; seeds were incubated for a period of 12 h for canola and 16 h for soybean and sunflower and, after drying, samples were submitted to Soxhlet extraction with hexane. Operational conditions (mechanical treatment, moisture content of the substrate and enzyme concentration and time of hydrolysis) and their effects on oil extractability were studied, as indicated above.

Mechanical treatment

This can be softer when followed by enzymatic treatment. The degree of flaking or particle size reduction is, however, a determining factor in the role of the enzymes. The effect on the oil extractability has been studied for canola seeds (Sosulski & Sosulski, 1990). Figure 17 illustrates the effect of the mechanical treatment on the average oil extractability of three canola cultivars, Westar, Regent and Tobin, treated with enzyme (data from Sosulski *et al.*, 1988). In all three cases there is a favourable effect of the enzymatic treatment on the oil extractability. Almost no oil was extracted from whole treated seeds. More oil is recovered when the seeds are ground before extraction; flaking the seeds allows the recovery of 25·3 % on control samples, and 41·4 % of total oil is recovered from enzymatically treated seeds.

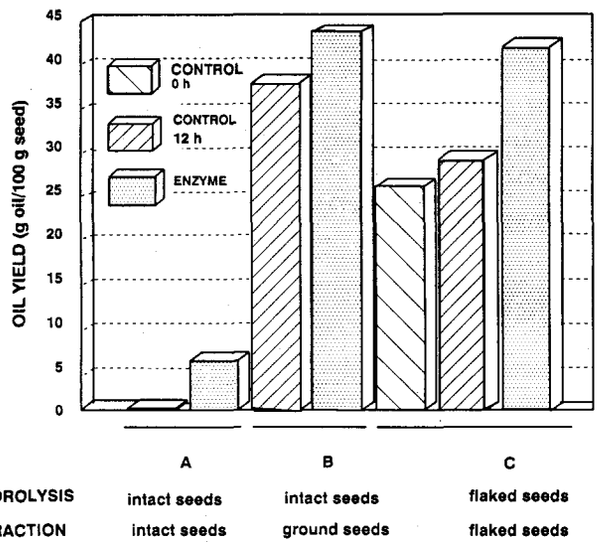


Fig. 17. Effect of the mechanical treatment on the average oil extracted from rape seed hydrolysed for 12 h; (A) intact seed hydrolysis and subsequent extraction for 14 h; (B) intact seed hydrolysis and ground seed extraction for 7 h; (C) flaked seed of canola (0·8 mm) are hydrolysed and extracted for 7 h.

Moisture

Figure 18 shows the combined effect of moisture (%) and enzyme concentration (g enzyme protein/100 g flakes) on the extractability reported as average oil extracted. The variety of *Brassica napus* used was Westar. The enzyme concentration was 0·12% (g enzyme protein/d.b.) and hydrolysis was carried out for 12 h at

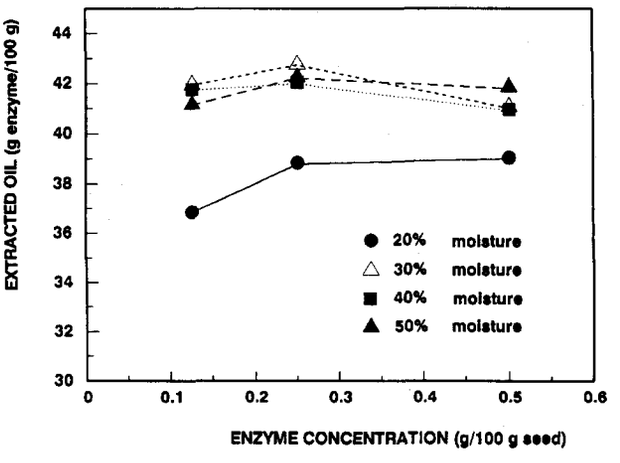


Fig. 18. Combined effect of moisture and enzyme concentration on the extraction of Westar canola (Sosulski *et al.*, 1988).

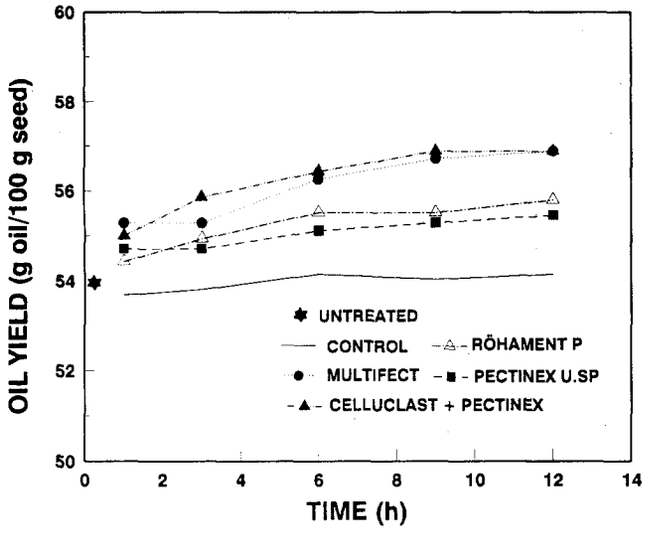


Fig. 19. Effect of the time of hydrolysis for sunflower kernels (2% (w/w), 40% moisture and ground before extraction for 3 h).

50°C with SP249. For concentrations below 0·35%, 30% is an adequate moisture.

Enzyme concentration

The cost of enzyme, as well as drying energy costs, are the most decisive economic factors of the process, so that the enzyme concentration has to be optimized together with the moisture percentage during the treatment. Although this variable clearly depends on the enzymatic formulation, an increase in the enzyme concentration allows a lower moisture, with the consequent sparing in drying energy, previous to the extraction as reported by Sosulski *et al.*, (1988). These authors found that for the selected moisture percentage, 30%, 0·125% (g enzyme protein/100 g flakes) was the optimum value, because no increase in oil yield was obtained at higher concentrations.

Time of treatment

This parameter significantly affects the extractability of the treated seeds and consequently the oil yield. Figure 19 shows the percentage of extracted oil from sunflower kernels untreated, treated with water (control) and treated with commercial enzymes at different reaction times. Although each formulation presents its particular behaviour, it can be concluded that times of around 8 h are adequate to enhance the extractability of the whole kernels (Domínguez *et al.*, 1992). Table 8 summarizes the effect of enzymatic treatment of oil seeds in the presence of reduced moisture on the oil extractability, measured as a percentage of oil extracted in Soxhlet for 3 h from the ground sunflower kernels.

Once the parameters that most markedly affect the process are optimized, the extraction process can be improved by pressing as Sosulski & Sosulski (1990) have shown working with flaked canola seeds. The throughput on the expeller was significantly increased

Table 9. Improvement of the pressing process of Westar canola after enzymatic treatment as compared to control (Sosulski & Sosulski, 1990)

Enzyme	Throughput (kg/h)	Oil flow rate (kg/h)	Efficiency of pressing (% of total oil)	Residual oil (% d.b.)
Control	18·0	6·4	78·7	16·8
Pectinase (extractase)	16·6	6·5	86·2	11·3
Cellulase (cellulase A)	21·8	8·9	89·3	9·1
Protease (fungal protease)	19·9	8·2	90·8	7·6
β-glucanase (finizym)	27·0	11·4	92·4	7·1
Multi-activity (olease)	25·8	10·9	92·6	6·8
Multi-activity (SP249)	25·8	11·2	93·6	6·5

from the control value of 18 kg/h to 25.8 kg/h for seed treated with multi-activity formulations. Thus, the yield of extracted oil was increased, and the residual oil in the presscake reduced, obtaining greater pressing efficiencies as Table 9 shows. As previously stated, the multi-activity enzymatic formulations are the most favourable during the pretreatment of the seeds when trying to improve the oil yield.

PRODUCT QUALITY

Oil

The oils obtained with enzymatic technology, stable enough to rancidity, show similar composition and structure to untreated ones. This technology was applied on an industrial scale for olive, and a pilot or semipilot scale for rapeseed and coconut; the improvement in the yield of oil was lower than in laboratory scale trials but other characteristics such as stability, acidity, and peroxide value were similar (Table 10). The quality of the oil (glyceride composition, acidity and stability) can be considered optimum. Oilcakes presented lower moisture values than controls and margins for treated samples had more volume and contained less oil (Alba, 1987). The raw oil recovered is clear and requires less purification to remove the degradation products obtained in conventional extraction processes. Aqueous enzymatically processed rapeseed yield a clear and light oil with a relatively low content of free fatty acids considering the high processing temperatures at pH 4·5.

Oil obtained by pressing from treated canola samples showed increased levels of free fatty acids, although the other characteristics were maintained (Sosulski & Sosulski, 1990). Peroxide values were low in all samples, although phosphorus levels were slightly increased in treated samples; degumming and refining decreased these values to less than 10 ppm. Oils from enzyme treated samples contained lower amounts of chlorophyll.

Table 10. Characterization of the oil from enzymatically treated oil fruits and seeds by different processes (several authors). In parentheses are indicated the limits of the standard values of each parameter corresponding to the fruits and seeds studied

Reference	Coconut Cintra *et al.* (1986)	Olive Alba *et al.* (1987)	Rapeseed			
			Aqueous Olsen (1987)	Solvent assisted Fullbrook (1984)	Reduced moisture Sosulski & Sosulski (1990)	Standard range
Saponification (mg KOH/g)	259 (251–264)	—	180–182	174	—	(168–181)
Unsaponifiables (mg/g)	—	11·2 (<1·5)	—	17·8	—	—
Iodine number		9 (7·5, –)	—	98	—	(94–120)
Free fatty acids	0·07 (—, 0·05)	0·26 (<3)	2·45–3·33	0·38	0·74	(–0·6)
Peroxide value (meq O_2/kg oil)	0·112 (–0·25) —	9·30 (<20)	2·36–2·41	9·62	0·40	(–10)
Refractive value	1·450 (1·448–1·450)	—	—	1·467	—	(1465–1469)

Meal

The rapeseed meal quality of treated samples has been compared with untreated samples. The analytical characterization of this product has been obtained separately for rapeseed oil and meal by aqueous processes and for canola (Canadian rapeseed cultivar) by pressing. Pilot plant data for the aqueous process (Olsen, 1986) show that the fat content in the meal is not as low as would be desirable and the solubilized protein was lost in the syrup; the proteinic meal has been considerably detoxified from glucosinolates and aromatic cholinesters. However only 80–90 % of the oil is recovered. Enzymatic pretreatment of canola increased meal quality by increasing digestibility, available lysine and by decreasing total fibre and glucosinolate contents (Sosulski & Sosulski, 1990).

CONCLUSIONS

There is a close relation between enzymatic action and the amount of released oil; enzymatically treated fruits or seeds show an increase in the oil yield in comparison to untreated samples. Regardless of the type of enzyme, the quality of the oil is good and its composition is not affected by enzymatic treatment (Duarte & Sameiro, 1979).

Although no reason has been given to explain this fact, the effectiveness of the treatment depends upon the seed. In the treatment of olive pastes the higher increments in oil yields are less than 2–3 g/100 g olive, nearly 20 % of the total oil initially present in the fruits, the increases registered in the extractability of the oil from seeds usually being higher. Treated olive pastes present a faster extraction rate and higher yields of recovered oil (Santos, 1978), as the rheologic modifications of the enzymatically-treated pastes favour the flow of liquids, reducing the pressing time and the residual oil. Also the oils from treated olive pastes are clearer than the ones from untreated pastes and no significant differences were found in organoleptic characteristics. Oil cakes from olive pastes present lower moisture and fat contents than untreated ones; also margins have lower amounts of solids and lower fat contents.

The enzymatic action is different depending on the seeds and within each kind on the cultivar. Therefore, an adequate strategy to efficiently carry out this enzymatic treatment depends mainly on the seed involved in the process, and also the extractive process influences the recovery of oil from different seeds. Water-extracted sunflower seeds give higher yields, in percentage of total extractable oil, after enzymatic treatment than peanut, which does not present a great difference between control and enzyme treated seeds (72 % versus 78 % for the latter, while 52 % versus 30 % for sunflower). For enzymatically-treated rapeseed in an aqueous process, 78 % is obtained versus 43 % for the control (Lanzani *et al.*, 1975). In the solvent-assisted aqueous process the efficiency of rape seed oil recovery can be significantly enhanced from 12·45 to 72·46 % of the total oil content, representing 60 % of increment (Fullbrook, 1984). Studies of the extractability of rape seed oil in the presence of reduced moisture associated with the conventional process, allow us to conclude that the enzymatic hydrolysis enhanced the oil extractability to 98·14%, that compared to 53·6 % for untreated Westar samples accounts for a 44·5 % of increment (Sosulski *et al.*, 1988).

Duarte & Sameiro (1979) concluded that, if economically feasible, the application of this enzymatic treatment would be of great interest to the oil extraction process, as it does not affect the oil quality. Olsen (1987) concluded that the aqueous enzymatic process, as an alternative to the pressing, can be performed on already-existing installations, which could be adapted without great investment. The cost of the enzymatic treatment was economically justified for olive oil; for rapeseed the higher cost of the oil (because only 80–90 % was recovered) is compensated by the higher quality of the meal.

Montedoro *et al.* (1975) verified a reduction in BOD and COD values of the waste waters obtained by the treatment with enzymes (with respect to untreated

effluents): 75 % reduction in BOD levels and 35–45 % in COD from enzymatically-treated olive pastes. This decrease is because treated olive mill wastewaters are less toxic and more susceptible to anaerobic digestion (Hamdi & Ellouz, 1992*a*,*b*).

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