The Use of Enzymes in the Processing of Oilseeds

PAUL D. FULLBROOK, Imperial Biotechnology Ltd., Kensington, London SW7 1NA, England

This paper reports work performed at laboratory scale. The work developed from an original project at the University of Reading in England to produce nutritive soluble protein hydrolysates from the waste seeds of melon fruit.

As the seeds contain ca. 30% w/w fatty material in addition to 50% crude protein (N× 5,7), it was considered necessary to produce a crude protein isolate from the seeds as a first step, which could then be used as starting material from which to produce the hydrolysate. Figure 1 indicates how this isolate was prepared (E-protein from the seed Egusi, a Nigerian word for melon seeds). Three fractions were obtained, the protein fraction being used for subsequent work. It was assumed that all of the oil material had been solvent extracted. The protein isolate hydrolysis was done at first using added proteolytic enzymes in the simple scheme illustrated in Figure 2. The reaction was maintained at constant pH and followed by chemical analysis of withdrawn samples, and the rate of addition of alkali during the reaction period.

However, what we found was that, as protein hydrolysis proceeded, extra oil was released from the crude isolate. The results of several experimental runs using several other enzyme systems are shown in Table I. Enzyme B1 not only solubilized 35% of the crude protein, and hydrolyzed 2.8% of the solubilized protein, but also released 16% of the total weight of the crude isolate as 'extra oil'. Enzymes from *Aspergillus* and *Trichoderma* sp. had similar effects. We then tried hydrolysis of the whole crushed seed with enzymes from *Aspergillus niger*, and obtained good solubilization of protein and higher yields of extractable oils.

Having demonstrated the principle, we then turned our attention to extraction of oil from more conventional oil seeds and chose soybean and rapeseed as illustrative material, using enzymes from the above source organisms.

The process procedure was essentially similar to that used previously, except that hydrolysis was done prior to product fractionation as indicated in Figure 3. The types of process investigated were aqueous hydrolysis followed by solvent extraction of the oil, and hydrolysis in the presence of solvent to extract released oil.

The main route to oil isolation in Figure 3 is shown by the bold lines and the dotted lines indicate byproduct isolation giving insoluble protein fraction or water solubles. The apparatus used was similar to that illustrated previously, except that a condenser was added to prevent solvent loss. The reaction was carried out over a 4-hr period and temperatures of 50 C and 63 C were used to allow maximum enzyme activity. A short heating period at 80 C was used to inactivate enzymes, prior to separation of fractions or oil extraction. The pH was chosen to be optimal for the enzymes under test, and maintained constant (but not optimized).

Results of yield of oil from oilseeds obtained using a simple aqueous hydrolysis with a mixed enzyme preparation from *Bacillus subtilis* is shown in Table II. Increasing the overall dosage of enzyme clearly caused a corresponding increase in oil yield. Yields could be considerably improved if hydrolysis was done in the presence of solvent, as illustrated by Figure 4. Maximal oil extraction was obtained with 3% enzyme levels, the solvent giving ca. 50% more oil yield for rapeseed.

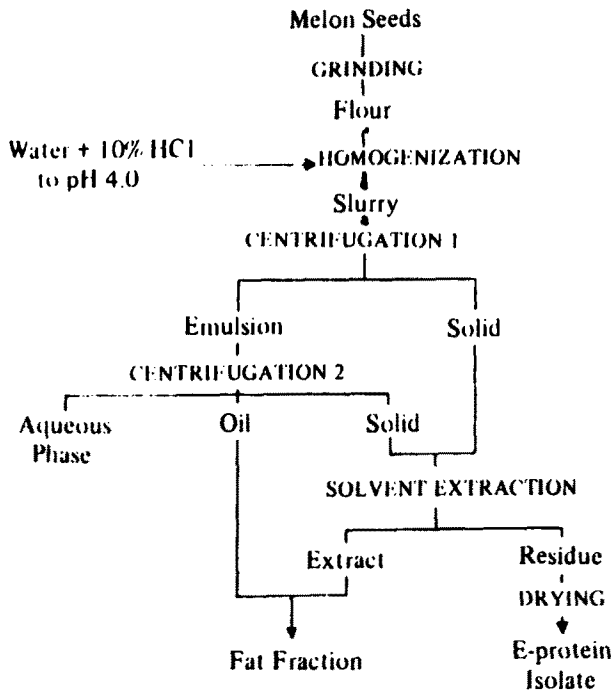

FIG. 1. Preparation of E-protein isolate—scheme of operations.

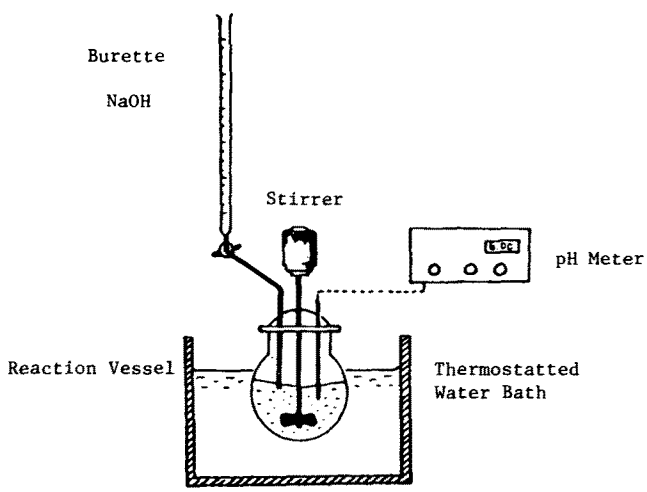

FIG. 2. Apparatus for laboratory preparation of protein hydrolysates.

We are seeking to optimize solvent:water:seed meal ratios and to investigate exactly why yields were improved, although our first indications are that it is a mechanical

TABLE I

Extraction of Melon Seeds—Results

Enzyme code	Source organism	Activity	% of raw material		
			Protein solubilized	Hydrolyzed	Oil released
B1	*Bacillus lichenformis*	α-Proteinase	35	2.8	16
T1	*Trichoderma harzianum*	Mixed	50	4.0	11
A1	*Aspergillus niger*	β-Glucanase	38	3.0	14
T2	*Trichoderma viridii*	Cellulase	43	3.4	14
A2	*Aspergillus niger*	Hemicellulase	47	3.5	19
A3	*Aspergillus niger*	Pectolytic	48	3.8	23
B2	*Bacillus subtilis*	Mixed	25	4.2	18

Enzymes B1, T1, A1 and T2 were used on E-protein isolate only. Enzymes A2, A3, and B2 were used on whole crushed seed.

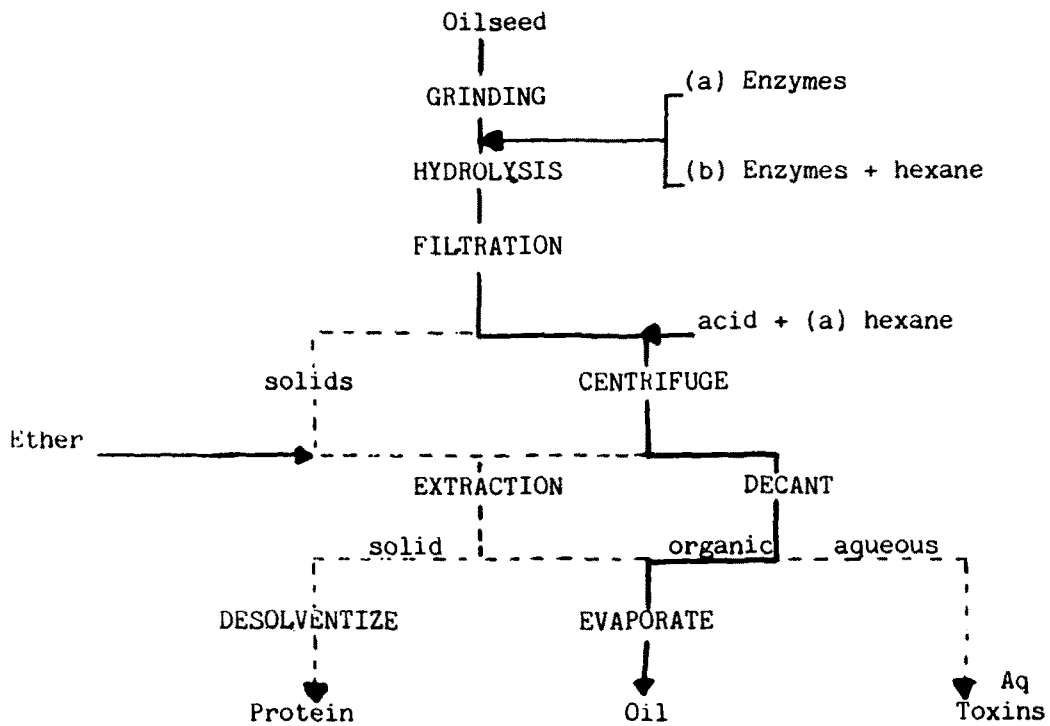

FIG. 3. Hydrolysis/extraction process—scheme of operations.

TABLE II

Oil Yield by Simple Hydrolysis

Enzyme concentration (% w/w basis)	Yield of oil obtained (%)	
	Rapeseed	Soybean
0	3.80	2.67
1	9.30	7.12
2	12.83	8.76
3	13.23	9.17

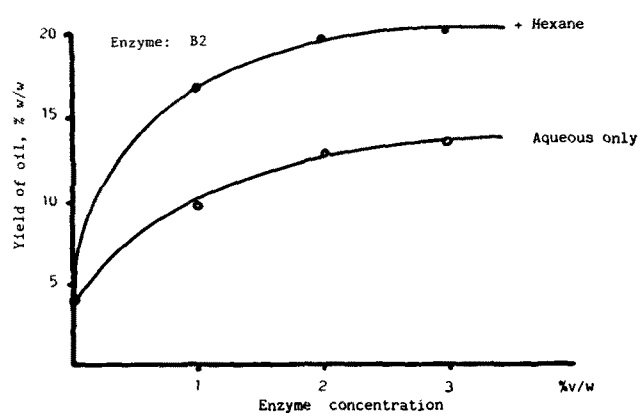

FIG. 4. Effect of solvent on yield of oil from rapeseed.

phenomenon due to oil binding to protein in the absence of solvent. It should perhaps be pointed out that in this and subsequent illustrations, although each curve is drawn using only 4 points, each point is the average of 5 separate experiments: e.g., the 9.3% point here is the average of values of 9.21, 9.32, 9.36 and 9.29 . . . , i.e., good reproducibility of experimental results.

Using different systems, higher yields of oil could be obtained (Fig. 5). In this and subsequent experiments, hydrolysis was done in the presence of solvent and results are expressed as oil yield both as a % of soxhlet extractable oil and on a w/w basis. Table III illustrates that enzyme preparations from *Aspergillus* give higher yield than from *Bacillus* sp. The yield of oil from soybeans was higher than for rapeseed, and ca. 90% yield was obtained, with 3% *Aspergillus* enzyme.

By plotting the increase in oil extracted as a function of reciprocal productivity against enzyme concentration (Fig. 6), a value of the efficiency constant for the enzyme for different oilseeds indicates their different degrees of extractability by the enzyme system. Similarly, different enzymes acting on a single oilseed substrate can be compared and quantified by different values of K, as the negative intercept on the x axis. We are working on this type of information to characterize the best systems on the various oilseeds. We realize that we have only demonstrated our results on a laboratory scale, but we are interested to see if the process could be improved and optimized and scaled up as an alternative to the current industrial processes for oil extraction. We are continuing this work to develop improved processes for higher oil yields, and have recently submitted a patent application.

The advantages of our process are:

- Energy and solvent usage is low—which must be of interest in these days of escalating mineral oil prices.
- Because it is a low temperature process, the protein is probably of a higher nutritive value—although we have yet to prove this.
- Aqueous processing of rapeseed will also remove phytic acid and other toxins from the residual protein, making it a more useful byproduct.
- Finally, the quality of the oil is good.

The process yields oil directly of a quality within codex specifications, so there is no need to refine the oil further, after extraction, which could mean a potential considerable economic saving.

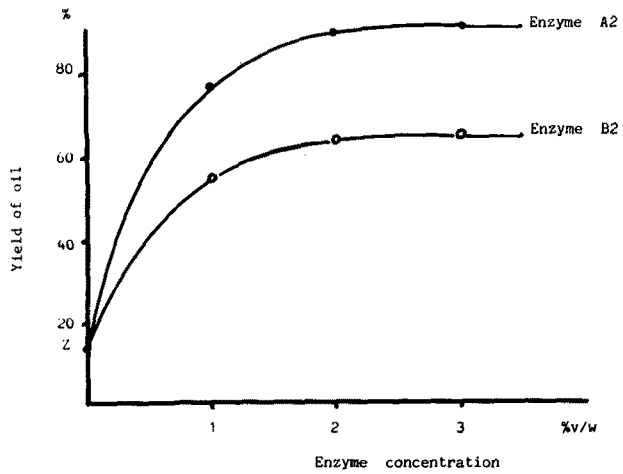

FIG. 5. Use of two enzyme systems in extraction of oil from soybean.

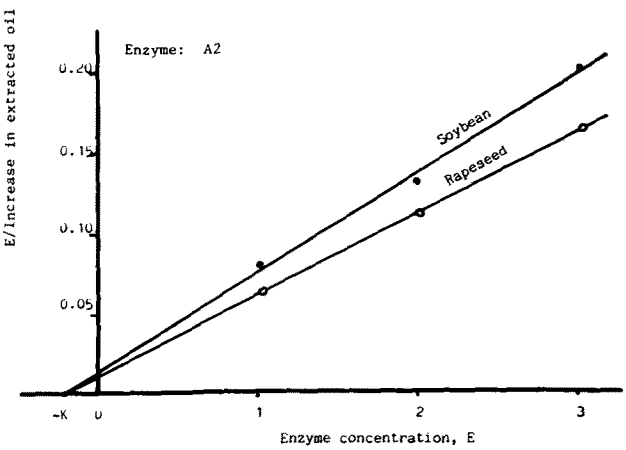

FIG. 6. Characterization of oil extractability.

TABLE III

Effect of Different Enzyme Systems on Oil Yield

Enzyme			Yield of oil obtained			
			Rapeseed		Soybean	
Producing organism	Type	Concentration used (% w/w)	a	b	a	b
		0	3.8	12.4	2.6	13.5
Bacillus		1	16.6	54.4	10.6	53.6
		2	19.8	65.0	12.4	62.2
		3	19.9	65.5	12.5	63.3
Aspergillus niger	1	1	18.5	60.6	14.2	71.7
		2	21.4	70.1	16.4	82.9
		3	21.5	70.5	16.5	83.8
Aspergillus niger	2	1	19.1	62.5	15.1	76.6
		2	21.9	71.1	17.5	88.6
		3	22.0	72.2	17.7	89.8

Yield expressed (a) % w/w of seedmeal; (b) % soxhlet extractable oil.