Using Enzymes for Oil Recovery from Edible Seeds

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Oilseeds and their products are the most valuable agricultural crops in the world trade with, ever-increasing demand for oil from edible oilseeds all over the world. India accounts for 9.6 per cent of the world’s oilseeds production. The demand for vegetable oils is increasing at 5 lakh t/y while the production is increasing at 2 lakh t/y only. The present demand over supply gap, in edible oils, is 1.8 million t needing to produce additional 5.4 million t oilseeds/y. Hydraulic, and expeller pressing, and solvent extraction are the three most common processes for oil recovery from oilseeds. Enzyme based oilseed processing technologies emerge as one of the most eco-friendly processing methods. The enzymes have specific mode of action, therefore, cellulase, hemicellulase and pectinase and even proteases are the most favourable enzymes for degrading the cell wall in oilseeds to loosen oil sacs embedded in the structures. The enzyme treatment has been found useful in conventional solvent extraction process also. Different factors like temperature, pH, moisture, grinding and size reduction of oilseeds are required by enzymatic processes which influence the efficiency of extraction, recovery of oil, that also helps maintain higher nutritive value. The usage of enzymes reduces environmental pollution with consequent reduction in BOD (Biological Oxygen Demand) and COD (Chemical Oxygen Demand) of the residues and wastewaters along with reduction in acid development and oxidation during further processing and storage. High cost and specificity of enzymes limit the enzyme usage for different oilseeds.

All over the world, an ever-increasing demand for oil from edible oilseeds is being witnessed. Since the beginning of history, people have made use of oils obtained from oilseeds and used principally as food. Oil constitutes a major portion of agricultural products. The sole objective is to increase productivity of oilseeds, in the wake of ever-increasing urbanization and industrialization.

India accounts for 9.6 per cent of world’s total oilseeds production from over 25 million ha of land, the largest area under oilseeds in the world. The average yield of oilseeds is only 900 kg/ha against the world average of 1,275 kg/ha and 2,500 kg/ha in USA.

Groundnut, rapeseed, mustard, castorseed, sesame, nigerseed, linseed, safflower, sunflower and soybean are the major oilseeds produced in the country with groundnut, rapeseed/mustard and soybean accounting for a major chunk of the output. The production of oilseeds in India rose dramatically from 108 lakh t in 1985-86 to 215 lakh t in 1993-94 with a target of 270 lakh t in 1998-99. The growth in oilseeds was 12 per cent per annum during 1985-86 to 1993-94 which dropped to just three per cent per annum during 1997-98.

The demand for vegetable oils is increasing at five lakh t/y while the production is increasing at two lakh t/y only. In the last six years, a demand over supply gap of 18 lakh t has been created and the gap stands at 1.8 million tonnes at present. In order to meet the gap, additional 5.4 million t/y oilseeds are required1.

The ever-increasing demand, the gap of demand over production, and increased production of oilseeds would necessitate a critical examination of look into oil extraction and processing technologies. It entails adoption of most modern technologies in extraction and processing of oils and also providing necessary technological inputs to the existing extraction and processing plants. Extraction technology, e.g. alternative solvents for extraction of oil including supercritical fluids (CO2), the enzymes assisted aqueous extraction and membrane technology for upgradation of protein quality, are the technologies that have a scope in the future2.

According to oil technologists all around the world, it is clear that more land will not be available for oilseed crop production. On the other hand, more pressure cannot be put on agricultural biotechnologists and scien-

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Table 1 — Enzymatic hydrolysis of oil seeds for enhancing oil extraction.

<table>
<thead>
<tr>
<th>Sl.no.</th>
<th>Material</th>
<th>Effect of hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>a Melon seeds</td>
<td>(i) Extra oil released</td>
</tr>
<tr>
<td></td>
<td>b Ground soyabean</td>
<td>(ii) Better oil quality</td>
</tr>
<tr>
<td></td>
<td>c Rape seeds</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>a Crushed Soyabean</td>
<td>Enhanced release of extractable oil</td>
</tr>
<tr>
<td></td>
<td>b Cotton Seeds</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c Castor Bean</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Canola Flakes</td>
<td>(i) Enhanced release of extractable oil</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(ii) Reduction in oil extraction time</td>
</tr>
<tr>
<td>4.</td>
<td>Soyabean</td>
<td>(i) Enhanced release of extractable oil</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(ii) Enhance oil recovery</td>
</tr>
<tr>
<td>5.</td>
<td>Groundnut</td>
<td>Enhance oil recovery</td>
</tr>
</tbody>
</table>

tists to increase crop yields. With rising values of oils, higher demand for quality in oil, coupled with several years of unfavorable climatic conditions in growing regions, there has been a noticeable increase in trials of enzyme based processing for a wider range of oilseeds. Enzyme based technologies have emerged as one of the most eco-friendly, in recent times.

A method for treatment of oilseeds with enzymes for softening and increasing the porosity of oilseeds to enhance the rate of oil recovery, has to be developed to minimise residual oil in oilseed cake and refining of high free fatty acid and dark colored oil. Besides increasing the efficiency of extraction, the method is environment friendly, e.g., a reduction of 75 per cent in BOD and 35-45 per cent in COD levels of wastewater were achieved after enzymatic treatment3, 4. The article aims at reviewing the results of different extraction processes and then examine the potential of the eco-friendly approach for oil recovery from oilseeds.

Structure of Oilseeds

For a better understanding of the possible role of enzymes, it is essential to consider the structure of oil-bearing materials. The oilseed cotyledon is the existence of discrete cellular organelles called lipid and protein bodies, which contains, respectively, most of the oil and protein in the grain. Protein bodies (aleurone grains) vary in size depending on the oilseeds, e.g., the average size of the protein body is 8-10 \( \mu \text{m} \) in soy and peanut5, and 2-20 \( \mu \text{m} \) in other oilseeds4, 6, 9. The protein bodies contain 60-70 per cent of the protein in oilseeds5. Lipid bodies (also known as spherosomes and oleosomes) are the principal repository sites of lipid reserves in oilseeds5, 11 which are 1-2 \( \mu \text{m} \) in most oilseeds5, 9, 10, although variation of 0.2-0.5 \( \mu \text{m} \) in soybean6, 12 to as large as 4 \( \mu \text{m} \) in cotton seeds13 have been observed.

Scanning electron microscopy (SEM) showed that lipid bodies of soybeans14 and peanuts5 are enmeshed in a kind of cytoplasmic network presumably composed of proteins14. The spaces between protein bodies in cotyledon cells are filled with lipid body and cytoplasmic network5, 12. The walls, which surround the cells, are primarily composed of cellulose, hemi-cellulose and lignin in addition to pectin15. Oil is usually inside of vegetative cells, linked with other macromolecules, such that partial hydrolysis of structural polysaccharide, the constituent of the cell wall and lipid body membrane of oilseeds15, 19 (Table 1) by enzymes makes the cell structure more permeable (Figure 1).

Oil Extraction Processes

There are many processes to extract oil with their associated merits and demerits (Table 2). Historically, the three most common processes for recovering oil from oilseeds are hydraulic pressing, expeller pressing and solvent extraction20, 21.

Hydraulic Pressing

Hydraulic pressing is the earliest process, which originated in Europe21. It is simple in operation, the ground seed material is placed in layers, with each layer separated from the other by a cloth. Pressure is applied, slowly
Table: 2 — Methods used in Oil Extraction

<table>
<thead>
<tr>
<th>Process of extraction</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical grinding</td>
<td>Conventional and easy operation</td>
<td>Oil remain in the cake</td>
</tr>
<tr>
<td>Aqueous extraction</td>
<td>Environmentally cleaner technology</td>
<td>Low in yield</td>
</tr>
<tr>
<td>Solvent extraction</td>
<td>Easy and instant</td>
<td>High solvent cost Uneconomical and Environment hazardous</td>
</tr>
</tbody>
</table>

Figure 1—Enzymatic hydrolysis of oilseeds wall

expeller can exert much greater pressure on the seedcake than a hydraulic press. The increased pressure permits recovery of a larger proportion of the oil present in oilseeds. Generally, 3-4 per cent oil is left in the cake when extracted with an expeller, compared to 4-6 per cent with a hydraulic press. Expellers vary in size, from machines that process 100 kg to more than 10 tonnes of oilseed / h.

**Solvent Extraction**

Solvent extraction of oil was a batch process in Europe in the latter half of the nineteenth century. Technological advances after World War I led to the development of continuous solvent extraction systems which proved to be excellent in processing oleaginous materials, including low oil content seeds. The modern solvent based extraction process consists of successive counter current wash extraction with hexane, of the previously cracked, flaked, ground or pressed oleaginous material. Hexane is then removed from oil, by evaporation and finally by vacuum distillation.

**Properties of Solvent Used in Oil Extraction**

Nearly all known oilseed extraction plants use hexane as the solvent, however, the industry is constantly looking for ideal solvent, one with high solubility at elevated temperatures and low solubility at ambient temperature which can be easily recovered from meal and oil, is plentiful, economical and finally that entails low energy costs, with high product yield, purity and market value. Solvent stability is the single most desired quality and it should not react with equipment.

Solvents, generally used in oil seed extraction, are hydrocarbon naphthas, trichloro ethylene, ethanol and ethanol-benzene. Recently the research focused on etha-
nol, isopropanol, methylene chlorides, acetone and hexane. However, in the last few decades, the light paraffinic petroleum fractions such as hexane, heptanes and pentanes were the most common solvents used. Hexane became the choice solvent as it evaporates easily and leaves no residual obnoxious odors or taste. Later the decision was further supported by Eaves and coworkers who investigated the extraction of cottonseed by five commercial solvents, viz, hexane, benzene, ethyl ether, acetone and butanone, and found that none compared favorably with hexane for the oil extraction. Solvent extraction is routinely used to extract oil from soybean, canola, sunflower, and other oilseeds.

**Physico-chemical Methods**

Elevated gas pressure is employed for transferring oil from particulate solids (oilseeds) to a transfer liquid (water) with the help of supercritical fluid (CO₂) which is used as a solvent for the displacement. Physical cracking of hull and beans is done as a pretreatment by heating to 60°C in a microwave dryer prior to oil extraction. Screw pressing is a physical force employed on vegetable oilseed, as a primary step prior to extraction in a common worm shaft rotating within a perforated barrel wall section and a non-perforated barrel wall section connected by an annular membrane with water injection nozzles, and then meal enters at inlet and oil is extracted through the wall. Other physico-chemical methods are used to increase porosity of oleaginous material such as rice bran, wheat mill feed, rapeseed, amaranth and other similar materials by stabilizing oil contained in them. Whereas removal of hull from oilseed by employing upward flows of gases for extended period of time such that the oilseed maintained within the plenum facilitates crisping of hull.

In another method oilseeds were treated with counter current flow of CO₂, ethane, ethene and/or propane at 40-110°C at pressures of 250-750 bar for 0.5-2.5 h with solvent ratios of 5-30 kg solvent/kg cake. Among the chemical methods, 2-25 per cent acetic acid (v/v) was used along with hexane to prepare ginned cottonseed for oil extraction by a solvent. As acetic acid in solvent increased, the concentration of total lipids, phospholipids and neutral oil in miscella also increased. The solubility of protein in 0.02 N NaOH did not decrease until the acetic acid used to prepare the meal increased to 4-10 per cent.

The major limitation of the conventional extrusion-expelling methods is the large residual oil left in the cake after extraction. Using hexane for soaking oilseeds, as a treatment, and subsequent recovery of hexane from the miscella (hexane and oil mixture) and from the marc (residue left after oil extraction) are both energy intensive processes that require extensive capital equipment.

Oil extraction by conventional mechanical methods do not ensure complete recovery of oil and the cake residues still contain 6-10 per cent oil. It leads to recurring loss of oil, running into millions of tones/yr. Oil extraction by different methods like soxhlet, automated soxhlet and sonication, microwave, SFE, ASE (accelerated solvent extraction) consumed 15-500 mL solvent/kg and average extraction time varied from 12 min to 48 h.
Table 3 — Parameters suitable for enzyme action on oil seeds.

<table>
<thead>
<tr>
<th>Oilseed</th>
<th>pH</th>
<th>Temperature, °C</th>
<th>Enzyme</th>
<th>Extracted oil, per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapeseed</td>
<td>6.6</td>
<td>70</td>
<td>Protease, α-1, 4</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>galacturonide acids</td>
<td></td>
</tr>
<tr>
<td>Soyabean</td>
<td>4.5</td>
<td>---</td>
<td>Proteolytic</td>
<td>86</td>
</tr>
<tr>
<td>Sunflower</td>
<td>5.0</td>
<td>Room temperature</td>
<td>Cellulase, α-1, 4</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>galacturonide acids</td>
<td></td>
</tr>
<tr>
<td>Peanut</td>
<td>4-10</td>
<td>60-65</td>
<td>Protease, Cellulase</td>
<td>74-78</td>
</tr>
</tbody>
</table>

Aqueous Enzymatic Oil Extraction (AEOE)

Aqueous extraction process (AEP) using water alone as the medium, was an alternative to solvent extraction of oil in 1950s as a safer and cheaper method. AEP used a distinctive principle compared to the usual solvent extraction process based on the capacity of oil to dissolve in and be extracted by a solvent. In AEP, as oil does not have high chemical affinity for the extraction medium, i.e., water, therefore, there was no chemical potential for oil dissolution.

Extraction of oil by AEP attracted attention for several years as it was based more on the insolvability of oil in water than on the dissolution of oil. The water soluble components of oil crops diffused in water rather than in oil, thereby releasing oil, previously bound in the original structure. The unit operations involved in AEP are:

(i) Size reduction (grinding) of seed/fruit material,
(ii) Extraction,
(iii) Solid-liquid separation,
(iv) Separation of oil rich phase,
(v) De-emulsification for further recovery of oil, and
(vi) Drying to remove moisture.

Grinding was carried out with a pestle and mortar, and the ground meal was boiled in water, liberating oil, which floated on to the surface. The oil was then carefully scooped out from the water surface, and dried to remove moisture.

In the modern aqueous process, centrifuges have replaced the gravity separation. For the application of AEP, different oilseeds require specific changes in conditions such as pH and temperature due to differences in chemical composition and the physical structure of oilseeds17-46(Table 3). It naturally makes sense to use all cell-wall degrading enzymes to facilitate the release of oil. The approach has several advantages, some inherent in the AEP, and some arising out of the use of enzymes.

(i) It nearly eliminated use of organic solvents resulting in (a) savings on the cost of such solvents, and (b) possibly low investments in infrastructure.

(ii) Simultaneous recovery of oil and protein fractions. It was one of the early driving forces which motivated people to explore AEP.

(iii) Quality of Oil—It is now well established that use of AEOE resulted in oil composition, which was very different from the one, obtained by 'conventional' means. A good illustrative example is the recent work by Ranalli et al.47 on olive oils where the oil obtained by enzymatic process had (a) higher contents of phenolics, tocopherols, trans-2-hexanal and other volatile aromatics, (b) enhanced oxidative stability, (c) lower turbidity values, and (d) higher ratios of 1,2 diglycerides/1,3 diglycerides, campesterol/stigmasterol and trans-2-hexanal/total aroma. Overall, better qualitative characteristics and higher oil yields were reported. 'Cytolase O' (Gist-Brocades, France) was the enzyme used in the process that mostly possessed pectinase and cellulase activities.

(iv) Degumming may not be needed.

Phospholipids, especially phosphatides, in oils, cause 'gumminess'. Lecithin and cephalin are common phospholipids found in edible oils. Soybean, corn, cottonseed and rapeseed are some of the major oils, which contain significant levels of phosphatides. Degumming is
Table 4 — Enzymatic extraction for different oil bearing material

<table>
<thead>
<tr>
<th>Seeds</th>
<th>Use level, per cent (w/w)</th>
<th>Enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapeseed</td>
<td>0.1 to 3</td>
<td>Pectinase, Cellulase</td>
</tr>
<tr>
<td>Soybean</td>
<td>0.2</td>
<td>Protease</td>
</tr>
<tr>
<td>Coconut</td>
<td>0.1</td>
<td>β-Glucanase, Pectinase, α-Amylase and Protease</td>
</tr>
<tr>
<td>Avocado</td>
<td>1.0</td>
<td>α-Amylase</td>
</tr>
<tr>
<td>Sunflower</td>
<td>1.5 (Ultrazyme)</td>
<td>Cellulase, α-Galacturinido-glicano hydrolase</td>
</tr>
<tr>
<td>Peanut</td>
<td>3.0</td>
<td>Cellulase, Protease</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>Cellulase, Protease, and Ultrazymes</td>
</tr>
</tbody>
</table>

Removal of phosphatides also removes bound iron with it and as such the oil obtained after enzymatic degumming is reported to be more stable. A new degumming process has been developed using a natural enzyme as a catalyst for phosphorus removal that can be used on all types and qualities of vegetable oils to reduce phosphorus to less than 10 ppm and thus potentially treated before physical refining.

The use of enzymes, from 0.1 to 3.0 per cent (w/w) in rapeseed, soybean, coconut, avocado, sunflower and peanut resulted in higher oil yields in AEP (Table 4). Selected enzymes that include cellulase, pectinase, protease, β-glucanase and α-amylose, when used on different types of oilseeds, resulted in higher oil extraction yields.

The Quality of Protein Residue

High temperatures involved in the conventional process are associated with decreased nutritional availability of some essential amino acids, e.g., lysine, due to Maillard reaction between free amino group in proteins and reducing sugars, resulting in decreased biological value of such proteins.

Ambient temperatures used in AEP avoided protein denaturation. 'Food grade', instead of 'feed grade', protein residues were obtained after AEP as: (i) it either inactivated or removed antinutritional factors present in oilseeds, (ii) it removed bitter, poisonous alkaloids from lupin seeds and non-gossipol pigments from cottonseed, (iii) it removed toxic goitrogenous sulfur compounds in the case of rapeseed, (iv) it also removed phytic acid and other toxins, and (v) caffeine and chlorogenic acids which cause sunflower seed meal to turn dark green or brown, were effectively removed in the aqueous process. Such polyphenols produced O-quinones, an extremely reactive class of compounds. Chemistry of the reaction of O-quinones with proteins and its nutritional consequences has also been reported.

Factors Affecting Oil Yield

Conditions required for oil extraction varied according to the oilseed composition and structure. Factors which influence the efficiency of extraction include grinding, size reduction of oilseeds, pH, moisture, temperature, time, solid:water ratio, degree of agitation and the number of extraction stages. Sometimes, just simple stirring was sufficient to obtain high yield as in the case of peanut and sunflower.
Grinding

Grinding has been considered a critical step in extraction process that directly affected oil yields. Smaller particle size allowed easier diffusion of water-soluble components leading to disintegration of the original structure and facilitated oil release; and it enhanced enzyme diffusion rates.

The grinding operation may be carried out either wet or dry depending on the oilseed. The choice between wet or dry grinding was dependent on several factors, such as initial moisture content, chemical composition and the physical structure of oilseed. In case of materials with high moisture content, e.g., coconut, wet grinding was more appropriate resulting in less than 5 per cent of oil in the residue after extraction. Thus avoiding additional intensive drying step before grinding. In case of materials with low initial moisture content like peanuts, rapeseed and soybean, dry grinding was more suitable. Excessive grinding favored cell rupture and increased the efficiency of oil extraction, however, it also produced smaller oil globules, which made de-emulsification more difficult. Insufficient grinding, on the other hand, resulted in loss of oil in residue.

Size Reduction of Oilseeds

The extraction of oil from oilseeds, either by mechanical pressing or by solvent extraction proceeded more efficiently, if the seed was first flaked or ground. Opinion was divided whether grinding or flaking was more effective in rupturing oil cells. Flaked oilseeds yielded a larger fraction of 'easily extractable' oil on treatment with solvents and a smaller fraction (usually 10-30 per cent of total oil) that was difficult to extract.

Moisture

Control of moisture was important for efficient pressing. Moisture content varied with oilseed and method used for pressing. Moisture control was also important in solvent extraction. Generally, intermediate moisture levels (4-7 per cent) resulted in efficient extraction of oil from oilseeds. Low moisture levels usually resulted in lower efficiency due to the lower solubility of phosphatides, while high moisture could cause swelling of proteins with subsequent reduction of flake porosity and solvent diffusion rate. As with pressing, moisture varied with the material being extracted, approximately 7-10 per cent for cottonseed and 8-12 per cent for soybean.

Temperature

It also showed considerable effect on oil extraction yields, though some authors considered it important in the hot water flotation process, and they ensured that boiling water was used for the extraction. However, prolonged boiling might not affect yield in certain oilseeds. The extraction temperature was not critical for protein recovery but important for oil extraction. The maximum oil recovery occurred between 40-60°C (Table 3).

Solid:Water Ratio

The highest possible solid:water ratios were desirable in the extraction step to obtain less stable emulsion and generate less effluent. However, to obtain the highest extraction rate and extraction yield, the use of large quantities of water was necessary. Recommended solid:water ratios are shown in Table 5.

SWOT Analysis of Oil Extraction

SWOT (Strength, Weakness, Opportunities and Threats) analysis has been an excellent, and fast tool for exploring the possibilities in initiating new programs. It has also been used as a decision making aid. The SWOT approach requires an internal survey to record strengths and weaknesses and an external survey to enlist threats and opportunities. It is a general tool, mostly used in the preliminary stages of decision-making and also as a precursor to strategic planning in a variety of applications.

Strengths and Weaknesses

These are internal (endogenous) characteristics that can be usually controlled or managed around and include...
Table 5 — Solid water ratio in extraction of oil from oil seeds

<table>
<thead>
<tr>
<th>S No.</th>
<th>Oilseeds</th>
<th>Solid water ratio</th>
<th>Oil yield*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Peanut*</td>
<td>1:5 to 1:12</td>
<td>89</td>
</tr>
<tr>
<td>2</td>
<td>Sunflower*</td>
<td>1:10</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>Soybean**</td>
<td>1:12</td>
<td>86</td>
</tr>
<tr>
<td>4</td>
<td>Rapeseed*</td>
<td>1:2.5 to 1:3.5</td>
<td>90</td>
</tr>
</tbody>
</table>

*based on the extracted oil from the seed.

factors such as geographic resources, climate, financial resources and the tax environment, human resources and skill information system, research and development capability, infrastructure/utilities, and the role of government and the regulatory environment.

Opportunities and Threats
These refer to external factors that enhance economic and human development. These factors include markets and trade, customer, industry trends, economic factors and technology.

The Strengths
Scientific and production innovations were applied which rapidly increased productivity in oilseeds. It would enable the industry to further expand and diversify its production base to capitalise on export market opportunities. Further, the enzymatic process has many advantages:

(i) It is eco-friendly, simple, and relatively inexpensive and produces little waste.
(ii) There is no need for degumming as the process is carried out in an aqueous medium, and phospholipids are also separated from oil.
(iii) It removes undesirable constituents presents in oilseeds.
(iv) It helped refine free fatty acid (FFA) and dark colored oils.
(v) It reduces BOD and COD levels, acid development, and oxidation during further processing and storage.
(vi) The use of enzymes has the potential to increase productivity, efficiency and quality in agro-industrial processing operation in many developing countries.
(vii) Enzymes enhance extraction and separation processes, eliminate toxic and anti-nutritional factors, catalyse carbohydrates, proteins, and lipids conversion through anti-oxidant and bio-catalytic activity.
(viii) It has reputations for supply of high quality, clean, dry products.

The Weakness

(i) Necessary enzymes are not available at low enough cost.
(ii) Capacity to produce, process and store quality oil, on a commercial scale, is currently limited.
(iii) Recovery of hexane is energy intensive process and requires extensive capital equipment.
(iv) There is insufficient work done to draw any definite conclusions on the possibility of using enzymes in softening seed coat and the impact of such a pretreatment on oil recovery.

The Opportunities

(i) A Technology Mission on Oilseeds (TMO) was established in May 1986 for harnessing the best of production, processing and management technologies.
(ii) Intensification of research efforts to increase production of oilseeds.
(iii) Increasing areas under non-traditional oilseeds crops.
(iv) Setting up of necessary processing and infrastructural facilities to keep pace with the production programme of oilseeds.
(v) Better incentive to producers through fixation of minimum support prices of major oilseeds.

To encourage industry modernization and technological upgradation, certain equipment considered necessary for modernization has been granted concessional rate of custom duty.

The Threats

(i) Rising costs of production and processing.
(ii) Government intervention in trade and production policies.
(iii) Reduction of R&D funding and other government support.
(iv) Technological barriers due to limited information available about most of the oilseeds.
(v) Conservative nature of oil extraction and processing industries.
(vi) There is excessive control and regulation by the Government which is difficult to justify in modern economy.

Enzyme Based Oil Extraction
Enzyme treatment is probably the most important in oil extraction as it digests the complex cell wall of oil-
seeds, altering permeability favouring oil extraction. After enzymatic treatment individual components, viz., protein, oil and polysaccharide could be conveniently separated with further processing. The treatment appeared to increase the productivity, efficiency and provide quality output in agro-industrial processing in many developing countries. Enzymes enhance extraction and separation processes, eliminate toxic and anti-nutritional factors, catalyse carbohydrate, protein and lipid conversion through their antioxidant and biocatalytic activities. The energy costs associated with processing were reduced, the nutritional quality and safety of foods improved, and the processing time were shortened. New products may be generated and alternative application for several agricultural product may be realized after enzymatic treatment.

Enzymatically treated oilseeds showed an increase in the oil yield in comparison to untreated samples. Regardless of the type of enzyme, the quality of the oil was good and its composition was not affected by the treatment. Enzyme may either be directly incorporated or may be immobilized on inert support matrices (immobilized enzymes) and allowed to act.

Enzymatic hydrolysis as a pretreatment of oil seeds have been shown to be effective for quick softening of seed coat which opened up oil cell walls and also broke up the complex lipoprotein and lipopolysaccharide molecules (not extractable for oil) into simple molecules releasing oil from lipid bodies enmeshed with carbohydrate and protein structures. Seed lipid bodies contained abundant proteins termed oleosins, which seem to play a major role in stabilizing these bodies. The structure of oleosins is generally the same for most oilseeds; it consists of low molecular weight proteins in the range of 15000 to 26000. The enzyme mediated oil extraction process would reduce loss of oil that normally remained in the seedcake after extraction (Figure 1).

The oilseed composition determined choice of enzymes. However, it was very difficult to compare results reported in different studies with a view to select the best enzyme combination for a given oilseed as most of the studies employed different extraction conditions. Different oil yields obtained with different enzymes for the same oil-seed reflected differences in the enzyme efficiency with respect to oil release. An enzyme system developed for one type of oilseed material could not be adopted to another oilseed. Also, the number of oilseeds tested so far has been very limited. In future, enzyme technology would play a big role in improving the yield and quality of oils.

It has been found that use of proteases, cellulases and hemicellulases helped release more oil both during solvent extraction as well as when expellers were used. Proteolytic enzymes mainly hydrolyzed proteins in cell membranes as well as inside the cytoplasm. The specificity of each carbohydrase, a rational choice of the enzyme for a given oilseed could only be made after gaining an understanding of the complex arrangement of polysaccharides in the cell wall. Primary cell walls of a variety of higher plants contained cellulose fibres to which strands of hemicelluloses are attached. Therefore, enzyme preparations capable of attacking cell walls would necessarily contain a mixture of cellulases, hemicellulases, pectinases and even proteases.

Enzyme mixtures gave better results compared to that with individual enzymes, e.g., the oil extraction yield from coconut was as high as 80 per cent with the combined treatment of polygalacturonase, ß-amylase and protease. Aqueous hydrolysis of dehulled rapeseed by a mixture of three cell wall degrading enzymes, pectinase, cellulase and hemicellulase increased permeability of cell wall allowing more efficient extraction of the oil. The effect of different carbohydrases was evaluated on the extraction time and yield of canola oil.

Oil extraction yields above 90 per cent were obtained using galactomannan combined with a polysaccharide enzyme-degrading complex. These enzymes degraded components of structural cell wall which contained mannan, galactomannan, arabinoxylagacton and cellulose. The recovery of oil from oilseeds could also be increased.

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulase&lt;sup&gt;55&lt;/sup&gt;</td>
<td>Trichoderma viridi</td>
</tr>
<tr>
<td>Hemicellulase</td>
<td>Aspergillus niger</td>
</tr>
<tr>
<td>Protease</td>
<td>Bacillus licheniformis</td>
</tr>
<tr>
<td>Extract</td>
<td>Bacillus subtilis</td>
</tr>
<tr>
<td>Cellulase&lt;sup&gt;66&lt;/sup&gt;</td>
<td>Sigma Chem. Co. USA</td>
</tr>
<tr>
<td>Hemicellulase</td>
<td>Sigma Chem. Co. USA</td>
</tr>
<tr>
<td>Extract&lt;sup&gt;68&lt;/sup&gt;</td>
<td>Aspergillus fumigatus</td>
</tr>
<tr>
<td>Cellulase&lt;sup&gt;68&lt;/sup&gt;</td>
<td>Humicola lanuginosa</td>
</tr>
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<td>Sigma Chem. Co. USA</td>
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</tbody>
</table>

Table 6 — Enzyme involved in pretreatment of oil seeds and their sources
by addition of microbial enzymes. Enzymes obtained from thermophilic moulds were much superior due to greater thermostability. Because of this, higher ambient temperature (45 to 60 °C), in industrial processes could be used with less contamination and cooling costs. Various crude enzymes have been isolated from different sources, viz, *Aspergillus fumigatus*, *Humicola lanuginosa*, and *Sporotrichum thermophile* and used in oil extraction.

The recovery of oil increased up to 2-5 per cent with *H. lanuginosa* in cottonseed while in sunflower and soybean oil it increased up to 4.2 per cent with *Aspergillus fumigatus*. The solvent oil extraction yield increased by around 20 per cent when kernels of shea tree were pre-treated with a mixture of protease and carbohydrates. In soybeans, the enhancement in expelled oil was up to 2.8 per cent of moisture free oil when pre-treated with crude enzyme preparations obtained from *Aspergillus fumigatus*. The gain corresponded to about 11.7 per cent more oil recovery, equivalent to 63.5 per cent of the total extractable oil. In groundnut kernels, pretreatment with cellulase resulted in 2.7 per cent more oil yield. Enzyme preparation from *Aspergillus* gave higher yield, and that from *Bacillus* solubilised 35 per cent crude protein and released 16 per cent of total weight of crude isolates as extra oil.

**Production of Enzymes for Enzyme Assisted Oil Extraction**

It is apparent from the foregoing that while the individual enzyme formulations, best suited for a particular system, had to be experimentally worked out, and generally proteases, cellulases, pectinases, hemicellulases and phospholipases were needed as obtained from various sources (Table 7). As the volumes of enzymes involved in the application are rather large, the cost of enzymes becomes a critical factor in the adaptation of this technology on a commercial scale. A large component of the high cost is due to downstream processing. The pretreatment of oilseeds and other such materials for loosening the structural matrix to facilitate oil recovery, apparently, does not demand high purity levels in enzyme preparations, it should, therefore, be possible to develop specific strategies for enzyme purification, which are efficient, economical and suitable for such applications. Less elaborate, toned down purification protocols with fewer steps and high selectivity might just be suitable.

Several relatively new techniques like expanded bed affinity chromatography, perfusion chromatography, three phase partitioning and affinity precipitation that emerged in recent years, along with some more well established approaches like two phase affinity extraction have been increasingly utilized to develop less costly procedures for obtaining enzymes. Use of fusion tags like oligohistidine residue to recover the largest protein via metal affinity has been increasingly used and appear to have the potential to prove useful in obtaining commercial grade enzymes for bulk applications like oil release.

It is quite possible that the three factors would combine in future to increase the use of enzyme in this area at an industrial level. First is, of course, the cost of enzymes. Second is the increase in oil yield upon enzyme treatment. However, environmental concerns in all probability will exert greater pressure for the adoption of enzyme assisted oil extraction by the trade in addition to the enumerated benefits. Also, the possibility of simultaneous oil and protein recovery, and energy saved by eliminating need for organic solvent stripping and further cost saved in process monitoring (as investment in volatile organic compound emission monitoring and control is not needed) may offset to a significant extent the, high cost of enzymes factor. Hence, one would actually see this green technology being increasingly used in oil extraction.
Conclusions
The use of enzymes has the potential to increase productivity, efficiency and quality of oil obtained. It requires a simple manufacturing base with low capital investment and relatively small amounts of energy consumption in comparison to other methods of oil recovery and processing.

Although basic information on most enzymatic processes is available, it is generally restricted to laboratory-scale studies. Enzymes and enzyme-catalyzed processes, however, need to be more fully explored and exploited in the light of benefits that may accrue from their use. Systematic process engineering investigations and economic evaluation of these processes appear necessary before venturing into scale-up studies.

Acknowledgements
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References
29. Lingasesedar, Apparatus and method for the extraction of vegetable oils, U S Pat 5680812, 28 October 1997.


