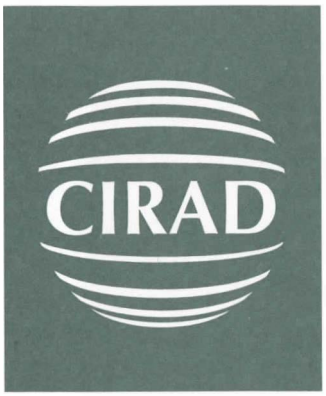
AVOC LAURIC OILS SYMPOSIUM



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NEW TRENDS IN BIOPROCESSING OF FATS AND OILS POSSIBLE APPLICATIONS TO LAURICS

Jean GRAILLE and Michel PINA

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Jean GRAILLE and Michel PINA
Lipotechny Laboratory- Tree Crops Department - CIRAD
PO BOX 5035
F 34032 MONTPELLIER CEDEX - FRANCE
Tel: (33) 67 61 58 81 - Fax: (33) 67 61 55 15

The use of enzymes in organic chemistry has become a vast multidisciplinary field; its merits in terms of practical applications or fundamental approaches have become increasingly clear over the years.

Biochemists have long known how to describe biological reactions based on *in vitro* reactions carried out under near-natural conditions, but although orthodox biochemistry is of fundamental importance for understanding biological environments, we cannot ignore non-conformists who have opened up the way for bio-organic chemistry and, especially for what concerns us most, those who are developing lipid biotechnology which, in practical terms, means providing industrialists with effective and ingenious bioprocesses.

The basic concept developed by unconventional chemists or biochemists is based on the fact that, in theory, there is nothing to prevent our considering enzymes as simple catalysts that are appropriate for numerous reactions that may seem somewhat exotic to traditional biochemists but are perfectly routine for bio-organic chemists. Such catalysts can offer numerous advantages, particularly specificity, regioselectivity, enantioselectivity, and the ability to catalyse inverse reactions of reference biological reactions and to catalyse reactions between molecules that are different from reference molecules.

In this article, "enzyme" signifies enzymes in their *sui generis* state and those attached to artificial or natural supports; by natural supports we mean the cells themselves, devitalized or not, growing or not, and dried plant latex powders, such as those from *Carica papaya* (papain) and from *Hevea brasiliensis*, bromelain from *ananas sativus*, lipases from defatted castor beans, or from pepper corns, or soybean lipoxygenase, or almond betaglucosidase, etc.

Although we cannot lay claim here to an exhaustive review of all aspects of lipid biotechnology, we shall endeavour to cover the basics of what is being done nowadays or what may be done in the future, with, wherever possible, concrete examples involving lauric oils at all levels of major industrial operations: oil milling, refining, processing and by-product valorization.

OIL MILLING

The profession has always endeavoured to bring about the best conditions for oil extraction from seeds and pulps. Mechanical processes alone do not result in total extraction and the process is very often completed with hexane, especially when seeds are involved. For several years, attempts have been made to determine why oil was systematically left in seeds and pulps. It is now known that despite thermomechanical treatment of oil-bearing tissues, not all the cells burst; heat treatment, such as sterilization in the case of palm oil, is designed, alongside lipase denaturation, to sterilize and detach palm kernels from the shells and help in dislocating the lignocellulosic and pectic structure that maintains the cohesion between oil-bearing cells; pressure is then applied to burst the weakened cells and expel the oil.

Many researchers then imagined that, starting from the premise that both the structure ensuring cohesion between cells and the cell walls themselves could be digested enzymatically, assisting conventional

extraction with an enzyme or a combination of appropriate enzymes should help to improve the extraction rate significantly.

Studies have now been going on for many years with cellulases, hemicellulases, pectinases, proteases and amylases, etc.

We would quote for example the work by LANZANI *et al.* (1) on rapeseed, sunflower and groundnut, FULLBROOK (2) on rapeseed and soybean, CINTRA *et al.* (3) on coconut oil, BUENROSTRO and LOPEZ-MANGUIA (4) on avocado, BOUVIER and ENTRESSANGLES (5) on palm oil, and lastly a great deal of work on olive, mostly by Italian and Spanish teams, notably LEONE *et al.* (6) and MARTINEZ-SUAREZ (7).

Enzyme-assisted extraction could come into its own if it were integrated into processes derived from a profound rethink of conventional oil mill processes; such a rethink is now prompted by various socio-economic parameters.

- Improving quality (risk of aflatoxin and polycyclic aromatic hydrocarbons in coconut products).

- Reducing production costs, for example by producing virgin coconut oil, which has a typical pleasing aroma and does not need refining. This gives a flavour oil, such as the walnut and olive oils produced by the European Union, or the red and virgin palm oils from West Africa (smallholder processes) or the red palm oil produced by UNITATA in Malaysia as a new health food rich in carotenes, tocopherols and tocotrienols.

- Environmental conservation and reducing the risks involved in the use of solvents such as hexane; many technologists are now thinking about abandoning traditional pressing and hexane extraction.

The products obtained would be of higher quality and cheaper to refine, and by-products such as presscake would be more satisfactory for animal and human consumption.

BHATTACHARYYA (8) is working in this field to obtain rice bran oil; also worth mentioning is the work by CHRISTENSEN (9) for rapeseed oil obtained without pressing or hexane extraction, but by fine grinding, resulting in a type of emulsion, followed by enzymatic treatment, then separation by centrifugation and drying; he has obtained 380 kg of oil, 450 kg of meal and 170 kg of molasses from 1,000 kg of seeds.

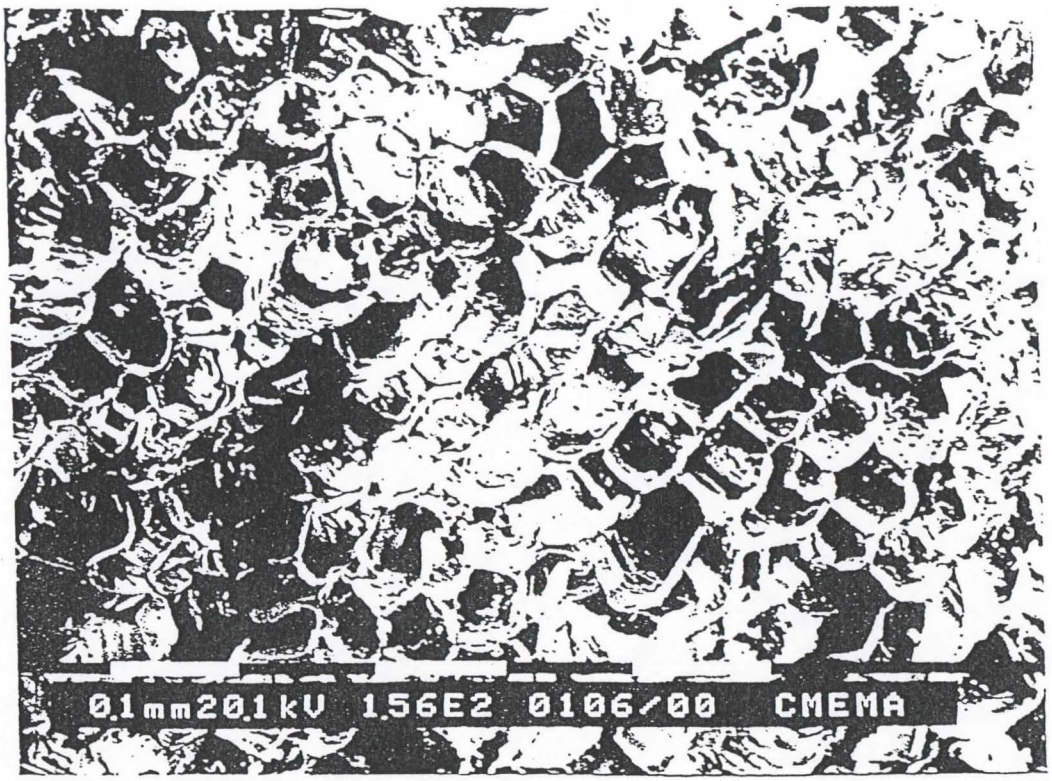
Lastly, as regards lauric oils in particular, we would mention the work by BERTRAND (10) on assisted extraction of coconut oil. This researcher has demonstrated that macerating the finely ground meat of ripe coconuts triggers the expression of an endogenous mannanase that destroys the mannan rich hemicellulosic tissue, thereby enabling oil extraction from the weakened oil-bearing cells.

The optimum conditions developed in the laboratory are as follows:

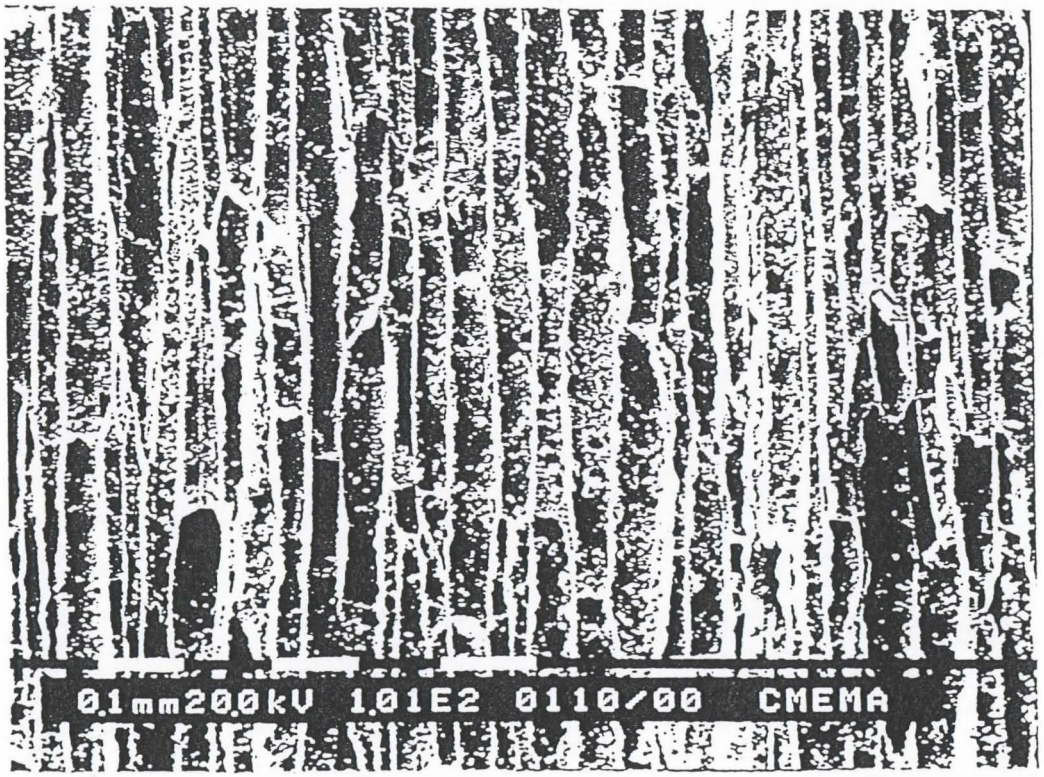
- maceration time: 1 to 2 hours,
- temperature: 30 to 50°C,
- pH: 5,
- water:meat ratio = 3 weight for weight,
- energetic stirring.

Observations under the electron microscope clearly revealed the effectiveness of the system (figures 1 and 2).

Figures 3, 4 and 5 show the structure of the cells, which are tubular with pentagonal or hexagonal cross-sections. Aleurone grains (storage proteins) can be seen dispersed in the aqueous phase and the oil globules.

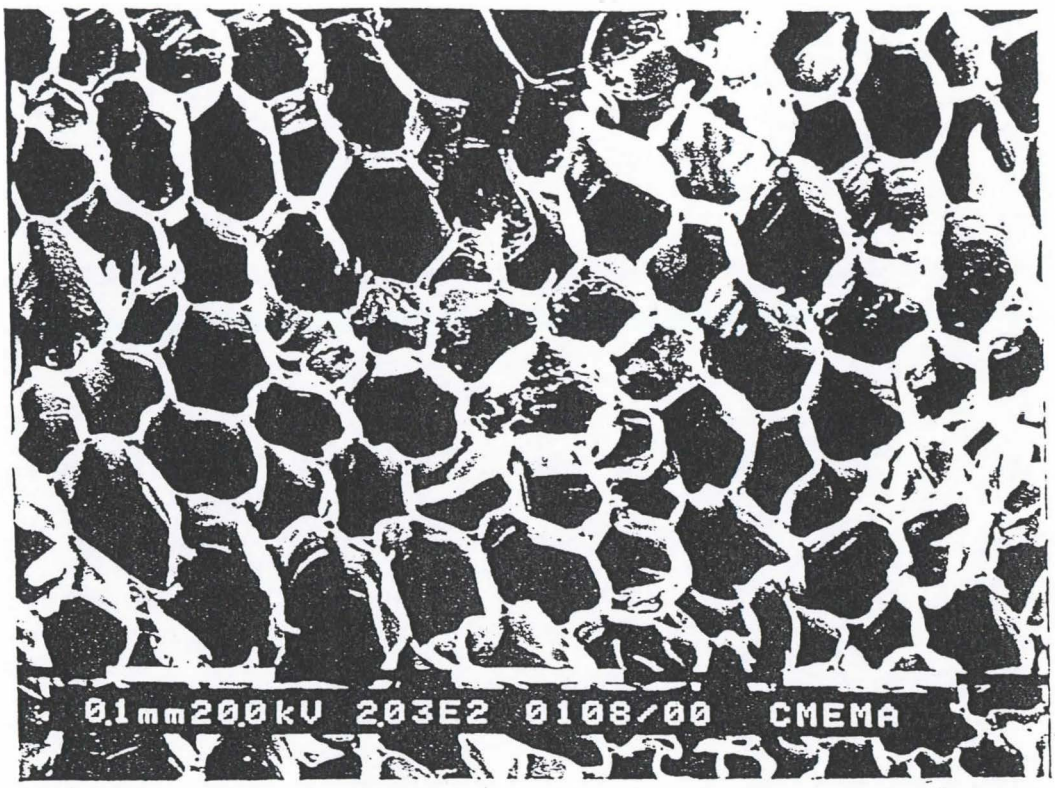


a) Cross section before maceration

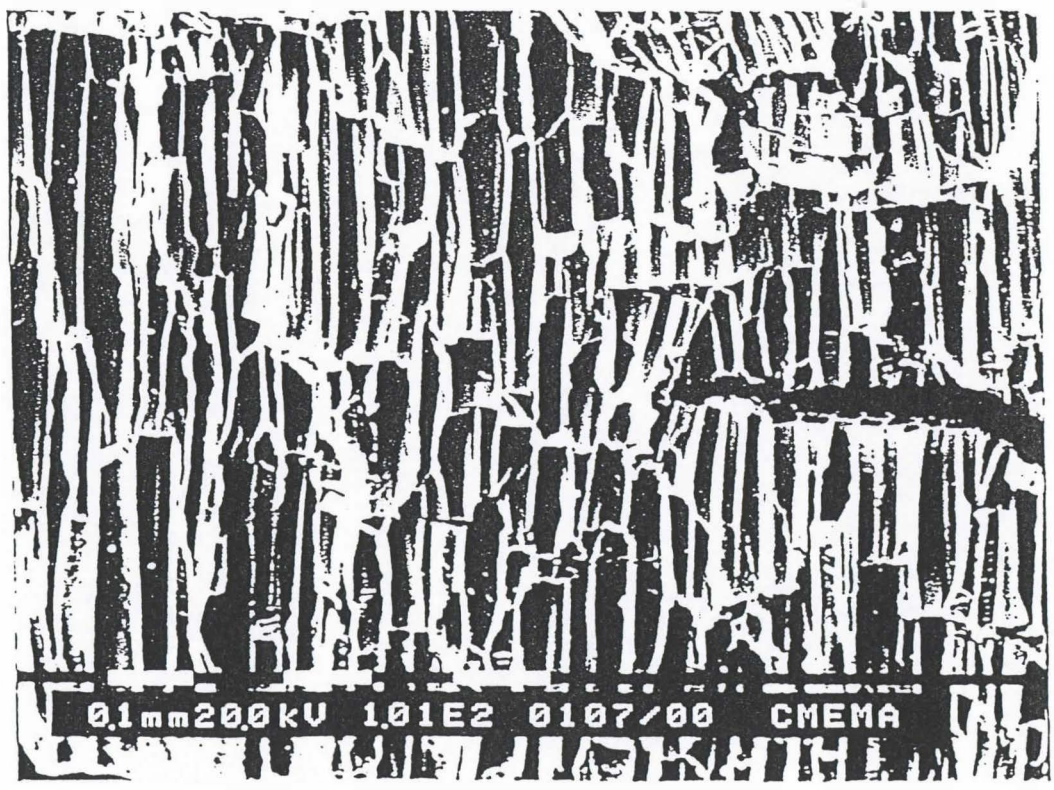


b) Longitudinal section before maceration

Figure 1: Scanning electron microscope image of coconut meat.



a) Cross section after maceration



b) Longitudinal section after maceration

Figure 2: Scanning electron microscope image of coconut meat

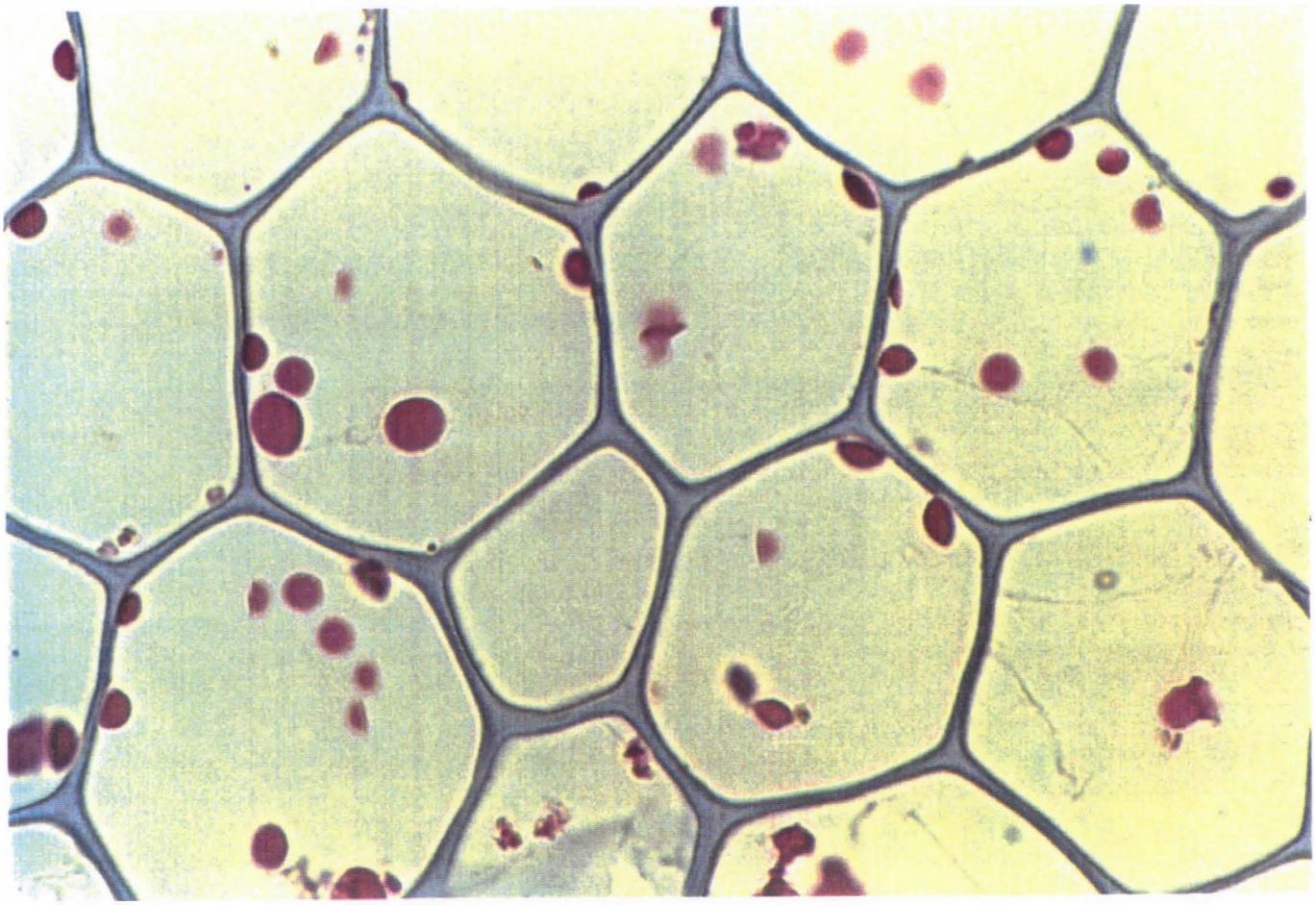


Figure 3: Light microscope image of a cross section of ripe coconut meat after double staining (x 160).

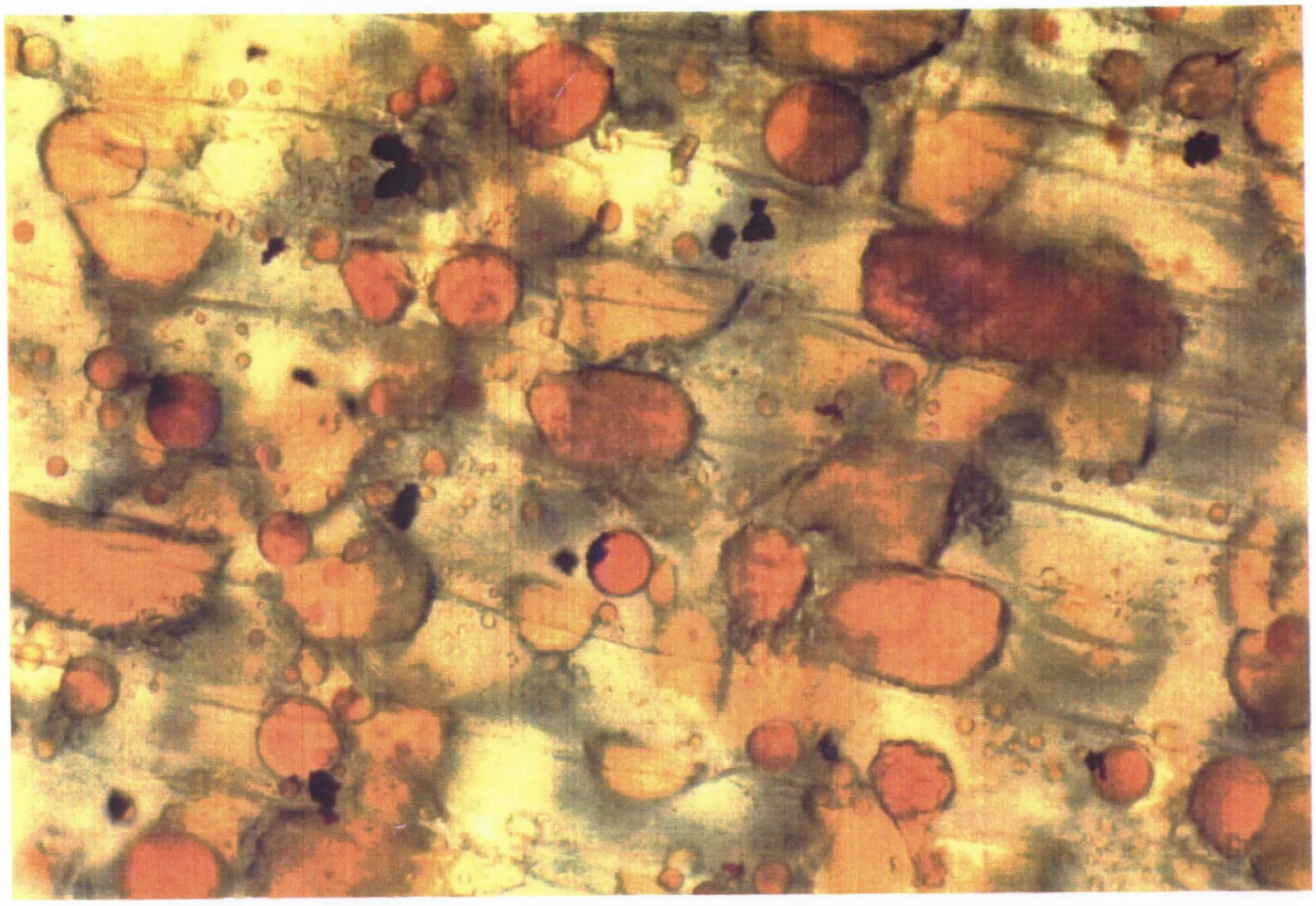


Figure 4: Light microscope image of a longitudinal section of ripe coconut meat after double staining (x 63).

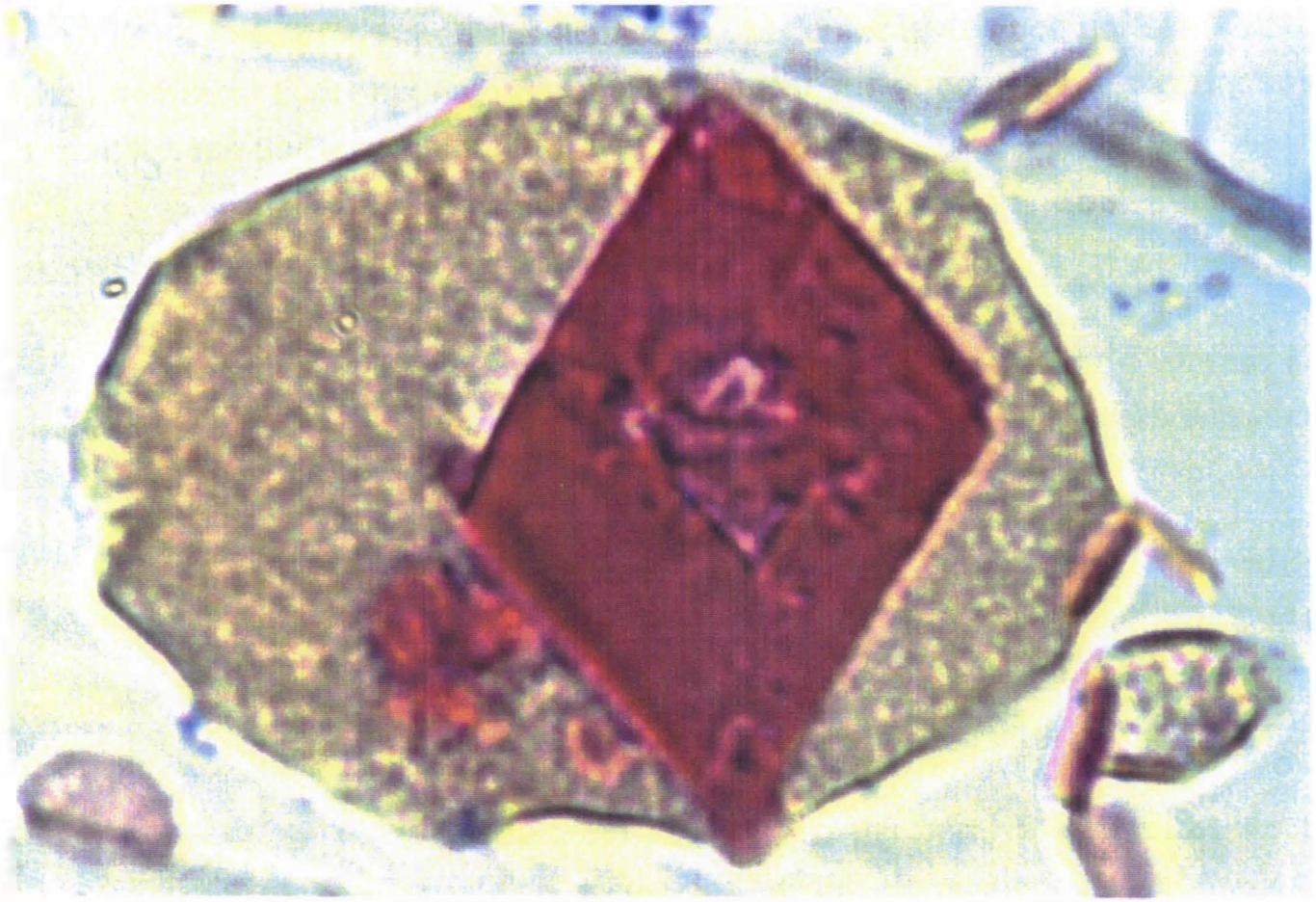


Figure 5: Magnified image of an aleurone grain showing proteic crystallite and globular proteins (x 500).

Pressure applied directly to grated coconut meat with a laboratory press gives an extraction rate of 40% of the total oil; after maceration the figure increases to 60%. These results suggest that colloidal grinding and maceration should result in high oil yields by simple processing in a three-phase centrifuge. This would be a new version of the wet process.

The result would be virgin coconut oil, an edible meal and a sugar-laden aqueous phase with many potential uses.

Unfortunately, it has not been possible to advance to a pilot stage in the field, for lack of resources and an interested industrial partner.

French industrialists are apparently prepared to get involved, since flavour-rich coconut products could tempt European Union consumers. CIRAD could play a major role in a wide-ranging operation to design suitable processes that give products with high nutritional and sensorial quality.

Enzyme-assisted extraction should not be seen as a pretext for setting the wet process against the dry process, they should be considered complementary, but it has to be admitted that operations upstream of the dry process very often leave much to be desired and need to be considerably improved to overcome, once and for all, those formidable pollutants aflatoxins and polycyclic aromatic hydrocarbons, whose total elimination by refining has to be guaranteed.

REFINING

Let us now look at what bioprocesses offer in terms of refining; remember first of all that chemical refining involves four traditional stages:

- mucilage removal or degumming, based on the principle of phospholipid elimination by water.
- neutralization, to remove free fatty acids using soda.
- bleaching with earth and/or plant or animal black.
- deodorization by steam treatment.

Bioprocesses can be useful at various levels in refining.

For degumming, phospholipids can be hydrolysed enzymatically with a phospholipase (11).

For neutralization, partial glycerides can be re-esterified by free fatty acids using a lipase in a non-aqueous medium.

In some cases, it is even possible to undertake bleaching if the colour is caused by chlorophyll, which can be hydrolysed with a chlorophyllase, releasing the coloured, water-soluble chlorophyllide fragment and the colourless, fat-soluble phytol fragment. This process is being tested on rapeseed oils, which are often rich in chlorophyllous pigments (12).

For lauric oils, which have a low phospholipid content and no chlorophyllous pigments, only enzymatic deacidification can be considered in the event of hyperacidity; i.e. for free fatty acid contents of over 5%.

Even today, crude copra oils with more than 5% acidity are unfortunately still frequent, due to poor copra preparation and storage conditions.

Physical refining is then virtually impossible; with chemical refining, losses are considerable and naturally higher than initial acidity. With palm kernel oils, high acidity batches are rarer, but they are not rare in Africa, which is why the laboratory has looked particularly closely at this question (13, 14, 15, 16); for example, we processed a batch of palm kernel oil with 8% acidity using an industrial lipase fixed to a support: lipozyme manufactured by NOVO INDUSTRI A/S.

An oil with 1.5% acidity was obtained after 15 hours' contact with stirring, under the conditions shown in figure 6. The oil was filtered to separate off the catalyst and could be refined without any difficulty, either chemically or physically.

Pressure: 20 mm Hg

Lipozyme amount: 5.5%

Temperature: 60°C

Water activity: $a_w = 0.43$

Time: 15 h

Figure 6:

Optimal conditions of neutralization with lipozyme IM20.

It would be worth testing lipozyme substitution with dried and ground *Carica papaya* latex (crude papain), which can easily be prepared at the lauric oil production site, to reduce production costs. It should be noted that in such a case, using bioprocesses could also induce papaya development, thereby generating new activities.

PROCESSING

a - Interesterification

One potentially very advantageous operation, given its many applications, is interesterification.

This operation is very common; it modifies the rheological properties of a given fat or oil, or blends. The process is used to obtain particular properties (softness, plasticity, hardness, spreadability straight from the fridge, etc.) for use in pastries, breads, ice creams, soft margarines, margarines, vanaspatis, ghees, etc.

It is often carried out by a chemical reaction at a temperature of around 100°C with a basic catalyst such as sodium methylate; in principle, the reaction is very rapid and complete in 30 minutes. It is essential that the reaction take place on refined substrates, otherwise the catalyst is rapidly poisoned by the impurities.

Chemical interesterification is characterized by totally random redistribution of fatty acids on the three positions of the glycerol.

1-3 regioselective interesterification (1-3 RI) catalysed by a 1-3 regioselective lipase limits random distribution to positions 1 and 3 without affecting position 2.

This technique, which has been extensively studied in the laboratory, has already been described in numerous publications (17, 18, 19, 20).

The bioprocess offers many advantages over the chemical process.

1- The composition of fatty acids in position 2 remains unchanged, and this position in the plant kingdom is usually rich in monounsaturated and polyunsaturated fatty acids (essential fatty acids); it is therefore of nutritional interest to keep them in this position, since the 2-monoglycerides derived from pancreatic digestion are the main conveyors of fatty acids through the wall of the intestines.

2- The formation of triglycerides with a high melting point generally seen in the chemical process is avoided or considerably reduced in the case of 1-3 RI.

3- As enzymatic reactions are slower, reaction kinetics are more effectively controlled. The reaction can be halted at any intermediate stage before total reaction, providing users with a wide range of products with different rheological properties.

4- Whilst chemical reactions require refined or anhydrous substrates, this is no longer necessary with 1-3 RI.

5- 1-3 RI requires relatively low temperatures: 35 to 60°C. Gains should therefore be made in quality.

6- Working on unrefined or only slightly refined substrates at a relatively low temperature makes for substantial energy savings.

In our studies on lauric oils, we tested 1-3 RI on two initial formulations:

70:30 palm oil:coconut oil

and 30:70 palm stearin:palm kernel oil.

1-3 RI was carried out with lipozyme as the catalyst, for reaction times ranging from 30 minutes to 4½ hours. Figures 7 and 8 show how the solid content varied according to the temperature as the reaction proceeded. The following results were seen:

- The interesterified products had a much lower solid content than the mixture prior to the reaction.

- The solid content of the interesterified products varied depending on the time spent in the reactor; it dropped steadily in line with the reaction time.

- At 37°C the solid content of the interesterified products fluctuated between 0 and 2%; in particular, it was nil after one hour's reaction for the product from the 70:30 palm oil:coconut oil mixture. For the product from the 30:70 palm stearin:palm kernel oil mixture, the solid content fell to 3 to 2% in 30 minutes and reached 0.4% after 4½ hours' reaction.

A comparison can be made with a hard margarine fat base and a soft margarine base. The solid contents were compared at 20, 30 and 37°C; the product from the 70:30 palm oil:coconut oil mixture after 4 hours reaction, could be likened to a soft margarine.

The 30:70 palm stearin:palm kernel oil mixture gave a hard margarine base after 4½ hours' reaction.

It is therefore easy to see the merits of this bioprocess, which could also be carried out with papain as the catalyst. A joint study with BIOTECH (University of Los Baños - Philippines) is under way; it is hoped that a cocoa butter equivalent can be obtained with this process, by reacting coconut oil with pili (*Canarium ovatum*) oil using papain as the catalyst.

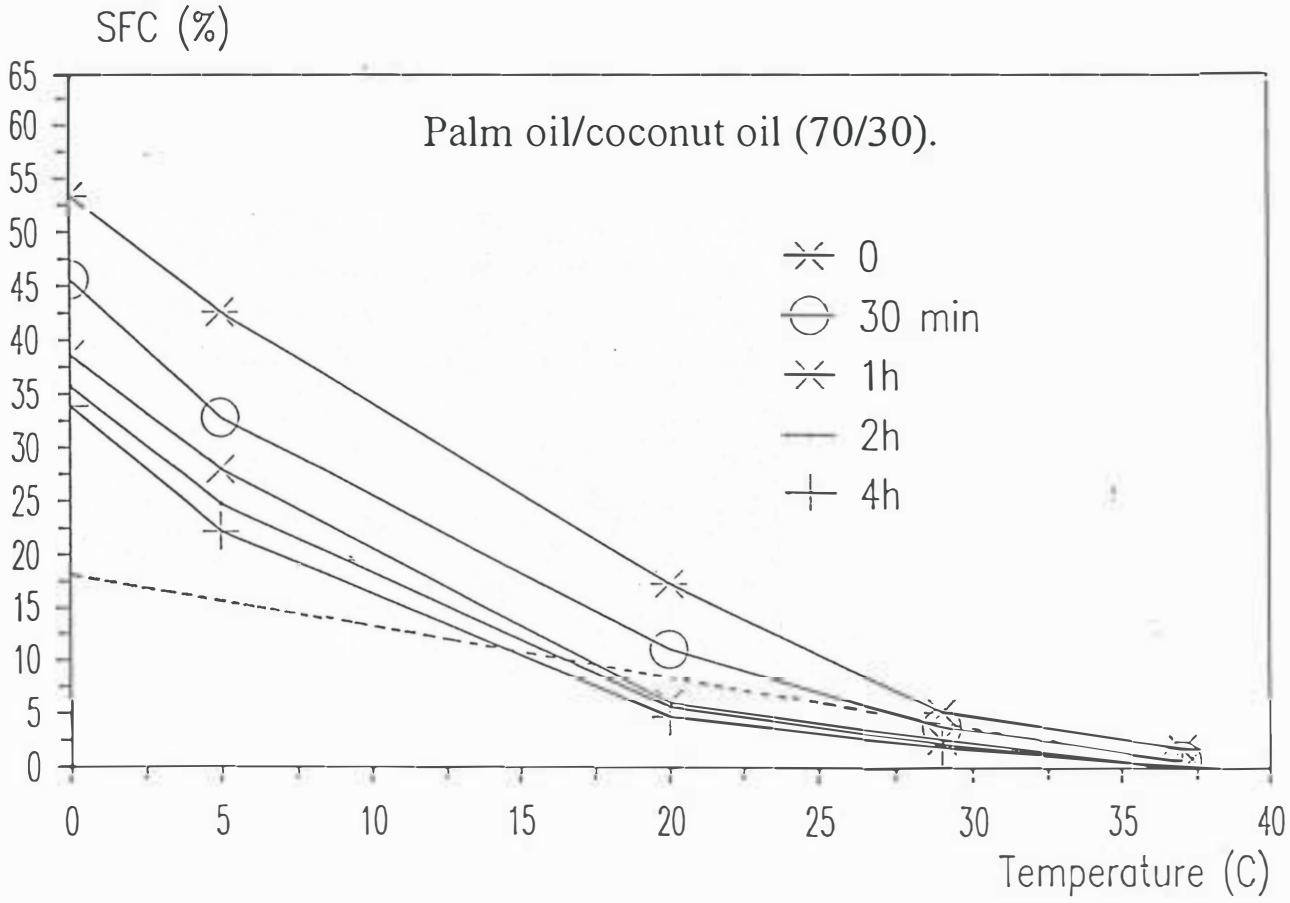


Figure 7: 1-3 Regioselective interesterification (1-3 RI) of palm oil and coconut oil (70/30).
SFC curves as a function of reaction time.
Comparison to a frigospreadable margarine (----).

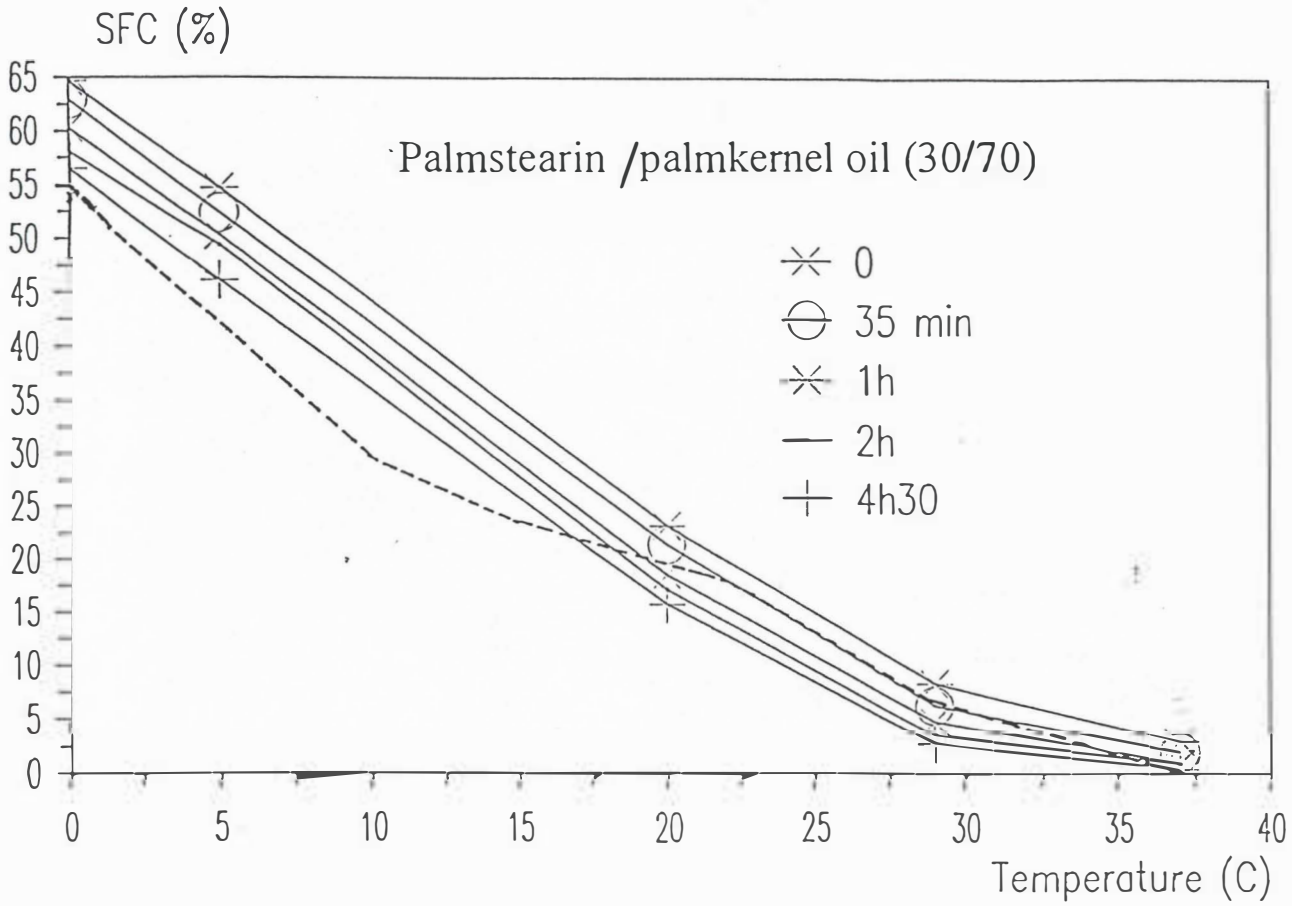


Figure 8: 1-3 Regioselective interesterification (1-3 RI) of palmstearin and palmkernel oil (30/70)
SFC curves as a function of reaction time.
Comparison to a firm pastry margarine (----).

b - Medium chain triglycerides (MCT)

Coconut oils can contain up to 20% short chain fatty acids; the TAC x BAO variety being studied by the PCA in Manila contains 0.7% C_6, 11.5% C_8 and 8.5% C_{10}.

MCT are medical specialities used in neonatal units for the nutrition of premature babies, and in geriatrics.

MCT are obtained by a chemical process involving hydrolysis of the coconut oil, separation of the C_6 to C_{10} fraction by distillation, re-esterification with the glycerol and, lastly, purification.

There are plans to test a bioprocess with the Chemistry Faculty of the University Los Baños, using papain again.

c - Presscake enrichment

A joint study has been undertaken by INRA, ORSTOM, CIRAD and the Mexico City Independent University, consisting in enriching copra presscake with proteins and probiotics (21).

Of a hundred or so strains of filamentous fungi selected, a dozen were capable of giving presscake with a protein content of around 35%.

The *Penicillum italicum* strain is economically advantageous, since for a tonne of dry matter containing 20 to 25% proteins, the culture of this fungus produces 830 kg of a product containing 34% proteins in terms of dry weight.

Moreover, the product was found to have a greater probiotic effect than a commercial probiotic widely used in cattle feeds.

In the case of presscake contaminated by aflatoxin and polycyclic aromatic hydrocarbons, it would obviously be ideal if the microorganism could destroy these two dangerous pollutants

Depending on the strains chosen, feeds can be prepared for cattle, sheep or poultry.

Contacts made with BIOTECH at the University of Los Baños have revealed skills in this field. Now seems to be the right time to implement a joint study in the true environment. Palm kernel cake can probably be enriched in the same way.

CONCLUSION

The few examples given in this account show that considerable technological progress can be made in the lauric oils field, in terms of product quality, elaboration of new products, and lower production costs.

Bioprocesses are too often considered to be over-sophisticated techniques and more costly than traditional processes.

In fact, the availability of very crude plant materials, papain being an excellent example, means that lauric oil producing countries have new technologies within their reach that could develop or strengthen other production activities at the same time, such as papaya cultivation with papain, or pineapple with bromelain, etc.

In this way, the economies of the sectors involved would receive a boost.

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