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Biological Modification of Oil Composition

B.K. TAN, S.H. ONG, N. RAJANAIDU and V. RAO, Palm Oil Research Institute of Malaysia, P.O. Box 10620, Kuala Lumpur, Malaysia

ABSTRACT

Prospects for modification of palm oil composition through oil palm breeding, tissue culture and enzyme-catalyzed transesterification are reviewed. Present emphasis in oil modification is toward greater unsaturation. The greatest prospect for this area lies in the interspecific hybridization of *E. oleifera* and *E. guineensis.* The target recommended is for a hybrid oil of iodine value above 72 having a palmitic acid content below 25% and an oleic acid content above 60%. It is noted that the variability of linoleic acid in the oil palm is limited regardless of species. The greatest contribution towards unsaturation, therefore, lies mainly in oleic acid. Tissue culture is seen as a potential propagating tool for selecting progenies of important crosses from the hybridization of *E. oleifera* and *E. guineensis,* while enzyme-catalyzed transesterification using a 1,3 specific lipase offers the possibility of enhancing the level of linoleic acid in palm oil. Besides breeding for unsaturation, production of palms giving oils of specific fatty acid or triglyceride types also may be possible ultimately.

INTRODUCTION

Malaysian palm oil and palm kernel originate mainly from the *tenera* variety of *Elaeis guineensis.* The oil palms in Malaysia originated from 4 seeds which came from Bogor, Indonesia, in 1848. Plant breeders have recognized the danger of too narrow a genetic base for the oil palm industry in Malaysia. Thus, several attempts have been made to expand the genetic range through selection of commercial and wild palms of *Elaeis guineensis* found in Zaire, Nigeria and the Cameroons (1). Seeds from the related species, *Elaeis oleifera,* also have been collected from South and Central America.

Palm oil is well endowed with properties which are suitable for many applications, notably in vanaspati, shortenings and margarines. In tropical countries its liquid fraction is used as a cooking oil. Its share in the salad and cooking oils category is limited, however, because of its composition. To enhance its usage in this category, efforts must be made to produce a more liquid palm oil through plant breeding. In recognition of this need, hybridization of palms belonging to *Elaeis guineensis* and *Elaeis oleifera* have been studied (2,3). Whereas past efforts of plant breeders have focused largely on improvements in yield, morphological and vegetatwe characteristics, there is growing emphasis on oil composition in breeding programs.

PROSPECTS FOR MODIFYING PALM OIL COMPOSITION

Present Status

World usage of oils and fats is divided equally between liquid oil and solid fat. While palm oil usage in the solid fat market is substantial, its share in the liquid oil market is limited because of its composition. Any emphasis on modifying composition should, therefore, be toward greater unsaturation of palm oil. Viewed in this perspective, it is relevant to discuss the composition of the major seed and olive oils in relation to palm oil. Table I shows the typical fatty acid composition of the major seed and olive oils. The following features are of interest when compared with palm oil.

(a) The total saturated acid content is below 20%, except for cottonseed oil.

(b) All the oils have greater than 70% unsaturated acids.

(c) Soybean, sunflower, cottonseed and corn oils have below 30% oleic acid and greater than 50% linoleic acid. Olive oil and rapeseed (low erucic type) have high oleic (60%) and low linoleic (20%) acid contents. Peanut oil has about equal percentages of oleic and linoleic acids.

(d) Soybean and rapeseed oils have a significant amount of linoleic acid.

(e) Iodine values of all the oils are above 80.

For comparison, note the composition of Malaysian palm oil. Rossell (5) has reported the fatty acid composition of oils from various areas. In general, the composition of palm oil falls into a narrow range regardless of geographical regions. Minor differences observed are due to plantings of mixed varieties rather than the predominance of a single variety. Palm oil commercially available today thus consists of an almost balanced proportion of saturated and unsaturated acids falling into a narrow range.

To compete with the seed oils for a major share of the salad and cooking oils market, the unsaturation level of palm oil must be increased. The question is, to what extent could the unsaturation be increased, and which unsaturated fatty acid should be increased. Noiret and Wuidart (7) have studied the variability of the fatty acid composition of *Elaeis guineensis* obtained from different origins and their hybrids (Table II). Their studies show that the level of

TABLE I

Fatty Acid Composition of Major Seed Oils and Olive Oil (%)

TABLE II

Fatty Acid Composition (mean values) of Palm oil Based on Origins and Hybrids (7)

TABLE III

Fatty Acid Composition of Otis from *E. oleifera, E. guineensis* and the Hybrid (6) (%)

Species	12:0	14:0	16:0	16:1	18:0	18:1		18:2 Total unsaturated
E. oleifera	\mathbf{tr}	0.1	30.2	0.2	1.0	53.5	14.9	68.6
E. oleifera X E. guineensis	tr	0.4	42.1	tr	1.0	46.7	9.8	56.5
E. guineensis var dura	\mathbf{r}	0.7	50.6	$\overline{}$	1.7	41.6	5.3	46.9

 $tr = trace.$

unsaturated acids in oil from *Elaeis guineensis* can be increased to 60%, but that to improve beyond this level, interspecific hybridization with *Elaeis oleifera* is necessary. Oils from *Elaeis oleifera* may be comparable in unsaturation to seed oils, but yields are uneconomically low. Table III shows the composition of a hybrid oil compared with oils from the parent palms. The fatty acid composition of oils from the hybrids is generally intermediate between those of the present palms. To ensure the desired unsaturation in the hybrid oil, selection of the parent palms with the highest unsaturation is necessary and can come about only through studies of the variability of *Elaeis guineensis* and *Elaeis oleifera* material collected. In assessing the variability for unsaturation, it is important also not to leave out palms which may give oils having specific fatty acids or triglycerides and which may find applications in specialty fats.

Variability in *Elaeisguineensis*

In 1973, a large scale collection of oil palm genetic material was made in Nigeria. Samples were collected at 45 sites throughout the country covering various climates and types of ecology. At each site, about 20 palms were sampled at random; this material was planted at Kluang, Johor in Malaysia (8). These palms now have been screened to measure the variability of fatty acid composition. Over 2,000 samples were taken and analyzed. The mean and range for individual fatty acids in the Nigerian population are given in Table IV, along with the levels observed for Malaysian and African palm oils computed by PORIM (4) and Institut de Recherches pour Des Huiles et Oleagineux (IRHO) (9) respectively.

It is obvious that the Nigerian palm population shows considerable variation in fatty acids when compared with

TABLE IV

alncludes laurie, palmitoleic, linolenic and arachidic acids.

TABLE V

Specific Fatty Acid Composition Patterns Observed in the Nigerian Population (%)

alncludes 12:0, 16:1, 18:3 and 20:0.

bRelative to the range observed for present palm oil.

TABLE VI

Types of Triglyceride Distribution Pattern Observed in the Nigerian Population

the PORIM and IRHO surveys and the results reported by Noiret and Wuidart (7). Of particular interest is the specific composition of oils from some of the palms, notably those which are high in stearic, palmitic, oleic or linoleic acids. Examples of these oils are given in Table V. Such palms could, in the future, be propagated by tissue culture to give specific types of palm oil tailored toward the requirements of the end-users.

The Nigerian palm population also has been observed to give oils having a triglyceride distribution of interest. Generally, 3 types of triglyceride distribution based on carbon number analyses by gas-liquid chromatography (GLC) have been noted (Table VI). While the type 1 triglyceride distribution is similar to the range now observed for palm oil, the other two types show marked differences. Type 2 shows a considerable enhancement of C52 and C54 triglycerides over present palm oil, while Type 3 exhibits a preponderance of C50 triglycerides. Type 2 triglyceride distribution generally is given by oils of low palmitic (less than 37%) or high stearic (greater than 7.0%) while the type 3 distribution is found in oils which are often high in palmitic (greater than 47%) or high in linoleic (greater than 12%). Type 1 distribution arises from a fatty acid composition which resembles that of present palm oil. The degree to which a fatty acid is considered high or low in content is relative to the present range observed for palm oil. It should be noted that though fatty acid and triglyceride distributions of interest have been observed in oils from some palms, the major problem stiU is striking a balance between obtaining an acceptable yield with the desired oil composition.

Variability in Elaeis oleifera

Initial results from the screening of *Elaeis oleifera* palms in PORIM are given in Table VII and compared to those reported by Meunier and Boutin (9). *Elaeis oleifera* palms produce oils with a very high percentage of unsaturated

acids ranging from 73 to 82%. In some instances, the oils obtained are comparable in composition to olive oil. It should be realized that, while the oils are of the desired composition, there are some problems related to the palms. Fruit set is poor and percentage parthenocarpy is high. Tam et al. (3) have reported the total fruit set (i.e fertile and parthenocarpic fruits) to be below 50%, with over 20% of the fruits parthenocarpic. Percentage oil to mesocarp generally is low, being half or less of that in *Elaeis guineensis.* Total oil to bunch figures reported are in the range of 2 to 4%, compared with 20 to 24% for *Elaeis guineensis* (3). Commercial cultivation of *Elaeis oleifera* is, therefore, not feasible. Although percentage parthenocarpy is high, there is no significant difference in the fatty acid composition of oils from fertile and parthenocarpic fruits (10).

Table VIII shows the triglyceride composition by carbon number of 2 *Elaeis oleifera* samples in comparison with palm oil and olive oil. Whereas the major triglycerides in palm oil are C50 and C52, the major groups in *Elaeis oleifera* oils are in the C52 and C54 triglycerides. These oils differ from olive oil by having a higher content of C52 and correspondingly lower C54 triglycerides. In screening *Elaeis oleifera* material, emphasis should thus be to look for *Elaeis oleifera* palms with the highest C54 triglycerides for hybridization with *Elaeis guineensis,* in order to increase the unsaturated triglycerides in the hybrids.

Variability in *Elaeis oleifera x Elaeis guineensis* **Hybrids**

To enhance the yield of palms giving unsaturated oils, hybridization of *Elaeis oleifera* with *Elaeisguineensis* is seen to be the best compromise. Table IX shows the fatty acid compositions for some hybrids. The variability of fatty acids in the hybrid palms is fairly high. Total unsaturated acids range from 52 to 76%, with the major acid being oleic acid. Parthenocarpy in the hybrids also is high, a characteristic which is inherited from *Elaeis oleifera.* Although the oil content is inferior to that of *Elaeis guineensis* palms, it is nevertheless much greater than that recorded for *Elaeis oleifera* palms. Yidds of 17% or higher of oil to bunch have been reported (3).

Ong et al. (11) have developed the co-dominance theory of *Elaeis* palm hybridization to predict the composition of oils from hybrid palms. We have found that, while most of the fatty acids and total unsaturation indicate additive or "co-dominance" effects, linoleic acid seems to be an exception. In this case, *Elaeis guineensis* seem to be dominant and dictates the level of linoteic acid in the hybrid. Figure 1 illustrates the inheritance of fatty acids in the hybrids. It will, therefore, be more worthwhile to screen *Elaeis guineensis* for linoleic acid than *Elaeis oleifera* when breeding for this particular acid.

Table X gives the triglyceride composition by carbon number of some hybrid palms screened. As with *Elaeis oleifera* the shift in the major triglyceride groups is from C50 and C52 in present palm oil to C52 and C54 in the hybrids. Compared with olive oil, the level of C52 triglycerides in the hybrids is still high and may be the factor preventing the achievement of cold stability properties similar to those found for olive and other liquid oils. The prospects for reducing the C52 triglycerides to levels found m olive and liquid oils may be very limited in view of the inherent high levels of these triglycerides in *Elaeis oleifera* and *Elaeis guineensis.* There is an apparent tendency in the oil palm to form C52 triglycerides at levels which are much higher than those found in olive and other liquid oils. Such a tendency merits further studies through an understanding of the biosynthesis of fatty acids and triglycerides in the oil palm fruit.

Tan et al. (12) have predicted that to achieve a hybrid

TABLE VII

Variation of Fatty Acid Composition *in E. oleifera* (n = 16)

alncludes 16:1, 18:3 and 20:0.

TABLE VIII

Triglyceride Composition of *E. oleifera (%)*

TABLE IX

Variation of Fatty Acid Composition *in E. oleifera × E. guineensis* Hybrids (n = **126)**

	PORIM	IRHO (9)	
Fatty acid (%)	Mean	Range	Range
14:0	0.4	$0.1 - 0.5$	$0.2 - 0.3$
16:0	33.3	$22.4 - 44.7$	$29.0 - 39.8$
18:0	2.6	$1.6 - 4.9$	$1.4 - 3.0$
18:1	50.5	$36.9 - 60.1$	$43.3 - 58.7$
18:2	12.2	$8.8 - 16.8$	$8.2 - 14.5$
Others ^a	1.0		

alncludes 16:1, 18:3 and 20:0.

oil which is similar in triglyceride composition to olive oil, a level of 8-13% palmitic and 70-80% of oleic acid will be required. Assuming the co-dominance of these acids from the parent palms, one would require an *Elaeis oleifera* palm of below 5% palmitic and an *Elaeis guineensis* palm of below 20% palmitic to produce such a hybrid. The fact that such levels of palmitic acid have so far not been observed in the 2 species poses a natural limitation toward the realization of this objective. A more realistic target would be for plant breeders to produce hybrid palms which give oils of iodine value around 72 and have a palmitic acid content of below 25% and an oleic acid content of above 60%. Hybrid oils having these characteristics when fractionated, will expand the usage of palm oil in the cooking and salad oils market.

Breeding Strategy in the Modification of Oil Composition

In view of the limited share of palm oil in the salad and cooking oils market, especially for temperate countries, priority at this stage in breeding is toward palms giving oils of higher unsaturation. In this context, screening for higher unsaturation in *Elaeis guineensis, Elaeis oleifera, the* hybrids and back-cross are important. Since palmitic and oleic acids

FIG. 1. Inheritance **of fatty** acids *in E. oleifera X E. guineensis.*

TABLE X

Triglyceride **Composition of** *E. oleifera X E. guineensis* Hybrids (n = 38)

Triglyceride (%)	Range	Palm oil (4) Range	Olive oil
C46	$0 - 1.1$	$0.4 - 1.1$	
C48	$0.9 - 8.9$	$4.5 - 10.3$	
C50	$11.1 - 25.5$	$39.3 - 44.7$	3
C ₅₂	$43.5 - 50.5$	$38.9 - 44.4$	28
C54	$21.8 - 44.7$	$6.7 - 11.9$	67
C56	$0 - 0.6$		

are negatively correlated in all the species (10), it should be possible to select for oleic acid at the expense of palmitic. Linoleic acid appears to be limited in *Elaeis guineensis* and *Elaeis oleifera.* In view of the dominance of *Elaeis guineensis* in this acid in the hybrid, it may be futile to breed for this fatty acid to enhance unsaturation. Nevertheless, the need to screen for *Elaeis guineensis* palms of the highest linoleic acid content should continue.

In breeding for greater unsaturation, it is noted that the tendency of the oil palm (whether *Elaeis guineensis, Elaeis oleifera* or hybrids) to form C52 triglycerides (mainly POO, PLO) may be the limiting factor toward achieving the unsaturation level observed for olive and the major seed oils. While the ultimate objective may be to produce unsaturation in palm oil equal to that of olive oil, the present recommendation, which is more practical, is to produce a hybrid oil of iodine value above 72 having a palmitic acid content below 25% and an oleic acid content above 60%. The yield in terms of bunches per hectare per year and oil yield per hectare also will have to be considered when breeding for unsaturation. Where the yield has to be slightly sacrificied at the expense of unsaturation compared with present yields of *tenera* palms, the question of a price premium must be considered because the cost will be higher.

Palms giving interesting oils of a specific fatty acid or triglyceride composition should be further enhanced in

order to expand the versatility of palm oil. Examples of such palms are those giving oils which are high in stearic or palmitic acids or in triglycerides such as oleodipalmitin (POP). Palm oils having low diglycerides are desired by the end-users, especially those in the confectionery fields. Hence, in breeding for palms giving a specific composition or unsaturation, the diglyceride aspect should not be overlooked.

TISSUE CU LTURE

Vegetative propagation of oil palms has been achieved using tissue culture methods (13,14). Early results reported by Jones (15) and Corley et al. (16) indicated a wide range of differences in oil composition between clones but high within-clone uniformity. Oil composition also was affected by both environment and genotype. The variability observed *in Elaeis guineensis, Elaeis oleifera* and hybrids points toward selection of parents within and between species for crossing to give palms producing oils of a specific composition. From selected progenies of such crosses, selection and propagation of individuals through tissue culture should be possible. In this context, selection of the interspecific hybrids and back-crosses with *Elaeis guineensis* offers the greatest scope for producing palms which give the desired oil in terms of both yield and composition.

Enzyme-Catalyzed Transesterification

Enzyme catalyzed transesterification gives fat products which are not obtainable by chemical interesterification methods. The use of microbial lipases for such interesterification has been described (17,18,19). Two areas are of potential application to palm oil. One is to alter the type of monounsaturated triglycerides from palm oil in which a palm mid-fraction is converted from a predominance of POP triglycerides to a mixture of POP, POS and SOS triglycerides. The other is to increase the linoleo-type of triglycerides in palm oil since linoleic acid is naturally limited in it. Figure 2 highlights the 2 application areas in which the 2 major triglycerides of palm oil, POP and POO, are involved and in which the interesterification is conducted using a 1,3 specific lipase as catalyst. Macrae (19) has

FIG. 2. Products formed by interesterification of 1,3-dipalmitoyl-2 monooleine (POP) and 1(3)palmitoyl-3-(1)oleoyl-2-monooleine (POO) with stearic and linoleic acids respectively using a 1,3-specific lipase as catalyst.

TABLE XI

Triglycerides Formed by Interesterification of a Mixture **of** Palm Midfraction (1.0 parts) and Stearic **Acid** (0.5 parts) Using *A. niger* Lipase as Catalyst (19)

aS=saturated fatty acid group; P=polumitate; St=stearate; O=oleate and Ln=linoleate.

TABLE XII

reported the conversion of a palm mid-fraction using a 1,3 specific lipase and stearic acid (Table XI). Successful implementation of enzyme-catalyzed transesterification of palm oil will supplement efforts of plant breeding toward producing specialty fats from the oil palm.

Kernel Oils

While great emphasis in breeding has been given to the mesocarp oil, less attention has been focused on the composition of kernel oils from *Elaeis guineensis, Elaeis oleifera* and the hybrids. Limited analyses of these oils have been conducted by Macfarlane et al. (2), Meunier et al. (20) and Tan et al. (21). Table XII shows the fatty acid composition of kernel oils from palms of *Elaeis oleifera,* hybrids and back-cross. *Eleais oleifera* kernel oils show lower lauric and higher myristic, oleic and linoleic acid content than *Elaeis guineensis oil.* The kernel oils of the hybrids, unlike the mesocarp oils, do not display an intermediate level in composition between the parent species. On the other hand, these oils and the back-cross show a composition closer to that of palm kernel oil *(Elaeis guineensis).*

Table XIII shows the triglyceride composition by carbon number of the various kernel oils. The major triglyceride groups range from C36 to C54 for *Elaeis oleifera* kernel oils. The absence of a major triglyceride group is unique, unlike in *Elaeis guineensis* kernel oil which has C36 as the major triglyceride. The distribution pattern of triglycerides for the hybrids and back-cross are in line with *Elaeis guineensis* oils. It should be stressed that the above observations are from a limited number of samples picked at random. Further studies from properly designed breeding programs are needed in order to obtain a better understanding of the inheritance of fatty acids and triglycerides in the kernel oils as a result of hybridization.

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Fatty Acid **Composition and** Iodine Value of Kernel oils from *E. oleifera,* **Hybrids** and Back-cross with *E. guineensis* (mean values)

aE.o. represents *Elaeis oleifera, E.g.o., E.g.p.* and *E.g.t.* represent unspecified *dura, pisifera* and *tenera* types of *Elaeis guine ensis, respectively; B.C. represents <i>Elaeis oleifera* back-cross.

TABLE XIII

aExpressed as normalized percentage peak area.

bNotation used is as given in Table Xll.

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