Histological Localization of Oil Palm Fruit Lipase

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Development of free fatty acids (FFA) and subsequent deterioration of palm oil quality can be attributed to lipase, an active hydrolytic enzyme present in the mesocarp of oil palm fruit. A histochemical method was used to localize this enzyme in the mesocarp. FFA released from endogenous glycerides were precipitated as lead salt. The deposits were located on the membranes of oil bodies. Enzymatic activity in the mesocarp was also studied under different conditions of fruit storage: ambient temperature, freezing, etc. The enzyme was active after freezing but less active under ambient conditions of fruit storage. Investigations on fruit development indicated that the enzyme activity started appearing between 16-20 weeks after anthesis (WAA) which synchronized with the beginning of lipid formation. These histochemical studies were supported with biochemical data on FFA.

KEY WORDS: *Elaeis guineensis*, lipase, mesocarp, oleosome, weeks after anthesis.

The fruit of oil palm *(Elaeis guineensis)* is a "drupe" consisting of a thin leathery exocarp surrounding the fibrous-fleshy mesocarp and a stony endocarp enclosing normally one but sometimes two or more kernels. Lipase (Glycerol ester hydrolase, E.C. 3.1.1.3) is an extremely active enzyme present in the mesocarp cells, which causes the release of free fatty acids (FFA). Quality deterioration of palm oil can be attributed to the action of this enzyme. Previous studies of lipase activity in oil palm mesocarp indicated that lipase was found only in the embryo of germinating palm seeds, and no activity was detected in either nongerminating seeds or in the mesocarp (1). Subsequent studies on the partial purification of lipase demonstrated its presence in the mesocarp (2). Histochemically, lipase activity has been studied in animal tissues by Gomori's Tween or lead salt method (3,4). The methodology used in this study is a modification of Gomori's. The *in situ* activity of lipase within mesocarp cells was reported to be accelerated by damage to mesocarp tissue by bruising, overripening, and other post-harvest changes (5). In this paper we focused on the distribution and localization of lipase and its substrate lipids in mature mesocarp and their formation during fruit development.

MATERIALS AND METHODS

Plant materials. Mature sound oil palm fruits of tenera variety (26 WAA) were collected from the Indian Council of Agricultural Research (ICAR) Plantations at Palode, Trivandrum. For development studies, inflorescences of tenera palms were randomly selected at anthesis and hand-pollinated.

Histochemical methods. Samples of known maturity were collected from selected palms. For controls, samples were immersed in water at 90°C for 30 min to inactivate the enzyme. For experimental samples, sections were taken immediately after collection. The fruits were cleaned, surface sterilized and washed in distilled water (2). Small pieces of mesocarp tissue were cut into thin sections of 10-15 μ thickness with a freezing microtome. The frozen sections were assayed according to Gomori (3,4) with two modifications. Firstly, frozen sections were incubated without substrate. Mature mesocarp tissue contains almost 50% palm oil glycerides, the endogenous substrate for oil palm lipase. Secondly, an incubation time of 10 min was sufficient for the maximum activity of lipase. Due to high activity of lipase and abrupt release of FFA which subsequently precipitated as lead sulphide, the sections were rapidly covered with black deposits. Therefore, controlled release of FFA was necessary to locate enzyme activity. The method adopted was to partially destroy the enzyme by heat. Mature fruits were immersed in hot water at 45°C for 30 min before sectioning. The localization of lipase activity was also studied by preparing agar-fatty layer gels of mesocarp oleosomes as described by Ory *et al.* (6) and Ory (7).

The formation of lipase and lipids during fruit development was studied at 4-28 WAA. Sudan black staining was adopted for the detection of lipids (8). Heat sensitivity of mesocarp lipase was examined in fruits blanched for 30 min at temperatures from 30-70°C. Lipolytic activity was also monitored during storage of mature fruits at ambient conditions and at -10°C for seven days.

Biochemical methods. Histochemical investigations were supported by estimating the FFA content in extracted oil samples by titration. The quantity of total lipids in developing fruit was estimated by the chloroform-methanol procedure (9).

RESULTS AND DISCUSSION

Figure 1 demonstrates the activity of lipase in the mesocarp tissue *in situ*. Upon lipolysis of endogenous triglycerides, FFA are produced that form insoluble calcium soaps. The calcium is subsequently replaced by lead ions which are then converted into lead sulphide (10,11). The black deposits visible in Figure 1 are due to the formation of lead sulphide which confirms the release of FFA. For controls (Fig. 2) the fruit was blanched in boiling water for 30 min to inactivate the enzyme. No black deposits formed—confirming the release of FFA due to the action of lipase in the experimental samples. Endogenous triglycerides were obviously utilized by the enzyme because external substrate was not supplied. It was also observed that even 10 min incubation was sufficient to detect enzyme activity. These observations with regard to substrate and time of incubation are quite different from those of Gomori's for animal lipase (4). The presence of a high amount of triglycerides, the natural substrate, and active lipase in the mesocarp would explain the differences. These histochemical observations were further supported by quantitative determination of FFA. When the fresh fruit was homogenized permitting the enzyme to contact endogenous lipid, the

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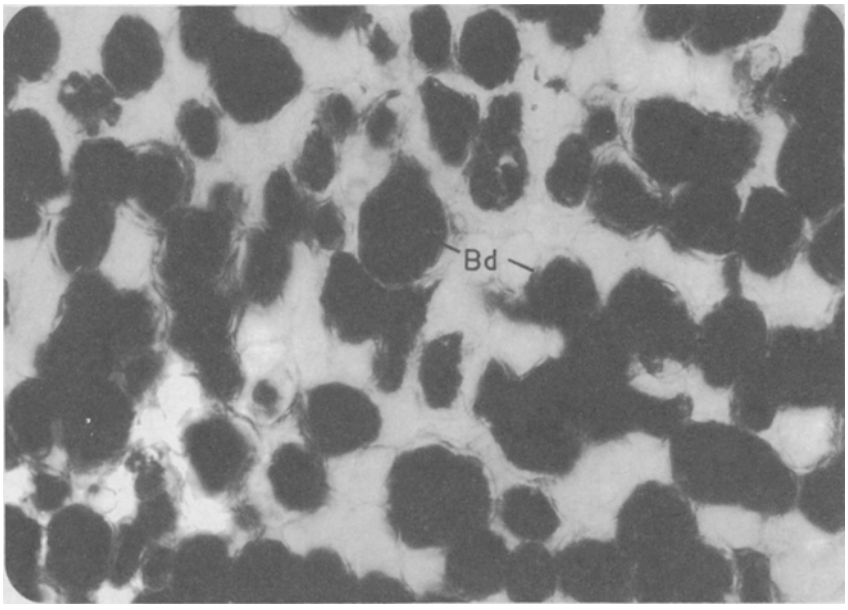

FIG. 1. Light microscopy of mesocarp tissue. The cells show black deposits (Bd) of lead sulphide which, in turn, confirms the release of FFA by the *in situ* activity of lipase (modified lead salt method) × 180.

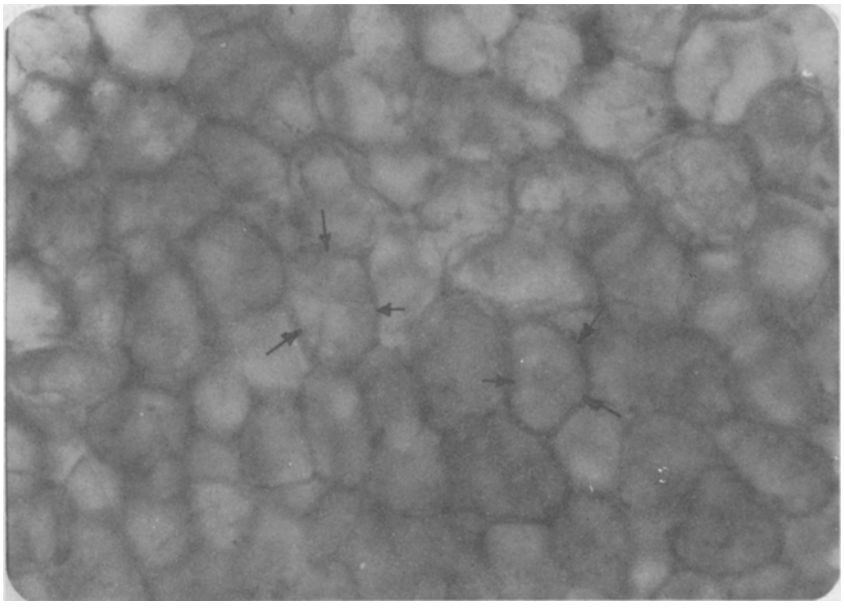

FIG. 2. The mesocarp cells (arrows) of blanched control fruit have no black color × 200.

FFA rose to 35% while the blanched control contained only 0.5%.

The above conditions could not be used for the localization of this enzyme due to complete blackening (Fig. 1). It was observed that enzyme activity could be limited to a manageable level of around 3% FFA by subjecting the fresh fruit to partial blanching (45°C for 30 min). The sections of partially blanched fruits were prepared as before, and the results are shown in Figure 3. The black deposits were limited to the peripheral regions of the oleosomes, suggesting that the sites of activity coud be on oleosome membranes. Using TEM, Ory *et al.* (6) and Ory (7) demonstrated that lipase is associated with the spherosome membranes of the resting castor bean. Similar enzyme-membrane association had been reported for *Crambe abyssinica* seeds (12).

In the case of oil palm mesocarp, lipase activity has been reported to be associated with the fatty layer during the isolation of enzyme (2). It has also been observed that the FFA content increases in the process of post-harvest handling, overripening, freezing and other mechanical injuries to the fruit. This indirect evidence coupled with observations by histochemical methods presented here suggests that lipase oil palm mesocarp is closely associated

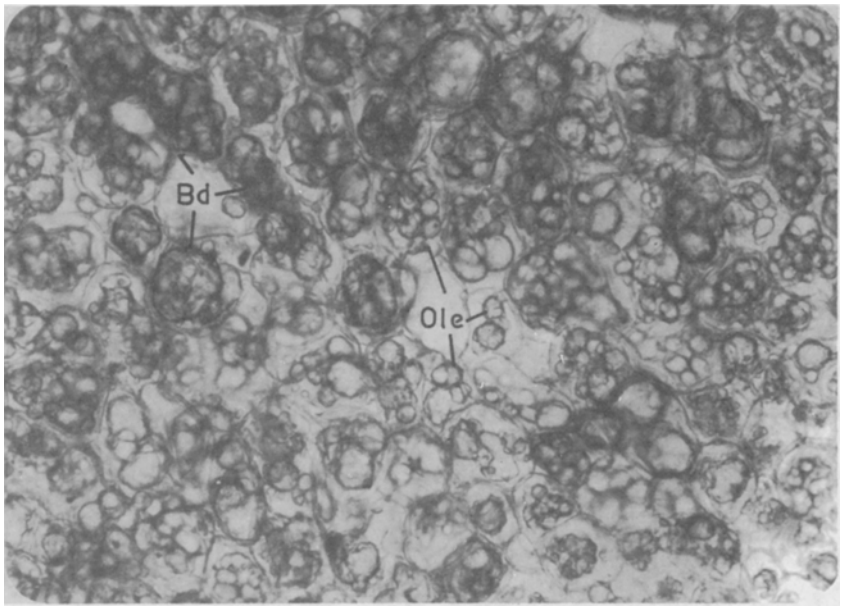

FIG. 3. The mesocarp tissue of a partially blanched fruit. The cells display the black deposits (Bd) limited to the peripheral regions of the oleosomes (Ole) × 180.

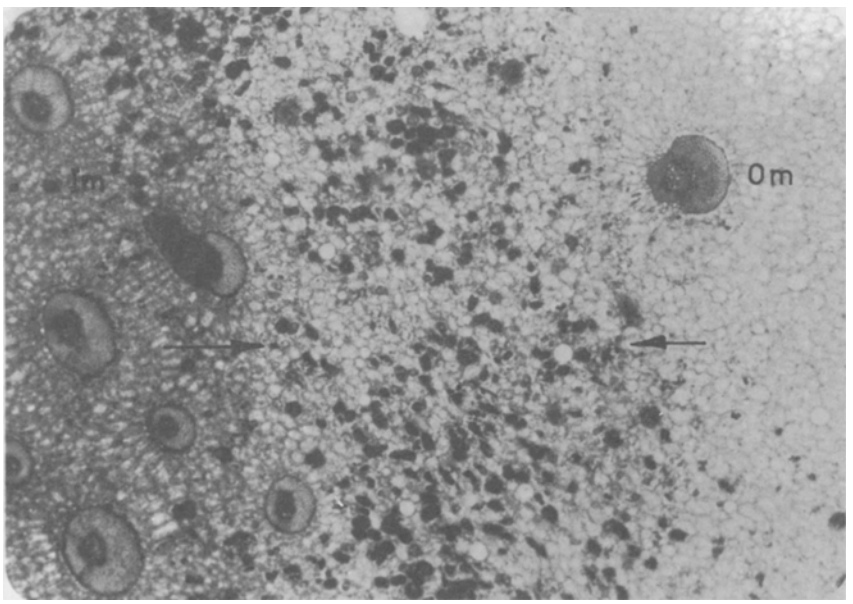

FIG. 4. Mesocarp tissue of oil palm at 16 WAA. The cells display scattered black deposits in the middle mesocarp (arrows). Inner mesocarp (lm), Outer mesocarp (Om) × 40.

with oleosomes. Oil-bearing organelles in oleaginous plant tissues are variously described as spherosomes (13) and oleosomes (14). Considering the specific function, i.e., oil storage, it is appropriate to term these oil-bearing bodies as oleosomes for oil palm mesocarp. Because the mesocarp oil reserve is not utilized during seed germination, the physiological significance of active lipase is unknown.

The formation of enzyme activity during fruit development was studied histochemically, and the results are presented in Figures 4 and 5. No lipase activity was observed until 12 WAA. The sections for 16 WAA exhibited scattered black deposits in the middle mesocarp region indicating appearance of lipolytic action (Fig. 4). The 20 WAA sections showed uniform black deposits throughout the entire mesocarp (Fig. 5). Studies of lipid formulation for corresponding samples showed that most of the lipids were formed between 16 and 20 WAA. It was possible that lipase activity prior to 16 WAA was not detected due to the absence of palm oil glycerides. This was examined by supplying external substrate in the medium (4). No activity was observed.

The quantitative estimation of mesocarp lipids for all development stages showed that the lipid content at 16

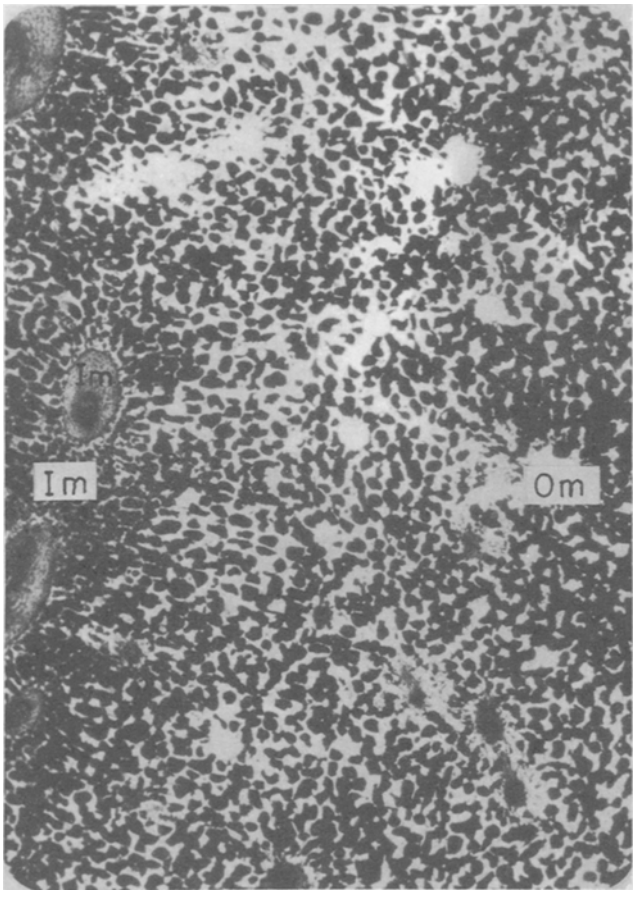

FIG. 5. Oil palm fruit tissue at 20 WAA. The cells exhibit uniform black deposits throughout entire mesocarp. Inner mesocarp (Im), Outer mesocarp (Om) × 40.

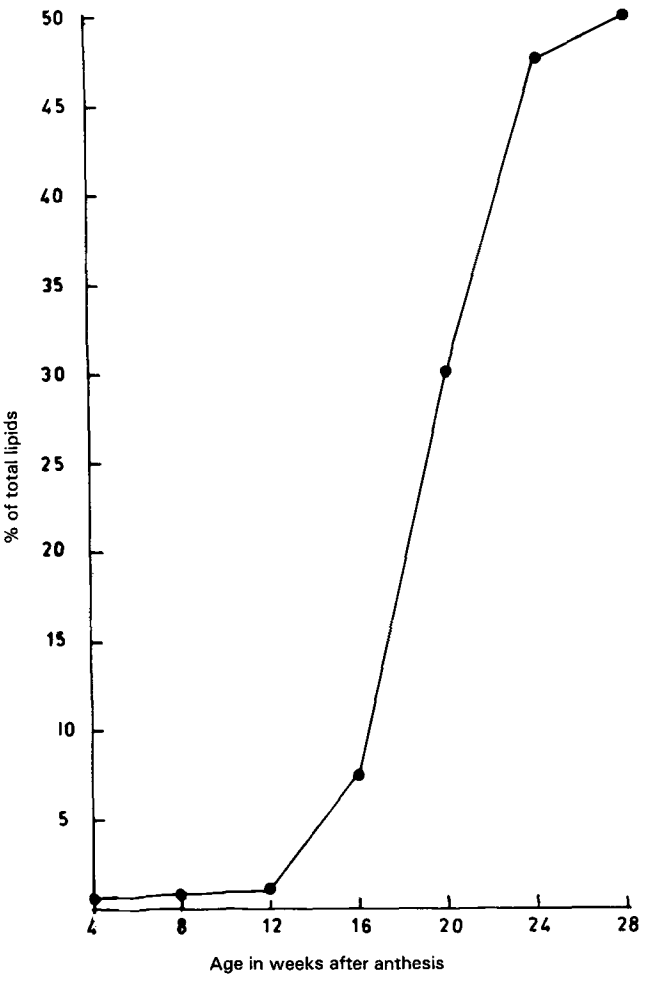

FIG. 6. The % of total lipids synthesized at various stages of fruit development.

WAA was 7% and subsequently reached a maximum of 48% at 24 WAA (Fig. 6). Chemical estimation of lipid formation in developing oil palm fruit was studied by a few workers. The general pattern of lipid synthesis was that a rapid phase of lipid synthesis occurred in the last quarter of fruit maturation (15,16). The results reported here are comparable with earlier reports regarding lipid formation. However, histochemical evidence for lipase and lipid formation in oil palm mesocarp has not been reported before. It is interesting to note that lipid formation followed the same pattern for lipase. Lipase and lipid therefore originated simultaneously at the middle mesocarp and advanced toward the inner and outer regions.

Lipase activity was markedly influenced by temperature. Fresh and blanched fruits were stored at -10°C for seven days, and histochemical studies and chemical estimation of lipids were done at one-day intervals. Untreated fruits showed active lipolysis, whereas blanched samples were inactive. FFA contents were 32% and 0.5% for the untreated and blanched samples, respectively, throughout the storage period. Samples stored under ambient conditions did not show appreciable FFA release as indicated by histochemical and chemical estimations for seven days. It seems that the substrate was made accessible to the enzyme by freezing, possibly due to disruption of the oleosome membranes.

Fresh fruits were subjected to blanching temperatures ranging from 30-70°C so that the effect of temperature on lipase activity could be observed. Activity readily decreased after 40°C and a complete loss occurred at 50°C.

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