Measurement of Lipase Activity in Rubber *(Hevea brasiliensis)* Seed

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ABSTRACT: Lipase activity in para rubber *(Hevea brasiliensis)* seed was measured by monitoring the release of free fatty acid by lipolysis of the endogenous lipid in a crushed sample of seed incubated at 37.5°C for 30 min. Free fatty acid was determined colorimetrically by a modified copper soap method. Fresh seeds showed the highest lipase activity. Drying the seeds at 60°C inactivated the enzyme. Drying of the seed at this temperature may be useful as a pretreatment for extraction of oil from the seed.

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KEY WORDS: Lipase, oil extraction, para rubber.

Para rubber *(Hevea brasiliensis)* seed is a potential raw material for vegetable oil. Rubber seed oil has been found to be nontoxic (1) and could be used for industrial purposes and as a trial oil in animal experiments (2,3). The oil could also be used for human nutrition (3). It is generally recognized that the seed contains a lipolytic enzyme, which makes oil refining difficult (4). Rubber seeds are pretreated for milling and, consequently, oil is extracted at different temperatures (5,6). This preconditional treatment reduces the moisture content, prevents the seed from rapid deterioration, and possibly inactivates the lipase enzymes (7). Lipase activities in different seeds have been measured either (i) titrimetrically by incubating the defatted powdered sample with an external oil substrate and titrating the release of free fatty acid with alcoholic potassium hydroxide (8) or (ii) colorimetrically by measuring the release of free fatty acids (FFA) (9).

This paper describes a simple colorimetric method of measuring lipase activity in para rubber *(H. brasiliensis)* seed, and the procedure is applicable for crushed samples of up to 1 g. Lipase activity was measured in fresh seed and in seed dried at 60°C.

MATERIALS AND METHODS

Rubber trees grown at the Faculty of Agriculture Farm, University of Nigeria (Nsukka, Nigeria), were used as sources for seed collection. The colorimetric copper soap method, described by Shipe *et al.* (9) for milk, was adopted for the detection of FFA in the seeds. Single whole seeds (decordicated), weighing 3.61–4.21 g, were soaked for 1 min with 40–60 mL of 1 N HCl and then extracted with 10 mL of a mixture of chloroform/heptane/methanol (CHM) (49:49:2, vol/vol/vol) for 2 min in a Pyrex centrifuge tube and then allowed to stand for 1 min. After filtration, the residue was re-extracted with 10 mL of CHM for another minute. The contents were transferred to a filter and washed with 50 mL of the same solvent system.

Five mL of the extract was mixed with 2 mL cupric nitrate/triethanolamine (9), centrifuged at 2,000 × *g* for 15 min, and 4 mL of the upper layer was mixed with 0.1 mL of sodium diethyldithiocarbamate solution (9). The absorbance was measured at 440 nm within 1 h with a PyeUnicam Sp 500 spectrophotometer (Philips Electronics, Ojuta Lagos, Nigeria). Crushed samples of fresh rubber seeds, up to 1 g, were similarly extracted with adjustment of acid and dilution of the sample. FFA content was expressed as mM/g of sample.

To determine the lipase activity with the endogenous triacylglycerol substrate, individual seeds were soaked in 2 mL of phosphate buffer pH 6.2 to give a moisture content of 50–100% (10) and were crushed in a mortar to ensure complete cell fracture. The samples were incubated for 30 min at 37–38°C in a water bath (Grant), acidified with 0.1 mL of 1 N HCl, and the FFA were extracted in the solvent mixture as described earlier. The procedure for incubation was applied to full seed, longitudinally disected seed (half seed), mascerated seed, and crushed seed.

RESULTS AND DISCUSSION

The method uses the endogenous lipid as substrate in the determination of lipase activity in the oilseed (10). Para rubber *(H. brasiliensis)* contains 40–45% fat, of which more than 80% is triacylglycerol (11). The optimum pH and temperature of purified lipase enzyme have been established (12). Complete extraction of FFA by different solvents, including CHM and acidification with HCl, has been reported as an effective method of releasing bound and entrapped FFA from plant cells (10).

Figure 1 shows the development of FFA in para rubber *(H. brasiliensis)* seed over a period of 24 h after inbibition of buffer and incubation of thinly sliced 1-g samples. One was a freshly harvested sample, and one a freshly harvested sample

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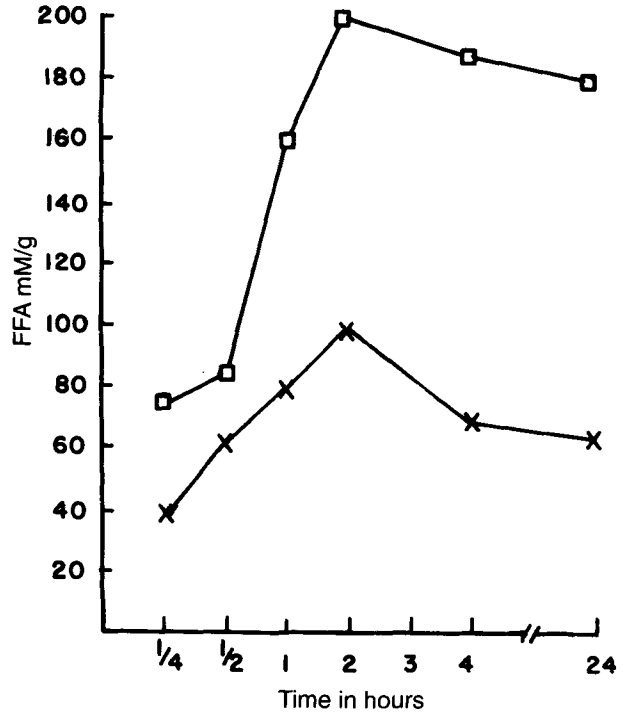

FIG. 1. Development of free fatty acid (FFA) during incubation at pH 6.2 up to 24 h. □, Freshly harvested rubber seeds; X, freshly harvested rubber seeds dried at 60°C.

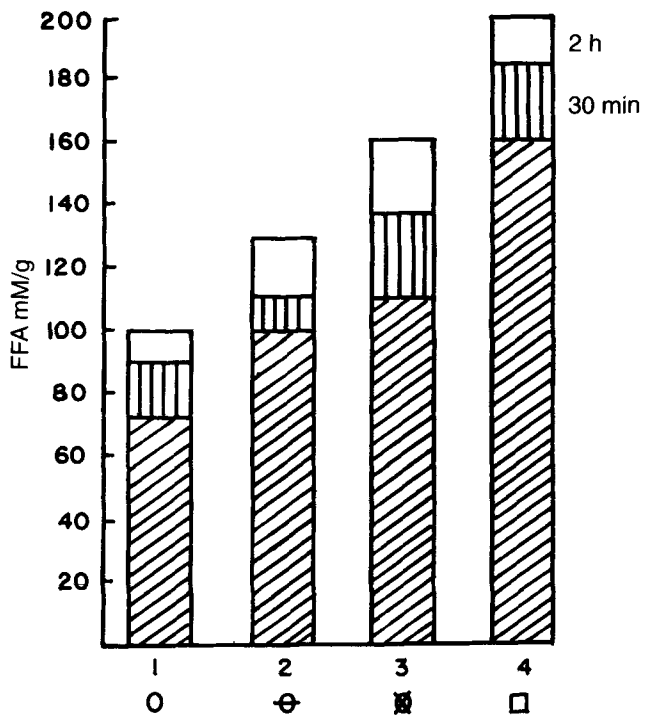

FIG. 2. Effect of cell fracture on lipase activity. Sample 1, intact whole seed; sample 2, seed halved into two longitudinal sections; sample 3, mascerated seed; and sample 4, crushed seed. Lower bar represents initial FFA content; middle bar represents development of FFA in 30 min; top bar represents further development of FFA up to 2 h. See Figure 1 for abbreviation.

dried for 24 h at 60°C. Each value of FFA represents an average of 1 g thinly sliced seed. Maximal development of FFA occurred at 2 h of incubation at 37.5°C, with no marked significant changes in FFA up to 24 h.

Lipase enzymes in many oilseeds are localized in the aleorone layer of the grain, whereas the lipids are dispersed in the sub-aleorone layer and the endosperm (10,13). Anatomical studies have shown that the lipids of para rubber *(H. brasiliensis)* are located in the sub-aleorone layer and the endosperm (7). Therefore, a complete cell fracture was essential for the lipase to act. The importance of cell fracture was demonstrated by determining the development of FFA during incubation of intact whole seed (sample 1); longititudinally dissected seed (half seed) (sample 2); mascerated seed (sample 3); and crushed seed (sample 4). All samples (Fig. 2) were soaked in phosphate buffer (pH 6.2) and incubated for 30 min.

Samples 3 and 4 showed an increase in FFA content between 136–184 mM/g during an incubation time of 30 min. No further significant increase was noted in sample 4 after 2 h. Variation in the FFA content in individual seeds within the same sample was determined by separately analyzing 10–20 seeds. The mean value (mM/g) with standard deviation for the samples was 184.5 ± 24.8.

Further studies are still being carried out to determine the optimum temperature of lipase activity and FFA content of damaged and fungus-infested rubber seed because deterioration of the seed has been reported to have an effect on the acid value and, hence, the FFA of rubber seed (14). However, it is interesting that lipase activity in fresh rubber seeds can be partially inactivated at 60°C and justifies the drying of the seed at this temperature. Pretreatment of rubber seeds at higher temperature has been reported; however, our experience is that drying the seeds at a higher temperature polymerizes the oil and gives it a dark color.

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