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Edited by James Cock, Reginald MacIntyre, and Michael Graham

The International Society for Tropical Root Crops in collaboration with Centro Internacional de Agricultura Tropical
International Development Research Centre
United States Agency for International Development
PROCEEDINGS
of the
FOURTH SYMPOSIUM
of the
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TROPICAL ROOT CROPS SYMPOSIUM

Tropical water should lower the temperature to about 46–47 °C. Sulfuric acid is used to bring the initial pH to 3.5. The large fermentor is now ready for inoculation with the starter culture produced in the same medium in the small fermentor (300 l). This inoculum is about 6.7% of the volume of the culture in the large fermentor. The fermentation is run for about 20 h, during which time the temperature is maintained by means of a temperature controller that actuates a solenoid controlled water valve to regulate the flow of cooling water at ambient temperature.

Once the culture completes its growth, part of the biomass can be saved as a starter culture for a second batch. The remainder of the biomass is harvested by means of a roller-press device designed for this process.

The biomass produced by this process will be mixed with more cassava or any other appropriate ingredient to lower the protein content to the desired level, for feeding as a moist ration to growing pigs. Because the material would spoil if stored wet, it is preferable to obtain a stable dry product for experimental purposes. The material becomes dark and hard when oven dried, but it can be dried by exposure to the sun and air.


Lipase Activity and the Conversion of Fat to Carbohydrate in Cassava

Frederick Narrey

The activities of lipase, isocitrate lyase, and malate synthetase were investigated in cassava. The enzymes were present in mature dry seeds. Their activities increased gradually during the initial phase of germination. In the postgermination period of growth in the dark, however, the activities of these enzymes increased rapidly, and reached their peaks at the period of maximum carbohydrate synthesis and storage, which nearly coincided with the period of maximum lipid degradation. This indicated that the fat-carbohydrate mechanism in cassava involves the key enzymes of the glyoxylate cycle.

Lipids and proteins form the major constituents of the storage reserves in mature cassava seed kernels. Free fatty acids are completely absent, and carbohydrates occur to a minor extent (Narrey et al. 1973). The germination of cassava seeds is accompanied by a gradual degradation of lipids and a rapid breakdown of proteins. However, during the


postgermination phase of growth, a rapid degradation of lipids occurs and results in the mobilization of approximately 62% of total storage lipids within 14 days. Meanwhile, de novo protein synthesis is initiated, resulting in a relatively large turnover of proteins (Nartey et al. 1974).

The rapid degradation of lipids during the postgermination period is concurrent with the accumulation of carbohydrates. This offers evidence for the operation of a fat-carbohydrate mechanism in cassava during germination and growth in the dark, which is further stimulated by illumination.

Oleaginous seeds mobilize and utilize fat for carbohydrate synthesis or respiration during germination and growth. In *Ricinus communis*, germination is accompanied by hydrolysis of triglycerides to free fatty acids, followed by their conversion to carbohydrate. However, in *Citrus vulgaris* and *Elaeis guineensis*, lipids are not converted to carbohydrate, but are largely respired.

During germination and growth of oleaginous seeds, which convert fat to carbohydrate, the activities of lipase, the enzyme that catalyzes the hydrolysis of lipids, increase with time. In *Ricinus communis*, germination is not only accompanied by an increase in the acid lipase activity, which is present in mature resting seeds, but also by the formation of a neutral and an alkaline lipase. Concurrent with the increases in lipase activities, the activities of the key enzymes of the fat-carbohydrate mechanism — isocitrate lyase and malate synthetase — also increase. Thus the increased activities of these enzymes lead to the rapid mobilization and utilization of lipids for carbohydrate synthesis and storage until the total lipid content is greatly diminished.

In our studies on lipid and carbohydrate metabolism in cassava (*Manihot esculenta* Crantz) seeds and seedlings, it was found that most of the storage lipids are rapidly converted to carbohydrate in the postgermination phase of growth (Nartey et al. 1974). It was therefore considered of interest to investigate the changes that occur in the activities of lipase, isocitrate lyase and malate synthetase, to provide more evidence for the operation of a fat-carbohydrate mechanism via the glyoxylate cycle.

**Results and Discussion**

Seeds and seedlings were either finely ground in a mortar with pestle or pressed in the french press with 0.2 M Tris buffer pH 7.0 containing 0.05 M cysteine and 0.01 M EDTA for lipase isolation, and with 0.067 M phosphate buffer pH 7.6 for isocitrate lyase and malate synthetase isolation. The brei was passed through four layers of muslin and centrifuged at 270 g for 30 min to remove cell debris. The cloudy supernatant was recentrifuged at 10,800 g for 30 min to obtain an upper layer fat-pad fraction containing most of the lipase activity, an infranatant fraction containing most of the isocitrate lyase and malate synthetase activities, and a precipitate that was discarded. The two fractions were partially purified by extraction with ether, followed by dialysis and lyophylization.

**Lipase Activity in Mature Dry Seeds:**

**pH Optima**

Mature dry seeds had lipase activity, which increased with imbibition of water. The enzyme had two pH optima, depending on the method of preparation. Enzyme preparations obtained by grinding showed an optimum at pH 7.0, whereas preparations from the french press showed an optimum at pH 9.0. This indicates the presence of two enzymes, one functioning as a neutral lipase, and the other as an alkaline lipase. Both types of enzymes have been identified in germinating castor bean and Douglas Fir seeds (Ching, 1968, Muto and Beevers, 1974).
Cassava lipase catalyzed the hydrolysis of a variety of oils, including endogenous *Manihot* oil, and exogenous *Manihot* oil, soybean oil, cotton seed oil, and olive oil. Fig. 1 shows cassava seed lipase activity on endogenous *Manihot* oil. In a typical experiment, the lipid-enzyme fat-pad was isolated intact, dialyzed, and lyophylized. A portion of the product thus obtained was homogenized in phosphate buffer pH 8.0, and the hydrolysis of ester bonds followed.

**Substrate Specificity**

Fig. 2. Electronmicrographs of thin sections through: (A) the endosperm of mature dry cassava seed showing cells filled with lipid bodies; (B) the endosperm of a 10-day-old etiolated seedling showing accumulation of starch in the plastids; and (C) the cotyledon of an 18-day-old seedling showing decreasing lipid bodies and increasing accumulation of starch.
Fig. 3. Changes in isocitrate lyase and malate synthetase activities in cassava as a function of germination and growth in the dark. Activities were determined spectrophotometrically.

Cassava lipase isolated from imbibed seeds gave the following results (μmole ester hydrolyzed in 30 min): Manihot oil 0.40; soybean oil 0.45; cotton seed oil 0.40; olive oil 0.75; triacetin 0.05; tributyrin 0.12; triolein 0.30; and trilinolein 0.50. The cassava lipase catalyzed the hydrolysis of Manihot and soybean oil at nearly equal rates. This is probably due to the nearly identical concentrations of triglycerides of linoleic, oleic, and palmitic acids present in Manihot and soybean oil (Nartey and Moller 1973). Although the enzyme showed a broad substrate specificity, it was more active on triolein and trilinolein. This indicates a substrate preference of triglycerides of long chain fatty acids with one or more double bonds.

Changes in Lipase Activity During Germination and Growth

Lipase activity in cassava seeds increases with germination and reaches a peak after 9 days. After 18 days, another peak is reached which coincides with the period of maximum lipid degradation. Fig. 2 illustrates this tendency at the cellular level and shows that lipid bodies present in mature dry seeds were hydrolyzed after 10 days, giving rise to the accumulation of some carbohydrate in plastids; after 18 days most of the lipids disappeared, giving rise to more carbohydrate.

Isocitrate Lyase and Malate Synthetase

As stated earlier, both soluble carbohydrates and starch begin to accumulate after 9 days. Accordingly, the enzymes concerned with the conversion of fat to carbohydrate were investigated during the period of germination and growth in the dark. It was found that dry seeds possess low activities of isocitrate lyase and malate synthetase. However, on germination, their activities increased, reaching a peak at approximately 15 days, nearly coinciding with the period of maximum degradation of triglycerides by lipase. Fig. 3 illustrates the activities of the key enzymes of the glyoxylate cycle in cassava seeds germinating and growing in the dark.

The above data gives further evidence that the operation of the fat-carbohydrate mechanism in cassava seeds during germination and growth involves the key enzymes of the glyoxylate cycle, namely, isocitrate lyase and malate synthetase. Together with lipase, the activity of which also increases several fold with germination and reaches a peak in the postgermination period of 9–18 days, these enzymes give rise to carbohydrate for storage and metabolic processes.

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