Incidence of Ripening and Chilling Injury on the Oxidative Activities and Fatty Acid Compositions of the Mitochondria from Mango Fruits

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ABSTRACT

The succinate oxidation capacities of mitochondria isolated from mango fruits (Mangifera indica L.) stored at 4, 8, 12, and 20 C were investigated during storage. In normally ripening fruits (at 12 and 20 C) the oxidative capacities increased during the first 10 days and then decreased slowly. At lower temperatures (4 and 8 C), the fruits showed chilling injury symptoms, after about 10 days of storage and the succinate oxidation capacities of mitochondria decreased progressively. Plots of succinate oxidation capacities as against storage temperature showed a marked discontinuity between 12 and 8 C, only when chilling injury was observed on fruits stored at low temperature.

The variations of mitochondrial fatty acid composition during the storage of fruits at different temperatures were also investigated. A marked decrease of the molar ratio palmitoleic acid/palmitic acid, the predominant fatty acids in mitochondrial lipids, was observed to accompany both the succinate oxidation decrease and the induction of chilling injury.

The induction of chilling injury has been related to temperature-induced changes in the molecular ordering of membrane lipids (13–15, 21). In chilling-sensitive plants, membrane lipids would exhibit a phase transition from a liquid-crystalline structure above the transition temperature, ranging usually around 10 to 12 C, to a rigid gelled structure at lower temperature.

This phase transition is a complex phenomenon and Arrhenius plots of the oxidative activity of mitochondria from chilling-sensitive plants as against temperature have been shown to be in fact triphasic, with discontinuities at about 12 and 28 C. The two temperatures at which discontinuities occur mark the limits of the lipid phase transition zone (22). On another hand, the fluidity of membrane lipids has been related to the degree of unsaturation of their component fatty acids (14).

None of the above observations had been established with the mango. We have therefore investigated both the oxidative capacities and the fatty acid compositions of isolated mitochondria from mango fruits stored at different temperatures to determine whether the onset of chilling injury could be related to an alteration of membrane lipids at low temperature.

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MATERIALS AND METHODS

Mango fruits (Mangifera indica L., cv. Amélie) were grown in Mali (Africa) and harvested at a mature green stage. Immediately after picking the fruits were sent by air to the laboratory in France. These green mango fruits have been stored at four different temperatures: on one hand 20 and 12 C, these temperatures allowing both a normal ripening of fruits; on the other hand, 8 and 4 C, these relatively low temperatures inducing both apparent chilling injury symptoms on the fruits, after about 10 or 15 days, respectively, at 4 or 8 C.

Mitochondria were isolated in the cold (0–4 C), after storage of the mangoes in the cold chamber for one night (14–15 hr). Pulp fragments were excised from the parenchyma of fruits. The homogenizing medium consisted of 0.6 m mannitol, 5 mM cysteine hydrochloride, 1 mM MgCl2, 10 mM KCl, 10 mM EDTA, 1 g/100 ml of PVP, 1.5 mg/ml of BSA, and 0.1 mM phosphate buffer adjusted to pH 7.5. Fifty g of mango pulp was homogenized in a blender with 200 ml of the homogenizing medium. The crude mixture was then infiltrated under vacuum for 10 min and ground again by means of a roller mill (9). The resulting juice was filtered through Miracloth, homogenized in a Potter apparatus, and centrifuged at 3,000g for 5 min. The supernatant was centrifuged at 17,000g for 15 min. The pellet, resuspended in a washing medium identical to the homogenizing medium except for PVP, cysteine, and KCl, was spun down at 12,000g for 10 min. The final mitochondrial pellet was suspended in 2 ml of the washing medium.

Oxygen uptake was measured polarographically (16) by means of a Gilson apparatus fitted with a Clark electrode. Measurements were made at 25 C, using 1 to 1.5 ml of mitochondrial protein, in the following respiratory medium: 0.5 m mannitol, 5 mM KCl, 0.5 mM EDTA, 10 mM KH2PO4, 10 mM Tris buffer (pH 7.2), and 0.75 mg/ml of BSA. Succinate and ADP were added to yield final concentrations of 0.1 m and 0.6 mM, respectively.

Analysis of mitochondrial fatty acids was by means of GLC as described previously (17). Proteins were determined by the method of Gornall et al. (7).

RESULTS AND DISCUSSION

Oxidative Capacities of Mitochondria Isolated from Fruits Stored at Different Temperatures. Considerable difficulty was encountered during the initial attempts of isolation of an actively respiring mitochondrial fraction from mango fruits because of the highly acidic nature of the fruit pulp and also because of the
abundance of tannins in the cell vacuoles. These difficulties have been partially overcome by using PVP in the grinding medium, as recommended by Hulme et al. (8). Succinate oxidation rates obtained with our preparations (10-22 nmol of O₂ min⁻¹ mg⁻¹ of protein at 25 C) are of the same order of magnitude as those reported previously for mango mitochondria by Patwardan (20) although we did not add any exogenous Cyt c in our mitochondrial preparations, which Patwardan did to activate his preparations.

More recently, Baqui et al. (3) also produced similar succinate oxidation rates (17-43 nmol of O₂ min⁻¹ mg⁻¹ of dry matter) for isolated mango mitochondria respiring in a medium containing Cyt c, NAD, and thiamine pyrophosphate. Our mango mitochondrial preparations appeared loosely coupled with respiratory control ratios ranging from 1.2 to 1.3.

The variations during storage of the oxidative capacities of the mitochondria isolated from mango fruits stored at four different temperatures are shown in Figure 1. In the case of fruits ripening normally, at 20 or 12 C, the succinate oxidation capacities increased during the first 10 days and then decreased slowly. A marked increase was observed at 20 C, corresponding to a marked climacteric rise in the respiration of entire fruits (4, 10, 11).

At lower temperatures, the fruits showed chilling injury symptoms after about 10 days of storage and the succinate oxidation capacities of isolated mitochondria did not show any increase during storage but instead a progressive decrease to lower values of the order of 5 nmol of O₂ min⁻¹ mg⁻¹ of protein.

The effect of storage temperature on the oxidative capacities of mitochondria from both normally ripening and chilling stored fruits is summarized in Figure 2. The succinate oxidation capacities of mitochondria showed an exponential decrease with decreasing storage temperature (from 20 to 4 C) for the first 10 days of storage during which no symptom of injury could be observed (Fig. 2A). It must be emphasized that the oxidation capacities of mitochondria were measured at a constant temperature (25 C) and that the observed decrease must result from modifications of the organelles during storage. This hypothesis is reinforced by the fact

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**Figure 1.** Variations, during storage, of the succinate oxidation capacities of mitochondria isolated from mango fruits, stored at 4, 8, 12, and 20 C. Each point is the average of three independent assays carried out as indicated under "Materials and Methods." Double arrows on the 8 and 4 C graphs indicate the appearance of chilling injury symptoms (C.I.).
that the plots showed a marked discontinuity between 8 and 12°C during the last days of storage (after about the 20th day) corresponding to the period of obvious development of chilling injury on fruits stored at low temperatures (Fig. 2B). This discontinuity, apparently correlated with the development of chilling injury, strongly suggests some modification of mitochondrial membrane composition in injured fruits.

In connection with these results, it was interesting to determine whether modifications of mitochondrial lipids occurred during the storage of fruits at different temperatures.

**Fatty Acid Composition of Mitochondrial Lipids.** The major fatty acids of mango mitochondria are palmitoleic (36.7 mol/100 mol), palmitic (23.7%), oleic (18.1%), linoleic (7.5%), and linolenic (6.7%) acids. The fatty acid composition of mitochondrial lipids was found essentially similar to that of the entire pulp tissues as yet analyzed by others (1, 2, 5, 6, 19).

The variations of mitochondrial fatty acid compositions during the storage of fruits at different temperatures were investigated (Fig. 3). The fatty acids having 16 carbon atoms presented the most remarkable change in correlation with the development of chilling injury. In the case of healthy fruits, ripening at 20 or 12°C, the molar ratio of palmitoleic acid to palmitic acid increased with ripening and this was due primarily to a fall in the proportion of palmitic acid in mitochondrial lipids at the end of the storage period (from 32 to 26%). When chilling injury developed on fruits, at 8 and 4°C, the molar ratio of palmitoleic acid to palmitic acid...
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FIG. 3. Variations, during storage, of the fatty acid compositions of mitochondria isolated from mango fruits stored at 4, 8, 12, and 20 C. C.I.: appearance and development of chilling injury.

decreased continuously, primarily because of a diminution of palmitoleic acid in mitochondrial lipids at the end of the storage period.

CONCLUSIONS

Changes in respiration of some tropical plants in response to chilling storage have been investigated (12, 18). Respiratory activities of mitochondria prepared from chilled sweet potatoes were decreased (23) and we have found again this phenomenon in mango mitochondria. We have been able to correlate this decrease with a remarkable change in the fatty acid composition of mitochondrial lipids in fruits stored at low temperature. The molar ratio of palmitoleic acid to palmitic acid was dramatically depressed, as compared with mitochondrial lipids from healthy fruits, which would certainly induce important changes in the fluidity of mitochondrial membranes. It is tempting to attribute the lowering of the oxidative capacities of mitochondria from chilling injured fruits to the modifications of membrane flexibility resulting from changes in fatty acid compositions.

LITERATURE CITED

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