generally the most important in controlling whiteness of bean seed-coats. Heterosis effects were also significant in the inheritance of bright-white-seed-coats, indicating the importance of dominance genetic effects. Significant reciprocal differences were attributed to maternal effects. In addition to the factors proposed earlier for the seed-coat whiteness character, nuclear genic influences of the female parent probably are involved in the observed reciprocal differences.

The seed-coats of ‘Bulgarian White’ had the brightest white color, and this cultivar had high general combining ability. Korban (5) has shown that ‘Bulgarian White’ also has high seed-coat cracking resistance and that it has a high ability to transmit this trait in crosses.

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Phosphorylase, Phosphatase, α-Amylase Activity and Starch Breakdown during Ripening of ‘Marmelo’ Banana [(Musa acuminata Colla) x (Musa balbisiana Colla) ABB Group] Whole Fruit and Thin Slices

Adimilson B. Chitarra
Departamento de Ciência dos Alimentos, Escola Superior de Agricultura de Lavras, 37.200 Lavras, MG, Brazil
Franco M. Lajolo
Departamento de Alimentos e Nutrição Experimental, FCF, USP, Caixa Postal 30786, 01000, São Paulo, SP, Brazil

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Abstract. Starch hydrolysis, phosphorylase, phosphatase and α-amylase activity of ‘Marmelo’ banana (Musa acuminata Colla x Musa balbisiana Colla, ABB group) during ripening of whole fruit and thin slices infiltrated with water (controls), cycloheximide and actinomycin D were investigated. Phosphatase and α-amylase activity of whole fruit increased during the climacteric while phosphorylase decreased as starch degradation proceeded. Starch degradation had a different temperature coefficient than that of α-amylase activity when the storage temperature was raised from 20 to 25°C and stopped when 25% of the starch was still present. Activity of α-amylase in slices infiltrated with water, increased before the onset of the climacteric as a result of injury and at the same time starch was being hydrolyzed. Cycloheximide inhibited this induced activity increase but not starch breakdown or the increase of activity which occurs during the climacteric. Actinomycin D did not inhibit the α-amylase increase and its effect was a general retardation of ripening.

Starch hydrolysis is one of the most conspicuous changes that characterizes ripening of some climacteric fruits. This change is more evident in banana where starch concentration drops in a few days from an average of 22% in the preclimacteric phase to less than 1% at the climacteric peak (8). Phosphorylase and amylase have been detected in many storage tissues of many fruits but the role of each in the ripening process is not clear. In spite of the importance of these reactions as furnishing energy for other changes of ripening, very little is known of the mechanisms involved. They are also attractive pathways as a model to study some possible ripening controls. Young et al. (21) noticed a rise of α-amylase activity of banana during ripening but their results did not provide a conclusion about its involvement in starch hydrolysis. They also detected the presence of β-amylase and phosphorylase in all stages of ripening but were not able to assay these enzymes due to the presence of enzyme inhibitors in the preclimacteric phase. Yang and Ho (20) suggested phosphorylase might be more important than amylase based on the observation of an increased

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activity during the climacteric. A similar observation was made by Surendranatham and Nair (18) but for earlier stages of ripening. Singh and Sanwall (15, 16, 17) partially purified three phosphorylase forms from ripe bananas and observed different biochemical characteristics but did not investigate the behavior of these enzymes during ripening.

Areas and Lajolo (1) reported starch degradation associated with phosphorylase action during ripening of 'Dwarf cavendish' (Musa acuminate Colla, AAA group). Banana 'Marmelo', a hybrid (ABB group) that incorporates 2 genomes from a different species, Musa balbisiana Colla, was studied here to relate phosphorylase, amylase and phosphatase with starch breakdown. This banana has an abnormal behavior: the peel does not become clear yellow, aroma and texture do not undergo their usual changes, and starch drops only to 5% during ripening at normal temperature and stabilizes, indicating some inhibitory mechanisms at this point which makes this cultivar an interesting model for study. Effects of certain metabolic inhibitors on these enzymes are also reported.

**Materials and Methods**

Fruit of 'Marmelo' (12, 14) were obtained at the preclimacteric stage (125 days after flowering) from the Agronomy School orchard at Lavras, Minas Gerais, Brazil, when the fruit reached a maturity stage between “full three quarters” and “full” (measured on the central fruit of the second hand of a bunch).

Fruit were washed with 2% sodium hypochlorite solution followed by distilled water and stored in respiration chambers (3 kg/ chamber, about 12 fingers taken from adjacent hands) with a flow of humidified air (7 liters/hr) at 20°C and 30°C for ripening experiments. Three chambers (desiccators with 30 cm diameter x 20 cm height) were used for each temperature. Relative humidity in the chambers was not measured but the air passed through 20% KOH, 5% Hg(ClO4)2, and 7% KOH solutions. About 60 g of 5 mm thick slices taken from the central part of several fruit from the same hand were used for infiltration experiments in jars (1 liter) with an air flow of liter/hr at 20°C. Three jars were used for each treatment. Infiltration of metabolic inhibitors was conducted at 100 mm Hg pressure until 10% weight increase was achieved. Slices were immersed in sterilized water (controls) actinomycin D was measured using C02 Infra Red Analyzer (Analytical Development Co.).

About 1 g of banana pulp was homogenized with 4 ml of 0.5 N NaOH in a Potter-Elvehjem homogenizer and centrifuged at 3,000 g for 15 min. The supernatant was neutralized with 0.5 N acetic acid and the volume made up to 100 ml. This solution was used for starch determination after precipitation with 80% ethanol and hydrolysis with amyloglucosidase obtained from Sigma (7 units/ml starch) for 2 hr. In 0.2 M acetate buffer (pH 4.8) (1). Nelson's method (6) was utilized for determination of soluble sugars in the same extract before starch precipitation.

Extraction of phosphorylase and phosphatase was done in 0.1 M citrate buffer (pH 6.3) containing 0.6% PVP, 2mM EDTA, 2mm cysteine hydrochloride at 4°C in a Potter-Elvehjem homogenizer. The resulting suspension, 3 g of banana/10 ml of extracting medium, was centrifuged at 4°C at 60,000 g for 1 hr and the supernatant was used as the source of the 2 enzymes. Phosphorylase activity was determined according to Lee's method (5) in the direction of synthesis. Inorganic phosphate produced was measured by Fiske-Subbarow's technique (4). Phosphatase activity was determined according to Deleo and Sacher (3) with p-nitrophenol estimated by reading at 400 nm on a Beckman Acta III spectrophotometer. One unit of enzyme (either phosphorylase or phosphate) activity was assumed to be equivalent to the production of 1µmol inorganic phosphate per min.

Amylase was extracted in a citrate phosphate buffer 0.1 M (pH 6.6) containing 0.6% PVP and 0.02 M cysteine hydrochloride. The homogenate was centrifuged at 20,000 g. Activity was evaluated in 1 ml aliquots to which were added 10 mg amylochrome (starch substrate labeled covalently with Remazol brilliant Blue R, specific for α-amylase) obtained from Hoffman-La Roche, Inc. and 1 ml water incubated at 37°C for 2.5 hr. (10). Four ml of 0.1 M potassium biphthalate solution were then added to inhibit further reaction and the suspension was centrifuged at 2,000 g for 10 min. Readings made at 620 mm in a Model Acta III Beckman spectrophotometer.

Electrophoresis was conducted in polyacrylamide gel according to Ornstein and Davis (7) using 7% monomer and 0.05% amyllopectin in the column. Phosphorylase bands were revealed by incubating the columns for 14 hr in a medium containing 0.025 M EDTA and 0.025 M glucose-1-phosphate in 0.5 M citrate buffer (pH 6.3), followed by iodine (0.2% I2 — 2% KI solution) staining. Glucose-1-phosphate and EDTA were omitted from the incubation medium for demonstration of amylase bands.

**Results and Discussion**

*Studies with whole fruit.* Changes observed in starch and soluble (reducing and non-reducing) sugars content during ripening of 'Marmelo' banana at different temperatures are shown in Table 1. An increase from 20° to 25°C greatly accelerated the onset of climacteric and starch breakdown but the process stopped when 5% starch was still present at both temperatures. A lower starch level, 3.3%, was reached at 30°C but the fruit were clearly overripe and

![Table 1. Starch, reducing (expressed as glucose) and non-reducing (expressed as sucrose) soluble sugars evolution during ripening of bananas at 20°, 25°, and 30°C. Results expressed as percent fresh weight.](image-url)
Fig. 1. Changes of respiration, starch content, α-amylase, phosphatase and phosphorylase activity of whole fruits stored at 20° and 25°C.

- ○ Respiration (mg CO₂/kg · hr)
- □ Starch % (fresh wt)
- △△ α-amylase (I.U./g fresh wt)
- ● Phosphorylase (U/g fresh wt)
- ■ Phosphatase (O.D./g fresh wt · min)

starting to deteriorate. By contrast, starch in ‘Dwarf Cavendish’ is almost completely, 99.7%, transformed during the climacteric (8).

Reducing sugar (presumably glucose + fructose) accumulation also seemed to precede formation of non-reducing (presumably sucrose) sugars. This again is different from ‘Dwarf Cavendish’ in which sucrose does not follow but accumulates first and faster than glucose + fructose as starch breakdown proceeds (1). These results for ‘Marmelo’ should be confirmed with a more specific method of sugar analysis than the ones used here.

The sugar content of ‘Marmelo’ is about 30% lower than for ‘Dwarf Cavendish’ as a result of incomplete starch transformation which explains at least partially the former’s different taste.

Behavior of phosphorylase, phosphatase and α-amylase during ripening at 20° and 25°C are depicted in Fig. 1. Phosphorylase activity was constant during the preclimacteric phase but dropped after the climacteric peak and paralleled the decrease in starch content. Phosphorylase activity shows a different pattern in ‘Dwarf Cavendish’ (1), where it shows a rate 2- to 4-fold higher and a 50% increase at the onset of climacteric just before the beginning of starch breakdown, then falls to the previous value after the disappearance of the starch. This behavior was taken as indicative of a possible involvement of this enzyme in starch – sucrose transformation via sucrose synthetase (1). The behavior observed in ‘Marmelo’ seems at first to rule out the participation of phosphorylase in the breakdown process but rather suggests at least a partial involvement of α-amylase which showed a sharp increase of more than 100% in 24 hr (Fig. 1). Similar results with amylase activity were obtained by Young et al. (21).

The fast phosphorylase inactivation which parallels starch disappearance at 20°C was confirmed at 25°C (Fig. 1) and also at 30°C (not shown), indicating it has the same temperature dependence as starch hydrolysis. Phosphorylase is present in the amyloplast hence these results are understood as an indication of the liberation of the enzyme from the amyloplast as a result of granule degradation and its subsequent inactivation in the cytoplasm (19).

The observed increase in α-amylase activity at 20°C also occurred at 25° but it was delayed in relation to the beginning of starch transformation (Fig. 1). In fact the starch present was 50% degraded when the amylase activity increase at 25°, while both processes were almost simultaneous at 20°. The different temperature coefficients for the processes of starch degradation and α-amylase increase, plus a degradation of starch prior to the induced increase in α-amylase activity (at 25°), raise doubts as to the exclusive involvement of this enzyme. The behavior of phosphatase, as with α-amylase was similar to that found in ‘Dwarf Cavendish’, there being a sharp increase in activity during the climacteric, as previously observed by other authors (3).

Studies with metabolic inhibitors. The significance of protein synthesis in relation to ripening has been object of controversy. An increased rate of radioactive amino acid incorporation into proteins has been measured in many other fruits as ripening commences (11), a fact that has been interpreted as being the synthesis of enzymes important in the ripening process. Immunochemical
Evidence of "de novo" synthesis has also been obtained (13). Recently, Brady and O'Connel (2) suggested ethylene induced an increase in the rate of turnover of many enzymes rather than a specific protein which would lead to ripening.

The controls (slices infiltrated with water) showed as expected the typical injury response for respiration (9), an increase of CO$_2$ production due to wound infiltration followed by a decrease to a normal value and a second rise corresponding to the climacteric (Fig. 2, 3). The response included increase of $\alpha$-amylase activity and the concomitant triggering of starch degradation process even before the climacteric, a result of the injury.

In consequence, starch was already 50% transformed at the beginning of the climacteric (day 4). Cycloheximide (a translation inhibitor) clearly blocked $\alpha$-amylase-induced synthesis but not starch degradation which proceeded at a similar rate, thus indicating non-existence of casual relationship between the 2, as suggested earlier (Fig. 1). It is interesting to observe cycloheximide did not inhibit the $\alpha$-amylase increase that occurred later during the climacteric peak.

Phosphatase was inhibited in relation to the control at the same time, confirming its known dependence upon protein synthesis (3) and indicating the effectiveness of cycloheximide. One can speculate whether there are different controls for $\alpha$-amylase induced during injury or during ripening.

Cycloheximide treatment of 'Marmelo' slices did not significantly alter the timing of ripening, the climacteric onset being about 4 days for the controls and 3 days for cycloheximide-treated slices. 'Dwarf Cavendish' slices treated with cycloheximide usually showed, however, a clear anticipation of the climacteric and ripening (1, 9).

Actinomycin D (a transcription inhibitor) gave a reaction as observed for the usual banana (1, 9) but extended the onset of the climacteric from 5 to 13 days and extended the time for starch hydrolysis correspondingly but also did not inhibit it or the injury-induced $\alpha$-amylase increase (Fig. 2).

One possible interpretation is there were stable mRNAs present for $\alpha$-amylase synthesis. An interesting observation was the rate of increase in soluble sugars was slower with cycloheximide and also for actinomycin D even though starch hydrolysis proceeded at the same rate as in the water (controls) and cycloheximide-treated slices. This was taken as indicative of the partial suppression of a sucrose synthesis mechanism and formation of a different sugar(s) not detected by the technique used for sugar determination. Further studies are under way to confirm this hypothesis. Electrophoresis profiles of $\alpha$-amylase and phosphorylase from slices in the preclimacteric and climacteric (ripened) stages are shown in Fig. 4. Ripening caused the appearance of a new band (region a), enlargement of some (region b) corresponding to the increase of activity and disappearance of others (region c). Cycloheximide, but not actinomycin D, was able to change the profiles causing the inhibition of synthesis of the band a and c. Cycloheximide (Fig. 2) but not actinomycin D (Fig. 3) was able to inhibit an increase in $\alpha$-amylase during injury, thus it is possible to conclude the band of region c (inhibited by cycloheximide) is possibly related to the injury effect and band a (not inhibited by actinomycin) to the climacteric phase. Band of region c (inhibited by cycloheximide) may possibly be related to the injury effect and band a (not inhibited by actinomycin) to the climacteric phase. Phosphorylase did not show different patterns during ripening nor a clear effect from the inhibitors.

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One can conclude from these results with whole fruit and slices that α-amylase activity increases during ripening and in response to injury. There is no strict dependence, however, of starch breakdown on increased activity of α-amylase.

Phosphorylase activity drops during the climacteric and ripening but could still be involved in the process of starch transformation since its activity would be enough for the observed rate of starch degradation. Phosphorylase behavior, sugar formation, final starch level and effect of cycloheximide are aspects that differentiate 'Marmelo' (ABB group) from 'Dwarf Cavendish' (AAA group) bananas.

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Response of Pear to Inbreeding

R. L. Belt and Jules Janick

Department of Horticulture, Purdue University, West Lafayette, IN 47907

R. H. Zimmerman

U.S. Department of Agriculture, Science and Education Administration, Beltsville Agricultural Research Center, Beltsville, MD 20705

T. van der Zwet

U.S. Department of Agriculture, Science and Education Administration, Appalachian Fruit Research Station, Kearneysville, WV 25430

R. C. Blake

U.S. Department of Agriculture, Science and Education Administration, Ohio Agricultural Research and Development Center, Wooster, OH 44691

Abstract. No consistent trend towards increased inbreeding has existed within the U.S. Department of Agriculture pear breeding program over 17 years of crosses based on the mean inbreeding coefficient and the percentage of non-inbred progenies. Selections did not tend to be more or less inbred than the population of all seedlings. There was consistent, but small, trend towards a reduction in seedling vigor with increased levels of inbreeding as measured by 5th year stem diameter. A significant positive, but small, association between increased inbreeding and improved flavor, grit, and texture were observed, even after correction for the effects of parental values for these characters. Limited inbreeding does not adversely affect improvement of fruit quality and appears to be of some benefit in facilitating selection of favorable alleles.

Inbreeding of naturally cross-pollinated species usually results in a loss of plant vigor due to the expression of deleterious recessive genes in the homozygous state. When the genetic base of a breeding program is very narrow, however, inbreeding may be difficult to avoid. A limited number of parents have been extensively used, for example, in the breeding of raspberries (15), peaches (11), cherries (9), and, to a lesser extent, apples (8).

Brown (8) noted a reduction of seedling vigor, and a concomitant increase in seedling mortality, associated with the recurrence of certain parents in the pedigrees of apples. He concluded, however, that a limited amount of backcrossing to one or a few outstanding parental genotypes may be useful in intensifying certain quantitatively inherited characters. Morrow and Darrow (15) observed decreased vigor as a result of limited inbreeding in strawberry. The method would be especially useful in situations where highly desirable levels of a character are present in only a few genotypes. Larger progenies, however, may have to be produced in order to compensate for the deleterious effects of inbreeding on seedling survival and vigor.

Outcrossing is the norm in natural pollination of pears. Gametophytic self-incompatibility effectively excludes selfing within the genus Pyrus. In the pear breeding program of the U.S. Department of Agriculture, the pedigrees of many early selections include 'Bartlett', 'Doyenne du Comice', 'Roi Charles de Wurtzburg', 'Seckel', and a few other outstanding cultivars. The selections have, in turn, been used extensively as parents in subsequent crosses. A decision to continually sample from a small or inbred...