

Effect of Gibberellic Acid on Ripening of Strawberry Fruits (*Fragaria annanassa* Duch.)

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Abstract. Effect of GA₃ on postharvest ripening in strawberry fruit was evaluated through different biochemical parameters. Strawberry slices at different ripening stages were incubated with GA₃. A significant decrease on respiratory activity depending on GA₃ concentration was obtained. Also GA₃ was applied to whole and deached fruit at white and green ripening stages. Our results show that GA₃ has an inhibitory effect on strawberry fruit ripening, evidenced by a decrease in the respiratory activity and a delay in anthocyanin synthesis and chlorophylls degradation.

Regulation of ripening is fairly known in climacteric fruits in which ethylene plays a key role as a ripening promotor. In contrast, ethylene has only a minor effect in nonclimacteric fruits like strawberry (Given et al. 1988a, Abeles and Takeda 1990). At present, hormonal regulation of strawberry fruit ripening is not fully understood. It has been demonstrated that the achenes produce the key hormones responsible for fruit ripening (Nitsch 1950). These hormones are probably auxins. They speed up the receptacle growth during the early stages of the development process (Southwick and Poovaiah 1987). During later stages of ripening, these hormones produce a decrease in the activity of phenylalanine ammonia-lyase (PAL, E.C.4.3.1.5), which, in turn, result in a decrease in the amount of anthocyanin. Auxins also lead to a delay in chlorophylls degradation (Given et al. 1988a,b,c). The role of gibberellins in the regulation of fruit ripening has also been the subject of a number of studies. It has been observed that gibberellic acid (GA₃) causes a delay in

ripening. Application of GA₃ delayed chlorophyll degradation in citrus (Biale 1978, Abdel-Gawad and Romani 1974) and mango fruits (Khader et al. 1988). GA₃-induced reduction in amylase and peroxidase activities were also observed in mango fruits (Khader et al. 1988). GA₃ also delayed the softness and color change in cherries (Facteau et al. 1985). The respiratory climateric was inhibited by GA₃ application in apricot (Abdel-Gawad and Romani 1974) and tomato (Babbitt et al. 1973) fruits. The object of this study was to examine the effect of exogenously applied GA₃ on strawberry fruits harvested during the early stages of ripening. The effect of GA₃ were evaluated by measuring CO₂ production and detecting changes in pigment levels (chlorophylls and anthocyanins).

Materials and Methods

Plant Material and GA₃ Application

Strawberries used (*Fragaria annanassa*, Duch, Selva) were representative of different ripening stages and were obtained from local producers. Fruits were classified depending on external color: green, white, and 25% red. A *green fruit* is defined as one that has completed its growth but has not yet changed its color, a *white fruit* has just started its color change, and a *25% red fruit* is one which has 25% of its surface with red color. The fruits were thoroughly washed with water, then with a 1% NaClO solution and, finally, three times with sterilized distilled water. Subsequently, they were dried under a laminar flow in sterile conditions. For certain experiments, achenes were removed using sterilized tweezers. Sterile solutions of 1, 0.5, and 0.25 mM GA₃ in 0.053 M citric buffer, 0.094 M Na₂PO₄H adjusted to pH 4.5, containing 2% dimethylsulfoxide (DMSO) were applied to whole fruit with cotton swabs. Treated fruits were placed in trays, covered with a polyolefinic film microperforated with 2 holes (6.25 cm²) per square inch and incubated in the dark at 20°C for 3 days. Samples were collected periodically and stored at -70°C until analysis.

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Determination of Respiratory Activity

Strawberry slices, 2–3 mm thick, were cut horizontally from the central part of the fruit. Three or four slices (approximately 2 g) were placed in hermetically sealed flasks, containing approximately 5 ml of sterile solutions of 0.053 M citric buffer, 0.094 M $\text{Na}_2\text{PO}_4\text{H}$ (pH 4.5) with 0–0.5 mM of GA_3 . The flasks were incubated in the dark at 20°C for 3 days. Gas samples were extracted periodically, and the level of CO_2 concentration measured. The analysis was performed in a Shimadzu GC-6A model gas chromatograph equipped with silica gel and molecular sieve 5A columns and a thermal conductivity detector. The carrier gas was H_2 . Respiratory activity was expressed as microliters of CO_2 produced per gram of tissue per hour.

Extraction and Estimation of Pigment Content

Anthocyanins. Frozen fruits were ground to fine powder and a portion (0.3 g) was extracted in 3 ml of methanol containing 1% HCl, stirred and centrifuged (2000g, 15 min) at 4°C. Supernatant anthocyanin concentration as pelargonidin 3-glucoside, was determined in a Shimadzu spectrophotometer UV 150-02 at 510 nm using a molar absorptivity coefficient of 36000 l/cm.mol (Woodward 1972).

Chlorophyll. Frozen fruits were ground to fine powder and a 500-mg portion was extracted in 5 ml of acetone at –20°C, stirred, and centrifuged (2000g, 15 min) at 4°C. The concentration of chlorophylls was determined spectrophotometrically at 645, 652, and 663 nm (Bruinsma 1963).

Results Analysis

Experiments were performed at least three times. Respiratory activity experiments were carried out with three to four slices in each flask, with three replications for each hormone concentration. Experiments for estimation of pigment content were performed with, at least, 20 fruits for each hormone concentration. Variance analysis (ANOVA) was used for statistical data evaluation. Mean comparison was determined with the Least Significant Difference (LSD) test at a significance level (α) of 0.05.

Results and Discussion

Numerous biochemical and physiological changes take place during fruit ripening. The production of ATP and other energy-rich component in ripening is provided by the respiration process. Thus, a higher rate of respiratory level is associated with a higher rate of ripening. In the present work the respiratory activity of strawberry fruit slices was evaluated at green, white, and 25% red stages of ripening during 3 days of incubation as described in the Materials and Methods section. Results are shown in Fig. 1. A statistical decrease of respiratory activity was ob-

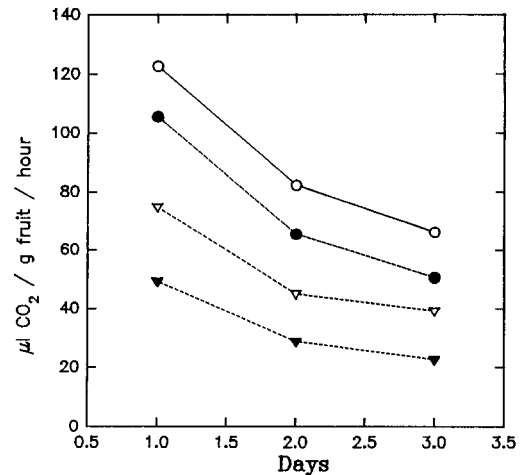


Fig. 1. Respiratory activity of strawberry slices from fruits harvested at different stages of ripening. Slices were incubated in the dark for 3 days at 20°C in 0.053 M citric buffer, 0.094 M $\text{Na}_2\text{PO}_4\text{H}$ adjusted to pH 4.5. $\text{LSD}_{0.05} = 10.3$. ○ = green, ● = white, ▽ = 25% red, ▼ = 25% red treated with 0.5 mM GA_3 .

served during storage. It corresponded to a decrease of 35–40% from the first to the second day of incubation and another 15–20% from the second to the third day of incubation. Moreover, CO_2 production decreased with the ripening stage evidencing a lower metabolic activity.

The effect of GA_3 on the respiratory activity was also studied. Hormones commonly have a physiological effect in the range of 10^{-5} to 10^{-9} M. However, when hormones are used for technological purposes, for instance to regulate fruit ripening, they are applied at higher concentrations: 10^{-4} to 10^{-5} M (Ben-Arie and Ferguson 1990, Facticeau et al. 1985, Hinderer et al. 1984, Ben-Arie et al. 1986). These high concentrations are necessary in part to assure an adequate diffusion into the tissue and overcome effects of degradation. Considering this fact and in order to analyze the feasibility of the application of GA_3 to regulate the rate of strawberry ripening, we decided to study the effect of the hormone at the 10^{-4} to 10^{-5} M level. The respiratory activity of strawberry slices corresponding to ripening stage of 25% red incubated with 0.5 mM GA_3 is shown in Fig. 1. This treatment leads to a decrease of CO_2 production compared to the control at the same ripening stage. It was also verified that the rate of inhibition was approximately constant during the incubation period. Similar results (not shown) were obtained for white and green fruit.

The effect of different levels of gibberellic acid on respiratory activity was analyzed by computing the ratio between the respiratory activities of slices treated with GA_3 (C) and control slices without GA_3

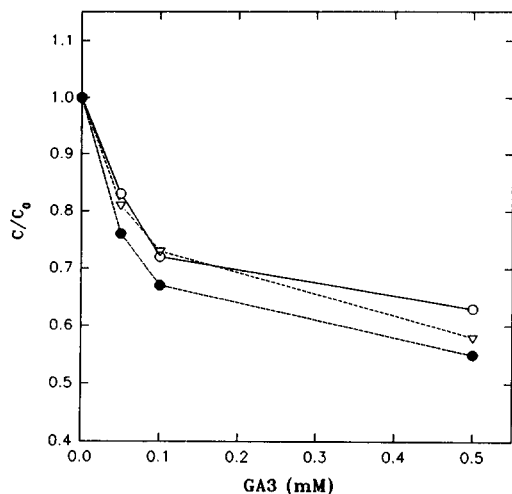


Fig. 2. Effect of GA₃ concentrations on the ratio between the respiratory activities of slices treated with GA₃ (C) and control slices without GA₃ (Co) in the same period. Strawberry slices obtained from fruits harvested at different stages of ripening and incubated in the dark for 3 days at 20°C in 0.053 M citric buffer, 0.094 M Na₂PO₄H adjusted to pH 4.5. LSD_{0.05} = 0.156. ○ = green, ● = white, ▽ = 25% red.

(Co) in the same period. As shown in Fig. 2, increasing amounts of GA₃ led to a decrease in CO₂ production. A 30% decrease of respiratory activity was obtained at a concentration of 0.1 mM of GA₃. The dose-response curve was nonlinear and tended to level off at higher GA₃ concentrations. This suggested a saturation of GA₃ receptor at high-GA₃ levels. However, it should be mentioned that the effects observed may be a useful pharmacological effect of supraoptimal levels of GA₃ not entirely related to any physiological effect. When the effect of different GA₃ concentrations on the respiratory activity of strawberry slices at different ripening stages was analyzed, it was found that the inhibitory effect of the hormone was similar for all stages (Fig. 2).

Loss of chlorophylls associated with degradation and/or synthesis of anthocyanins are useful parameters to investigate the fruit ripening process. We examined the effect of GA₃ on pigments content of whole strawberry fruits. Figure 3 shows a photograph of strawberry fruits nontreated and treated with GA₃ (1 mM) and incubated at 20°C for 3 days as described in Materials and Methods. GA₃ delayed the ripening of treated fruits compared with the control fruits. The color change was more evident in nontreated fruits. The anthocyanin and chlorophyll contents of control and GA₃-treated strawberry fruits were measured during the course of 3 days incubation at 20°C. Normally, green fruits increased their anthocyanin contents after the third

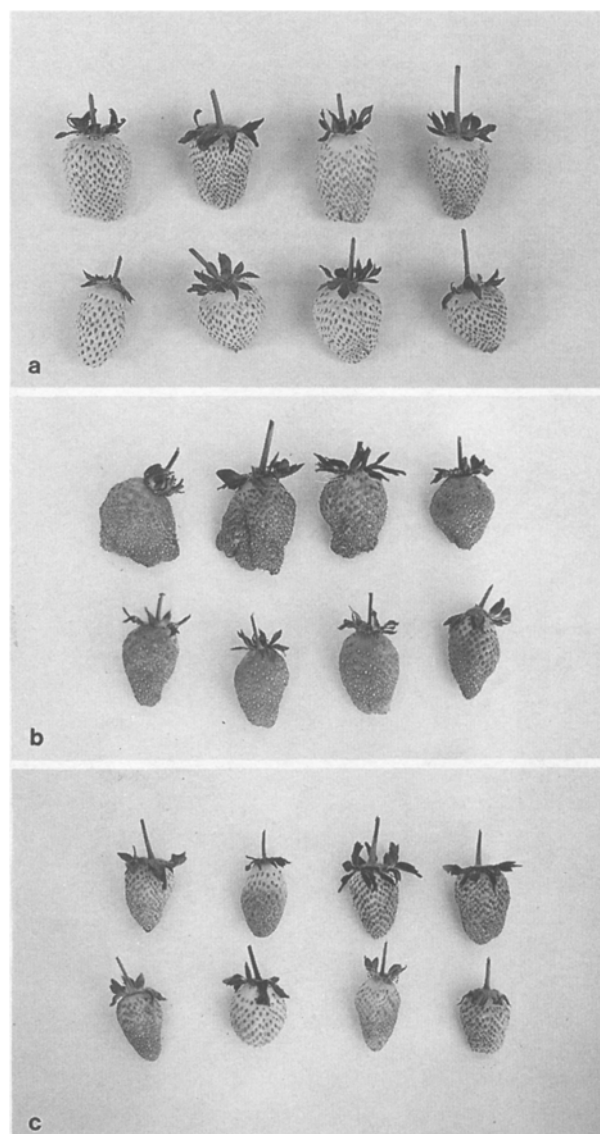


Fig. 3. Photograph of white strawberries at harvest (a), white strawberries nontreated (b), and treated with GA₃ 1 mM (c). Both (b) and (c) were incubated for 3 days at 20°C as described in Materials and Methods.

day of incubation reaching 50–75% of the surface red color, while white fruits increased their anthocyanin contents after the third day of incubation reaching 75–100% of the surface red color (Table 1). In accordance with other authors (Given et al. 1988a, Cheng and Patrick 1991), a marked increase in anthocyanin concentration was observed during the last stage of ripening. Exogenous application of GA₃ caused a delay in anthocyanin production of 30–35% (Fig. 4a). This effect was statistically significant at the second and third days of incubation. The same effects were observed for both ripening

Table 1. Effect of incubation for 3 days at 20°C on the levels of anthocyanins and chlorophylls in strawberry fruit

Fruit stage	Anthocyanins ^a		Chlorophyll a ^b		Chlorophyll b ^b	
	Day 0	Day 3	Day 0	Day 3	Day 0	Day 3
Green	6.0 ± 0.4	83 ± 14	10.5 ± 0.6	4.3 ± 0.3	4.1 ± 0.7	2.6 ± 0.2
White	8.2 ± 1.2	280 ± 24	7.6 ± 1.1	2.7 ± 0.2	2.6 ± 0.2	1.0 ± 0.1

^a Expressed as nanomoles of anthocyanins per gram fresh weight ± σ .

^b Expressed as micrograms of chlorophyll per gram fresh weight ± σ .

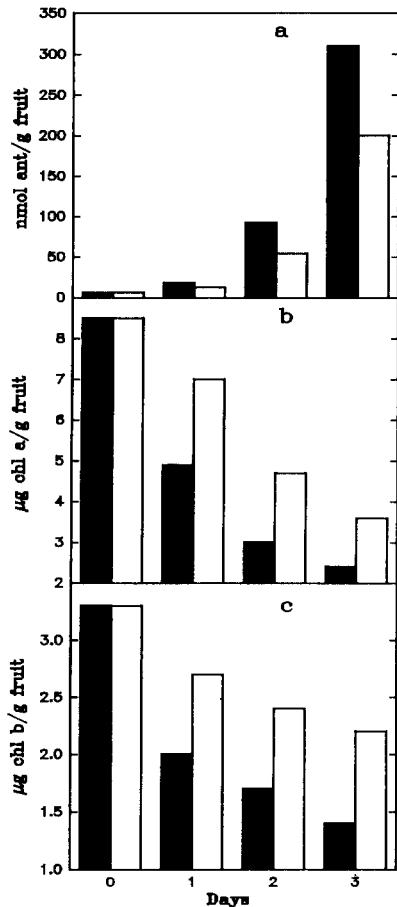


Fig. 4. Changes in pigment content of white strawberry fruits nontreated and treated with 1 mM GA₃ and incubated for 3 days at 20°C. (a) Anthocyanins. LSD_{0.05} = 8.31 (b) chlorophyll a. LSD_{0.05} = 0.23 (c) chlorophyll b. LSD_{0.05} = 0.13. ■ = nontreated, □ = treated.

stages used as starting plant material: white and green fruit. Similar effects were reported by other authors working with different systems. GA₃ was found to delay color production and anthocyanin synthesis in cherries (Facteau et al. 1985), and to stop the anthocyanins synthesis at chalcone synthase and PAL level in cellular cultures of carrots (Hinderer et al. 1984).

At the same time, during ripening a disorganiza-

tion of the chloroplast occurs and consequently, chlorophyll degrades. Working under the same condition described for anthocyanin studies, nontreated green and white fruits decreased their chlorophylls contents as shown in Table 1. Exogenous treatment with GA₃ caused a delay in the degradation of chlorophylls, both Chl a (Fig. 4b) and Chl b (Fig. 4c). In control and GA₃-treated fruits, the degradation rate of Chl a was higher than the one of Chl b. However, the inhibition produced by GA₃ was the same for both kinds of chlorophylls. This GA₃ inhibition was statistically significant from the first day of incubation. As occurred for anthocyanins, inhibition of chlorophylls degradation was observed in both green and white fruit. The effect of GA₃ on chlorophyll degradation has been studied by Khader et al. (1988) on climacteric fruit such as mangos and by Biale (1978) on nonclimacteric fruit such as citrics. In both cases GA₃ reduced chlorophylls degradation, similar to the effect described here.

On the assumption that achenes produce auxins (Nitsch 1950, Southwick and Poovaiah 1987) a series of experiments were performed in which fruit were deachened in order to evaluate the effect of removing that source of auxin and subsequent interference. GA₃ was applied as described previously for the whole fruit experiments. Control fruits were also deachened. Results were similar to those obtained before. The synthesis of anthocyanin was inhibited by 30–35% and the degradation of chlorophyll a and b was delayed in deachened and GA₃-treated fruit compared to deachened control fruit. A remarkable aspect of these observations was that assayed concentrations of GA₃ (0.25–1 mM) led to the same inhibitory effect, and no significant differences were found.

We conclude that application of GA₃ results in an inhibition of the general metabolic activity, evidenced through a decrease in the respiratory activity of the three ripening stages assayed. Postharvest application of GA₃ inhibited 30–35% the color development of green and white fruit; it also delayed chlorophylls a and b degradation. This inhibitory activity of GA₃ can take place in the presence and/or absence of the hormone produced by the

achenes. It is likely that, GA₃ may be acting in some way on the enzymes thought to be involved in this process.

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