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Effect of Exogenous Application of Gibberellic Acid on Color Change and Phenylalanine Ammonia-lyase, Chlorophyllase, and Peroxidase Activities during Ripening of Strawberry Fruit (*Fragaria* × *ananassa* Duch.)

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Abstract. The effect of exogenously applied gibberellic acid (GA_3) on the postharvest color change of strawberry fruit was evaluated through their external color and surface color parameters. A significant delay on color evolution was observed in fruits treated with GA_3. The evolution of activities of phenylalanine ammonia-lyase (PAL), chlorophyllase, and peroxidase was also analyzed. PAL activity increased during strawberry ripening, but in fruits treated with GA_3 the increase in such activity was slower, and, probably as consequence, the development of red color was delayed. Moreover, the activity of chlorophyllase and peroxidase, enzymes possibly involved in chlorophyll metabolism, decreased during strawberry ripening. However, a delay was observed in the decrease of such activities in GA_3-treated fruits.

Key Words. Strawberry fruit—Gibberellic acid—Ripening—PAL—Chlorophyllase—Peroxidase

Color change is an important parameter in evaluating the ripening process in fruits in addition to being a key factor in determining their commercial quality. In strawberries, color change, the loss of the green color and the appearance of the red color, is the result of the synthesis of anthocyanin (Cheng and Breen 1991, Given et al. 1988a, 1988b, Woodward 1972). These compounds are flavonoids and are synthesized from the aromatic amino acid phenylalanine. Two key enzymes were described for the synthesis of these compounds: phenylalanine ammonia-lyase (PAL) and chalcone synthase. In the particular case of PAL, it has been demonstrated that this enzyme is synthesized de novo during ripening of the strawberry cv. Brighton (Given et al. 1988c).

During fruit ripening, chloroplasts and their thylakoid membranes disorganize, and a rapid degradation of chlorophylls takes place which causes the loss of green color. There is still no clear explanation for this biochemical process, although previous authors have mentioned several enzymes possibly related with it, such as magnesium dechelatase, lipoxygenase, peroxidase, and chlorophyllase (Orthoefer and Dugan 1973, Langmeier et al. 1993, Shioi et al. 1991, Yamauchi and Hashinaga 1992). Chlorophyllase catalyzes the hydrolysis in vitro of the bond between the phytol group and the 7-propionic acid residue of the ring D of the tetrapyrrolic nucleus of chlorophylls and pheophytins (Amir-Shapira et al. 1987, McFeeters et al. 1971, Yamauchi et al. 1991). However, several papers indicate that this process would also occur in vivo, in a reaction that is regarded as a possible first stage in the degradation of chlorophylls (Amir-Shapira et al. 1987, Purvis and Barmore 1981, Yamauchi et al. 1991).

Peroxidase is distributed widely in the plant kingdom and presents numerous physiologic roles, some of which, for instance the degradation of chlorophylls or auxins, might be of importance in strawberry ripening. It was demonstrated that peroxidase is able to catalyze the oxidation in vitro of chlorophylls in the presence of H_2O_2 (Kato and Shimizu 1985), whereas in some fruits, it was suggested that peroxidase would be involved directly in the degradation of chlorophylls (Yamauchi and Hashinaga 1992, Yamauchi et al. 1991).

Abbreviations: PAL, phenylalanine ammonia-lyase; GA_3, gibberellic $acid_3$; PVPP, polyvinylpolyprrolidone; CEAU, chlorophyllase enzymic activity unit; PEAU, peroxidase enzymic activity unit; LSD, least significant difference.

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Strawberries ripen quickly, and the passage from mature green to overripe fruits attached to the plant takes about 5–6 days under normal conditions (20°C) (Manning 1993). Consequently, methods that can improve their storage life could be useful for their marketing.

Gibberellins are used in commercial fruit production to modify fruit set and to delay maturation and ripening (Ben-Arie and Ferguson 1991). Several authors have observed that the exogenous addition of gibberellic acid (GA_3) retards chlorophyll degradation in citrus (Biale 1978), apricot (Abdel-Gawad and Romani 1974), and mango (Khader et al. 1988). Moreover, GA_3 delays the color change in cherries (Facteau et al. 1985) and tomatoes (Babbitt et al. 1973, Ben-Arie et al. 1995).

In a previous work we showed that external application of GA_3 in strawberries led to a decrease in the respiratory activity of tissue discs and retarded anthocyanin accumulation and chlorophyll degradation (Martínez et al. 1994). In the present work we analyze the effect of the same exogenous application of GA_3 on the development of strawberry surface color and on the activities of some enzymes possibly related to such development (PAL, chlorophyllase, and peroxidase).

Materials and Methods

Plant Material and Hormonal Treatment

Strawberries used (*Fragaria* × *ananassa* Duch. cv. Selva) were from producers of the La Plata region. Fruits were harvested in different ripening stages and classified visually according to their external coloration as mature green, white, 25% red, 50% red, 75% red, 100% red, and ripe. The mature green is a stage in which the fruits have reached their full size but have not yet changed their color. In the 25, 50, 75, and 100% red stages the percentages indicate the proportion of the external surface which has become red.

Fruits were washed thoroughly with water and then with 2% w/v NaClO, and finally washed three times with sterile distilled water.

The hormonal treatment was done with solutions of 10^{-3} M GA_3 in buffer (0.053 M citric acid, 0.094 M Na_2PO_4H, pH 4.5) with 2% v/v dimethyl sulfoxide. The solutions were applied to the whole fruit surface by cotton swabs.

Fruits were put on trays and covered by a 15-μm-thick polyolefinic film with two 1.1-mm-diameter perforations per square inch. The trays so prepared were stored at 20°C for 3 days, and samples were extracted at 24, 48, and 72 h.

Determination of Surface Color

The objective parameters of surface color (*L*, *a* and *b*) were determined in a HunterLab colorimeter. For each fruit the surface color was measured in three different zones of its surface, and the values so obtained were averaged.

Extraction and Dosage of the Enzymic Activities

PAL. About 20 g of fruit was homogenized in an Omnimixer at 4°C with 4 volumes of buffer of the following composition: 0.1 M $Na_2B_4O_7 \cdot 10\ H_2O$, 5 mM 2-mercaptoethanol, 2 mM EDTA, 3% w/v PVPP, pH 8.8. The mixture was left for 1 h at 4°C under magnetic stirring and then centrifuged at 10,000 × *g* for 20 min at 4°C. The enzymic activity was measured in the supernatant by the method described by Zucker (1965), with the following reaction mixture: 0.03 M $Na_2B_4O_7 \cdot 10\ H_2O$, pH 8.8, 0.01 M L-phenylalanine, 1 ml of enzymic extract, in a total volume of 3 ml. This mixture was incubated at 30°C, and the reaction was evaluated through the increase of optical density at 290 nm caused by production of *trans*-cinnamic acid.

The enzymic activity unit (kat) was defined as the amount of enzyme required to produce 1 mol of *trans*-cinnamic acid/s under these conditions. The specific enzymic activity was calculated as enzymic activity/mg of protein.

Chlorophyllase. About 20 g of fruit was homogenized in an Omnimixer at 4°C with 3 volumes of buffer of the following composition: 0.038 M Na_2HPO_4, 0.062 M NaH_2PO_4, 0.5% v/v Triton X-100, 1 mM cystein, 1% w/v PVPP, pH 7.0. The mixture was left for 1 h under magnetic stirring at 4°C, then centrifuged at 9,200 × *g* for 15 min at 4°C. The chlorophyllase enzymatic activity was measured in the supernatant. Substrate consumption was measured using a modified version of a technique originally described by McFeeters (McFeeters et al. 1971), for which the following reaction mixture was employed: 0.08 M Na_2HPO_4, 0.02 M NaH_2PO_4, 0.15 v/v Triton X-100, 18.4 μM spinach chlorophyll, 16% v/v acetone, pH 7.8. The total volume was 12.2 ml, and the working temperature was 40°C. The reaction mixture was prepared in the absence of extract, and its temperature was allowed to stabilize for 2–3 min in a thermostatic bath. Then 2.8 ml of enzymic extract was added, and the mixture was homogenized, after which zero time was considered. Duplicate samples of 2 ml each were taken at this initial time and then periodically up to 60 min. Each sample was poured into a 5-ml mixture of hexane and acetone (7:3 v/v) and cooled in ice water (0°C). The resultant system was stirred vigorously until emulsion formation, then allowed to rest in the dark at 4°C for 10 min and centrifuged afterward at 9,000 × *g* for 5 min at 4°C. The top hexanic layer, which contained the chlorophyll, was taken to determine the absorbance (*A*) at 663 nm.

The chlorophyllase enzymic activity unit (CEAU) was defined as the amount of enzyme needed to cause a decrease of 0.001 *A* units/min at test conditions. The specific enzymic activity was calculated as CEAU/mg of protein.

Peroxidase. About 20 g of fruit was homogenized in an Omnimixer at 4°C with 4 volumes of buffer of the following composition: 0.04 M Na_2HPO_4, 0.06 M NaH_2PO_4, 0.01% v/v Triton X-100, 1 M NaCl, 1% w/v PVPP, pH 7.0. The system was left under stirring for 1 h at 4°C and then centrifuged at 8,000 × *g* for 15 min. The enzymic activity was dosed in the supernatant previously obtained using the following reaction mixture: 0.02 M Na_2HPO_4, 0.08 M NaH_2PO_4, 20 mM guaiacol, 4 mM H_2O_2; enzymic extract: 300 μl, total volume: 3 ml. The mixture was incubated at 30°C and pH 6.0, and the enzymic activity was determined by measuring the increase in *A* at 470 nm.

The peroxidase enzymic activity unit (PEAU) was defined as the amount of enzyme required to increase 0.001 *A* units in 1 min at test conditions. The specific enzymic activity was calculated as PEAU/mg of protein.

Preparation of Chlorophyll

Two or three spinach leaves were crushed in an Omnimixer with 50–60 ml of acetone previously cooled to 4°C. The suspension so obtained was vacuum filtered through filter paper, and the total chlorophyll

concentration was measured on the filtrate by the method of Bruinsma (1963).

Dosage of Proteins

The protein concentration in the enzymic extracts of PAL, chlorophyllase, and peroxidase was determined by the modified Lowry method (Potty 1969) using a wavelength of 750 nm.

Experimental Design and Analysis of Results

The tests were done at least in triplicate, and not less than 20 fruits were used in each treatment. To evaluate the results statistically, analysis of variance (ANOVA) was used. Comparison of means was done by the least significant difference (LSD) test at a significance level of 0.05 (α).

Results and Discussion

Development of Color

Fruits in the ripening stages mature green, white, and 25% red were used to study the effect of GA_3 on color change. Gibberellins commonly have a physiologic 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^{-3} to 10^{-5} M (Ben-Arie and Ferguson 1991, Ben-Arie et al. 1986, 1995, Facteau et al. 1985, Hinderer et al. 1984). These high concentrations are necessary in part to assure an adequate diffusion into the tissue and overcome effects of degradation. Similarly, auxins are considered the principal hormones in the regulation of strawberry fruit development and ripening, but it requires 10- to 100-fold higher concentrations of exogenously applied auxins to elicit the response compared with other systems (Reddy et al. 1990). Moreover, in a previous work (Martínez et al. 1994), it was already demonstrated that the exogenous application of 1 mM GA_3 was effective in delaying anthocyanin synthesis and chlorophyll degradation. Considering these facts we decided to study the effect of an exogenous application of GA_3 at the 10^{-3} M level.

Fruits were incubated at 20°C for 3 days, this maximum incubation time being used because longer periods lead to significant damage in the fruits. Moreover, the experiments done on fruits in ripening stages earlier than mature green showed a defective color development, significant spoilage, and fruit browning. The development of color in fruits after 3 incubation days, expressed as percentage of red surface, is presented in Table 1. For the ripening stages used, the development of color was delayed noticeably in those fruits treated with GA_3. However, the use of GA_3 in fruits at advanced ripening stages, later than 25% red, was ineffective in delaying color development (data not shown).

Table 1. Evolution of external color in control (without GA_3) and treated (with 1 mM GA_3) fruits after incubation of 3 days at 20°C.

Initial ripening stage	Control	Treated
Ripe green	50–75% red	25–50% red
White	75–100% red	50–75% red
25% red	100% red-ripe	75–100% red

The evolution of color in fruits was also evaluated by measuring their surface color with a colorimeter. To this end, white fruits were used as starting material; they were treated with 1 mM GA_3 and then incubated for 3 days at 20°C. In parallel, control (untreated) fruits were also incubated at the same conditions. The resulting surface color parameters *L*, *a*, and *b*, are shown in Table 2.

The parameter *L*, which is a measure of fruit brightness, experienced a small decrease over the incubation period at 20°C. This loss of brightness was also described by other authors (Collins and Perkins-Veazie 1993) for fruits at commercial ripening stage (75–100% red) incubated at various temperatures.

The most noticeable change was that of parameter *a*, which indicates the green-red equilibrium. The value of *a* was low for the initial white fruits and increased considerably for control fruits incubated for 3 days. However, under the same incubation conditions, this increase was less in fruits treated with GA_3, indicating the weaker development of color in the surface. On the other hand, the parameter *b*, which indicates the yellow-blue equilibrium, showed only a small decrease. Although the decrease was less marked in fruits treated with GA_3, differences with the controls were significant (α = 0.05).

The combination of the last two parameters, expressed as the ratio of *a* to *b*, is another way to observe variations. In control fruits, the ratio after incubation increased substantially to a value some eight times as high as it was in the initial white fruits, whereas in treated fruits, the ratio increased only five times, which indicates that the development of surface color was retarded by about 35–40%.

The evolution of surface color was also assessed by the ratio of *a* to *b* in other fruits such as citrus and tomato (Babbit et al. 1973, Purvis and Barmore 1981). For the last fruit, an increase in the ratio *a*/*b* was observed during ripening, a process that was retarded by the exogenous application of GA_3 (Babbit et al. 1973).

The external color of strawberries depends on the chlorophyll and anthocyanin contents, so a delay in the change of this color in fruits treated with GA_3 shows a close relationship with a similar delay in the degradation of chlorophylls and in the synthesis of anthocyanin ob-

Table 2. Evolution of surface color parameters from initial ripening stage white for control (without GA_3) and treated (with 1 mM GA_3) fruits during incubation of 3 days at 20°C.

Parameter[a]	Initial value	Final value	
		Control	Treated
L_a	72.83	61.40	66.54
a_b	0.73	4.18	2.95
b_c	4.20	3.17	3.62
a/b_d	0.17	1.34	0.81

[a] Parameters: a, $LSD_{0.05}$ = 0.63; b, $LSD_{0.05}$ = 0.33; c, $LSD_{0.05}$ = 0.25; d, $LSD_{0.05}$ = 0.20.

served previously in GA_3-treated fruits (Martínez et al. 1994).

PAL

As described in a previous work (Martínez et al. 1994), the in vitro incubation of strawberries at 20°C causes a noticeable development in the surface color and an increase of the anthocyanin content. Besides, bearing in mind the close relationship between the enzymic activity of PAL and the anthocyanin content, we decided to study the evolution of the activity of PAL during ripening in vivo as a starting point for the subsequent analysis of the effect of GA_3 on this enzyme.

The evolution of enzymic activity/g of fruit and the specific enzymic activity of PAL during ripening are showed in Fig. 1. The activity of PAL is very low in the mature green, white, and 25% red stages, and then increase considerably from the 50% red stage. The enzymic activity and specific enzymic activity values obtained here were similar to those mentioned previously in the literature for other strawberry varieties such as Brighton (Given et al. 1988a, 1988b, Hyodo 1971) and Tilikum (Cheng and Breen 1991). For the latter variety, the authors reported two peaks of maximum enzymic activity during the development, the last peak being coincident with anthocyanin synthesis during fruit ripening. The increase of PAL activity during ripening takes place at the same time as that of other enzymes involved in the synthesis of anthocyanin, such as UDP-glucose:flavonoid-3-*O*-glucosyltransferase (Given et al. 1988b).

Taking into account the delay of color development and anthocyanin synthesis caused by GA_3 it was decided to evaluate the effect of GA_3 on the enzymic activity of PAL. GA_3 was applied to whole fruits in ripening stages mature green, white, and 25% red, with a GA_3 concentration of 1 mM. Fig. 2*a* shows the enzymic activity/g of fruit and the specific activity of PAL in mature green fruits as initial ripening stage over 3 days. In this period, the enzymic activity of PAL in control fruits increased about 7–8 times with respect to its initial value, whereas

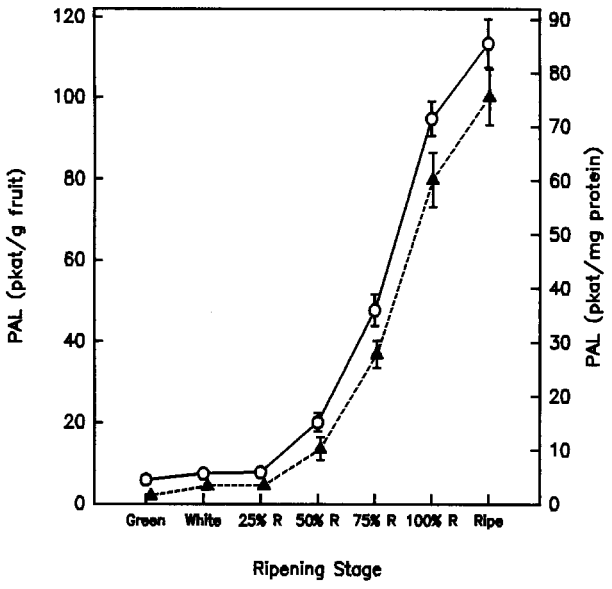

Fig. 1. Evolution of PAL activity during ripening of strawberry fruits. ○, enzymic activity (pkat/g of fruit); ▲, specific activity (pkat/mg of protein). *Vertical bars* indicate ± S.E. when it exceeds the size of the symbol.

such an increase was only 4–5 times in treated fruits. After 3 days, the enzymic and specific activity values were consistent with those observed in fruits 25–50% red (treated) and 50–75% red (controls). Therefore, both enzymic and specific activities of control fruits were approximately 35–40% above those of treated fruits.

The evolution of enzymic and specific activity of white fruits was much more marked. Fig. 2*b* shows that enzymic activity had a 12 and 8 times increase in control and treated fruits, respectively, over the incubation of 3 days. In addition, values obtained were consistent with the ripening stages reached, that is, 75–100% red for controls and 50–75% red for treated fruits. Hence, the delay observed for the increase in enzymic and specific activities was similar to that obtained for fruits of the initial ripening stage mature green.

Finally, for 25% red fruits as initial ripening stage, the evolution of activities of PAL for control fruits and GA_3-treated fruits is exhibited in Fig. 2*c*. The graph shows that, in this case, the increase of both enzymic and specific activities was slower than those of white fruits. The enzymic activity increased only 6 and 5 times in control and treated fruits, respectively, after 3 days. Moreover, the specific activity values reached after this period were below those of fresh fruits in a similar ripening stage. In spite of that, the enzymic and specific activity values were about 15–20% lower in treated fruits than they were in control fruits.

The activity of PAL in strawberries may decrease because of the action of different substances such as *L*-α-

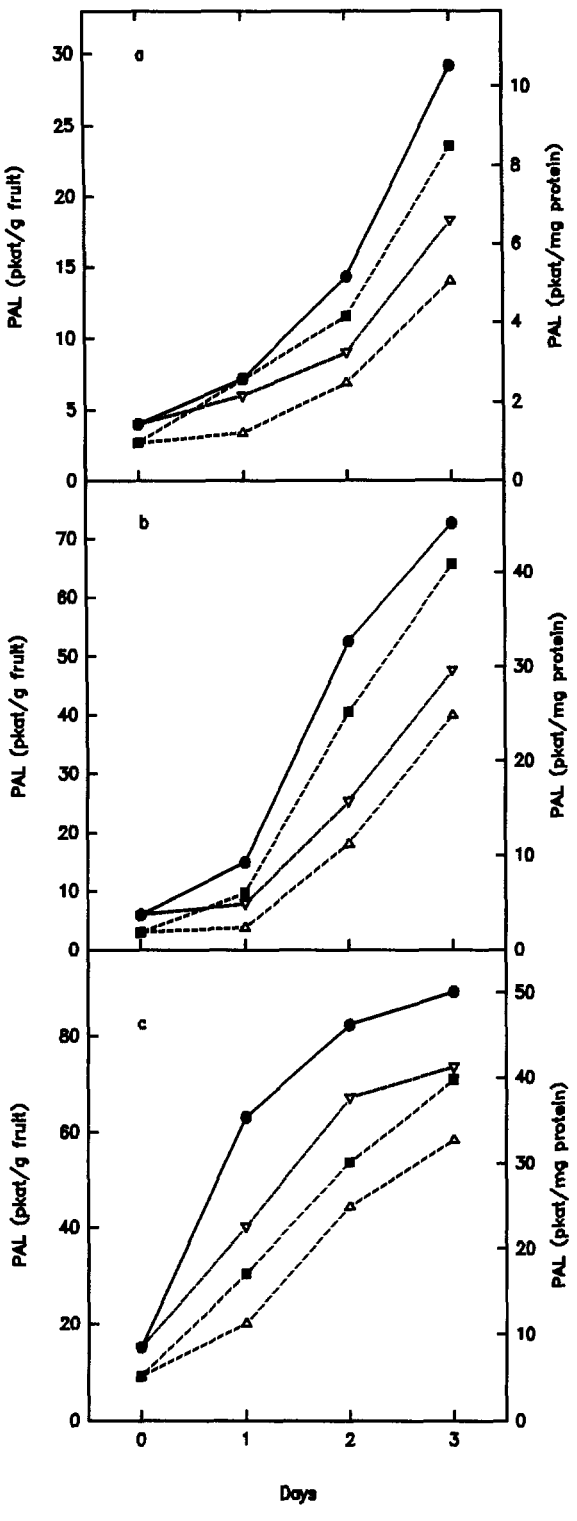

Fig. 2. Evolution of PAL activity of strawberry fruits incubated for 3 days at 20°C. Initial ripening stage: *a*, mature green; *b*, white; *c*, 25% red. Enzymic activity (pkat/g of fruit) $LSD_{0.05}$ = 4.05; ●, control; ▽, treated with 1 mM GA_3. Specific activity (pkat/mg of protein) $LSD_{0.05}$ = 2.31: ■, control; △, treated with 1 mM GA_3.

aminooxy-β-phenylpropionic acid or the synthetic auxin naphthaleneacetic acid (Given et al. 1988a). The use of this compounds inhibited the activity of PAL and, consequently, delayed the synthesis and accumulation of anthocyanins. Moreover, the presence of GA_3 in cellular cultures of carrot caused a decrease in the activity of PAL and chalcone synthase, which resulted in a lower accumulation of anthocyanins in the culture (Hinderer et al. 1984).

As a consequence, the results obtained in the present work would indicate that the delay in the color development and anthocyanin production in strawberries treated with GA_3 would be due to inhibition or retardation of the increase of PAL activity. Such delay would be dependent, in turn, on the ripening stage of fruits at the beginning of the experiment.

Chlorophyllase

The enzymic activity of chlorophyllase was evaluated in vitro in fruits of ripening stages mature green and white, which were treated with 1 mM GA_3. Fruits were incubated at 20°C, and the enzymic activity was measured over 3 days. It was observed that the enzymic activity of mature green fruits was higher than that of white fruits and that the enzymic activity decreased during the incubation period (Fig. 3). These values were similar to those observed previously during the in vivo ripening of strawberries (Martínez et al. 1995). The specific enzymic activity of fruits treated with GA_3 was about 15–20% higher than that of control fruits during the whole incubation period. Such a percentage difference was observed in the experiments done with both mature green fruits and white fruits. These results indicate that fruits treated with GA_3 had a slower decrease in the specific activity of chlorophyllase.

The enzymic activity of chlorophyllase increases during the senescence of leaves of oats and barley (Rodríguez et al. 1987, Sabater and Rodríguez 1978) although tobacco leaves show a decrease of activity during this period (Yamauchi and Watada 1991). In citrus, the activity of chlorophyllase increases at the same time as the chlorophyll content decreases, particularly when they are treated with ethylene (Amir-Shapira et al. 1987, Yamauchi et al. 1991). However, in the regreening of Valencia oranges, both chlorophyllase activity and chlorophyll content increase at one time, indicating a possible biosynthetic activity of chlorophyllase (Aljuburi et al. 1979). In many instances, chlorophyllase was considered as having a degrading-type activity, but it would not be the main enzyme involved in the process, and its action would be associated with that of other enzymes (Thomas and Janave 1992, Yamauchi and Watada 1991, Yamauchi et al. 1991). Thus, chlorophyllase could be involved both in the biosynthesis and the degradation of chloro-

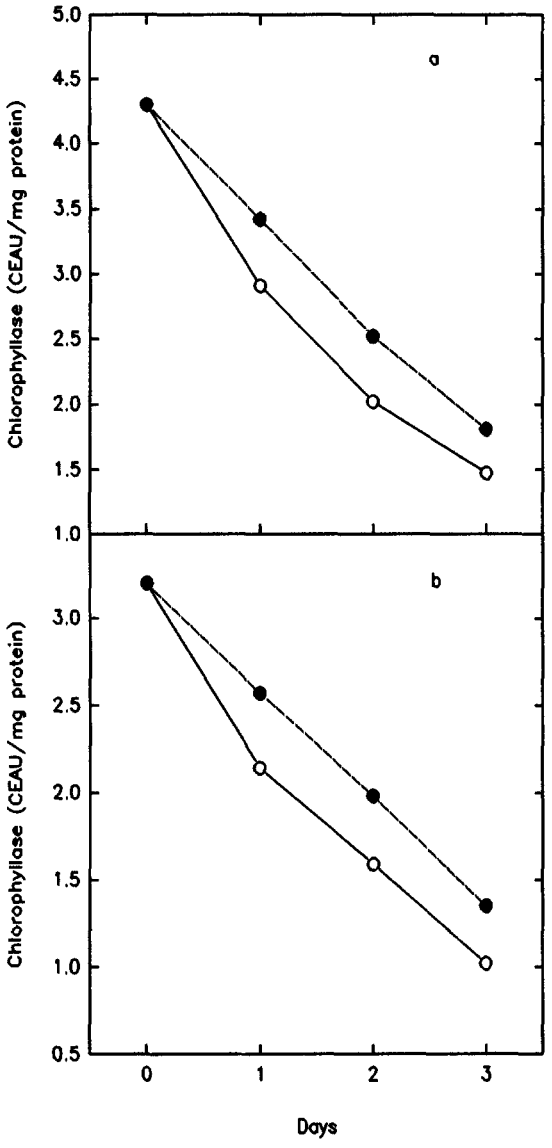

Fig. 3. Evolution of chlorophyllase specific activity of strawberry fruits incubated for 3 days at 20°C. Initial ripening stage: *a*, mature green; *b*, white. ○, control; ●, treated with 1 mM GA_3 $LSD_{0.05}$ = 0.143.

phylls. In our case, a notable parallelism between the evolution of chlorophyll content, as was mentioned previously (Martínez et al. 1995), and that of chlorophyllase activity was observed, so this might indicate a biosynthetic action of the enzyme. Therefore, treatment of strawberry fruits with GA_3 would lead to higher chlorophyllase activity levels and, as a consequence, stronger biosynthesis of chlorophylls in less ripe fruits. A similar action was observed during the senescence of barley. In this grain, the exogenous application of kinetin retarded chlorophyll degradation, mainly because of a higher activity of those enzymes involved in the chlorophyll biosynthesis (Hukmani and Trepathy 1994, Kuroda et al. 1990).

As the degradation mechanism of chlorophylls is not fully explained yet, particularly in strawberries, further studies about the activity and function of these and other enzymes involved are needed.

Peroxidase

The enzymatic activity of peroxidase was measured in vitro in fruits of ripening stages mature green and white, which were treated with 1 mM GA_3. Fruits were incubated at 20°C, and the enzymic activity was measured for 3 days. The enzymic activity of mature green fruits was higher than that of white fruits, and in both cases, the enzymic activity decreased slowly during the incubation period, indicating a lower enzymic activity in riper fruits (Fig. 4). In a similar fashion, the peroxidase activity decreased during ripening in fruits attached to the plant (Civello et al. 1995).

Peroxidase activity was higher (about 10%) in strawberries treated with GA_3 than it was in control fruits (Fig. 4). Therefore, bearing in mind that fruits treated with GA_3 had a general retardation of ripening, they also showed a delay in the decrease of peroxidase enzymic activity. A similar effect has been observed during the senescence of barley leaves treated with kinetin, since this hormone retarded chlorophyll degradation at the same time as it increased the activity of total peroxidase. However, it has also been observed that kinetin retarded the increase of a cationic isoenzyme that would be directly related to chlorophyll degradation (Kuroda et al. 1990). On the contrary, peroxidase activity increased during the ripening of mango, and the application of GA_3 delayed such an increase (Khader et al. 1988).

It has been demonstrated that the peroxidase-induced degradation of chlorophylls requires the presence of cofactors such as simple phenols (Huff 1982, Kato and Shimizu 1985) or flavonoid compounds (Yamauchi and Hashinaga 1992). The enzymic activity of PAL increased during ripening, leading to the accumulation of flavonoids, which, in turn, could increase the degrading activity of peroxidase on chlorophylls. On the other hand, even though peroxidase activity decreased during the incubation period, its value was considerable in ripen fruits. Then, it can be considered that the degrading activity of peroxidase on chlorophyll may be amply favored by the increase of phenol acting as cofactors, an alternative that could have a greater effect than a variation in peroxidase content itself. We observed that strawberry peroxidase is able to degrade chlorophylls, but its action depends strongly on the presence of phenols in the reaction medium (data not shown). Thus, the reduced chlorophyll degradation observed in strawberry fruits treated with GA_3 would be due to a lesser activity of PAL, which would cause a lower amount of the flavonoids used by peroxidase as cofactors for chlorophyll degradation.

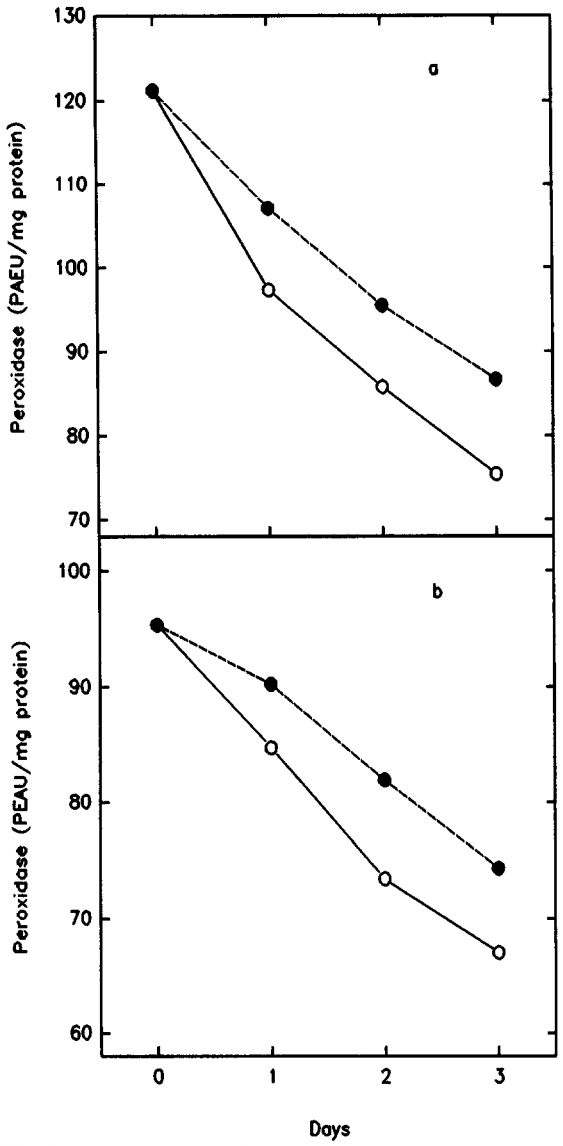

Fig. 4. Evolution of peroxidase specific activity of strawberry fruits incubated for 3 days at 20°C. Initial ripening stage: *a*, mature green; *b*, white. ○, control; ●, treated with 1 mM GA_3 $LSD_{0.05}$ = 2.31.

Conclusions

Strawberry fruits exogenously treated with GA_3 show a delay in the development of surface color which could be caused by a delayed increase in PAL activity. Moreover, some enzymes possibly involved in chlorophyll metabolism, such as chlorophyllase and peroxidase, decrease their activity during strawberry ripening, and the exogenous application of GA_3 retards such a decrease. In a previous paper we showed that a similar GA_3 treatment delayed the accumulation of anthocyanins and the degradation of chlorophylls (Martínez et al. 1994). Consequently, postharvest GA_3 treatments delay several factors involved in strawberry fruit ripening and could be especially useful for improving their postharvest life.

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References

Abdel-Gawad H, Romani RJ (1974) Hormone-induced reversal of color change and related respiratory effects in ripening apricot fruits. Physiol Plant 32:161–165

Aljuburi H, Huff A, Hsheih M (1979) Enzymes of chlorophyll catabolism in orange flavedo. Plant Physiol 63(suppl):73

Amir-Shapira D, Goldschmidt EE, Altman A (1987) Chlorophyll catabolism in senescing plant tissues: In vivo breakdown intermediates suggest different degradative pathways for *Citrus* fruits and parsley leaves. Proc Natl Acad Sci USA 84:1901–1905

Babbitt JK, Powers MJ, Patterson ME (1973) Effects of growth regulators on cellulase, polygalacturonase, respiration, color, and texture of ripening tomatoes. J Am Soc Hort Sci 98:77–81

Ben-Arie R, Bazak H, Blumenfeld A (1986) Gibberellin delays harvest and prolongs storage life of persimmon fruits. Acta Hort 179: 807–813

Ben-Arie R, Ferguson B (1991) Ethylene production by growing and senescing pear fruit cell suspension in response to gibberellin. Plant Physiol 95:943–947

Ben-Arie R, Mignani I, Greve C, Huysamer M, Labavitch J (1995) Regulation of the ripening of tomato pericarp discs by GA_3 and divalent cations. Physiol Plant 93:99–107

Biale JB (1978) On the interface of horticulture and plant physiology. Annu Rev Plant Physiol 29:1–23

Brooks JL (1986) Oxidase reactions of tomato anionic peroxidase. Plant Physiol 80:130–133

Bruinsma J (1963) The quantitative analysis of chlorophylls *a*, and *b*, in plant extracts. Photochem Photobiol 2:241–249

Cheng GW, Breen PJ (1991) Activity of phenylalanine ammonia-lyase (PAL) and concentrations of anthocyanins and phenolics in developing strawberry fruit. J Am Soc Hort Sci 116:865–869

Civello PM, Martínez GA, Chaves AR, Añón MC (1995) Peroxidase from strawberry fruit (*Fragaria ananassa*, Duch): Partial purification and some properties. J Agric Food Chem 43:2596–2601

Collins JK, Perkins-Veazie P (1993) Postharvest changes in strawberry fruit stored under simulated retail display conditions. J Food Qual 16:133–143

Facteau TJ, Rowe KE, Chestnut NE (1985) Firmness of sweet cherry following multiple applications of gibberellic acid. *J Am Soc Hort Sci* 110:775–777

Given NK, Venis MA, Grierson D (1988a) Hormonal regulation of ripening in the strawberry, a non-climacteric fruit. Planta 174: 402–406

Given NK, Venis MA, Grierson D (1988b) Phenylalanine ammonia-lyase activity and anthocyanin synthesis in ripening strawberry fruit. J Plant Physiol 133:25–30

Given NK, Venis MA, Grierson D (1988c) Purification and properties of phenylalanine ammonia-lyase from strawberry fruit and its synthesis during ripening. J Plant Physiol 133:31–37

Hinderer W, Petersen M, Seitz HU (1984) Inhibition of flavonoid biosynthesis by gibberellic acid in cell suspension cultures of *Daucus carota* L. Planta 160:544–549

Huff A (1982) Peroxidase-catalyzed oxidation of chlorophyll by hydrogen peroxide. Phytochemistry 21:261–265

Hukmani P, Trephathy B (1994) Chlorophyll biosynthetic reactions during senescence of excised barley (*Hordeum vulgare* L. cv IB 65) leaves. Plant Physiol 105:1295–1300

Hyodo H (1971) Phenylalanine ammonia-lyase in strawberry fruits. Plant Cell Physiol 12:989–991

Kato M, Shimizu S (1985) Chlorophyll metabolism in higher plants. VI. Involvement of peroxidase in chlorophyll degradation. Plant Cell Physiol 26:1291–1301

Khader SE, Singh BP, Khan SA (1988) Effect of GA_3 as a post-harvest treatment of mango fruit on ripening, amylase, and peroxidase activity and quality during storage. Sci Hort 36:261–266

Kuroda M, Ozawa T, Imagawa H (1990) Changes in chloroplast peroxidase activities in relation to chlorophyll loss in barley leaf segments. Physiol Plant 80:555–560

Langmeier M, Ginsburg S, Matile P (1993) Chlorophyll breakdown in senescent leaves: Demonstration of Mg-dechelatase activity. Physiol Plant 89:347–353

Manning K (1993) Soft fruit. In Seymour GB, Taylor JE, Tucker GA (eds) Biochemistry of fruit ripening. Chapman and Hall, pp 347–377

Martínez GA, Chaves AR, Añón MC (1994) Effect of gibberellic acid on ripening of strawberry fruit (*Fragaria ananassa* Duch.). J Plant Growth Regul 13:87–91

Martínez GA, Civello PM, Chaves AR, Añón MC (1995) Partial characterization of chlorophyllase from strawberry fruit (*Fragaria ananassa,* Duch.). J Food Biochem 18:213–226

McFeeters RF, Chichester CO, Whitaker JR (1971) Purification and properties of chlorophyllase from *Ailanthus altissima* (tree-of-heaven). Plant Physiol 47:609–618

Orthoefer FT, Dugan LR (1973) The coupled oxidation of chlorophyll *a,* with linoleic acid catalyzed by lipoxidase. J Sci Food Agric 24:357–365

Potty VH (1969) Determination of protein in the presence of phenols and pectins. Anal Biochem 29:535–539

Purvis AC, Barmore CR (1981) Involvement of ethylene in chlorophyll degradation in peel of citrus fruits. Plant Physiol 68:854–856

Reddy A, Jena P, Mukherjee S, Poovaiah B (1990) Molecular cloning of cDNAs for auxin-induced mRNAs and developmental expression of the auxin-inducible genes. Plant Mol Biol 14:643–653

Rodríguez MT, González MP, Linares JM (1987) Degradation of chlorophyll and chlorophyllase activity in senescing barley leaves. J Plant Physiol 129:369–374

Sabater B, Rodríguez MT (1978) Control of chlorophyll degradation in detached leaves of barley and oat through effect of kinetin on chlorophyllase levels. Physiol Plant 43:274–276

Shioi Y, Tatsumi Y, Shimokawa K (1991) Enzymatic degradation of chlorophyll in *Chenopodium album.* Plant Cell Physiol 32:87–93

Thomas P, Janave MT (1992) Effect of temperature on chlorophyllase activity, chlorophyll degradation and carotenoids of Cavendish bananas during ripening. Int J Food Sci Tech 27:57–63

Woodward JR (1972) Physical and chemical changes in developing strawberry fruits. J Sci Food Agric 23:465–473

Yamauchi N, Hashinaga F (1992) Chlorophyll degradation by peroxidase in ethylene-treated satsuma mandarin fruits. J Jap Soc Cold Pres Food 18:167–172

Yamauchi N, Hashinaga F, Itoo S (1991) Chlorophyll degradation with degreening of kabosu (*Citrus sphaerocarpa* Hort. ex Tanaka) fruits. J Jap Soc Hort Sci 59:869–875

Yamauchi N, Watada AE (1991) Regulated chlorophyll degradation in spinach leaves during storage. J Am Soc Hort Sci 116:58–62

Zucker M (1965) Induction of phenylalanine deaminase by light and its relation to chlorogenic synthesis in potato tuber tissue. Plant Physiol 40:779–785