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Regulation of Ethylene, ACC, MACC Production, and ACC-Oxidase Activity at Various Stages of Maturity of Apple Fruit and the Effect of Exogenous Ethylene Treatment

Zur Regulation der Ethylen-, ACC- und MACC-Produktion sowie der ACC-Oxidase-Aktivität während unterschiedlicher Reifestadien der Apfelfrucht und ihre Beeinflussung durch exogene Ethylenbehandlung

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Summary

The regulation of ethylene production of apple fruit (*Malus domestica Borkh.*, cv. 'Golden Delicious') at different maturity stages was investigated by measuring the concentration of 1-aminocyclopropane-1-carboxylic acid (ACC), the immediate precursor of ethylene, its conjugation to N-malonyl-ACC (MACC), and its oxidation by ACC-oxidase to ethylene. The concentration of these metabolites was determined in the peel and pulp of the fruits. Both ACC and ACC-oxidase were rather low in the peel and in the pulp and increased only slightly before the onset of ripening, indicated by the start of autocatalytic ethylene production. In contrast, MACC levels were considerably higher in the peel and pulp tissue. With the start of autocatalytic ethylene production and the onset of the respiratory climacteric, a concomitant rapid increase in ACC, MACC and ACC-oxidase was detected. However, this increase very much depended an the maturity stage at which the fruits were harvested: those harvested very early did not attain similar high levels/activities compared to those harvested close or even after the beginning of the climacteric.

Even a continuous treatment with saturating concentrations of exogenous ethylene did not overcome the inability of prematurely harvested fruit to produce "sufficient" ACC and ACC-oxidase activity and thus autocatalytic ethylene production of these fruit was very much delayed.

There was considerable compartmentalization of ACC, MACC and ACC-oxidase between peel and pulp of the fruit. The ACC-oxidase activity was e.g. considerably higher in the pulp then in the peel, whereas ACC and particularly MACC were higher in the peel.

The experiments revealed that the pulp rather than the peel played a dominant role in the regulation of autocatalytic ethylene production and subsequent ripening.

Zusammenfassung

Es wurde die Regulation der Ethylenproduktion von Apfelfrüchten (*Malus domestica* Borkh., cv. 'Golden Delicious') in unterschiedlichen Reifestadien untersucht. Hierzu wurde die Konzentration von 1-Aminocyclopropan-1-Carboxylsäure (ACC), die unmittelbare Vorstufe von Ethylen, ihre Konjugation zu N-Malonyl-ACC (MACC), und ihre Oxidation zu Ethylen durch ACC-Oxidase in der Fruchtschale und im Fruchtfleisch analysiert. Sowohl ACC als auch die ACC-Oxidase-Aktivität war niedrig sowohl in der Fruchtschale als auch im Fruchtfleisch und nahm vor der Induktion der Fruchtreife lediglich geringfügig zu. Im Gegensatz dazu war das MACC Niveau in der Fruchtschale und im Fruchtfleisch deutlich höher. Mit dem Start der autokatalytischen Ethylenproduktion und der Induktion des respiratorischen Klimakteriums wurde eine gleichzeitige schnelle Zunahme von ACC, MACC und ACC-Oxidase festgestellt. Allerdings hing diese Zunahme sehr von dem Reifestadium ab, in dem die Früchte geerntet worden waren. Sehr früh geerntete Früchte erreichten auch nicht annähernd so hohe Niveaus/Aktivitäten dieser Metaboliten wie Früchte, die kurz vor oder während des Klimakteriums geerntet wurden.

Sogar eine kontinuierliche Behandlung mit gesättigten Konzentrationen von exogenem Ethylen konnte die Fähigkeit prä-klimakterisch geernteter Früchte „ausreichend" ACC und ACC-Oxidase-Aktivität zu produzieren nicht wesentlich erhöhen und demzufolge wurde die autokatalytische Ethylenproduktion dieser Früchte stark verzögert.

Zwischen Fruchtschale und Fruchtfleisch besteht eine wesentliche Kompartimentierung von ACC, MACC und ACC-Oxidase. Die ACC-Oxidase-Aktivität, z.B., war wesentlich höher im Fruchtfleisch als in der Fruchtschale, während ACC und insbesondere MACC in der Fruchtschale in höherer Konzentration vorlagen.

Die Experimente zeigten, dass nicht die Fruchtschale sondern das Fruchtfleisch eine dominante Rolle in der Regulation der autokatalytischen Ethylenproduktion und der darauf folgenden Reifeprozesse spielt.

Introduction

Ripening of climacteric fruits, such as apple, is characterized by a rapid rise in respiration and autocatalytic ethylene production. In these fruits, ethylene is considered the trigger because the application of exogenous ethylene induced ripening and inhibition of ethylene biosynthesis prevents ripening (McGlasson

et al. 1978). In higher plants, ethylene biosynthesis follows the path: methionine → S-adenosylmethionine → ACC (1-aminocyclopropane-1-carboxylic acid) → ethylene (ADAMS and YANG, 1979). The final step is catalyzed by the enzyme ACC oxidase (previously known as ethylene forming enzyme – EFE) (YANG and HOFFMAN 1984). It was assumed that the ACC oxidase is membrane bound and requires some structural or compartmental association to be active (GUY and KENDE 1984, ANDREW et al. 1986) until VERVERIDIS and JOHN (1991) demonstrated complete recovery of ACC oxidase activity as a soluble enzyme from melon fruit (*Cucumis melo*). Thereafter, this was also demonstrated in apple (KUAI and DILLEY 1992, FERNANDEZ-MACULET and YANG 1992), pear (VIOQUE and CASTELLANO 1994), avocado (MCGARVEY and CHRISTOFFERSON 1992) and banana (MOYA-LEON and JOHN 1994).

ACC, the direct precursor of ethylene in higher plants (ADAMS and YANG 1979) can be regulated by the respective rate of synthesis and conversion to ethylene as well as by conjugation to N-malonyl-ACC (MACC) (YANG and HOFFMAN 1984), which was identified as a major metabolite of ACC in higher plants (AMRHEIN et al. 1981, HOFFMAN and YANG 1982). Therefore, the rate of ethylene production could be affected by at least three reactions: the conversion of S-adenosylmethionine to ACC by ACC synthase, ACC to ethylene by ACC oxidase and ACC to MACC by ACC-malonyltransferase. Ethylene, on the other hand, can regulate the activity of ACC oxidase (LIU et al. 1985a), ACC synthase (BUFLER 1984) and ACC-malonyl-transferase (LIU et al. 1985a, b).

The role of the peel and the pulp tissue in starting autocatalytic ethylene production and ripening of intact fruit could be different. It was suggested e.g. that in bananas ACC oxidase activity in the pulp is a key factor initiating autocatalytic ethylene production during ripening (DOMINGUEZ and VENDRELL 1993). For apple fruit such an investigation has not been conducted.

In the present study we investigated the evolution of ethylene production, ACC oxidase activity and the levels of free and conjugated ACC both in the peel and in the pulp tissue from apple fruits harvested at various stages of maturity. In addition, the response to exogenous ethylene treatment during ripening was investigated.

Materials and Methods

Plant materials

Apple fruits (*Malus domestica* Borkh., cv. 'Golden Delicious') were obtained in two seasons from the experimental station of the University of Hohenheim at Stuttgart, Germany. The fruits were harvested at various stages of maturity at 10-day intervals. The harvest dates and the internal ethylene concentration (IEC) of fruits at corresponding harvest dates are shown in Table 1.

Experimental set up for ethylene treatment and gas measurements

Immediately after harvest, uniform apple fruits were selected and placed in ten 5 L glass jars (1.5 kg fruits jar^{-1}). The jars were placed in a temperature-regulated water bath and exposed to a continuous and constant flow of moist air (Control; Co.) or moist air enriched with 100 μL L^{-1} C_2H_4 (Ethylene Treatment; ET) for up to 10 days at 25 °C. Ethylene was premixed in cylinders with nitrogen and the desired concentration of ethylene was provided by appropriate mixing with the air stream entering the jars.

Internal gas samples from the core of fruits were obtained with a syringe after immersing the fruit in water. Autocatalytic ethylene production was determined by taking gas samples from the air stream leaving the glass jars. Ethylene concentrations of the gas samples were determined by conventional gas chromatography. Autocatalytic ethylene production of fruits treated with continuous exogenous ethylene (100 μL L^{-1}) was determined by subtracting the ethylene concentration of the in-going air stream from that of the out-going air stream.

Analysis of ACC oxidase activity, ACC and MACC contents

During each experimental period samples of fruits from each harvest were taken at 2-days intervals. At each sampling time, one out of 3 replicate jars of fruits (1.5 kg) per treatment was removed from the water bath and internal ethylene concentration (IEC) was determined. Subsequently, the fruits were washed and peeled using a potato peeler. The peel and segments of pulp from the equatorial regions of each fruit were separated, quickly frozen in liquid nitrogen and subsequently lyophilized. The lyophilized peel and pulp were then ground to a fine powder together with dry ice and stored at –20 °C until required for the analysis of ACC oxidase, ACC and MACC.

a. Extraction and assay procedure of free and conjugated ACC

One g dry peel or pulp powder was added to a centrifuge tube containing 10 mL of 80% (V/V) methanol and mixed for 30 seconds using a vortex mixer. After 30 min of extraction at ambient temperature, the homogenate was centrifuged at 18000 rpm and the supernatant evaporated to dryness. The dried residue was dissolved in 5 mL water and served as the aqueous extract for the determination of ACC and MACC.

ACC was determined according to LIZADA and YANG (1979). Total ACC (free and conjugated ACC) was measured from the aqueous extract hydrolyzed with 5 N HCL at 100 °C for 3 h, according to the method of HOFFMAN et al. (1982) and MACC calculated by subtracting free ACC. Results were given in nmol g dw^{-1}.

b. Extraction and assay procedure of ACC oxidase activity

The extraction ACC oxidase was modified from the method of FERNANDEZ-MACULET and YANG (1992). One g dry peel or pulp powder was added to 10 mL of extraction buffer containing 0.1 M Tris-HCl (pH 7.7), 30 mM sodium ascorbate and 5% (w/v) insoluble polyvinylpolypyrrolidone (PVPP). After mixing using a vortex mixer for 20 seconds, the homogenate was

Tab. 1. The sampling dates of apple fruits and the corresponding internal ethylene concentrations (IEC), ethylene productions, ACC oxidase activities, ACC and MACC concentrations.
Erntetermine der Äpfel und endogene Ethylenkonzentrationen (IEC), Ethylenproduktionen, ACC-Oxidase-Aktivitäten, ACC- und MACC-Konzentrationen.

	Fruit Tissue	1st Harvest (19.09.96)	2nd Harvest (29.09.96)	3rd Harvest (09.10.96)	4th Harvest (19.10.96)
IEC (μL L^{-1})		0.01 ± 0.01	0.04 ± 0.01	0.11 ± 0.02	13.5 ± 2.50
C_2H_4 (μL kg^{-1} h^{-1})		0.01	0.01	0.03	26.62
ACC-oxidase	peel	0.33 ± 0.03	0.53 ± 0.11	0.80 ± 0.16	3.02 ± 0.67
(nl C_2H_4 h^{-1} g dw^{-1})	pulp	4.36 ± 0.76	5.91 ± 0.36	6.40 ± 1.39	17.81 ± 1.13
ACC	peel	0.90 ± 0.06	1.51 ± 0.11	2.66 ± 0.42	57.82 ± 4.90
(nmol g dw^{-1})	pulp	0.76 ± 0.05	1.10 ± 0.19	2.26 ± 0.32	47.19 ± 7.25
MACC	peel	8.63 ± 0.57	15.55 ± 2.71	18.51 ± 1.50	42.98 ± 3.56
(nmol g dw^{-1})	pulp	7.97 ± 0.91	8.80 ± 0.89	9.16 ± 1.36	12.73 ± 2.91

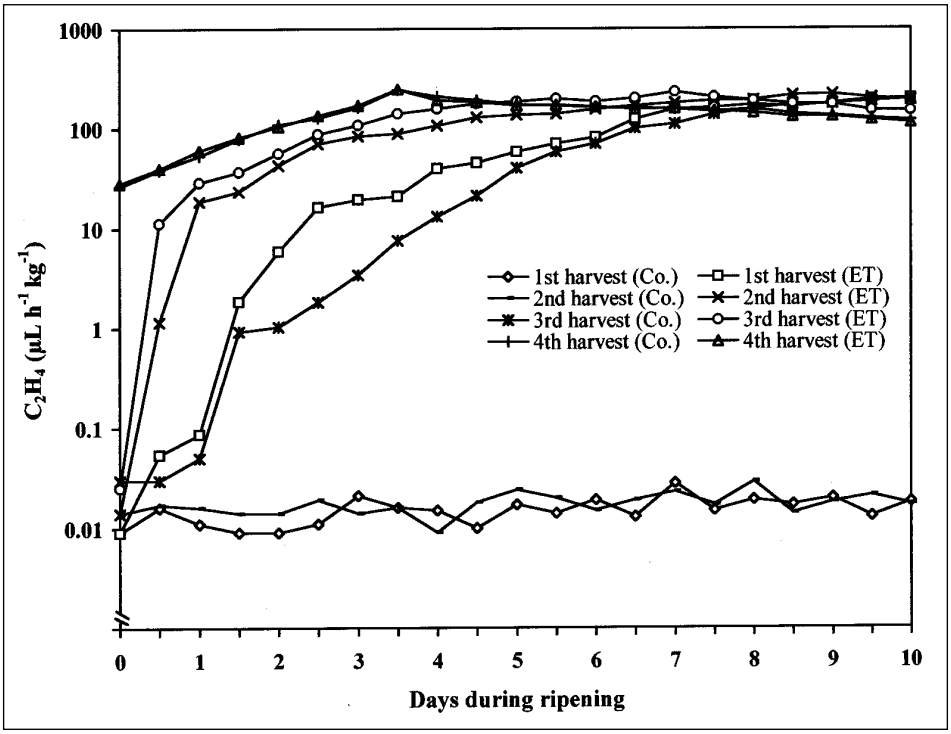


Fig. 1. Autocatalytic ethylene production during ripening at 25 °C of apple fruits harvested at various stages of maturity and effects of exogenous ethylene treatment (100 μL L^{-1}). (Co. = control, ET = ethylene treatment).
Autokatalytische Ethylenproduktion von Äpfeln verschiedener Reifegrade bei 25 °C und nach exogener Ethylenbehandlung (100 μL L^{-1}). (Co. = Kontrolle, ET = Ethylenbehandlung).

placed an ice, extracted for 1 h and then centrifuged at 22000 rpm for 25 min at 0–4 °C. The supernatant (crude extract) was used for enzyme assays. The ACC oxidase activity was determined according to KUAI and DILLEY (1992). Results were given in nl C_2H_4 g dw^{-1} h^{-1}.

All experiments were conducted over two seasons (1995 and 1996) and the results obtained from both years were similar. Only those obtained in 1996 are presented here.

Results

Ethylene production, IEC, ACC oxidase activity, ACC and MACC concentration during maturation and ripening on tree

Tab. 1 shows clearly that fruits at the first and second harvest were preclimacteric, and fruits at the third harvest were just before climacteric, whereas fruits at the forth harvest had already entered ethylene climacteric, indicated by the IEC. During the preclimacteric stage, the fruits produced only just detectable amounts of ethylene. After induction of ethylene climacteric, however, a sharp increase in autocatalytic ethylene production occurred (fourth harvest).

ACC oxidase activities, ACC and MACC levels increased in the preclimacteric phase but the increases were much slower compared to those in the climacteric phase, both in the peel and in pulp tissue. Pulp tissue showed considerably higher ACC oxidase activity than peel tissue during maturation and ripening. In contrast, ACC and particularly, MACC levels were higher in the peel than in the pulp (Tab. 1).

Ethylene production during ripening after the various harvests and response to exogenous ethylene treatment

The evolution of ethylene during ripening after the various harvests and effects of exogenous ethylene treatment are shown in Fig. 1. The fruits from the first

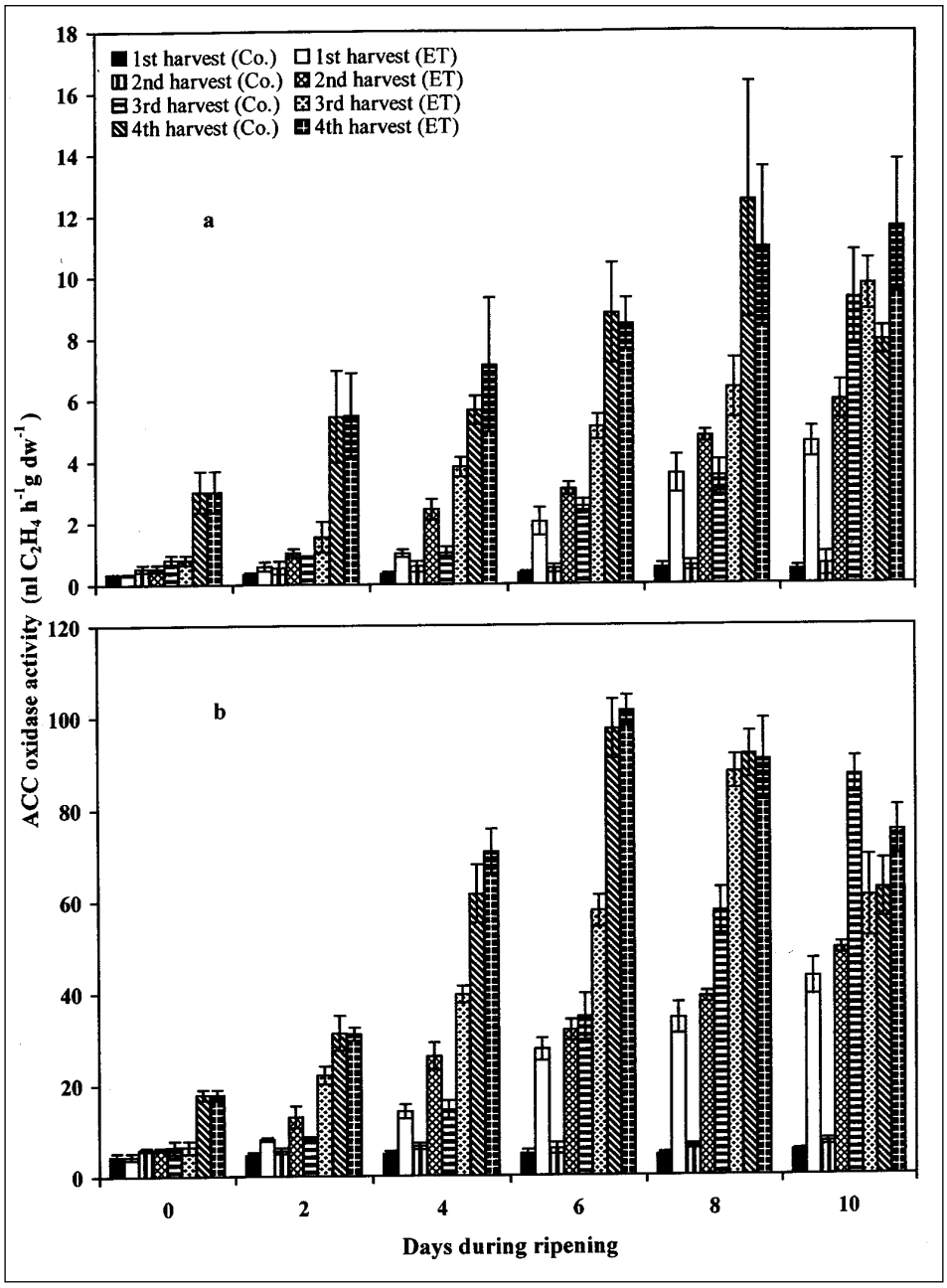


Fig. 2. Changes in ACC oxidase activity in peel (a) and pulp tissue (b) during ripening at 25 °C of apple fruits harvested at various stages of maturity and effects of exogenous ethylene treatment (100 μL L^{-1}). Each value represents the mean ± SE of ACC oxidase activity of 3 replicates (Co. = control, ET = ethylene treatment).
Änderungen der ACC-Oxidase-Aktivität in Schalen (a) und Fruchtfleischgewebe (b) von Äpfeln verschiedener Reifegrade bei 25 °C nach exogener Ethylenbehandlung (100 μL L^{-1}). Die ACC-Oxidase-Aktivität wird als Mittelwert ± SE aus 3 Wiederholungen dargestellt.

and second harvests produced only just detectable amounts of ethylene until the end of the experiments. Fruits from the third harvest, which were already in an advanced stage of maturity, produced detectable levels of ethylene (1.03 μL kg^{-1} h^{-1}) after two days at 25 °C and then entered the climacteric phase. The peak of ethylene production, however, was delayed and was probably not reached even after 10 days of ripening. The ethylene production of the fruits from fourth harvest increased sharply and attained a peak of ca. 245 μL kg^{-1} h^{-1} after 3–4 days and then decreased sharply.

Comparing ethylene treated (ET) fruits with control (Co.)-fruits, it is obvious that exogenous ethylene treatment stimulated autocatalytic ethylene production of preclimacteric harvested fruits. Fruits from the first harvest did not produce significant autocatalytic ethylene until after approximately. two days of treatment, while fruits from the second and third harvests responded already after 24 and 12 h, respectively. Ethylene production from fruits from the fourth harvest which were already in a climacteric stage at harvest, resembled very much that of fruits from the same harvest without ethylene treatment (Fig. 1). This effect, however, was different for the various maturity stages, with fruits from the first harvest being much less responsive than those of the second and then the third.

ACC oxidase activity during ripening after the various harvests and effects of exogenous ethylene production

Changes in ACC oxidase activities during ripening at 25 °C of the peel and the pulp of fruits harvested at various stages of maturity and effects of exogenous ethylene treatment are shown in Fig. 2a–b. The ACC oxidase activity in the peel was very low and increased only slightly before ripening started. In the pulp, ACC

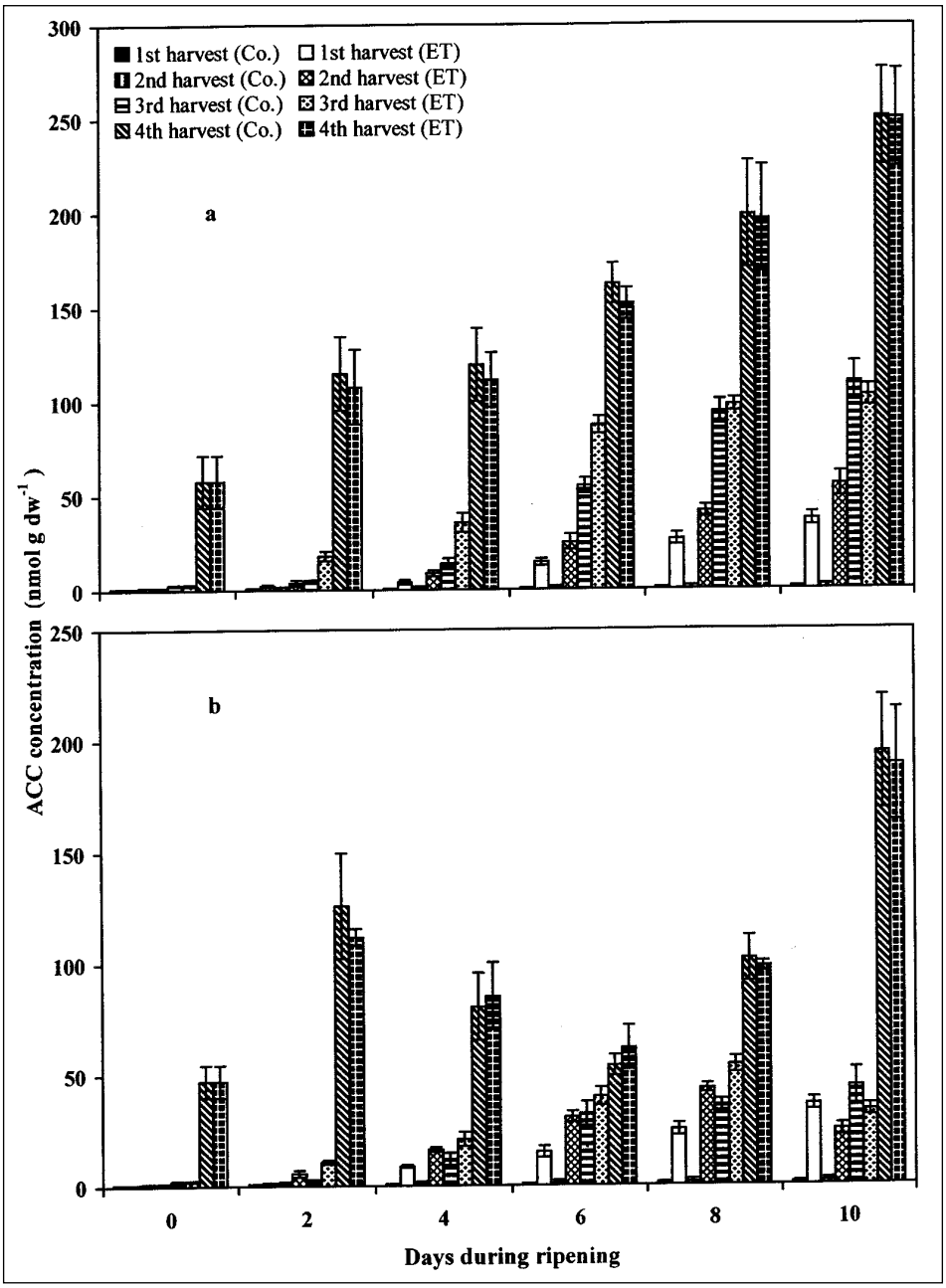


Fig. 3. Changes in ACC concentration in peel (a) and pulp tissue (b) during ripening at 25 °C of apple fruits harvested at various stages of maturity and effects of exogenous ethylene treatment (100 μL L^{-1}). Each value represents the mean ± SE of ACC concentration of 3 replicates (Co. = control, ET = ethylene treatment).
Änderungen in der ACC-Konzentration in Schalen (a) und Fruchtfleischgewebe (b) von Äpfeln verschiedener Reifegrade bei 25 °C nach exogener Ethylenbehandlung (100 μL L^{-1}). Die ACC-Konzentration wird als Mittelwert ± SE aus 3 Wiederholungen dargestellt.

oxidase activity was higher but also increased only slightly. After onset of ripening (fourth harvest), ACC oxidase activity increased quickly, particularly in the pulp. As in preclimacteric fruits, ACC oxidase activity in the pulp of the climacteric fruits was consistently much higher than that in the peel.

It is obvious that ACC oxidase activities both in the peel and in the pulp of preclimacteric fruits were strongly stimulated by exogenous ethylene treatment, but much less than during the two earlier harvests. In fruits of all four harvests, ACC oxidase activity in the peel increased steadily during ripening and the earlier the harvesting of the fruit, the lower the ACC oxidase activity. In the pulp, ACC oxidase activity behaved similarly but a decline for the fourth harvest was seen after 6 days of exogenous ethylene treatment. The peak of ACC oxidase activity occurred subsequently to the ethylene peak (Fig. 1 and 2). In the fruits of all four harvests, ACC oxidase activity in the pulp was considerably higher than that in the peel.

Changes of free ACC levels during ripening after the various harvests and effects of exogenous ethylene treatment

Changes in free ACC levels during ripening at 25 °C and effects of exogenous ethylene treatment are shown for the peel (Fig. 3a) and pulp (Fig. 3b) of fruits harvested at various stages of maturity. The ACC levels both in the peel and in pulp of preclimacteric fruits (first and second harvest) increased only slightly during the ripening period of 10 days. After the onset of ripening, indicated by production of autocatalytic ethylene, however, the ACC levels increased sharply, particularly in the peel of fruit from the third and fourth harvests. In the pulp, ACC increased steadily only within fruits

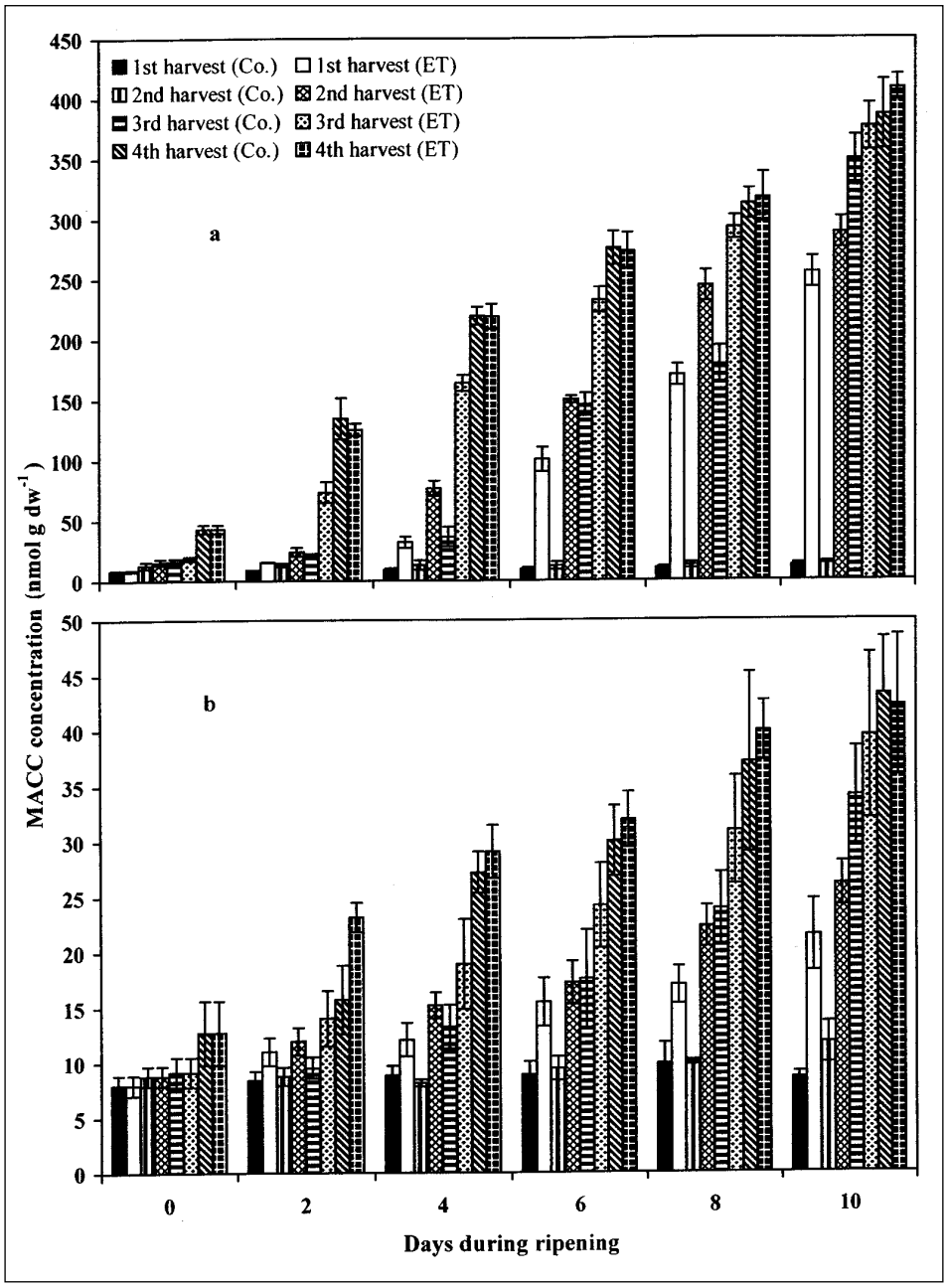


Fig. 4. Change in MACC concentration in the peel (a) and pulp tissue (b) during ripening at 25 °C of apple fruits harvested at various stages of maturity and effects of exogenous ethylene treatment (100 µL L^{-1}). Each value represents the mean ± SE of MACC concentration of 3 replicates (Co. = control, ET = ethylene treatment).
Änderung der MACC-Konzentration in Schalen (a) und Fruchtfleischgewebe (b) von Äpfeln verschiedener Reifegrade bei 25 °C nach exogener Ethylenbehandlung (100 µL L^{-1}). Die MACC-Konzentration wird als Mittelwert ± SE aus 3 Wiederholungen dargestellt.

from the third harvest, whereas in fruits from the fourth harvest, the ACC concentration was more variable.

In the C_2H_4-treated fruits from all four harvests, the ACC contents in the peel increased steadily until the end of the experiments. In the pulp, ACC induction by ethylene was less pronounced and more erratic compared to that in the peel. It is particularly noteworthy that the ACC contents, both in the peel and in the pulp of fruits harvested early, were considerably less stimulated by ethylene than in the more mature fruit from the third and especially fourth harvests.

Conjugated ACC (MACC) levels during ripening after the various harvests and response to exogenous ethylene production

Figure 4 shows changes in MACC concentrations during ripening at 25 °C in the peel and in the pulp of fruits harvested at various stages of maturity and effects of exogenous ethylene treatment. Both the peel and the pulp of preclimacteric fruits contained significant MACC concentrations but very low free ACC levels and ACC oxidase activity. Before the commencement of autocatalytic ethylene production, however, the increases in MACC were very slow. After the onset of ripening, MACC levels, both in the peel and in the pulp, increased steadily and rapidly, particularly in the peel. Interestingly, MACC levels were higher in the peel and lower in the pulp than the free ACC levels during the late climacteric phase (Fig. 3 and 4).

The results show clearly that, as in autocatalytic ethylene production, ACC oxidase activities and free ACC levels, exogenous ethylene treatment strongly stimulated the formation of MACC in the pulp and, particularly, in the peel of preclimacteric fruits. Nevertheless, MACC levels, both in the peel and in the pulp

of early harvested fruits, were consistently lower than those in late harvested fruits.

Discussion

Apple fruits harvested considerably before their climacteric rise in ethylene production exhibit, even after exogenous ethylene treatment, a much lower rate of ripening, as can be seen e.g., from Fig. 1. One of the factors possibly responsible for this low ripening process could be the low concentration of ACC, both in peel and pulp, which could prevent or limit autocatalytic ethylene production (SITRIT et al. 1986, DOMINGUEZ and VENDRELL 1993, MAGNE and LARHER 1995). This suggestion is supported by the finding that ACC oxidase activity, the enzyme converting ACC to ethylene, was sufficiently high, at least in the pulp, and could not be considered as a rate limiting factor, which confirms results of SITRIT et al. (1986) for avocado and BUFLER (1986) for apples. It has been suggested that in preclimacteric fruit, ACC synthase is restricted by a repressor that is destroyed in the course of ripening or ethylene treatment (HOFFMAN and YANG 1982). Although preclimacteric fruits contained significant amounts of MACC, both in peel and pulp (Fig. 4a–b), the ACC concentration (Fig. 3a–b) and ethylene production (Fig. 1) were low and almost undetectable. This indicates that MACC is not a storage form for ACC and, if at all, only slowly releases ACC for ethylene production (HOFFMAN et all 1983, KNEE 1985). Thus, malonylation of ACC must rather be seen as an inactivation process (YANG and HOFFMAN 1984) and may participate in the regulation of ethylene production in preclimacteric as well as in climacteric fruit. In the later fruits, the concentration of MACC increased steadily. This is not in accordance with results of AMRHEIN et al. (1982) that stated that climacteric apple fruit do not conjugate ACC.

The lag period of about 1 day observed between the peak of free ACC content and the peak of ethylene production (Fig. 3 and 1) indicates that the enzymatic system oxidizing ACC to ethylene is not sufficiently active in preclimacteric apples and a certain time is required before the enzyme becomes fully efficient. This is further verified by data showing that the peak of ACC oxidase activity in the pulp is subsequent to the peak of ethylene production (Fig. 2a and 1). The increase in ACC at the beginning of fruit ripening may be a result of a new synthesis of ACC synthase, although the possibility of the activation of this enzyme can not be excluded because ethylene treatment enhanced ACC synthase activity in ripening apples (BUFLER 1984). The second increase in ACC level in the pulp occurs as fruit becomes overripe (Fig. 3b) and is accompanied by a decreasing ethylene production (Fig. 1) and ACC oxidase activity (Fig. 2b). This suggests that the ACC oxidase is impaired as the tissue senesces. In this postripening phase, ACC would accumulate as long as ACC synthesis is not impaired to the same extent.

Exogenous ethylene treatment stimulated autocatalytic ethylene production, ACC oxidase activity and ACC and MACC formation of preclimacteric fruit but the earlier the harvesting of preclimacteric fruits, the weaker the response to the exogenous ethylene. The ACC oxidase activity in 'Golden Delicious' apple fruit was increasingly stimulated by ethylene as fruit development progressed (CHOI et al. 1994). Together with these data, this suggests that ACC concentration is not the sole and possibly not even the most important factor limiting autocatalytic ethylene formation and ripening. Sensitivity of fruit to exogenous or endogenous ethylene is obviously an equally important factor for immature fruit. The ACC oxidase activity together with ACC concentration seems to be a good marker to monitor this sensitivity, because their response to ethylene is low as long as the fruit is immature. In the peel, ACC oxidase activity increased steadily (Fig 2a) and the ACC content was sufficiently high during the course of ripening (Fig. 3a), but ethylene production of intact fruit decreased in the overripe stage (Fig. 1). This suggests that ACC oxidase activity in the peel is not responsible for autocatalytic ethylene production of intact fruit. This, coupled with the suggestion that the increase in ACC oxidase activity in the peel may be induced by ethylene produced in the pulp (LIU et al. 1985a, BUFLER 1986), suggests that the pulp rather than the peel plays a decisive role in starting the autocatalytic ethylene production and ripening.

A slow rate of ripening after harvesting immature fruit is desirable for a good keeping quality and transport capability of apple fruit. These fruit remain firmer and more juicy than fully mature fruit. For this reason, apple fruits are often harvested too early with the negative result that the production of aroma volatiles is impaired (SONG and BANGERTH 1996) which often leads to consumer complaints. To avoid impaired volatile production, ACC oxidase in the pulp could be used as a marker to identify fruit at a maturity stage that ripens after harvest at a rate slow enough to guarantee sufficient transport/keeping quality but fast enough to produce acceptable aroma volatiles.

Literature

ADAMS, D. O. and S. F. YANG 1979: Ethylene biosynthesis: Identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. Proc. Natl. Acad. Sci. USA **76**, 170–174.

AMRHEIN, N., H. SCHNEEBECK, H. SKORUPKA and S. TOPHOF 1981: Identification of a major metabolite of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid in higher plants. Naturwissenschaft **68**, 619–620.

AMRHEIN, N., F. BREUNING, J. EBERLE, H. SKORUPKA and S. TOPHOF 1982: The metabolism of 1-aminocyclopropane-1-carboxylic acid. In: P. Wareing (ed.), Plant Growth Substances, Academic Press, p. 249–258.

ANDREW, J. R. P., J. T. BORLAKOGLU and P. JOHN 1986: Activity of ethylene-forming enzyme in relation to plant cell structure and organization. J. Plant Physiol. **125**, 207–216.

BUFLER, G. 1984: Ethylene-enhanced 1-aminocyclopropane-1-carboxylic acid synthase activity in ripening apples. Plant Physiol. **75**, 192–195.

BUFLER, G. 1986: Ethylene-promoted conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene in

peel of apple at various stages of fruit development. Plant Physiol. **80**, 539–543.

CHOI, S. J., G. BUFLER and F. BANGERTH 1994: Ethylensensitivität von Apfel- und Tomatenfrüchten während ihrer Entwicklung. Gartenbauwissenschaft **59**, 154–158.

DOMINGUEZ, M. and M. VENDRELL 1993: Ethylene biosynthesis in banana fruit: Evolution of EFE activity and ACC levels in peel and pulp during ripening. J. Hort. Sci. **68**, 63–70.

FERNANDEZ-MACULET, C. J. and S. F. YANG 1992: Extraction and partial characterization of the ethylene-forming enzyme from apple fruit. Plant Physiol. **99**, 751–754.

GUY , M. and H. KENDE 1984: Conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene by isolated vacuoles of Pisum sativum L. Planta **160**, 281–287.

HOFFMAN, N. E. and S. F. YANG 1982: Enhancement of wound-induced ethylene synthesis by ethylene in preclimacteric cantaloupe fruits. Plant Physiol. **69**, 317–322.

HOFFMAN, N. E., S. F. YANG and T. MCKEON 1982: Identification of 1-(malonylamino)cyclopropane-1-carboxylic acid as a major conjugate of 1-aminocyclopropane-1-carboxylic acid, an ethylene precursor in higher plants. Biochem. Biophys. Res. Com. **104**, 765–770.

HOFFMAN, N. E., J. R. FU and S. F. YANG 1983: Identification and metabolism of 1-(malonylamino)cyclopropane-1-carboxylic acid in germinating peanut seeds. Plant Physiol. **71**, 197–199.

KNEE, M. 1985: Metabolism of 1-aminocyclopropane-1-carboxylic acid during apple fruit development. J. Exp. Bot. **36**, 670–678.

KUAI, J. P. and D. R. DILLEY 1992: Extraction, partial purification and characterization of 1-aminocyclopropane-1-carboxylic acid oxidase from apple fruit. Postharvest Biology and Technology **1**, 203–211.

LIU, Y., N. E. HOFFMAN and S. F. YANG 1985a: Promotion by ethylene of the capability to convert 1-aminocyclopropane-1-carboxylic acid to ethylene in preclimacteric tomato and cantaloupe fruits. Plant Physiol. **77**, 407–411.

LIU, Y., L. Y. SU and S. F. YANG 1985b: Ethylene promotes the capability to malonylate 1-aminocyclopropane-1-carboxylic acid and D-amino acids in preclimacteric tomato fruits. Plant Physiol. **77**, 891–895.

LIZADA, M. C. C. and S. F. YANG 1979: A simple and sensitive assay for 1-aminocyclopropane-1-carboxylic acid. Analyt. Biochem. **100**, 140–145.

MAGNE, C. and F. LARHER 1995: Changes in ethylene-producing capacity of apple fruit tissues during natural or induced senescence. J. Hort. Sci. **70**, 307–314.

MCGARVEY, D. J. and R. E. CHRISTOFFERSON 1992: Characterization and kinetic parameters of ethylene-forming enzyme from avocado fruit. J. Biol. Chem. **267**, 5964–5967.

MCGLASSON, W. B., N. L. WADE and J. ADATO 1978: Phytohormones and fruit ripening. In: D. S. LETHAM, P. B. GOODWIN and T. J. V. HIGGINS (editors), Phytohormones and Related Compounds – A Comprehensive Treatise II. Elsevier/North-Holland Biomedical Press, Amsterdam, Oxford, New York, p. 447–493.

MOYA-LEON, M. A. and P. JOHN 1994: Activity of 1-aminocyclopropane-1-carboxylate (ACC) oxidase (ethylene-forming enzyme) in the pulp and peel of ripening bananas. J. Hort. Sci. **69**, 243–250.

SITRIT, Y., J. RIVO and A. BLUMENFELD 1986: Regulation of ethylene biosynthesis in avocado fruit during ripening. Plant Physiol. **81**, 130–135.

SONG, J. and F. BANGERTH 1996: The effect of harvest date an aroma compound production from 'Golden Delicious' apple fruit and relationship to respiration and ethylene production. Postharvest Biol. Technol. **8**, 259–269.

VERVERIDIS, P. and P. JOHN 1991: Complete recovery in vitro of ethylene-forming enzyme activity. Phytochem. **30**, 725–727.

VIOQUE, B. and J. M. CASTELLANO 1994: Extraction and biochemical characterization of 1-aminocyclopropane-1-carboxylic acid oxidase from pear. Physiol. Plant. **90**, 334–338.

YANG, S. F. and N. E. HOFFMAN 1984: Ethylene biosynthesis and its regulation in higher plants. Ann. Rev. Plant Physiol. **35**, 155–189.

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