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THE EFFECT OF ETHYLENE ON THE PHYSIOLOGY OF RIPENING OF APPLE FRUITS AT HYPOBARIC CONDITIONS

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RÉSUMÉ

L'entreposage de pommes sous une pression réduite (L P S) retarde la maturation, comparativement à la conservation au froid ou en atmosphère contrôlée (CA). Comme la teneur en O_2 est la même en CA et en LPS, l'éthylène doit jouer un rôle même à basse température et à basse tension d'oxygène. Pour étudier cet effet de l'éthylène plus en détail, des pommes ont été entreposées à 3 °C (témoins), à 3 °C et 100 Torr de pression (LPS) et à 3 °C et 100 Torr de pression avec une fourniture continue d'éthylène (LPS, C_2H_4). Dans ces conditions, l'éthylène causait une chute de la fermeté et stimulait en outre d'autres phénomènes de la maturation, mais pas tous. La dégradation de la chlorophylle par exemple était à peine influencée. Après environ 4 semaines d'entreposage, les fruits traités par l'éthylène montraient un accroissement de l'émission de CO_2 comparativement aux fruits LPS. Après des temps croissants d'entreposage, la respiration de ces fruits continuait d'être stimulée. En outre, le traitement par l'éthylène induisait un accroissement important de la perméabilité des tissus et une activité accrue de plusieurs enzymes telles que enzyme malique, phosphatase acide ou PAL. Les résultats indiquent que dans ces conditions d'entreposage, l'éthylène peut affecter la maturation des pommes en modifiant la perméabilité des membranes aussi bien qu'en accroissant l'activité des enzymes.

SUMMARY

Storing apple fruits at low pressure retards ripening compared to cold or CA storage. Because the O_2 content in CA and LPS was similar, this indicates that ethylene plays a role event at low temperatures and at a low O_2 tension. To investigate this ethylene effect in more detail apples were stored at 3 °C (control), 3 °C and 100 Torr pressure (LPS) and 3 °C, 100 Torr pressure plus a continous ethylene supply (LPS, C_2H_4). Under these conditions C_2H_4 caused a decrease in fruit firmness and also stimulated other ripening phenomena, but not all. Chlorophyll breakdown e.g. was hardly influenced. After about 4 weeks in storage the ethylene treated fruits showed an accelerated output of CO_2 compared with the LPS fruits. With increasing storing time respiration of these fruits was further stimulated. Beside this, ethylene treatment induced a strong increase in tissue permeability and a higher activity of several enzymes, such as malic enzyme, acid phosphatase or PAL. The results indicate that under these storing conditions ethylene may affect ripening of apples by changing membrane permeability as well as by increasing enzyme activities.

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The question whether ethylene can accelerate ripening of apples even at low temperature and reduced oxygen tensions is a matter of controversy (e.g. FIDLER, 1950; FORSYTH et al., 1969; BLANPIED, 1972; STOLL, 1972). In a previous experiment we showed that ripening of apples stored at hypobaric conditions was delayed compared to storage in conventional CA— or cold storage (BANGERTH, 1973). As the partial pressure of oxygen in LPS and CA-storage was similar, it was presumed, that under these conditions ethylene had affected ripening even though the temperature was 3 °C and the oxygen content 4 %. To investigate this problem in more detail, low pressure storage seems to be a good method, since it is possible to keep the endogenous ethylene level of stored fruits below a physiological active value and therefore excluding ethylene as a ripening factor (see BURG and BURG, 1966).

The following investigation was set up to explore the effect of ethylene under the above mentioned conditions on different ripening parameters of apples as well as on possible reasons for this ethylene effect.

1. Materials and methods.

The experimental set up for LPS trials was similar to that used by BURG and BURG (1966) and was described in detail by BANGERTH (1973). Three different treatments were carried out:

1) One desiccator with 8 kg of fruits (Golden delicious) was kept at 100 Torr pressure and 3 °C throughout the experiment (LPS). The air flow through the system was about 4,5 l/h, which makes an air exchange of 1/5 per hour.
2) The conditions in the second desiccator were identical to the first one, with the exception that 500 ppm of ethylene were continuously added to the air stream (LPS, C_2H_4).
3) In the third desiccator the fruits were kept at normal air pressure (ca. 760 Torr), with the same air exchange as in desiccators one and two (control).

Three different harvest times were used (5.10.1973; 19.10.1973; 26.10.1973) to investigate whether picking time is of significance for the effect of ethylene under these conditions. Fruits from harvest time one had an endogenous ethylene content of 0,11 ppm, which indicates that they were in the preclimacteric stage. Those from harvest time two and three had already reached the climacteric.

Respiration of fruits (CO_2 release) and ethylene synthesis were measured throughout the duration of the experiment, using a method developed by BANGERTH and STREIF (BANGERTH, 1973). A special designed cold trap was inserted between storage container and vacuum pump and immersed in liquid air. Almost 85 % of CO_2 and C_2H_4 coming from the storage container were frozen out in this trap at 100 Torr pressure. After thawing, CO_2 and ethylene in the trap were measured by GLC using a TCD for CO_2— and FID for C_2H_4 determinations.

Fruits were removed from the storage containers at intervals indicated below and each analysis was done on at least 8 fruits.

Decrease of fruit firmness was followed with an Italian effe gi penetrometer and expressed as kg/cm². Chlorophyll analysis was made by homogenizing fifty 1 cm² pieces of fruit skin in 50 ml aceton plus 250 mg $CaCO_3$. After, vacuum filtration absorbance was measured at 665 nm. Titratable acidity was determined after ethanol extraction by titration with 0,01 N NaOH to pH 7,0. Changes in tissue permeability were followed up by measuring the conductivity in 30 ml 0,4 M mannitol solution in which 3 g of tissue slice were incubated (1 cm diameter and 2 mm thick). The slices were shaken at 25 °C and readings taken every hour. At the end of the experiment the slices were frozen together with the incubation solution, thawed in boiling water and after equilibration conductivity was measured again (= maximal conductivity). The time it took to reach half maximal conductivity was used as a measure for tissue permeability. Aceton powder was prepared for malic enzyme and acid phosphatase analysis. The enzymes were extracted from the aceton powder with an extraction medium consisting of 0,1 M Tris buffer, 0,35 M mannitol and 5×10^{-4} M EDTA at a pH of 8,5. After one hour extraction at 2 °C the suspension was centrifuged for 30 min at 46000 g. Malic enzyme was determined according to the method of DILLEY (1966) and acid phosphatase was measured as described by BERGMEYER (1963).

2. Results.

Harvest time did not affect the difference between fruit ripening in LPS or LPS,C_2H_4 storage. Therefore only the results of harvest time I fruits will be dealt with here.

Within 15 days after the start of the experiment, respiration (CO_2-release) of the control fruits was already twice as high as that of LPS- and LPS,C_2H_4 stored fruits. Until that time the addition of ethylene had no effect on the respiration of the stored fruits. After about 5-6 weeks in storage, however, ethylene treatment caused a remarkable rise in CO_2 output of the LPS,C_2H_4 fruits as compared to LPS fruits. A progressive increase in ethylene stimulated respiration could be observed, as storage time advanced (Fig. 1). The same trend in respiration was found with fruits harvested at time II and III.

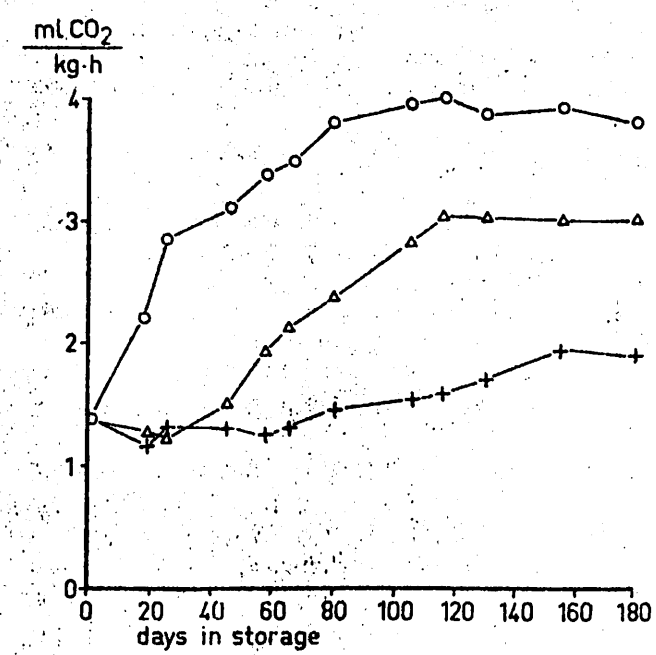

FIG. 1. — CO_2-release of control (○ —— ○), LPS (+ —— +) and LPS, C_2H_4 (△ —— △) fruits during storage.

Ethylene production could only be determined for the control and the LPS fruits, because the ethylene content added to LPS,C_2H_4 was not constant enough to measure the additional ethylene released by the fruits. As can be observed from figure 2 ethylene production of control fruits rose very quickly after the start of the experiment and exceeded 70 µl/kg/h after 6 weeks in storage. LPS fruits on the other hand did not surpass 2 µl/kg/h, which indicates that they did not reach the stage of autocatalytic ethylene production.

Under hypobaric conditions ethylene had an effect not only on respiration, but also on fruit firmness. Figure 3 shows that after about 7 weeks in storage the LPS,C_2H_4 fruits showed a small but significant drop in fruit firmness when compared to LPS storage. Fruits stored in LPS romained firm for a long time. Those treated with ethylene, however, showed a remarkable decrease in fruit firmness as storage time proceeded and at the end of the experiment pressure readings from LPS,C_2H_4 fruits were similar to those of the control fruits.

Decrease in titratable acidity also was delayed when the fruits were stored at hypobaric conditions (Fig. 4), and here again treatment with ethylene partially neutralized this LPS effect.

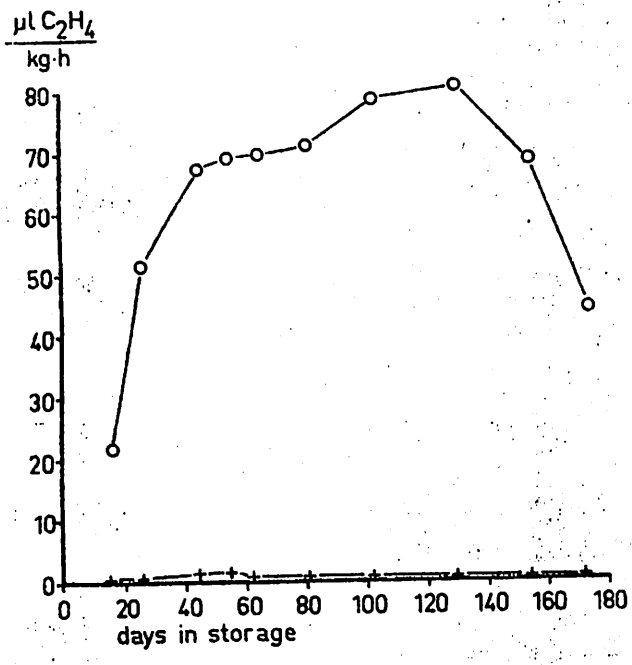

FIG. 2. — Ethylene synthesis of control (O —— O). and LPS (+ —— +) fruits during storage.

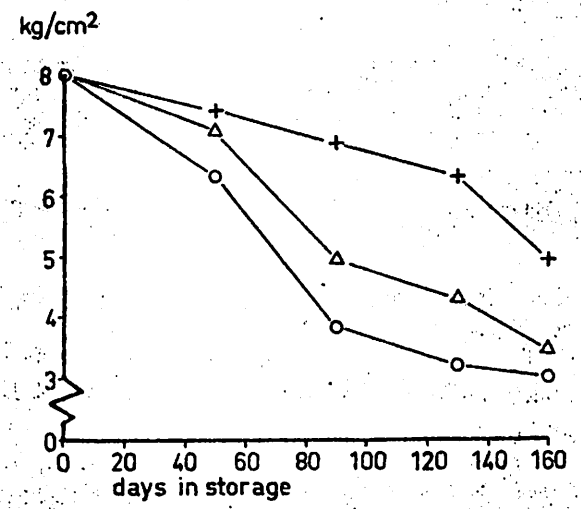

FIG. 3. — Decrease in fruit firmness during storage. Control (O —— O), LPS (+ —— +), LPS, C_2H_4 (Δ —— Δ).

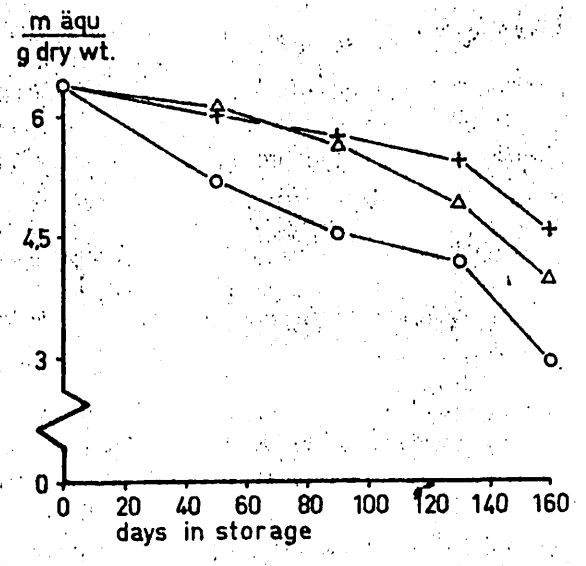

FIG. 4. — Loss of titratable acidity of control (O —— O) LPS (+ —— +), and LPS, C_2H_4 (Δ —— Δ) fruits.

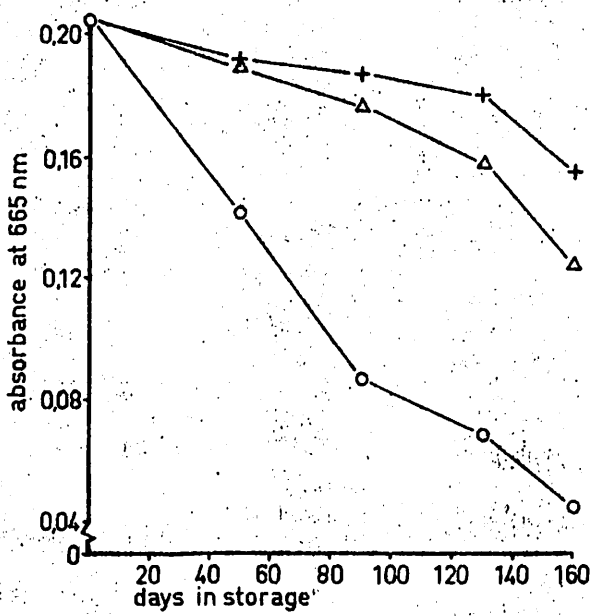

FIG. 5. — Reduction in chlorophyll content of control (O —— O), LPS (+ —— +), and LPS, C_2H_4 (Δ —— Δ) fruits.

In contrast to respiration, acidity and especially fruit firmness, chlorophyll breakdown during storage was only slightly affected by the addition of ethylene (Fig. 5). This was especially true for the first three quarters of the storage period. Only after about three months in storage did the chlorophyll content in ethylene treated fruits drop significantly below the values obtained for LPS fruits.

An increase in *de novo* synthesis of enzymes and/or higher enzyme activities are often made responsible for the observed increase in respiration and for the acceleration of other ripening phenomena of stored fruits. As could be shown by a number of investigators (e. g. FRENKEL et al., 1968; DILLEY, 1970; HULME et al., 1971) malic enzyme is specifically involved in the CO_2 production of pome fruits during ripening. Another enzyme (or groupe of enzymes) which cannot be correlated with certain ripening phenomena but nevertheless is involved in many metabolic processes is acid phosphatase. RHODES and WOOLTORTON (1967) detected an increase in the activity of an acid phosphatase during the climacteric of apple fruits. We therefore determined, in fruits stored for three months, the activities of these specific and nonspecific enzymes, in order to investigate whether the observed effects of ethylene on fruit ripening could be explaned by a higher activity of certain enzymes. Table I shows that in fruits treated with ethylene the activities for malic enzyme as well as for acid phosphatase are indeed much higher than in LPS fruits.

As early as in 1928 BLACKMAN and PARIJA discussed the possibility that changes in permeability may be involved in fruit ripening. Until recently this subject was a matter of controversy (for a complete treatise see SACHER 1973). The same is true for the possibility that ethylene may exert part of its effect on fruit ripening via an influence on membrane permeability. BURG (1968) and ABELES (1972) in discussing this problem came to the conclusion that there is no evidence to justify an effect of ethylene on permeability.

To see whether ethylene had influenced membrane permeability under the conditions of hypobaric storage, we measured the half maximal conductivity in the isotonic bathing solution of tissue disks. Table II shows that ethylene treatment had a very remarkable effect on membrane permeability. Half maximal conductivity was reached after about 3 hours in tissue disks from ethylene treated fruits, respectively, however, after 8,5 hours in disks from LPS fruits (sampling time : 15.12.1973). The same situation was noticed in fruits of harvest time II and III. The fact that 1/2 maximal conductivity was reached earlier in LPS, C_2H_4 — than in control fruits, in spite of their retarded ripening stage, was surprising. A possible explanation for this is that these fruits were treated from the beginning with high ethylene concentrations. Autocatalytic ethylene production of the control fruits in the first part of the storage period was, however, possibly not high enough to induce the same effect. For some reasons ion leakage seems to decline after prolonged storage in overripe fruits (see also LEWIS and MARTIN, 1969) and values obtained at this time (5.2.1974) are therefore confusing. In spite of the lack of evidence for an ethylene effect on membranes (see above) our results indicate that such an effect possibly exists. This would be in agreement with recent experiments on other plant material where a change in membrane permeability after ethylene treatment was observed (STACEWICZ-SAPUNCAKIS et al, 1973; BANGERTH, 1974). As in the enzyme activity experiments, however, exact time course experiments will be necessary for a more complete understanding of the significance of membrane changes in fruit ripening.

TABLE I

The activity of malic enzyme and acid phosphatase prepared from fruits stored for 3 months

	Enzyme units/100 mg aceton powder*	
	ME	acid phosphatase
Control	7 200	450
LPS	4 100	240
LPS, C_2H_4	6 080	360

* One enzyme unit represents that amount of enzyme giving a change in optical density of 0,001/min.

TABLE II

Changes in permeability during storage

Days in storage	Time (h) to reach half maximal conductivity		
	Control	LPS	LPS, C_2H_4
0	14,3	..	..
41	8,1	11,2	7,2
72	4,2	8,7	2,9
101	3,2	4,8	3,1
130	6,0	3,2	3,5

3. Discussion.

The results obtained during the course of these experiments clearly show that ethylene can accelerate ripening of apples even at low temperatures and low partial pressures of oxygen. However, not all ripening parameters investigated seem to be affected to the same degree by an ethylene treatment. Whereas fruit firmness was obviously very responsive to 500 ppm of ethylene under these conditions, chlorophyll breakdown showed little response until the last part of

the storage period. This is in accordance with results obtained by other invcstigators. PRATT and GOESCHEL (1969) reported different maximum and minimum ethylene requirements for respiration, pigment changes and softening of tissue in « Honey Dew » melons and WANG et al. (1972) found a lower ethylene requirement for fruit softening than for respiratory activity.

The fact that ethylene can react at low temperatures and low O_2-tensions does not mean that fruits will synthesize autocatalitic ethylene under these conditions at a rate high enough to promote fruit ripening. Several investigations have indeed shown that this is not the case for apples picked in the preclimacteric stage (see e. g. MEIGH and REYNOLD, 1969; HULME et al., 1971; SFAKIOTAKIS and DILLEY, 1973) and our figure (Fig. 2) supports their results. Whether it will be possible to pick all fruits in this stage and to store them immediately in CA, however, is another question.

Judged from the progress in ripening the storage pressure of 100 Torr was not low enough to keep the fruits in the desired stage of maturity. We used 100 Torr because our method to measure CO_2 release was limited to this pressure (BANGERTH, 1973). In a parallel experiment, fruits from harvest time I were stored at 50 Torr. After 5 months in storage no marked drop in fruit firmness nor in chlorophyll content could be observed in these fruits. Membrane permeability, as judged from conductivity measurements, was especially low (1/2 max conductivity in about 12 hours) and so were the activities for malic enzyme and acid phosphatase.

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Discussion

M. Marcellin. — Dans les expériences hypobares, l'action de C_2H_4 endogène n'est-elle pas facilitée par l'accroissement de la perméabilité des pores du fruit, celle-ci étant très largement accrue, comme on le sait, à très basse pression ?

M. Bangerth. — It is well known that the diffusion of gases, including ethylene, out of the fruit and into the fruit is increased with the reduction of the total pressure. The relation is almost proportional.

M. Bruinsma. — I wonder why you applied a concentration of 500 ppm of ethylene which is unusually high to exert a hormonal effect. This might lead to abnormal effects in, for example, permeability experiments.

M. Bangerth. — The C_2H_4 concentration used was not high, compared e.g. with the concentration in a CA storage. At low temperatures and low pO_2 high C_2H_4 concentrations are necessary to observe an effect, and we used 500 ppm just to be on the safe side. As far I know no toxic effects of high C_2H_4 concentrations could be observed on fruits.