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MODIFIED ATMOSPHERE PACKAGING OF APPLE FRUIT FOLLOWING CONTROLLED ATMOSPHERE STORAGE

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MODIFIED ATMOSPHERE PACKAGING OF APPLE FRUIT FOLLOWING CONTROLLED ATMOSPHERE STORAGE.

By

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ABSTRACT OF A DISSERTATION

By

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Retardation of rapid deterioration of apple fruit following controlled atmosphere storage was achieved by gas atmosphere modification. At warm temperature (20°C), O_2 at 3% and 1.5% significantly reduced fruit softening and weight loss compared to fruits held in air. This beneficial effect is valuable for apple fruit stored under these conditions immediately out of CA or after exposure to low temperature in air for several days. Fruit at 3% O_2 had a volatile compounds profile similar to that of fruit ripened normally in air. Fruit kept below 3% O_2 for prolonged periods of up to 3 weeks exhibited a profile of volatiles symptomatic of alcoholic fermentation.

Modeling studies showed that both the empirical and mathematical approach were applicable in developing modified atmosphere packaging (MAP) guidelines for apple fruit. Both methods yielded similar results for predicting fruit weight or film characteristics for desired oxygen concentration at steady state.

The quality of apple fruit from MAP was affected by the gas steady-state attained in the package. Packages comprised of less permeable film contained very low concentrations of O_2 and relatively high concentrations of CO_2. These conditions were found to be associated with dull and yellow color of fruit and ethanol production when CO_2 within the packages was not scrubbed. Packages composed of films with an intermediate permeability to O_2 tended to retain flesh

firmness, reduce weight loss and keep good appearance and good organoleptic quality of fruit. Volatiles produced by fruit from MAP were similar in type and relative magnitude to those produced by fruit transferred immediately to air after CA storage. Differences in the pattern of volatiles produced by the fruit between those in less permeable packages and those kept in air may be attributed to low oxygen and relatively high CO_2 established in the packages which delay normal ripening.

TO MY WIFE FATIMA FOR HER LOVE, SACRIFICES AND CONTINUOUS HELP THROUGHOUT.....

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Guidance Committee

This disseration was written in accordance with departmental and university regulations. The dissertation body is divided into three sections. All sections are written in the style of the Journal of the American Society for Horticultural Science.

SEARCH FOR KNOWLEDGE AS FAR AS IN CHINA

Muslim Hadith

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LITERATURE REVIEW

The benefits of controlled atmosphere storage of fruit and vegetables have been recognized since the classic studies of Kidd and West (1932) in England. The concept of controlled atmosphere storage is to retard the rate of fruit ripening by limiting substrate availability and through product inhibition by reducing oxygen availability for respiration and increasing carbon dioxide concentration in the atmosphere surrounding the fruit. This is normally done while the fruits are under refrigeration. Gas storage of apples, as it was conceived and termed by the English researchers, grew out of attempts to prolong the period of marketability of English grown apples and those transported by ship from abroad beyond the duration achievable by refrigerated storage in air. Moreover, the storage duration of certain cultivars was limited by the occurrence of physiological disorders which were aggravated at storage temperatures of 0°C. When these cultivars were stored in air at 3 to 4°C to avoid this form of chilling injury, fruit ripening progressed more rapidly than when stored at 0 to 1°C. This led Kidd and West to investigate gas atmosphere modification as a means to delay ripening. While much has been learned from storage and fruit physiology studies during the past 50 years, the effects of controlled atmosphere on fruit metabolism are still not fully understood (Fidler and North, 1969; Kader, 1985).

The successful development of gas storage for apples in England quickly led to its application for pears and spawned studies with other fruits as well (Kidd and West, 1932). At present, CA storage facilities are widespread in most important apple and pear producing areas in the world. Although they are not grown locally, some cultivars of apples are found in markets all year round. Despite the widespread benefits of extending shelf life and preserving quality, the use of CA is still mainly limited to apples and pears. The reasons are partly due to economic factors, such as construction and operating costs and, partly due to limitation of knowledge and technology to extend the application of CA to other

crops. Additionally, certain commodities do not lend themselves to CA storage due to physiological limitations.

In general apple fruit were stored under 2 to 3% O_2 and 3 to 5% CO_2 at 0° to 3°C according to cultivar requirements of temperature. In the past 10 years there has been a world-wide trend toward the use of lower oxygen concentrations to further increase the length of the storage season of apples and pears. Concentrations of oxygen of 1 to 2% (Lange, 1985; Lange and Fica, 1985; Lau, 1985; Little and Tugwell, 1985; Dilley, 1987; 1990; Sharples and Stow, 1982; Truter and Eksteen, 1987) are now commonly employed. Despite the fact of prolonging storage life, maintaining appearance and keeping flesh firmness of apples at acceptable levels for long durations, it is commonly reported that apples are often subject to rapid deterioration and loss in quality after removal from CA storage. The degree and rapidity of poststorage quality deterioration are related to the cultivar, storage conditions and duration of storage.

The problem of accelerated deterioration of quality after storage can be remedied by extending the period of atmosphere modification. Interest is growing in the use of packaging technology to modify the fruit's environment during the poststorage period. The object is to control levels of oxygen, carbon dioxide, humidity and in some cases, ethylene, which is a biologically active gaseous plant hormone that promotes fruit ripening.

Theoretically, a modified or controlled atmosphere environment can be created and maintained within packaged units by using plastic films of various thicknesses and gas permeability. Reliably achieving and maintaining a desired atmosphere is more readily accomplished if the product is maintained at a desirable and constant temperature. However, temperature is often not well controlled during distribution of the product to market after storage. Therefore, an optimal package system must be designed to function satisfactorily over a fairly

wide temperature range (Kader, 1985).

The package unit is a dynamic system and product quality is dependent upon fruit respiration and film permeation. Respiration and permeation will establish a steady state between the gases in the package and the surrounding environment. Using a package in the fashion is termed modified atmosphere packaging (MAP); The film package also provides protection against moisture loss and maintains product appearance (Bussel and Kenigsberger, 1975; Sonsino, 1986). Two of the most important considerations in MAP are the build-up of CO_2 and depletion of O_2. When these exceed product tolerance, off-flavors, discoloration, textural changes, increased decay, and general deterioration of the commodity can occur. Humidity in the package is also an important factor as high humidity allows moisture condensation as temperature fluctuates and this favors decay development for some commodities (Zagory and Kader, 1988).

Several attempts have been made to describe the relationship between O_2 and CO_2 concentrations within the package, film characteristics, and physiology of the commodity. Until recently, the approach has been mostly empirical for different commodities such as apples (Hardenberg and Anderson, 1966, Tolle, 1962, 1971, Jurin and Karel, 1963, Smith et al., 1987), pears (Deily and Rizvi, 1981), strawberries (Saguy and Mannheim, 1973), bananas (Karel and Go, 1963; Daun et al., 1973), oranges (Ben-Yehoshua, 1985; Ben-Yehoshua and Shapiro, 1986), tomatoes (Cameron et al., 1989, Geeson et al., 1982, Kawada, 1982), and peppers (Bussel and Kingsberger, 1975, Risse et al., 1987). Most of the early investigations stressed the difficulties encountered in obtaining the desired atmospheric conditions within sealed packages. The relationship between O_2 and CO_2 concentrations and film permeabilities have been studied by Tomkins (1962, 1963, 1967). Jurin and Karel (1963) developed a graphical approach to predict gas concentration inside the package at equilibrium. Several authors have

recommended the use of holes in the packages or the use of unsealed packages for storage (Marcellin, 1974; Scott and Tewfik, 1947) but such packages may not give adequate control of moisture loss and limited control of oxygen and carbon dioxide. However, developments in plastic engineering and new packing technology in the past decade have made it possible to design and create a more predictable modified atmosphere for fruits and vegetables in hermetically sealed packages.

Henig (1972) was the first investigator to develop a differential equation to characterize the changes in concentrations of gases within polymeric film packages for produce. Only a few researchers have used a mathematical approach to design and optimize packages (Prince, 1980; Henig and Gilbert, 1975; Jurin and Karel, 1963; Boylan-Pett, 1986; Cameron et al., 1989). Many physiological and biochemical aspects of the response of fruits under CA conditions have been investigated (Kader, 1985; Wolfe, 1985; Zagory and Kader, 1988).

Although many perishables may retain good appearance and condition during CA storage and immediately upon removal at the ideal low temperature, they may undergo rapid loss in quality shortly after transfer to air at warmer temperatures experienced in distribution to market. In other instances, fruits, while they appear sound, may fail to ripen properly and to develop characteristic taste, flavor and aroma following CA storage (Hatfield and Patterson, 1974). Smith et al. (1987) reported that 'Discovery' apples ripened and deteriorated rapidly even after short-term CA storage. Bangerth, (1983) found that 'Golden Delicious' apple fruit stored at low oxygen levels failed to develop full flavor and aroma after removing them from CA storage, although other quality factors were acceptable. Both problems of deterioration and with flavor and aroma development remain largely unresolved (Lidster et al., 1983; El-Hadi et al., 1985).

Research on apple aroma began early in this century. Compounds which

contribute to apple flavor and aroma include alcohols, aldehydes, ketones and esters (White, 1950, Huelin, 1952, Meigh, 1957). Although more than 300 compounds have been identified as volatiles produced by apples, some do not contribute to flavor and aroma at the concentrations normally found (Dimick et al., 1983). The use of gas chromatography coupled with mass spectrometery and infrared spectral analysis permitted the identification of components not previously known to affect the aroma of apple wine (Matthews et al., 1962) and apple juice (Macgregor et al., 1964). In 'Golden Delicious' apple, Flath et al. (1969) identified and organoleptically evaluated hexanol, trans-2-hexenal and ethyl-2-methylbutyrate. The concentration of trans-2-hexenal was correlated with the quality of apple essence (Koch and Schiller, 1964). Drawert et al. (1969) reported that this compound did not occur in significant concentration in whole apples but was formed rapidly during crushing. Williams and Knee (1977) identified 4 methoxyallyl-benzene in all the cultivars they tested, and this compound contributed to aroma. The compound was produced biosynthetically from sugar via shikimic acid and from phenylalanine (Dimick et al., 1983). The authors concluded that apple flavor requires the presence of esters with molecular weights of 100 and 130.

Guadagni et al. (1971) studied changes in the volatiles produced by apples in cold storage and found that most of the increase in volatiles during storage consisted of esters such as ethyl acetate, ethyl propionate, ethyl-2-methyl propionate, 2 methyl propyl acetate, ethyl butyrate, butyl acetate, pentyl acetate, ethyl-2-methyl butyrate, and butyl propionate. Moreover, the apple fruit skin was the main source of these compounds and maximum production rate was associated with the climacteric phase of ripening. Dirink et al. (1984a,b) found that during ripening of 'Golden Delicious' apple fruit at room temperature total esters increased up to 14 days then declined, but the level remained higher than

in fruit freshly harvested.

Williams and Knee (1977) reported that low ester production of controlled atmosphere fruit was due to a lack of precursors and a low level of esterifying enzymes. High esterase activity and/ or high diffusion rates of esters may contribute in the reduction of esters (Knee and Hatfield, 1981). Ester formation in the fruit is derived largely from acids and alcohols (Knee and Hatfield, 1981, Yamashita et al., 1977). Huelin (1952) working with 'Granny Smith' apples, considered aldehydes to be intermediates in the conversion of alcohols to acids. Low molecular weight aldehydes, ketones, alcohols and acids are derived from the oxidation of unsaturated fatty acids. Apples treated with acetic, propionic, butanoic and di-carboxylic acids convert these compounds into esters or alcohols by beta-oxidation (Dirink et al., 1984a; Knee and Hatfield, 1981).

The rate of volatile production is directly affected by storage temperature (Kidd and West, 1939). Greevers and Doesburg (1965) reported that fruits stored either at 10 or 15 °C produced more volatiles than fruits kept at 3 °C.

The maturity of the fruit at harvest plays a major role in flavor development. Cheraghi (1988) observed that immature or overipe apples did not develop full flavor and attributed this to lack of ripening enzymes in immature fruits and to senescence processes in fruits harvested too ripe. Brown et al. (1966) showed that fruits picked early produced lower levels of non-ethylenic volatiles than those harvested late.

Fidler and North (1969) observed that increasing the concentration of CO_2 and lowering O_2 in the atmosphere surrounding apple fruit reduced the rate of volatile production and this was attributed to respiration rate reduction. Low oxygen concentration in CA storage had a greater effect than the CO_2 level on respiration rate reduction (Patterson et al., 1974). Similar results were obtained by Lidster et al. (1983) using 1 % and 1.5 % O_2 atmosphere. They concluded

that shortage of oxygen in the atmosphere surrounding the fruits resulted in reduction of aroma due to lack of substrate or inhibition of enzymes. Knee and Hatfield (1981) attributed low ester production under low O_2 concentrations to decreased levels of alcohols which serve as precursors of esters and aldehydes in apples. The same authors (1976) found that apple fruits stored under 1 % and 2 % O_2 at 3.5 °C for 12 months developed less aroma components than those stored in air even after transferring the CA stored fruit to ambient air. El-Hadi et al. (1985), however, reported that 'McIntosh' apple fruit from long term CA storage gained ability to produce the flavor components after 2 to 3 weeks at 20 °C. These investigators also indicated that not all of the compounds studied were affected similarly by CA. Similar findings were reported by Lidster et al. (1985) for 'McIntosh' apples. The time required for fruits to regenerate flavor following CA storage depended on the temperature at which they were held and the length of the storage period (Hatfield and Patterson, 1974). In other studies, apple fruit ripened for 7 and 14 days at 21 °C were of good flavor after 7 days and poor flavor after 14 days (Brown et al., 1966).

Several methods and techniques for extraction and identification of volatiles have been developed. Dynamic headspace, static headspace and non odor volatile analysis (N.O.V.A) were described as effective techniques to study flavor (Liu, 1985; Dimick et al., 1983; Cheraghi, 1988). Despite numerous articles published through the 1980's on apple flavor components, the science of flavor development is still poorly understood.

Hexanal, trans-2-hexenal and ethyl 2-methylbutyrate are thought to be the most important apple aroma constituents (Flath et al., 1969). Use of the headspace technique indicated that 4-methoxyallyl benzene was also a good contributor to characteristic flavor (Mannito et al., 1974). Cheraghi (1988) reported that several compounds identified by headspace analysis of 'Empire'

apple fruit appeared to have a significant effect on flavor. The author reported 24 esters, 7 aldehydes, 13 alcohols, 2 ketones and 5 other miscellaneous compounds (Table 1). Ethyl propionate, methyl butanoate, ethyl butanoate, methyl-2-methylbutyrate, ethyl-2-methyl butanoate, propyl acetate, ethyl acetate, butyl acetate, pentyl acetate, butanal, 2-methyl butanol, hexanal, butanol, hexanol and 2-pentanol were major constituents of flavor.

The objective of the present study was to develop a model package for Empire and McIntosh apple fruits to maintain and preserve quality for a simulated marketing period for fruits following CA storage. Other purposes of the study were to relate the length of storage time to the equilibrium gas concentration inside the package and to fruit firmness retention, weight loss and apple flavor changes during MAP storage.

Table 1. Aroma compounds identified using the dynamic headspace method for 'Empire' apple fruit (Chiraghi, 1988).

ESTERS	ALDEHYDES	ALCOHOLS
Methyl acetate	2-Methyl butanal	1-Butanol
Ethyl acetate	2-Hexanal	2-Methyl-1-butanol
Propyl acetate	Pentanal	3-Methyl-1-butanol
Butyl acetate	Isopentanal	2-Methyl-1-propanol
Pentyl acetate	Phenyl acetaldehyde	1-Pentanol
Hexyl acetate	Butanal	2-Pentanol
Isopentyl acetate	Heptanal	3-Pentanol
Ethyl phenyl acetate		2-Methyl-1-pentanol
Ethyl propinoate		1-Hexanol
Methyl butanoate		2-Hexanol
Ethyl butanoate		3-Hexanol
Methyl-2-methyl butanoate		(Z)-3-Hexen-1-ol
Ethyl-2-methyl butanoate		(E)-3-Hexen-1-ol
Propyl butanoate		
Butyl butanoate		
3-Methylbutyl butanoate		KETONES
2-Methylbutyl-2-methyl butanoate		
Ethyl formate		2-Pentanone
Butyl formate		3-Heptanone
Methyl pentanoate		
Ethyl-2-methyl pentanoate		
2-Methylbutyl pentanoate		
Ethyl hexanoate		
Butyl hexanoate		

LITERATURE CITED

1. Ben-Yehoshua, S. 1985. Individual seal-packaging of fruit and vegetables in plastic film. A new postharvest technique. HortScience 20:32-37

2. Ben-Yehoshua, S. and S. Shapiro. 1986. Effects of shrink seal-packaging with cross-linkedpolyolefin films on keeping quality of various citrus fruits. J. of Plastic, Film and Sheeting 2:255-257.

3. Boylan-Pett, W. 1986. Modified Atmosphere Packaging for Tomato. MS Thesis, Michigan State Univ., East Lansing, MI, U.S.A.

4. Bussel, J. and Z. Kenigsberger. 1975. Packaging Green Bell peppers in selected permeability films. J. Food Sci. 40:1300-1303

5. Cameron, A.C, W. Boylan-Pett, and J.J. Lee. 1989. Design of Modified Atmosphere Packaging Systems: modeling oxygen concentrations within sealed packages of tomato fruits. J. Food Sci., 54(6):1413-1416

6. Chiraghi, T.S. 1988. Effect of harvest date and ethylene concentration in controlled atmosphere storage on the quality of Empire apples. PhD Dissertation, Dept. of Food Science and Human Nutrition, Mich. State Univ., East Lansing, MI, U.S.A.

7. Daun, H., S.G. Gilbert, Y. Ashkenazi, and Y. Henig. 1973. Storage quality of bananas packaged in selected permeability films. J. Food Sci. 38:1247-1250.

8. Deily, K.R. and S.S.H. Rizvi. 1981. Optimization of parameters for packaging of fresh peaches in polymeric films. J. Food Process Engineering 5(1):23-41.

9. Dilley, D.R. 1987. Recommended storage conditions for Michigan apples. Postharvest Laboratory Dept. Hort., Michigan State, East Lansing, M.I., U.S.A.

10. Dilley, D.R. 1990. Application of air separator technology for controlled atmosphere storage of apples. Ed. J. Fellman. Proceedings of the 5th Controlled Atmosphere Storage Research Conference. Wenatchee, WA. June 14-15, 1:409-418.

11. Dimick, P.S., J.C. Hoskin, and T.E. Acree. 1983. Review of apple flavor-state of the art. CRC Crit. Rev. Food Sci. Nutr. 181:387-394.

12. Dirink, P., H. De Pooter, G. Willaert, and N. Schamp. 1984a. Application of a dynamic headspace procedure in fruit flavor analysis. In: Analysis of volatiles methods applications, P. Schreier (Ed), p. 381. W. de Gryter, New York.

13. Dirink, P.J., A.H. Kutom, and N.M. Schamp. 1984b. Quantitative dynamic headspace analysis for objective measurement of rancidity in oil. In: Progress in flavor research. J. Adda (Ed). p. 505. Elsevier Scientific Publishing Co., Amsterdam, The Netherlands.

14. Drawret, F., W. Heimann, R. Emberger, and R. Tressel. 1969. Gas chromatographic investigation of vegetable aroma. II. Concentration, separation and identification of apple flavor components. J. Chromatogr. 2:57-61

15. El-Hadi, Y., F. W. Liu, T.E. Acree, and R. Butts. 1985. Odor active volatiles in McIntosh apples stored in simulated low ethylene controlled atmosphere. Proceedeings of the Fourth National Controlled Atmosphere Conference. North Carolina State Univ. Raleigh pp. 70-81

16. Fidler, J.C. and C.J. North. 1969. Production of volatile organic compounds by apples. J. Sci. Fd. Agric. 20:521-524.

17. Flath, R.A., D.A. Black, D.G. Guadagni, W.H. McFadden, and T.H. Schultz. 1967. Identification and organoleptic evaluation of compounds in apple essence. J. Agric. Fd.Chem.15:29-35.

18. Flath, R.A., D.A. Black, R.R. Forrey, G.M. McDonald, T.R. Mon, and R. Teranishi. 1969. Volatiles in Gravenstein apple essence identified by GC-MS. J. Chromatogr. Sci. 7:508-512.

19. Geeson, J.D., K.M. Browne, K.Maddison, J. Shephered, F.Guaraldi. 1982. Modified atmosphere packaging to extend the shelf life of tomatoes. J. Food Technol. 1985, 20, 339-349.

19. Greevers, G., and J.J. Doesburg. 1965. Volatiles of apples during storage and ripening. J. Food Sci. 30:412-415.

20. Guadagni, D.G., J.L. Bomben, and J.S. Hudson. 1971. Factors influencing the development of aroma in apple peel. J. Sci. Fd. Agric. 22:110-114.

21. Hardenberg, R.E and R.E. Anderson. 1966. Polyethylene box liners for storage of Golden Delicious apples. USDA Mktg. Res. Rpt. No 461 A.R.S. U.S.D.A. 35p.

22. Hatfield, S.G., and B.D. Patterson. 1974. Abnormal volatile production by apples during ripening after controlled atmosphere storage. Colloques Internationaux CNRS (Paris), 238:57-62.

23. Henig, Y.S., and S.G. Gilbert. 1975. Computer analysis of the variables affecting respiration and quality of produce packaged in polymeric films. J. Food Sci. 40:1033-1035.

24. Huelin, F.E. 1952. Volatile products of apples. III. Identification of aldehydes and ketones. Aust. J. Sci. Res. B. 5:328-331.

25. Jurin, V. and M. Karel. 1963. Studies on control of respiration of McIntosh apples by packaging methods. Food Tech. 17(6):104-108.

26. Kader, A.A. 1985. Modified atmosphere and low pressure systems during transport and storage. In: A.A. Kader (ed) Postharvest technology of horticultural crops. Univ. Calif. Div. Agric. and Nat. Res. Publi. 3311:58-64.

27. Karel, F., and C. Go. 1964. Control of respiratory gas. Mod. Pack. 37(6):123-125.

28. Kawada, K. 1982. Use of polymeric films to extend postharvest life and improve marketability of fruits and vegetables-Unipack: Individually wrapped storage of tomatoes, oriental persimons and grapefruit. In" Controlled Atmospheres for the Storage and Transport of perishable Agricultural Commodities" ed. D.G. Richardson and M, Meheriuk, p.87. Timber Press, Beaverton, Oreg.

29. Kidd, F., and C. West. 1932. Gas storage of tomatoes. Gt. Brit. Dept. Sci. Indus. Res. Food Invest. Bd. Rept. 209-211.

30. Kidd, F., and C. West. 1939. The production of volatiles by apples: effects of temperature, maturity, technique of estimation, etc. Rept. Food Invest. Bd (Gt. Brit.). p.136.

31. Knee, M., and S.G. Hatfield. 1981. The metabolism of alcohols by apple fruit tissue. J. Sci. Food Agric. 32:593-600.

32. Koch, J. and H. Schiller. 1964. Beitrag zur kenntnis des aplfelaromas. Z. Lebensm. Unters. Forsch. 125:364-368.

33. Lange, E. 1985. Recent advances in low O_2 and low ethylene for apples in Poland. Proceedings of the 4th National Controlled Atmosphere Research Conference, North Carolina State Univ., Raleigh, U.S.A. pp. 295-307.

34. Lange, E. and J. Fica. 1982. Storage of Spartan, Melrose and Idared apples in ultra-low oxygen controlled atmospheres. Fruit Sci. Rpt (Skierniewce, Poland) 9:123.

35. Lau, O.L. 1985. Storage responses of four apples cultivars to low O_2 atmospheres. Proceedings of the fourth National Controlled Atmosphere Research Conference, North Carolina State Univ. Raleigh, U.S.A. p. 43-56.

36. Lee, J.L. 1986. The design of controlled or modified packaging systems for fresh produce. School of Packaging, Mich. State Univ., MI, U.S.A.

37. Lidster, P.D., H.J. Lightfoot, and K.B. McRae. 1983. Production and regeneration of principal volatiles in apples stored in modified atmosphere and air. J. Food Sci. 48:400-402.

38. Lidster, P.D., K.B. McRae, and E.M. Johnson. 1985. Retention of apple quality in low oxygen storage followed by standard controlled atmosphere regimes. J. Amer. Soc. Hort. Sci. 110:755-759.

39. Little, C.R. and B.L. Tugwell. 1985. Storage construction for effective ultra-low oxygen storage in Australia. Proceedings of the fourth National Controlled Atmosphere Research Conference, North Carolina State Univ. Raleigh pp: 281-289.

40. Liu, F.W. 1985. Low ethylene controlled atmosphere storage of McIntosh apples. In: Ethylene and plant development. T.A. Roberts and G.A. Tucker (Ed), p. 385. Butterworths, London.

41. Marcellin, P. 1974. Conservation des fruits et legumes en atmosphere controlée, à l'aide de membranes de polymeres. Revue Gen. Froid 3:217-223.

42. Matthews, J.S., H. Sugisawa, and D.R. McGregor. 1962. The flavor spectrum of apple-wine volatiles. J. Food Sci. 27:355-357.

43. McGregor, D.R., H. Sugisawa, and J.S. Matthews. 1964. Apple juice volatiles. J. Food Sci. 29:448-451.

44. Meigh, D.F. 1957. Volatile compounds produced by apples. II. Alcohols and esters. J. Sci. Food. Agric. 8:313-317.

45. Patterson, B.D., S.G. Hatfield, and M. Knee 1974. Residual effects of controlled atmosphere storage on the production of volatile compounds by two varieties of apples. J. Sci. Food. Agric. 25:843-848.

46. Prince, T.A. 1980. Design and function of a modified atmosphere package for precooled tulip bulbs. PhD Dissertation, Mich. State Univ., East Lansing, MI, U.S.A.

47. Risse, L.A., D. Chen, and W.R. Miller. 1987. Chilling injury of film-wrapped and conditioned Bell peppers during cold storage. Trop. Sci 27:85-90

48. Saguy, I. and C.H. Mannheim. 1973. Prolonging shelf life of strawberries by packaging in selected plastic films. Bull. Inter. Inst. Refrig. Annexe 1973-3: 149-152.

49. Sharples, R. O. and J.R. Stow. 1982. Recommended conditions for the storage of apples and pears. Report of the East Malling Research Station for 1981,pp.199-202.

50. Scott, L.E., and S. Tewfik. 1947. Atmospheric changes occurring in film-wrapped packages of vegetables and fruits. Proc. Amer. Soc. Hort. Sci. 49:130-136.

51. Smith, S.G., J.Geeson, and J. Stow. 1987. Production of modified atmospheres in deciduous fruits by the use of films and coatings. HortScience 22:772-776.

52. Tolle, W.E. 1962. Film permeability requirements for storage of apples. USDA Tech. Bull. 1257.

53. Tolle, W.E. 1971. Variables affecting film permeability requirements for modified atmosphere storage of apples. USDA Tech. Bull. 1422.

54. Tomkins, R.G. 1962. The conditions produced in film packages by fresh fruits and vegetables and the effect of these conditions on storage life. J. Appl. Bact. 25(2):290-307.

55. Tomkins, R.G. 1963. The effects of temperature, extent of evaporation, and restriction of ventilation on the storage life of tomatoes. J. Hort. Sci. 38:335-347.

56. Tomkins, R.G. 1967. Assessing suitability of plastic films for prepackaging fruit and vegetables. Food Manufacture 42:34-38

57. Truter, G.J., and G.J. Eksteen. 1987. Storage potential of apples under controlled atmosphere storage at ultra-low oxygen concentrations. Sagtevrugteboer (South Africa), pp. 141-145.

58. White, J.W. 1950. Composition of a volatiles fraction of apples. Food Res. 15:68-72

59. Williams, A.A., and M. Knee. 1977. The flavor of Cox's Orange Pippin apples and its variation with storage. Pro. Assoc. Appl. Biol. 87:127-131.

60. Wolfe, F.H. 1983. Modified atmosphere packaging and storage of perishable foods. Overview of theory and basics. Agriculture and forestry bulletin vol. 7(2):40-42.

61. Yamashita, I., V. Nemoto, and S. Yoshikawa. 1977. Studies on flavor development in strawberries. 4. Biosynthesis of volatile alcohol and esters from aldehyde during ripening. J. Agric. Fd. Chem. 25:1165-1174.

62. Zagory, D. and A.A. Kader. 1988. Modified Atmosphere Packaging of Fresh produce. Food Technology 42(9):70-74.

SECTION I

LOW OXYGEN STORAGE OF 'EMPIRE' AND 'McINTOSH' APPLE FRUIT AT WARM TEMPERATURE FOLLOWING CONTROLLED ATMOSPHERE STORAGE

INTRODUCTION

Successful storage of several apple (*Malus domestica* Borkh.) cultivars at low oxygen (LO) and ultra low oxygen (ULO) concentrations has been demonstrated (Lange and Fica, 1982; Lau, 1983, 1985). Although fruits stored under these conditions were reported to be sensitive to internal browning and other physiological disorders, some researchers reported that LO storage followed by standard CA conditions improved quality of apples (Lidster et al., 1985). Other researchers have advocated intermittent warming during CA in order to circumvent disorders caused by metabolic imbalances and chilling injury (Wade, 1979).

Smith et al. (1987) reported that modified atmosphere packaging (MAP) at 20°C improved the storage life and preserved quality of 'Discovery' apples pre-stored under short-term CA conditions. The CO_2 and O_2 concentrations obtained in the packages made from 25 and 35 μm low density polyethlene (LDPE) films were (3-5% CO_2 and 5-6% O_2). Lower concentrations of both O_2 and CO_2 were not tried at warm or ambient temperatures.

The objective of this study was to determine the effect of low concentrations of oxygen on ripening and quality of 'Empire' and 'McIntosh' apples from CA storage when they were subsequently stored at various O_2 levels at 20°C. This was to simulate the O2 levels that may occur in packages during the marketing period. Fruit ripening was evaluated by flesh firmness retention and fruit quality was assessed organoleptically and by physiological disorder incidence. Flavor and aroma development was assessed by analysis of the volatiles produced by the fruit in air at 20°C after various durations at different O_2 levels.

MATERIALS AND METHODS

'Empire' and 'McIntosh' apples were harvested from commercial orchards near Grand Rapids, Michigan. Maturity parameters evaluated included the starch index, flesh firmness and internal ethylene. The bulk of the fruit of each cultivar were judged to be fully mature yet preclimacteric with respect to internal ethylene levels at the time of harvest (.07 to .37 $\mu l.l^{-1}$). Flesh firmness (lbs) was measured with an Effigi penetrometer using a 10 mm diameter tip. The flesh firmness values were then converted to Newtons (N). Unless otherwise noted, 10 fruits were employed per replicate with a single measure of firmness made on the pared surface of each fruit. The fruits were stored at the Michigan State University Horticultural Experiment Station at Clarksville under CA conditions of 1.5% O_2, 1.8% CO_2 and 95% relative humidity at a storage temperature of 1°C and 3°C, respectively, for 'Empire' and 'McIntosh'. Storage conditions were established within 4 days of harvest and maintained using a Prism Alpha nitrogen gas generating system from Permea Inc., Monsanto Co., St. Louis, Missouri. This system employs hollow-fiber membrane gas separator technology to remove O_2 from air, purifying the remaining N_2 (Dilley, 1987, 1990). Fruit were stored in CA for 4 months. These CA storage conditions arrested ripening development during storage as judged by the fact that virtually no decrease was observed in flesh firmness from harvest.

Respiration Rate Studies. The respiration rate of the apples was determined at 20°C in air and at two low levels of O_2. Three gas mixtures were employed: air (21% O_2 in N_2; 1.5% O_2 in N_2; and 3% O_2 in N_2. Gas mixtures were prepared by blending the respective gases from high pressure sources of pure gases with the aid of flowmeters and a mixing chamber. These experiments were done with 'Empire' and 'McIntosh' apples stored at 1°C in air for different

periods after removal from CA. For each treatment, 12 wide mouth jars (ca. 2 L in capacity) containing 5 apples of ca. 130 g each were employed for each of the three gas mixtures. The jars were tightly sealed with a screw lid equipped with inlet and outlet ports. The inlet was connected to a capillary flow board providing the respective gas mixture and the outlet was vented to the room. The concentration of O_2 at the inlet and outlet ports to the jars was monitored with a portable Servomex oxygen analyser, Model 570A from Servomex Ltd., Sussex England. The CO_2 concentration was monitored at the outlet port using a portable infrared ADC gas analyser, Model SB300, from the Analytical Development Company Ltd., England. Gas mixtures were humidified by passing them through water before entering a capillary flow board which distributed the gas mixture to the jars of fruit at a flow rate of 50 to 60 cc/min. The respiration rate study was conducted twice; once upon removing the fruit from CA storage and again after the fruits had been held in air at 1°C for one and two weeks. Respiration rate (CO_2 production) was determined by closing the jars for an interval of 1 hour each day and measuring the CO_2 which accumulated in the headspace. This was done by removing gas samples via a syringe and needle through a septum in the jar lid. Carbon dioxide was measured with an infrared gas analyzer (ADC model SB300) employing N_2 as a carrier gas. In this procedure the gas sample is injected as a pulse into the N_2 carrier gas through a section of latex rubber tubing leading to the detector cell. The infra detector signal was recorded as peak height on a strip chart recorder as the CO_2 (now diluted by the carrier gas) passes through the detector cell. The nitrogen carrier gas flow rate was adjusted to provide a linear output from the IR detector cell that was proportional to the CO_2 in the gas sample or CO_2 standards injected. Carbon dioxide and O_2 in the respirometer jars was also verified by gas chromatography.

The respiration rate studies were conducted for 3 consecutive weeks. Each week, fruit from 4 jars at each O_2 level at 20°C were removed and analyzed for weight loss, flesh firmness and flavor analysis. Texture, flavor and off-flavor development were evaluated organoleptically.

Fruit Volatiles Study: Fruit volatiles were determined using a dynamic headspace sampling technique. Samples were taken for analysis of volatiles production at 20°C within one day of removal of fruits from CA storage and after holding them in air for 7 and 14 days at 1°C. Analyses were also made at weekly intervals after holding fruits at various O_2 levels at 20°C over a three week period. At each sampling fruits (ca. 1 kg) were placed in a 10 l glass desiccator and flushed continuously with air at a flow rate of 50 cc/min at 20°C. The outlet port of the desiccator was fitted with an adsorption tube which consisted of pyrex glass tubing (6 mm dia x 10 cm) filled with 0.3 g of 80/100 mesh Tenax-GC (ANSPEC, Ann Arbor, MI) plugged at both ends with silanized glass wool. A 5 hour adsorption period was employed. After Adsorption the Tenax GC tubes were kept at -20°C until analyzed. The volatiles production studies were conducted at weekly intervals for 3 weeks at 20°C.

The volatiles on the Tenax adsorbant were extracted with isopentane. The solvent (2 ml) was added by a micropipette to the adsorption tubes which were fixed to test tubes with a rubber septum. The tube assembly was centrifuged for 5 min at 1500 g to collect the isopentane extract. The extract was transferred rapidly to a small vial which was closed tightly and stored at -20°C for subsequent GC analyses.

Gas Chromatographic Analysis of Volatiles: Chromatographic analyses were performed with a Hewlett Packard GC Model 5850 A at the MSU School of Packaging. The GC was equipped with a flame ionization detector and a bonded fused silica capillary column (0.25 mm x 60 m) coated with Carbowax 20 M

(Supelco Inc, Bellefonte, PA). The gas carrier (He) flow rate was 28 cc/ min. The temperature program was 30°C for 1 min and followed by a rate of 2°C/min up to 180°C. Sample volumes of 1 μl were injected. Data were recorded on HP digital recorder Model 3392 A. Some of the volatiles (Table 1) were tentatively identified based on retention times of known standards prepared by adding 0.1 μl of the known standard to 1 ml of isopentane.

Data were analyzed by analysis of variance employing the computer statistical package COSTAT. Means were separated by Student-Newman-Keuls test at 5% where appropriate.

These experiments were conducted after 3, 4 and 5 months of controlled atmosphere storage as described above. Only the results after 4 months of CA storage are presented herein as the results were typical of those reported.

RESULTS AND DISCUSSION

'Empire' fruit at harvest time had an internal ethylene concentration ranging from 0.01 to 0.3 $\mu l\ l^{-1}$ with an average flesh firmness of 85 N. 'McIntosh' at harvest time had internal ethylene levels ranging from 0.08 to 0.7 $\mu l\ l^{-1}$ with an average flesh firmness of 70 N. After 4 months in CA storage, the 'Empire' and 'McIntosh' fruit had both retained about 94 % of their flesh firmness values at harvest. No physiological disorders were apparent. The respiration rate of 'Empire' and 'McIntosh' apples after CA storage is shown in Figure 1 indicating that the fruit were still preclimacteric upon removing them from CA storage.

Fruit transferred to 1.5 or 3% O_2 at 20°C within 1 day of removing them from CA retained flesh firmness values significantly higher than those transferred to air at 20°C for 'Empire' (Figure 2) and for 'McIntosh' (Figure 3) after 1 and 2 weeks. This was also true for fruit held in air at 1°C for 7 or 14 days after removing them from CA beginning after 1 week at 20°C. Fruit kept at 1.5% O_2 at 20°C retained firmness better than those kept at 3% O_2 after being held in air for 7 days. This effect was less evident for fruit kept in air at 1°C for 14 days prior to placing them under a low O_2 regime. Fruit kept at 1.5 and 3% O_2 at 20°C retained flesh firmness values after 3 weeks that were about equal to or higher than those of fruits held in air for only 2 weeks at 20°C even when the fruits were first held in air at 1°C for as long as 2 weeks after CA storage.

The flesh softening of fruits transferred to air immediately after CA storage indicates that the development of the physiological and biochemical capacity for cell wall degradation had occurred during storage. This capacity was readily expressed and accelerated when the fruits were supplied with adequate oxygen at a favorable

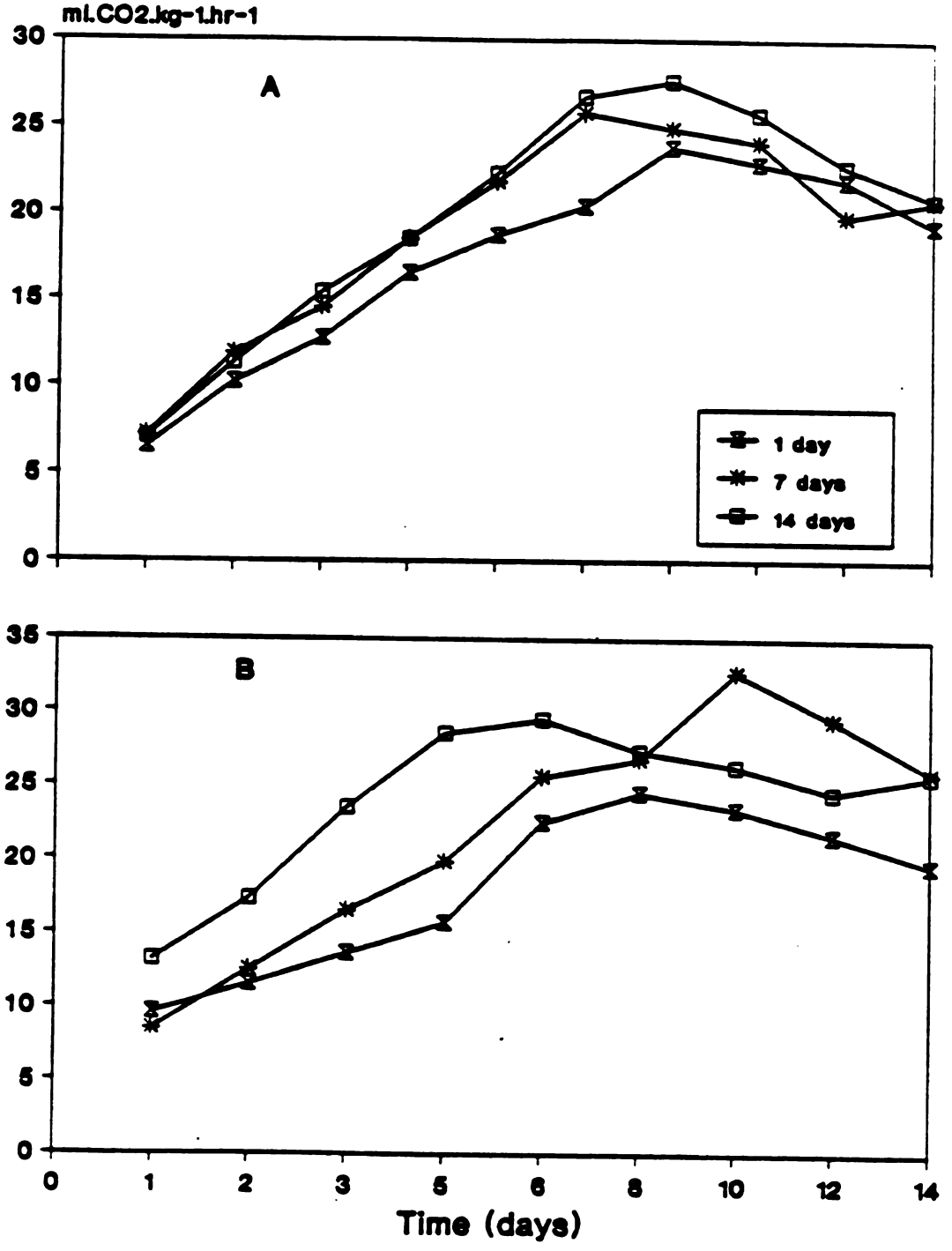


Figure 1. Respiration rate (ml CO_2 kg^{-1} hr^{-1}) of 'Empire' (A) and 'McIntosh' apple fruit at 20°C after various durations in air at 1°C following controlled atmosphere storage.

Figure 2. Change in flesh firmness (N) of 'Empire' apple fruit held at 1.5, 3 and 21 % O_2 at 20°C. Fruits were placed under these regimes after 1 (A), 7 (B) or 14 (C) days at 1°C in air.

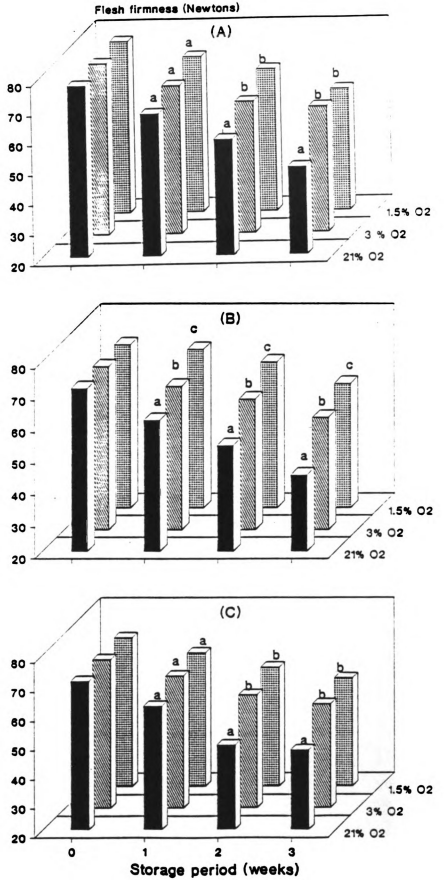
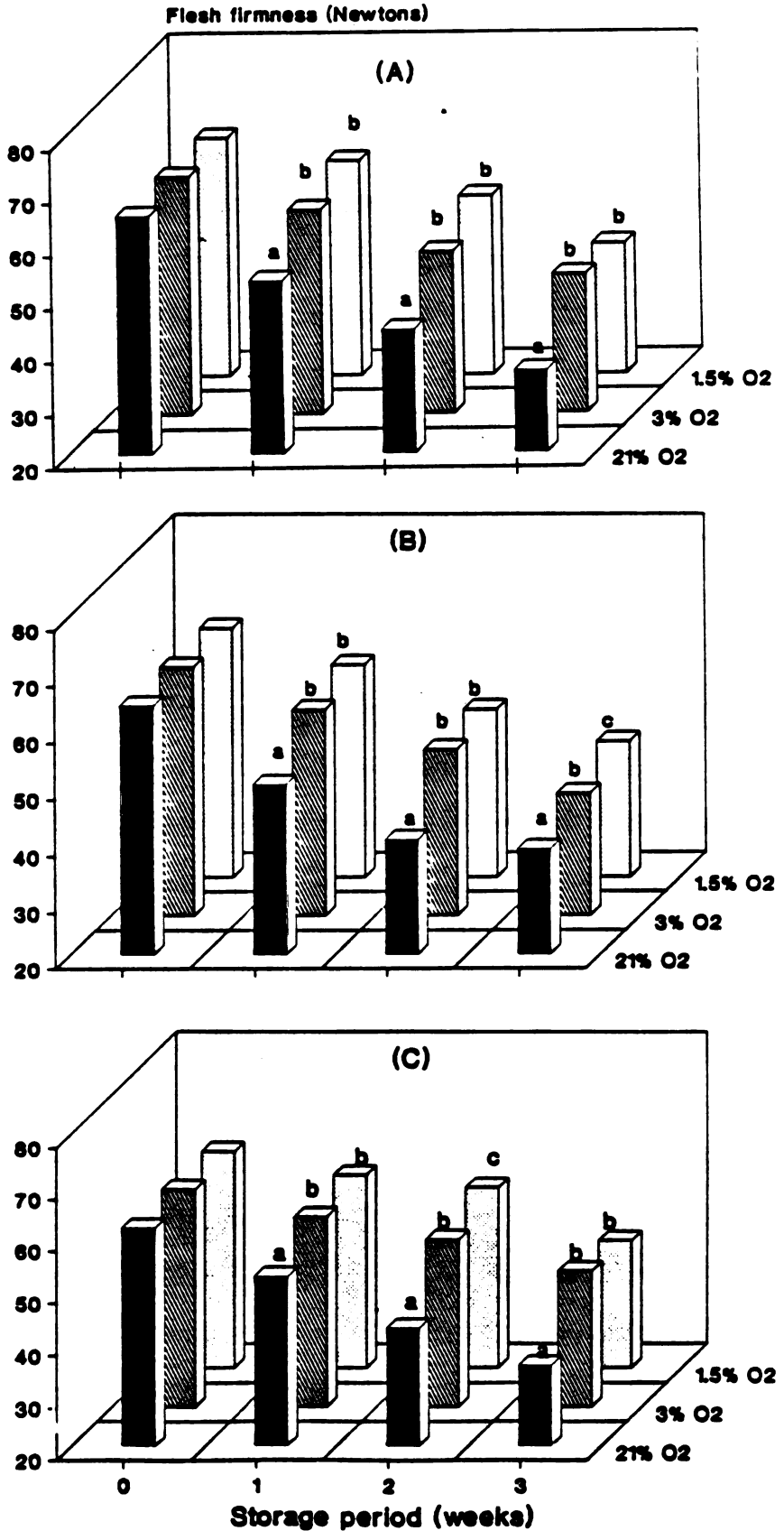

Flesh firmness (Newtons)
(A)
(B)
(C)
80
70
60
50
40
30
20
1.5% O2
3 % O2
21% O2
0
1
2
3
Storage period (weeks)

Figure 3. Change in flesh firmness (N) of 'McIntosh' apple fruit held at 1.5, 3 and 21 % O_2 at 20°C. Fruits were placed under these regimes after 1 (A), 7 (B) or 14 (C) days at 1°C in air.


Flesh firmness (Newtons)
(A)
(B)
(C)
80
70
60
50
40
30
20
1.5% O2
3% O2
21% O2
0
1
2
3
Storage period (weeks)

temperature for these reactions to proceed. Limiting the oxygen availability to 3% or below, although at a warm temperature of 20°C, significantly arrested the process of softening.

The role of oxygen in cell wall softening may be indirect rather than direct. Cell wall softening is presumed to be a consequence of hydrolytic enzyme activity as opposed to oxidative enzyme activity. It may be argued that oxygen limitation reduces the availability or quantity of the hydrolases. Oxygen is required for energy to sustain synthesis of these proteins. Moreover, oxygen is required for the action of ethylene which is in some manner involved in the expression of gene activity which determines the nature of the proteins synthesized. Evidence for this model comes from studies where ethylene action has been inhibited in fruits which have begun to ripen even in the presence of adequate oxygen.

Fruit weight loss was also reduced significantly under low oxygen conditions following storage compared to that for fruits ventilated with air (data not shown). However, no significant differences were observed between 1.5 and 3% O_2. The relative humidity was ostensibly the same at all oxygen levels by humidification of the gas mixture supplied to fruits. The greater weight loss found for fruits ventilated with 21% O_2 may be attributed to a higher respiration rate.

The quality of fruit was evaluated subjectively at weekly intervals upon removing samples for flesh firmness evaluation. No off-flavors or off-odors were observed from the fruits at the different oxygen levels over the 3 week period at 20°C and the fruits did not develop decay.

The results of these experiments at 20°C indicate beneficial effects of low oxygen concentration in the range of 1.5 to 3% in delaying ripening following CA storage as judged subjectively by organoleptic evaluation and objectively by flesh firmness retention. This data indicates that modified atmosphere packaging of

'Empire' apples may be useful in delaying softening and quality deterioration after CA storage providing these low O_2 regimes are achievable in MAP.

Fruit Volatiles Production: The nature, magnitude and complexity of fruit volatiles produced at 20°C in air was examined for 'Empire' and 'McIntosh' fruit after removing them from CA storage and holding them in air at 1°C for 1 day prior to the analysis. Volatiles were collected after 1 day at 20°C. This was also done at weekly intervals for 3 consective weeks for fruits kept in air and at 1.5 and 3% O_2 and then transferring them to air at 20°C for collection of volatiles as described earlier. Some, but not all, of the volatile components collected from the fruits were tentatively identified based on retention times and profile analyses of known standards.

Figure 4 is a chromatographic profile of known standards used for tentative identification of volatiles collected from the apples.

One day after removing apples from CA storage, fruit of 'Empire' (Figure 5) and 'McIntosh' (Figure 9) cvs. both exhibited a low rate of production of volatiles characteristic of flavor and aroma indicating that fruit ripening had not advanced during CA storage. Particularly relevant is the low production of ethyl-2-methylbutyrate, hexanal and 1-hexenol in this respect.

A general complexity in the nature of components produced and an increase in magnitude is seen for volatiles from 'Empire' and 'McIntosh' apple fruit in Figures 6c to 8c and 10c to 12c, respectively, as the duration of time in air at 20°C was extended over a three week period.

With fruit kept at 3% O_2 at 20°C for periods up to 3 weeks and then transferred to air for measurement of volatiles, a general pattern of increase in complexity and magnitude of components is also seen to occur but at a slower rate than was observed for fruits kept in air. This is seen for 'Empire' in Figures 6b to 8b and for 'McIntosh' in Figures 10b to 12b.

Fruit kept at 1.5% at 20°C for up to 3 weeks and then transferred to air for measurement of volatiles exhibited a similar lag in production of volatiles as was observed at 3% O_2 in comparison to fruit kept in air. This is seen for 'Empire' in Figures 6a-8a and, for 'McIntosh' in Figures 10a-12a. Whereas the nature and magnitude of many of the volatiles produced by fruits held at 1.5% O_2 was generally similar to that of fruits held in 3% O_2 the complexity was somewhat greater and this may reflect the accumulation of intermediates as a result of incipient fermentation metabolism. Some tissues of the fruit while at 1.5% O_2 showed low O_2 injury symptoms.

Ethyl acetate and ethyl propionate were found to be present at high concentration in fruits examined at different storage periods. Upon removal from CA storage ethyl acetate was the dominant compound among those tentatively identified in both 'Empire' and 'McIntosh' apples. Ethyl 2-methylbutyrate an important aroma component, was found at low concentration after 7 days in low oxygen treatments but increased significantly at the 14 and 21 day assessment periods for 'Empire' apples and remained low in 'McIntosh'. This pattern was also apparent for other compounds, hexanal, ethyl hexanoate as well as trans-3-hexen-1-ol, 1-hexenol and cis-3-hexen-1-ol, which also contribute to apple flavor and aroma. These components were almost nonexistant the first day after removing the fruits from CA but increased considerably after 7 days and through 21 days at 20°C. Hexanal was present at a very low concentration after 7 days and remained low even after 3 weeks. Several other compounds appeared shortly after exposure to air at 20°C and then decreased thereafter. This may be explained by the fact that these compounds serve as precursors of other components responsible for aroma and other substances produced by the fruit.

The difference in time of onset of high production rates for volatiles observed between the fruits kept in air and those held at low O_2 levels is probably

due in part to the delay in the development of ripening process and on the lower rate of respiration at the low O_2 levels. Low oxygen, alone or in association with high carbon dioxide may affect several metabolic reactions in the fruit tissue. A similar pattern of volatiles production has been reported by many researchers and this was related to the climacteric rise and fruit ripening (Brown et al., 1966; Cheraghi, 1988). Greevers and Doesburg (1964) found that fruit stored at 10 or 15°C produce more volatiles than fruit at lower temperatures. The effect of low oxygen concentration in the atmosphere may reduce substrate availability or result in incompletely oxidized metabolites by restricting the activity of oxidative enzymes (Patterson et al., 1974; Lidster et al., 1983). This would certainly be true of lipoxygenase which has a relatively low affinity for O_2 and is involved in the oxidation of unsaturated C_{18} fatty acids. Moreover, lipoxygenase activity is known to increase as apples ripen (Rhodes, 1983).

Although quantitative analysis for each compound was not performed, peak heights can serve in a relative manner to assess quantities of the compounds separated. For several of the compounds, increases of 4 to 5-fold or more were found over the 21 day period at 20°C. Dirink et al.(1984) reported that the sum of esters increased during ripening up to 14 days and then decreased. This was seen for the fruits kept in air.

Atmospheric conditions of low oxygen with or without carbon dioxide would tend to delay fruit ripening and senesence processes at high temperature and at low temperatures. However, more research is needed to elucidate the optimum concentration and the length of storage period acceptable without limiting flavor development of fully ripe fruits and the onset of fermentation.

No major qualitative differences in the make-up of volatile profiles were found between 'Empire' and 'McIntosh' apple fruit held at low oxygen concentration at high temperature. Therefore fruit volatiles changes are due mostly to ripening

process. Any condition that slows the ripening process would be expected to limit production of fruit volatiles responsible for fruit aroma.

This study was conducted at the relatively high temperature of 20°C which is often experienced in marketing apples. The results indicate that fruit of both 'Empire' and 'McIntosh' held at this temperature at 3% O_2 may develop typical types and quantities of volatiles known to contribute to flavor and aroma. Fruit held at 1.5% at this temperature for prolonged periods up to 3 weeks may exhibit a profile of volatiles symptomatic of alcoholic fermentation and this should be avoided. The fruit volatiles data along with the favorable effects of low O_2 levels at 20°C on flesh firmness retention indicates that MAP of apples following CA storage may offer beneficial effects on quality preservation. It must stressed however, that these studies were conducted with apples from CA storage that resulted in very limited ripening development during storage; holding fruit from CA storage or otherwise where ripening has been allowed to advance and subjecting them to low O_2 levels at 20°C might be detrimental.

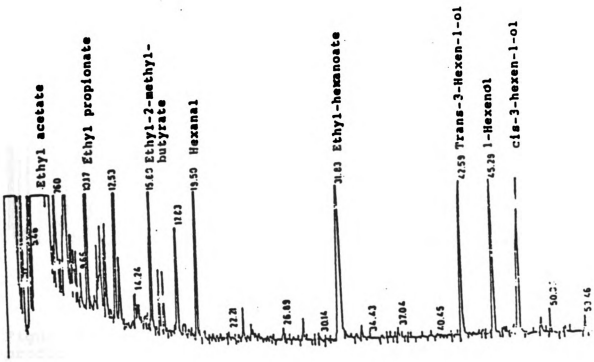


Figure 4. Gas chromatographic profile of volatiles tentatively identified from apple fruit.

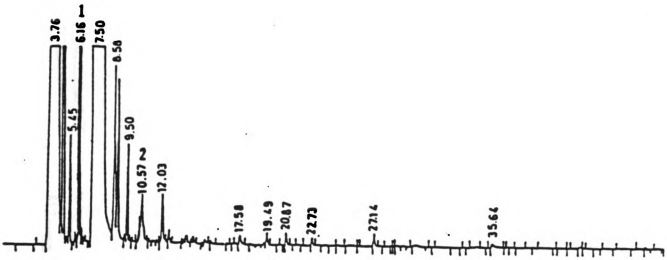


Figure 5. Gas chromatographic profile of volatiles produced by 'Empire' apple fruit from CA after 1 day in air at 20°C, 1. ethyl acetate and 2. ethyl propionate.

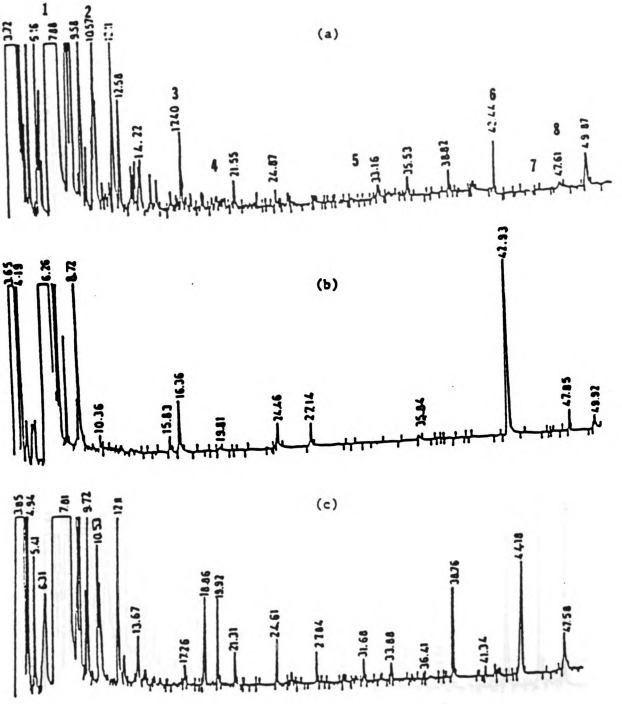


Figure 6. Gas chromatographic profile of volatiles produced by 'Empire' apple fruit from controlled atmosphere storage after 7 days in differeent oxygen regimes, 1.5% (a), 3% (b) and 21% (c) at 20°C. 1. ethyl acetate, 2. ethyl propionate, 3. ethyl-2-methyl butyrate, 4. hexanal, 5. ethyl hexanoate, 6. trans-3-hexen-1-ol, 7. 1-hexenol and 8. cis-3-hexen-1-ol.

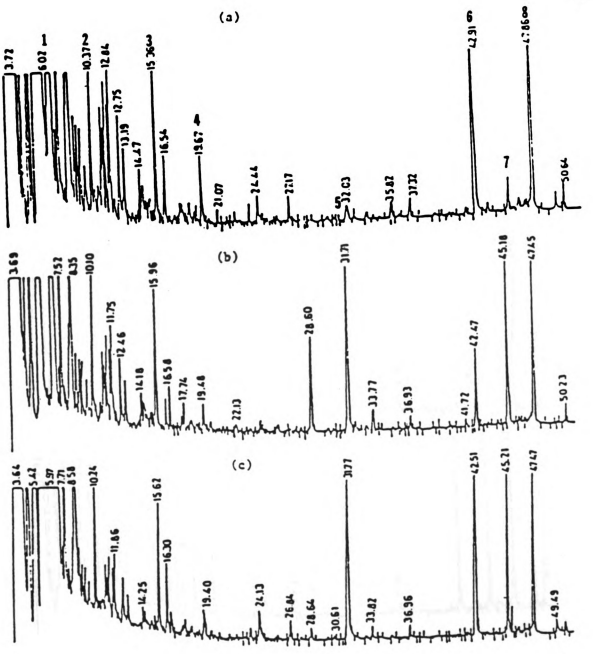


Figure 7. Gas chromatographic profile of volatiles produced by 'Empire' apple fruit from controlled atmosphere storage after 14 days in different oxygen regimes, 1.5% (a), 3% (b) and 21% (c) at 20°C. 1. ethyl acetate, 3. ethyl propionate, 3. ethyl-2-methyl butyrate, 4. hexanal, 5. ethyl hexanoate, 6. trans-3-hexen-1-ol, 7. 1-hexen-ol and 8. cis-3-hexen-1-ol.

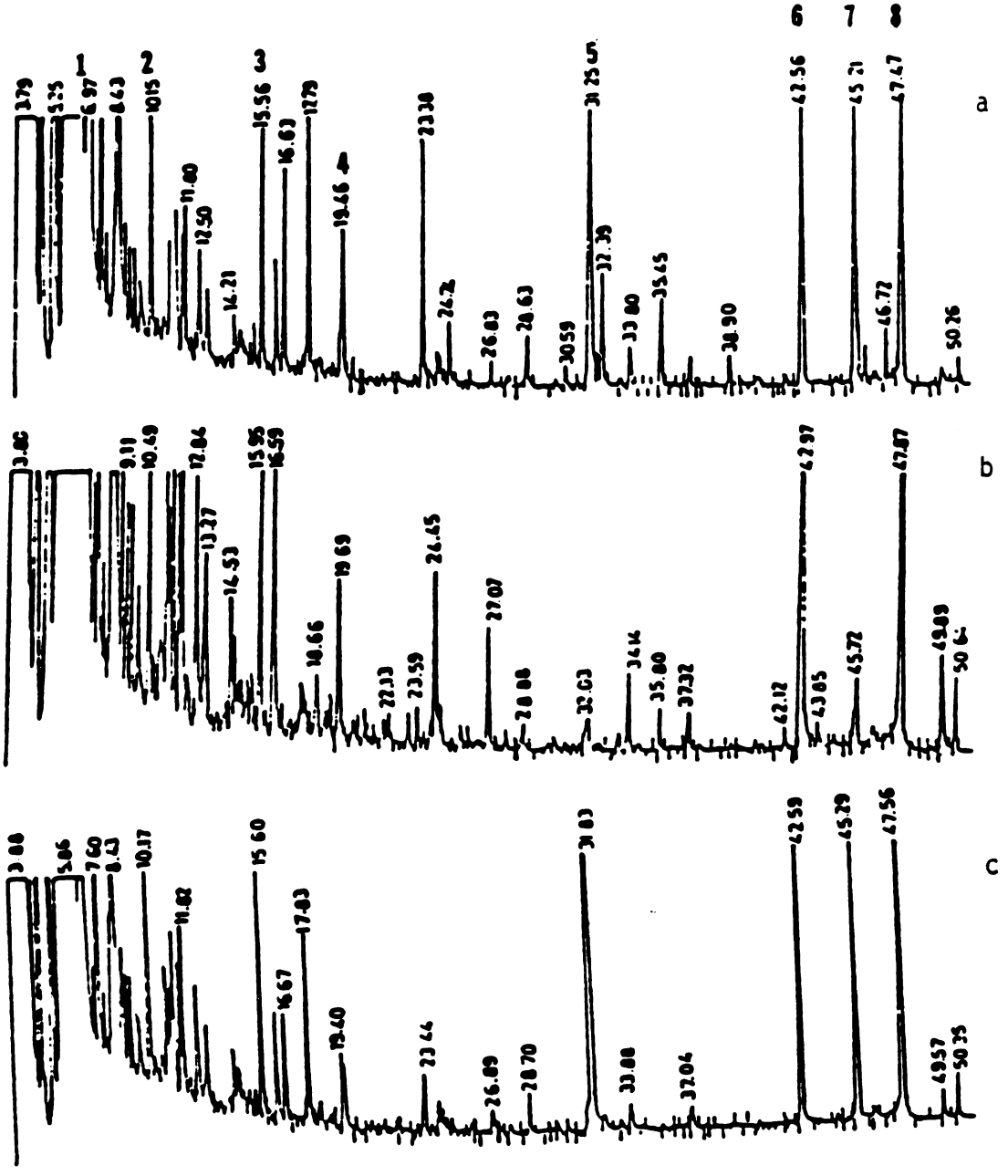


Figure 8. Gas chromatographic profile of volatiles produced by 'Empire' apple fruit from controlled atmosphere storage after 21 days in different oxygen regimes, 1.5% (a), 3% (b) and 21% (c) at 20°C. 1. ethyl acetate, 2. ethyl propionate, 3. ethyl-2-methyl butyrate, 4. hexanal, 5. ethyl hexanoate, 6. trans-3-hexen-1-ol, 7. 1-hexenol and 8. cis-3-hexen-1-ol.

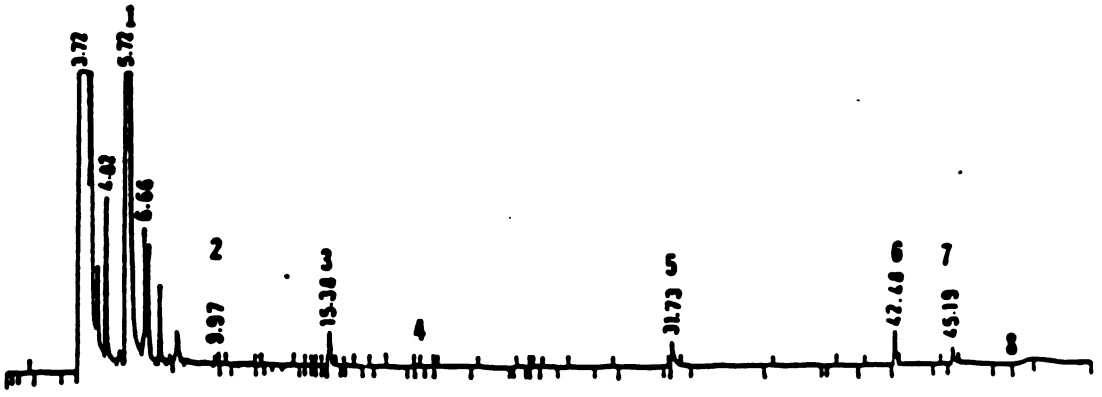


Figure 9. Gas chromatographic profile of volatiles produced by 'McIntosh' apple fruit from controlled atmosphere storage after 1 day in air at 20°C. 1. ethyl acetate, 2. ethyl propionate, 3. ethyl-2-methyl butyrate, 4. hexanal, 5. ethyl hexanoate, 6. trans-3-hexen-1-ol, 7. 1-hexenol and 8. cis-3-hexen-1-ol.

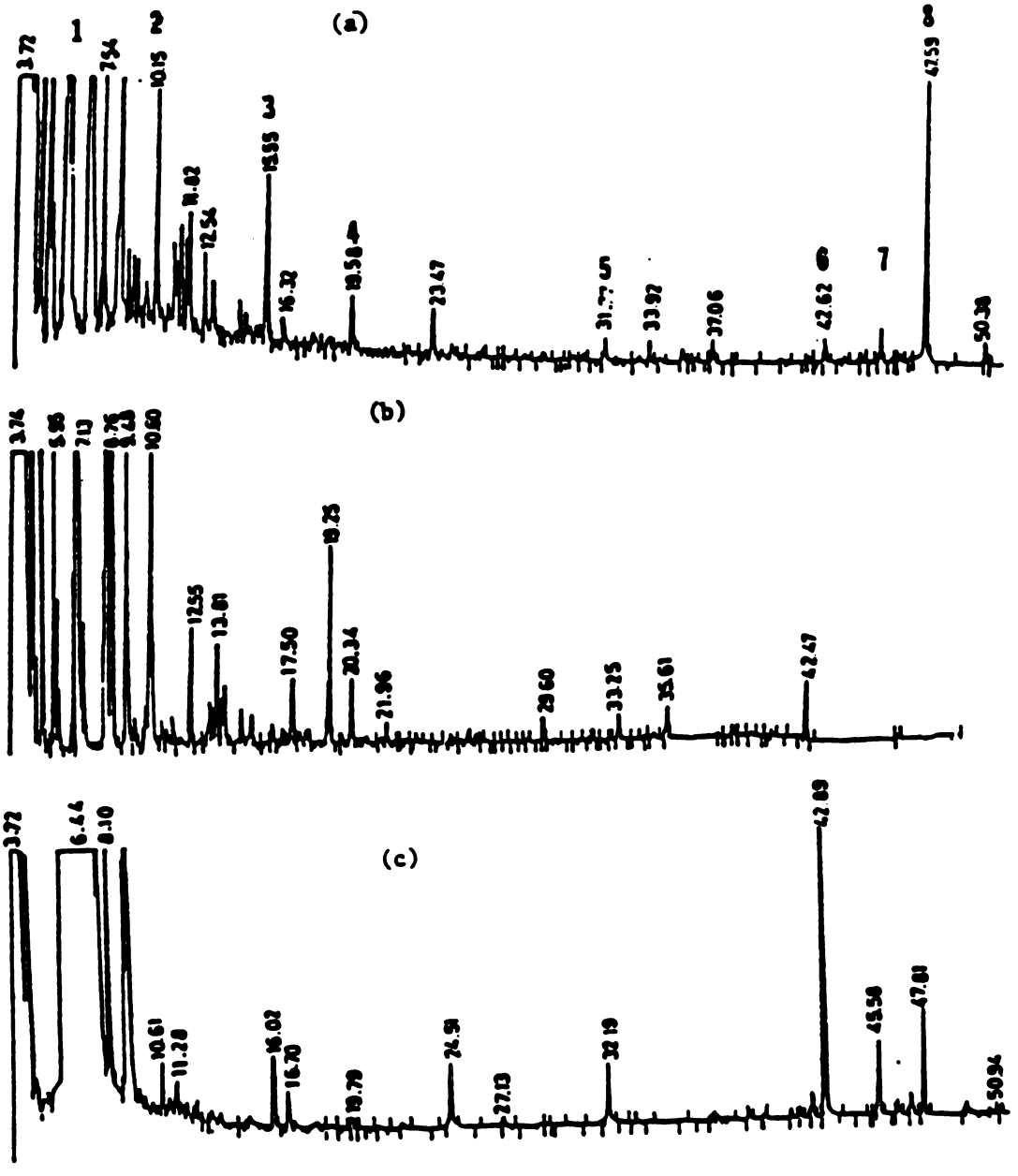


Figure 10. Gas chromatographic profile of voltiles produced by 'McIntosh' apple fruit from controlled atmosphere storage after 7 days in different oxygen regimes, 1.5% (a), 3% (b) and 21% (c) at 20°C. 1. ethyl acetate, 2. ethyl propionate, 3. ethyl-2-methyl butyrate, 4. hexanal, 5. ethyl hexanoate, 6. trans-3-hexen-1-ol, 7. 1-hexenol and 8. cis-3-hexen-1-ol.

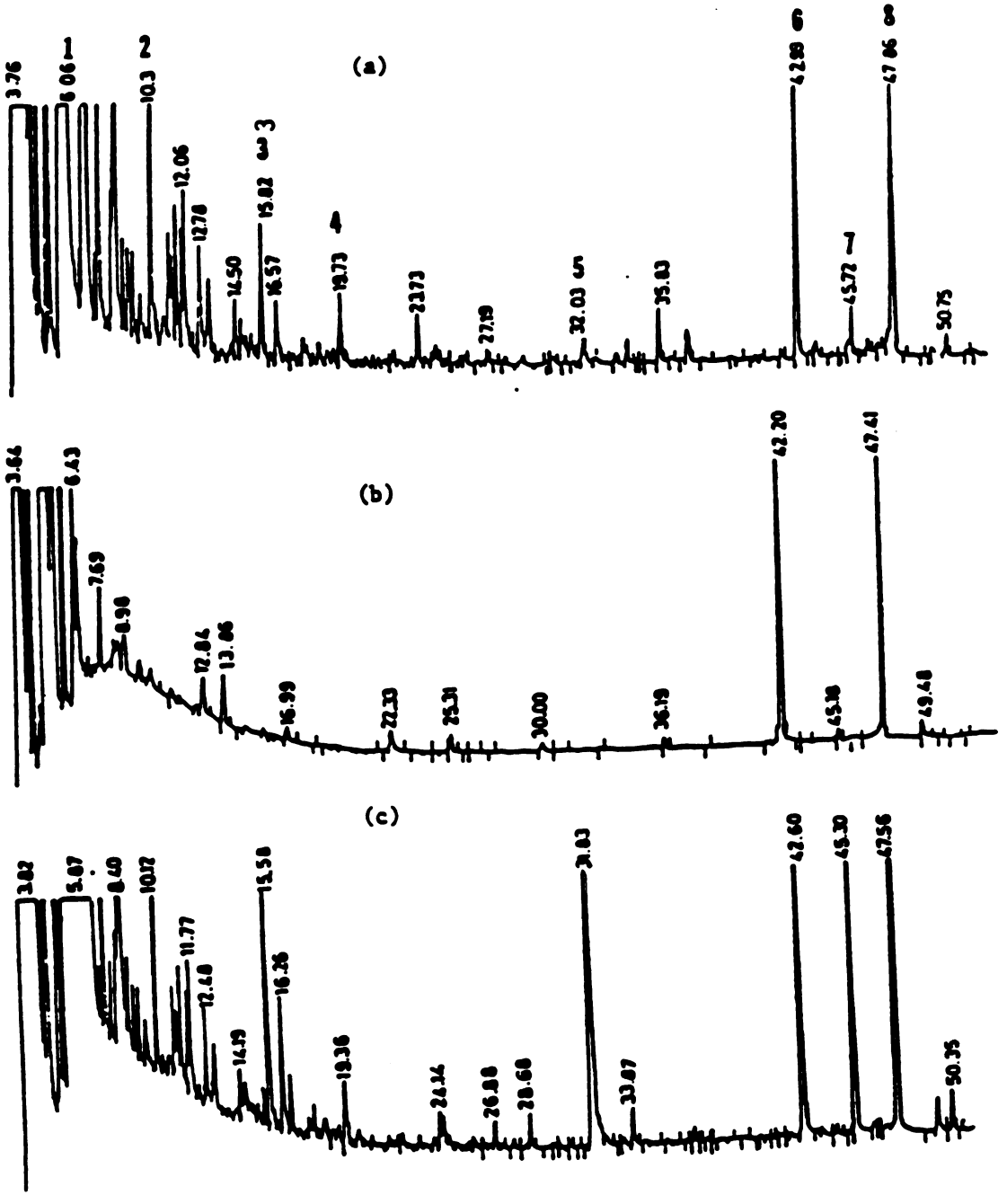


Figure 11. Gas chromatographic profile of volatiles produced by 'McIntosh' apple fruit from controlled atmosphere storage after 14 days in different oxygen regimes, 1.5% (a), 3% (b) and 21% (c) at 20°C. 1. ethyl acetate, 2. ethyl propionate, 3. ethyl-2-methyl butyrate, 4. hexanal, 5. ethyl hexanoate, 6. trans-3-hexen-1-ol, 7. 1-hexenol and 8. cis-3-hexen-1-ol.

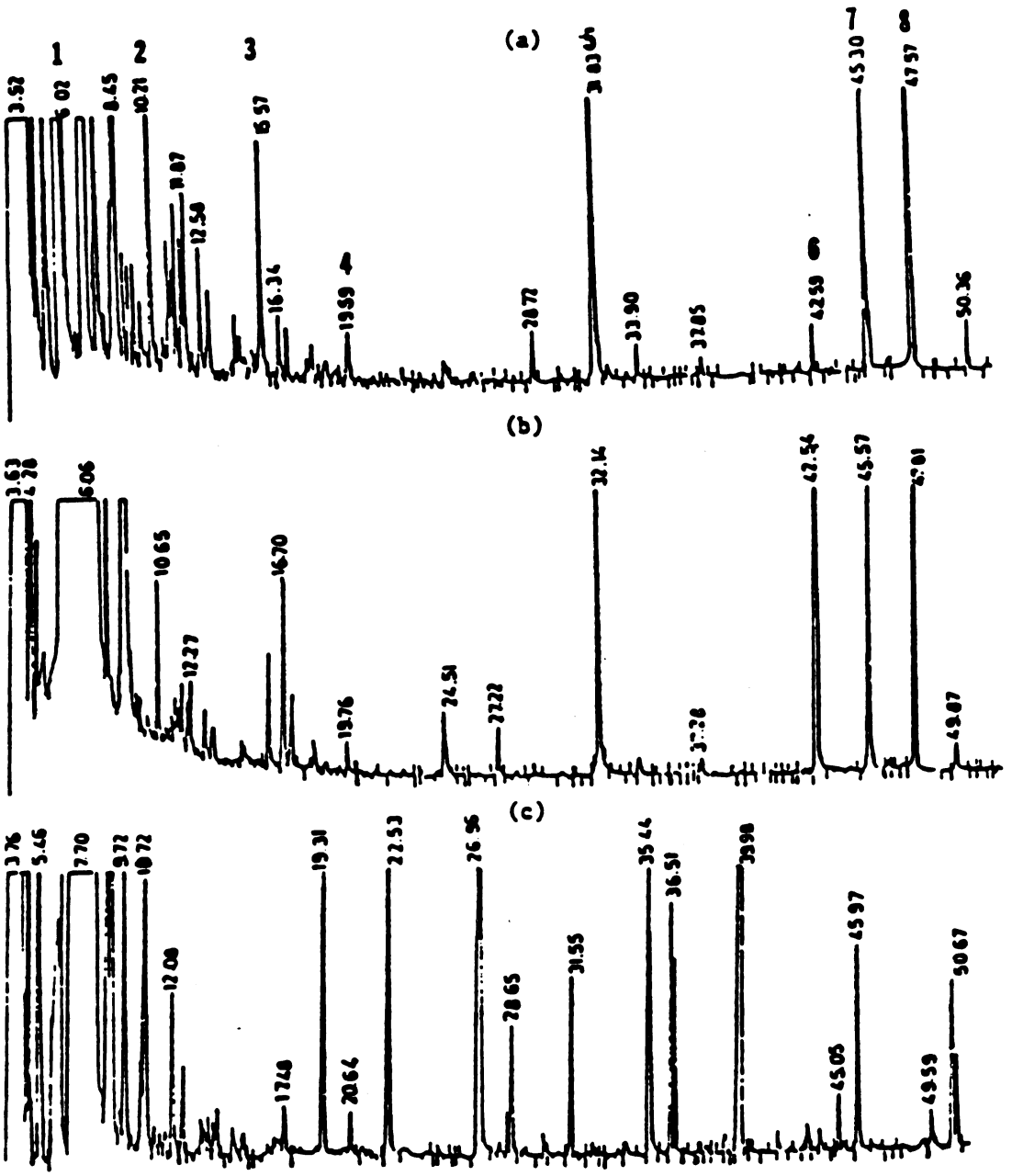


Figure 12. Gas chromatographic profile of volatiles produced by 'McIntosh' apple fruit from controlled atmosphere storage after 21 days in different oxygen regimes, 1.5% (a), 3% (b) and 21% (c) at 20°C. 1. ethyl acetate, 2. ethyl propionate, 3. ethyl-2-methyl butyrate, 4. hexanal, 5. ethyl hexanoate, 6. trans-3-hexen-1-ol, 7. 1-hexenol and 8. cis-3-hexen-1-ol.

LITERATURE CITED

Brown, D.S., J.R. Buchanan, and J.R. Hicks. 1966. Volatiles from apples fruits as related to variety, maturity, and ripening. J. Amer. Soc. Hort. Sci. 88:98-102.

Chiraghi, T.S. 1988. Effect of harvest date and ethylene concentration in controlled atmosphere storage on the quality of Empire apples. PhD Dissertation, Dept. of Food Science and Human Nutrition, Mich. State Univ., East Lansing, MI, U.S.A.

Dilley, D.R. 1987. Recommended storage conditions for Michigan apples. Postharvest Laboratory, Dept. Hort., Michigan State, East Lansing, M.I., U.S.A.

Dilley, D.R. 1990. Application of air separator technology for controlled atmosphere storage of apples. Ed. J. Fellman. Proceedings of the 5th National Controlled Atmosphere Research Conference. Wenatchee, WA. June 14-15, 1:409-418.

Dirink, P., H. De Pooter, G. Willaert, and N. Schamp. 1984a. Application of a dynamic headspace procedure in fruit flavor analysis. In: Analysis of volatiles methods applications, P. Schreier (Ed), p. 381. W. de Gryter, New York.

Greevers, G. and J.J. Doesburg. 1965. Volatiles of apples during storage and ripening. J. Food Sci. 30:412-415.

Lange, E. and J. Fica. 1982. Storage of Spartan, Melrose and Idared apples in ultra-low oxygen controlled atmospheres. Fruit Sci. Rpt (Skierniewce, Poland) 9:123.

Lau, O.L. 1983. Effects of storage procedures and low oxygen and carbon dioxide atmospheres on storage quality of Spartan apples. J. Amer. Soc. Hort. Sci. 108(6):953-957.

Lau, O.L. 1985. Storage responses of four apples cultivars to low O_2 atmospheres. Proceedings of the 4th National Controlled Atmosphere Research Conference pp.43-56.

Lidster, P.D., K.B. McRae, and E.M. Johnson. 1985. Retention of apple quality in low oxygen storage followed by standard controlled atmosphere regimes. J. Amer. Soc. Hort. Sci. 110:755-759.

Patterson, B.D., S.G. Hatfield, and M. Knee. 1974. Residual effects of controlled atmosphere storage on the production of volatile compounds by two varieties of apples. J. Sci. Food. Agric. 25:843-848.

Rhodes, M.J.C. 1983. Enzyme activities and postharvest change In. "Postharvest Physiology and Crop Preservation". (Ed) M. Lieberman, Plenum Publ. Corp., New york, N.Y. pp. 99-121.

Smith, S.G., J.Geeson, and J. Stow. 1987. Production of modified atmospheres in deciduous fruits by the use of films and coatings. HortScience 22:772-776.

Wade, N.L. 1979. Physiology of cool-storage disorders of fruits and vegetables. Low Temperature Stress in Crop Plants: The role of the membrane, pp. 81-96. Academic Press, New York.

SECTION II

MODELING FOR MODIFIED ATMOSPHERE PACKAGING OF APPLE FRUIT

INTRODUCTION

For many years, concern has been expressed by researchers and commercial users about the rapid decline in the quality of CA apples experienced during marketing of apples after removal from controlled atmosphere storage. Moreover, CA storage at very low O_2 levels to maximize retardation of ripening has been attributed to causing loss of capacity of fruits to ripen properly and produce volatiles characteristics of aroma and flavor. Many techniques and strategies have been adopted for large commercial stores that permit maintenance of fruit throughout the year. However, exposure of post CA apples to ambient air and warm temperatures rapidly hastens ripening and subsequent loss of quality. These conditions can be encountered at the market and warehouses levels. Ripening and loss of quality were delayed by modified atmosphere packaging (MAP) techniques for 'Discovery' apple fruit (Smith et al., 1987). They found that MA within sealed packs helped to maintain flesh firmness and reduce weight loss and had no deleterious effects. The beneficial effect of low O_2 on post-CA storage of 'Empire' and 'McIntosh' apple fruit was confirmed section I of this dissertation. Other researchers succeeded to maintain and prolong shelf life of some commodities for several weeks, Cameron et al. (1989), Kawada (1982) for tomatoes, Smith et al. (1987) for apple fruit.

The eventual concentration of gases inside the package is the result of an equilibrium reached between the fruit respiration and gas permeation through the plastic film at a given temperature. MAP is not recent and for more than three decades scientists have been developing this technology for different commodities. The potential for MAP has increased rapidly as now a wide range of films with specific permeability characteristics for gas and water transmission are available (Barmore, 1987). Availability of absorbers or adsorbers of O_2 , CO_2, water vapor

and several other volatiles, provides an important means to attain desired conditions within the package unit. Actually there is possibility to incorporate fungicides during manufacturing in the film structure (Zagory and Kader, 1988).

Designing a MAP model requires an understanding of the relationship between physiological characteristics of the commodity and its surrounding environment. Zagory and Kader (1988) stressed that designing a model of MAP, should take into account several parameters including: how changes in O_2 and CO_2 affect respiration rate, the permeability of the film to O_2 and CO_2 , the effect of temperature on film permeability, the headspace of the package, the optimal atmospheric conditions for storage of the produce and, the resistance of the commodity to gas diffusion.

Since the early 1960's researchers recognized the significance of the steady state gas concentration reached in hermetically packaged produce and mathematical approaches were attempted to optimize model packages (Cameron et al. 1989; Hayakawa et al., 1975; Henig, 1975; Henig and Gilbert, 1975; Prince et al. 1982). Despite many beneficial and encouraging results obtained by different researchers, optimizing the model was never achieved in an applicable manner. The success of MA packaging storage technique depends upon the understanding of the complex interactions between produce physiology, package material characteristics and the environment and the effect of the resultant equilibrium atmospheres on several biochemical and physiological aspects of the stored commodity (Kader, 1986).

Two different methods were employed in the current study to optimize and develop a technique to describe the influence of a number of factors on the equilibrium gas atmospheres in packages of apple fruit. The first method was to monitor and analyze oxygen depletion in closed systems as adopted from Cameron et al. (1989); the second was based on the manipulation of steady-state

concentrations of oxygen attained from MAP trials with various fruit weights packaged in different LDPE films with various thicknesses at 20°C.

MATERIALS AND METHODS

Fruits employed: 'Empire' apple fruit were harvested from a commercial orchard near Grand Rapids, MI. Fruit were stored in CA storage at the Clarksville Horticultural Experiment Station under 1.5% O_2 and 1.8% CO_2 with a relative humidity of ca. 96%. Fruits for the oxygen depletion study were stored for 104, 133 and 156 days under CA storage and for MAP trials 133 days. Fruit free of defects were sorted into size classes and randomized. Flesh firmness was measured for duplicate 10 fruit samples measuring firmness of the pared surface on opposite sides of the fruit using an 11 mm diameter tip.

Oxygen depletion studies: For each oxygen depletion test, a single fruit of known weight was placed in a ca. 500 ml wide mouth jar with 5 g of MgO as a CO_2 scrubber contained in a 5 x 5 cm sealed Tyvek pouch. The jar lid was sealed tightly with a screw ring. The void volume was calculated by substracting the fruit weight (grams) from the volume of the empty jar (cc). The oxygen concentration in the jar was monitored by inserting an oxygen electrode probe (Beckman Oxygen Analyzer Model 0260) fixed to the lid through a 2.5 cm dia. hole fitted with a rubber stopper. The probe was connected to an IBM personal computer for automatic data recording at 15 min intervals. The oxygen probe was calibrated to known oxygen concentrations before and after each test. The jar was submerged in a controlled temperature water bath at 20°C. The oxygen concentration in the headspace surrounding the fruit declined as O_2 was consumed by the fruit from 21% to ca. 0%. The time required to reach 0% varied with fruit weight and thus with void volume. The oxygen depletion test was repeated 2 to 3 times after equilibrating the fruit in air before each determination. More than 20 fruits were analysed in this manner. Data were converted to % of oxygen on an hourly basis prior to performing curve fitting with

a computer using the Plotit statistical package.

MAP studies: Three low density polyethylene (LDPE) films were obtained from Dow Chemical Co, Midland, Michigan, U.S.A., of thicknesses of 25.4, 44.4 and 50.8 μm corresponding to 1, 1.75 and 2 mil respectively. Various weights (75 to ca. 1000 g) of fruit were placed in packages with a surface area of 1250 cm^2. The permeability constant for oxygen of the different films was calculated for the same surface area (Table 1). The bags were sealed with a heat sealer machine, Model 420 from Audion Elekto, Holland. A scrubber for CO_2 was placed in each package and consisted of 5 g of MgO in 5x5 cm Tyvek pouch. Packages were then stored at 20°C and ca. 90% relative humidity. Concentrations of O_2 and CO_2 were monitored daily until equilibrium was reached. Gas was sampled by withdrawing 1 ml samples of gas from the package headspace using plastic syringes equipped with a 25g 1/2" hypodermic needle. The needle was inserted through a silicone rubber septum fixed to 1 cm^2 piece of polyethylene electrical tape on each bag. Samples were analyzed for O_2 as described in section I of the dissertation.

Table 1. Film thickness, surface area and permeability constant of the different LDPE films used.

Film thickness (cm)	SA (cm^2)	$P^Y \cdot A/DX$ ($cc\ hr^{-1}\ atm^{-1}$)
25.4×10^{-4}(1 mil)	1250	40.25
44.4×10^{-4}(1.75 mil)	1250	23.00
50.8×10^{-4}(2 mil)	1250	20.12

SA = surface area of 25 x 25 cm for each side of the bag.

[Y] Measured permeability to oxygen for 1 mil (25.4 μm) thick LDPE film equals 500 cc . mil^{-1} . 24 hr^{-1} . 100 in^{-2} . atm^{-1} (Boylan-Pett, 1986).

Testing the model using MA packages: The steady state that is reached within the MAP units requires an understanding of Fick's law governing gas diffusion through the film barrier.

Equilibrium was considered to be established when the oxygen concentration within the package declined to a fairly constant value. Each value represents an average of 3 successive readings at 24 hr intervals. Data were then used for modeling technique based on the flux of oxygen through film which is expressed by Fick's law as follows:

$$J_{O2}^{film} = (P_{O2} \cdot A \cdot DX^{-1}). ([O_2]_{atm} - [O_2]_{pkg}) \qquad [1]$$

Where;

J_{O2}^{film} = movement of O_2 per unit time through the film ($cm^3\ hr^{-1}$)

P_{O2} = permeability of the film to O_2 ($cm^2\ hr^{-1}\ atm^{-1}$)

A = surface area (cm^2).

DX^{-1} = thickness of the film (cm)

$[O_2]_{atm}$ = partial pressure of oxygen in air and,

$[O_2]_{pkg}$ = partial pressure of O_2 inside the package (atm).

It was assumed that the oxygen uptake represents the amount of the oxygen that passes through the fruit skin. At equilibrium the amount of oxygen that passes through the film should equal the flux of oxygen into the fruit. The latter is then expressed as follows:

$$J_{O2}^{fruit} = RR_{O2}([O_2]_{pkg}) \cdot W \qquad [2]$$

Where; J_{O2}^{fruit} = flux of O_2 per unit time into the fruit ($cm^3\ hr^{-1}$)

$RR_{O2}([O_2])$ = O_2 uptake ($cm^3\ kg^{-1}\ hr^{-1}$) and,

W = weight of fruit (kg).

Combining Equations (1) and (2) allowed calculation of the rate of respiration (O_2 uptake) at equilibrium as follows:

$$RR_{O2}([O_2])_{pkg} = \frac{P_{O2} \cdot A \cdot DX^{-1} \cdot ([O_2]_{atm} - [O_2]_{pkg})}{W} \qquad [3]$$

In MAP trials since the permeability to oxygen, surface area, film thickness and the oxygen gradient in atm at equilibrium were known, calculation of respiration rate was straightforward from Equation (3).

RESULTS

Oxygen depletion in closed system:

Examples of oxygen depletion curves obtained in closed jars over time for 3 different removal periods are illustrated in Figures 1a, 1b and 1c, respectively for 104, 133 and 156 days of CA storage. The best fit equation curve type with $r^2 = 0.999$ or higher has the following form:

$$[O_2] = a \cdot (1 - e^{-(b + c \cdot t)})^d \qquad [4]$$

Where; $[O_2]$ = concentration of oxygen (%), t = time (hr) and,

a, b, c and d are arbitrary constants, tabulated their values are presented in Table 2.

To calculate the respiration rate; the first approach was to measure the difference in O_2 level over short intervals and convert this to a per hour basis. Values obtained represent the O_2 concentration (%) change over the testing time (t) and can be characterized by $d[O_2] \cdot dt^{-1}$. The rate of O_2 depletion is a function of void volume and fruit weight, the values in % were multiplied by jar void volume (ml) and divided by weight of fruit (kg) yielding rate of respiration as expressed in the following equation:

$$RR_{O2} = d[O_2] \cdot dt^{-1} \cdot V \cdot W^{-1} \qquad [5]$$

Where; V = void volume (ml) and W = fruit weight (kg).

Figure 1. Typical oxygen depletion curves in closed system over time at 20°C for apple fruit after 104 (A), 133 (B) and 156 (C) days in CA storage. All curves have been fitted to the following equation $Y = a[1-e^{(-(b + c \cdot t)^{**}d)}]$. Constants values were given in Table 2. All R^2 values equal 0.999 or higher.

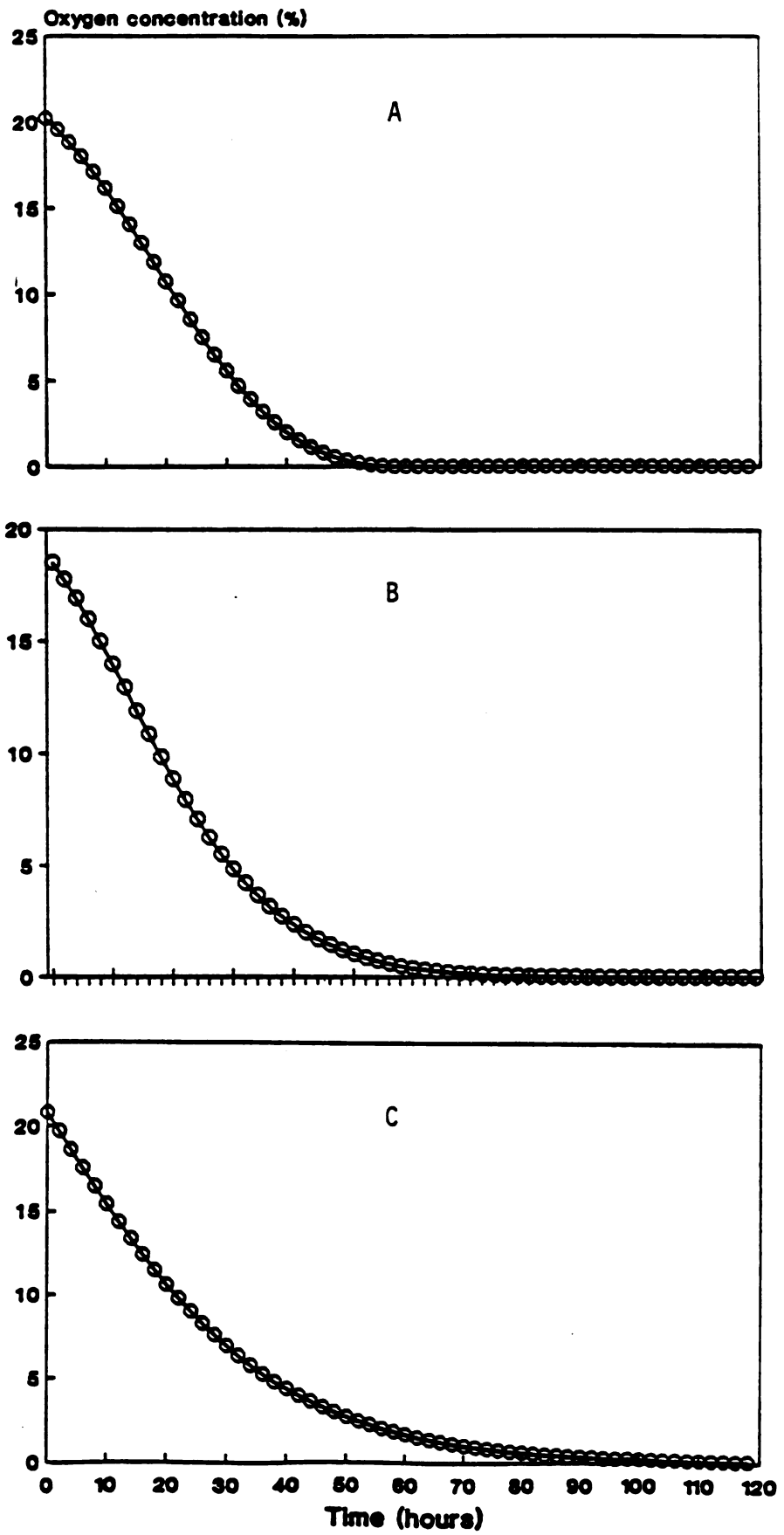

Oxygen concentration (%)
25
20
15
10
5
0
A
20
15
10
5
0
B
25
20
15
10
5
0
C
0
10
20
30
40
50
60
70
80
90
100
110
120
Time (hours)

Table 2. Individual fruit weight, void volume and constant values for the O_2 depletion best fit curve[Z] equation $Y = a \cdot (1- e^{-(b + c \cdot t)^d})$ after 104, 133 and 156 days in CA storage.

Sorage period	104 days	133 days	156 days
Fruit	1	2	3
Fruit weight (gm)	137.4	138.6	138.9
Void volume (cc)	357.6	361.2	361.1
Constant values			
a	29.421	21.2907	23.6013
b	1.0012	1.071005	1.24774
c	-0.000294	-0.00645	-0.02003
d	171.007	10.3515	3.00814

[Z] All values of $r^2 = 0.999$ or higher.

Data were then plotted vs time (Figure 2) and fitted to an equation having the form:

$$RR_{O2} = a\cdot(1- e^{(-b\cdot X)})^c \qquad [6]$$

Where $X = [O_2]$

Values of constants and r^2 of the equation are presented in Table 3.

The second approach followed to describe respiration rate was based on the first derivative of the best fit equation of O_2 depletion and was conducted largely according to that described by Cameron et al. (1989). The principle is to fit O_2 concentration data vs time to an equation as shown in Figure 1 having equation 4 form.

Values of constants are presented in Table 2. The first derivative of Equation 4 represents the rate of change in O_2 percent as a function of time and giving the following equation:

$$d[O_2]/dt = acd\cdot[(b+c\cdot t)^{d-1}]\cdot[e^{-(b+c\cdot t)^d}] \qquad [7]$$

From Equation 5 and 8, the respiration rate (ml kg^{-1} hr^{-1}) is calculated:

$$RR_{O2} = acd.V.W^{-1}.[(b+c.t)^{d-1}].[e^{-(b+c.t)^d}] \qquad [8]$$

When solving this equation for the same time (t) value, oxygen concentration and corresponding respiration rate can be determined, respectively, from Equation 4 and (8). Typical data are plotted in Figure (3).

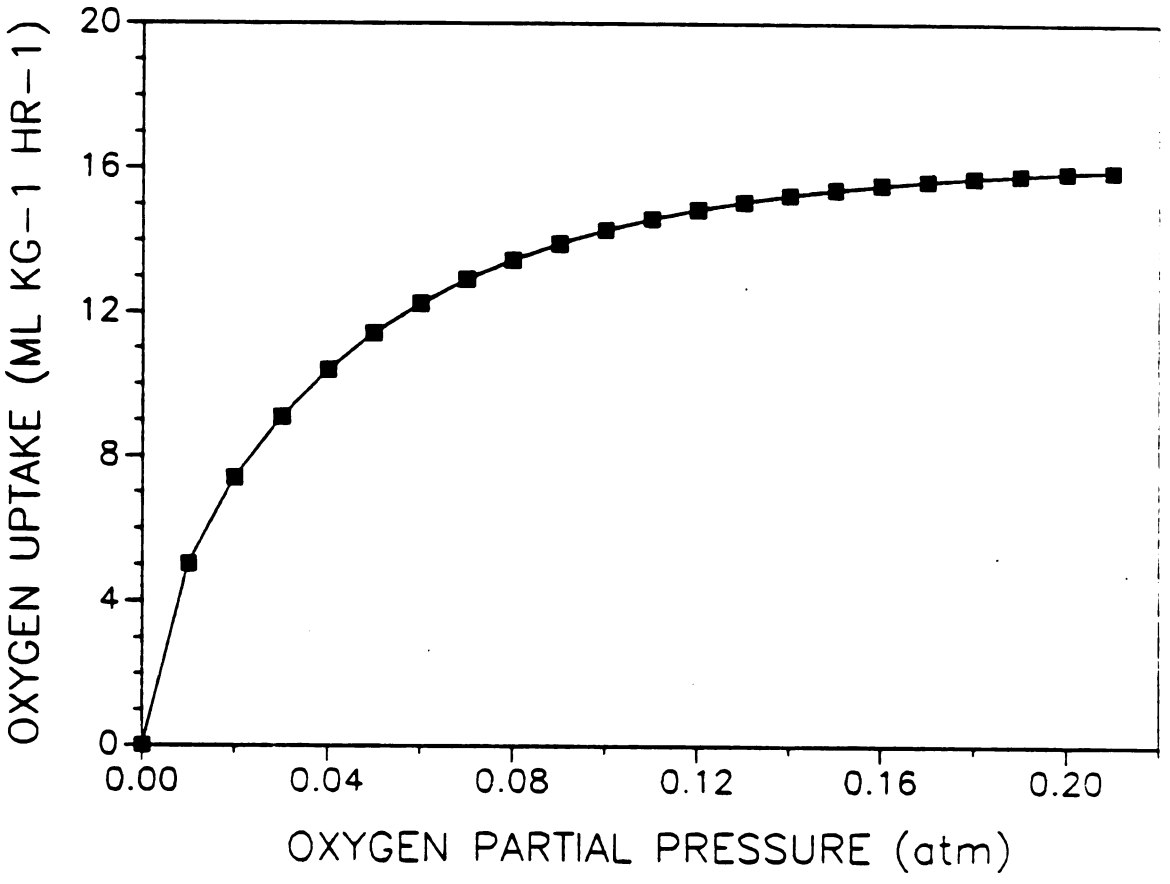


Figure 2. Plot of oxygen uptake (ml kg^{-1} hr^{-1}) in closed system versus oxygen partial pressure (atm). $Y = a\cdot(1-e^{(-b\cdot X)c}$. Values of constants are 16.2316, 17.0696 and 0.63384 respectively for a, b and c.

Table 3. Values for constants of the best fit curve of equation $Y = a(1 - e^{-(b \cdot X)})^c$ describing oxygen uptake (ml kg^{-1} hr^{-1}) as a function of oxygen concentration in closed system and in MAP trials at 20°C.

	Closed system[z]		MAP trials
Fruit number	1	2	
Constants			
a	16.2316	24.5417	17.5764
b	17.0696	15.2347	18.7800
c	0.6334	0.8683	0.67330
r^2	1.000	0.999	0.873

[z] Fit curve of the difference of subsequent data (1) and (2) first derivative approach.

Fitting the rate of respiration led to a curve with following equation that has the same form as in the first approach and illustrated by Equation (6). Constant values are given in Table 3 (2).

After substitution and rearrangement of this new Equation (6) into Eq. (3), weight of fruit can be expressed as follows:

$$W = \frac{P \cdot A \cdot DX^{-1} \cdot ([O_2]_{atm} - [O_2]_{pkg})}{a \cdot (1 - e^{-b \cdot [O2]})^c} \qquad [9]$$

This relationship first used by Cameron et al. (1989) determines fruit weight according to parameters of the package system as illustrated in Figure 4.

Model Testing:

The above models were tested using apple fruit sealed in packages of known permeability. The procedure for the determination of optimum fruit weight consisted of empirical packaging trials using various fruit weights. The concentration of oxygen within sealed packages, at steady state, decreases with increasing fruit weight and film thickness (Figure 5) and this relationship was characterized by the following equation:

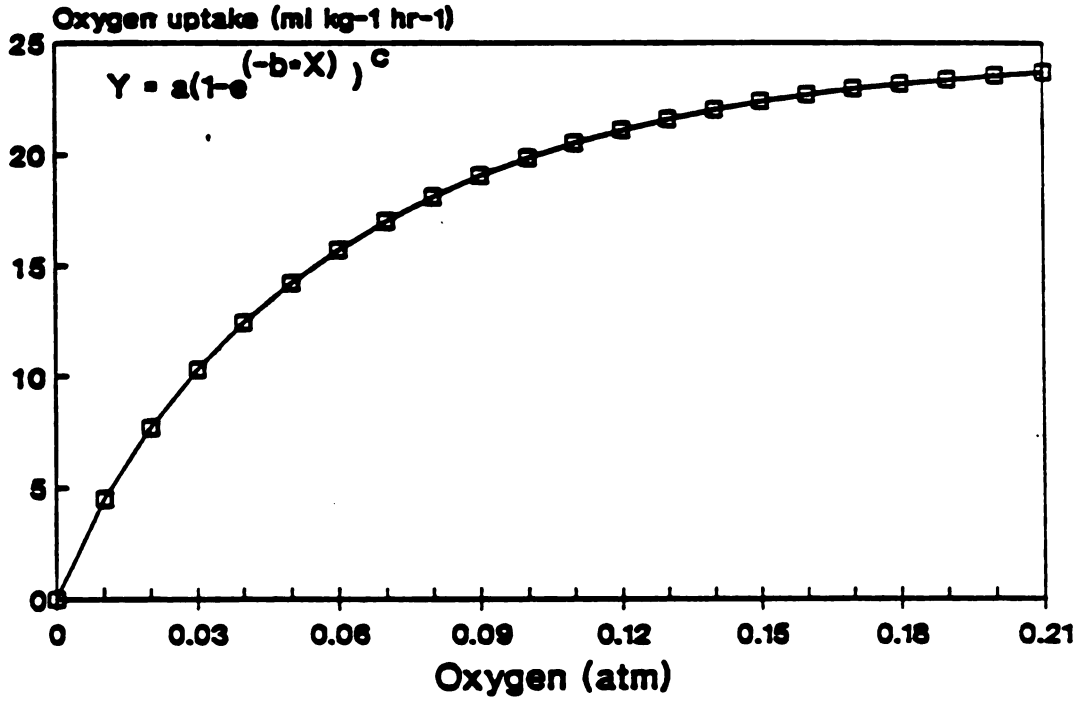


Figure 3. Plot of the first derivative of oxygen depletion curve in closed system versus oxygen concentration. Values of constants are 24.5417, 15.2347 and 0.8686 respectively for a, b and c.

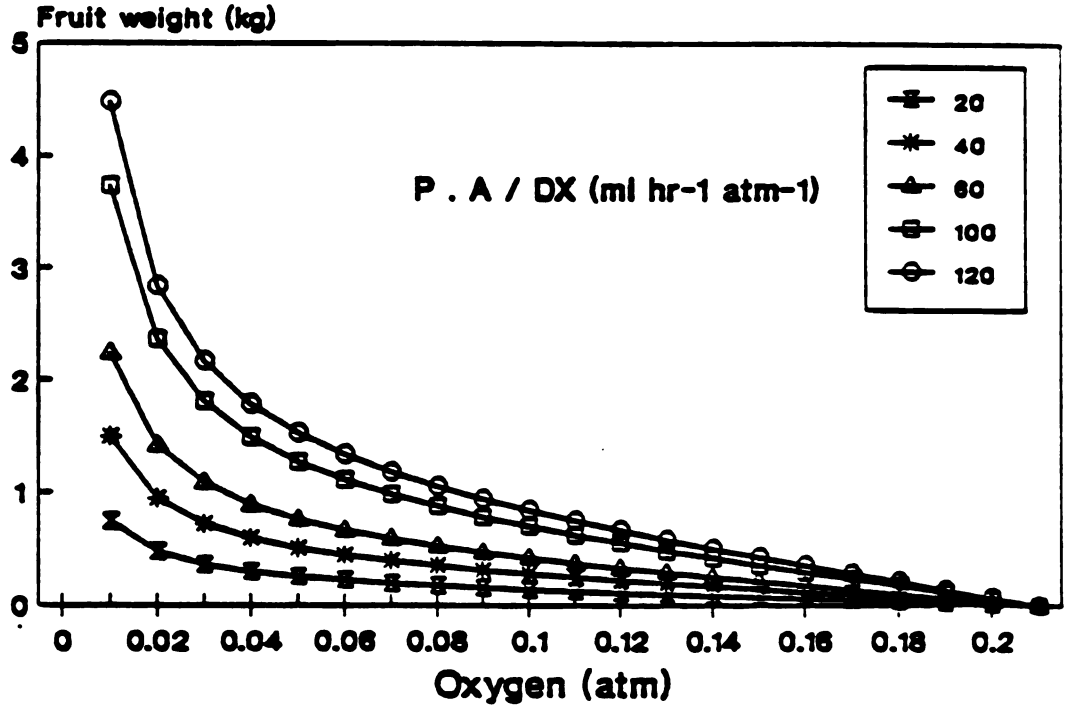


Figure 4. Predicted weight of apple fruit for a range of film characteristics that would generate desired O_2 (atm) in the sealed package at 20°C.

$$[O_2] = a \cdot e^{(b \cdot fw)} + c \qquad [10]$$

Where; $[O_2]$ = oxygen concentration (%), fw = fruit weight (kg) and, a, b and c are arbitrary constants.

Tabulated values of constants are presented in Table 4.

The calculated respiration rates from Equation (3) were plotted vs oxygen gradient and fitted to an equation curve for all 3 films combined as illustrated in Figure 6. The best fit equation was similar to the one obtained in closed system and had the form of Equation (6). Constants values were given in Table 5.

After substituting Equation (6) into Equation (3) and rearranging the equation in order to express film characteristic ($P \cdot A \cdot DX^{-1}$) the relationship yielded to:

$$P.A.DX^{-1} = \frac{a \cdot (1 - e^{-b \cdot [O2]})^c \cdot W}{([O_2]_{atm} - [O_2]_{pkg})} \qquad [11]$$

The difference between Equation (9) and (11) is rearrangement of different parameters. Equation 11 was used to generate data of film characteristics needed for a given fruit weight and desired oxygen concentration at equilibrium. Predicted data are presented in Figure 7.

Table 4. Values for constants of equation ($Y = a \cdot e^{(b \cdot X)} + c$) of oxygen concentrations in the package as a function of fruit weight for different LDPE films at 20°C.

	Constants		
Film thickness (μm)	a	b	c
25.4	0.225034	-0.32677E-02	0.2196E-02
44.4	0.21763	-0.533321	0.010094
50.8	0.2648669	-0.792680E-2	0.11095E-02

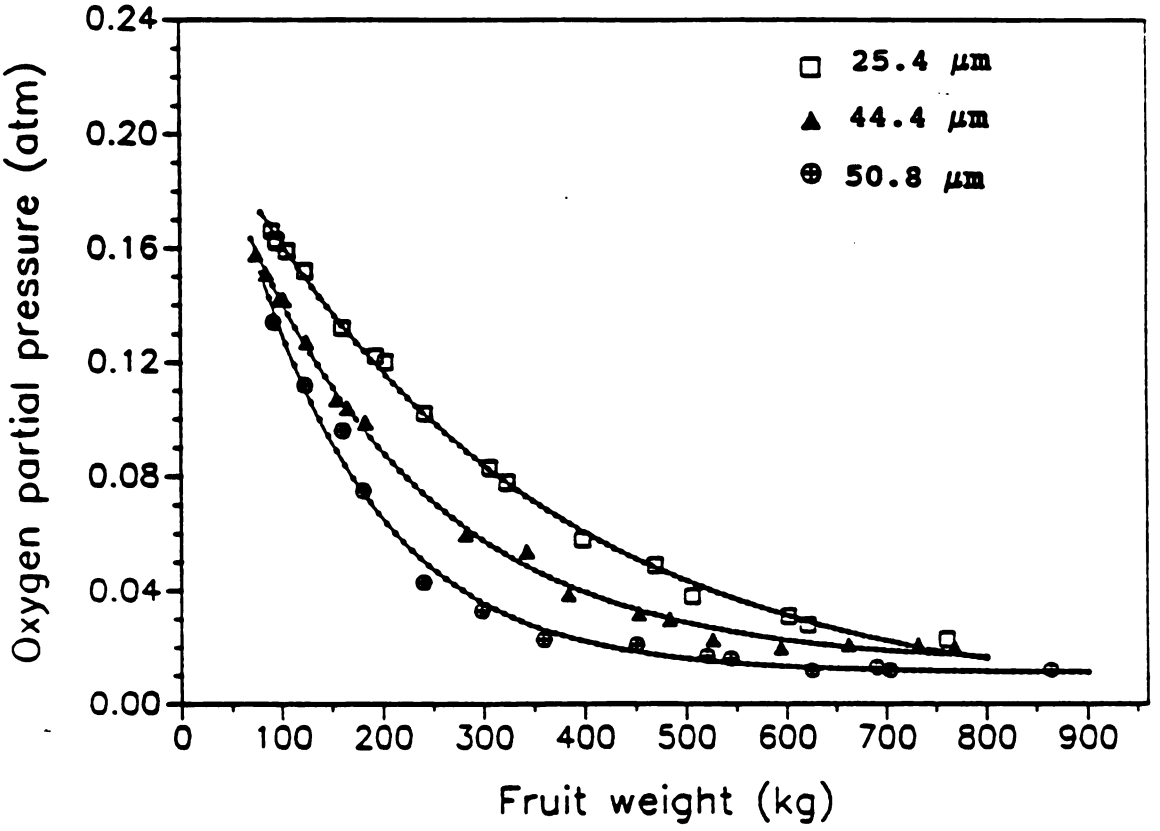


Figure 5. Oxygen concentration in different packages made from different LDPE films and the best fit curve at steady state for different 'Empire' apple fruit weights at 20°C.

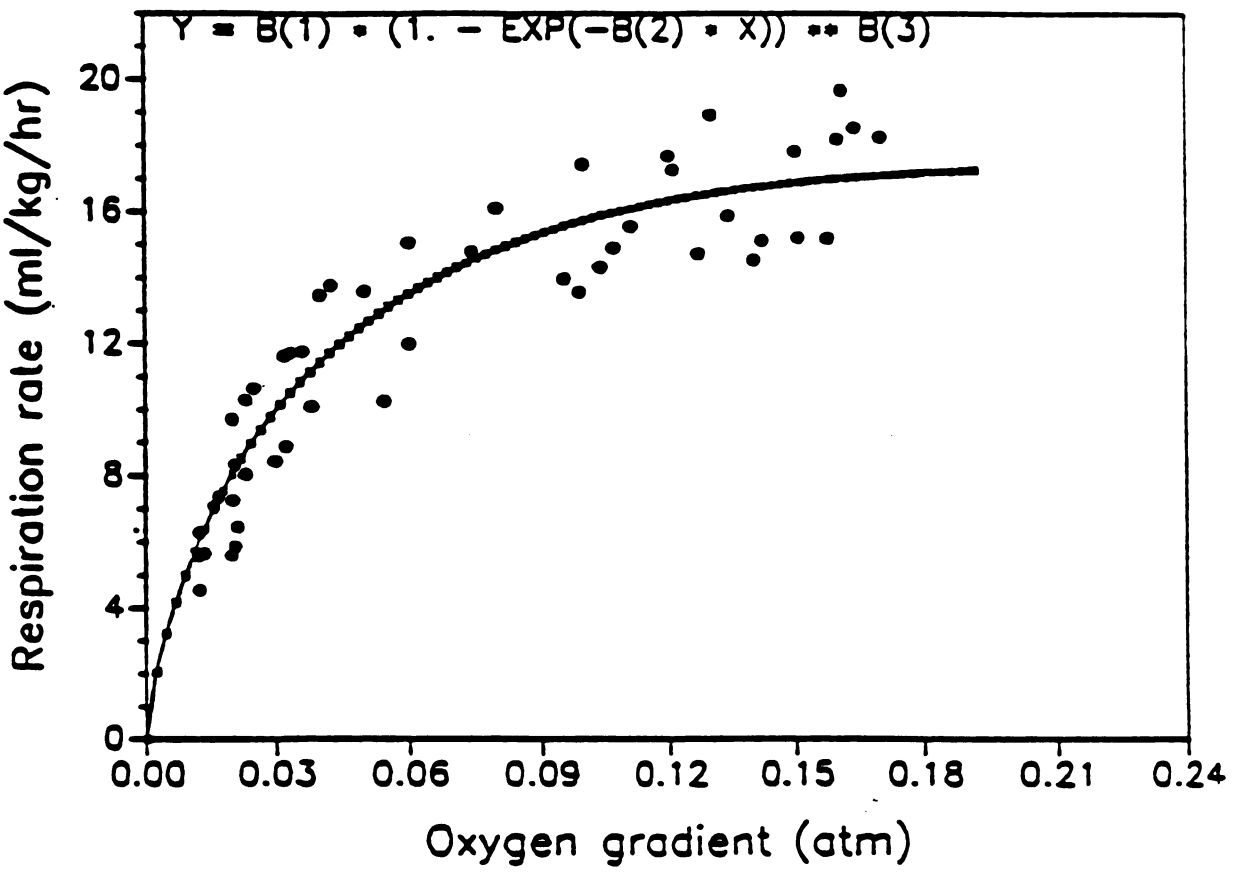


Figure 6. Oxygen uptake (ml kg_1hr^{-1}) of 'Empire' apple fruit from packages as a function of oxygen partial pressure at 20°C and the average data best fit curve for 3 films.

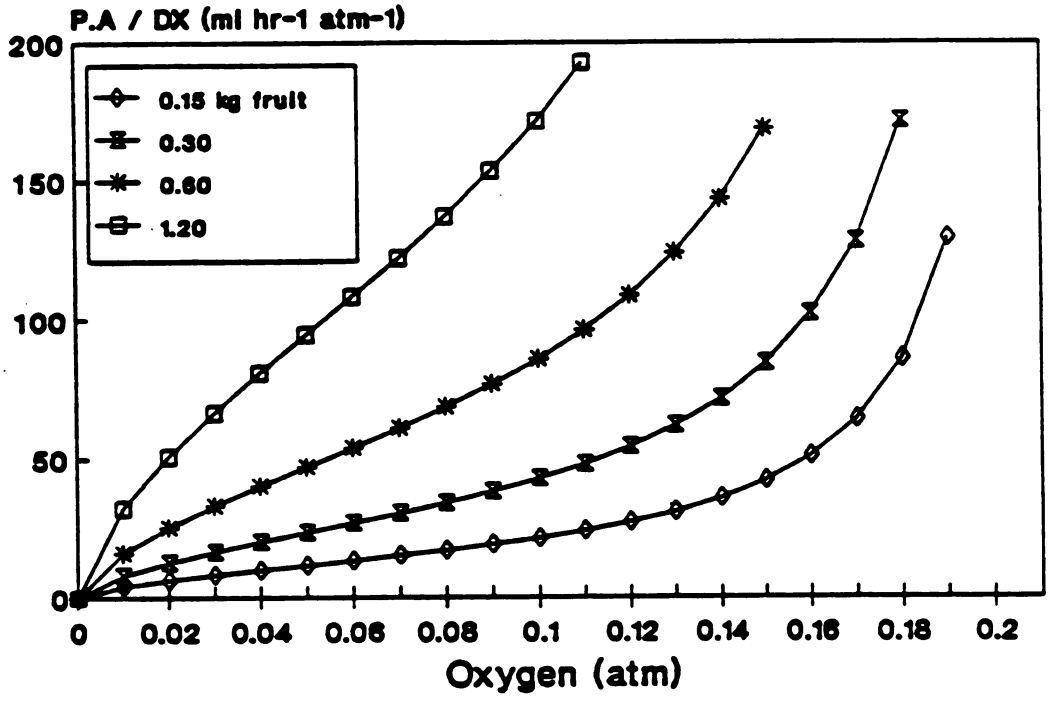


Figure 7. Predicted film characteristics required to establish a desired oxygen concentration for various apple fruit weights in a sealed package at 20°C.

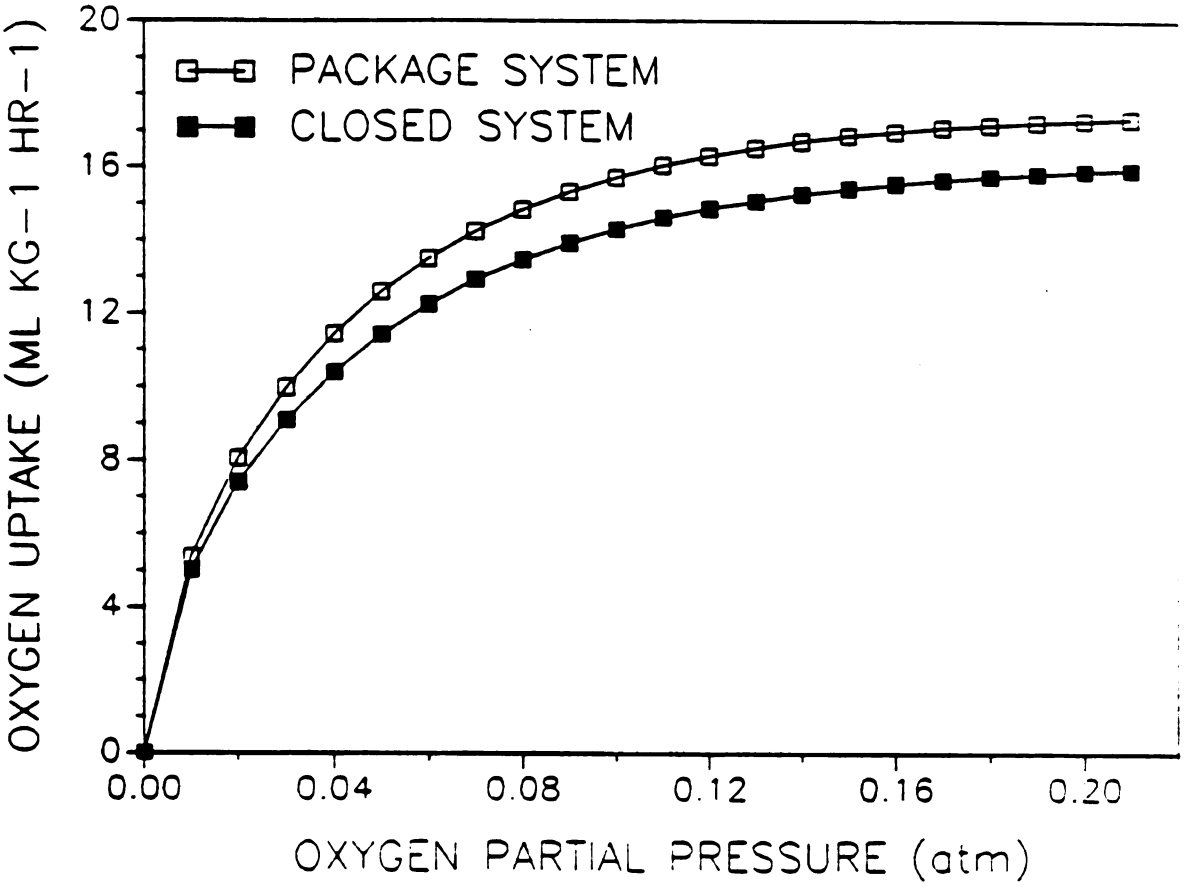


Figure 8. Plot of oxygen uptake (ml kg^{-1} hr^{-1}) versus oxygen partial pressure (atm) for closed system and package system. $Y = a\{1-e^{(-b\,fO2)c}$. Values of constants are given in Figure 2 for closed system and in Table 3 for package system respectively for a, b and c.

Table 5. Values for constants of equation ($Y = a \cdot (1 - e^{(-b \cdot X)})^c$ describing respiration rate as a function of oxygen gradient for each film and their average fit curve at 20°C.

	Constants values		
Film thickness (μm)	a	b	c
25.4	19.43273	15.64932	0.54026
44.5	15.72387	19.99732	0.798078
50.8	15.18553	58.81798	1.529614
Average curve	17.5764	18.782	0.673

DISCUSSION

The technique of oxygen depletion in a closed system as described for tomatoes by Cameron et al. (1989) permitted measurements of oxygen concentration without interference of errors or leaks associated with conventional sampling of the headspace. Oxygen depletion curves obtained in this study for apples were closely similar to what was found for tomatoes (Cameron et al. 1989). Results obtained from packaging trials showed similar oxygen uptake values with relatively small differences due to the permeability of each film. The best fit equation curve for MA packaging trials showed a similar pattern to that obtained with the closed system as illustrated in Figure 8. It was assumed in both methods that carbon dioxide has no or little effect on oxygen at steady state and this was supported by other research data shown in Section III of this work. The respiration rate is oxygen concentration dependent; the lowest values were obtained when the oxygen was low in packages with high fruit weight or with film less permeable to oxygen. Better understanding of the effect of oxygen on respiration rate and the minimum O_2 concentration in the package to prevent fermentation is needed in packaging research.

Data generated from Equation (9) and (11), for fruit weight and film characteristics and presented in Figures 4 and 7, constitute an interesting means to design the package model for apple fruit. For instance, if apples should be stored at 0.06 atm of oxygen at 20°C, Figure 4 shows that fruit weight should be ca. 0.30, 0.60 and 0.90 kg, respectively, for 20, 40 and 60 ml hr^{-1} atm^{-1} of film permeability constants for O_2. For 1.2 kg of apples stored at 0.04 and 0.06 atm of O_2, Fig. 7 indicates that film characteristics should be, respectively, 80 and 110 ml O_2 $hr^{-1}atm^{-1}$. For the latter example, if 4 apples were packed in 800 cm^2 film, $P.DX^{-1}$ varies from 10×10^{-1} and 13.75×10^{-1} cm, respectively, for 0.04 and 0.06 atm of O_2. This also indicates that there is a wide range of flexibility between the film characteristics and fruit weights that may be choosen to achieve

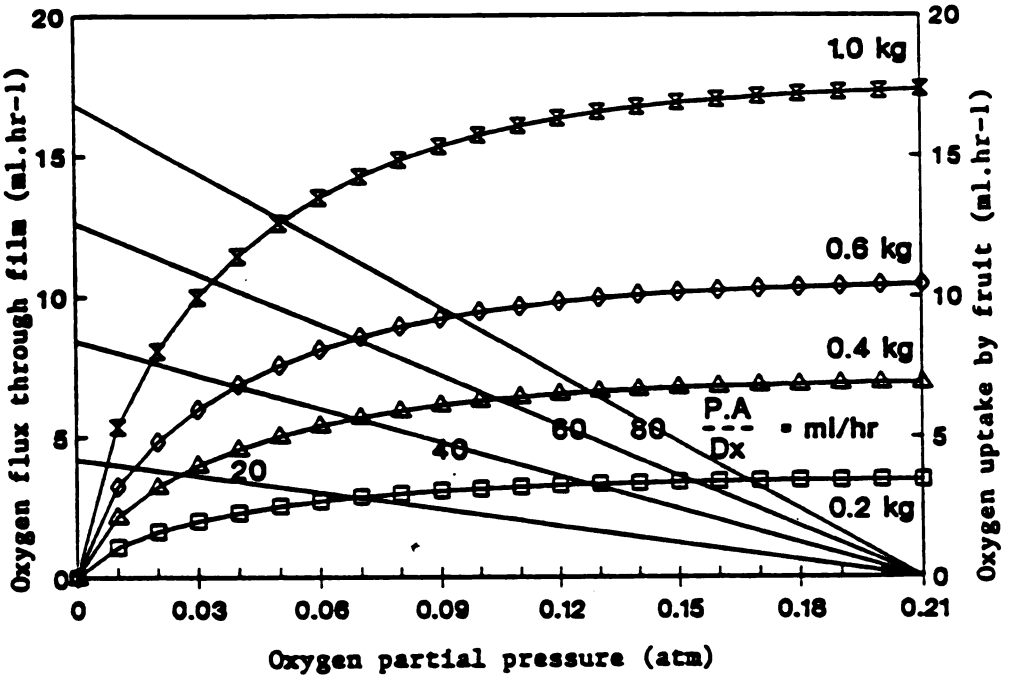


Figure 9. Plot of the rate of oxygen flux and rate of oxygen uptake by the apple fruit as a function of oxygen partial pressure (atm) in the package, film permeability characteristics (ml hr^{-1}) and apple fruit weight (kg). Each intersection between oxygen flux (solid line) and oxygen uptake (symbol) indicates the O_2 concentration at steady state for the film/fruit weight combination.

recommended conditions of storage of the commodity. Figures 4 and 7 show that increasing fruit weight and film thickness reduces the concentration of oxygen in the package. As the fruit weight in the package increases so also does oxygen depletion rate. Packages more permeable to O_2 are required to achieve the desired steady state oxygen partial pressure within sealed packages as product weight increases (Figure 9).

The results of this study shows that the empirical and mathematical approach are both applicable in developing packaging guidelines for fruits and vegetables. The flexibility obtained between film characteristics and fruit weight is wide and supports the feasability of developing a package system for some commodities (Cameron et al. 1989).

LITERATURE CITED

1. Barmore, C.R. 1987. Packing technology for fresh and minimally processed fruits and vegetables. J.Food Qual. 10: 207-217

2. Boylan-Pett, W. 1986. Modified Atmosphere Packaging for Tomatoes. MS. Thesis Michigan State University East Lansing MI.

3. Cameron, A.C., W. Boylan-Pett, and J.J. Lee. 1989. Design of Modified Atmosphere Packaging Systems: Modeling oxygen concentrations within sealed Packages of Tomato fruits. J. Food Sci. 54 (6): 1413-1416 & 1421.

4. Hayakawa, K., Y.S. Henig, and S.G. Gilbert. 1975. Formulae for predicting gas exchange of fresh produce in polymeric film package. J. Food Sci. 40: 186.

5. Henig, Y.S. 1975. Storage stability and quality of produce packaged in polymeric films, p. 144-152. In " Postharvest biology and handling of fruits and Vegetables. N.F Haard and D.K. Salunke (Ed) A.V.I. Westport, CN.

6. Henig, Y.S. and S.G. Gilbert. 1975. Computer analysis of the variables affecting respiration and quality of produce packaged in polymeric films. J. Food Sci. 40:1033.

7. Kader, A.A. 1986. Biochemical and physiological basis effects of controlled and modified atmospheres on fruits and vegetables. Food Technol. 40(5): 99-103.

8. Kawada, K. 1982. Use of polymeric films to extend postharvest life and improve marketability of fruits and vegetables-- Unipack: Individually wrapped storage of tomatoes, oriental persimmons, and grapefruit, p. 87-89. In "Controlled Atmosphere for the Storage and Transport of Perishable Agricultural Commodities" D.G. Richardson and M. Meheriuk (Ed) Oregan State Univ. School of Agr. Symp. Series. 1.

9. Prince, T.A., R.C. Herner and S.W. Gyszley. 1982. Storage of precooled tulip bulbs in controlled atmosphere packages. In "Controlled Atmospheres for the Storage and Transport of Perishable Agricultural Commodities" D.G. Richardson and M. Meheriuk (Ed), p. 77 Timber Press, OR.

10. Smith, S.G., J. Geeson and J. Stow. 1987. Production of modified atmospheres in deciduous fruits by the use of films and coatings. HortScience 22:772-776.

11. Zagory, D. and A.A. Kader. 1988. Modified Atmosphere Packaging of Fresh Produce. Food Technology 42:(9) 70-74 & 76-77.

SECTION III

MODIFIED ATMOSPHERE PACKAGING OF 'EMPIRE' APPLE FRUIT FROM CONTROLLED ATMOSPHERE STORAGE

INTRODUCTION

Controlled atmosphere (CA) storage of fruits to extend their seasonal availability is well documented in the literature. Except for apples and pears, CA storage is not widely used despite successful studies carried out in many countries around the world for different commodities, including bananas (McGlasson and Wills, 1971), cabbage (Geeson et al., 1977), oranges (Ben-Yehoshua, 1985), tomato (Kidd and West, 1932; Kawada, 1982; Dennis et al., 1979). Common CA conditions recommended, and widely used for several years, for apple storage were 3% oxygen and 5% carbon dioxide with some variance depending upon cultivar. In the early years of CA storage, these conditions were obtained by allowing fruit respiration to modify the storage atmosphere; in many countries this is still the practice with CA rooms of approximatively 100 - 200 ton capacity. Once the desired level of O_2 is reached, the O_2 and CO_2 levels are monitored and scrubbers are used to control CO_2 and the rooms are ventilated with air to maintain the desired O_2 concentration. In recent years, 3% O_2 : 5% CO_2 concentrations have been largely abandoned for many cultivars and now there is a world-wide trend to use Ultra Low Oxygen (ULO). O_2 concentrations as low as 1% and for some cultivars, lower CO_2 concentrations even to 1% CO_2 further prolong the storage period and keeping quality of some apple and pear cultivars. Several researchers, (Chen, 1985; Chen et al., 1985; Lange, 1985; Lau, 1985; Lidster et al., 1985) reported, independently, good results with oxygen below 2% and CO_2 kept around 1 to 1.5% in order to prevent anaerobic conditions and CO_2 injuries. ULO storage conditions are now widely used in most areas without major problems. Dilley (1987) recommended CA storage of Michigan apples at 1.5% O_2 and 3% CO_2. In England, Sharples and Stow (1982) reported that the use of 1.25% O_2 and 1% CO_2 permitted storage of 'Cox's Orange Pippin' apples for up to 9 months rather than 2.5 to 3 months in air. The authors reported that

the same conditions were recommended for storage of 'Idared' and 'Jonagold'. Truter and Eksteen (1987) in South Africa, working with 3 apple varieties ('Golden Delicious', 'Starking' and 'Granny Smith'), concluded that 1% O_2 and 1% CO_2 gave particularly good results in maintaining quality of apples after 9 months of CA storage. One of the major concerns of postharvest researchers on quality of fruit from CA is the suppression of fruit aroma and flavor (Bangerth, 1984). Despite the ability of the fruit stored at these low O_2 atmospheres to attain quality characteristics after exposure to ambient air at 0°C for 2 to 3 weeks (Lau, 1985), fruit firmness declines rapidly after removal from CA (Smith et al., 1987).

Obtaining and maintaining low concentrations of gases in CA rooms is sometimes a difficult and expensive practice for commercial stores. In order to preserve high quality fruit during distribution of fruit to market after CA the fruit must be maintained under refrigeration and moved to market as quickly as possible. Modified atmosphere packaging (MAP) offers a means for quality preservation that may be used as a supplement to or an alternative for refrigeration that may be employed for fruits at harvest or from CA storage. The principle of MAP is to create modified concentrations of oxygen, carbon dioxide and relative humidity within the headspace surrounding the commodity using film plastic. This technique was introduced for bulk transport and transit for the first time in the 1950's for holding horticultural produce and is still in limited use today.

MAP has been the subject of numerous articles and publications in the last decade (Kader, 1986). In theory, MAP in consumer units employing a polymeric film, a steady state concentrations of O_2 and CO_2 will be established inside package units due to fruit respiration and film permeation to O_2 and CO_2. Gas concentration at steady state is an important variable in extending the storage

period of fruits by MAP. Commodities may differ with respect to the optimum gas concentrations for maximum shelf life and quality preservation. Several researchers have investigated the use of polymeric films in order to create favorable atmospheric conditions for produce (Henig and Gilbert, 1975; Smith et al. 1985, 1987; Laurie et al.,1989; Allen and Allen, 1960; Tolle, 1962; Scott and Tewfik, 1947; Boylan-Pett, 1986) and the list is extensive for several commodities. Anaerobic conditions or very poor quality of the commodity was reported by Scott and Tewfik (1947), due possibly to insufficient permeability of films for O_2 and CO_2 and thus they advocated perforation of plastic films to overcome low O_2 concentration and build-up of CO_2. Today, most polyethylene bags for apple fruit have perforations.

Most of the early work in MAP was based on empirical methods. Some authors recognized the significance of developing predictive equations for desired gases at steady state and developed formulae or equations to optimize the package atmosphere. This methodology was first attempted by Jurin and Karel (1963) and Karel and Go (1964) for bananas. They developed a graphical approach for the model. Henig (1972) developed differential equations that estimated O_2 and CO_2 concentrations within the package unit. However, the optimization was not fully achieved and the model had some limitations. Film permeability characteristics were not included in the computer solution. Hayakawa et al. (1975), working with tomatoes, generated, from Henig and Gilbert's 1975 model, a mathematical approach and algebraic formulae that characterized the gas exchange of the tissue. The authors succeeded in developing their model. Other researchers succeeded in developing other techniques that characterize commodity respiration rate, film characteristics and other parameters to optimize their models (Cameron et al., 1989; Prince, 1980). Several patents have been issued covering the use of polymeric films to extend the

preservation period of perishable fruits and vegetables; most notable among them was the 'banavac' patents of the United Fruit Company (Badran, 1969)

The objective of this study was to evaluate the effects of atmospheric modification by MAP on quality retention of 'Empire' apple fruit following CA storage and to characterize the effects of films of different permeabilities at various storage temperatures.

MATERIALS AND METHODS

'Empire' apple fruit were harvested from a commercial orchard in the Grand Rapids, Michigan, area on Sept. 19, 1986. The fruit were preclimacteric with respect to ethylene production. Approximately 20 percent of the apple fruit had internal ethylene concentrations between 0.1 and 0.2 $\mu l.l^{-1}$ with the remainder of the fruits having lower levels. The average flesh firmness at harvest was 84 N, and the starch index was 4. Based on these maturity parameters, the fruit were judged to be of ideal maturity and physiological development for CA storage. These guidelines are based upon previous investigations with 'Empire' apple fruit (Fica et al.,1987) with provision that the fruit are subsequently cooled and placed in CA within 7 days of harvest.

The apple fruit were stored under CA at the Michigan State University Clarksville Horticultural Experiment Station. The CA rooms had a capacity of 2,500 bushels (approximately 50 tons). CA conditions of 1.5% O_2, 1.8% CO_2, greater than 96% RH were achieved within 4 days of harvest by using a Prism Alpha nitrogen generating system from Permea, Inc. (Monsanto Co, St Louis, MO). Storage temperature was 0°C. The nitrogen is used to purge the oxygen from CA rooms. This system was also used as a scrubber to purge carbon dioxide from the room to maintain CO_2 below 2% (Dilley, 1987, 1990) circumventing the need of other CO_2 scrubber systems.

Apple fruits were removed from CA after 4 months, and were kept overnight at 20°C after which they were randomly sorted into experimental sub-samples of sound fruit. A sample of 10 fruits was used to determine flesh firmness and another 10 fruits for CO_2 production at the temperatures employed for the packaging study to determine respiration rate.

Four low density polyethylene (LDPE) films of various thicknesses (25.4, 44.4, 50.8 and 76.2 μm corresponding to 1, 1.75, 2 and 3 mil respectively) from

Dow Chemical, Midland, Michigan, were tested at 5 different temperatures (0, 5, 10, 15, and 20°C). Bags of 25 x 25 cm with a surface area of 1250 cm^2, were made in the laboratory using an impulse heat sealing machine Model 420 from Audion, Holland. Four uniform fruits, each with an average weight of 130 $\pm$ 3 g were placed in each bag. Each treatment and control (unsealed bags) were comprised of 6 replications. A Tyvek pouch (5 x 5 cm dimension) containing 5 g $\pm$ 0.01g of magnesium oxide was used as a carbon dioxide scrubber and this was placed inside the bags unless otherwise stated. The packages were then stored in controlled temperature chambers where the relative humidity was kept at approximately 90%.

ANALYTICAL PROCEDURES

Oxygen and carbon dioxide. The concentrations of O_2 and CO_2 inside the packs were monitored in triplicate at two day intervals until steady-state was established. Steady-state was considered to be reached when the O_2 or CO_2 concentration in the packages stabilized. Gas sampling was done by withdrawing 1 ml samples of gas from the package headspace using plastic syringes equipped with a 25ga 1/2" hypodermic needle. The needle was inserted through a silicone rubber septum fixed to a 1.6 cm x 2 cm piece of polyethylene electrical tape on each bag. Carbon dioxide was measured with an infrared gas analyzer (ADC model SB300) employing N_2 as a carrier gas. In this procedure, the gas sample is injected as a pulse into the N_2 carrier gas through a section of latex rubber tubing leading to the IR detector cell. The IR detector signal is recorded as peak height on a strip chart recorder as the CO_2 now diluted by the carrier gas, passes through the detector cell. The nitrogen carrier gas flow rate was adjusted to provide a linear output from the IR detector cell proportional to the CO_2 in the gas sample or CO_2 standards injected. Carbon dioxide and O_2 in the packages

were also determined as needed by gas chromatography.

Ethanol evolution. At weekly intervals, 2 one ml samples of gas were withdrawn from the packs as described earlier for O_2 and CO_2, and injected into a Varian GC (Model 3400) equipped with a flame ionization detector. Helium was used as carrier gas. Ethanol standards were prepared to known concentrations ($\mu l.l^{-1}$) in the headspace vapor of an ethanol/water solution in a thermostatically controlled water bath. This was employed for calibration of the GC and determination of the ethanol concentration in the packages. The retention time for ethanol was 1.80 min. GC conditions for operation and detection were as follows: column, injection, and detector temperatures 135, 135 and 250°C respectively. The column support was Poropak Q 80/100 mesh (2 mm x 60 cm).

Flesh firmness. Flesh firmness was determined weekly with 8 uniform fruits (2 package units) with an average diameter of 65 to 70 mm. An Effigi penetrometer with an 11 mm diameter tip mounted on a drill press stand was employed. Readings were taken on the pound-force scale. Measurements were made on two opposite sides of each fruit on the pared surface after removing the skin. The two readings were averaged and converted to Newtons (N) prior to data analysis.

External and internal disorders. Visual observations for the presence of superficial disorders were made for each fruit before flesh firmness determination. After measuring flesh firmness, the fruits were sectioned equatorially and examined for internal disorders and anomalies.

Fruit volatiles determination. Volatiles produced by the fruits were determined at each assessment period, before being used for flesh firmness analysis and internal disorders assessment. Fruits from two packs with an average total weight of 1 kg, were placed in a 10 L desiccator which was flushed

continuously with air at a rate of 50 cc/min at 20°C for 5 hours. Fruit volatiles were collected using a 6 mm x 10 cm glass tube containing Tenax-GC (80/100 mesh) from ANSPEC, Ann Arbor, MI, as an adsorber. At the completion of the adsorption period the tubes were stored at -20°C until analyzed. Volatiles were extracted from the Tenax adsorbant with isopentane. The solvent (2 ml) was added by micropipette to the adsorption tubes which were fixed to test tubes with a rubber septum. The assembly was centrifuged for 5 min at 1500 x g to collect the isopentane extract. GC analysis was performed on aliquots of the isopentane solution with a Hewlett Packard GC, Model 5850 A, equipped with flame ionization detector. The column was a 0.25 mm x 60 m capillary column coated with Carbowax 20M. The carrier gas was He at 28 ml/min. The temperature was held at 30°C for 1 min then increased to 180°C at 2°C/min.

Data analysis. Analysis of variance was used to analyze the results and means were compared by the Student Newman-Keuls test at 5% when apropriate. Analysis were performed with a computer statistical package Costat.

RESULTS

Production rates of CO_2 at different temperatures after 4 months in CA storgae were given in Figure 1. As expected the rate of production is function of temperature.

In the absence of a CO_2 scrubber, carbon dioxide concentrations at equilibrium increased with temperature from 0 to 20°C for all film thicknesses employed (Figures 2 and 3). For the 25.4 μm films the values ranged from 2.7 to 5.1 % at 0 and 20°C, respectively; while for 76.2 μm films the values were 3.4 to 12.7 %, respectively, over the same temperature range. For the 44.4 and 50.8 μm films the CO_2 values followed the same trend but were intermediate in magnitude and in relation to film thickness.

Including a CO_2 scrubber in the package reduced the steady state level of CO_2 to similar levels for all film thicknesses at a given temperature. Moreover, the CO_2 levels were low and similar and increased only slightly over the temperature range of 0 to 20°C. The values ranged from 1.8 to 2.1% CO_2 at steady state at 0°C for packages made of film 25.4 μm and 76.2 μm thick, respectively. Even using the 76.2 μm film, the CO_2 level ranged from 2.1 to only 2.9 over a temperature range of 0 to 20°C; and 2.9% was the highest CO_2 value observed among all packages containing a CO_2 scrubber. This value (2.9 %) is less than one/fourth the level of CO_2 at steady-state (12.4%) found for the same film without a scrubber when package units were held at 20°C. Carbon dioxide levels as high as 12% can damage fruits of many apple cultivars, whereas CO_2 levels less than 3 % are normally considered safe over a wide temperature range.

These data clearly demonstrates the value of including an effective CO_2 scrubber within the package. The CO_2 scrubber prevented the accumulation of CO_2 above 3% over a temperature range of 0 to 20°C where fruit respiration may be expected to quadruple. It must be stressed that this interpretation is based

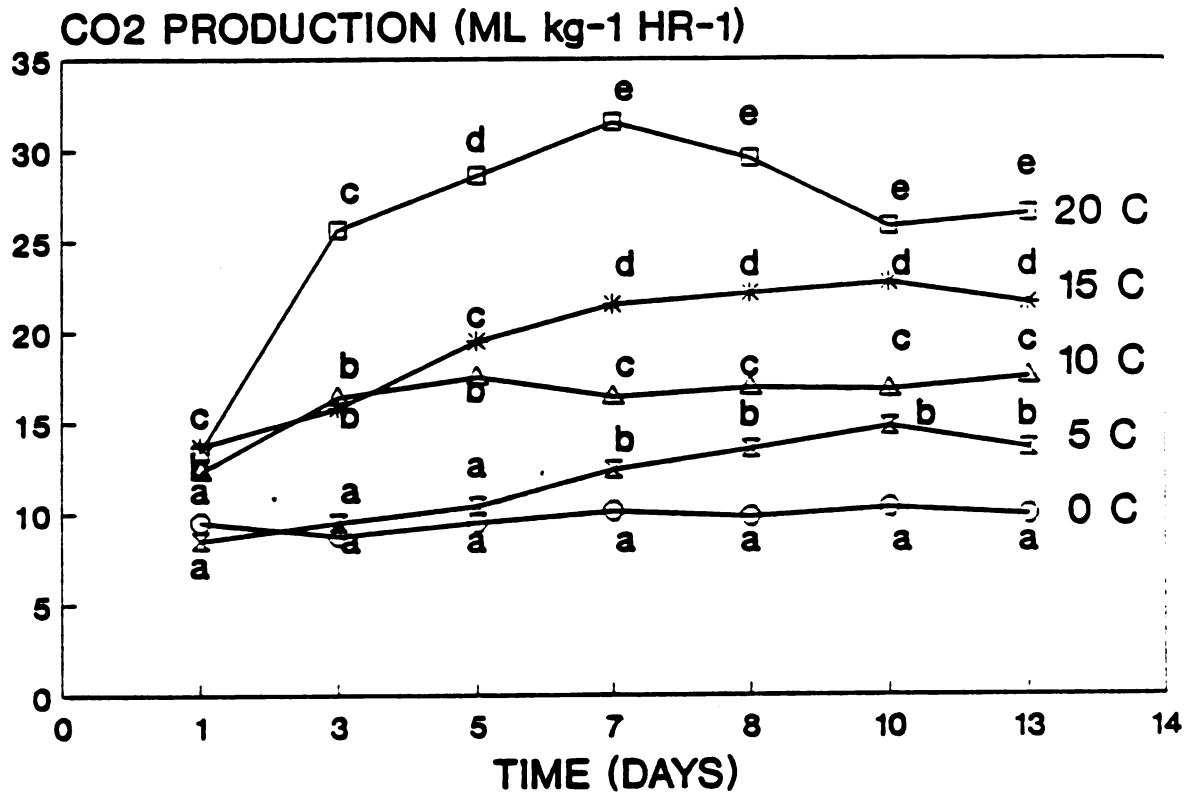


Figure 1. Production rates of carbon dioxide for 'Empire' apple fruit in semi-closed system at various temperatures. Fruits were held in CA for 4 months. Values followed by the same letter for each time (days) are not significantly different at p = 0.05.

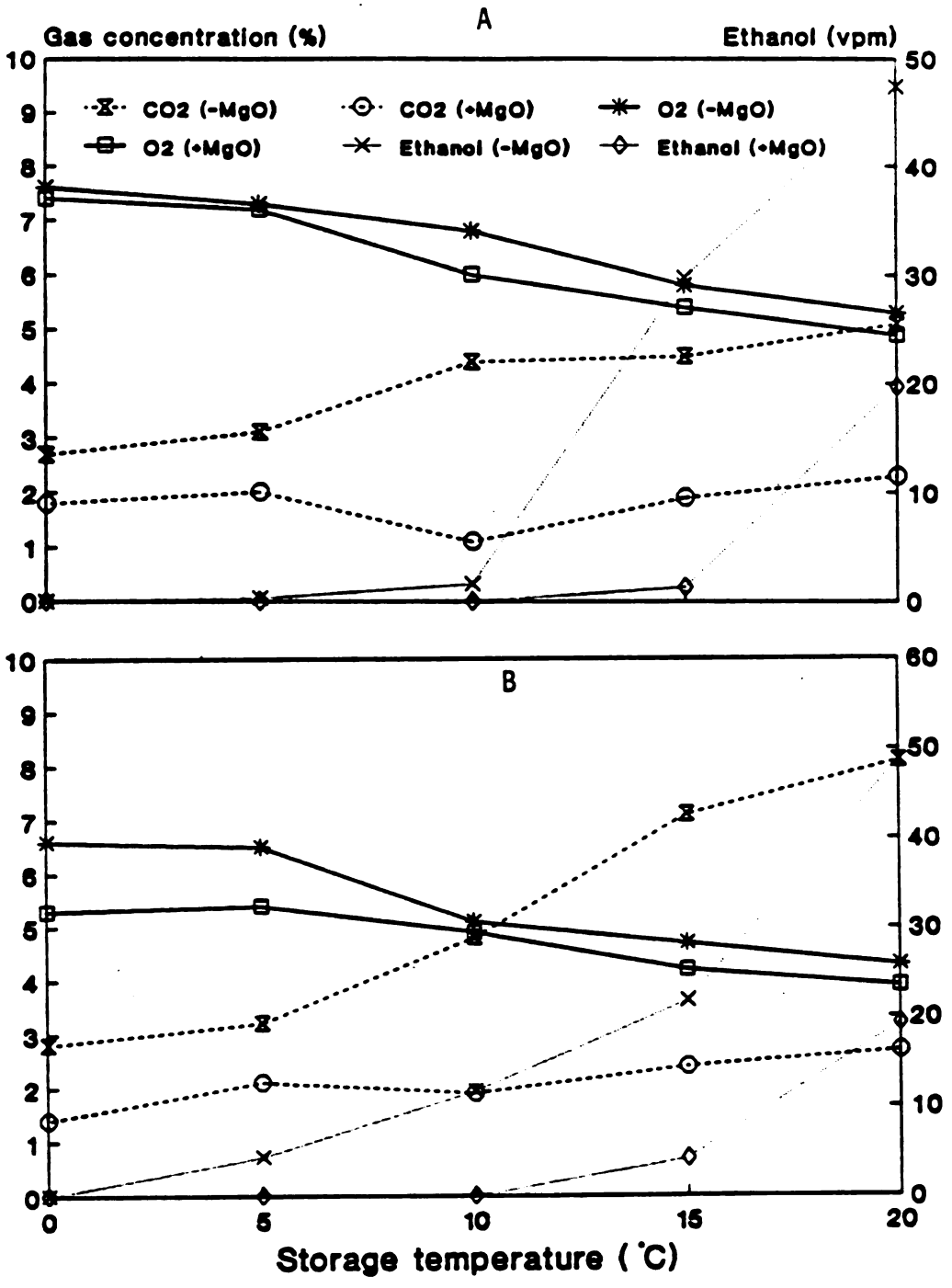


Figure 2. Gas concentrations of oxygen and carbon dioxide (%) and ethanol (vpm) at steady state within packages containing 'Empire' apple fruit made from 25.4 (A) and 44.4 (B) μm LDPE films. Fruits were held at various temperatures for 3 week period following 4 months of CA storage.

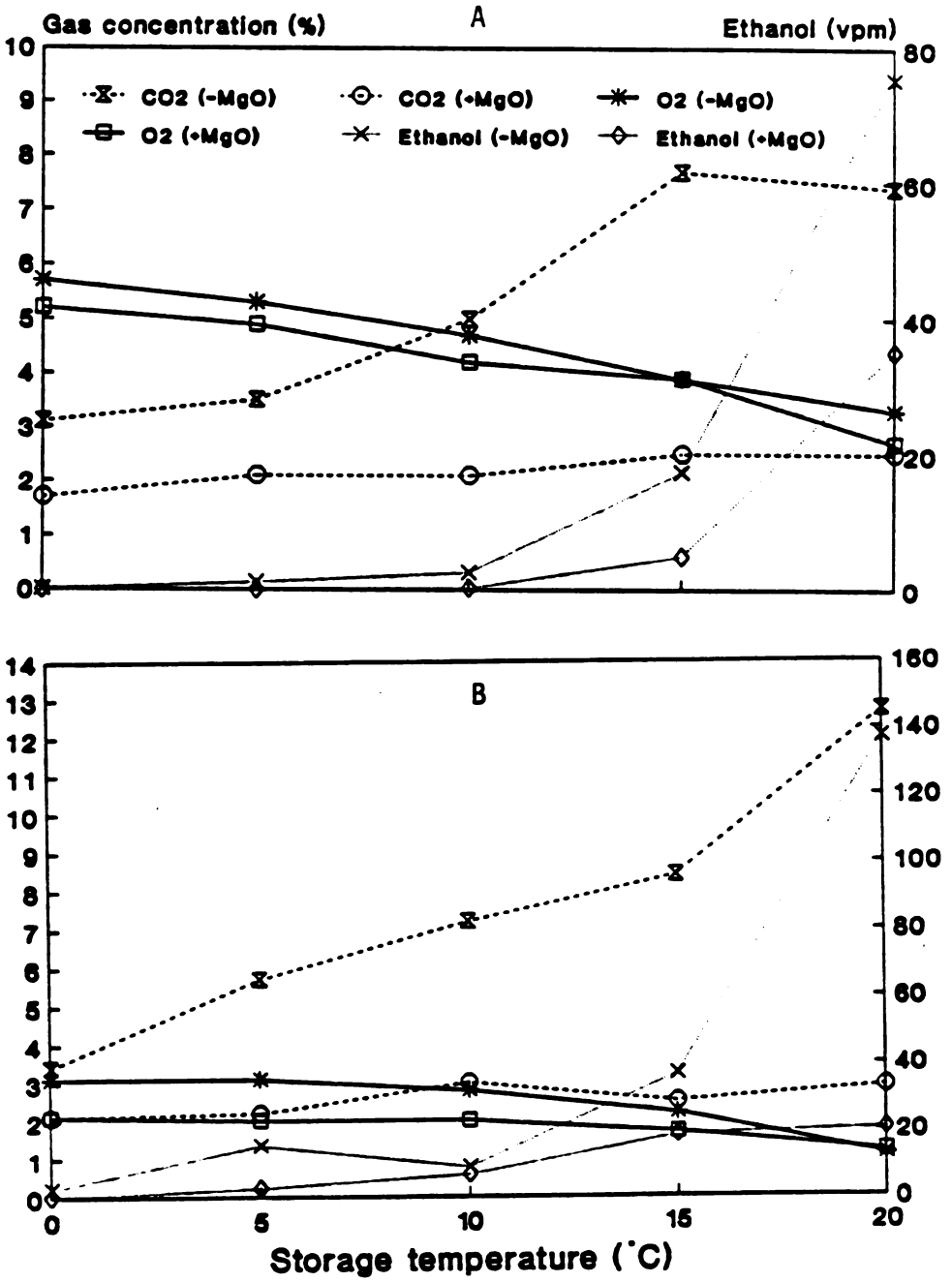


Figure 3. Gas concentrations of oxygen and carbon dioxide (%) and ethanol (vpm) at steady state within packages containing 'Empire' apple fruit made from 50.8 (A) and 76.2 (B) μm LDPE films. Fruits were held at various temperatures for 3 week period following 4 months of CA storage.

upon steady-state values for CO_2 and if the neutralizing capacity of the scrubber was exceeded by long periods at warm temperatures, CO_2 eventually accumulated to steady-state values similar to those observed without a scrubber.

Oxygen concentrations at steady-state in packages of 'Empire' apple fruit without a CO_2 scrubber were found to decrease as the temperature was raised from 0 to 20°C for all film thicknesses employed (Figures 2 and 3). For the 25.4 μm film the values ranged from 7.6 to 3.1% at 0 and 20°C, respectively; while for the 76.2 μm film the O_2 values were 3.1 to 1.1 %, respectively, over the same temperature range. The O_2 values for the 44.4 and 50.8 μm films were intermediate in magnitude as expected.

The presence of a CO_2 scrubber in the package consistently lowered, albeit insignificantly, the steady-state O_2 values reached with packages of all film thickness and over virtually all temperatures in comparison to O_2 steady-state values reached without a CO_2 scrubber.

The CO_2/O_2 ratios at steady-state within the packages were found to increase with temperature and with film thickness for packages with or without a CO_2 scrubber (Figure 4). For the 25.4 μm film the ratio values rose from 0.4 to 1.0 at 0 and 20°C, respectively; while for the 76.2 μm film the values were 1.1 to 4.7, respectively, over the same temperature range. For the 44.4 and 50.8 μm films the ratios followed the same general trend and were intermediate in magnitude between the thinner and thicker films. The CO_2/O_2 ratios for the packages with CO_2 scrubber were generally about half as large as for packages without CO_2 scrubber. An example of the change in O2 and CO2 within sealed packages as a function of time at 20°C for the 76.2 μm film is shown in Figure 4.

Ethanol vapor was found to accumulate in the packages with or without a CO_2 scrubber as the temperature increased above 15°C (Figures 2 and 3). This effect was generally less with a CO_2 scrubber with some notable exceptions with

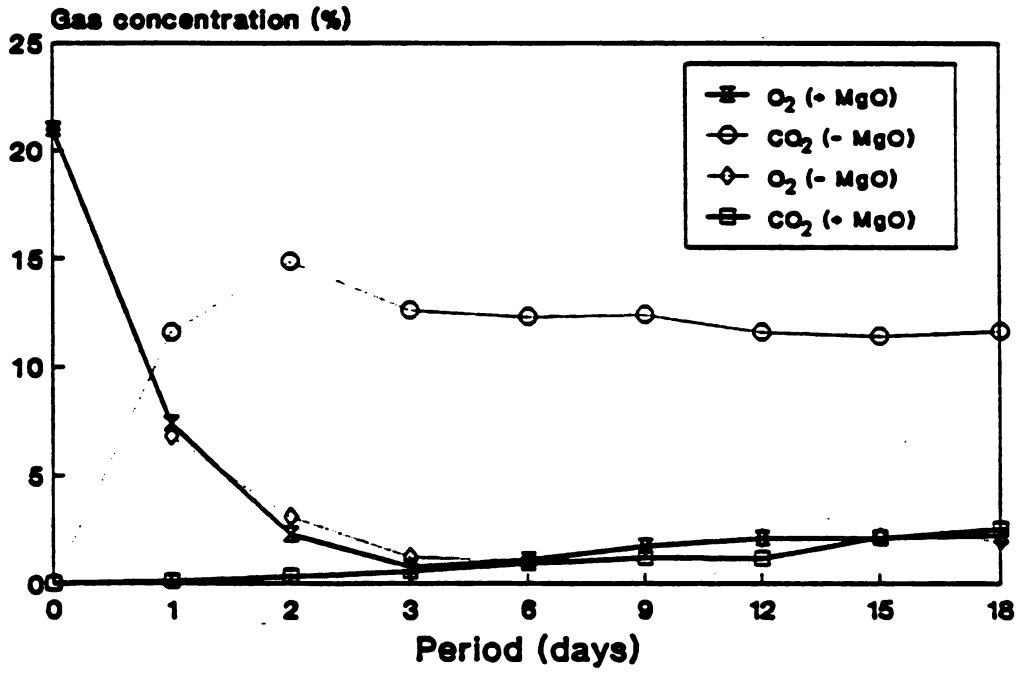


Figure 4. Changes in the concentrations of oxygen and carbon dioxide within packages containing 'Empire' apple fruit made from 76.2 μm LDPE film, with or without MgO and held at 20°C.

the 25.4 μm film. Ethanol accumulation increased at a given temperature as film thickness increased from 25.4 to 76.2 μm. The highest value recorded was 137 $\mu l.l^{-1}$ for the thickest film at the warmest temperature. With the exception of values taken at 20°C no values for ethanol exceeded $\mu l.l^{-1}$.

Flesh firmness. Fruit firmness data are presented in Tables 1a to 3a for packages without CO_2 scrubber and in Tables 1b to 3c for packages with CO_2 respectively, for 1, 2 and 3 week storage periods. At low temperatures of 0 and 5°C no difference in values of firmness was observed between unsealed and packaged fruit. However, increasing temperature up to 20°C and storage length up to 3 weeks led to significant firmness loss in unsealed packages. Similarly, no difference was found between different film thicknesses at these temperatures during the first two weeks of storage. After that, apple fruit from packages made of 25.4 and 44.4 μm LDPE films had higher firmness than 50.8 and 76.2 μm films. This was also true when fruit were stored at higher temperatures of 10 to 20°C. Including CO_2 adsorbant in the package had no significant effect on firmness retention.

External and internal disorders. At each removal period, fruit from different packs were inspected visually for any symptom of disorders. Some fruit kept at high temperature (20°C) and in the least permeable films had O_2 steady state levels near 1% O_2 and showed surface bleaching symptomatic of anaerobic injury. However, no internal browning or breakdown of the fruit was observed upon cutting the fruit in half. Eating quality of these fruits was acceptable after exposure to ambient air for 1 hour or more. Fruit from packages did not shrivel and the weight loss was significantly lower ($P \leq 0.05$) for packaged vs fruits in unsealed packages (6.73%). Although the weight loss of fruits was slightly higher for CO_2 scrubbed packs (1.65%) in comparison to those not scrubbed (1.32%) after a one week period, the pattern remained similar for both scrubbed and not

scrubbed packs. Weight loss (%) over the three week storage period was relatively low; 2.19% and 1.96%, respectively, for scrubbed and not scrubbed.

Fruit volatiles in MAP. Fruits which had been packaged eventually produced volatiles similar in type and quantities to those produced by 'Empire' apple fruit held under 1.5% and 3% O_2 at 20°C in the study conducted earlier. These fruit were from the same CA storage lot held under conditions of 1.5% O_2 and 1.8% CO_2 for 4 months at 0°C and did not begin to produce volatiles significantly until they had been transferred to air at 20°C for 7 days. This indicates that these CA conditions for a period of 4 months delayed ripening. The pattern of volatiles production of fruits kept in air from 1 through 21 days at 20°C is shown in Figs 5a to 5d. It is evident from the progression of the nature, magnitude and complexity of volatile components produced from day 1 through day 21 that the major components that contribute to flavor and aroma developed as the fruit progressed from an unripe to fully ripe then overripe condition.

Figures 5a to 5d will serve as the guide for comparing the volatiles produced by the apple fruit packaged in various films. Only the data on volatiles produced by fruit held at 20°C is presented. This is considered to be the most strigent comparison and extreme conditions expected to be encountered in handling 'Empire' in MAP. It should also be noted that some packages became nearly anaerobic over the 3 week period at 20°C so anomalies among volatiles between these and those fruits kept in the strickly aerobic environment might be expected and noteworthy with respect to normal flavor and aroma development. The Figures for volatiles produced by fruit with and without a CO_2 scrubber are provided as CO_2 accumulation to levels above 5% may be expected to cause some metabolic effects.

The first general impression to be gained from volatiles produced by MAP fruit is that fruit volatiles never reached the complexity nor magnitude of

production compared to fruit kept in air over the 3 week period. This can be seen comparing Figure 5d for air vs Figure 6c through Figure 9c for MAP fruit in the various film thicknesses. The fruits kept in air for 3 weeks at 20°C were obviously overripe and quite senescent by this time so many of the volatiles were likely being derived from extensive membrane degradation associated with advanced ripening. However, even after the shorter durations, the air stored fruit showed a more complex pattern of volatiles than fruit from packages as seen in Figures 5b and c vs Figures 6a and b through Figures 9a and b for MAP fruit.

The second general effect observed was that fruit in MA packages with a CO_2 scrubber generally exhibited a more complex pattern of volatiles than MAP fruit without a scrubber. This may reflect the retarding effect of CO_2 accumulation on ripening development. For fruit held for 21 days in MAP with 76.2 μm films without a CO_2 scrubber, the volatiles pattern (Figure 9c) is quite complex and this may be indicative of a CO_2 induced metabolic disturbance perhaps linked to fermentation as the O_2 level in these packages was near 1% O_2 and CO_2 was over 12%.

Table 1a. Flesh firmness[z] (N) of 'Empire' apple fruit[y] from CA storage after holding at various temperatures and packaged without a CO_2 scrubber in LDPE films of various thickness for 1 week.

	TEMPERATURE OF STORAGE (°C)				
	0	5	10	15	20
FILM THICKNESS (μm)					
25.4	73.5ab	73.8a	73.1c	72.7b	73.3a
44.4	72.1a	70.6a	66.9ab	65.2a	63.7b
50.8	75.3b	74.3a	64.2a	64.3a	62.8b
76.2	73.4ab	72.6a	67.9b	66.5a	68.2b
UNSEALED	71.3a	72.3a	66.1ab	64.2a	62.3b
Mean	73.1	72.7	67.6	66.6	66.0
$LSD_{0.05}$	02.21	02.73	02.39	03.03	02.45

[z] Data followed by the same letter within columns are not significantly different at p = 0.05

[y] Flesh firmness at removal from CA was 84 N.

Table 1b. Flesh firmness[z] (N) of 'Empire' apple fruit[y] from CA storage after holding at various temperatures and packaged with a CO_2 scrubber in LDPE films of various thickness for 1 week.

	TEMPERATURE OF STORAGE (°C)				
	0	5	10	15	20
FILM THICKNESS (μm)					
25.4	74.2ab	74.0a	72.8b	73.0b	71.5a
44.4	71.6a	71.9a	69.4ab	65.8a	64.3a
50.8	76.4b	75.3a	68.4ab	63.6a	63.4a
76.2	71.4a	71.6a	69.8ab	65.3a	67.6a
UNSEALED	71.3a	72.2a	66.1a	64.2a	62.3a
MEAN	72.9	73.0	69.3	66.4	65.8
$LSD_{0.05}$	03.18	02.98	04.31	06.19	07.80

[z] Data followed by the same letter within columns are not significantly different at p = 0.05

[y] Flesh firmness at removal from CA was 84 N.

Table 2a. Flesh firmness[z] (N) of 'Empire' apple fruit[y] from CA storage after holding at various temperatures and packaged without a CO_2 scrubber in LDPE films of various thickness for 2 weeks.

	TEMPERATURE OF STORAGE (°C)				
	0	5	10	15	20
FILM THICKNESS (μm)					
25.4	69.9a	68.7c	66.6b	70.0b	67.9c
44.4	70.1a	64.7b	64.3ab	63.0a	60.3a
50.8	69.4a	65.2b	59.4ab	67.8b	61.3a
76.2	68.6a	65.8b	63.2ab	63.3a	65.1b
UNSEALED	67.2a	62.2a	61.5ab	61.8a	59.7a
MEAN	69.0	65.3	63.0	65.2	62.8
$LSD_{0.05}$	03.06	02.09	03.82	03.69	01.61

[z] Data followed by the same letter within columns are not significantly different at p = 0.05

[y] Flesh firmness at removal from CA was 84 N.

Table 2b. Flesh firmness[z] (N) of 'Empire' apple fruit[y] from CA storage after holding at various temperatures and packaged with a CO_2 scrubber in LDPE films of various thickness for 2 weeks.

	TEMPERATURE OF STORAGE (°C)				
	0	5	10	15	20
FILM THICKNESS (μm)					
25.4	70.2ab	69.7b	68.1c	68.1b	66.5a
44.4	71.3b	66.3ab	65.5bc	62.7a	63.4a
50.8	68.6ab	65.6ab	62.6ab	66.7ab	63.7a
76.2	69.4ab	65.2ab	59.4a	63.4a	61.3a
UNSEALED	67.2a	62.2a	61.5ab	61.8a	59.7a
MEAN	69.3	66.2	63.3	64.5	63.0
$LSD_{0.05}$	02.76	04.48	03.31	03.71	05.09

[z] Data followed by the same letter within columns are not significantly different at p = 0.05

[y] Flesh firmness at removal from CA was 84 N.

Table 3a. Flesh firmness[z] (N) of 'Empire' apple fruit[y] from CA storage after holding at various temperatures and packaged without a CO_2 scrubber in LDPE films of various thickness for 2 weeks.

	TEMPERATURE OF STORAGE (°C)				
	0	5	10	15	20
FILM THICKNESS (μm)					
25.4	68.2b	66.3d	65.1c	62.6d	60.2c
44.4	68.6b	62.8bc	58.3b	56.8bc	55.6b
50.8	67.8b	64.2cd	55.4a	54.3b	57.8c
76.2	64.3a	60.5ab	55.9a	58.8c	58.3c
UNSEALED	63.5a	58.9a	54.6a	51.9a	48.3a
MEAN	66.5	62.5	57.8	56.9	56.0
$LSD_{0.05}$	01.99	02.30	02.31	02.56	02.03

[z] Data followed by the same letter within columns are not significantly different at p = 0.05

[y] Flesh firmness at removal from CA was 84 N.

Table 3b. Flesh firmness[z] (N) of 'Empire' apple fruit[y] from CA storage after holding at various temperatures and packaged with a CO_2 scrubber in LDPE films of various thickness for 2 weeks.

	TEMPERATURE OF STORAGE (°C)				
	0	5	10	15	20
FILM THICKNESS (μm)					
25.4	67.4ab	67.3c	64.7c	71.7c	60.5c
44.4	69.5b	63.2b	60.5b	53.8ab	51.6b
50.8	65.9ab	61.6b	56.9a	56.4b	58.4c
76.2	65.7ab	62.3b	53.2a	52.3a	60.2c
UNSEALED	63.5a	58.9a	54.6a	51.9a	48.3a
MEAN	66.4	62.6	58.0	55.2	55.8
$LSD_{0.05}$	03.09	02.54	03.1	03.11	02.37

[z] Data followed by the same letter within columns are not significantly different at p = 0.05

[y] Flesh firmness at removal from CA was 84 N.

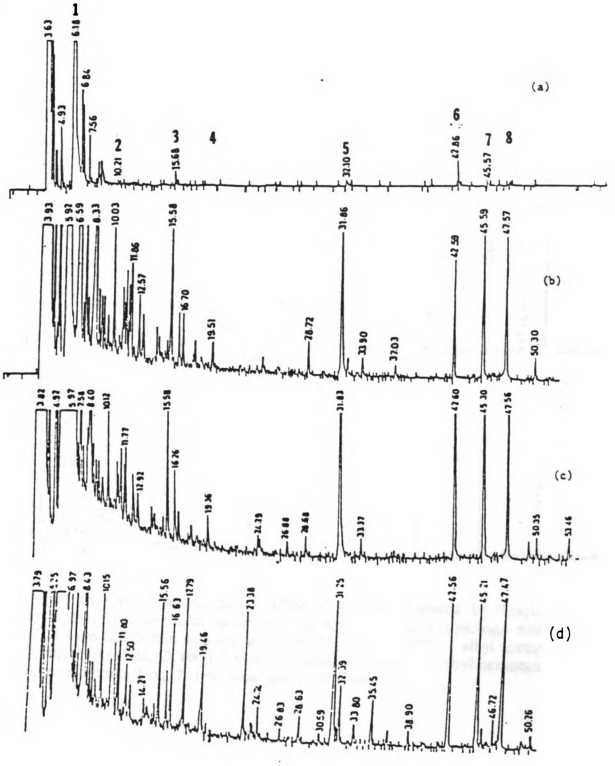


Figure 5. Gas chromatographic profile of volatiles produced by 'Empire' apple fruit from controlled atmosphere storage after 1 day in air at 20°C. 1. ethyl acetate, 2. ethyl propionate, 3. ethyl-2-methyl butyrate, 4. hexanal, 5. ethyl hexanoate, 6. trans-3-hexen-1-ol, 7. 1-hexenol and 8. cis-3-hexen-1-ol.

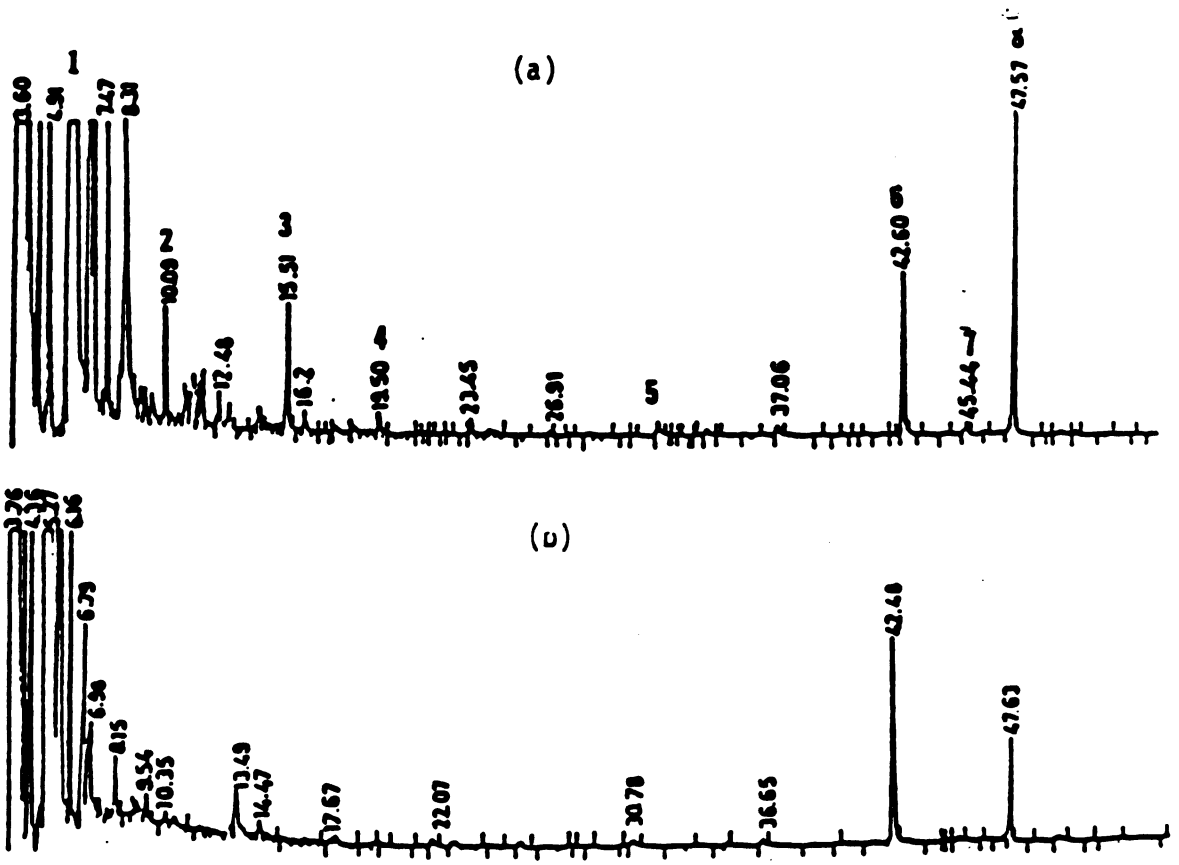


Figure 6a. Gas chromatographic profile of volatiles produced by 'Empire' apple fruit from controlled atmosphere storage after 7 days in package made of 25.4 μm LDPE film with (A) or without MgO (B) at 20°C, 1. ethyl acetate, 2. ethyl propionate, 3. ethyl-2-methyl butyrate, 4. hexanal, 5. ethyl hexanoate, 6. trans-3-hexen-1-ol, 7. 1-hexenol and 8. cis-3-hexen-1-ol.

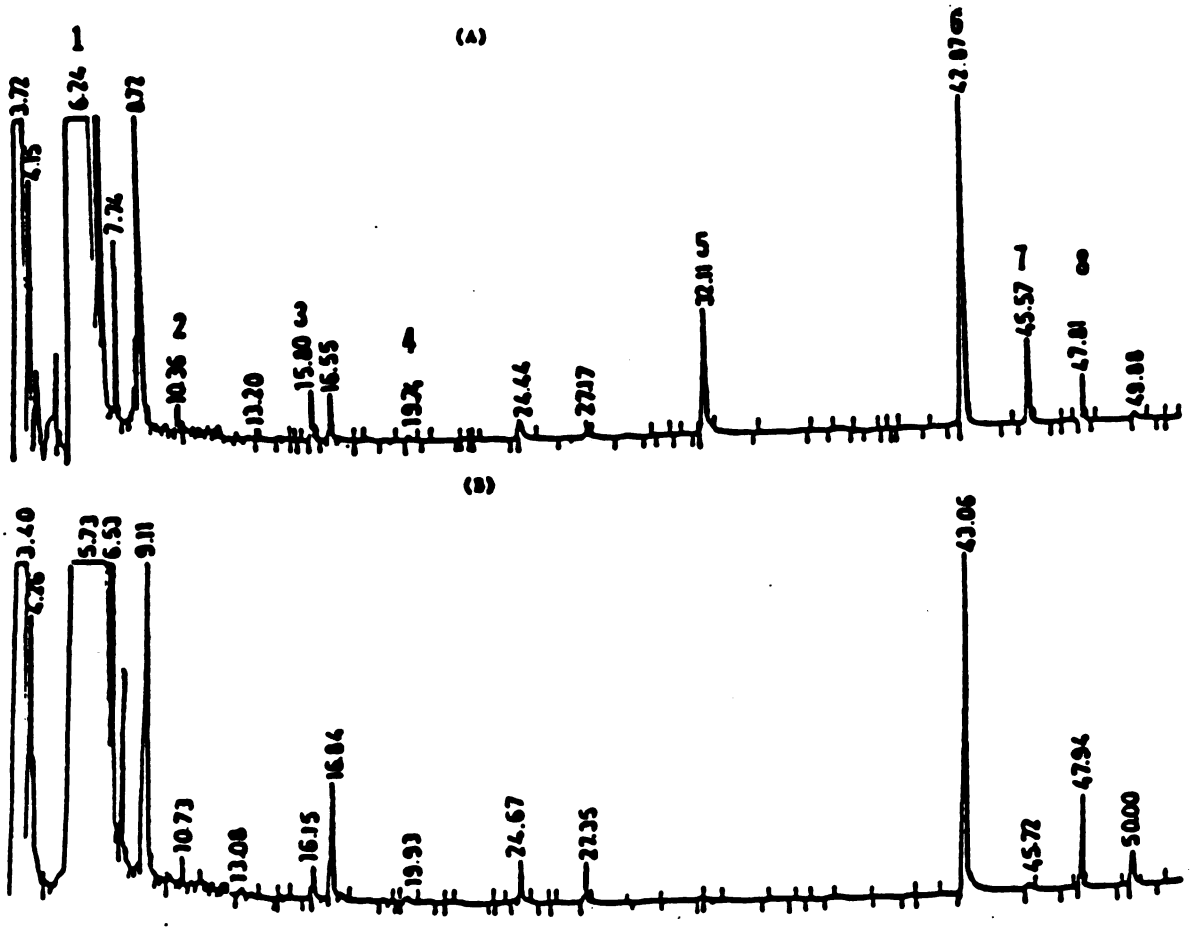


Figure 6b. Gas chromatographic profile of volatiles produced by 'Empire' apple fruit from controlled atmosphere storage after 14 days in package made of 25.4 μm LDPE film with (A) or without MgO (B) at 20°C, 1. ethyl acetate, 2. ethyl propionate, 3. ethyl-2-methyl butyrate, 4. hexanal, 5. ethyl hexanoate, 6. trans-3-hexen-1-ol, 7. 1-hexenol and 8. cis-3-hexen-1-ol.

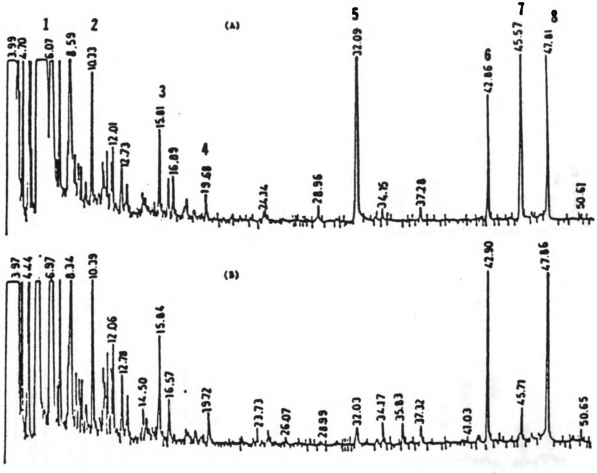


Figure 6c. Gas chromatographic profile of volatiles produced by 'Empire' apple fruit from controlled atmosphere storage after 21 days in package made of 25.4 μm LDPE film with (A) or without MgO (B) at 20°C, 1. ethyl acetate, 2. ethyl propionate, 3. ethyl-2-methyl butyrate, 4. hexanal, 5. ethyl hexanoate, 6. trans-3-hexen-1-ol, 7. 1-hexenol and 8. cis-3-hexenol.

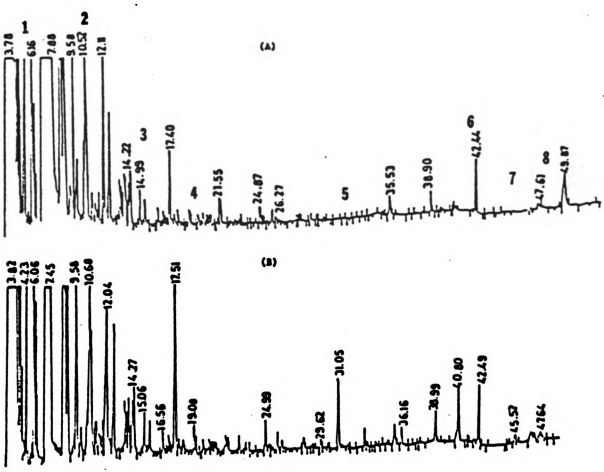


Figure 7a. Gas chromatographic profile of volatiles produced by 'Empire' apple fruit from controlled atmosphere storage after 7 days in package made of 44.4 μm LDPE film with (A) or without MgO (B) at 20°C, 1. ethyl acetate, 2. ethyl propionate, 3. ethyl-2-methyl butyrate, 5. ethyl hexanoate, 6. trans-3-hexen-1-ol, 7. 1-hexenol and 8. cis-3-hexen-1-ol.

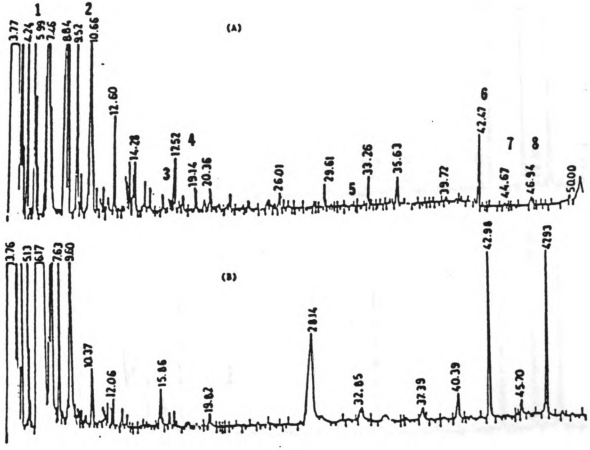


Figure 7b. Gas chromatographic profile of volatiles produced by 'Empire' apple fruit from controlled atmosphere storage after 14 days in package made of 44.4 μm LDPE film with (A) or without MgO (B) at 20°C. 1. ethyl acetate, 2. ethyl propionate, 3. ethyl-2-methyl butyrate, 4. hexanal, 5. ethyl hexanoate, 6. trans-3-hexen-1-ol, 7. 1-hexenol and 8. cis-3-hexen-1-ol.

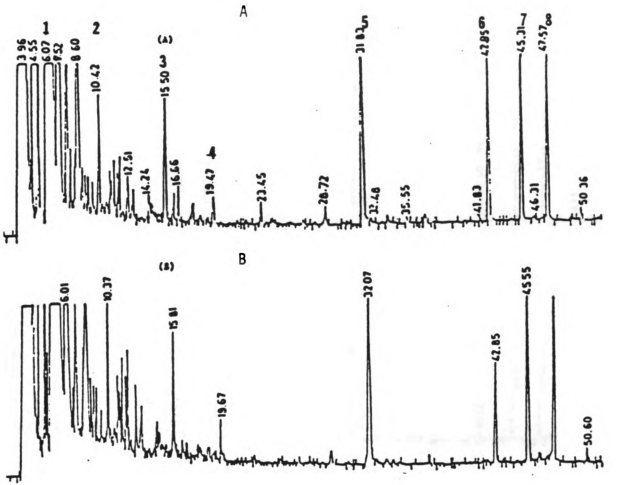


Figure 7c. Gas chromatographic profile of volatiles produced by 'Empire' apple fruit from controlled atmosphere storage after 21 days in package made of 44.4 μm LDPE film with (A) or without MgO (B) at 20°C, 1. ethyl acetate, 2. ethyl propionate, 3. ethyl-2-methyl butyrate, 4. hexanal, 5. ethyl hexanoate, 6. trans-3-hexen-1-ol, 7. 1-hexenol and 8. cis-3-hexen-1-ol.

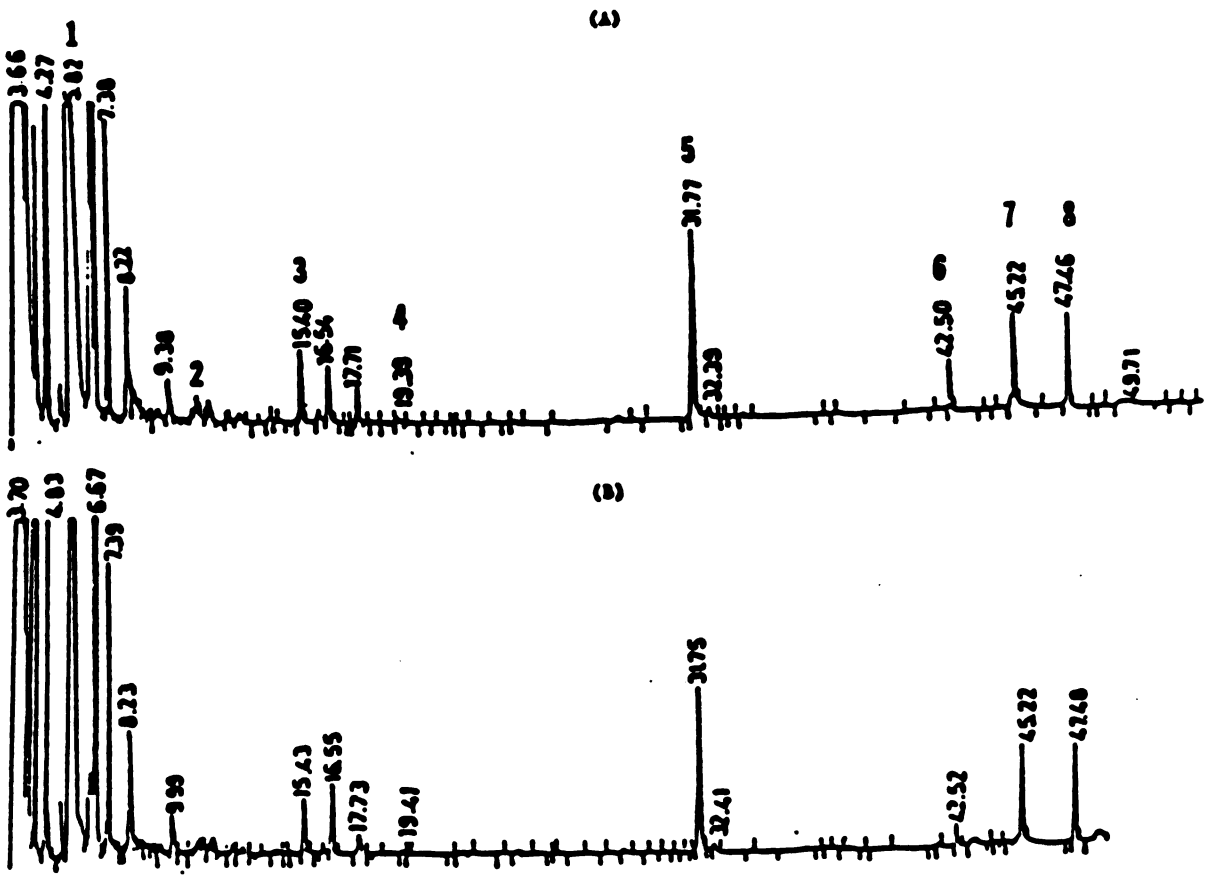


Figure 8a. Gas chromatographic profile of volatiles produced by 'Empire' apple fruit from controlled atmosphere storage after 7 days in package made of 50.8 μm LDPE film with (A) or without MgO (B) at 20°C, 1. ethyl acetate, 2. ethyl propionate, 3. ethyl 2-methyl butyrate, 4. hexanal, 5. ethyl hexanoate, 6. trans-3-hexen-1-ol, 7. 1-hexenol and 8. cis-3-hexen-1-ol.

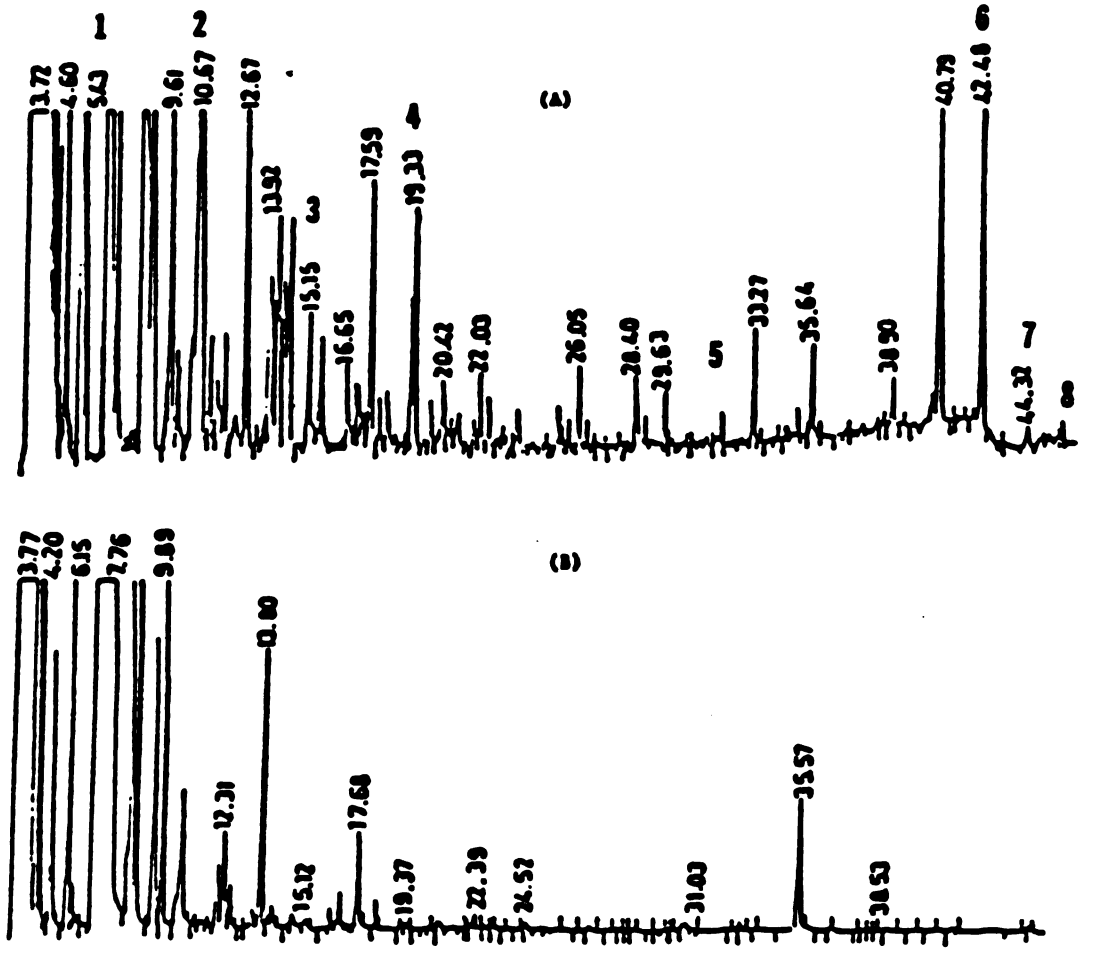


Figure 8b. Gas chromatographic profile of volatiles produced by 'Empire' apple fruit from controlled atmosphere storage after 14 days in package made of 50.8 μm LDPE film with (A) or without MgO (B) at 20°C, 1. ethyl acetate, 2. ethyl propionate, 3. ethyl-2-methyl butyrate, 4. hexanal, 5. ethyl hexanoate, 6. trans-3-hexen-1-ol, 7. 1-hexenol and 8. cis-3-hexen-1-ol.

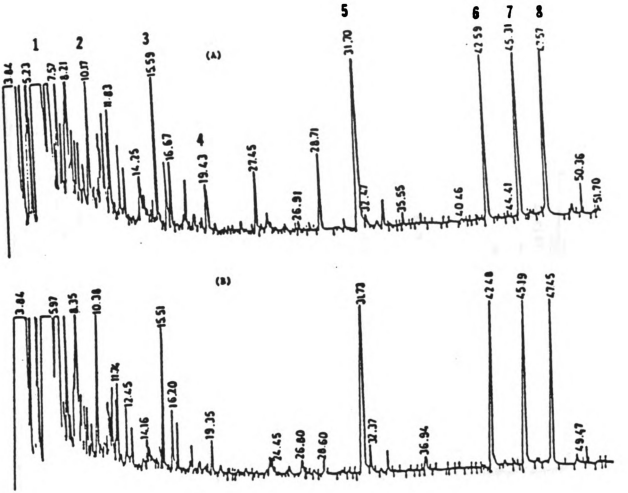


Figure 8c. Gas chromatographic profile of volatiles produced by 'Empire' apple fruit from controlled atmosphere storage after 21 days in package made of 50.8 μm LDPE film with (A) or without MgO (B) at 20°C. 1. ehtyl acetate, 2. ethyl propionate, 3. ethyl-2-methyl butyrate, 4. hexanal, 5. ethyl hexanoate, 6. trans-3-hexen-1-ol, 7. 1-hexenol and 8. cis-3-hexen-1-ol.

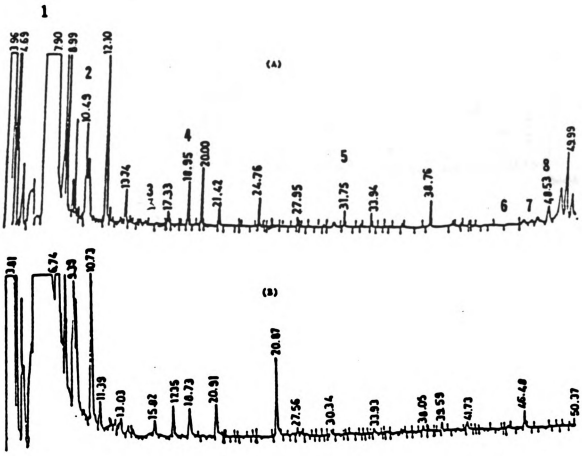


Figure 9a. Gas chromatographic profile of volatiles produced by 'Empire' apple fruit from controlled atmosphere storage after 7 days in package made of 76.2 μm LDPE film with (A) or without MgO (B) at 20°C. 1. ethyl acetate, 2. ethyl propionate, 3. ethyl-2-methyl butyrate, 4. hexanal, 5. ethyl hexanoate, 6. trans-3-hexen-1-ol, 7. 1-hexenol and 8. cis-3-hexen-1-ol.

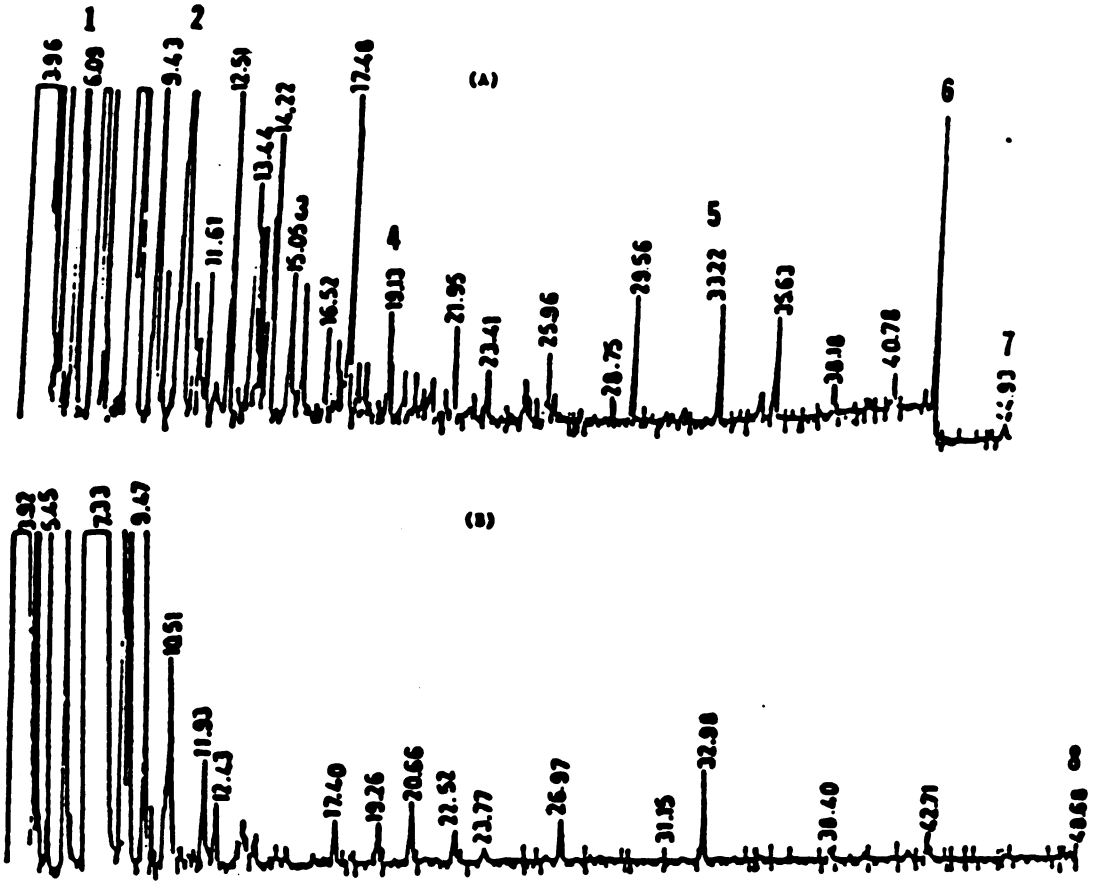


Figure 9b. Gas chromatographic profile of volatiles produced by 'Empire' apple fruit from controlled atmosphere storage after 14 days in package made of 76.2 μm LDPE film with (A) or without MgO (B) at 20 °C. 1. ethyl acetate, 2. ethyl propionate, 3. ethyl-2-methyl butyrate, 4. hexanal, 5. ethyl hexanoate, 6. trans-3-hexen-1-ol, 7. 1-hexenol and 8. cis-3-hexen-1-ol.

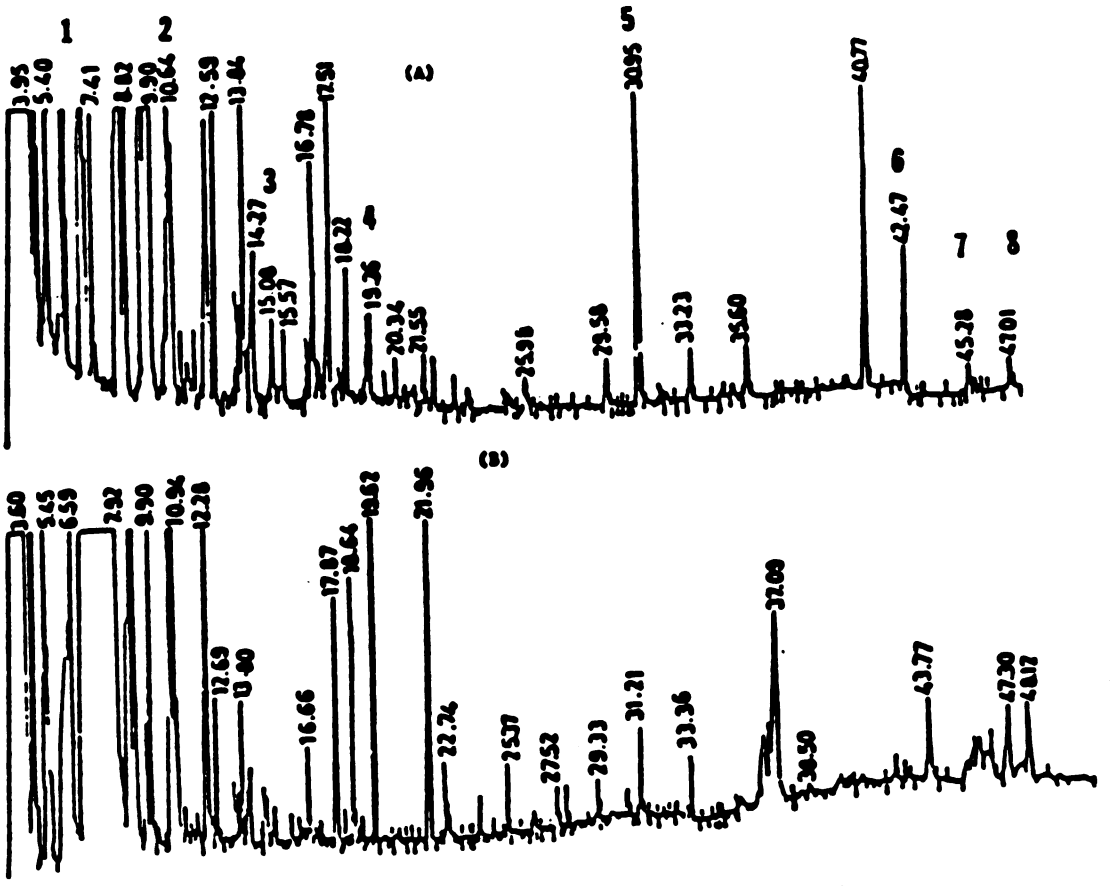


Figure 9c. Gas chromatographic profile of volatiles produced by 'Empire' apple fruit from controlled atmosphere storage after 21 days in package made of 76.2 μm LDPE film with (A) or without MgO (B) at 20°C. 1. ethyl acetate, 2. ethyl propionate, 3. ethyl-2-methyl butyrate, 4. hexanal, 5. ethyl hexanoate, 6. trans-3-hexen-1-ol, 7. 1-hexenol and 8. cis-3-hexen-1-ol.

DISCUSSION

The gas concentration inside MAP units containing 'Empire' apple fruit without a CO_2 scrubber showed a rapid decline of oxygen and an accumulation of CO_2 during the first 2 to 3 days of storage. This rate of change was progressively greater as the temperature was increased from 0 to 20°C and as film thickness increased from 25.4 to 76.2 μm. The CO_2 concentration rose to a maximum of 15% with the 76.2 μm film at 20°C and then stabilized at a fairly constant lower level over a 3 week period. This behavior is typical and is related to the rate of CO_2 production in relation to the film permeation rate for CO_2 (Tomkins, 1962).

The presence of MgO as a CO_2 scrubber only slightly altered the pattern of O_2 reduction in packages. The explanation for this may lie with the lower levels of CO_2 obtained in packages with a scrubber. Carbon dioxide, a product of respiration, is known to inhibit respiration via a feed-back mechanism. Comparison of the data of CO_2 and O_2 in Figures 2 and 3 reveals that as the CO_2 level increased with temperature in packages without a CO_2 scrubber the O_2 level did not generally decrease as much as in packages with a scrubber. A slightly lower respiration rate would reduce the O_2 gradient and result in slightly higher steady-state O_2 values in packages without CO_2 scrubbers with a given film thickness. Another factor that may contribute to this observation may be the slight reduction in void volume in packages with the CO_2 scrubber. Packages with less void volume would be expected to come to steady-state O_2 levels more quickly than packages with larger void volumes. It is unclear however, that high CO_2 obtained in packages without the adsorbant slows the respiration and thus O_2 at steady state remains high.

Steady-state concentrations of O_2 and CO_2 in packages were determined by fruit weight and respiration, by film characteristics and by temperature. The levels of O_2 and CO_2 attained are roughly proportional to film thickness and

temperature of storage and the trend is illustrated in Figure 4. Steady-state was reached fastest in the least permeable film; with equilibrium reached within 3 to 4 days at 20°C compared to about 10 days at 0°C (data not shown). This behavior is due to film permeability characteristics which are temperature dependent. The effect of temperature was very significant, reflecting both the higher rate of respiration as temperature increased and the temperature effect on film permeation. The combined effect affects the establishment of equilibrium conditions within the package units. Steady-state values will differ between packages at different temperatures unless the rate of diffusion through the film barrier is affected by temperature to the same extent as respiration. However this is not the case, the steady-state concentrations at lower temperatures were, respectively, high for oxygen and low for carbon dioxide. The steady state values reached changed as the temperature increased and, as a result, the level of O_2 decreased and CO_2 increased in the package.

Film permeability considerably affected the steady-state concentrations of O_2 and CO_2. High CO_2 concentrations (15%) and very low O_2 (less than 1%) were obtained with 76.2 μm thick film at 20°C as shown in Fig. 5. However, these conditions tended to stabilize at equilibrium. Similar observations were reported for other commodities: Boylan-Pett (1986) and Geeson et al. (1985) for tomatoes; Smith et al. (1987) for apples and Prince (1982) for tulip bulbs.

The use of a CO_2 scrubber in the packages tended to suppress the accumulation of CO_2. Eventually, however, a burst of CO_2 occured as the Mg0 was neutralized. This was true for all treatments but varied in time in temperature and in film thickness. This pattern was correlated to temperature and may be explained by the saturation of the chemical adsorbant as reported earlier (Boylan-Pett, 1986). Tomkins (1966) found that the level of CO_2 in the packages did not significantly affect the O_2 assimilation. However, in this

study, the O_2 equilibrium value was generally lower in packages with a CO_2 scrubber which kept CO_2 at lower steady state values. This is consistent with high CO_2 levels suppressing O_2 uptake by the fruit. The CO_2 absorbant tended to also limit water condensation in the packages. This was also seen in preliminary experiments (data not shown) using 15 g vs 5 g of MgO per package with 0.5 kg of fruits wherein this was associated with a higher weight loss of fruit. However, the adsorption isotherm of the MgO scrubber was not determined nor was the actual humidity within the packages, although some packages showed water condensation on the inner surface of the film.

Ethanol vapor accumulated inside packages dependent upon temperatures and this was independent of the presence of a CO_2 scrubber. Ethanol accumulation in the bags was positively correlated to film thickness. Increasing both temperature and film thickness resulted in the greatest ethanol accumulation (Figures 2 and 3). As the concentration of O_2 at equilibrium was considered to be above the extinction point for alcoholic fermentation yet ethanol accumulated, it is speculated that apple fruit while in CA may produce ethanol and was released at warm temperature also CO_2 content inside the packages may have had a direct effect on ethanol production. It is generally recognized that high levels of CO_2 can induce fermentation of plant tissu. Ethanol levels were higher at high CO_2 levels in packages at similar O_2 levels. Ethanol levels were generally lower with the MgO CO_2 scrubber in the package and this is consistent with this interpretation. Oxygen values at equilibrium at 15 and 20°C may have been below the extinction point of alcoholic fermentation for some tissues of the fruit before reaching steady state and this would allow ethanol production. Correlation coefficients of ethanol concentration vs O_2 level were very significant and varied from ($r = 0.57$ to $r = 0.93$). The coefficients were calculated for each film at different temperatures with or without the use of magnesium oxide (data not

presented).

Ethanol accumulation in the package is symptomatic of alcoholic fermentation by the fruit tissue. Cases where ethanol accumulated to high levels were associated with low O_2 levels and/or high CO_2 levels. High levels of CO_2 can induce fermentation even at relatively adequate O_2 levels (above the extinction point) which normally would not support alcohol formation. This may explain the lower ethanol levels found in packages with a CO_2 scrubber at equal or nearly equal O_2 levels at similar temperatures.

The accumulation of CO_2 in the package is partly a consequence of fruit respiration from aerobic processes at O_2 levels above the extinction point of fermentation. And at O_2 levels below the extinction point of fermentation, CO_2 would be derived increasingly from alcoholic fermentation as O_2 is depleted in the tissue. The actual extinction point of fermentation for the fruit as a whole entity is not a single fixed value but rather a term that should be considered at a subcellular level; namely at the mitochondrial level where the CO_2 is converted to water in oxidative phosphorylation. Cytochrome oxidase, the terminal electron acceptor in respiration has a very high affinity for O_2 and is considered to be saturated at O_2 levels much lower than 1%. Thus, fruit cells near the surface of fruits in 1% O_2 may still be respiring aerobically while those deep within the fruit flesh may become deprived of O_2 by gas diffusion limitation in relation to oxygen demand and progressive depletion of O_2 consequentially results in anaerobic respiration or fermentation (Lougheed, 1987).

The results of this study showed also that good retention of flesh firmness, reduced weight loss, good external appearance, and good organoleptic quality of fruits was maintained by modified atmosphere packaging. Greater beneficial effects of MAP were found at the higher storage temperatures of 15 and 20°C than at the lower temperatures of 0 and 5°C. At 20°C fruits in the least

permeable film tended to retain firmness during the first week, but subsequently firmness decreased with increasing period of storage to levels similar to those of the other films.

Fruits that had been packaged produced volatiles similar in type and relative quantity to those produced from fruits held under the continuous flow CA system studied in Section I. These fruits were from CA storage at 1.5% with 1.8% CO_2 for 4 months at 0°C and did not begin to produce volatiles significantly until they had been transferred to air at 20°C for 7 days. This indicates that these CA conditions delayed ripening development for a period of 4 months. Modified atmosphere packaging of these apples, in the different thicknesses of LDPE films, altered the rate of volatile production subsequent to returning the fruit to air. Moreover, this was found to be influenced by the temperature. Fruits from all packaging treatments tested showed a delay in flavor and aroma development compared to apples kept in air and this may be attributed to atmospheric conditions developed inside packages that reduced respiration rate and ripening. Fruits kept in air showed a significant increase in volatile components after 7 days compared to that of fruits from the other treatments. A maximum volatile production by fruit in air was attained after 14 days and continued to increase up to 21 days. However, fruit packaged in different film thicknesses had their maximum production of volatiles at 21 days. The lag in time observed, again may be attributed to the ripening delay by modification of atmospheric conditions surrounding the fruit.

Volatile components, of which several are known to contribute to the characteristic flavor and aroma of Empire, apples were directly related to fruit ripening development stage (Chiraghi, 1988). At the time of harvest fruit were mature but unripe and thus lacked in aroma. After 4 months exposure to the controlled atmosphere conditions employed in these studies, the fruit remained

unripe. Following subsequent storage in air for one week, volatiles which are associated with ripening began to be produced in significant amounts; whereas, for those from fruits kept under MAP conditions for 7 to 14 days the rate of volatiles production was slower than that for non packaged fruits subsequent to transferring the fruit to air. Eventually the MAP fruits produced volatiles similar in type and relative magnitude to those produced sooner by fruits transferred immediately to air after CA storage. Differences observed in the pattern of volatiles produced by MAP and air-stored fruits may be attributed to the low oxygen and relatively high CO_2 established in the packages which delayed the normal ripening response.

CO_2 scrubbing did not affect the pattern of volatiles. Moreover, fruits in MAP with 76.2 μm films without a CO_2 scrubber and held for 3 weeks at 20°C showed an abnormal profile than fruit keep in air. This may be associated with low O_2 and relatively high CO_2 reached in these packages. This was not seen for other fruits in MAP with 76.2 μm films at lower temperature or in MAP of other films at all temperatures.

Accumulation of CO_2 and reduction of O_2 in the atmosphere surrounding fruits achieved by MAP of fruits from CA storage reduced fruit respiration, ethylene action and thus ripening. These results indicate that the beneficial effects of MAP on keeping quality of apples may be realized even at relatively high temperatures of 15 to 20°C.

LITERATURE CITED

Allen, A.S., and Allen, N. 1950. Tomato film findings. Mod. Pack. 23(6):123-126.

Bangerth, F., G. Bufler and, H. Halder-Doll, 1984. Experiments prevent ethylene biosynthesis and/or action and effects of exogenous ethylene on ripening and storage of apple fruits.In "Ethylene: Biochemical, Physiological, and Applied Aspects," Y.Fuchs and E. Chalutz (ed), P.291. Martinus Nijhoff/Dr. Junk, The Hague.

Ben-Yehoshua, S. 1985. Individual seal packaging of fruit and vegetables in plastic film. A new postharvest technique. HortScience 20 : 32-37

Boylan-Pett, W. 1986. Modified Atmosphere Packaging of tomato fruit. MS Thesis, Mich. State Univ., East Lansing, MI, U.S.A.

Badran, A.M. 1969. Controlled atmosphere storage of green bananas. U.S. Patent 3,450,542.

Cameron, A.C., Boylan-Pett, W., and J.L. Lee. 1989. Design of Modified Atmosphere packaging systems; modeling oxygen concentrations within sealed packages of tomato fruits. J. Food Sci. 54 (6):1413-1416.

Chen, P.M. 1985. Some advantages and disadvantages of low oxygen storage of red Delicious apples. Postharvest Pomology Newsletter 3:10-13.

Chen, P.M., Olsen, K.L., and M. Meheriuk. 1985. Effect of low oxygen atmosphere on storage scald and quality preservation of "Delicious" apples. J. Amer. Soc. Hort. Sci. 110:16-20.

Dennis, C., K. M. Browne and F. Adamicki. 1979. Controlled atmosphere storage of tomatoes. Acta Hort. 93:75-83

Dilley, D.R. 1987. New Developments in Scrubbing Technology for Controlled Atmosphere storage. The Great Lakes Fruit Growers News. May, 1987.

Dilley, D.R. 1988. Recommended storage conditions for Michigan apples. Postharvest Laboratory Mich. State University, East Lansing, MI. USA.

Dilley, D.R. 1990. Application of air separator technology for controlled atmosphere storage of apples. Ed. J. Fellman. Proceedings of the 5th controlled atmosphere storage Research Conference. Wenatchee, WA, June 14-15 vol. 1:409-418.

Geeson, J.D., K. M. Browne, K. Sheppard and J.K. Guaraldi. 1985. Modified Atmosphere packaging to extend the shelf life of tomatoes. J. Food Technol. 20:339-349

Hayakawa, K., Y.S. Henig, and S.G. Gilbert. 1974. Formulae for predictin gas exchange of fresh produce in polymeric film package. J. Food Sci. 40:186-191.

Henig, Y.S. 1972. Computer analysis of the variable affecting respiration and quality of produce packaged in polymeric films. PhD Thesis Rutgers University, New Brunswick, NJ. U.S.A.

Henig, Y.S., and S.G. Gilbert. 1975. Computer analysis of the variables affecting respiration and quality of produce packaged in polymeric films. J. Food Sci. 40:1033.

Jurin, V., and M. Karel. 1963. Studies on control of respiration of McIntosh apples by packaging methods. Food Tech. 17(6):104-108.

Kader, A.A. 1986. Biochemical and physiological basis for effects of controlled and modified atmosphere on fruits and vegetables. Food Tech. 40(5):99-104.

Karel, M., and J. Go. 1964. Control of respiratory gases. Mod. Pack. 37(6):123-127,190,192.

Kidd, and West, C. 1932. Gas storage of tomatoes. Gt. Brit. Dept. Sci. Indus. Res. Food Invest. Bd. Rept. 209-211.

Lange, E. 1985. Recent advances in low O_2 and low ethylene for apples in Poland. Proceedings of the 4th National Controlled Atmosphere Research Conference. pp. 295-307.

Lau, O.L. 1983. Effects of storage procedures and low oxygen and carbon dioxide atmospheres on storage quality of "Spartan" apples. J. Amer. Soc. Hort. Sci. 108(6):953-957.

Lau, O.L. 1985. Storage responses of four apples cultivars to low O_2 atmospheres. Proceedings of the fourth National Controlled Atmosphere

Research Conference, pp. 43-56

Laurie, G.H. and B.E. Mackey. 1989. Permeability of flexible polymer films used to wrap citrus fruits to the fumigants ethylene dibromide and methyl bromide. J. Amer. Hort. Sci. 114(1):86-90

Lidster, P.D., k.b. McRae and E.M. Johnson. 1985. Retention of apple quality in low-oxygen storage followed by standard controlled atmosphere regimes. J. Amer. Soc. Hort. Sci. 110:755-759.

Little, G.R. and B.L. Tugwell. 1985. Storage construction for effective ultra-low oxygen storage in Australia. Proceedings the fourth National CA Research Conference, 281-289.

Lougheed E.C. 1987. Interactions of oxygen, carbon dioxide, temperature and ethylene that may induce injuries in vegetables. HortSci. 22:791-794.

McGlasson, W.B., and R.B.H. Wills. 1971. Effects of oxygen and carbon dioxide on respiration, storage life and organic acids of green bananas. Aust. J. Biol. Sci. 24:1103.

Prince, T.A. 1980. Design and function of a modified atmosphere package for precooled tulip bulbs. PhD Dissertation, Mich. State Univ., East Lansing, MI.

Scott, L.E., and S. Tewfik. 1947. Atmospheric changes occurring in film-wrapped packages of vegetables and fruits. Proc. Amer. Soc. Hort. Sci. 49:130.

Sharples, R.O. and J.R. Stow. 1982. Recommended conditions for the storage of apples and pears. Report of the East Malling Research Station for 1981, 199-202.

Smith, S.M.; D.S. Johnson, J. Chesworth, J. Mori. 1985. Short term storage of Discovery apples. J.Hort. Sci. 60, 207-214

Smith, S., J. Geeson and J. Stow. 1987. Production of modified atmospheres in deciduous fruits by the use of films and coatings. HortScience 22:772.

Tolle, W.E. 1962. Film permeability requirements for storage of apples. USDA Tech. Bull. 1257.

Truter, G.J., and G.J. Eksteen. 1987. Storage potential of apples under controlled atmosphere storage at ultra-low oxygen concentrations. Sagtevrugteber. (South Africa) pp. 141-145.

GENERAL CONCLUSION

The results from the dynamic atmosphere storage studies conducted at the relatively high temperature of 20°C, which is often experienced in marketing of several horticultural comodities, indicated that apple fruit of both 'Empire' and 'McIntosh' varieties held at this temperature and low oxygen concentrations retained flesh firmness better and had reduced weight loss than fruit held in air over the 3 week period test. Fruit held at 3% O_2 developed typical profiles of volatiles known to contribute to aroma as in the air. However, although 1.5% O_2 for prolonged periods up to 3 weeks retained better flesh firmness, fruit exhibited a profile of volatiles symptomatic of the onset of fermentation and these conditions should be avoided for long exposure periods. The low oxygen concentrations employed were similar to those found in MAP suggested that MAP may offer a beneficial effect on maintaining quality of apple fruit after removal from controlled atmosphere storage.

A predictive technique to achieve establishment of desired conditions of oxygen in sealed package was developed. The oxygen depletion method and MAP trials yielded similar equations of prediction of weight and film characteristics. Although more refinement may be needed for this technique, similarity of results between empirical and mathematical approachs was encouraging and shows their applicability as guidelines in the area of modified atmosphere packaging research for apple fruit.

MAP trials in different LDPE films showed that fruits kept in films made of 25.4 μm LDPE ripened more rapidly than those in thicker films (i.e, 76.2 μm). This is due probably to the permeability of the film and thus to the relatively high steady state concentration of oxygen that resulted in these package units. The effect was more noticeable at high temperatures of 15 and 20°C than at 5 and 10°C. Low oxygen and high carbon dioxide tended to delay fruit ripening and

deterioration.

Fruits packaged with 76.2 μm LDPE films and held at 20°C for up to 3 weeks accumulated ethanol and exhibited a volatiles profile symptomatic of fermentation. This was correlated with low oxygen and high carbon dioxide levels within the package. Fruits in films with intermediate permeability to O_2 retained more flesh firmness and weight than fruits kept in air and developed the capacity to produce typical types and relative quantities of volatiles but with a lag time estimated of about 1 week. This indicates that MAP of apple fruit following CA storage may extend the poststorage preservation period by at least 1 week relative to storage in ambient air.

It must stressed however, that at removal from controlled atmosphere, apple fruit used in these studies showed very limited ripening development during storage. Holding fruit from CA storage or otherwise where ripening has been allowed to advance and subjecting them to low O_2 i.e 1.5% level at 20°C should not be undertaken.