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Changes of Volatiles from Apple Fruits during Maturity and Storage

Part. I. Identification and Determination of Volatiles in the Fruits

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사과 成熟 및 貯藏中 香氣成分의 變化

第一報. 果實 成熟中 香氣成分의 同定 및 定量

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초 록

우리 나라에서 비교적 생산량이 많은 사과과실 3품종(Fuji, 홍옥, 국광)을 대상으로 향기성분을 분리 및 동정하고, 또한 성숙시기별로 향기성분의 함량변화를 측정하였다. 향기성분은 30종 이상 분리되었고 그 중 ester 23종, alcohol 9종 및 aldehyde 2종을 동정하였다. 성숙중 향기성분의 종류와 함량은 증가하였으며 1-butanol, isobutyl butyrate, 2-pentanol, ethyl valerate 및 hexanal 등의 함량이 많았다.

Introduction

The volatile compounds produced by plant tissues are believed to be produced by cell metabolism and there is much evidence showing that at least some are products of the energy producing metabolic process of cell respiration.[1~5] Identification of these metabolic products would aid in determining their route of biosynthesis and how they affect tissue metabolism.[6~9]

Identification studies of plant tissue volatiles have been conducted for decades. Brooks *et al.*[10] believe scald is caused by accumulations of certain gases produced by apples in their life processes. Gane[11] identified ethylene as the gas in the emanations from ripe apples which has striking physiological effects on plant tissues. Power *et al.*[12] used steam distillation to remove and concentrate apple volatiles from the cortex and epidermal tissues separately and then identified acetaldehyde and ethyl alcohol and show evidence for the presence of some methyl alcohol in the cortex. White[13] isolated furfural, three aldehydes and ten alcohols from McIntosh and Stayman Winesap apples by concentration and fractionation, but esters as such were not identified. Turk *et al.*[14] used cold traps and activated carbon to collect the volatiles from apples, and Henze *et al.*[15] analysed an extract from activated carbon and, using chiefly White's methods,[13] identified methanol, ethanol, isopropanol, n-butanol, a C_5-alcohol, n-hexanol, formic, acetic, propionic, n-butyric, valeic and n-caproic acids. Flath *et al.*[16~17] analysed volatile aroma mixtures extracted from Delicious and Gravenstein apple essence and identified fifty-six and sixty-six compounds of the extracts by combination GC-MS, respectively.

In Korea, many workers[18~22] have reported on weight loss, firmness and chemical compositions of apples, but quantitative information relating to the volatiles of apples during maturity and storage has appeared few in the literature. This work was undertaken to measure qualitatively and quantitatively the volatile components from 3 varieties of apples during maturity.

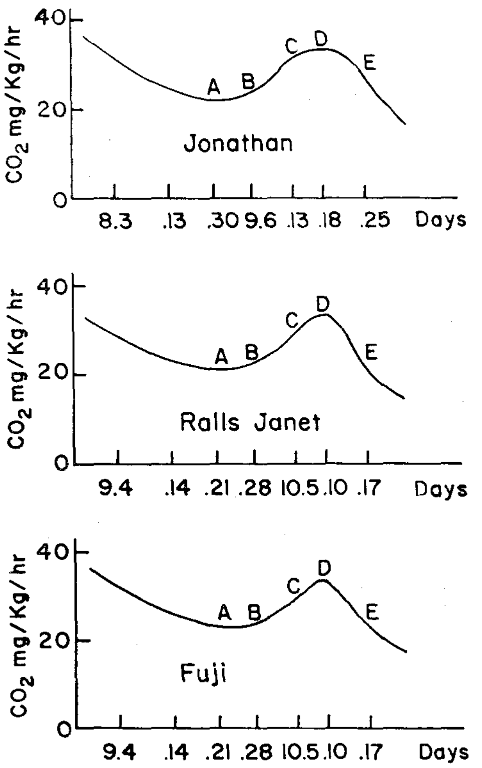

Fig. 1. Changes of carbon dioxide evolution in Jonathan, Ralls Janet and Fuji apples during maturity.

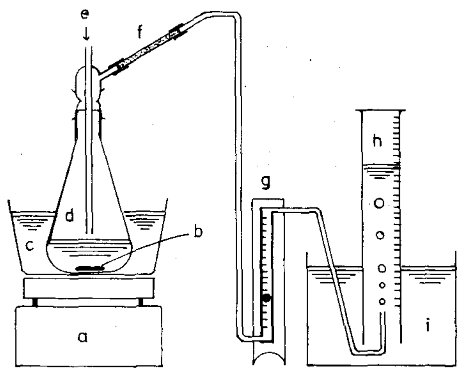

Fig. 2. Apparatus for trapping headspace flavor.
a: Magnetic stirrer, b: Magnetic bar, c: Water bath, d: Flask, e: Nitrogen gas, f: Flavor trap, g: Flow meter, h: Measuring cylinder, i: Glass jar.

Materials and Methods

Raw materials: Fuji, Ralls Janet and Jonathan apples from an orchard near Taegu, Gyeongbug were picked at the stages of A,B,C,D and E according to the rate of CO_2 production by Biale's method[23](Fig. 1).

Sample preparation: For Tenax GC trapping of apple volatiles by the methods of Shimoda *et al.*[24] and Simon *et al.*[25] as shown in Fig. 2, six replications of each sample were prepared by removing the peel, core and seed from 5 apples to supply a desired ample weight (ca. 100g), and grinding these samples in a Waring blendor with an equivalent weight of distilled water until pureed (approximately 30 sec). Each replicate with 0.1 m*l* of 0.2% 1,2-dichloroethane and cyclohexanol as internal standard was then transferred to a 300 m*l* boiling flask was held at 30°C water both Sample flasks were held for 15 min and then volatiles were collected on Tenax GC traps for 25 min under a nitrogen flow of 20 m*l*/min bubbled through the mixture. Traps were prepared with 100 mg of Tenax GC between glass wool plugs in a small-bore glass column. After sampling, traps were backflushed at room temperature 2 min, sealed. and refrigerated at -15°C, and volatiles were eluted with 300 μl diethyl ether by shake in a Vortex mixer for 2 min and 1,000 rpm centrifugation for 1 min. Gas chromatography and gas chromatography-mass spectrometry were performed on 2 and 5 μl injections.

Gas chromatography: Gas chromatography: was carried out using Shimadzu Model GC-6A gas chromatograph equipped with flame ionization detectors and 3m×3mm glass columns packed with 10% PEG 20M on 60 : 80 mesh Chromosorb W and programmed at 3°C/min from 60 to 165°C with a nitrogen flow of 30 ml/min.

Gas chromatography-mass spectrometry: The GC-MS apparatus consisted of a Varian 3700 gas chromatograph equipped with a 20 m SE-54 (glass capillary column 0.25 mm i.d.) linked to Varian MAT 212 mass spectrometer and MAT 188 data system. The operating conditions for the GC-MS coupling were: temperature programming 30~240°C at 10°C/min; 1ml He/min; separator oven and interconnecting lines 250°C; ion source temperature 250°C; ionization voltage 70 eV; emission current 1mA.

Identification and determination of volatiles: Volatiles in headspace collections were identified by gas chromatography with authentic materials to give symmetrical peak enhancement and were found to give identical spectra when recorded under the same conditions on the integrated GC-MS system. Determination of each component in the collected headspace volatiles was calculated on the basis of peak areas by data processor for chromatography (Shimadzu. C-E1B).

Results and Discussion

Identifying volatiles

Table 1 shows the 34 compounds identified in

Table 1. Identification of volatile components in apples

RT	Compounds	Means of identification	RT	compounds	Means of identification
2.77	Isopropyl acetate	RT,MS	7.76	Propyl butyrate	RT,MS
2.78	Ethyl acetate	RT,MS	7.78	Isopentyl acetate	RT,MS
3.28	Isopropanol	RT,MS	8.18	Ethyl valerate	RT,MS
3.44	Isobutyl acetate	RT,MS	8.£0	Butyl propionate	RT,MS
3.50	Ethanol	RT,MS	8.40	1-Butanol	RT,MS
3.70	Methyl butyrate	RT,MS	9.10	Isobutyl butyrate	RT,MS
3.90	Isopropyl propionate	RT,MS	9.78	Methyl hexanoate	RT,MS
4.20	Ethyl propionate	RT.MS	10.15	Isopentyl propionate	RT,MS
4.30	Propyl acetate	RT,MS	10.96	Butyl butyrate	RT,MS
5.48	Propanol	RT,MS	11.34	2-Hexenal	RT,MS
5.60	Propyl propionate	RT,MS	12.20	1-Pentaonl	RT,MS
6.30	Butyl acetate	RT,MS	13.13	Hexyl acetate	RT,MS
6.52	Hexanal	RT	15.07	Pentyl butyrate	RT,MS
6.56	Isobutyl propionate	RT,MS	16.20	1-Hexanol	RT
6.75	Isobutanol	RT,MS	18.40	trans-2-Hexenol	RT,MS
7.06	Isobutyl isobutyrate	RT,MS	19.10	Butyl hexanoate	RT,MS
7.64	2-Pentanol	RT,MS	23.26	Pentyl hexanoate	RT,MS

RT: identified by retention time (min).

MS: tentatively identified by mass spectrometry.

3 varieties of apples harvested at the post-climacteric stage (Stage E). They are arranged in order of their elution from a 10% PEG 20M on Chromosorb W column. However, among 34 compounds, methyl butyrate and ethyl propionate in Fuji, methyl butyrate and propanol in Jonathan and ethyl propionate in Ralls Janet were not detected during the GC-mass spectrometry runs.

Hulme[26], Huelin[27] Grevers *et al.*[28], and Drawert *et al.*[29] reviewed various aspects of research on apple volatiles, including evidence as to their nature, identification, and relation to apple metabolism. Earlier works were, as mentioned by Smock *et al.*,[30] and Gane,[11] usually concerned with total volatiles, presumably due to difficulties in analytically separating various components. Recently, gas chromatography and GC-mass spectrometry have given impetus to detailed investigations of fruit volatiles even though it has certain limitations.[16,17,31] In this work, Identified compounds were similar to the results reported by Flath,[16,17] Brown *et al.*,[32~34] Strackenbrock,[35] Matthews *et al.*,[36] MacGregor *et al.*,[31] Schultz,[37] Gascó *et al.*,[38] Schreier,[39] Goliás,[40] and Oishi.[41]

Volatiles and fruit maturity

The contents of the volatiles in Fuji, Ralls Janet and Jonathan apples harvested at the stages of A,B,C,D and E are shown in Tables 2, 3 and 4.

The volatiles of Fuji apple as shown in Table 2 were increased their kinds and contents in proportion to maturity,vand among 17 compounds could have more content than other volatiles produced by Fuji, ethyl valerate, 1-butanol, 2-pentanol, hexanal and isobutyl butyrate were most prominent.

Table 2. Changes of the volatiles in Fuji apple during maturity (μg/100g)

volatiles	Stages				
	A	B	C	D	E
2-Pentanol	26.3	26.8	38.0	41.5	46.9
1-Butanol	13.4	36.5	53.8	56.3	58.9
1-Pentanol	7.6	16.2	18.0	23.1	24.1
1-Hexanol	4.8	5.7	12.0	15.6	21.3
trans-2-Hexenol	tr	4.6	15.3	15.4	17.3
Ethyl acetate	tr	3.2	4.6	4.8	5.6
Isobutyl propionate	7.8	15.2	17.0	18.6	23.2
Ethyl valerate	23.2	27.6	33.8	42.8	59.3
Isobutyl butyrate	7.8	32.3	37.0	39.5	42.4
Isopentyl propionate	tr	1.2	1.4	8.6	16.9
Butyl butyrate	1.3	4.7	7.6	8.2	9.5
Hexyl acetate	7.8	13.1	16.8	17.9	21.5
Pentyl butyrate	6.9	12.7	16.0	17.2	17.4
Butyl hexanoate	9.3	12.4	16.2	18.2	18.3
Pentyl hexanoate	tr	4.7	11.4	15.6	19.8
Hexanal	4.2	24.1	34.0	36.7	44.8
2-Hexanal	tr	6.7	13.2	19.6	20.2
kinds of the Volatiles	21	23	23	27	37

tr means less than 0.05μg/100g.

Table 3. Changes of volatiles in Ralls Janet apple during maturity (μg/100g)

Volatiles	Stages				
	A	B	C	D	E
2-Pentanol	15.7	23.4	27.5	28.3	30.4
1-Butanol	24.1	38.6	40.1	44.3	45.7
1-Pentanol	8.3	17.2	19.0	19.4	19.6
1-Hexanol	5.7	6.8	17.4	18.3	23.2
trans-2-Hexenol	tr	tr	6.2	8.4	9.7
Ethyl acetate	tr	1.2	3.0	3.2	3.4
Isobutyl propionate	6.3	13.2	15.0	15.4	16.3
Ethyl valerate	13.4	17.6	21.4	30.2	34.3
Isobutyl butyrate	11.3	37.6	44.2	48.4	53.7
Isopentyl propionate	4.8	12.3	13.6	13.8	15.2
Butyl butyrate	3.8	4.7	5.2	6.2	7.6
Hexyl acetate	8.2	9.8	11.5	13.4	14.1
Pentyl butyrate	4.3	8.7	10.2	11.3	12.6
Butyl hexanoate	8.7	9.6	12.0	13.2	17.9
Pentyl hexanoate	4.7	6.8	13.1	14.5	15.6
Hexanal	1.2	15.3	20.2	23.6	27.6
2-Hexenal	tr	7.9	11.6	13.2	14.3
Kinds of the volatiles	18	20	22	29	35

tr means less than 0.05μg/100g.

Among volatiles produced by Ralls Janet, isobutyl butyrate, 1-butanol, ethyl valerate and 2-pentanol were contained more than others as shown in Table 3. They were components most closely associated with maturity.

The relation of fruit maturity to production of volatiles in Jonathan (Table 4) was similar to that evidenced in Fuji and Ralls Janet (Tables 2 and 3). Maximum production of each volatile occurred the more mature the apples. The content of each volatile was much in order of 1-butanol, isobutyl butyrate, 2-pentanol, isobutyl butyrate, 2-pentanol and ethyl valerate.

As shown in Tables 2, 3 and 4 the amount of volatiles given off by apples was greatly dependent on the degree of maturity. The greatest amount of volatiles in Fuji, Ralls Janet and Jonathan was produced at the stage E. The quantity of volatiles produced was lower at the stages A and B than at the stages C,D and E, and in Ralls Janet and Jonathan than in Fuji. Guadagni *et al.*[42] reported that harvest maturity, variety and storage temperature greatly affected the rate and extent of volatiles production. Neubeller *et al.*[43,44] reported that production of each volatile from Golden Delicious during maturity in 1971~1973 was increased. Brown *et al.*[32] noted that the production pattern of the volatiles changed dynamically and characteristically during ripening, but was conditioned by the degree of maturity of the fruit at harvest. This result is comptible with reports[31,33,43,44] that maximum production of volatiles from fruits occurred the more mature the fruit.

Abstract

Volatiles from *Mallus pumila Miller Var.*

Table 4. Change of the volatiles in Jonathan apple during maturity (μg/100g)

Volatiles	Stages				
	A	B	C	D	E
2-Pentanol	4.7	24.3	35.1	36.4	37.3
1-Butanol	16.4	38.2	50.1	53.1	54.7
1-Pentanol	10.9	14.7	15.8	16.2	17.9
1-Hexanol	2.7	19.4	22.4	26.8	27.2
trans-2-Hexenol	4.7	9.6	15.0	16.8	16.9
Ethyl acetate	tr	1.3	4.2	4.7	5.4
Isobutyl propionate	10.2	14.8	16.9	17.5	18.3
Ethyl valerate	7.3	17.3	21.0	27.6	35.5
Isobutyl butyrate	6.9	21.4	40.0	42.7	46.3
Isopentyl propionate	2.4	6.8	13.8	16.5	18.3
Butyl butyrate	3.2	4.7	6.8	6.9	8.4
Hexyl acetate	8.6	10.3	12.0	15.6	15.7
Pentyl butyrate	5.4	7.6	13.2	13.4	24.8
Butyl hexanoate	1.4	6.4	5.8	7.2	8.8
Pentyl hexanoate	2.3	8.7	10.2	11.6	14.3
Hexanal	4.2	27.3	30.9	32.4	36.8
2-Hexenal	1.3	9.7	12.5	14.6	15.5
Kinds of the volatiles	19	19	21	28	32

tr means less than 0.05μg/100g.

Fuji, Ralls Janet and Jonathan during maturity were examined. The volatiles of apples were collected by the headspace trapping method using. Tenax GC and identified by gas liquid chromatography and GC-mass spectrometry. Among over 34 GLC peaks, 23 esters, 9 alcohols and 2 aldehydes were identified. The quantity of volatiles produced was increased in proportion to the degree of maturity, and 1-butanol, isobutyl butyrate, 2-pentanol, ethyl valerate and hexanal were major.

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References

1. Knee, M.: J. Sci. Food Agr., 22 : 365(1971).
2. Knee, M.: J. Sci Food Agr., 22 : 368(1971).
3. Knee, M.: J. Sci. Food Agr., 22 : 371(1971).
4. Sharples, R.O.: Acta Hort., 34 : 421(1972).
5. O'loughlin, J.B.: Scientia Hort., 3 : 75(1975).
6. Fidler, J.C. and North, C.J.: J. Hort. Sci., 43 : 421(1968).
7. Wills, R.B.H. and Scriven, F.M.: Phytochem., 18 : 785(1979).
8. Murata, T. and Minamide, T.: Plant & Cell Physiol., 11 : 857(1970).
9. Fidler, J.C. and North, C.J.: J. Hort. Sci., 46 : 245(1971).
10. Brooks, C., Coaley, J.S. and Fisher, D.F.: J. Agr. Res., 16 : 195(1919).
11. Gane, R.: Nature, 134 : 1008(1934).
12. Power, F.B. and Chesnut, V.K.: J. Amer. Chem. Soc., 42 : 1509(1920).
13. White, J.W.: Jr. : Food Res., 15 : 68(1950).
14. Turk, A., Smock, R.M. and Taylor, T.I.: Food Technol., 5 : 58(1951).

15. Henze, R.E., Baker, C.E. and Quackenbush, F.W.: J. Agr. Food Chem., 2 : 1118(1953).
16. Flath, R.A., Black, D.R., Forrey, R.R., McConald, G.M., Mon, T.R. and Teranishi, R.: J. Chromatogr. Sci., 7 : 508(1959).
17. Flath, R.A., Black, D.R., Guadagni, D.G., McFadden, W.H. and Schultz, T.H.: J. Agr. Food Chem., 15 : 29(1967).
18. Sohn, T.H.: Ph. D. thesis 'Youngnam University, Taegu, Korea (1975).
19. Park, N.P,, Choi, E.H. and Lee, O.H.: J. Ker. Soc. Hort, Sci,, 7 : 15(1970).
20. Park, N.P., Choi, E.H. and Kee, O.H.: J. Kor. Soc. Hort Sci., 8 : 51(1970).
21. Ko, K.C.: J. Kor. Soc. Hort. Sci., 8 : 41 (1970).
22. Lee, D.S., Woo, S.K. and Yang, C.B.: Korean J. Food Sci. Technol. 4 : 134(1972).
23. Biale, J.B.: Ann. Rev. Plant Physiol., 1 : 183(1950).
24. Shimoda, M,, Yamasaki, K. and Osajima, Y.: J. Agr. Chem. Soc. Japan, 54 : 271(1080).
25. Simon, P.W., Lindsay, R.C. and Peterson, C.E.: J. Agr. Food Chem., 28 : 549(1980).
26. Hulme, A.C.: Advances in Food Res., 8 : 297(1958).
27. Huelin, F.E.: Austral, J. Sci. Res., Series B, 5 : 328(1952).
28. Grevers, G. and Doesburg, J.J.: J. Food Sci., 30 : 412(1965).
29. Drawert, F., Heimann, W., Emberger, R. and Tressl, R.: Chem. Mikrobiol. Technol. Lebensm., 1 : 201(1972).
30. Smock, R.M. and Neubert, A.M.: In 'Apples and apple products', Intersci. Publ. Inc., New York(1950).
31. MacGregor, D.R., Sugisawa, H. and Matthews, J.S.: J. Food Sci., 29 : 448(1964).
32. Brown, D.S., Buchanan, J.R. and Hicks, J.R. Proc. Amer. Soc. Hort. Sci., 88 : 98 (1966).
33. Brown, D.S., Buchanan, J.R. and Hicks, J.R.: Hilgardia, 39(2) : 37(1968).
34. Brown, D.S., Buchanan, J.R. and Hicks, J.R.: Proc. Amer. Soc. Hort. Sci., 93 : 705 (1968).
35. Strackenbrock, K.H.: Erwerbsobstbau, 3 : 147(1961).
36. Matthews, J.S., Sugisawa, H. and MacGregor, D.R.: J. Food Sci., 27 : 355(1962).
37. Schultz, T.H., Flath, R.A., Black, D.R., Guadagni, D.G., Schultz, W.G. and Teranshi, R.: J. Food Sci., 32 : 279(1967).
38. Gascó, L., Barrera, R. and de la Cruz, F.: J. Chromatogr. Sci., 7 : 228(1969).
39. Schreier, P., Drawert, F. und Mick, W.: Lebensm. -Wiss. u. -Technol., 11 : 116(1978).
40. Goliás, J., und Nevâk, J.: Nahrung, 23 : 393(1979).
41. Oishi, S.: J. Home Economics of ʃ apan, 27 : 566(1976).
42. Guadagni, D.G., Bomben, J.L. and Hudson, J.S.: J. Sci. Food Agr., 22 : 110(1971).
43. Neubeller, J. und Buchloh, G.: Erwerbsobstbau, 15 : 120(1973).
44. Buchloh, G. und Neubeller, J.: Erwerbsobstbau, 16 : 69(1974).