A STUDY OF THE FLAVOR COMPONENTS OF ONIONS

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by

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ABSTRACT

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A Study of the Flavor Components of Onions

Major Professor: Lucien J. LaCroix

Canned samples of twenty varieties of onion (Allium cepa) were studied with an electron capture gas chromatograph. Various chromatograms were obtained from both the petroleum ether extract and headspace samples of these varieties. A striking varietal difference in the flavor components was demonstrated in the chromatogram patterns.

About twelve components were readily detected and separated in each chromatogram of petroleum ether extract of each onion variety. Of these, two seem to be important in determining flavor. Five components were detected in the headspace samples of the canned onions. Only one of the components was assumed to be of some importance in relation to pungency rating. Further investigations on these components would be of practical interest.

A statistically significant correlation coefficient ($r = 0.46$) was found between the pyruvate test of fresh onion samples and the organoleptic evaluation of the pungency of canned onion samples of these twenty varieties.

The combined heights of peaks 4 and 7 from the hexane extract of canned onions were positively correlated ($r = 0.66$) with pungency as determined by the organoleptic test.

The successful application of a gas chromatographic method in the analysis of the flavor components of onion varieties is fully described. With further research the practical use of gas chromatographic methods for routine evaluation of onion pungency on a commercial basis is envisaged.

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INTRODUCTION

The study of onion flavor is of both practical and academic importance. Chemists are interested in isolating, identifying and characterizing the flavor components of onion. By means of preparative methods chemists might be able to synthesize the desirable flavors for commercial uses. Food technologists are in need of a reliable and objective method for the measurement of onion flavors in order to select highly flavored raw onions and maintain a standard level of quality. Plant breeders are concerned with the development of either mild or pungent varieties to meet the demands of the food industry. With the development of suitable methods of pungency rating, a series of standard grades could be established for the commercial trade.

During the last thirty years, numerous methods for measuring onion flavor have been described in the literature. The determination of total volatile reducing substances and the measurement of total sulfur have been utilized to evaluate the pungency of onions. However, these methods are not widely accepted as a means of standardizing the quality of raw onions or as a tool for routine evaluation of onion flavor. The two main drawbacks of these methods are that they do not always agree well with the taste panel evaluation and the procedures involved

are lengthy and therefore not suitable for routine analysis. In recent years, Schwimmer and Weston (1961) suggested a method for estimating the enzymatic development of pyruvic acid in onion as a measure of its pungency. A highly significant correlation was found between the amount of the enzymatically produced pyruvic acid in the juice of comminuted onions and olfactory threshold concentration of the juice (Schwimmer and Guadagni, 1962). Gas chromatography has been used successfully in separation, isolation and identification of onion flavor components (Carson and Wong, 1961a). The development of the technique of electron capture gas chromatography may enable the food technologist to accurately evaluate the intensity of onion flavor on the basis of quantitative and qualitative results obtained by this procedure.

Preliminary results (LaCroix et al. 1966) showed a striking varietal difference of the flavor components of onions using electron capture gas chromatography. The investigation herein described and discussed was an extension of this gas chromatographic technique in the evaluation of flavor of commercial processed onions. Also, an attempt was made to isolate the individual components and to establish their olfactory responses, so that the characteristic chromatogram of each variety might be related to pungency as evaluated by organoleptic tests.

REVIEW OF LITERATURE

It is a well known fact that oils of *Allium*, prepared by steam distillation of ground tissues, have an inherent, characteristic, pungent odor (Jones and Mann, 1963). Results from recent investigations on the isolation, identification and characterisation of the flavor components revealed that organic sulfur compounds are the major components responsible for this property (Carson and Wong, 1961a; Jones and Mann, 1963; Bernhard *et al*. 1964; Saghir *et al*. 1964; Jacobson *et al*. 1964).

In the nineteenth century Semmler (1892) postulated the presence of allyl n-propyl disulfide in garlic oil. Many years later Challenger and Greenwood (1949) isolated n-propyl thiolfrom onion volatiles by absorption with mercuric cyanide. Niegisch and Stahl (1956) then identified n-propyl thiol and n-propyl disulfide from the raw onion volatiles. There was no indication of the presence of allyl n-propyl disulfide in their study. Recently Carson and Wong (1961a) identified several sulfur compounds in the onion volatiles and extracts, through the use of gas chromatography, infrared spectrophotometry and chemical derivative methods. They identified n-propyl thiol, dimethyl disulfide, methyl n-propyl trisulfide, dimethyl trisulfide, methyl n-propyl disulfide, di-n-propyl disulfide and di-n-propyl trisulfide. Neither monosulfides nor allyl n-propyl disulfide were detected in their study.

More recently, Bernhard et al.(1964) reported the presence of diallyl monosulfide and allyl alcohol in Allium volatiles. Oaks et al. (1964) analysed the sulfur compounds from garlic headspace and hexane extract with electron capture/flame ionization dual channel gas chromatography and detected the presence of methyl mercaptan and the mono, di and trisulfides of dimethyl, methyl allyl and diallyl. Methyl n-propyl disulfide was found in trace amounts. No ethyl sulfide was reported in either onion or garlic in their paper. From their studies, they suggested that methyl and n-propyl sulfides are the principal constituents in onion whereas allyl and methyl sulfides are the main constituents in garlic. A similar conclusion was also reported by Saghir et al. (1964) in the study of the distribution of mono and disulfides in the common Allium food species. They indicated that garlic volatiles contain largely allyl radicals and only small amounts of methyl and n-propyl radicals. Onion, on the other hand, apparently contains mainly methyl and n-propyl radicals and only small amounts of allyl radicals.

In the last few years, a mechanism which leads to the liberation of the characteristic odor from the comminuted onion tissues has been established. Evidence has been accumulated to suggest that the characteristic odor

of onions arises as a result of the interaction of S-substituted L-cysteine sulfoxide derivatives and the phosphopyridoxal enzyme alliinase or L-cysteine sulfoxide lyase when the onion tissues are injured or disintegrated (Carson and Wong, 1961b; Virtanen and Spare, 1961; 1962; Kupiecki and Virtanen, 1960; Schwimmer et al. 1960; 1961; Schwimmer and Mazelis, 1963).

The first derivative of S-substituted L-cysteine sulfoxide was isolated from garlic and identified as S-allyl L-cysteine sulfoxide which is also called alliin. When alliin is treated with a preparation of garlic containing the enzyme alliinase it gives rise to allicin (diallyl thiosulfinate), pyruvate and ammonia according to the following equation (Cavallito et al. 1944a, b; 1945; Stoll and Seebeck, 1951):

$$2CH_2{=}CH{-}CH_2{-}\overset{\overset{O}{\uparrow}}{S}{-}CH_2{-}CH{-}COOH + H_2O \xrightarrow{\text{alliinase}} CH_2{=}CH{-}CH_2$$

$$\overset{\overset{O}{\uparrow}}{S}{-}S{-}CH_2{-}CH{-}CH_2 + 2CH_3COCOOH + 2\ NH_3$$

Allicin, unlike the odorless alliin, has the typical odor of fresh garlic. It is, however, unstable and volatile. After heating, it gives rise to the sulfides (Carson and Wong, 1961a).

Following the discovery of alliin and allicin in garlic, S-methyl L-cysteine sulfoxide (MCSO), S-n-propyl L-cysteine sulfoxide (PCSO) (Virtanen and Matikkala,1959a)

and cycloalliin (3-methyl 1,4 thiazane 5-carboxylic acid 1-oxide)(Virtanen and Matikkala, 1959b) have been isolated from onion. The phosphopyridoxal enzyme alliinase (Schwimmer et al. 1960) and L-cysteine sulfoxide lyase (Schwimmer et al. 1961) have been found in onion. These enzymes have been characterized by Schwimmer and Mazelis (1963) and Schwimmer (1963).

Numerous methods for measuring the pungency of garlic and onions have been established during the past thirty years. Platenius (1935) and Kohman (1952) determined the onion pungency by measuring the volatile sulfur evolved by steam distillation. Although a highly significant correlation was found between the dry weight and onion pungency by this technique, its main drawback was that it did not offer a rapid and sensitive method for routine work (Wilkens,1964). Lang et al. (1944) and Faber (1949;1957) evaluated the pungency of onion and garlic oil on the basis of total reducing substances. However, this method did not always agree too well with the subjective response of a taste panel (Wilkens,1964). Currier (1945) developed a photometric method for the estimation of volatile sulfur in onion as a measure of its pungency.

In recent years, Schwimmer and Weston (1961) published a paper in which they suggested a rapid method for

estimating the enzymatic development of pyruvic acid in onion as a measure of its pungency. A highly significant correlation between the amounts of enzymatically produced pyruvic acid in the juice of the comminuted onion and the olfactory threshold concentration of the juice was found (Schwimmer and Guadagni, 1962). Schwimmer and Weston (1961) also used this pyruvate method to grade the onion pungency of different varieties. They found that weak onions gave 2 to 4 μ moles; moderate onions 8 to 10 μ moles and strong onions 15 to 20 μ moles of pyruvate per g of onion. More recently the pyruvate method has been extended to evaluate the pungency of commercially dehydrated onion powder (Schwimmer et al.1964).

Schwimmer and Weston (1961) suggested other methods based on properties of the sulfur volatiles, such as their antibiotic activity (Virtanen and Matikkala 1959b); their reaction with N-ethyl maleimide (Carson and Wong, 1959a; Schwimmer et al. 1960) and their reaction with thiamine to form ultraviolet light absorbing analogs of allithiamine (Morgan, 1946).

Organoleptic tests have been used in subjective evaluations of food flavors (Little, 1957). One of these methods has been employed to evaluate onion pungency and it is well correlated with the pyruvate method (Schwimmer and Guadagni,1962). In addition, this method has also been

used in association with gas chromatography to establish the odor thresholds of some organic compounds in food flavors (Guadagni et al.1963). It has been suggested that the smell sensation might be used directly to evaluate the odor strength of each component as it escapes from the column (Burr, 1964) or the detector (Hartmann, et al. 1963) of a gas chromatograph.

Within the last five years, gas chromatography has been used to a greater extent to study the volatile components of garlic (Bernhard et al.1964; Saghir et al. 1964; Oaks et al. 1964; Schultz and Mehrmann, 1965) and onions (Wilkens,1964; Carson and Wong,1961a; Saghir et al. 1964; Mackay et al. 1961; LaCroix et al. 1966).

Of these studies, Oaks et al.(1964) demonstrated the advantages of using electron capture gas chromatography to study the sulfur compounds of garlic. Saghir et al. (1964) reported on the distribution of sulfides in the flavors of some common Allium species. LaCroix et al. (1966) examined the flavors of six onion varieties by using electron capture gas chromatography and found that there appeared to be varietal differences in the flavor components of onions. Their finding has substantially supported the statement given by Platenius (1941) that the inherent characteristic of varieties had the most pronounced influences on pungency. The extension of the

gas chromatographic method in the study of flavors of commercially processed onions could be of great importance.

INSTRUMENTATION

DESCRIPTION OF GAS CHROMATOGRAPH AND ITS OPERATING CONDITIONS

A Wilkens Aerograph Model 660 gas chromatograph equipped with an electron capture detector cell containing a 250 mc titrium source was used in this study. The column was stainless steel, 10 ft x 1/8 in. O.D, packed with acid washed, silanized chromosorb W, 60/80 mesh, coated with 5% carbowax 20M. Nitrogen gas was used as a carrier gas. A Leeds & Northrup Speedomax H, 1 mv recorder equipped with a disc chart integrator was connected to the chromatograph.

Operating conditions for gas chromatographic analysis of petroleum ether extracts and headspace samples of onion varieties are shown in Table 1.

TABLE 1. Operating Conditions for Gas Chromatographic Analysis of Onion Samples.

	Petroleum ether extracts	Headspace
Sample size	1.8 - 2.4 µl	1.0 ml
Injector temp.	190°C	168°C
Detector temp.	174°C	172°C
Column temp.	142°C	73°C
N_2 flow rate	23.8 ml/min	32.3 ml/min
Chart speed	30 in/hr.	30 in/hr.

MATERIAL

ONION SAMPLES AND THEIR PREPARATIONS

Samples of twenty onion varieties (Table 2) were canned by Campbell Soup Company. The procedure for preparing onion samples is outlined as follows:

One-quarter sections of peeled onion bulbs (15-20 bulbs) were bulked and blended for two minutes in a Waring blendor. Samples of each variety weighing 5g, 7g, 10 and 15g were topped with hot 1% salt solution, leaving one quarter inch for headspace. Finally, cans were sealed and processed at 250°F, 15 psi for 30 minutes.

All steps were done as quickly as possible to reduce the volatile fraction loss to a minimum (estimated 2-3 minutes to weigh, top off with salt solution and seal can).

TABLE 2. Onion Varieties* used in the Preparation of Processed Samples for Gas Chromatographic Studies.

White Granite	Early Yellow Globe	Elite
Empire	Autumn Spice	Abundance
Encore	Autumn Splendor	Harvest Pak 'A'
Aristocrat	Fiesta	W 45
Early Harvest	Australian Brown	Elba
Sanpak 'A'	Experimental Hybrid #4	Pacesetter
Copper Gem	Pronto	

* All varieties were grown from onion sets.

METHODS

(A). PETROLEUM ETHER EXTRACT

Canned samples (10g) of eighteen onion varieties as listed in Table 2 (Experimental Hybrid #4 and Copper Gem were not included) were extracted for chromatography. The content of each can (approximately 280 ml) was divided into duplicate samples. Each of the samples was extracted repeatedly in a separatory funnel with 100 ml of petroleum ether (bp 30- 42°C). The petroleum ether was obtained by fractional distillation of commercial Skelly F (bp 30-60°C). Extraction was repeated until no detectable peaks appeared in the chromatogram. The extracts were combined, concentrated to a small volume by vacuum evaporation, then diluted to 10 ml with petroleum ether. A mixture of sample and reference compound (diallyl disulfide) in a proportion of 2 to 1 (v/v) was prepared for each duplicate sample. An aliquot of the mixture and of the sample alone, for each variety, were chromatographed under identical conditions.

(B) HEADSPACE

Headspace from canned samples (7.5g) of the twenty onion varieties (Table 2) were studied. A hole was pierced through the surface of the top of each can with a can piercing device (Figure 1). Di-n-propyl disulfide (1.0 ul) was injected into the can then a 1.0 ml sample of headspace was withdrawn with a one-milliliter hypodermic syringe

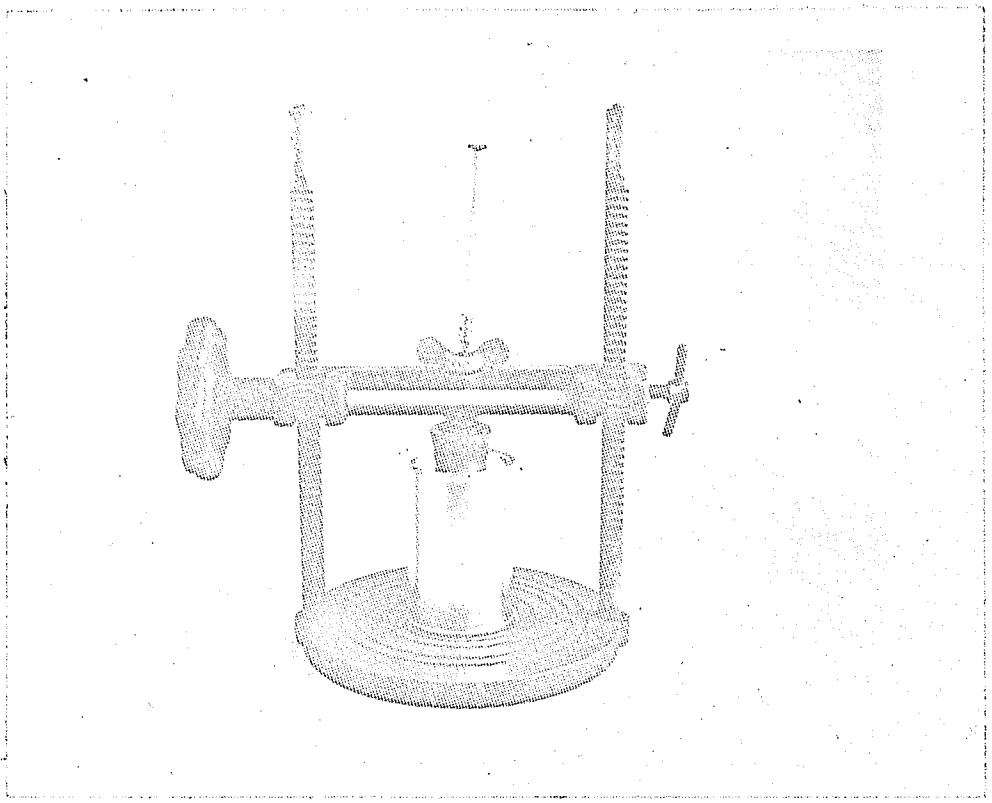


FIGURE 1. A modified can piercing device for headspace sampling.

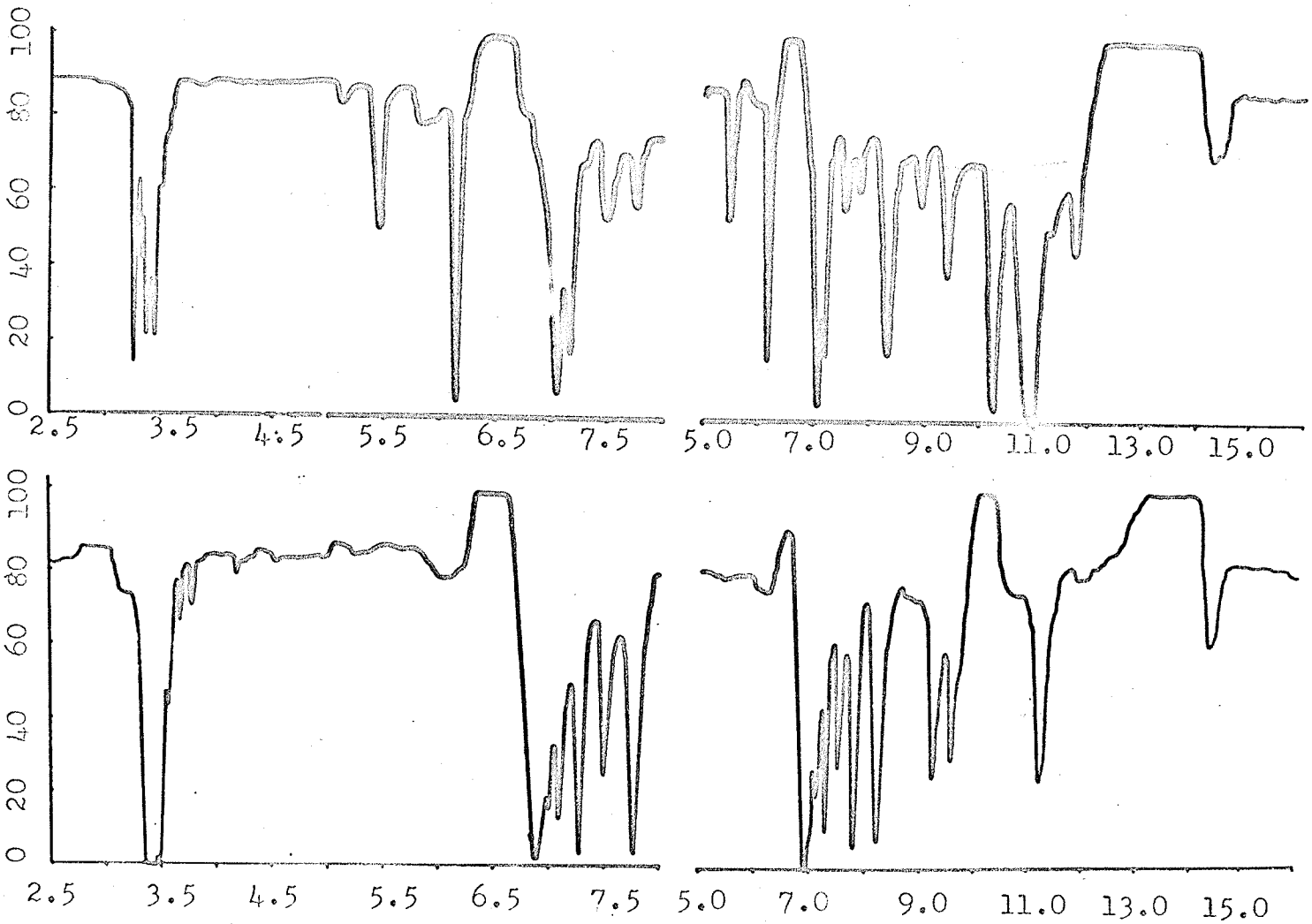
through a rubber septum in the centre of the can piercing device. The mixture of headspace gases (including the di-n-propyl disulfide reference) was injected directly into the gas chromatograph. The reproducibility of this technique for each canned sample was tested.

(C) REFERENCE COMPOUNDS

Diallyl disulfide and di-n-propyl disulfide were used as reference compounds in petroleum ether extracts and headspace samples respectively. The retention for each peak recorded is relative to these compounds. Diallyl disulfide (Eastman Kodak Company, Rochester 3, New York) and di-n-propyl disulfide (K & K Laboratories, Inc., Plainview, New York) were purified with a Wilkens Aerograph A-90-P gas chromatograph. A copper column of 6 ft x 1/4 in. OD, packed with 10% carbowax 20M coated on 60/80 mesh chromosorb W was used for this purpose. The collected fractions were identified with a Perkin Elmer Model 237 grating infrared spectrophotometer. The infrared spectra shown in Figures 2 and 3 were obtained from the repurified diallyl disulfide and di-n-propyl disulfide respectively. These spectra appeared to be identical to those in the literature (Jacobsen et al. 1964).

FIGURE 2. (Upper) Infrared Spectrum of Chromatographically Purified Diallyl Disulfide.

FIGURE 3. (Lower) Infrared Spectrum of Chromatographically Purified di-n-propyl Disulfide.


Transmittancy (%)
0
20
40
60
80
100
2.5
3.5
4.5
5.5
6.5
7.5
5.0
7.0
9.0
11.0
13.0
15.0
Wavelength (microns)

RESULTS AND DISCUSSION

(A). PRELIMINARY STUDY ON THE FLAVOR COMPONENTS OF SIX ONION VARIETIES.

Preliminary results (LaCroix et al. 1966) of a gas chromatographic study of flavor components of six onion varieties have shown the striking varietal differences of onion flavor components. In addition, chromatograms of one variety grown under different environmental conditions gave the same patterns.

(B). GAS CHROMATOGRAPHIC STUDY OF CANNED SAMPLES OF TWENTY DIFFERENT ONION VARIETIES

Twenty different varieties (Table 2) of onion (Allium cepa) were canned and chromatographed by the methods previously described. Chromatograms of these twenty varieties were compared and attempts were made to correlate gas chromatographic results with taste panel tests. If such a correlation was obtained then a method would be available to the food technologist to select the best variety for canning.

(C). REPRODUCIBILITY OF RETENTION TIME AND PEAK HEIGHT OF REFERENCE COMPOUND

Four individual samples of 1.1 µl diallyl disulfide in petroleum ether were analysed by gas chromatography under conditions identical to those used for analysis of petroleum ether extracts. Reproducibility of retention

time and peak height of this reference compound is shown in Figure 4 and Table 3.

TABLE 3. Reproducibility of Retention Time and Peak Height of Diallyl Disulfide

Injection	Peak height (cm.)	Retention time (min.)
1st	7.70	2.590
2nd	7.80	2.590
3rd	7.70	2.580
4th	7.70	2.580
Mean	7.73	2.585

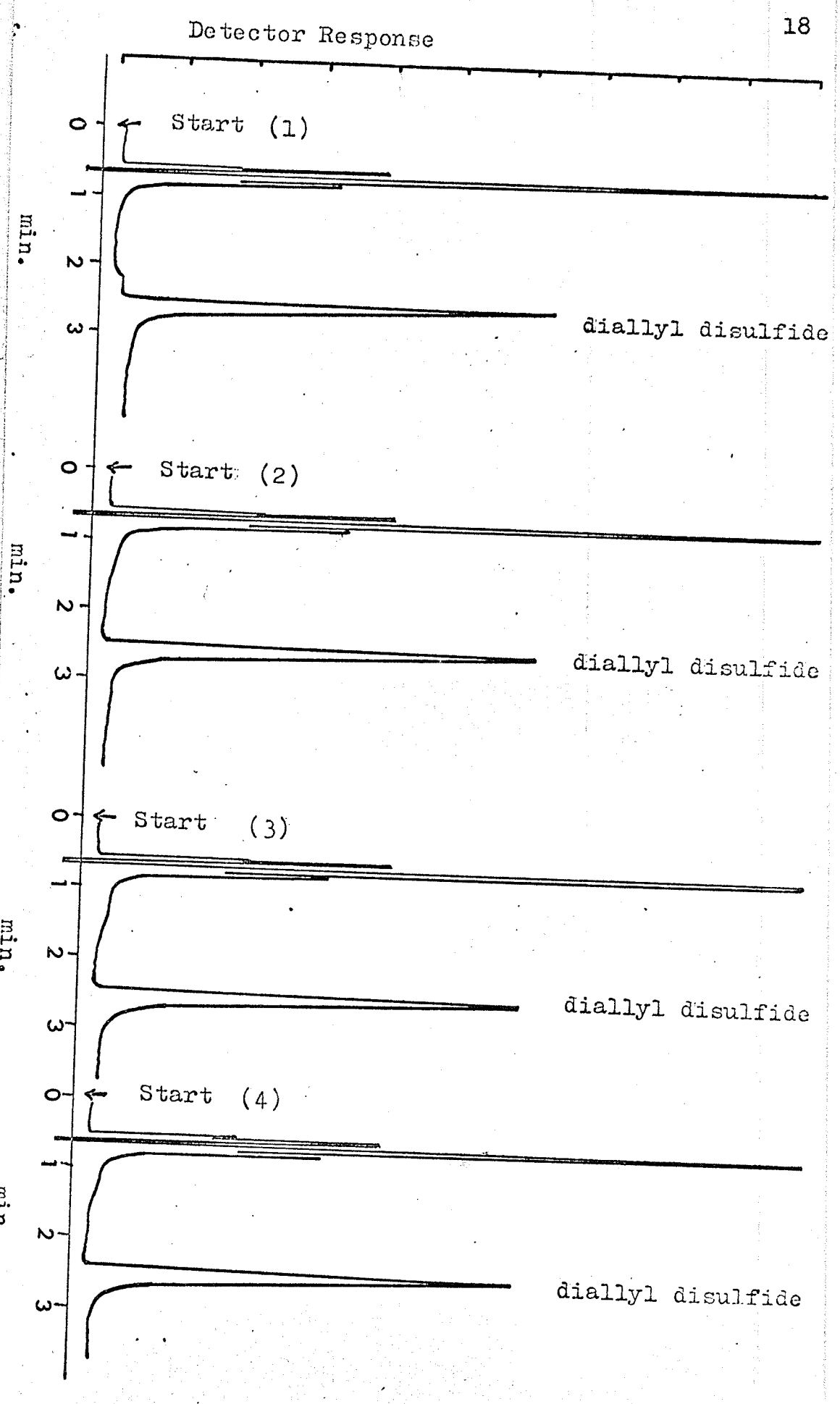
The absolute standard deviation (σabs) and relative standard deviation (σrel) of peak height and retention time of diallyl disulfide were calculated as follows:

$$\sigma \text{ abs} = \sqrt{\frac{(x-m)^2}{n-1}} \qquad \sigma \text{ rel} = \frac{\sigma \text{ abs}}{m} \times 100$$

σ abs of peak height = $\pm$ 0.0503 σ rel of peak height = 0.65%

σ abs of retention time = $\pm$ 0.0057 σ rel of retention time = 0.22%

FIGURE 4. Chromatograms of Four Injections of Diallyl Disulfide.


Detector Response
Start (1)
0
1
2
3
min.
diallyl disulfide
Start (2)
0
1
2
3
min.
diallyl disulfide
Start (3)
0
1
2
3
min.
diallyl disulfide
Start (4)
0
1
2
3
min.
diallyl disulfide

(D). REPRODUCIBILITY OF CHROMATOGRAM PATTERNS

Chromatograms of duplicate samples of petroleum ether extracts of Yellow Globe onions (Figure 5) and Empire onions (Figure 6) demonstrated excellent agreement of patterns between duplicate samples. Reproducibility of the relative retention of each individual peak can also be obtained from these chromatograms.

The calculated relative retentions of individual peaks in Figures 5 and 6 are listed in Table 4.

(E). CHROMATOGRAMS OF PETROLEUM ETHER EXTRACTS OF CANNED ONION SAMPLES

Chromatograms of canned onion samples with and without reference, for each variety are shown in Figures 5 - 22.

In these chromatograms, reference peaks are represented by 'R' and other peaks are labelled with 1, 2, 3 etc. Peaks that exhibit identical relative retentions in each chromatogram are labelled with the same numbers. The peaks that appear before the number one peak are solvent peaks. Besides these solvent peaks, five to twelve readily detectable components were recorded in each sample. Since these components were separated in a higher temperature range than headspace components, they might be assumed to be responsible for taste to a greater extent than odor.

FIGURE 5. Chromatograms of Duplicate Samples of Early Yellow Globe onions.

(A) Chromatogram of the sample

(B) Chromatogram of the sample with reference

(C) Chromatogram of the duplicate sample

(D) Chromatogram of duplicate sample with reference.

Abbreviations used in these figures are:

16x, 4x, 2x and 1x refer to attenuation.

'R' indicates reference peak.

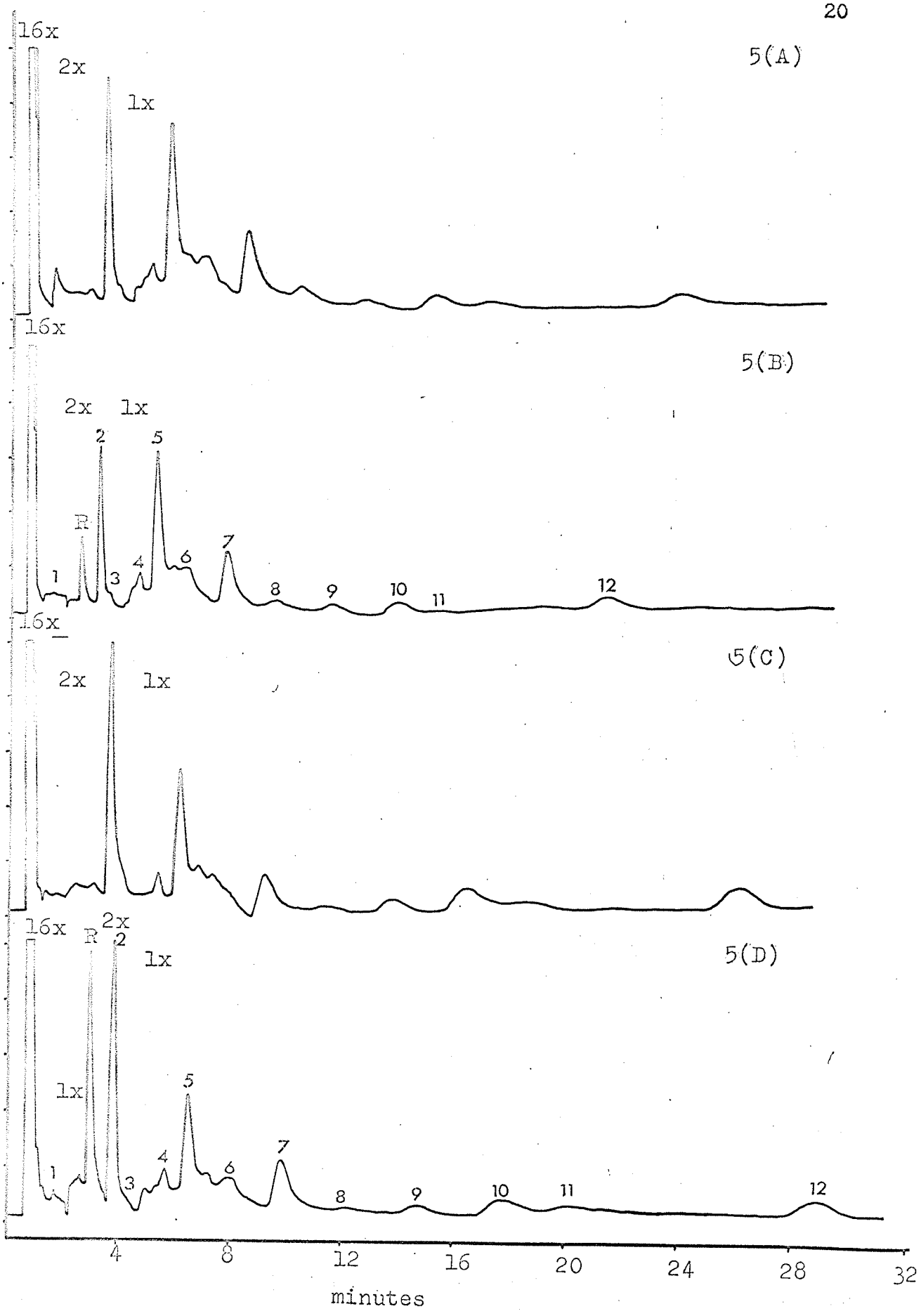

16x
2x
1x
5(A)
16x
5(B)
2x 1x
R
1
2
3
4
5
6
7
8
9
10
11
12
16x
5(C)
2x
1x
16x
R
2x
2
1x
5(D)
1x
1
3
4
5
6
7
8
9
10
11
12
4
8
12
16
20
24
28
32
minutes

FIGURE 6. Chromatograms of the Duplicate Samples of Empire Onions.

(A) Chromatogram of the sample with reference

(B) Chromatogram of the sample

(C) Chromatogram of the duplicate sample with reference

(D) Chromatogram of the duplicate sample.

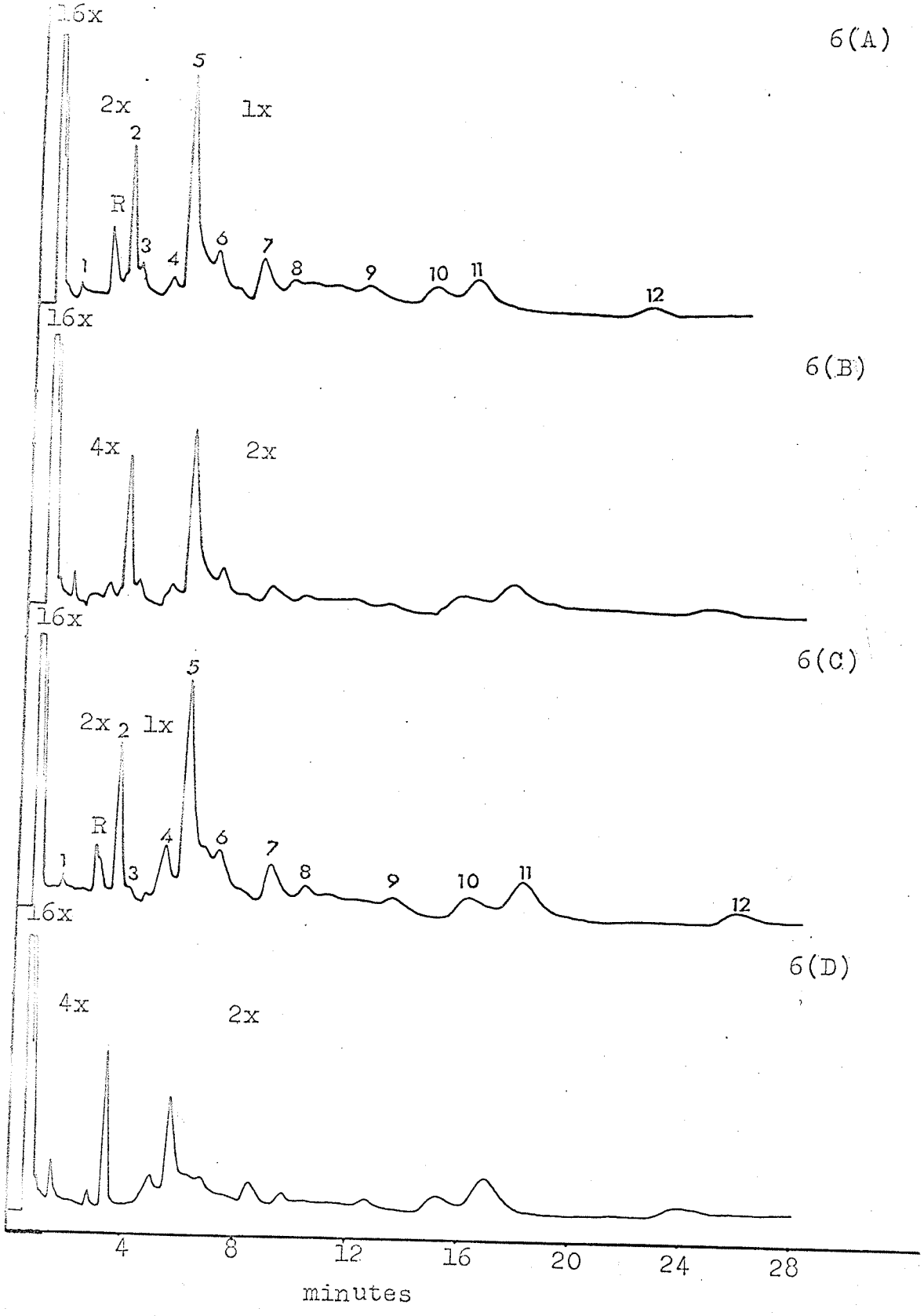

16x
6(A)
5
2x
1x
2
R
3
4
6
7
8
9
10
11
12
1
16x
6(B)
4x
2x
16x
6(C)
5
2x
2
1x
R
4
6
7
8
9
10
11
12
1
3
16x
6(D)
4x
2x
4
8
12
16
20
24
28
minutes

TABLE 4. Relative Retentions of the Numbered Peaks in Chromatograms of Petroleum Ether Extracts.

Peak No.	Relative retentions and σ abs	σ rel (%)
1	0.576 ± 0.0206	3.576
2	1.283 ± 0.0099	0.771
3	1.452 ± 0.0338	2.396
4	1.893 ± 0.0378	1.996
5	2.160 ± 0.0616	2.851
6	2.579 ± 0.0971	3.765
7	3.231 ± 0.1299	4.020
8	3.817 ± 0.2476	6.786
9	4.883 ± 0.1913	3.917
10	5.784 ± 0.2636	4.557
11	6.559 ± 0.3041	4.636
12	9.151 ± 0.6741	7.366
R	1.000	

FIGURE 7. (A) Chromatogram of White Granite Onions with Reference

(B) Chromatogram of White Granite Onions

FIGURE 8. (A) Chromatogram of Autumn Spice Onions with Reference

(B) Chromatogram of Autumn Spice Onions.

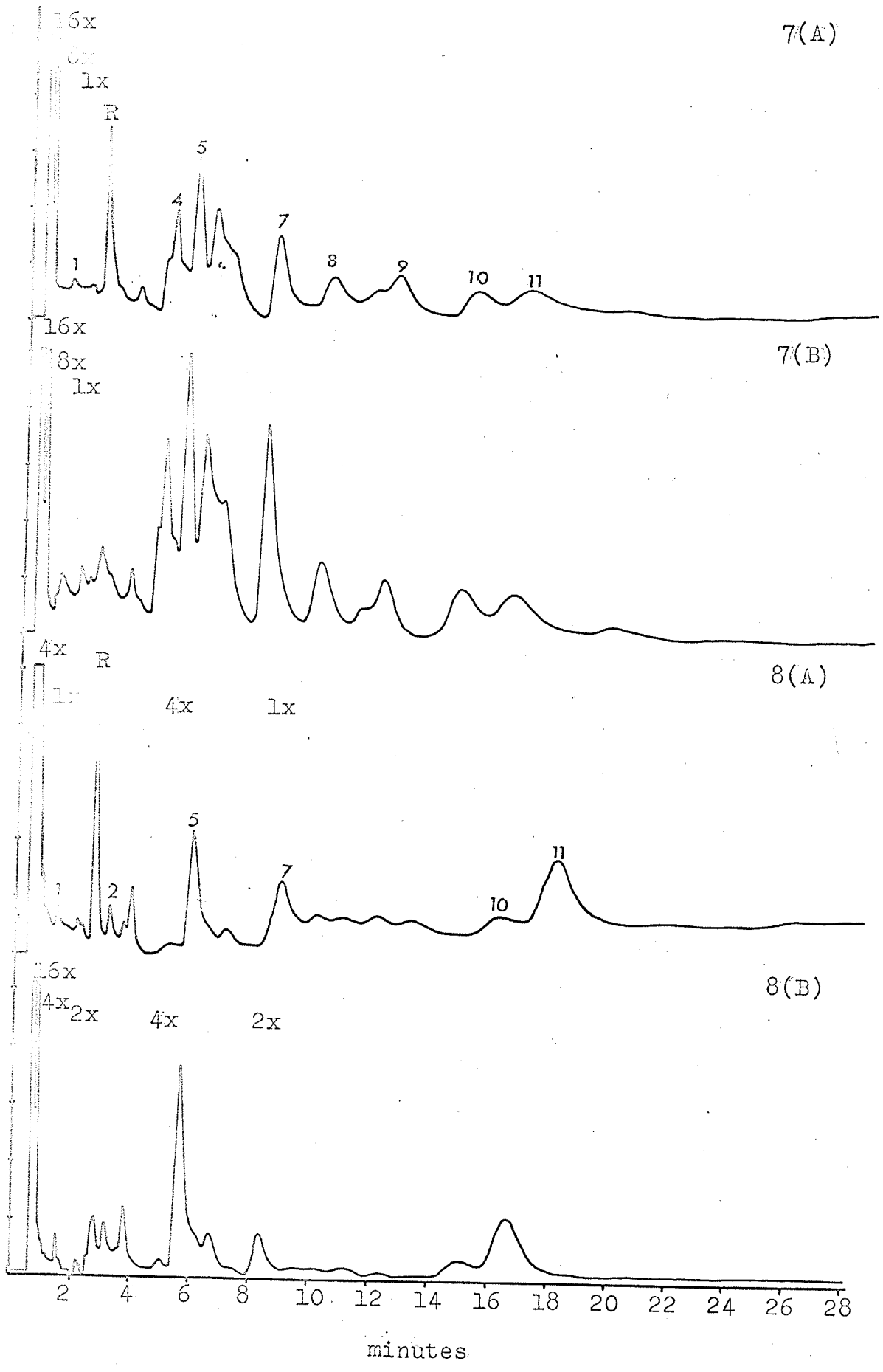

16x
1x
R
1
4
5
7
8
9
10
11
7(A)
16x
8x
1x
7(B)
4x
R
4x
1x
2
5
7
10
11
8(A)
16x
4x
2x
4x
2x
8(B)
2
4
6
8
10
12
14
16
18
20
22
24
26
28
minutes

FIGURE 9. (A) Chromatogram of Encore Onions with Reference

(B) Chromatogram of Encore Onions

FIGURE 10. (A) Chromatogram of Abundance Onions with Reference

(B) Chromatogram of Abundance Onions

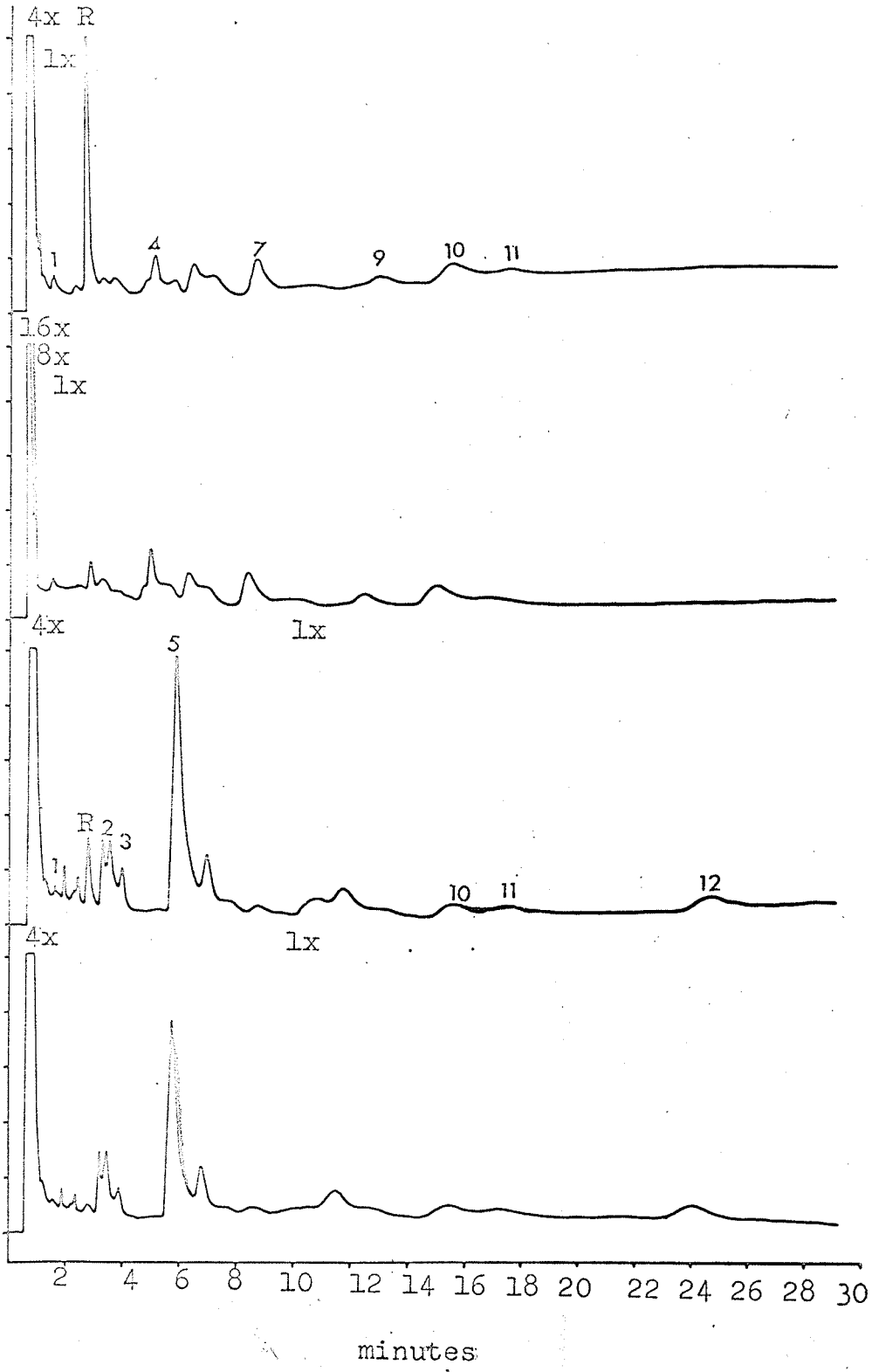

4x R
1x
1
4
7
9
10
11
9(A)
16x
8x
1x
9(B)
4x
1x
5
10(A)
R
2
3
1
10
11
12
4x
1x
10(B)
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
minutes

FIGURE 11. (A) Chromatogram of Harvest Pak 'A' Onions with Reference

(B) Chromatogram of Harvest Pak 'A' Onions

FIGURE 12. (A) Chromatogram of Autumn Splendor Onions with Reference

(B) Chromatogram of Autumn Splendor Onions

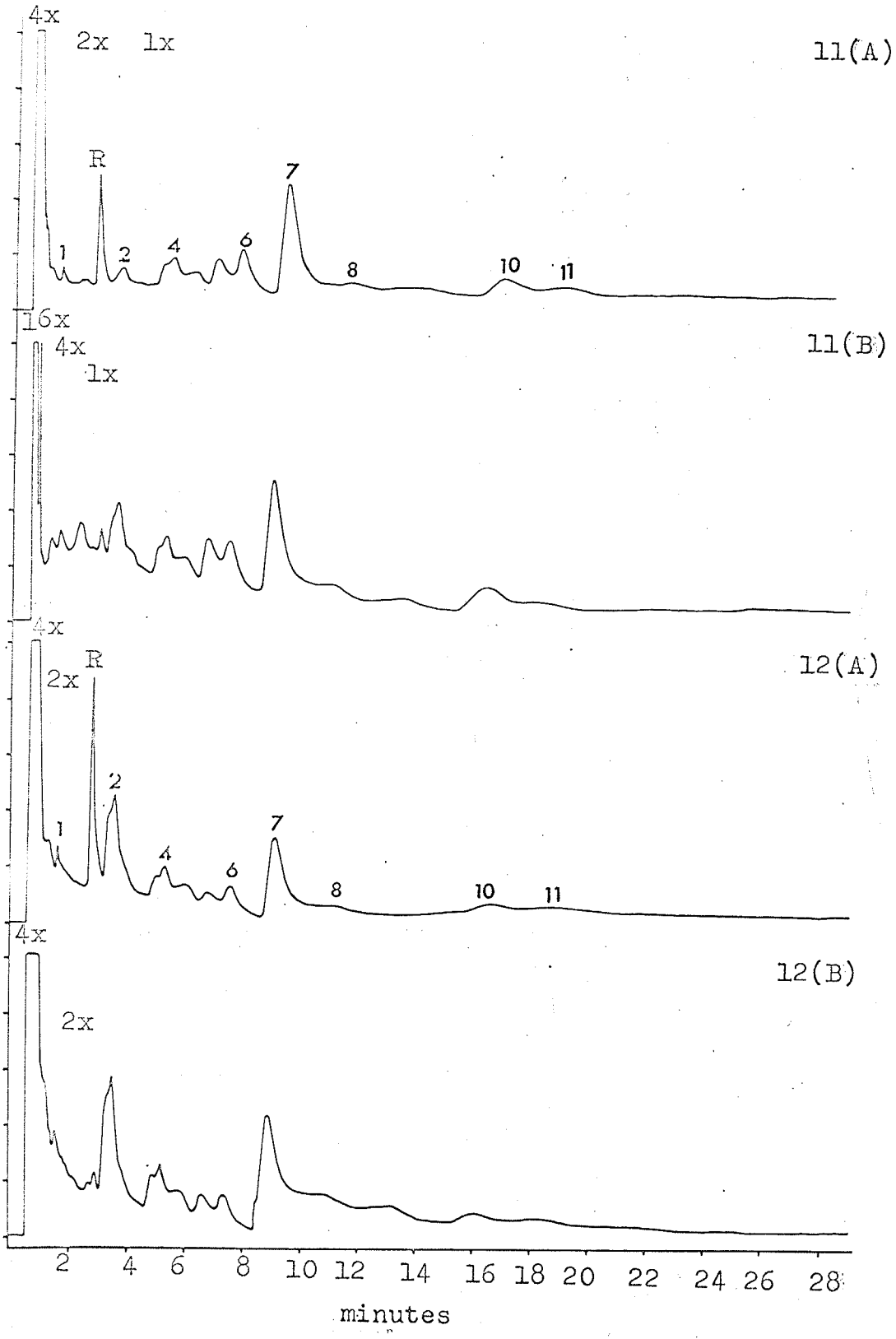

4x
2x
1x
11(A)
R
1
2
4
6
7
8
10
11
16x
4x
1x
11(B)
4x
2x
R
12(A)
1
2
4
6
7
8
10
11
4x
2x
12(B)
2
4
6
8
10
12
14
16
18
20
22
24
26
28
minutes

FIGURE 13. (A) Chromatogram of Fiesta Onions with Reference

(B) Chromatogram of Fiesta Onions

FIGURE 14. (A) Chromatogram of Aristocrat Onions with Reference

(B) Chromatogram of Aristocrat Onions

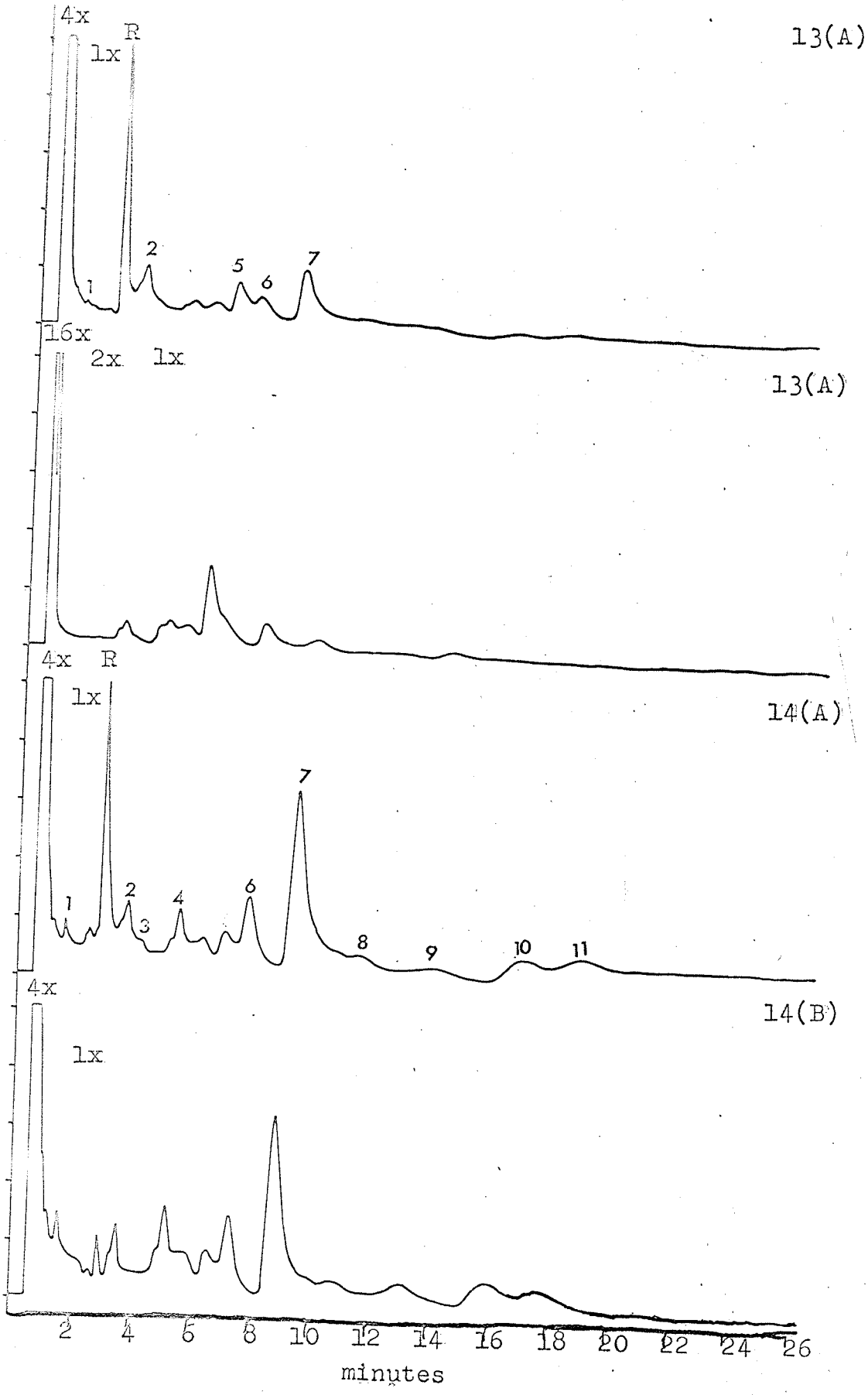

4x
1x
R
1
2
5
6
7
13(A)
16x
2x
1x
13(A)
4x
R
1x
1
2
3
4
6
7
8
9
10
11
14(A)
4x
1x
14(B)
2
4
6
8
10
12
14
16
18
20
22
24
26
minutes

FIGURE 15. (A) Chromatogram of Australian Brown Onions with Reference

(B) Chromatogram of Australian Brown Onions

FIGURE 16. (A) Chromatogram of Elite Onions with Reference

(B) Chromatogram of Elite Onions

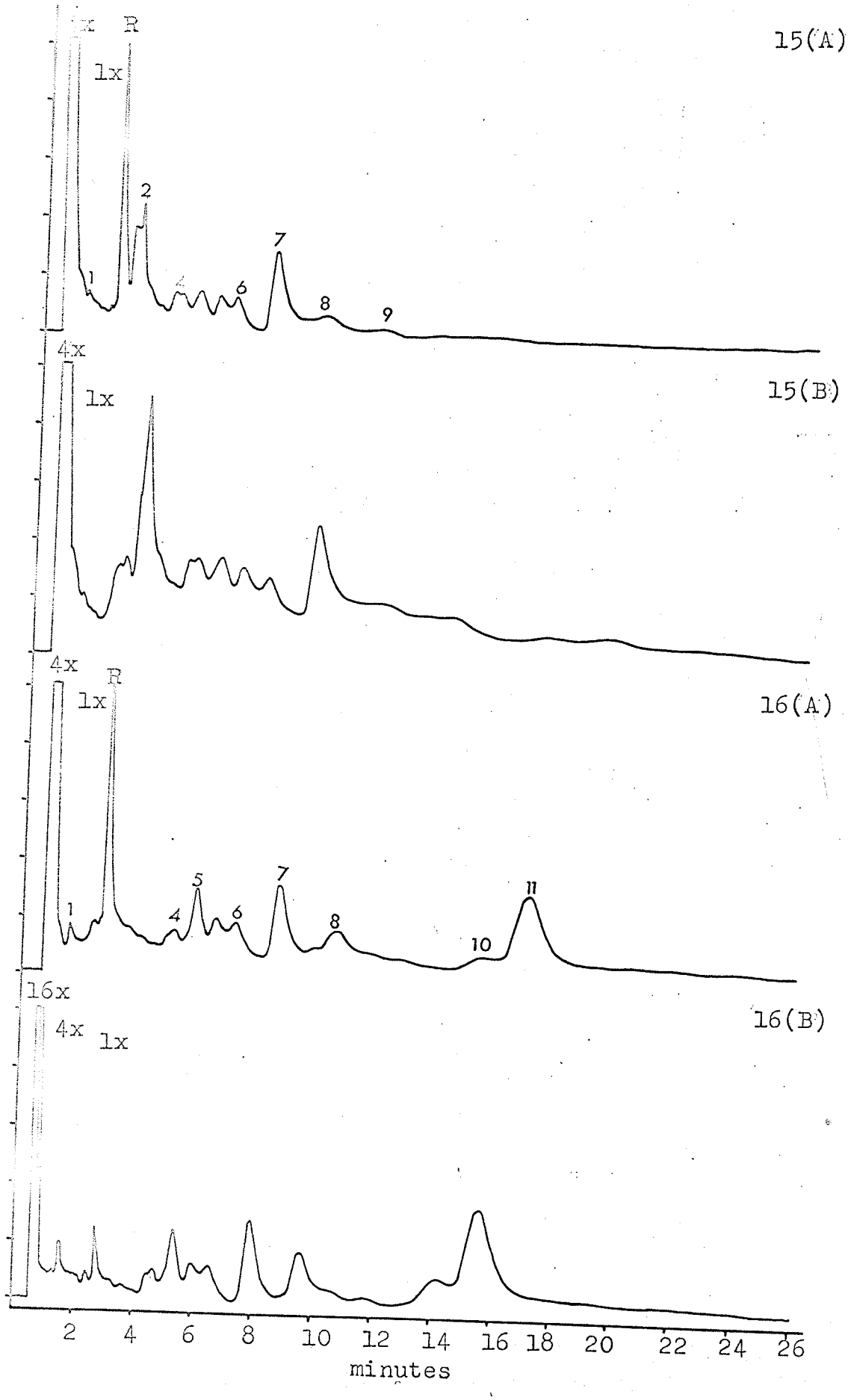

15(A)
1x
R
1x
2
1
4
6
7
8
9
4x
1x
15(B)
4x
1x
R
16(A)
1
4
5
6
7
8
10
11
16x
4x
1x
16(B)
2
4
6
8
10
12
14
16
18
20
22
24
26
minutes

FIGURE 17. (A) Chromatogram of Early Harvest Onions with Reference

(B) Chromatogram of Early Harvest Onions

FIGURE 18. (A) Chromatogram of W 45 Onions with Reference

(B) Chromatogram of W 45 Onions

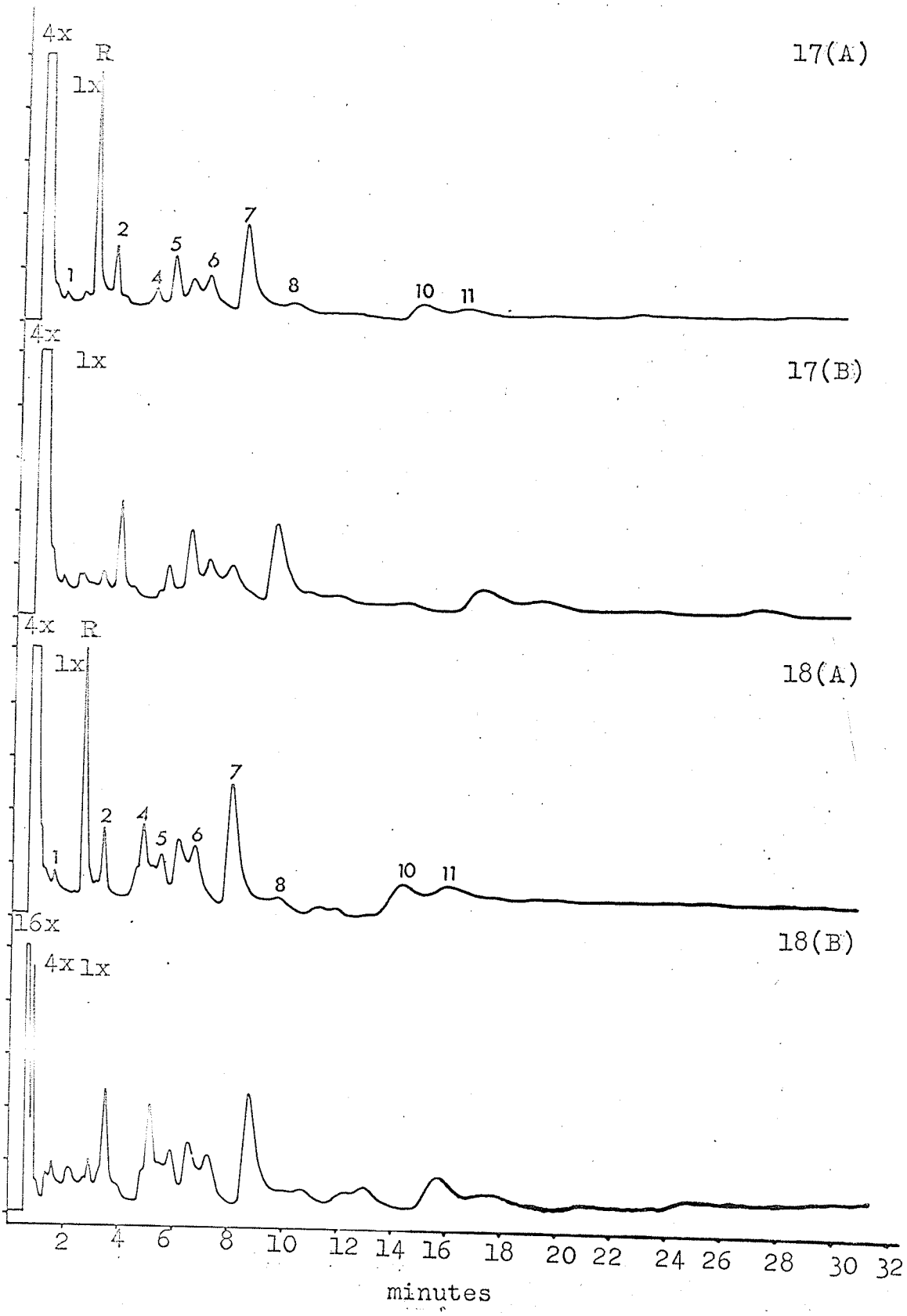

4x
R
1x
17(A)
1
2
4
5
6
7
8
10
11
4x
1x
17(B)
4x
R
1x
18(A)
1
2
4
5
6
7
8
10
11
16x
4x
1x
18(B)
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
minutes

FIGURE 19. (A) Chromatogram of Sanpak 'A' Onions with Reference

(B) Chromatogram of Sanpak 'A' Onions

FIGURE 20. (A) Chromatogram of Pacesetter Onions with Reference

(B) Chromatogram of Pacesetter Onions

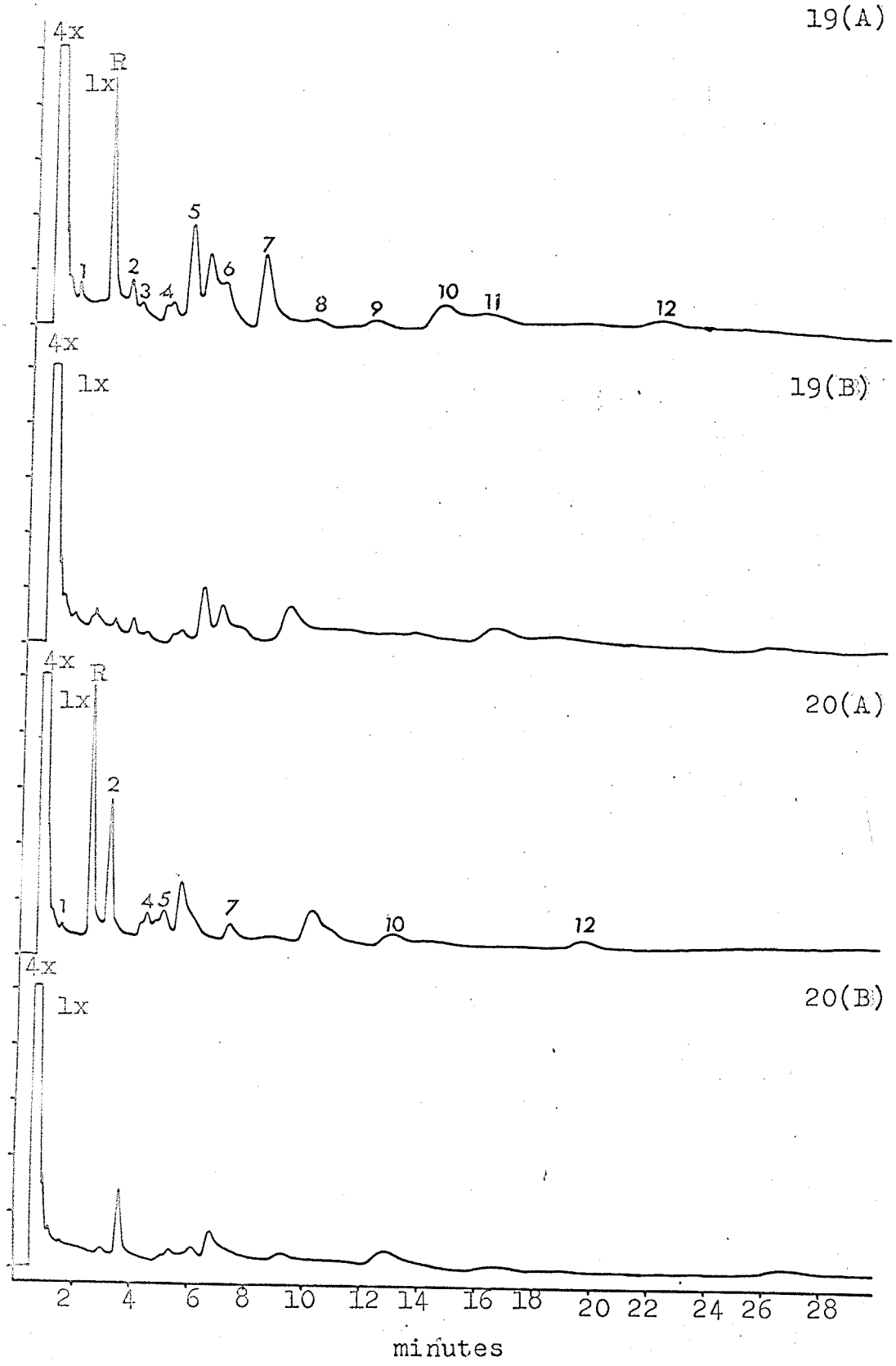
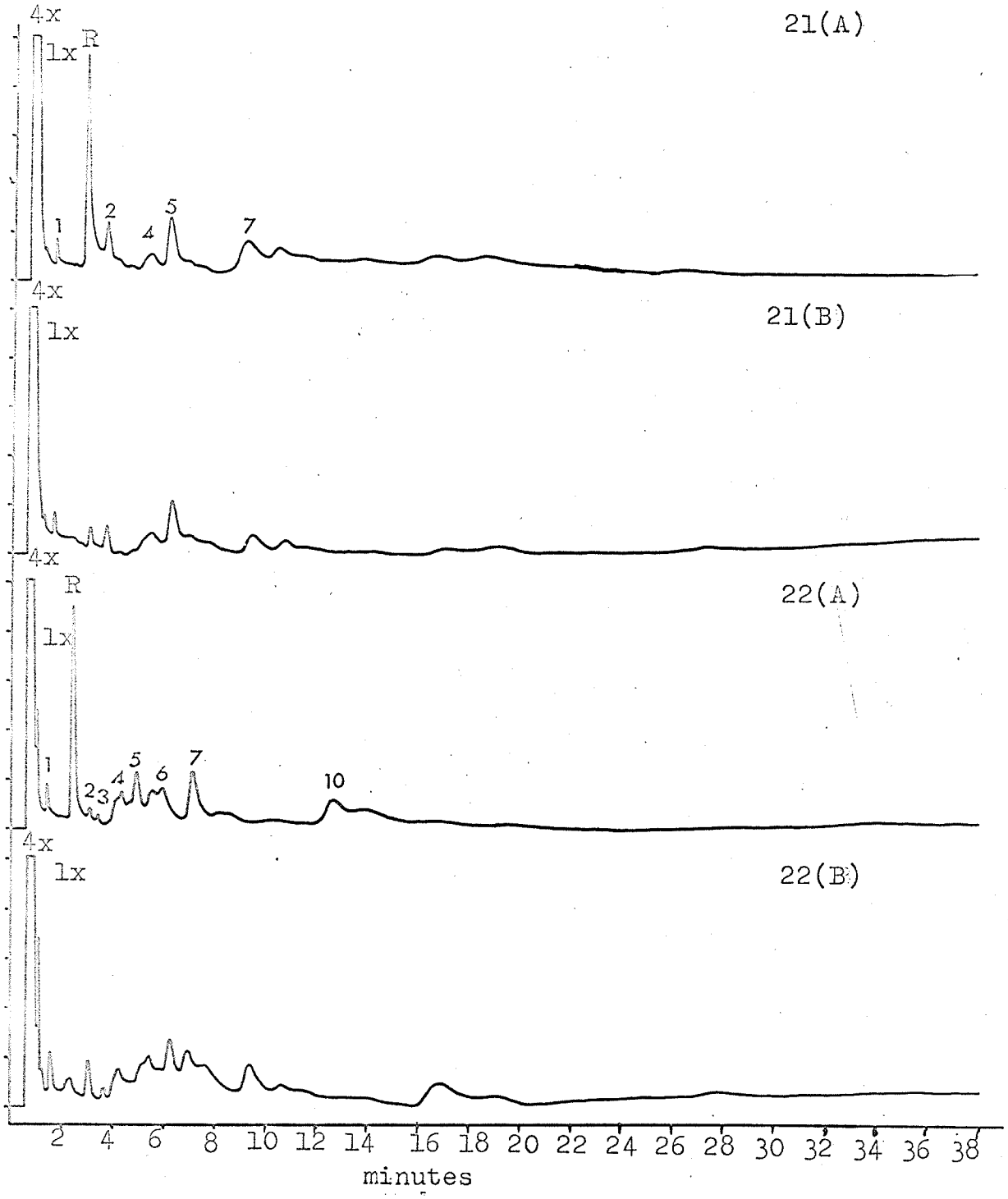

19(A)
4x
1x
R
1
2
3
4
5
6
7
8
9
10
11
12
19(B)
20(A)
20(B)
2 4 6 8 10 12 14 16 18 20 22 24 26 28
minutes

FIGURE 21. (A) Chromatogram of Pronto Onions with Reference

(B) Chromatogram of Pronto Onions

FIGURE 22. (A) Chromatogram of Elba Onions with Reference

(B) Chromatogram of Elba Onions


4x
1x
R
1
2
4
5
7
21(A)
4x
1x
21(B)
4x
R
1x
1
2
3
4
5
6
7
10
22(A)
4x
1x
22(B)
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38
minutes

Although data regarding the relative contribution to flavor of equivalent amounts of the individual components is lacking, comparison can be made of the chromatogram patterns, based on the organoleptic evaluation presented in Table 6.

By observation, it appeared that components in the peak range from 1-7 may be the most important to the flavor of these canned samples. Among these seven peaks, peak 4 and 7 seem to be predominate. The sum of the peak heights is related ($r = 0.66$, significant at $p = 0.05$) to the order in the pungency rating obtained from the organoleptic test. Whereas, a rather lower correlation coefficient ($r = 0.54$, significant at $p = 0.05$) between the heights of peak 7 in these chromatograms and the organoleptic test of these samples was obtained.

From this information, the isolation, identification and organoleptic evaluation of peaks 4 and 7 may be of primary concern. Similar studies on the other flavor components in the canned onion samples would be of considerable importance.

In addition, if the organoleptic coefficients of the individual components were established, an attempt to correlate the sum of products of peak area and the organoleptic coefficients of these components with the onion

pungency evaluated by organoleptic tests would therefore be possible.

(F). REPRODUCIBILITY OF CHROMATOGRAM PATTERNS OF HEADSPACE SAMPLES

Reproducibility of the headspace technique is shown in chromatograms of Figures 23 and 24. In these chromatograms obtained from two injections of headspace samples of each variety, a close agreement in the retention of each individual peak was obtained.

Relative retention of each of the seven peaks shown in chromatograms of headspace samples were calculated and listed in Table 5.

TABLE 5. Relative Retentions of the Seven Peaks Recorded in Headspace Samples.

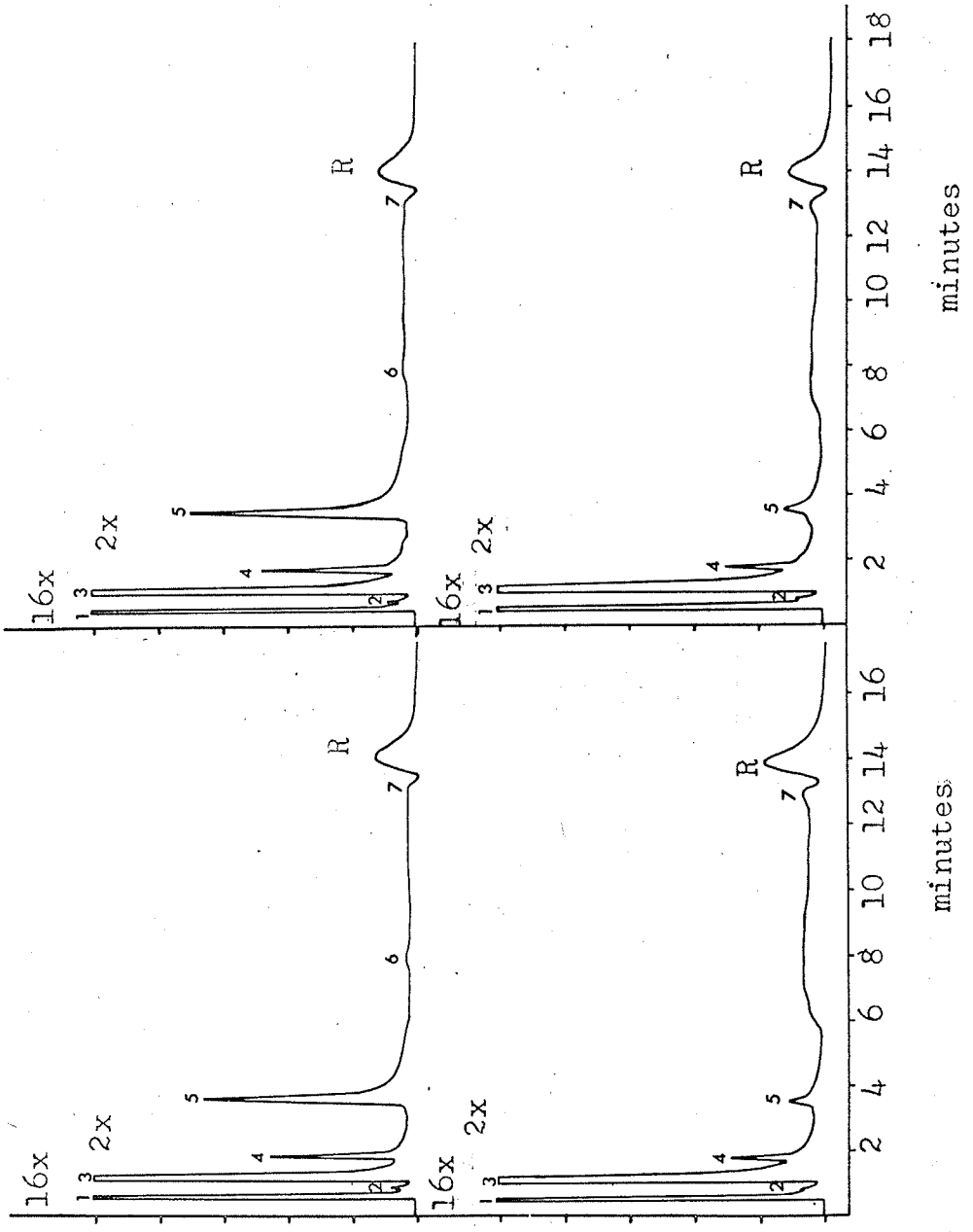
Peak No.	Relative retentions and σ abs	σ rel (%)
1	0.039 $\pm$ 0.0000	0.000
2	0.058 $\pm$ 0.0008	1.379
3	0.080 $\pm$ 0.0015	1.875
4	0.127 $\pm$ 0.0018	1.417
5	0.252 $\pm$ 0.0029	1.150
6	0.555 $\pm$ 0.0014	0.252
7	0.930 $\pm$ 0.0052	0.559
R	1.000	

FIGURE 23. (Upper left and right)

Chromatograms of Two Injections for Headspace Sample of Aristocrat Onions

FIGURE 24. (Lower left and right)

Chromatograms of Two Injections for Headspace Sample of Autumn Splendor Onions


16x
2x
1
3
2
4
5
6
7
R
2 4 6 8 10 12 14 16 18
minutes

(G). CHROMATOGRAMS OF HEADSPACE SAMPLES OF CANNED ONION SAMPLES

Chromatograms of headspace samples of twenty onion varieties are shown in Figures 23 - 42. Reference peaks are indicated by 'R' and the rest of the components are numbered by the order of effluence. The number one peak shown in every chromatogram of these headspace sample is an air peak. Five to six flavor components were recorded in each of these chromatograms. Chromatograms of headspace samples gave the lower boiling components which might be important to the smell and/or taste of these canned samples.

Comparison was made on the basis of the organoleptic and pyruvate tests with the chromatogram patterns. It appears that there is no significant relationship between the apparent relative peak height of the components present in headspace samples with pyruvate or organoleptic tests. However, readily detectable amounts of peak 7 were present in a number of strong onion varieties such as White Granite, W 45 and Autumn Splendor. From this it is assumed that this component may be important for flavor.

An equilibrium exists between the sulfur compound present as a gas in the headspace and that compound dissolved in the liquid fraction of a canned onion sample.

FIGURE 25 (Upper left) Chromatogram of Headspace sample of White Granite Onions.

FIGURE 26 (Center left) Chromatogram of Headspace sample of Early Yellow Globe Onions.

FIGURE 27 (Lower left) Chromatogram of Headspace Sample of Empire Onions.

FIGURE 28 (Upper right) Chromatogram of Headspace Sample of Autumn Spice Onions.

FIGURE 29 (Center right) Chromatogram of Headspace Sample of Abundance Onions.

FIGURE 30 (Lower right) Chromatogram of Headspace Sample of Encore Onions.

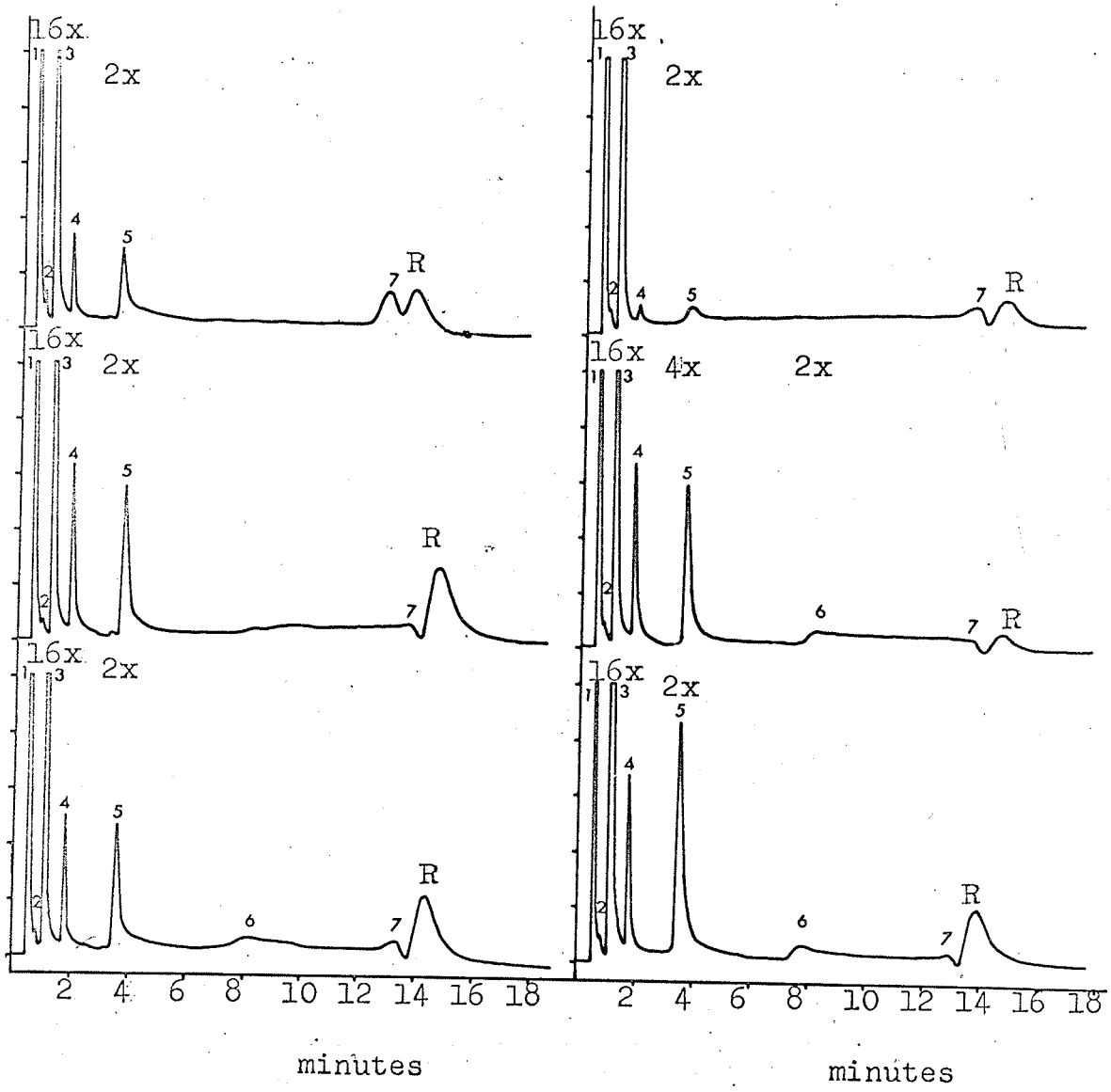

16x
2x
4x
R
1
2
3
4
5
6
7
2 4 6 8 10 12 14 16 18
minutes
minutes

FIGURE 31 (Upper left) Chromatogram of Headspace Sample of Harvest Pak 'A' Onions

FIGURE 32 (Center left) Chromatogram of Headspace Sample of Fiesta Onions

FIGURE 33 (Lower left) Chromatogram of Headspace Sample of Elite Onions

FIGURE 34 (Upper right) Chromatogram of Headspace Sample of Australian Brown Onions

FIGURE 35 (Center right) Chromatogram of Headspace Sample of W 45 Onions

FIGURE 36 (Lower right) Chromatogram of Headspace Sample of Early Harvest Onions

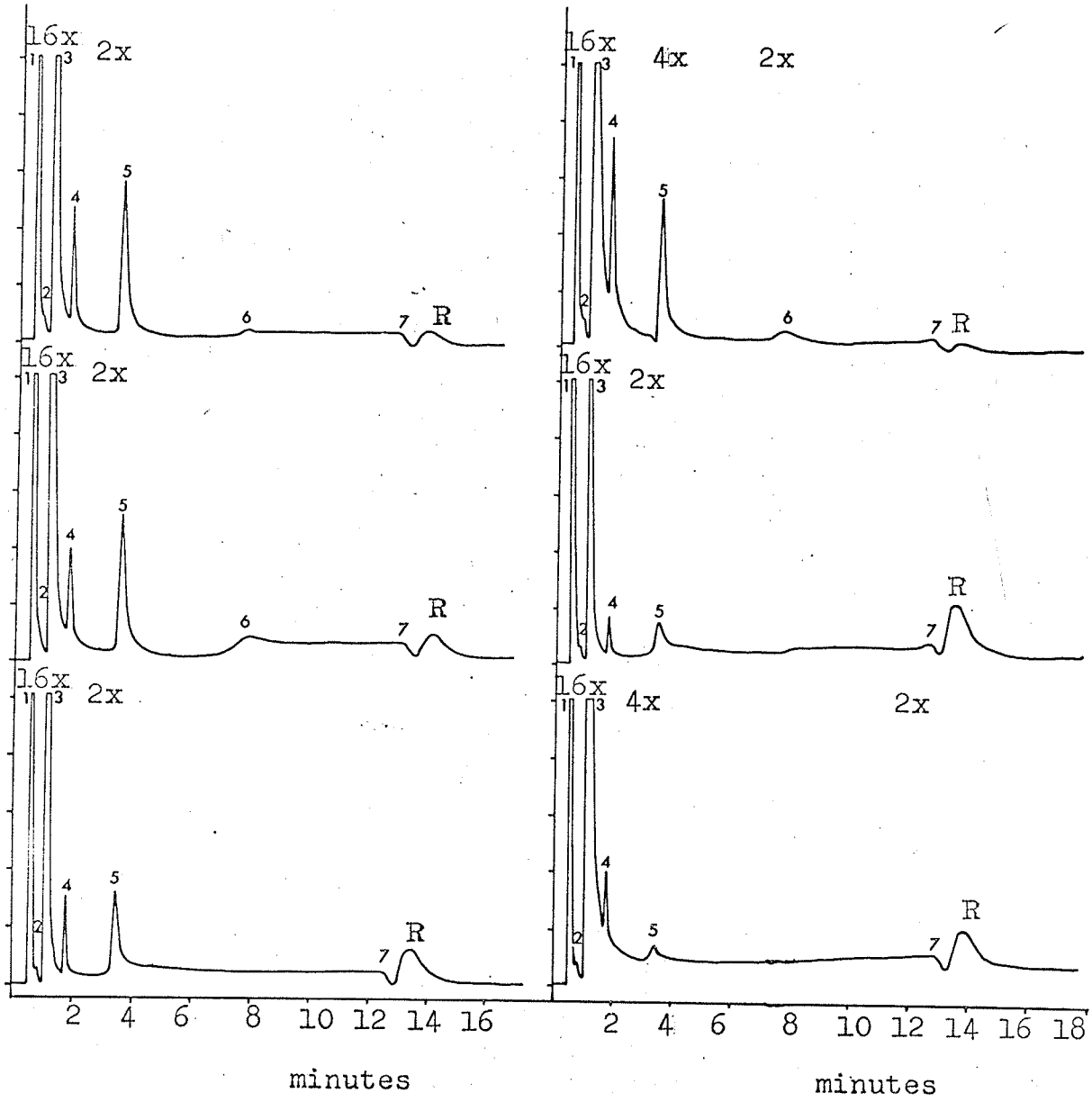

16x
2x
4x
1
2
3
4
5
6
7
R
2 4 6 8 10 12 14 16
minutes
2 4 6 8 10 12 14 16 18
minutes

FIGURE 37 (Upper left) Chromatogram of Headspace Sample of Elba Onions

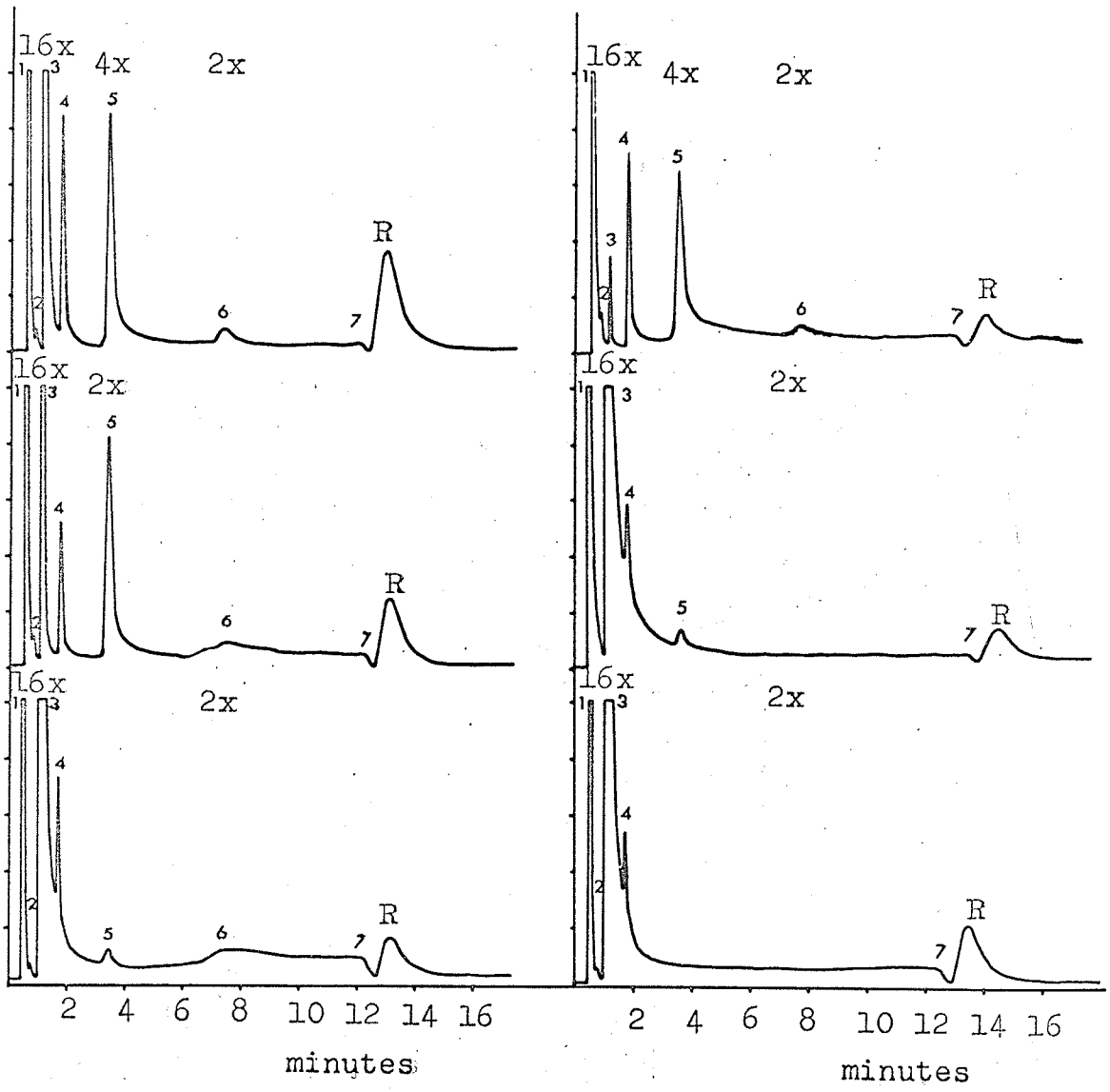
FIGURE 38 (Center left) Chromatogram of Headspace Sample of Experimental Hybrid #4 Onions

FIGURE 39 (Lower left) Chromatogram of Headspace Sample of Sanpak 'A' Onions

FIGURE 40 (Upper right) Chromatogram of Headspace Sample of Pacesetter Onions

FIGURE 41 (Center right) Chromatogram of Headspace Sample Copper Gem Onions

FIGURE 42 (Lower right) Chromatogram of Headspace Sample of Pronto Onions


16x
4x
2x
1
2
3
4
5
6
7
R
2 4 6 8 10 12 14 16
minutes

The percentage of the compound existing in the gaseous state may be insignificant and yet a minute amount may result in a significant sensation of small. However, due to variability in volatility, partition coefficient and odor strength of the various sulfur compounds, gas chromatographic determinations of the petroleum ether extract are likely more important indicators of onion pungency than determinations of headspace.

Nevertheless, further investigation on the identification and organoleptic evaluation of headspace components, may aid in determining the relationship of these compounds to those in the petroleum ether extract. Such data would be useful in establishing a procedure for the gas chromatographic evaluation of onion pungency.

(H). RESULTS FROM THE ORGANOLEPTIC EVALUATION OF ONION PUNGENCY

Results shown in Table 6 are from the organoleptic evaluation of onions which were tested at the Quality Control Laboratory of Campbell Soup Company in Toronto. Four judges were asked to rank the varieties by comparing them to the standard variety, Early Yellow Globe.

(I). RESULTS FROM THE PYRUVATE TEST OF ONION PUNGENCY

Schwimmer and Weston (1961) published a paper describing the pyruvate method for evaluating onion pungency.

TABLE 6. Results from the Organolpetic Evaluation of Onion Pungency.

Variety	Organoleptic evaluation % of standard variety (mean of four judges)
W 45	185.5
White Granite	166.0
Autumn Splendor	114.1
Experimental Hybrid #4	110.3
Encore	109.7
Aristocrat	100.0
Elite	100.0
Early Harvest	100.0
Harvest Pak 'A'	96.6
Abundance	95.4
Fiesta	93.2
Empire	91.2
Australian Brown	91.2
Copper Gem	91.2
Autumn Spice	82.4
Pacesetter	74.7
Sanpak 'A'	73.5
Pronto	73.5
Elba	71.3

A highly significant correlation was found between the amount of enzymatically developed pyruvate present in the juice of comminuted onion and the olfactory threshold concentration of the juice (Schwimmer and Guadagni, 1962). In their paper they also showed that a highly significant correlation existed between the total pyruvate and the threshold concentrations of the twenty lots of onions. The pyruvate method was recently utilized to evaluate the pungency of commercially dehydrated onion powder (Schwimmer et al. 1964).

The pyruvate method was used in this study to evaluate the pungency of fresh onion samples of twenty onion varieties. The results obtained from the pyruvate method are listed in Table 7.

Results from the organoleptic evaluation of canned samples and the pyruvate method for estimating the pungency of these twenty onion varieties (fresh onions) were compared. A correlation coefficient $r = 0.46$ (significant at $p = 0.05$) was computed. These results lend support to the data presented by Schwimmer and Guadagni (1962) and also suggest that the relative pungency of onion varieties remains unchanged with this type of processing.

TABLE 7. Results from the Pyruvate Method of Evaluating Onion Pungency

Variety	Pyruvate concentration* (μ moles/ml)
White Granite	36.25
Copper Gem	35.50
W 45	33.75
Australian Brown	32.50
Early Yellow Globe	31.90
Elba	31.00
Fiesta	30.50
Early Harvest	29.00
Harvest Pak 'A'	29.00
Encore	28.40
Autumn Spice	28.30
Autumn Splendor	27.50
Empire	26.90
Aristocrat	26.80
Pronto	26.80
Abundance	25.00
Sanpak 'A'	24.80
Pacesetter	24.00
Experimental Hybrid #4	23.50
Elite	22.00

* The values presented in this table are the means of two determinations. Duplicates with a variation greater than 6% were discarded.

GENERAL DISCUSSION AND CONCLUSION

The gas chromatographic method for analysis of onion flavor components described in this investigation is rapid, sensitive and reliable. An excellent reproducibility of chromatogram patterns, of relative retentions and of peak heights indicated that this method has an advantage when used in quantitative and qualitative analysis of onion flavor. The extremely sensitive and selective electron capture detector can in some cases omit the clean up procedure in the preliminary sample preparation. By combining the solvent extraction and headspace techniques, one is able to isolate most of the volatile components of a wide boiling range from any given onion sample. Furthermore the carbowax column which gave a complete separation for mercaptans and sulfides (Carson and Wong,1959b) has given a successful separation for the flavor components of onion varieties. Further modification and addition of some ancillary devices to this instrument would make it more suitable for routine work.

The results of the preliminary study (LaCroix et al. 1966) showed a striking varietal difference among the flavor components of onions. The environemtnal conditions appear to have little effect on the varietal composition. Results of the present study on the flavor components of

canned onion samples also showed a striking varietal difference as revealed by the gas chromatographic method. Of the twenty onion varieties studied, no two varieties produced identical chromatogram patterns.

Results of the present study demonstrated that peaks 4 and 7 of the petroleum ether extracts were the predominate components contributing to the flavor of canned onion samples. A statistically significant correlation coefficient ($r = 0.66$) was found between the sum of the heights of peaks 4 and 7 and the organoleptic test of these samples, whereas a lower correlation coefficient ($r = 0.54$) was found between the peak height of peak 7 alone with the pungency rating of these samples. Of the components present in the headspace chromatograms, peak 7 appeared to be the only one which may have some importance in relation to flavor.

Results presented in gas chromatographic analysis of onions showed a large number of components which could have a relationship to flavor. It would be possible by further research to chemically identify and rate the odor strength of all of these components, however, further investigations of the components represented by the peaks previously mentioned may be the most fruitful initial undertaking.

An attempt to correlate these components with the pungency obtained from organoleptic evaluation could then

be made by isolating these chromatographically separated components and establishing an organoleptic coefficient for each. Eventually, the sum of the products of peak area and organoleptic coefficient of each component may be used as an index of the overall flavor of onion samples.

Information of this type would be of value to food technologists, chemists and plant breeders. Aware of the importance of the predominate flavor components in onion samples, food technologists could attempt to preserve these components as much as possible in the finished products. Plant breeders could attempt to develop varieties which contain large proportions of these components for canning. On achieving the technique of analysis of onion samples, plant breeders could attempt to evaluate objectively a particular flavor component content of breeding lines. Eventually, they might be able to breed for a specific grade of onion which will have a higher content of the particular components that determine the odor strength of that onion variety. Thus various grades of onions on the basis of the content of the flavor component could be selected to meet the need of the commercial market.

A statistically significant correlation coefficient ($r = 0.46$) was found between the amounts of the total pyruvic acid in the comminuted onion juice and the organo-

leptic evaluation of flavor of canned samples. This substantially supports the paper of Schwimmer and Weston (1961) that pyruvate measurement can be used to estimate onion pungency.

Saghir et al.(1964) did not succeed in their attempts to correlate the total sulfide peak area from the gas chromatograms with the enzymatically produced pyruvate. However, pyruvate was correlated with organoleptic tests (Schwimmer and Guadagni,1962). Saghir et al.(1964) claimed that this failure might indicate a lack of uniformity of sample preparation for chromatography or a variation in volatility from tissues, or it might arise from other causes.

According to the equation mentioned previously, pyruvic acid is evolved enzymatically through crushing of onion tissues and appears stoichiometrically with the amount of allicin formed. The allicin finally, gives rise to a number of sulfides which determine the pungency of a particular onion variety. Since the relative concentration of an equivalent amount of each of the sulfides to the pungency is different, the amount of precursor (allicin) is obviously not directly related to the total odor strength of its break down products (sulfides). Thus Saghir's attempts to correlate the total sulfide peak area with the amount of pyruvate produced in the onion juice did not succeed. In addition, the limitation of

the sensitivity of a hydrogen flame ionization detector to these sulfides may be a contributing factor to this failure.

Among all the methods available for the evaluation of onion pungency, organoleptic tests are the most reliable. However, organoleptic tests have a number of disadvantages in routine analysis. Laborious procedures are involved in the preparation of samples for these tests. A large number of samples and a number of judges are involved and the results are also influenced by individual judgements. The pyruvate method, on the other hand has a simple, standardized procedure for the sample preparation and for the pyruvic acid determination. However, as previously suggested, it is illogical to conclude that the measurement of a sulfide precursor should give an accurate evaluation of onion pungency. Therefore, the pyruvate method can be criticised on this basis and in fact the method has not found a general application in the commercial food processing industry.

The gas chromatographic method for the analysis of flavor compounds in vegetables, fruits and food products has been well developed during the last few years, for it has the great advantage of being simple, sensitive and rapid. Gas chromatographic methods have proven to be successful in the application to analysis of the flavor components

of fresh or canned onion samples. However, a standard procedure for rating pungency of onion varieties has not as yet been established and widely accepted.

SUMMARY

Electron capture gas chromatography was utilized to study the flavor components from the petroleum ether extracts and headspace of canned onion samples. Excellent reproducibility of the chromatogram patterns, relative retentions and peak heights by this method was demonstrated.

Chromatogram patterns of petroleum ether extracts and headspace of canned samples of twenty onion varieties revealed a striking varietal difference in the flavor components.

Several predominate flavor contributing components were found in petroleum ether extracts and headspace samples by comparing these chromatograms with the results obtained from organoleptic and pyruvate tests. A positive correlation ($r = 0.66$) between the sums of heights of peaks 4 and 7 (hexane extract) and the organoleptic rating suggested that a relatively small number of components may be important for flavor. These results encourage the belief that the gas chromatographic procedure may become a useful routine procedure for onion flavor evaluation. Plans for further isolation, organoleptic evaluation and peak area correction of each flavor component are proposed in this study. Theoretically a highly significant correlation between the total correlated peak areas and organoleptic test would be expected.

A statistically significant correlation coefficient ($r = 0.46$) was found between the pyruvate test of fresh onion samples and the organoleptic evaluation of the pungency of canned onion samples of these twenty varieties.

Conventional methods of onion pungency evaluation were compared and criticized. Further research on the application of gas chromatographic method to study the onion components and to evaluate the onion pungency was was strongly recommended.

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