mg. per liter for linaloöl and 12.6 mg. per liter for α-terpineol, their approximate concentrations in peel juice. Three out of 14 described linaloöl as conferring a bitter flavor, but only one out of 11 thought α-terpineol bitter when each was added singly. However, when 8 mg. of linaloöl and 3 mg. of α-terpineol were added together to 1 liter of juice, the taste was judged objectionable and bitter. These concentrations were about one third those of the peel juice used which contained about 23 mg. of linaloöl and 8.5 mg. of α-terpineol per liter. However, peel juice is much more bitter than can be accounted for by its content of these alcohols, but they do appear to contribute to its total taste effect.

As noted in the experimental section it is necessary to neutralize orange juice before distillation to prevent conversion of *d*-limonene to terpineol. If sodium or potassium hydroxide is used for this purpose, uncontrollable foaming results, probably because of the soluble soaps formed from the lipide matter of the juice. Calcium hydroxide, which forms insoluble soaps, avoids this difficulty.

Conclusion

Linaloöl and α-terpineol have been identified as highly flavored constituents of orange peel juice. A method has been devised for their determination in orange juices and oils. Part of the linaloöl may come from other locations in the peel than the peripheral oil cells. Taste tests were conducted to establish the taste thresholds, levels at which they became objectionable, and flavor character. These substances added individually were not particularly bitter but, when added together, they were. It was not possible, however, to account for a major part of peel juice bitterness on the basis of linaloöl and β-terpineol contents.

Acknowledgment

The author wishes to thank Gordon Fisher of the Naval Stores Station at Olustee, Fla., for running the infrared curves by which linaloöl and α-terpineol were identified. Thanks are due to M. K. Veldhuis of this station for suggestions concerning applications.

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Received for review September 8, 1960. Accepted December 28, 1960. The use of trade names in this article is for identification only and implies no endorsement of manufacturer or product. U. S. Fruit and Vegetable Products Laboratory is one of the laboratories of the Southern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

ONION FLAVOR AND ODOR

Enzymatic Development of Pyruvic Acid in Onion as a Measure of Pungency

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Pyruvic acid appears enzymatically in onion tissue disintegrated by comminution. Over 95% of the maximum amount of pyruvic acid is produced within 6 minutes after the start of comminution. The total amount produced appeared to depend on the generally accepted degree of pungency of the onion lot investigated. Weak onions produced 2 to 4 μmoles, those of intermediate strength 8 to 10 μmoles, and strong onions 15 to 20 μmoles of pyruvic acid per gram of onion. The enzymatic basis of the method, as well as its relation to other methods of estimation of pungency, is discussed.

A SURVEY of recent literature on the chemical and enzymological properties of the onion strongly suggests that its pungency arises as a result of the interaction of *S*-substituted L-cysteine sulfoxide derivatives and enzymes of the alliinase type when the integrity of the onion tissue is destroyed by comminution or other means:

$$RS\overset{\uparrow}{O}CH_2CHNH_2COOH + H_2O \dashrightarrow [RSOH] + NH_3 + CH_3COCOOH$$

(pyruvic acid)

The presumed unstable initial products, RSOH [sulfenic acids (*22*)], can then react in several ways to form sulfur-containing odoriferous substances which impart the characteristic pungency to homogenates of onion.

The existence of such amino acids in the onion is evidenced by paper chromatographic identification (*19, 23*) and more recently by the actual isolation and positive identification of *S*-methyl-L-cysteine sulfoxide (MCSO) and *S*-propyl-L-cysteine sulfoxide (PCSO) (*25*) from onion, and of cycloalliin (*24*) (3-methyl-1,4 - thiazane - 5 - carboxylic acid - 1-oxide). The existence in onion of the appropriate enzyme and its partial purification have recently been reported (*20*). Evidence exists that a substantial part of the volatile reaction products of the hypothetical sulfenic acid intermediates are present in onion homogenates as methyl and propyl esters of methyl and propyl thiosulfinic acids ($RS\overset{O}{\overset{|}{S}}R$). Thus, they react with thiamine to give rise to allithiamine analogs (*14*); with *N*-ethyl maleimide after appropriate extraction procedures (*4, 20*); and after heating, give rise to families of alkyl di- and trisulfides which have been identified after separation by gas chromatography (*3*). In addition, the following volatile sulfur-containing compounds have been reported to be present in onion volatiles: *n*-propanethiol (*3, 6, 16*); *n*-propylthioaldehyde (*10*); hydrogen sulfide and sulfur dioxide (*4, 16*).

The presence of relatively large

Table I. Development of Pyruvate in Onion Slurries

	Method[a]					
	A	B	C	A	B	C
	μmoles Pyruvate/G. Onion					
	Red Bermuda			Southport White Globe		
Test	7.1	6.0	4.5	19.8	16.7	16.9
Control	3.2	2.0	0.9	3.8	2.5	1.6
Difference	3.9	4.0	3.6	16.0	14.2	15.3
Variation (±)	0.9	0.2	0.1	0.2	0.1	0.2

[a] Described in text under Materials and Methods.

amounts of pyruvic acid in onion was first detected qualitatively by Bennet (*2*). Morgan (*15*) proved its presence by isolation of its 2,4-dinitrophenylhydrazone (DNPH) from an unheated macerate and demonstrated that it arises enzymatically from precursors. Vilkki (*23*) showed that these precursors are converted to both pyruvate and ammonia by onion juice. Schwimmer *et al.* (*20*) demonstrated that alliinase of onion produces pyruvate and ammonia in amounts equivalent to that of the disappearance of the substrate as well as less than the stoichiometric amounts of thiosulfinates.

The above summary of recent investigations on the mechanism of production of volatile flavor components suggests several approaches toward the objective evaluation of pungency of onions. The most direct over-all approach would be the determination of volatile sulfur. This was used in the pioneering work of Platenius (*17*). He determined sulfur (as barium sulfate after oxidation with bromine) in a distillate after acid hydrolysis of the onion at high temperatures for prolonged periods. Currier (*7*) improved this method by using lower temperatures and by converting sulfur eventually to methylene blue, thus affording in effect a colorimetric determination of sulfur. Kohman (*11*), aware of the enzymatic nature of the production of the sulfur volatiles, employed steam distillation of onion macerates in a special apparatus, followed by conversion of the volatile sulfur components to barium sulfate. There appears to be a highly significant correlation between dry weight and pungency (*18*).

Other methods which might be used on the basis of present knowledge of the properties of the sulfur volatiles are: antibiotic activity (*25*); reaction with *N*-ethyl maleimide (*3, 20*); reaction with thiamine to form analogs of allithiamine (*15*) which possess characteristic ultraviolet absorption spectra (*8*); and gas chromatography (*20*).

As an alternative to estimation of sulfur volatiles, the determination of increases in ammonia or pyruvic acid in macerates suggests itself. The large amounts of endogenous ammonia render the determination of ammonia unattractive. On the other hand the determination of pyruvic acid as a measure of flavor has already been applied to garlic by Jäger (*9*) and more recently to Mexican varieties of garlic (*1*).

In the present investigation, it has been demonstrated that onion homogenates from different varieties and lots of onion produce pyruvic acid in varying amounts, which appear to correlate well with the generally accepted pungency of these varieties.

Materials and Methods

Source of Onions. All but one lot of the onions investigated were obtained from local commercial sources at the time they first appeared on the market. The lot of Southport White Globe onions (strain Sunspice) (Basic Vegetable Products, Inc.) had been stored at 34° F. for 8 months prior to analysis.

Preparation of Onion Macerates. Duplicate batches, each consisting of at least four onions weighing between 400 and 700 grams, were blended with equal volumes of water for 3 to 5 minutes at room temperature (test sample). Between 10 and 20 minutes after completion of the blending, to each of duplicate 5-gram aliquots of the slurry were added 5 ml. of trichloroacetic acid. After at least 1 hour, the slurry was filtered through Celite on a Büchner funnel and washed with water to a total volume of 200 ml. The heated control onions were treated in the same way, except that the onions were first heated to destroy enzyme activity by radiofrequency energy (λ = 12 cm.) for 5 minutes in an electronic oven (Radarange, Raytheon Corp.) and then extracted as for the test lots of onions.

Determination of Pyruvic Acid. Method A. The method is essentially that used by Schwimmer *et al.* for the determination of alliinase activity (*20*). It determines total 2,4-dinitrophenylhydrazine-reacting carbonyls. To 1 ml. of filtrate was added 1 ml. of 0.0125% 2,4-dinitrophenylhydrazine in 2*N* HCl and 1 ml. of water. After 10 minutes at 37° C., 5 ml. of 0.6*N* NaOH were added and absorbance was measured with an Evelyn colorimeter (420-mμ filter, 10-ml. setting, reagent blank set at 0 absorbance). The calibration curve obtained using sodium pyruvate as standard is shown in Figure 1, *A*. Here are plotted micromoles of pyruvate in the final chromogenic solution *vs.* absorbance. This method was used routinely for the determination of enzymatically liberated pyruvic acid in the different onion varieties. Figure 2 shows that at least 95% of the maximum of pyruvate developed within 6 minutes after the beginning of the comminution process.

Method B. This method, which measures the concentration of the DNPH of pyruvic acid after differential extraction to remove unreacted hydrazine derivative and nonpyruvate hydrazones, is based on the method developed for garlic by Jäger (*9*) and modified by Alfonso and Lopez (*1*). The onion filtrate, diluted to 50 ml., was treated with excess 0.2% 2,4-dinitrophenylhydrazine in 2*N* HCl (1.4 ml. for the control, 3 ml. for test filtrate). After standing at room temperature for 1 hour, the solution was extracted according to the scheme shown in Figure 3 and the final ammoniacal extract was diluted to 50 ml. After 1 to 10 dilution in 2*N* ammonia, the absorbance of the presumably pure pyruvate hydrazone was measured at 370 mμ (Beckman spectrophotometer; 1-cm. light path; reference cell, 2*N* ammonia). A calibration curve using highly purified recrystallized DNPH of pyruvic acid (212° C.) dissolved in 2*N* ammonia is shown in Figure 1, *B*. Actually, recovery of standard sodium pyruvate with the extraction procedure employed amounted to considerably less than 100% and was variable. Hence it was necessary to carry through a sodium pyruvate control along with the onion filtrates and to apply the appropriate correction factor.

Method C. This method is based on the oxidation of reduced diphosphopyridine nucleotide (DPNH) by the pyruvate of the onion extracts in the presence of excess of crystalline lactic dehydrogenase (*12*). To 5 grams of slurry were added 5 ml. of 0.10*N* HCl. An aliquot of the filtrate (0.05 to 0.1 ml.) was added to a lactic dehydrogenase reaction mixture containing 0.033*M* phosphate buffer, 0.2 μmole of DPNH, and 0.05 ml. of lactic dehydrogenase (Worthington Biochemical Corp., diluted 1 to 10) in a total of 3 ml. The absorbance at 340 mμ was followed until no further change occurred. The decrease in absorbance due to oxidation of DPNH was then used to calculate the amount of pyruvate in the reaction mixture. Stoichiometric relation between standard pyruvate added and DPNH oxidized was obtained only when freshly prepared solutions of DPNH were used (Figure 1, *C*).

Results

Comparison of Pyruvic Acid Methods. California Early Red and Southport White Globe varieties were

Table II. Development of Pyruvate in Onion Homogenates

Variety or Type	Pyruvate, μmoles/G. Test	Control	Diff.	Variation (±)
White Globe (cooking)	22.1	2.4	19.7	0.8
Yellow Globe	20.3	3.7	16.6	1.1
South port Red Globe	20.2	4.1	16.1	1.0
Southport White Globe	19.8	3.8	16.0	0.3
White Grano	11.8	2.1	9.7	0.6
Scallions, mid-section	9.7	1.8	7.9	0.2
White Bermuda Hybrid	10.0	2.2	7.8	0.6
"Strong" scallions, bulb	6.4	1.9	4.5	0.1
California Early Red	7.1	3.2	3.9	0.3
"Weak" scallions, bulb	3.9	1.9	2.0	0.3

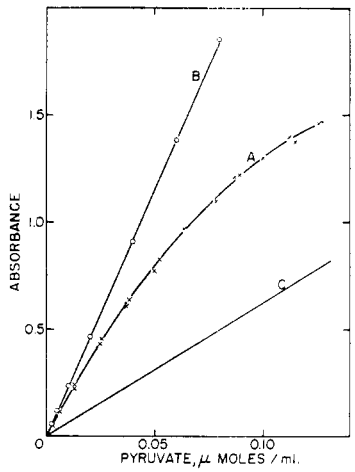

Figure 1. Calibration of pyruvic acid methods described in text (Materials and Methods)

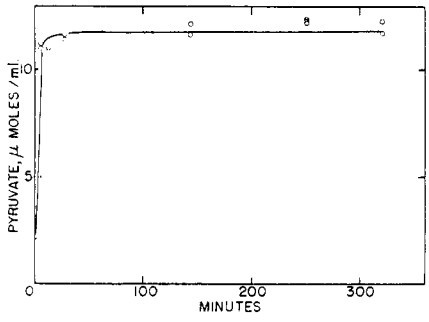

Figure 2. Development of pyruvic acid in a homogenates of White Grano onions

selected as representative of weak and strong onions, respectively. Table I shows that the amounts of apparent pyruvate produced enzymatically (difference between test and control) were approximately the same for the three methods. Since Methods B and C

Table III. Concentration of Onion Components Related to Pungency

Determination or Components	Lit.	Concn. μmoles/G. Onion
Pyruvic acid	Present (7, 16, 19)	2–19
Total volatile sulfur		2–5
Steam-distilled sulfur	(11)	2–7
NCSO + PCSO	(22)	1.6–1.9
MCSO + PCSO + cycloalliin	(22, 23)	5–11
Total S in methanol[a] extract	..	18
Pyruvic acid–garlic	(1, 8)	47–63

[a] Present in Southport White Globe onion used in present studies (5).

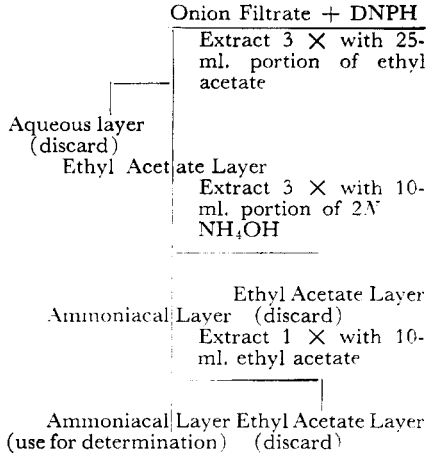

Figure 3. Scheme for extraction of pyruvic 2,4-dinitrophenylhydrazone

are designed to be specific for pyruvic acid, it may be concluded that the enzymatic increase in 2,4-dinitrophenylhydrazine–reacting carbonyl can be largely attributed to pyruvic acid. However, the quantity of carbonyl-containing components (Method A) of the heated controls, calculated as pyruvic acid, was two to three times higher than that found by the lactic dehydrogenase method (C).

Pyruvate Content of Different Onion Varieties. Table II lists the varieties in order of decreasing amount of enzymatically produced pyruvate as determined by Method A. White Globe cooking onions produced the greatest amount of pyruvic acid (19 μmoles per gram) and informal estimation of its pungency ranked it as the strongest of the onions used in the present investigations. The other Globe onions were also pungent and produced almost as much pyruvate (16 μmoles). Intermediate in the amount of pyruvate produced and also in estimated pungency were the White Grano, White Bermuda Hybrid, and mid-sections of the so-called scallions (8 to 10 μmoles per gram). California Early Red, adjudged to be an extremely mild onion, developed a maximum of 4 μmoles of pyruvic acid per gram (13). This ranking of pungency is in the same order as that assigned by Magruder *et al.* (13) and also by various commercial seed catalogs for the varieties described in these sources. The bulb section of a relatively strong bunch of scallions developed 4.5 μmoles, whereas a mild bunch developed only 2 μmoles of pyruvic acid per gram. The mid-section of the immature onions sold on the market as "scallions" is more pungent than the bulb. There appears to be no relation between pungency and the apparent pyruvate content of the heated controls (2 to 4 μmoles of pyruvic acid per gram of onion). Only one half or less of this value represents true pyruvate.

Discussion

The results demonstrate that the development of carbonyl components in onion macerate can be largely attributed to pyruvic acid. The amount of this acid found in the present experiment is of the same order of magnitude as that found by Morgan (15) (12 μmoles per gram). Since the amount of pyruvic acid developed appears to bear a fairly reasonable relation to the degree of pungency, its determination by Method A may afford a rapid and relatively simple alternative to the laborious and time-consuming methods used heretofore. For routine use, it may be possible to simplify the method even further by use of a vegetable and fruit juicer and elimination of the heated control. The variation in the amount of pyruvate in these controls probably does not exceed detectable differences in pungency.

Table III compares the range of pyruvic acid found in the present investigations with the amounts of other onion components which may bear some relation to flavor. The range of pyruvate exceeds that of the other methods designed to test the pungency of onions. While this suggests that the pyruvic acid method is more sensitive in this regard, the problem of the discrepancy between maximum volatile sulfur and the pyruvate remains.

From the intensity of ninhydrin spots corresponding to MCSO and PCSO on paper chromatograms, Virtanen and Matikkala (25) estimated that these two components of onions amounted to a maximum of 2 μmoles per gram of onion (Table III). If the pyruvic acid developed in onion macerates does indeed arise as the result of alliinase-type action, we must conclude that substrates other than these two amino acids exist in onions. One possible source of such a progenitor could be cycloalliin, isolated from methanolic extracts of onions by the

same investigators (*24*). Results in this laboratory (*5*) show that at least 30% of the total sulfur of such methanolic extracts can be accounted for as cycloalliin. Table III shows that the total amount of these three amino acids raises the value to the range found by the other methods. Furthermore, Virtanen and Matikkala (*26*) have reported the presence of sulfur-containing γ-glutamyl peptides, which could conceivably give rise enzymatically to pyruvic acid, possibly from noncyclic precursors to cycloalliin. The amount of pyruvic acid developed in garlic macerates (*1, 9*) averages about five times that found in onions.

Acknowledgment

The authors thank Mendel Mazelis for performing the pyruvic acid assays by the lactic dehydrogenase method.

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Received for review September 2, 1960. Accepted January 10, 1961. Mention of specific products does not imply recommendation by the Department of Agriculture over others of a similar nature not mentioned.

VEGETABLE DETERIORATION

N^6-Benzyladenine, a Senescence Inhibitor for Green Vegetables

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Green vegetables deteriorate steadily and rapidly after harvest. Protein content declines, and chlorophyll content decreases in close proportion. The loss of quality inevitable in such produce is minimized by rapid, careful handling and the best possible storage conditions. Kinetin-like chemicals delay senescence of green vegetables and offer an additional means of reducing quality losses in perishable crops. N^6-benzyladenine applied to green vegetables delays visual manifestations of senescence that occur during storage. This senescence inhibitor was effective in maintaining the green color and appearance of lettuce, endive, escarole, Brussels sprouts, sprouting broccoli, mustard greens, radish tops, celery, parsley, green onions, and asparagus.

GREEN VEGETABLES deteriorate steadily and rapidly after harvest. Protein content declines, and chlorophyll content decreases in close proportion, as shown by detached-leaf studies (*2–4*). Loss of quality is minimized by rapid, careful handling and good storage conditions. However, the finding by van Overbeek (*6*) that kinetin-like chemicals delay senescence of green vegetables offers an additional means of reducing quality losses in these perishable crops.

This paper reports the results of a study of the effect of N^6-benzyladenine (known experimentally as SD 4901) on the rate of deterioration of several green vegetables, with stress on response rather than on the biochemical modes of action.

A series of experiments was conducted in 1958–1960 to test the effect of dosage, methods, and time of application of N^6-benzyladenine on the rate of deterioration of several vegetables. Two types of holding tests were run: those in which the vegetables were harvested, trimmed, packed as gently as possible, and held at 40° or 70° F.; and a series with vegetables held 8 days at 40° and then transferred to 70° F., thus simulating an 8-day transit period from California to New York, and a following period in retail outlets. All vegetables were harvested from commercial fields in the central coastal district of California. Each treatment consisted of ten matched heads, stalks, buds, etc., depending on the vegetable. Samples were at market