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Comparison of Three Analytical Methods for Measurement of Onion Pungency

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ABSTRACT

Three analytical methods (gas chromatography, pyruvic acid, and thiosulfinate) were used to measure pungency in onion, and their efficiencies in classifying thirteen short-day onion cultivars were evaluated. The gas chromatography method showed a significant correlation with the pyruvic acid method, but not with the thiosulfinate method. The gas chromatography method, measuring sulfur volatiles developed in the headspace of blended samples, was able to measure many kinds of volatile compounds and was sensitive in detecting small differences of sulfur volatiles. However, this method was slow and difficult to automate. The pyruvic acid method was relatively easy to perform and estimated total pungency, but could not detect different flavor compounds. The thiosulfinate method was affected by experimental conditions and results could not be reproduced. We suggest using the gas chromatography method as an alternative to the pyruvic acid method for more information on specific sulfur volatiles and for detailed assessment of onion pungency.

RESUMEN

Se usaron tres métodos analíticos (cromatografía de gases, ácido pirúvico, y tiosulfinato) para medir la acrimonia en cebolla, y se evaluó su eficiencia en la clasificación de 13 cultivares de cebolla de día corto. El método de cromatografía de gas mostró una correlación significativa con el método de ácido pirúvico, pero no con el método de tiosulfinato. El método de cromatografía de gases, consistente en la medición de los sulfuros volátiles desarrollados en el espacio entre la muestra licuada y la cubierta de la licuadora, fue capaz de medir muchas clases de compuestos volátiles y fue sensible para la detección de pequeñas diferencias de sulfuros volátiles. Sin embargo, este método fue lento y difícil de automatizar. El método de ácido pirúvico fue relativamente más fácil de realizar y estimó la acrimonia total, pero no pudo detectar diferentes compuestos saborizantes. El método de tiosulfinato fue afectado por las condiciones experimentales y los resultados no pudieron ser reproducidos. Sugerimos el uso del método de cromatografía de gases como una alternativa al método de ácido pirúvico para obtener más información sobre sulfuros volátiles específicos y una evaluación más detallada de la acrimonia de la cebolla.

Additional index words. Allium cepa, gas chromatography, pyruvic acid, thiosulfinate, sulfur volatiles.

Onions and the many different *Allium* species are chemically characterized by their organic sulfur compounds, which give off the characteristic aroma and pungency (Whitaker, 1976). Pungency and odor of onions develop rapidly when tissues are damaged by cutting or maceration. This reaction occurs when alliinase, confined in vacuoles, comes in contact with flavor precursors in the cytoplasm (Lancaster and Boland, 1990). Flavor precursors collectively known as S-alk(en)yl cysteine sulfoxides include methyl-, propyl-, propenyl- and allyl- cysteine sulfoxides (Fenwick and Hanley, 1985). Concentration and composition of these precursors are species specific. For example, S-1-propenyl cysteine sulfoxide and S-2-propenyl (allyl) cysteine sulfoxide are predominant flavor precursors in onion and garlic, respectively. The alliinase-catalyzed reaction produces as many as 30 different sulfur volatiles, such as mono-, di-, trisulfides or intermediate sulfur compounds, thiosulfinate, and pyruvic acid (Lancaster and Boland, 1990).

Onion pungency can be estimated indirectly by measuring one of the compounds produced in the enzymatic reaction. Various analytical methods can be used, including pyruvic acid, gas chromatography, and thiosulfinate tests. Measuring the flavor precursor levels is also suggested to estimate onion pungency (Lancaster and Boland, 1990), but is not widely used because analysis is difficult.

The pyruvic acid test is one of the oldest methods used to determine pungency (Bennett, 1945; Schwimmer and Weston 1961; Wall and Corgan, 1992). This method measures enzymatically developed pyruvic acid from blended onion juice. Although it measures total flavor, this method provide no information about relative amounts of individual flavor precursors or final flavor volatiles (Lancaster and Boland,

1990). It is generally accepted that the higher the pyruvic acid content, the hotter the onion (Schwimmer and Weston 1961; Wall and Corgan, 1992). Modification of this method for a fast analysis has been reported (Randle and Bussard, 1993; Yoo et al., 1995).

The gas chromatography method measures amounts of gas volatiles produced when the onion tissue is disrupted by blending (Saghir et al., 1963; Bernhard, 1968; Galetto and Patrick, 1970; Boelens et al., 1971; Freeman and Whenham; 1975, Mazza et al., 1980; Saito et al., 1989). Because gas volatiles are unstable and continuously changing, the sampling procedure must be standardized. Disulfides are the major sulfur compounds in freshly blended onion tissues (Whitaker, 1976).

The thiosulfinate test measures the color reaction produced by thiosulfinates and N-ethyl maleimide (Carson and Wong, 1959; Nakata et al., 1970; Freeman and McBreen, 1973). This reaction is unstable and color fades rapidly (Nakata et al., 1970). Modifications of this method have also been reported (Freeman and McBreen, 1973; Thomas et al., 1992).

Although the pyruvic acid test has been used extensively to classify onion cultivars for pungency, to date there are no reports on the comparison of these three methods and their efficiencies in classifying cultivars for pungency and flavor. This study was performed to compare gas chromatography, pyruvic acid, and thiosulfinate methods for pungency determination in onion and to assess how effectively these methods detect cultivar differences.

MATERIALS AND METHODS

Plant materials. Nine commercial onion cultivars (Rio Bravo, Contessa, Granex 33, Granex 429, Henry's Special, Redbone, Sweet Savannah, TG 1015Y, and TG 502) and four test hybrids (XPH 6022, XPH 6023, XPH 6028, and XPH 6068) from Seminis Vegetable Seeds (Saticoy, Calif.) were used in this study. Onion cultivars were chosen to represent sources of low, intermediate, and high pungency levels. The onions were grown in a commercial field in the Rio Grande Valley, Texas, and harvested in April, 1993. Onion bulbs were field cured for three days in burlap bags and stored at 4°C until analyzed. Before analysis, onions were removed from cold storage and kept at 24°C overnight. Ten onion bulbs per replication with three replications (total 30 bulbs) were analyzed in each cultivar in a randomized complete design (Table 1).

Gas chromatography method. A 1 cm thick cross section was cut from the equator of the onion bulb. A 50 g sample was blended with 100 g of water in a domestic blender using a 500 ml plastic container for 1 min. A 0.5 ml headspace sample was withdrawn and injected into a Perkin-Elmer gas chromatograph (GC) (Model 8500) equipped with a sulfur flame photometric detector. Total sulfur volatile content in the headspace was measured as total peak area expressed in electronic units (x 10^6 E.U.) of a Hewlett-Packard integrator (Model 3395). A glass column (2 mm i.d. x 2 m long) packed with 8% carbowax 150 on 80 to 100 mesh chromosorb WAW-DMCS (Supelco, Bellefonte, Pa.) was used. Oven temperature was raised from 100 to 190°C for 3 min and held for 0.5 min. Injector and detector temperatures were 250°C. The carrier gas was helium at a flow rate of 30 ml · min^{-1}. Sulfur volatiles were identified by standard compounds (methyl propyl, dipropyl, propyl propenyl disulfides) or comparing retention time of peaks (thiopropanal S-oxide) (Mazza et al., 1980).

Pyruvic acid method. A method by Schwimmer and Weston (1961) was used to measure pyruvic acid. The remaining homogenate from the GC method was filtered and the extract kept in a 7 ml vial for 30 min before freezing at -20°C. Frozen samples were thawed and diluted 57-fold with distilled water. One ml of the diluted sample was mixed with 1 ml of 2,4-dinitrophenyl hydrazine. Samples were thoroughly mixed and kept in a 37°C water bath for 10 min followed by the addition of 5 ml of 0.6 N NaOH. Sample absorbency at 420 nm was measured by using a spectrophotometer. Pyruvic acid

Table 1. Evaluation of onion pungency in 9 commercial cultivars and 4 test hybrids by using gas chromatoraphy, pyruvic acid, thiosulfinate methods.

Cultivar	Pyruvic acid concentration (Rank)	GC Peak area (Rank)	Thiosulfinate concentration (Rank)
	(μmoles•ml^{-1})	(E.U. X 106	(A_{515nm} x 10^{-3})
Rio Bravo	7.9±2.1 a (1)	57.1±27.7[z]abc[y](3)	36±16 ab (7)
Granex 429	6.8±2.2 ab (2)	56.4±27.1 ab (4)	51±17 a (1)
XPH 6022	6.7±2.8 ab (3)	63.1±27.7 a (1)	46±27 a (2)
Sweet Savannah	5.9±2.4 ab (4)	58.8±28.8 ab (2)	35±13 ab (8)
Granex 33	5.7±1.9 ab (5)	54.1±26.2 abc (5)	45±25 a (3)
Henry's Special	5.6±1.9 ab (6)	47.9±21.6 abcd (6)	20±14 ab (12)
XPH 6068	5.4±2.0 ab (7)	35.8±20.8 bcd (8)	43±20 a (5)
TG 1015 Y	5.4±2.2 ab (8)	27.2±16.5 d (11)	45±16 a (4)
TG 502	4.6±1.2 ab (9)	46.4±23.3 abcd (7)	10±8 b (13)
XPH 6023	4.6±1.6 ab (10)	26.5±15.6 d (12)	23±14 ab (10)
Redbone	4.1±2.3 b (11)	24.4±14.2 d (13)	42±14 a (6)
XPH 6028	3.9±1.2 b (12)	32.3±17.3 cd (10)	23±9 ab (11)
Contessa	3.9±1.4 b (13)	34.0±17.8 bcd (9)	27±13 ab (9)

[z]Data are shown as mean ± SD.

[y]Mean separation in each column by Duncan's multiple rage test ($P \leq 0.05$).

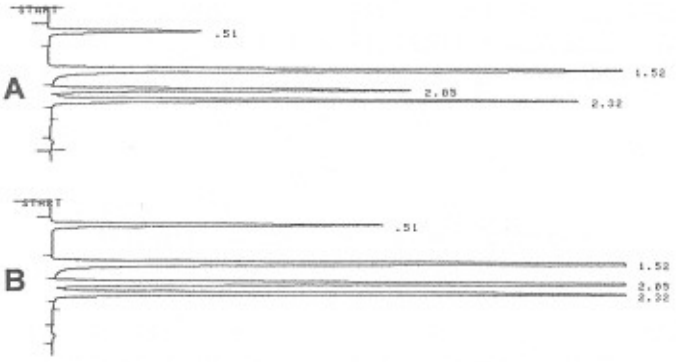

Fig. 1. Representative gas chromatograms of mile (A, peak area 31.8 x 106 E.U. and pyruvic acid 1.9 µmoles·ml-^{1}) and hot (B, peak area 51.0 x 106 E.U. and pyruvic acid 4.3 µmoles·ml^{-1}) onions in cv. TG 502. Identified peaks are thiopropanal S. oxide (0.51 min), methyl propyl disulfide (1.52 min), dipropyl disulfide (2.05 min), and propyl propenyl disulfide (2.32 min).

concentrations in samples were calculated using regression of a sodium pyruvate standard.

Thiosulfinate method. The method described by Nakata et al., (1970) was followed. The same onion extract from the pyruvic acid method was used. One ml of 5-fold diluted extract with cold water was mixed with 1 ml of 0.05 M N-maleimide, 0.25 M KOH and 1% ascorbic acid. The mixtures were kept in an ice bath for 10 min and absorbency at 515 nm was recorded. Since no standards were available, absorbency units per ml of onion extract were used.

Statistical analysis. Correlation, treatment means and SE were calculated using the SAS general linear model (Cary, N.C.).

RESULTS AND DISCUSSION

Gas chromatography method. Four major sulfur volatiles, including thiopropanal S-oxide, methyl propyl, dipropyl, and propyl propenyl disulfides (Fig. 1) were separated and identified. Composition and concentration of each compound varied between cultivar and individual bulb. Comparisons between mild and hot onions clearly demonstrated that pungent onions contained greater amounts of sulfur volatiles than milder onions (Figs. 1a and 1b). Quantification of individual or total sulfur volatiles was not attempted because thiopropanal S-oxide standard was unavailable and the volatile compounds rapidly changed in both concentration and composition.

To obtain reproducible results using the GC method, careful standardization of the sampling procedure is a prerequisite. Mazza et al. (1980) reported that the degree of tissue maceration, incubation temperature, and timing of samples were important factors required to obtain reproducible results. In this study, the gas chromatography method was performed standardizing most conditions, including plant part used (equatorial cross section), tissue disruption time (1 min), and disruption method (blending). It took 6 min to run each sample and about 70 samples could be analyzed daily. Consistent peak areas were obtained when the same onion samples were tested.

Pyruvic acid method. Average pyruvic acid concentrations ranged from 3.9 to 7.9 µmoles · ml^{-1} (Table 1). The development of simplified pyruvic acid procedures for pungency evaluation as those described recently by Randle and Bussard (1993) and Yoo et al., (1995) have made this method easy and fast to perform. About 200 frozen extract samples were processed daily.

Thiosulfinate method. The thiosulfinate method was found to be unreliable because color development was highly influenced by time, temperature and other conditions. Reproducible results were difficult to obtain. This method measures thiosulfinates, which are intermediate compounds in onion extract and are eventually converted to many disulfides. Therefore, the thiosulfinate quantity cannot be measured precisely. This method was considered the least reliable. As with the pyruvic acid method, 200 samples were processed daily.

Comparison of gas chromatography and pyruvic acid methods. The pyruvic acid method was significantly correlated (r^2 =0.78**) with the gas chromatography method (Fig. 2, A). However, correlation between the pyruvic acid and thiosulfinate method (r^2 =0.52^{NS}) (Fig. 2, B) and between the gas chromatography and thiosulfinate method (r^2 =0.34^{NS}) were nonsignificant.

These results disagree with those reported by Saghir et al., (1963) who were unable to find a correlation between aliphatic disulfide and pyruvic acid concentration. According to Whitaker (1976), this discrepancy is normal and expected, because although disulfides and pyruvic acid are products of the alliinase-catalyzed reaction, the reactivity of these compounds is different. Schwimmer and Guadagni (1968) suggested that disulfides and pyruvic acid are produced via the same enzyme but that the formation of disulfides is unstable, increases with time and occurs after the formation of pyruvic acid. However, in this study, we could find a fairly consistent relationship between these two methods by standardizing the GC method.

Evaluation of cultivars. The cultivars analyzed showed

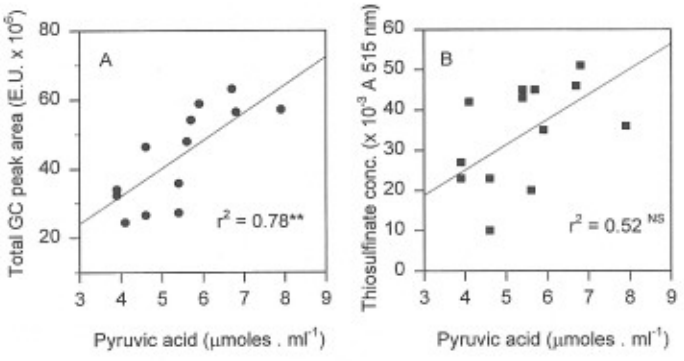

Fig. 2. Correlation analysis between pyruvic acid concentration and GC peak area (A) and thiosulfinate concentration (B) in 9 commercial cultivars and 4 test hybrid onions. NS, ** Nonsignificant or significant at $P \leq 0.01$ (**), respectively.

significant differences using the gas chromatography, pyruvic acid and thiosulfinate methods. A large variation in the magnitude and order of the cultivars was observed between analysis methods (Table 1). However, evaluation of cultivars for pungency using the gas chromatography and pyruvic acid method indicated some consistency (Table 1).

After comparing three analytical methods used to measure pungency in onions, the GC method was thought to be a sensitive and efficient method in detecting variations in the relative amounts of sulfur volatiles. This method could detect variation in the levels of flavor compounds according to the plant part analyzed. Outer scales and stem usually have lower flavor precursor concentrations than inner leaves and the same pattern was observed between the top and bottom parts of the bulb (Freeman and Whenham, 1975).

Although gas chromatography method was preferred over the pyruvic acid method, based on its sensitivity and specificity, this method also measured the secondary products of the enzyme reaction. The relative contribution of these sulfur compounds to overall flavor and aroma is unknown (Whitaker, 1976). Automation using the GC test was not possible, nor did it provide direct information about contents or ratios of compounds in the onion matrix (Kallio and Salorinne, 1990).

The pyruvic acid test has been one of the most widely used methods for pungency determination since the early 1960s. Several authors (Schwimmer and Guadagni, 1962; Wall and Corgan, 1992) have suggested using the pyruvic acid method for pungency screening in onion. However, after testing different cultivars by this method, it was found that the variation in pyruvic acid concentration in certain cultivars (Granex 33, Granex 429, Henry's Special, TG 1015Y, TG 502, etc.) was so great that it was difficult to distinguish clearly between these cultivars. This method was unable to detect differences of individual flavor precursors or final flavor volatiles in onions.

We have evaluated onion pungency using three representative methods and propose using gas chromatography as an alternative method to the conventional pyruvic acid method. The GC method provides additional information on the specific sulfur volatile composition of onion cultivars or breeding lines which cannot be provided otherwise by the pyruvic acid method. However, the use of any particular screening method depends on the purpose of the research and availability of economic and plant material resources.

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