Sampling Procedures to Estimate Flavor Potential in Onion

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Abstract. Twenty bulbs from each of 10 onion (*Allium cepa* L.) cultivars and one mass population were harvested from two locations and evaluated for three traits associated with flavor quality. Variance components for soluble solids content (SSC), pyruvic acid concentration (PAC), and percent S were calculated, and sampling schemes required to detect specific differences among treatment means were determined. In general, a five-bulb sample and four replications were sufficient to detect desired differences for SSC and PAC, whereas percent S required a larger sample size and more replications.

Flavor in onions is expressed on cellular disruption through the interaction of water-soluble carbohydrates and specific S compounds. While flavor is influenced by genetics and the environment, little published information exists on the genetic control of flavor or how the environment influences flavor (Lancaster and Boland, 1990). When designing experiments to test the effect of treatments on flavor or when selecting individuals in a breeding program for superior flavor characteristics, it is important to have an estimate of the inherent variability of the cultivars or populations under investigation. In heterogeneous species such as onions (Dowker, 1990), estimating the inherent variability would establish reliable sample sizes and appropriate replication needed to detect specific differences among means at stated statistical levels. In a breeding program where individual bulbs are selected without replication, identifying estimates of detectable differences among bulbs would be advantageous. This study was conducted to determine optimum sample size and replication of onions for soluble solids content (SSC), enzymatically developed pyruvic acid concentration (PAC), and total S percentage (percent S).

Data were obtained from 10 cultivars and one mass population grown in the field and in the greenhouse at the Univ. of Georgia, Athens. Cultivars were short- or intermediate-day types and included F₁ hybrids and open-pollinated cultivars. The mass population was derived from a single cycle of random mating using 18 short-day parents of differing genetic background. Seed was sown in October in flats and grown in the greenhouse to obtain transplants. Twenty-five visually uniform plants of each entry were transplanted into greenhouse and field locations 40 days after sowing. In the greenhouse, plants were grown in 0.75-liter pots containing Fafard #3 artificial medium (Fafard Corp., Anderson, S.C.) and were fer-

tilized with full-strength Hoagland's (1950) solution twice a week. Plants were supplemented with tap water as needed. Night and day temperatures were set at 16 and 28C, respectively. In the field, plants were grown on 1.1-m centered raised beds. Two rows were transplanted per bed. Within and between row spacing were 7.5 and 20 cm, respectively. Plants were top dressed with 112 kg calcium nitrate per hectare at transplanting and at 6 and 12 weeks after transplanting (336 kg·ha³ total). Plots were hand weeded and irrigated as needed.

The onions were grown to maturity. A cultivar was considered mature when the foliage lodged on 80% of the plants. The bulbs were then harvested and the foliage and roots severed. The bulbs were cured in paper bags at ambient greenhouse temperatures for 7 days, and stored at room temperature for 30 days before quality analysis.

Individual bulbs were quartered longitudinally for quality analysis. SSC was determined from duplicate samples of individual bulb quarters using a Kernco (Kernco Corp., Tokyo, Japan) hand-held refractometer. Juice was obtained by placing a single bulb quarter in a tincture press (Univ. of Georgia design). Enzymatically produced PAC, which is a measure of pungency, was determined from the methods of Schwimmer and Weston (1961) using two of the four bulb quarters. Total percent S determinations were made from the remaining bulb quarter using the procedures of Jones and Isaac (1972) on a Leco Sulfur Analyzer (Leco Laboratory Equipment Corp., St Joseph, Mich.).

Data were analyzed using a completely randomized two-factor model and the pro-

grams of SAS (Cary, N.C.). An arcsin transformation was performed on percentage data. Nontransformed data are reported.

The determination of sample size and replications needed to detect differences between means were calculated from the following equation (Cochran and Cox, 1957): $\delta = \sqrt{2/r}\sigma(t\alpha_{,df} + t_{2(1-P),df})$, where δ = desired detectable differences, r = number of replications per treatment, σ = standard error per unit, t = values from 2-tailed student t table for the estimate of the error variance, σ = desired probability level (0.05), df = degrees of freedom for σ^2, P = power of the test (0.80). Values of σ^2 (variance of a treatment mean) were estimated for varying numbers of bulbs per cultivar (b) using the following equation: $\sigma^2 = \sigma^2_b/b$, where σ^2_b = variance among bulbs within cultivars.

Location and cultivar differences were significant for all traits measured (Table 1). The location × cultivar interaction was nonsignificant for SSC and PAC but was significant for percent S. Comparing the two locations, cultivar means for SSC were higher under field conditions than under greenhouse conditions. Cultivar means and SD for PAC and percent S, however, were higher in the greenhouse than in the field (Table 2).

Increased precision can only be achieved with replication over locations when the cultivar × location interaction is large for a trait. Since the cultivar × location interaction in this investigation was nonsignificant for SSC and PAC, the optimum number of replications and samples within a location can be calculated to detect a desired difference among cultivars. The desired detectable difference then depends on the particular researcher's objectives and the type of material being evaluated. For SSC, a detectable difference of 1% would be useful. This level of sensitivity could be achieved with four replications and a five-bulb sample or with two replications and a 10-bulb sample (Table 3). If, however, a detectable difference of 0.5 SSC were desired, one would need a minimum of between five and 10 replications with a 10-bulb sample or five replications with a 15-bulb sample. The decision then becomes one of cost. Quality analysis often is most cost effective with decreased replication and increased sample size. The saving is in time, labor, and chemicals.

A similar number of replications and bulb sample sizes are needed to detect l- and 0.5-µmol/g fresh weight differences in PAC (Table 3). Two replications and a five-bulb sam-

Table 1. Analysis of variance of a completely randomized two-factor design for SSC, percent S, and PAC in onion bulbs. Twenty bulbs were harvested from 10 onion cultivars and one mass population grown in a greenhouse and in the field.

Source	df	SSC		Percent S		Pyruvate	
		MS	αᶻ	MS	α	MS	α
Location(L)	1	180.5	0.001	12.61	0.001	637.20	0.001
Cultivar(C)	10	182.1	0.001	0.40	0.001	18.33	0.001
LC	10	1.8	NS	0.07	0.001	0.53	NS
Among bulbs within C	418	1.1		0.03		0.61	

ᶻSignificance level of F statistic.

Table 2. Means and SD for SSC, percent S on a dry weight basis, and PAC (μmol/g fresh weight) for 10 onion cultivars and one mass population grown under greenhouse or field conditions.

	Field						Greenhouse					
	SSC		Percent S		Pyruvate		SSC		Percent S		Pyruvate	
Cultivar	Mean[z]	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Willamette Sweet	9.0	0.7	0.28	0.06	7.50	1.17	8.0	0.7	0.65	0.15	10.13	1.18
Z238	8.2	0.8	0.32	0.05	5.86	0.74	7.2	0.8	0.72	0.12	7.93	0.71
Granex 33	7.6	0.6	0.45	0.06	6.66	0.88	6.6	0.6	0.63	0.10	9.00	0.92
Granex 429	7.7	0.5	0.22	0.04	6.10	0.86	6.7	0.5	0.44	0.08	8.58	0.78
Rio Bravo	9.0	0.6	0.33	0.05	5.87	0.52	8.0	0.6	0.71	0.11	8.04	0.40
Sweetex	8.8	0.3	0.48	0.10	6.78	0.98	7.6	0.3	0.93	0.19	9.40	1.00
Aviv	12.1	1.0	0.26	0.05	5.78	0.38	10.1	0.9	0.60	0.11	7.97	0.35
Sintese 39	12.9	1.7	0.25	0.06	7.04	0.66	11.9	1.5	0.64	0.15	9.28	0.68
JP8	8.9	1.2	0.24	0.05	6.69	0.52	7.8	1.2	0.55	0.12	9.04	0.46
JP70001	8.2	1.2	0.23	0.03	6.20	0.40	6.2	1.2	0.54	0.07	8.79	0.51
MCL1	14.0	2.0	0.65	0.15	7.24	0.57	12.1	2.1	0.67	0.09	10.02	1.32
Mean	9.7	2.4	0.31	0.21	6.52	0.92	8.4	2.2	0.67	0.42	8.93	1.08

[z]Mean of 25 plants.

Table 3. Estimated detectable differences (δ) for SSC, PAC, and percent S at the 5% significance level when varying the number of bulbs per cultivar and replications for 10 onion cultivars and one mass population.

	Bulbs/cultivar				
Replications	20	15	10	5	1
	SSC (%)				
1	0.945	1.091	1.337	1.890	4.228
2	0.668	0.772	0.945	1.337	2.989
3	0.546	0.630	0.772	1.091	2.441
4	0.473	0.546	0.668	0.945	2.114
5	0.423	0.488	0.598	0.845	1.890
10	0.299	0.345	0.423	0.598	1.337
20	0.211	0.244	0.299	0.423	0.945
	PAC (μmol·g^{-1})				
1	0.690	0.796	0.975	1.379	3.085
2	0.488	0.563	0.690	0.976	2.181
3	0.398	0.460	0.563	0.796	1.781
4	0.345	0.398	0.488	0.690	1.542
5	0.308	0.356	0.436	0.617	1.379
10	0.218	0.252	0.308	0.436	0.976
20	0.154	0.178	0.218	0.308	0.690
	Percent (dry wt)				
1	0.141	0.163	0.199	0.282	0.629
2	0.010	0.115	0.141	0.199	0.445
3	0.081	0.094	0.115	0.163	0.363
4	0.070	0.081	0.010	0.141	0.314
5	0.063	0.073	0.089	0.126	0.282
10	0.044	0.051	0.063	0.089	0.199
20	0.032	0.036	0.044	0.063	0.141

ple were sufficient to detect a 1-μmol difference, while at least four replications and a 10-bulb sample were needed to detect a 0.5-μmol difference.

More replications and bulbs were needed to detect desired differences for percent S than for the other two variables. To detect differences of <0.1, five replications and a sampling of 10 bulbs or three replications and 15 bulbs were needed (Table 3). To detect a difference of <0.05 (» 10% of the population mean) would require 10 replications and >20 bulbs sampled.

When breeding onions for improved characteristics, single-bulb selection with no replication is a common methodology in the early stages of improvement. The sensitivity to detect differences, using the above equations, decreases as the number of bulbs and replication per cultivar decreases and is lowest with single bulbs and no replication. Therefore, using these data one may detect a difference of 4.2% SSC, 3.09 μmol PAC, and 0.63% S among two individual bulbs ($\alpha = 0.05$ and $P = 0.8$) in a breeding program with variance components similar to those in the experiment reported (Table 3).

The short- and intermediate-day cultivars in this experiment were selected to represent a range of potential inherent variation encountered by researchers. Sampling procedures presented here, however, are appropriate only if variance components are known or are presumed to be similar to those reported here.

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