The Improvement of Flavor in a Program of Carrot Genetics and Breeding

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The genetic improvement of vegetable quality is a complex task which requires the concerted efforts of several disciplines. The ultimate needs to be satisfied are those of the consuming public. Therefore, long-range plans for improvement must include a determination of positive and negative quality attributes of relevance to nutritional needs and sensory preferences of consumers. Horticultural characteristics for large-scale production and marketing must also be maintained while quality improvements are being made. When quality attributes to be altered have been determined and means of simultaneously fulfilling production criteria established, improvement can proceed.

For reasonable genetic gains in quality to be made, attributes under consideration should be readily measurable using simple techniques, they should be stable or at least predictable over the usual range of growth, storage, and processing conditions, and inheritance patterns of attributes should be known. Finally, methods for incorporating the improved attributes into the existing genetic, physiological and cultural system must be determined.

Unfortunately, the environmental and genetic variability observed with many quality factors is either complex or unknown. Furthermore, the biological components of quality are difficult to assess (e.g. flavor, protein efficiency ratio), so that consumer panels or other complex biological assays must be used to measure quality.

Carrots (Daucus carota L.) are of considerable nutritional significance because they are the most important vegetable source of provitamin A in the U.S. diet, providing 14% of the total (17). Varietal and seasonal differences in carrot flavor have been reported since the introduction of carrots to Europe from the Mideast in the 12th century (3). This report details some of the particular problems and potential solutions for the genetic improvement of carrot quality.

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Perspective of Quality in U.S. Carrot Production and Improvement

Carrot Production. The ability to improve the organoleptic and nutritional quality of carrots by changing production and distribution requirements is best understood by considering some statistics and regulations of production. The majority of carrots grown in the U.S. are for the fresh market which accounts for over 80% of the per capita consumption. The remainder is for processed carrots (25). Marketable yield for fresh market carrots is a function of root size, shape, and external appearance. Eating quality is not a factor in grading fresh vegetables for sale, even though it may be very important for repeat sales and for establishing consumer preferences.

Carrot Genetics and Breeding. The ability to introduce quality improvement as a major objective in carrot breeding is easier to understand with an overview of the numbers and operations of such programs. Three federal or state programs and several private seed firms currently perform all of the breeding and genetic improvement for carrots in the U.S. Therefore, no single research program can confine all of its efforts to improving culinary quality. The trend for new cultivars is toward hybrid carrots, which provide a more uniform, predictable crop for yield and quality than does the older method of open-pollinated varietal improvement.

The U.S.D.A. carrot improvement program currently includes approximately 50 inbred carrot lines, and annually tests several hundred hybrid crosses and numerous segregating populations. In addition to culinary and nutritive quality parameters, characteristics being improved include root color, shape, and size, disease resistance, root storage ability, uniformity of flowering, and seed production. All of these characteristics must be maintained at an acceptable level while attempting to improve quality.

One feature of the carrot life cycle which must be considered is its biennial habit. Carrot roots must be refrigerated for up to 8 weeks after harvest before they will flower and consequently set seed, and only the crown third of the root is needed for seed production. This allows laboratory and sensory analysis to be made on the remaining portion of the root while the cold treatment is underway, and only selected roots are used to produce seed.

Carrot Quality Improvements. The lack of definite eating quality standards, and the difficulty of quickly and easily assessing carrot quality makes the grading of culinary quality in carrots as they come from production areas to the consumer unfeasible. This places the burden of quality improvement on altered production practices, varieties with genetically improved quality over a wide range of environments, or both.

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Since acceptance of new cultivars depends on a favorable response from growers, short-term breeding and genetic goals tend to be producer-directed. This is satisfactory for most consumer needs, but as stated above, culinary and nutritive traits can be overlooked. Long-range improvement programs should also direct research efforts to consumer needs. GRAS (generally regarded as safe) regulations for nutrients and toxicants set by the Food and Drug Administration further emphasize consumer welfare (17).

Thus, the improvement of carrot quality can be a major objective if the breeder is willing to pursue long-term research goals while concurrently placing adequate emphasis on marketable yield. With such a program, the steps for quality improvements are to define important quality attributes, ascertain the feasibility of their improvement, and establish techniques for screening the large number of samples that must be tested.

Steps in the Improvement of Carrot Quality

Choosing Quality Attributes to be Tested. The choice of nutritional and toxic metabolites to be considered and methods of analysis are largely dictated by health scientists. As a result, carotenoid (9), myristicin and falcarinol (30) levels have received some attention in carrots. Choosing sensory characteristics is more difficult, and requires an accurate assessment of influential attributes along with identification of suitable terms for communication. In the case of carrots, undesirable and variable flavors are commonly mentioned culinary quality attributes needing improvement. Bitter flavor, often developing in stored carrots, results from the synthesis of "isocoumarins" in the presence of ethylene (6, 22). More recently, the need for sweeter and less harsh ("turpentiney", "oily") flavor has been recognized by carrot growers as desirable, and formal sensory evaluations have demonstrated that panelists can distinguish between extremes for these attributes (18, 20).

Determining Means for Implementing Quality Improvement. Culinary quality improvement cannot be attributed exclusively to selection of preferred genotypes. Altered cultural practices and controlled atmospheric storage have resulted in flavor improvement in many fruits and vegetables, including bitterness in carrots, without creating new genotypes (10). Thus, it must be determined how strongly genetic and non-genetic sources of variation contribute to improved culinary quality. This determination should be made over the range of environments prevalent in important growing areas, storage conditions, and genetic stocks available so that the magnitude of potential genetic change can be estimated.

For carrots, genetic variation for sensory attributes is substantially greater than environmental (climate and soil) variation (18, 20, 21). This indicates an opportunity for substantial improvements of culinary quality in a program of carrot genetics and breeding.

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Screening Techniques for Quality Factors in a Program of Genetics and Breeding. Important quality factors exhibiting genetic variation should be screened in every generation of the breeding cycle. The development of genetic stocks often requires ten to twenty years. Less important factors may only be screened at the beginning of the cycle, and again when the material is about to be released for public use. Simply inherited factors will need to be less frequently screened if the genetic purity of the original material is carefully controlled.

Components of nutritional quality are generally screened by accepted laboratory methods. Often modifications or shortcuts designed for the specific item to be samples are used to speed up such analyses [e.g. extremes in color of carrot closely estimate extremes in carotenoid levels (9)].

The ideal system for scoring culinary quality samples is with a trained, descriptive taste panel since this approximates the test made by the consumer. However, limitations of time and resources prohibit this approach with genetically segregating materials of a breeding program. For example, if the ten sweetest roots were sought in four different genetic or breeding stocks of 100 roots each, 400 roots would need to be scored by the panel. Since a typical descriptive sensory evaluation panel is comprised of 25 participants, and scores at most five samples at one sitting, the small available sample size and large number of roots to be scored would both rule out the use of panels for such preliminary screening. Sensory analysis is a well-established tool, however, for two other phases of this task: correlation of sensory and objective parameters (trained panel) and evaluation of the final product (consumer panel).

The ease of performing physical and chemical laboratory analyses in comparison to sensory evaluations has led to the use of selected laboratory (objective) data to predict sensory response. High correlations between selected objective data and sensory parameters are necessary, but adequate relationships have been determined in genetic variants of several fruits and vegetables, including grapes (15, 28), apples (27, 29), pears (26), raspberries (12), tomatoes (24), snapbeans (23), onions (16), and carrots (20). In addition to the relative ease of laboratory analyses, they offer greater speed and repeatability than sensory evaluation and they require much smaller samples. This latter feature is crucial where a sample usually consists of only one plant's root, fruit or leaves. Harsh flavor in carrots is related to levels of volatile terpene compounds, and a quantitative laboratory method for these compounds employing porous polymer trapping can be used to analyze up to 30 small (25 g) samples per day (19, 20). The use of an efficient volatile terpenoid analytical method also provides chemical information for determining the biogenetic pathways of these compounds. This information may suggest the most effective way to genetically fix these terpenoids at a desired level.

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Sensory/objective parameter correlations are of particular interest to flavor chemistry research where reconstitution or enhancement of an organoleptic response by a correlated compound or group of compounds provides support for correlations noted. For example, flavor notes of apple (8), blueberry (11), tomato (24), and orange juice (1, 2) have been reconstituted. Sweetness in carrots is enhanced by added fructose with no significant diminution of harsh flavor (18) whereas harsh flavor has been elicited in mild carrots by the addition of suitable levels of the major volatile terpenoids of carrot (Table I).

In this study a mixture of terpinolene, caryophyllene, γ-terpinene, limonene, and myrcene (70:14:9:5:2) was dissolved in an equal amount of propylene glycol, blended for 30 sec. at high speed in water to give a concentration of 1% terpenoids, and applied to carrot slices. The terpenoid mixture was administered as a 25 µl droplet between two 2.5 g carrot slices to raise the total terpenoid level of the mild inbred B9304 (10 ppm) by 50 ppm which was nearly that of the harsh inbred B0493 (68 ppm). Introduction of the terpene solution between carrot slices proved to be a successful means of providing a uniform flavoring release and mixing during assessment of the samples by panelists. The added terpene compound mixture yielded a harsh flavor intensity in the mild B9034 carrots similar to that of the harsh B0493 carrots. While not specifically evaluated, the harsh flavor quality of the naturally-flavored B0493 carrots was very similar to that of the artificially-flavored B9304 carrots. Perceived sweetness appeared to be suppressed by the higher levels of terpene compounds although the difference was not statistically significant. However, both harsh-flavored carrot samples were significantly less preferred than the mild-flavored B9304 samples indicating the important influence of volatile terpenoid concentrations on the culinary quality of carrots.

With the chemical analysis of 30 or more samples per day in the laboratory, it is possible to secure enough genetic data to assess the feasibility of improving quality parameters, to determine biosynthetic pathways of compounds important to quality, and to correlate objective data with sensory parameters. Furthermore, these methods can be used to screen genetic materials in the numbers needed in breeding for quality. It is not possible, however, to screen all stocks in such a program and still perform the operations necessary in a traditional program for improving marketable yield. A faster sampling technique must be used to accomplish this.

Several techniques are available to vegetable breeders for estimating quality factors rapidly, including refractive index for total sugar levels, specific gravity for dry matter content, visible color for pigment levels, dichloroindophenol for vitamin C levels (14), 2,4-dinitrophenylhydrazine for pungent compounds in onions (16), and IR reflectance for protein levels. Except total sugar measurements and pungent sulfur-containing compounds,

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Table I. Mean Scores for Descriptive Sensory Analysis of Mild and Harsh Carrots with Added Terpene Flavor Mixture

Raw Carrot Samples	Sample Attributes: Intensity of Harshness[a]	Intensity of Sweetness[a]	Overall Preference[b]
	(----------Mean scores[c]---------)		
Mild Inbred (B9304) + 1% Propylene Glycol	2.24 a	4.15 a	4.77 a
Mild Inbred (B9304) + Basic Terpene Mixture in 1% Propylene Glycol	4.33 b	3.67 a	3.19 b
Harsh Inbred (B0493) + 1% Propylene Glycol	4.68 b	3.60 a	3.16 b

[a]Scale: 1 = very weak; 7 = strong.

[b]Scale: 1 = dislike very much; 7 = like very much.

[c]a, b: Mean scores in same column followed by the same letter are not significantly different at 5% level; n = 30.

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high speed laboratory techniques are not available for most flavor compounds. This again leads to the use of sensory analysis for organoleptic factors, but with a different approach.

Trained testers and expert judges have long been used in the quality control of many foods, including dairy products, beer, wine, brandy, coffee, and soy sauce. For carrots, trained taste panels noted a difference between genetic stocks of over 1.9 units on a 7 point scale for harsh flavor, and over 1.0 units for sweetness and preference with samples from three locations (18). With this great a difference and a standard error of the mean at around 0.20, only 2 or 3 assessments need to be made to discriminate between such genetic stocks with a 95% level of confidence (4). Thus, there is a good opportunity for using 2 or 3 evaluators, or only one with multiple assessments, to score for extremes in flavor between carrot samples. Since each evaluation needs to be made with only 3-5 g slices, 3 determinations can be made from the middle one-third of even a small (30 g) root without destroying the crown portion needed for seed production.

The effectiveness of this screening method is being tested with carrot populations segregating for harsh flavor. One evaluator (P.W.S.) has scored roots in triplicate, and putative uniformly extreme populations will be tested by a standard sensory evaluation panel after several cycles of selection. When selection is performed for only one attribute, several samples can be tested per minute. Less fatigue is experienced if samples are not swallowed and carryover influences from harsher samples can be greatly reduced with a rinse of carbonated water. Periodic checks of sensitivity are made by testing slices of known mild samples. Typically, an acceptable repeatability (±15%) is realized for over 80% of the samples tested. Those samples not falling into this range are not included in further testing. With this system of quality screening, 400 carrot roots can be scored for one flavor attribute per day.

Current Status and Future Prospects for Improvement of Carrot Culinary Quality

Steps to improve the culinary quality of carrots include the reduction of bitterness by altered postharvest storage techniques and the reduction of harsh flavor by genetic selection. The 25-fold genetic variation in "isocoumarin" levels also suggests a potential for genetic improvement of bitterness (5, 6). Dominance for mild flavor over harsh flavor in an F_1 hybrid grown in nine environments (21) and in a range of F_1 hybrids grown in two different environments (Table II) suggest excellent prospects for improving the flavor of hybrid carrots by including at least one mild flavored parent in the hybrid. An investigation of the inheritance of sugar types and levels may also provide direction for the genetic improvement of carrot flavor (7).

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Table II. Harsh Flavor Intensity of Several Inbred and F_1 Hybrid Carrots Grown in Two Locations.[a]

Parent[b]	B10138	B9304	B6274	B524	B4367	B0493	B3615
	(Mean scores[c])						
B10138	1.2/1.0	1.2/1.0	1.5/1.0	2.0/1.0	2.1/2.3	2.5/1.5	2.2/2.6
B9304		1.2/1.0	1.3/1.0	2.2/1.2	2.3/2.0	2.9/3.0	2.4/2.0
B6274			2.1/1.5	2.1/1.4	2.1/1.5	2.9/1.5	4.7/4.8
B524				3.5/2.5	3.5/2.6	5.0/5.5	5.4/4.1
B4367					5.8/5.6	5.7/5.6	5.9/5.8
B0493						6.8/6.6	6.8/6.8
B3615							7.0/7.0

[a]Roots were grown at Zellwin Farms, Zellwood, FL (muck soil) and the Imperial Valley Field Station, El Centro, CA (sandy soil) during the winter (Oct., 1979-Feb. 1980).

[b]Scores for inbreds are on the diagonal.

[c]Scale: 1 - low intensity of harsh flavor; 7 = high intensity of harsh flavor; X/Y - FL score/CA score; n = 6 or 7.

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Future activities include the improvement of techniques for selecting quality attributes already identified, and to add other attributes. A faster laboratory method for screening the "iso-coumarins", volatile terpenoids, sugar types, and carotenoids will be valuable. Other flavor attributes of both fresh and processed carrots need consideration. The potential for genetic improvement of carrot fiber, which is a high quality nutrient (13) that may influence texture, also warrants investigation.

With a better knowledge of the inheritance of quality factors and with improved methods for screening them, the improvement of culinary and nutritive components in a program of carrot breeding and genetics is feasible. A proper perspective of quality and marketable yield must be maintained, but quality characteristics should be included in genetic improvement programs.

Abstract

The improvement of raw carrot flavor requires a determination of desirable and objectionable sensory attributes, an efficient means to assess these attributes or factors related to them, and an understanding of how flavor can be altered genetically and environmentally. Using a wide range of genetic stocks grown in several environments, sensory evaluation indicated substantial variation in carrot flavor due to environmental and, to a greater extent, genetic factors. Statistical analyses suggested that volatile terpenes and sugars could account for the observed sensory variation, and reconstitution experiments with authentic compounds supported these observations. Genetic experiments indicated dominance for both mild flavor and low volatile levels, to make the use of mild-flavored inbred lines advisable in producing hybrid carrots. Methods for screening large, segregating carrot populations for flavor improvement are available and readily applicable in a carrot breeding program.

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