Development of carrot texture phenotyping methodology for genetic analysis and crop improvement

Avelina Gaston[1], Julie Dawson[1], Philipp Simon[1,2]

[1] Department of Plant and Agroecosystem Sciences, University of Wisconsin—Madison, [2] USDA—ARS, Vegetable Crops Research Unit

LinkedIn:

Email: asgaston@wisc.edu

FUNDING: USDA-NIFA-OREI 2021-51300-34900

CIOA Carrot Improvement for Organic Agriculture

USDA ARS Agricultural Research Service

College of Agricultural & Life Sciences UNIVERSITY OF WISCONSIN-MADISON

Why study carrot texture?

Texture is a complex trait that is important for consumer appeal and found to be polygenic in fruit crops such as apple[1]. In carrot (*Daucus carota* ssp. *sativus* L.), texture has been studied for processing, storage, and mechanical harvest purposes but not in the context of genetic analysis or crop improvement. The crisp, juicy carrots that consumers prefer are too brittle for the mechanical harvesters used by large commercial farms[2], but they are highly desirable to smaller, hand-harvesting farms. Therefore, while organic fresh-market agriculture is the target of this project, we believe that identifying genetic variants for texture traits will have widespread impact. Accurate phenotyping methods have been developed for other crops, but organoleptic descriptions of the trait have been somewhat difficult to connect to instrumental assessments thus far. To gain a broader understanding of the complexity of carrot texture, in this study, we implemented semi-trained sensory paneling for further investigation into the relationship between consumer perception and instrument-derived response variables.

Consumers prefer crisp, juicy fresh-market carrots[2]

PYP Dark-6 (Organic Seed Alliance)

Specific aims of the project

I. Develop standard phenotyping protocol with high-resolution texture analyzers

II. Determine relationships of instrumental response variables to sensory perception (focus of this presentation)

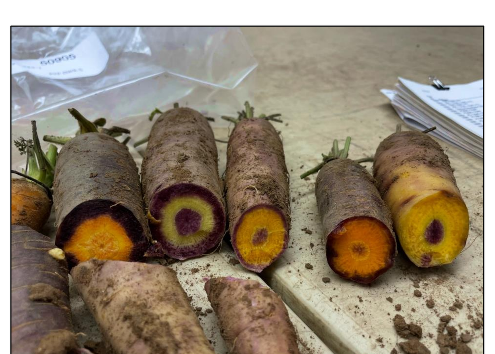
III. Fine tune methodology

IV. Apply methods to diverse germplasm: develop breeding & mapping populations & perform multi-environment genome-wide association analysis

Initial protocol development

To provide a foundation for studying carrot texture, we used high-resolution texture analyzers to evaluate multiple factors that may affect texture phenotyping, including sample positioning direction and location of puncture, as well as carrot handling variables. From these results, we developed a texture analysis protocol (below) that has been implemented in phenotyping more than 200 carrot accessions for a genome-wide association analysis across multiple environments.

PROTOCOL:



Topped carrots are stored in paper bags within polyethylene bags at 1C.

Remove from cold storage at least 4hr. prior to testing.

Slice 1-2cm-thick coins from midsection.

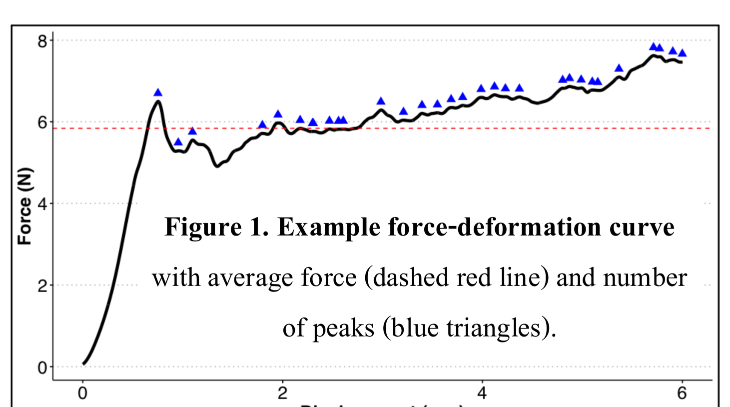
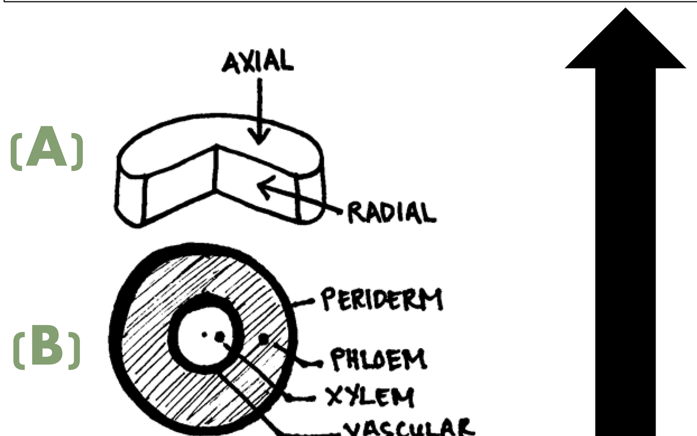
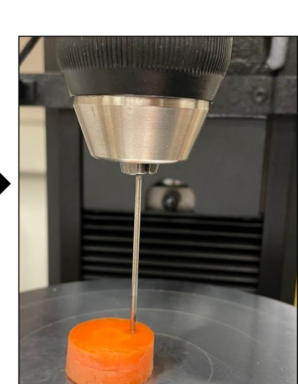


Figure 1. Example force-deformation curve with average force (dashed red line) and number of peaks (blue triangles).

Analyze the first 6mm of raw data to obtain various response variables.





Puncture coins in the axial direction (A) in phloem & xylem tissue (B) with the Instron 68SC-5 UTS (Instron®)

Test speed 2mm/s

Organoleptic methods

Training of volunteer sensory panelists

Most carrots of the extreme textures needed to train panelists are scarce and show high amounts of variation. Therefore, reference samples of vegetables with texture similar to carrot that could be eaten raw and showed uniformity were obtained from markets in Madison, WI. The panelists were trained with a standard method of biting (Figure 2) and scoring in two sessions on a set of seven vegetables (Table 1) that were shown to represent the full range of hardness and juiciness seen thus far, as carrot extremes of these variable attributes are scarce and exposure to them is minimal for most people.

Sensory panel evaluations of carrots

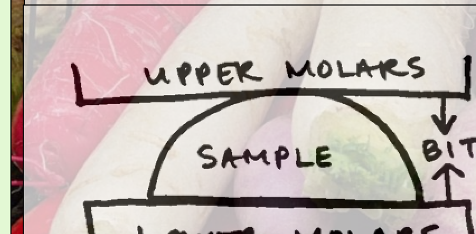
Six carrot breeding lines of varying textures were evaluated in a randomized experimental design in three duplicated sessions with an additional replication of two lines per session. Panelists present at each session were given eight samples to score.

Table 1. Reference samples used in training & their corresponding low, medium, and high sensory hardness & juiciness (right).

Reference	Hardness
Daikon Radish	1-2
Kohlrabi	2.5-3.5
Rutabaga	4-5

Reference	Juiciness
Rutabaga	1-2
Kohlrabi	2.5-3.5
Daikon Radish	4.5-5

Figure 2. Standard biting protocol (below).



References

1 Di Guardo, M. et al. 2017. Deciphering the genetic control of fruit texture in apple by multiple family-based analysis and genome-wide association. Journal of Experimental Botany. 68(7): 1451-1466.

2 Simon, P.W. et al. 2008. Carrot. In: Vegetables II: Fabaceae, Liliaceae, Solanaceae, and Umbelliferae. 327-357.

3 Bejaei, M., Stanich, K. & Cliff, M.A. 2021. Modelling and Classification of Apple Textural Attributes Using Sensory, Instrumental, and Compositional Analyses. Foods. 10(2): 384.

Table 2. Response variables correlated with sensory hardness

Response	Direction	Correlation (p-value)
Average force (N)	Radial	0.22 (0.0004**)
Slope 1.0mm (Nmm^{-1})	Radial	0.21 (0.0008**)
Maximum force (N)	Radial	0.21 (0.001*)
Slope 2.0mm (Nmm^{-1})	Radial	0.17 (0.008*)
Average drop in force (N)	Radial	0.14 (0.03*)
Average force (N)	Axial	0.18 (0.005*)
Slope 2.0mm (Nmm^{-1})	Axial	0.17 (0.009*)
Maximum force (N)	Axial	0.15 (0.0176*)

Table 3. Response variables correlated with sensory juiciness

Response	Direction	Correlation (p-value)
Number of peaks	Radial	0.34 (3.57×10^{-8} ***)
Expressed juice (%)	Radial	0.31 (5.30×10^{-7} ***)
Number of peaks	Axial	0.07 (0.258 n.s.)
Expressed juice (%)	Axial	0.12 (0.084 n.s.)

Instrumental methods

Figure 3 (top). Visual of axial (A) and radial (R) directions of puncture with a T372-29 .0625" cylindrical probe (Instron®) to obtain various response variables including average force (Fa, Newtons) from the first 6mm of force-deformation data.

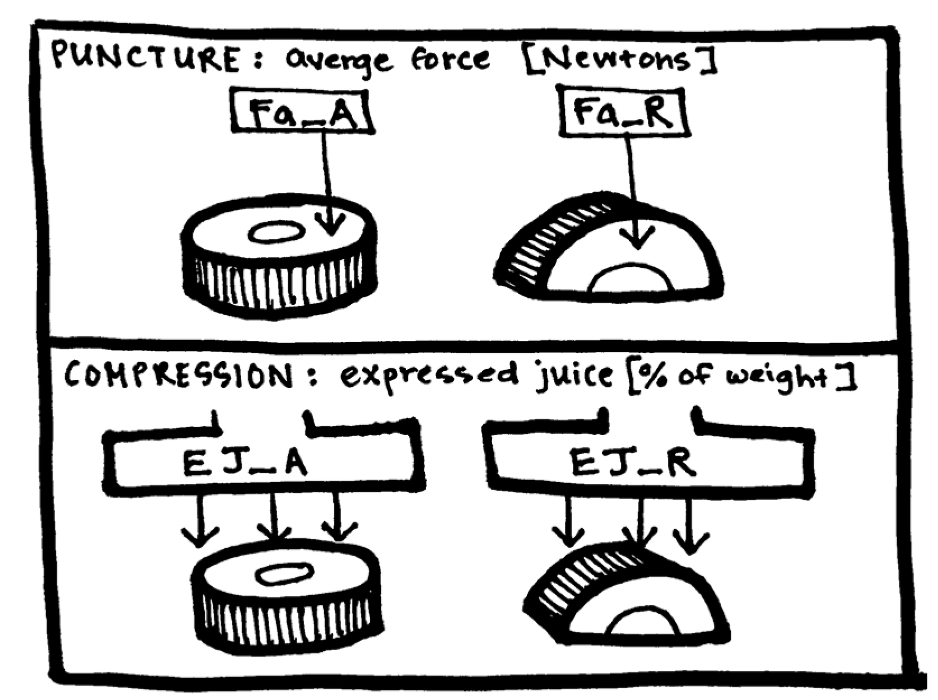
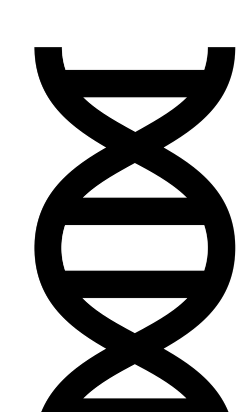
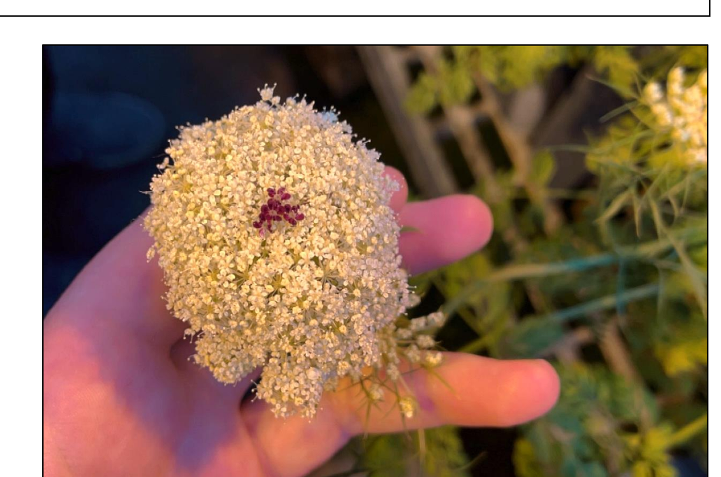


Figure 3 (bottom). Visual of axial (A) and radial (R) directions of compression for 8mm with a 2501-083 flat compression plate (Instron®) to obtain expressed juice (EJ). Coins were measured and weighed, placed between two filter papers, compressed, dabbed dry, then weighed again. EJ was calculated as a percent of the initial weight lost during compression. The method was adapted from a 2021 food science study on apple textural attribute modeling and classification[3].





Results & discussion

To determine the most relevant response variables for consumer perception of carrot hardness and juiciness, we performed principal component analysis (PCA) of 40 instrumentally-derived response variables, sensory data, and other covariates. Many response variables were found to have a significant association with sensory hardness (Table 2), but average force (Newtons) was consistently the strongest. While the radial direction of puncture (Figure 3) was better correlated with sensory perception, probably due to the sensory methods, measurement in the axial direction was still strong enough to justify its use (Figure 4). The logistics of measurement in the radial direction don't allow for separate measurement of phloem and xylem tissue; therefore, we concluded that phenotyping carrot hardness with average force over 6mm of puncture in the axial direction would be the most efficient, informative, and relevant method.

Expressed juice (EJ) was only significantly associated with sensory juiciness when compressing samples in the radial direction (Table 3, Figure 4). Interestingly, the number of peaks (count of local maxima) from 6mm of puncture was also correlated with juiciness (Table 3) and negatively correlated with hardness-related responses. Further investigation into other responses, such as NP and the average drop in force from each peak to its subsequent trough, may reveal meaningful relationships with sensory attributes like hardness, juiciness, crispness, or mealiness. Finally, some significant correlations were found between objective responses, sensory data, and dimensional covariates, such as coin diameter or depth (Figure 4). Therefore, a final composite experiment should include all the previously studied covariates.

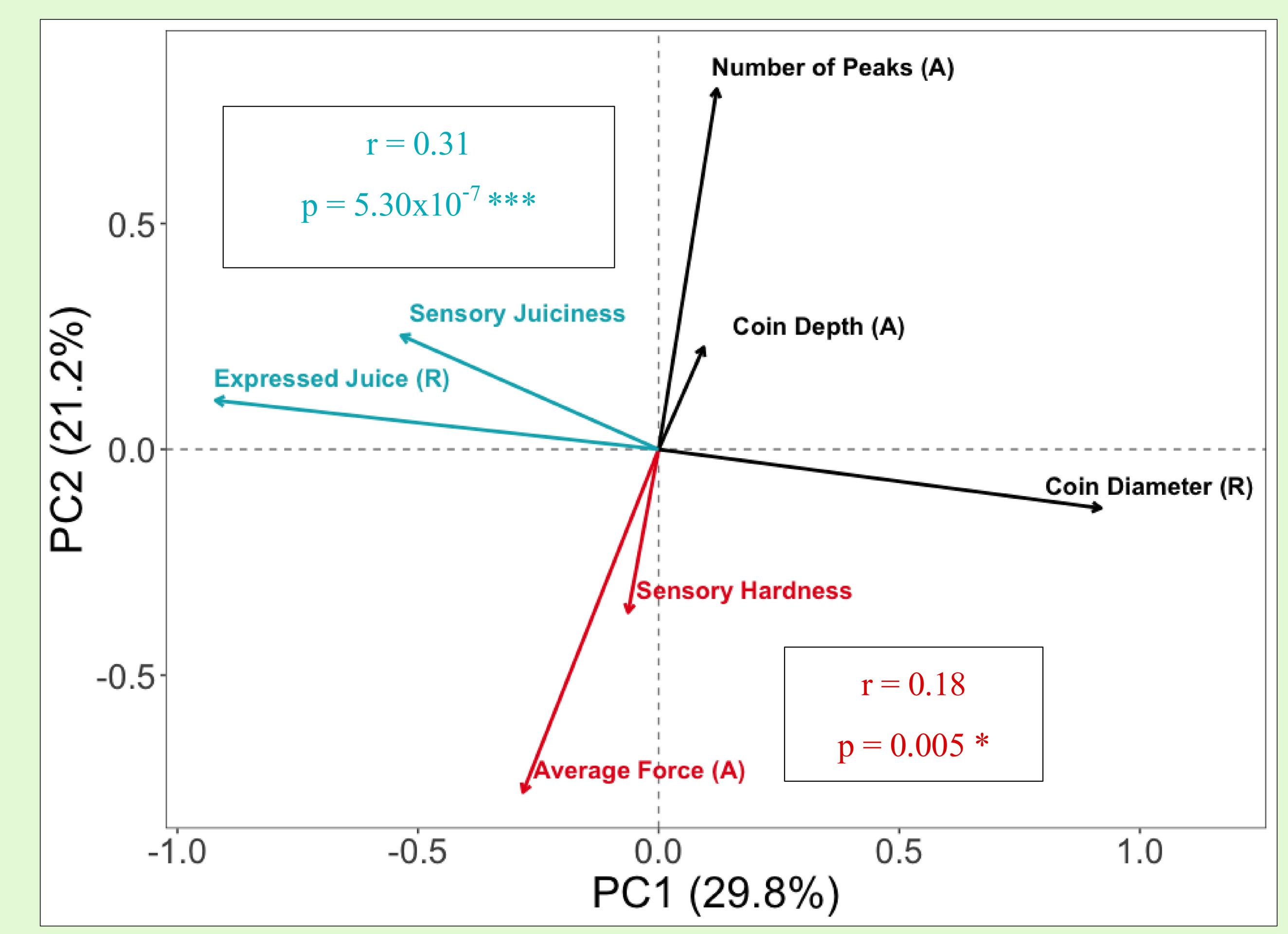


Figure 4. Principal component analysis of sensory panel evaluation data with the inclusion of additional covariates revealed significant association of sensory hardness with average force (red) from puncture in the axial (A) direction, and of sensory juiciness with expressed juice (blue) from compression in the radial (R) direction. Carrot coin depth for axial puncture and number of peaks—referring to a count of local maxima along the force-deformation curve—and coin diameter for radial compression contributed significantly to variation in the data and require further investigation.

Future directions

- Moving forward, we plan to refine the methodology by conducting a composite experiment with two texture analyzers, multiple instrument probes, multiple methods (test speed, strain, distance, etc.), and multiple carrot pedigrees of varying textures.
- As discussed in the above section, further exploration of the more elusive response variables such as number of peaks and dimensional covariates may help us gain insight into the complexity of carrot texture.
- We have already begun applying our methods of measuring carrot hardness and juiciness to a large set of diverse germplasm for genome-wide association mapping.
- Identification of extremes of carrot hardness and juiciness has allowed us to begin production of linkage mapping populations as well as breeding pools of varying textural attributes.