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Objective Method for Measuring Firmness of Iceberg Lettuce

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Abstract. **Traditional hand compression firmness scores of iceberg lettuce (*Lactuca sativa* L.) heads were compared with force-deformation data collected from parallel-plate compression tests conducted with a universal testing machine. Sample deformation was measured over a load range of 30 to 40 N. A quadratic response surface best described the relationship between hand firmness scores (1 to 5 scale) and three measurements of sample deformation (mm). Sample deformation was as precise as hand compression in measuring lettuce firmness, and it provided improved reproducibility by eliminating much of the human error. Although adequate for most firm heads, the predictive ability of the statistical model was weak for soft heads (when the hand firmness score was <2), and for heads with inconsistencies in firmness because of uneven leaf distribution. The minimum sample size required to determine accurately the mean firmness score (±0.5 units) of a population of harvested lettuce was ≈20 heads. This may be a disadvantage, since sampling one head requires ≈1.5 minutes. Overall, the instrument-based method measures lettuce firmness as precisely as the hand compression method, and provides a standardized, objective measurement for postharvest researchers when exchanging or reporting firmness data.**

Firmness is one of the main quality characteristics evaluated in iceberg lettuce because it denotes harvest maturity. A firm lettuce head is desirable because it indicates full development and minimizes the effects of handling stresses (Garrett et al., 1969). The standard method of lettuce firmness evaluation is a hand compression test, which ranks the firmness on a one to five scale (Kader et al., 1973). Correct application of this method requires the evaluator to be trained and exposed to a range of possible firmness values; however, even these precautions do not eliminate human error due to the subjectivity of the test. Sometimes it is difficult for two evaluators to apply subjective tests consistently, as the rating scale can shift with the user. This complicates the exchange of data among research groups. Also, an evaluator who is unfamiliar with the rating scale requires instruction from a person who is already skilled at the method. An instrument-based method may resolve some of these problems by providing a standardized, objective firmness measurement.

The firmness of an entire plant organ, such as a single fruit or a single leaf, is affected by a number of factors. Mean cell volume and number, tissue types (parenchyma, collenchyma, and sclerenchyma), and ratios of intercellular to cellular volumes affect the overall plant structure (Mohsenin, 1986). The elasticity, strength, and rigidity of plant tissue is attributed to the cell wall (Falk et al., 1958; Frey-Wyssling, 1952), whose rheological properties are derived from the crystal lattice of cellulose microfibrils and the supporting amorphous matrix of hemicelluloses, pectins, and lignin. Water content and cell turgidity also play major roles in tissue rigidity (Falk et al., 1958). Many of the previously mentioned characteristics are affected by cultivar, maturity, senescence, water loss, and temperature at the time of evaluation. Although these factors play a role, the volume of the air pockets within the head largely determines the firmness of whole iceberg lettuce heads. This is described sometimes as the solidity or puffiness of the head (Wurr et al., 1992), and is related to density of the head (Garrett et al., 1969).

Several instrument-based methods of firmness evaluation of whole heads have been developed using a force-deformation test, in which the sample is compressed under a load and the distance of compression (sample deformation) is measured. Garrett et al. (1966) employed a mechanical firmness tester as the selector-component of a mechanized lettuce harvester. In this method, the initial load compressed the outer leaves against or below the top of the head; then a further increase in load was used to derive a force-deformation curve. Later, this group developed a laboratory method (Garrett et al., 1969) evaluating the force-deformation curve over a force range of 10 to 40 N. Bourne (1982) suggested a parallel-plate compression test using an Instron universal testing machine (Instron Corp., Canton, Mass.), measuring the deformation that occurs as the load increases from 0.5 to 10.5 N. The two methods developed by Garrett et al. (1966, 1969) have an advantage in that they ignore the highly variable initial sections of the force-deformation curves, which usually reflect compression of the outer leaves only.

In general, simply transposing testing procedures and theories of nonbiological materials onto biological materials is not possible because of the additional complexity of the biotic components. One parameter derived from force-deformation tests of nonbiological materials is the elastic modulus: the ratio of stress to corresponding strain. For nonbiological materials this ratio remains proportional up to a limiting stress, but for biological materials the force-deformation curve is sigmoidal. Thus, the elastic modulus is dependent on the degree of deformation (Mohsenin, 1986). In addition, the deformation of biological materials is not elastic because a part of it is always unrecoverable. For these kinds of tests, the elastic modulus can be approximated by using a region of the stress-strain curve where the slope is nearly linear; even then, all experimental conditions should be specified.

Expanding upon the previous force-deformation studies, the objectives of this research were to relate the instrument-based evaluation of lettuce firmness to the hand compression method, to compare the precision of the two methods, and to evaluate the practical utility of the instrument-based method for postharvest research.

Materials and Methods

Iceberg lettuce cv. Ithaca 989 (Asgrow Seed Co., Tifton, Ga.) was used, since this cultivar manifests a wide range of firmness, depending on maturity. About 100 heads of lettuce representing all firmness categories were harvested on 22 July 1998 from the Agriculture and Agri-Food Canada muck-soil research farm in Sainte-Clotilde, Que. The lettuce was delivered promptly to the postharvest laboratory in St-Jean-sur-Richelieu, where it was held at 6 °C for 5 to 6 h. The heads were then acclimated to 20 °C and the wrapper leaves removed, leaving the first tightly held leaf attached. The heads were sorted into categories of firmness based upon the hand compression method (Kader et al., 1973), which

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scores firmness on a five-point scale: 1 = *soft*, easily compressed or spongy; 2 = *fairly firm*, neither soft nor firm, good head formation; 3 = *firm*, compact but may yield slightly to moderate pressure; 4 = *hard*, compact, and solid; and 5 = *extra-hard*, overmature, may have cracked midribs. The heads were divided into eight categories, with firmness scores of 1.0 to 4.5, in 0.5-unit increments.

A parallel-plate compression test was conducted using a universal testing machine (Lloyd Instruments Ltd., Fareham, England) equipped with a 100-N load cell (precision = 0.001 N) and horizontal compression plates. Force on the load cell and its vertical position during compression (precision = 0.01 mm) were monitored by computer. Before the method was finalized, preliminary tests were conducted using a variety of compression objects. The most effective compression tests employed plates with large surface areas because smaller circular probes (≈1 cm in diameter) and punches (≈5 cm in diameter) damaged the leaves.

Deformation tests were performed on the lettuce head while it rested in three positions: two side positions and one top position. For the first side position, the central axis of the head (that which intersected the butt and apical meristem, and corresponded to the direction of vertical growth in the field) was kept parallel with the compression plates and the head was rotated until the outer midrib was at a 15° angle to the force. This ensured that the load cell plate simultaneously contacted the outer midrib and another portion of the sample, to avoid crushing the midrib. After the first side position was tested, the head was rotated 90° (in the same direction) to the second side position, keeping the central axis parallel with the compression plates, and the second measurement taken. For the final (top) position the head was rotated 90°, aligning the central axis perpendicular to the surface of the compression plates, and the third measurement taken. Immediately prior to this final test, the butt was trimmed slightly to ensure that the sample rested securely in position. Trimming usually detached three or four leaves, but they were not removed from the head.

The load cell plate descended upon the sample at a speed of 60 $\text{mm} \cdot \text{min}^{-1}$. Once the compression force of the initial contact reached 1 N, the force was re-zeroed and the actual test began. At the maximum force of 40 N, the test was completed. The computer logged the distance (mm) the load cell descended, and calculated deformation of the sample over several 10-N spans of force. In the present study, deformation data collected over the 30- to 40-N span are presented because they best fit the statistical model. Deformations induced by forces of <10 N were influenced more by the compression of outer leaves and were less reliable. This is not surprising, as 10 N was the lower limit used by Garrett et al. (1969). Deformation data collected over the 10- to 20-N and 20- to 30-N spans provided trends similar to data collected over 30 to 40 N, but were more variable and did not fit the model well.

Statistical analysis. The number of samples per hand firmness category varied from six to 21. Regression analysis of the data was performed using the General Linear Models procedure of SAS (1985). Hand firmness (y) was regressed on various combinations of force deformation (x) in first-, second-, and third-order multiple regression models. From a full model comprising all the variables, the least significant variables were excluded one by one, readjusting the model each time. The significance of a variable's effect upon the model was tested at the 10% level.

The number of samples required to estimate the mean firmness (±0.5 units) of a future population of lettuce (with 95% confidence) was calculated with a *t* test, using a postulated value for the population standard error of the mean (σ^2) based upon the relation:

$$\left(t^2 \cdot \sigma^2\right) \big/ r < d^2 \qquad (1)$$

where r = the sample size required; d = the desired difference, 0.5 firmness units; and *t* = Student's *t*, at df = n – 6 and *P* = 0.05. To approximate the postulated σ^2 of a future population, the individual standard errors of observations [SE($\hat{y}$)] from this experiment (n = 98) were used, applying the equation:

$$\mathrm{SE}(\hat{y}) = \sqrt{\sigma^2 / n} \qquad (2)$$

which approximates the true relation between SE($\hat{y}$) and σ^2. The range of SE($\hat{y}$) values provides a range of σ^2 values in which one could expect to find the σ^2 of a future population.

Results and Discussion

The average deformation values of the top position (2.5 mm) were lower than those of the side positions (3.6 mm), while there was no difference between the two side positions ($P <$ 0.05, data not shown). As one would expect, a better model was developed when the average of the two side positions was used rather than measurements from only one of the side positions.

For the applied force range of 30 to 40 N used in the test, a quadratic response surface best described the relationship between the hand compression measurements and the instrument measurements of sample deformation (mm):

$$\begin{aligned}(\text{hand}) = \; & 5.85 - 0.640 \cdot (\text{sides}) - 0.674 \cdot (\text{top}) \\ & + 0.063 \cdot (\text{sides})^2 + 0.101 \cdot (\text{top})^2 \\ & - 0.075 \cdot (\text{sides} \cdot \text{top})\end{aligned}$$

where (hand) = hand compression measurements; (sides) = averaged deformations of the side positions; and (top) = deformations of the top position. All independent variables were significant at $P \leq 0.01$, except for (sides·top), which was significant at $P \leq 0.1$. The model fit the data quite well, as indicated by a reasonable coefficient of variation (16.1%) and a relatively high correlation coefficient ($R^2 = 0.783$).

Generally, the observed top position deformation of a head was 1.0 mm less than its averaged side position deformation. Thus, when plotted, most of the experimental values seemed to fall along the line where the top position deformation + 1 equaled the average side position deformation (Fig. 1). The model estimated with most certainty the firmness of heads that had small differences (<3.0 mm) between the deformations of the top and side positions. This was because the model was based chiefly upon data from heads that exhibited such small differences. Two-dimensional plots at fixed values of top position deformation showed a narrowing of the 95% confidence interval of the mean near values where top + 1 = sides (Fig. 2).

Very few data were collected in the region where firmness scores were <2, and thus the 95% confidence interval of the mean broadened substantially as deformation values exceeded 6 mm and firmness estimates were <2 (Fig. 2). Consequently, the model's most meaningful estimates were of firmness scores ≥2. Garrett et al. (1969) reported that soft heads, in particular, have discontinuities in their force-deformation curves because of relative slip between leaves and cracking of leaves. Although a part of the deformation of lettuce is always unrecoverable, slipping and cracking damages the heads to reduce the deformation recovery further. This probably distorted the second and third measurements of soft heads. However, for firm heads (with firmness scores ≥2) no differences ($P \leq 0.05$) were noted between the first and second side position deformations, and Garrett et al. (1969) asserted that compression measurements of firm heads were reasonably repeatable. Sampling more heads with firmness scores <2 may not enhance the model's predictive ability, since distorted second and third measurements and discontinuity due to slipping and cracking will increase the error associated with the evaluation of soft heads.

Some variation in the model was attributed to the protocol for the universal tester. Some heads had regions that were softer or harder than the rest of the head because of uneven leaf distribution. One compression with two hands was usually sufficient to sample an entire head so that its inconsistencies were considered in the evaluation of the overall firmness. However, the universal tester could only compress in one direction. The inability to provide an overall firmness evaluation was compensated for, but not completely eliminated, by incorporating measurements from several positions. Note that other methods only involved a single compression of the head (Bourne, 1982; Garrett et al., 1966, 1969). Since the hand compression method was subjective, its degree of repeatability was probably lower than that of the universal tester. Ironically, the present study requires an infallible human comparison to develop a close-fitting model. In reality, the true firmness categories of several heads probably were judged erroneously by the hand compression method, given that the experience of the evaluator was only a few seasons. In the hands of an expert evaluator, the relationship between the two methods may be much closer. Though inexperience may have reduced the fitness of the current model, it also supports the need for an objective test.

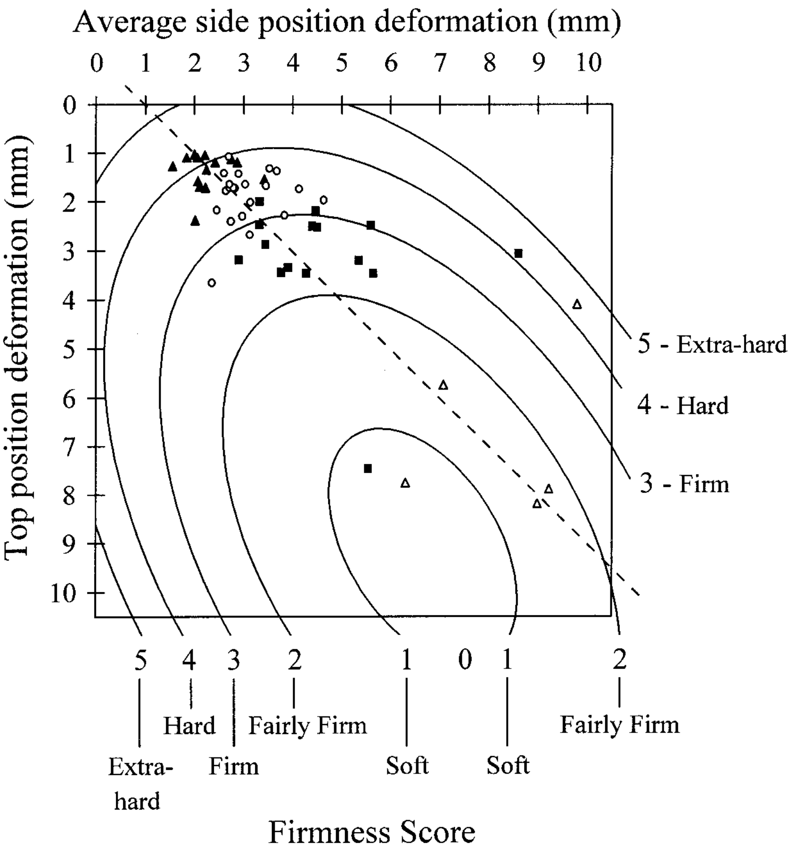

Fig. 1. Quadratic response surface that estimates firmness values (rings) from the deformations (mm) of top and average side positions, measured over the force span of 30 to 40 N. Individual points represent measurements of single heads that have been plotted according to their side and top position deformations. The symbols represent the heads according to their hand firmness scores: Δ = 1 (soft), ■ = 2 (fairly firm), ○ = 3 (firm), and ▲ = 4 (hard). To avoid cluttering, data points with observed hand firmness scores of 1.5, 2.5, 3.5, and 4.5 were omitted. The dashed line is where top position deformation + 1 = average side position deformation.

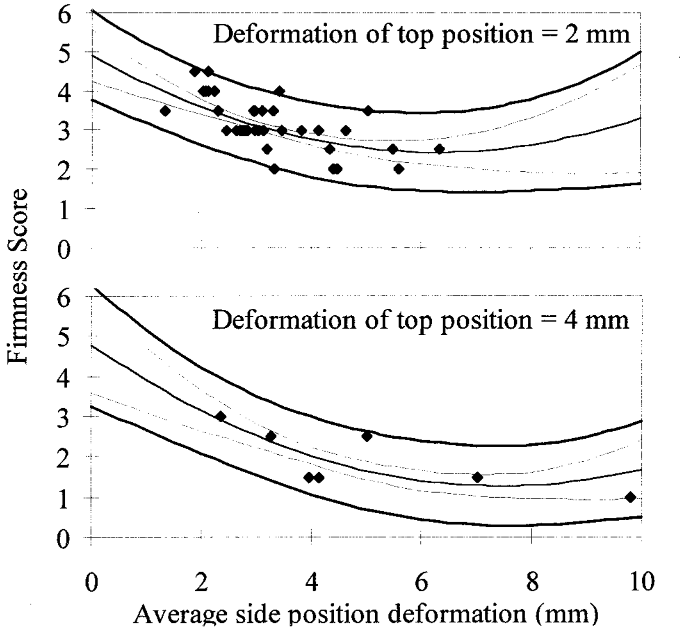

Fig. 2. Two-dimensional depictions of Fig. 1 at fixed values of top position deformation (mm). Firmness scores are plotted against the average deformation of the side positions, measured over the force span of 30 to 40 N. Individual points represent measurements of single heads with top position deformations equal to the fixed value (±0.5 mm), and were plotted according to side position deformations and hand firmness scores. On either side of the line that estimates the firmness are dashed (inner) lines demarcating the 95% confidence interval of the mean, and thick (outer) lines demarcating the confidence interval estimated to contain 95% of the observed values.

To measure the utility of the model, the number of samples required to determine the mean firmness score (±0.5 units) of a future group of lettuce was estimated. We assumed that the population's firmness was homogenous (if not, any method would require a large number of samples to produce narrow confidence intervals), and that all of the heads had firmness scores ≥2. Sample sizes of 20 and 30 adequately determined the firmness scores (±0.5 units) of samples whose σ^2 were ≈0.10 and 0.13, respectively (Table 1). When considering the present data with firmness scores ≥2, 83% and 92% of the observations had an SE($\hat{y}$) that approximated a σ^2 <0.10 and 0.13, respectively. Therefore, the firmness of future populations of lettuce should be correctly determined with sample sizes of 20 or 30. These estimates are conservative and no such estimation exists for the hand compression method described by Kader et al. (1973). Evaluating the error of a hand compression measurement is difficult, because there is no accepted standard for comparison. However, we assume that the factors that caused "erroneous" observations to deviate from our model (e.g., inconsistent regions within a head) adversely affect both methods; thus, attempts to evaluate the hand compression method may generate similar required sample sizes.

Other authors have not directly compared the instrument-based and hand compression methods; however, it is implied or assumed sometimes that the instrument-based method is accurate. Garrett et al. (1969) demonstrated a strong relationship between firmness and an experienced evaluator's judgment of a lettuce head's acceptability for harvest (acceptable/unacceptable). This provides a glimpse of the reliability of the parallel-plate compression method, since it was the sole method of evaluating firmness. Although the amount of literature directly related to this study is sparse, it seems to support our conclusion that an instrument-based method can provide an objective evaluation of firmness that is comparable to the traditional hand compression method.

The universal tester method has both weak and strong points. The prediction ability of the model is weak when the firmness score is <2, and this may or may not be improved by

Table 1. The number of samples required to estimate the mean firmness (±0.5 units) of a future population of lettuce (with 95% confidence), assuming firmness ≥2.0. The calculation of this number was based upon a *t* test that used a postulated value for σ^2. SE($\hat{y}$) from this experiment (n = 98) was used to approximate the postulated σ^2 values. Indicated are the percentage of observations (n = 86) in which SE($\hat{y}$) approximated a σ^2 less than the one used in the *t* test.

No. samples required	Postulated σ^2	Observations (n = 86) which approximated an σ^2 < σ^2 of test (%)
10	0.057	10
20	0.105	83
30	0.134	92
40	0.162	94
120	0.283	100

additional sampling of soft heads. Similarly, the model is less likely to evaluate accurately a head with large inconsistencies, which are sometimes manifested as large differences between the top and side deformations. Unfortunately, to estimate the firmness score within 0.5 units, the safest minimal required sample size is quite large (20 or 30). Although this is a consequence of the method's limitations, it has probably been exaggerated by the inconsistency of the hand compression method with which the instrumental method was compared. Another disadvantage is that one sample, consisting of three deformation tests, requires ≈1.5 min for evaluation. On the other hand, the instrumental method eliminates most human error and provides improved reproducibility. When exchanging or reporting data, the hand compression rating scale could be expressed relative to the scale of the instrument, and in situations of multiple evaluators or when consistency is extremely important, this method could be applied in place of the hand compression method. In addition, this method makes self-training possible for those who are unfamiliar with the hand compression method.

The method described provides a standardized, objective measurement of lettuce firmness, which is comparable (in units and precision) to the traditional hand compression method described by Kader et al. (1973). The instrument-based method could substitute for subjective ratings in laboratory studies in which selection of lettuce according to head firmness is required; however, its usefulness will be limited by the required sample size and time of sampling. Also, further testing is necessary to assess the effects of cultivar, growing season, field conditions, and tissue water status on force-deformation of harvested lettuce.

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