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A proposal for determining the flesh softening of peach and nectarine in postharvest through simplified targeted modeling

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ABSTRACT

Peach flesh softening is a continuous process that occurs mainly during postharvest. It allows fruit to reach the proper firmness for consumption. In this trial, it was evaluated different texture attributes of three nectarine cultivars and three peach cultivars over a 5-day period (day of harvest, and the first, second, third, and fourth days after harvest) at 20 °C using penetration and uniaxial compression tests. Through linear modeling with it was obtained the fixed and random effects and linear functions, where the slopes represent the flesh softening. Cultivars were segregated using contrast tests. It was found that the penetration test was more effective than the uniaxial compression test, as it allowed for the segregation of the genotypes into three clusters. One cluster, grouped the melting fleshed (*MF*) nectarines ('Andes Nec-1', 'Andes Nec-3', and 'Venus'), showed the fastest softening rate. The *MF* 'Sweet September' and the non-melting (*NMF*) 'Hesse' were grouped together, as they exhibited the lowest softening rate. Lastly, the *NMF* 'Andross' was alone, showing an average score. These results suggest that the different softening patterns cannot be fully explained by the *MF*/*NMF* classification. In order to facilitate the implementation of the protocol for determining softening, it was used a targeted model that focused on the most informative phase of the curve—namely, the first and third days of the evaluations. This simplified targeted model allowed to segregate the genotypes into the same original clusters, and it confirms that the analysis of only two points of evaluation is sufficient to characterize the softening rates of different peach cultivars.

1. Introduction

The texture of fruit, as an attribute of a particular fruit's entire sensory quality, is important when determining consumer preferences (Bonnin and Lahaye, 2013; Tunick, 2011). The factor that most affects peach texture is softening, which determines the sensory quality and shelf life of fruit in postharvest (Ghiani et al., 2011a,b). During this process, the cell walls forming the mesocarp are relaxed and solubilized (Hayama et al., 2006; Waldron et al., 2003), thus generating a smooth and melt texture in melting fleshed peaches (*MF*). On the other hand, non-melting peaches (*NMF*), even if they soften, remain relatively firm during postharvest (Brovelli et al., 1999; Lester et al., 1996; Yoshioka et al., 2010).

Measuring firmness in peach is useful for (1) determining the proper time to harvest (Infante, 2012), (2) monitoring postharvest fruit ripening (Zhang et al., 2010), and (3) as a parameter associated with acceptability and consumer preference (Delgado et al., 2013). The most common way to measure peach firmness is by penetrating or puncturing the flesh with a 7.9 mm probe (Infante et al., 2008). Although there are other rheological tests available, they are seldom used in fruit studies, even if they could be useful for determining other parameters of texture. Among these, single or uniaxial compression tests are commonly employed in the determination of food texture (Bourne, 2002). As it pertains to fruit, a uniaxial compression test involves pressing the sample – without destroying it – by employing a probe larger in diameter than the sample itself, thus resembling the action of gentle hand pressure placed on the fruit (Bourne, 2002). This type of analysis allows for multiple measurements of the same fruit on more than one occasion during postharvest.

In general, studies dealing with postharvest peach firmness show that this parameter is frequently associated with the content of soluble solids or with a certain background skin color; there is little research that has focused on how the flesh soften during postharvest (Cano-Salazar et al., 2013a,b; Cantin et al., 2010; Iglesias and Echeverria, 2009). Flesh softening is a continuous process, and, therefore, must integrate various measurements over time. Considering many different peach and nectarine cultivars on the market have unknown postharvest life spans, it is crucial to assess and classify the genotypes according to their softening

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patterns. The aim of this paper is to analyze and compare the softening patterns of different peach cultivars during shelf lives and to determine a simple and reliable methodology to measure their softening.

2. Materials and methods

2.1. Fruit sorting

The trial was conducted during the 2014 and 2015 seasons. It was harvested four *MF* cultivars and two *NMF* in physiologically ripe, pre-climacteric stage (Contador et al., 2011; Zhang et al., 2010). In the first season, it was analyzed the peach cultivars 'Andross' (*NMF*), and the nectarine 'Andes Nec-1' (*MF*), 'Andes Nec-3' (*MF*), and 'Venus' (*MF*). In the second season, it was analyzed the peach cultivars 'Hesse' (*NMF*) and 'Sweet September' (*MF*). Immediately after harvesting, the fruit were transported to the lab, and their ripeness levels were homogenized by sorting them in accordance with the chlorophyll absorbance index (I_{AD}), as measured on both cheeks of each fruit with a Da-Meter device (Sinteleia, Bologna, Italy).

The fruit were sorted using a range between 1 and 1.5 units of I_{AD} (Shinya et al., 2013). I_{AD} is an index that measures the difference in absorbance levels of chlorophyll at two wavelengths – A670 and A720 – which have been reported to have significant correlations with the flesh firmness and the angle hue color in peach, demonstrating that it is an effective, non-destructive tool for determining peach ripeness (Shinya et al., 2013; Ziosi et al., 2008). Lastly, the fruit were transferred to a ripening chamber at 20 °C and 90% RH for four days. Twenty fruit of each cultivar were separated for the uniaxial compression test, and the rest were withdrawn daily in batches of 15 fruit to perform the destructive penetration test.

2.2. Uniaxial compression test

Twenty fruit were subjected to uniaxial compression tests on the day of harvest and on the first, second, third, and fourth days after harvest. The measurements were made in the equatorial zone of each fruit using a TA.XT Plus Texture Analyser (Stable Micro Systems, Surrey, UK), and using a wide plunger (20 mm diameter) that deforms the whole fruit. The haul of the plunger ran 1 mm deep from the time it makes contact with the skin and at a speed of 5 mm s^{-1}. To avoid possible mechanical damage induced by the measurement procedure, the skin location where the plunger was in contact with the fruit was first marked with an indelible ink pen (Sharpie Fine Point Permanent) so that each measurement would be slightly separated from the former one. This evaluation allowed assessing the maximum force (N) of the uniaxial compression.

2.3. Penetration test

At harvest, and on the first, second, third, and fourth days after harvest, 15 fruit were evaluated on each day. After removing the epidermis with a scalpel, they were subjected to a puncture test with a 7.9 mm plunger using a TA-XT Plus texture analyzer (Stable Micro Systems, Surrey, UK). The penetration depth was 10 mm, and the probe ran at a constant speed of 5 mm s^{-1}. This evaluation allowed to assess the fruits' maximum force (N); final force (N); total area (area under the curve, or work performed by the probe to penetrate tissue in N m); maximum force area (area under the curve up to the maximum force in N m); linear distance (length of an imaginary line connecting all points of the curve); number of peaks; and young modulus (tissue elasticity in N m^{-1}).

2.4. Data analysis

To characterize the ripeness levels at harvest, ANOVA was performed, based on the variable maximum force (N), as measured by the penetration test with a 7.9 mm plunger. This test corresponds to the most common parameter used by the peach industry, which is assessed with a portable penetrometer. The maximum force means were separated by a Fishers Least Significant Difference (LSD) test (5%). Based on the penetration tests, and before the linear model was built, the most relevant flesh-softening parameter was chosen. Next, biplot principal component analysis (PCA) was performed, and this was analyzed using only the vectors and their relative significance according to their projections on the axes. The results of both tests (uniaxial compression and penetration) were adjusted to linear modeling with fixed and random effects. The model for analyzing the uniaxial compression test was the following:

$$Y_{ijkl} = \mu + D_i + V_J + (D \times V)_{ij} + f_k + t_l + \varepsilon_{ijkl},$$

where Y = maximum force (N); D = evaluation day (harvest, first, second, third, fourth day after harvest); V = cultivar; f = fruit; and t = season (2014; 2015). The day (D) and cultivar (V) were the fixed effects, while the fruit (f) and time (t) were the random effects that added unplanned variability to the model, and, in this case, could be calculated regardless of the experimental error ε. Thus the results of the predicted values are explained by the fixed factors of the model. As the penetration test analysis is a destructive test, it is not possible to estimate the variability of the force ascribable to the fruit, since daily 15 different fruit samples were measured, and, therefore, this variance is attributed to the experimental error ε; the model is the following:

$$Y_{ijk} = \mu + D_i + V_j + (D \times V)_{ij} + t_k + \varepsilon_{ijk},$$

where Y = maximum force (N); D = evaluation day (harvest, first, second, third, and fourth day after harvest); V = cultivar; and t = season (2014; 2015).

The linear functions of softening for each cultivar were generated based on these models. Following this, the slopes were analyzed, i.e., the loss of firmness over time, also known as the softening rate. In order to find the differences between the slopes, it was executed contrast analysis, which also allowed comparing groups of treatments for the *MF*/*NMF* typologies. The results of both models were analyzed; the best model was the one that was able to segregate more cultivars according to the softening rates. Further, it was calculated a new model, targeted at the most informative phase of the curve. Statistical analysis was completed using the InfoStat version 2014 statistical software (Grupo InfoStat, Córdoba, Argentina).

3. Results and discussion

3.1. Firmness at harvest

The optimum range of flesh firmness at harvest for *MF* peach varies between 45 and 53 N (Crisosto, 1994). In general terms, other studies indicate that the flesh firmness of *MF* and *NMF* peaches should be between 40 and 65 N, depending on the variety (Ghiani et al., 2011a,b). Accordingly, all varieties used in this trial were within the optimal range of firmness at harvest (Table 1).

3.2. Principal component analysis (PCA)

The variables that best represent the softening patterns of peach cultivars during their shelf lives are the following: Young modulus, final force, maximum force, and maximum force area. This is because their vectors were grouped together and follow the largest

Table 1
The maximum force of the flesh of three peach and three nectarine cultivars, measured through flesh penetration tests with a 7.9 mm plunger at the time of harvest.

Cultivars	Firmness (N)	
Andes Nec 1	54.82	A[*]
Andes Nec 3	57.15	A
Venus	56.83	A
Sweet September	47.96	B
Hesse	45.40	B
Andross	39.91	B

[*] Different letters indicate statistical differences, test de LSD (p-value < 0.05).

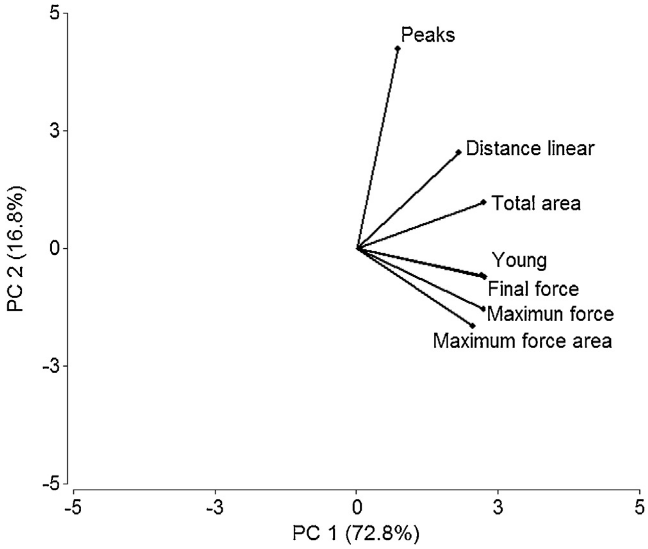

Fig. 1. Principal component analysis of the texture attributes that best represent the softening patterns of three peach and three nectarine cultivars during shelf life at 20 °C.

Table 3
Adjusted linear functions of the changes of flesh firmness over time for three peach and three nectarine cultivars, analyzed using penetration tests. The conditions of the trial were 20 °C and 90% RH.

Modeling		Function by cultivar		Flesh type[a]	
Firmness (Andes Nec 1)	=	52.91–11.58 Day	a[*]	MF	a
Firmness (Andes Nec 3)	=	55.70–11.58 Day	a	MF	
Firmness (Venus)	=	56.16–11.58 Day	a	MF	
Firmness (Sweet September)	=	52.91–3.27 Day	b	MF	
Firmness (Hesse)	=	46.49–2.39 Day	b	NMF	b
Firmness (Andross)	=	32.53–6.66 Day	c	NMF	

[*] Different letters indicate statistical differences as determined through mixed models and contrasts (*p*-value < 0.05).

[a] MF: melting flesh; NMF: non-melting flesh.

projection parallel to the main axis (first component) of the PCA (Fig. 1). There is also a high correlation among them due to the proximity of the vectors, thus all reflect the same phenomenon. However, according to Table 2, the variable maximum force (N) was the parameter that showed the greatest incidence, i.e., it is the one that best represents softening (Delgado et al., 2013; Zhang et al., 2010). The adoption of the idea of common initial firmness in peach is a complex issue when we deal with individuals belonging to the two classic typologies (*MF* and *NMF*), as it is well known that *NMF* genotypes exhibit slow softening, which allow them to be harvested in a more ripe stage (Sandefur et al., 2013). On the other hand, *MF* peaches should be harvested at a time of higher firmness due to their rapid postharvest softening, transitioning from firm flesh at harvest (40 N) to very soft flesh (less than 10 N) in just two days (Sandefur et al., 2013).

Concerning the softening patterns among the genotypes (Fig. 2), it is clear that between the day of harvest and the first day postharvest, it is not possible to observe differences between the cultivars because firmness levels can be erratic or imperceptible. The *NMF* 'Andross' is the only genotype showing some softening at this stage, but this variety also showed the lowest initial firmness. In general, from the first day of postharvest onward, it was observed evidence that some genotypes soften faster than others. This is especially made clear with the penetration test (Fig. 2A), where marked differences between the varieties can be observed. On the other hand, when the uniaxial compression test is used, all varieties appear to have rather similar and low slopes.

3.3. *Linear modeling the penetration test*

It was found the variable maximum force as obtained by the PCA was the parameter that best represents the process of softening; therefore, this was then considered for the further data analysis. Thus, linear functions were built for each genotype, in which the softening rate was calculated by the slope of the curve of firmness loss over time. It can be observed that the pattern of the relative order of the functions obtained for the cultivars is similar to the observed curves (Figs. 2 A and 3), so this is evidence that the models accurately reflect reality. The 'Andes Nec-1', 'Andes Nec-3', and 'Venus' cultivars exhibit similar behaviors (Fig. 3), which is confirmed via their slopes, which are equal ($-11.58\,\mathrm{N\,d^{-1}}$) (Table 3), which is confirmed via their slopes, which are equal ($-11.58\,\mathrm{N\,d^{-1}}$) (Table 3). It is worth noting that the 'Andes Nec-1' and 'Andes Nec-3' were developed through the same breeding program and that they are also quite similar to Venus. These three nectarine varieties show outstanding postharvest performance, maintaining fruit quality for more than 40 days in a cold chamber (0 °C), showing a rapid softening rate at 20 °C (Cano-Salazar et al., 2013a,b). The contrast analysis of the softening rates (Table 3) showed that the *NMF* peach cultivars softened slower than the *MF*; this behavior can be explained because these genotypes have either reduced or zero activity concerning the enzyme endo-polygalacturonase (Callahan et al., 2004; Lester et al., 1996), which is the enzyme that is largely responsible for decoupling the structural components of the cell wall that form the flesh. However, in terms of the softening patterns, this tra-

Table 2
Variables and their relative weights of the penetration test with 7.9 mm plunger considered in the principal component analysis of peach and nectarine cultivars.

Variable	Value for each component		Value per each PC		Weight
	PC1	PC2	PC1 (0.728)	PC2 (0.168)	
Maximum force	0.424	−0.241	0.308	−0.040	0.349
Total area	0.424	0.185	0.308	0.031	0.339
Maximum force area	0.387	−0.312	0.281	−0.052	0.334
Final Force	0.427	−0.115	0.310	−0.019	0.330
Young modulus	0.418	−0.108	0.304	−0.018	0.322
Linear distance	0.339	0.384	0.246	0.064	0.311
Peaks	0.137	0.799	0.099	0.134	0.233

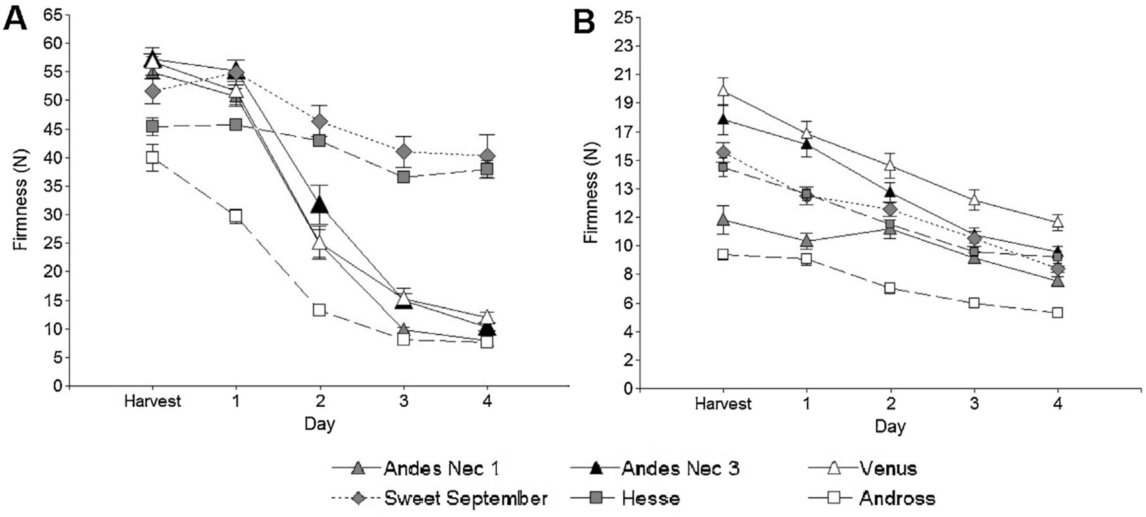

Fig. 2. Evolution of the flesh firmness of three peach and three nectarine cultivars kept in postharvest at 20 °C. The curves were built using flesh penetration tests with a 7.9 mm plunger (A), and through uniaxial compression of whole fruit with a 20 mm plunger (B).

ditional classification would hide some variability that lies inside either the *MF* or the *NMF* groups. This relates to how the three groups are formed: the *MF* nectarines 'Andes Nec-1', 'Andes Nec-3', and 'Venus' soften faster than the rest; the group formed by the *NMF* 'Hesse' and the *MF* 'Sweet September' show the slowest softening rates, and, finally, the *NMF* 'Andross' softens at an intermediate rate.

In prior research, it was reported that there is some variation in the softening pattern within the group of *MF* peaches, which highlights the difficulty of classifying peach texture (Mignani et al., 2006). Regarding the texture classification of peach, it was recently described that there is also a sub-group known as "slow melting flesh" (*SMF*), in which the individuals of this sub-group are characterized as having "semi-melting" flesh—maintaining firm and crispy flesh longer, but that, at some moment, they release ethylene and begin to soften (Ghiani et al., 2011a,b; Mignani et al., 2006). The main representative of this sub-group is the cultivar 'Big Top', which is a fruit that is less susceptible to bruising during postharvest (Ghiani et al., 2011a,b). In this research, the cultivar 'Sweet September' showed that it is able to maintain its flesh firmness to a greater extent than the other *MF* cultivars, thus resembling behavior similar to the *NMF* genotype.

3.4. Linear modeling the uniaxial compression test

The results achieved by modeling the uniaxial compression test are different from those observed in modeling the penetration test since the magnitudes of the slopes of this test are quite low, and lower than the ones observed with the penetration test (Tables 3 and 4). Graphically, both the observed phenomena and the calculated functions show similar curves (Figs. 2 B and 4). The contrast analysis allowed for only segregating the softening rate of the genotype 'Hesse' from all of the others (Table 4). Even if both tests (uniaxial compression and penetration) are able to differentiate the softening rates among the different *MF* and *NMF* peach cultivars (Tables 3 and 4), the results suggest that the penetration test has a greater power of segregation, as it can generate three groups with particular softening patterns, versus the compression test that is able to distinguish only one genotype from the others. The low segregation capacity of the uniaxial compression test can be explained by the nature of this type of non-destructive method, since the plunger compresses the fruit for a 1 mm haul, unlike the 10 mm penetration of the probe inside the flesh using the penetration test. With the penetration test, the maximum force represents

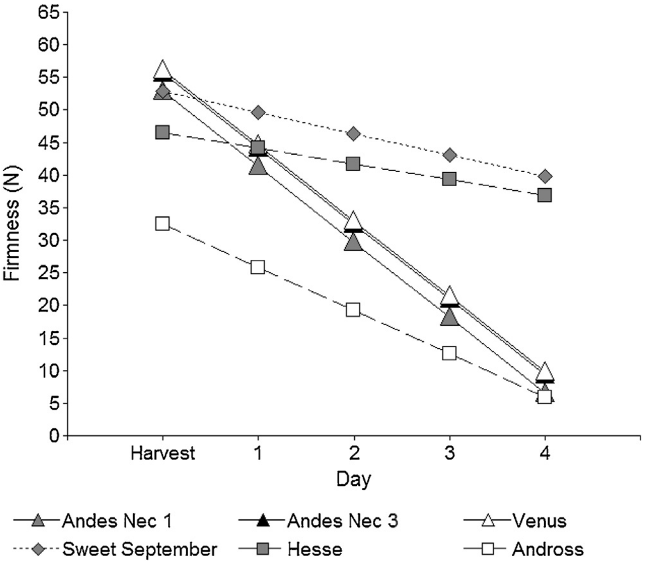

Fig. 3. The adjusted linear functions of the evolution of flesh firmness in postharvest at 20 °C of three peach and three nectarine cultivars analyzed using flesh penetration tests with a 7.9 mm plunger.

Table 4
Adjusted linear functions of the changes of the maximum force over time of three peach and three nectarine cultivars, analyzed using uniaxial compression tests. The conditions of the trial were 20 °C and 90% RH.

Modeling					
			Function by cultivar	Flesh type[a]	
Firmness (Andes Nec 1)	=	11.74–1.06 Day	a[*]	MF	a
Firmness (Andes Nec 3)	=	23.74–2.14 Day	A	MF	
Firmness (Venus)	=	19.66–1.15 Day	A	MF	
Firmness (Sweet September)	=	15.65–1.90 Day	A	MF	
Firmness (Hesse)	=	8.96–1.46 Day	B	NMF	b
Firmness (Andross)	=	9.21–1.06 Day	A	NMF	

[*] Different letters indicate statistical differences as determined through mixed models and contrasts (p-value < 0.05).

[a] MF: melting flesh; NMF: non-melting flesh.

the collapse of the tissue (Harker et al., 2010); on the other hand, the compression test is more closely related to tissue elasticity (Bourne, 2002; García-Ramos et al., 2005). However, the main technical advantage of the uniaxial compression test is that it allows using the same piece of fruit during repeated postharvest assessments, thus decreasing the high variability of the results that are characteristic of stone fruits. In this sense, it seems unreasonable to dismiss this approach entirely. Rather, this analysis can be evaluated using longer compression hauls of the probe (over 1 mm) while avoiding reaching the threshold that may cause irreversible tissue damage; this could capture more information regarding the fruit texture. Moreover, this test could be used as an analogous methodology to the manual compression of fruit, as it is a very useful aid in packaging lines for sorting and discarding fruit, and also widely used by consumers when choosing fruit (Pallottino et al., 2013). It is also important to note that, because the material that is tested is firm, the manual compression test is inefficient, and thus, its utility is restricted to fruit near consumption ripeness (Harker et al., 2010).

3.5. Targeted linear modeling of the penetration test

Considering that the penetration test allows building the best type of modeling for flesh softening, a new targeted model was generated based on this test. It is well known that the softening curve of peach during postharvest fits a logistic function (Lurie et al., 2013; Rizzolo et al., 2009; Zerbini et al., 2011). In it, both the beginning and end phases of the curve show asymptotic behavior: they show zero or minimal softening from harvest to the first day postharvest, as well as from the third day onward; further, between the first and third days postharvest, there is a rapid, linear loss of flesh firmness (Fig. 2A). For this reason, the targeted model was built to consider only two single points of the whole process—those that indicate when the most significant changes occur (Table 5). Comparing the results of the targeted modeling with the one that includes all of the days of sampling (day of harvest, and first, second, third, and fourth day after harvest), one can observe that the pattern of the relative order of the functions is similar (Figs. 3 and 5), but with the targeted modeling, the magnitude of the observed slopes are higher for all genotypes compared with the functions resulting from the original model of 5 days (Tables 3 and 5). This confirms that it is during this period when the fastest softening rate occurs. When the softening rates are contrasted, it can be observed that the results are equal to the original modeling, based on the five sampling times (Tables 3 and 5), thus generating the same three groups described above. The texture of the fruit is described in terms of mechanical force, deformation, and elasticity (Bourne, 2002; Harker et al., 2010), and in the case of peach, flesh firmness is a critical attribute, both because it describes its postharvest life and because it affects the quality and perception of the fruit by consumers (Abbott, 1999; Shewfelt, 1999).

Recently, the softening rate of peach has been highlighted as one of the most important traits in terms of the development and selection of new cultivars in a breeding program scheme because of the great interest in improving this feature by producers and marketers (Gallardo et al., 2012). However, measuring the firmness of the flesh and determining the softening rate in peach has also become a major challenge due to high intra- and inter samples variability observed with stone fruits. The results of this study indicate that, from a practical point of view, targeted modeling can more efficiently determine softening rates, and thus reduce costs and simplify the phenotyping of the phenomenon. Usually, when a peach cultivar is evaluated on the day of harvest, the flesh temperature is uneven, affecting to reach precise measurements. In this

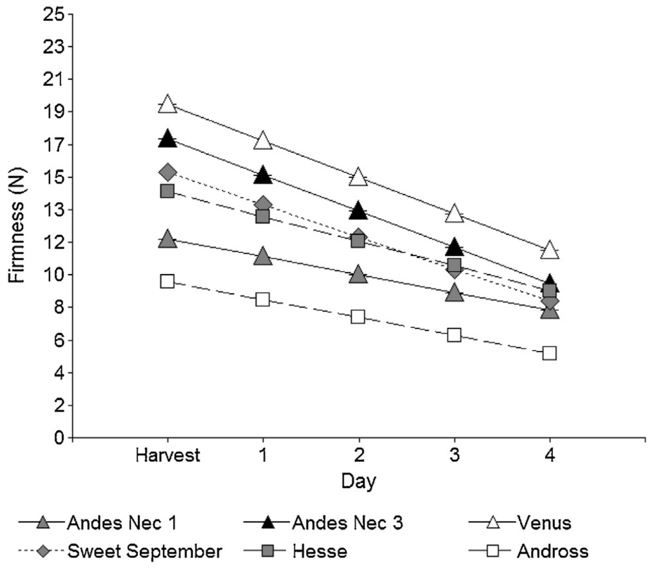

Fig. 4. Maximum force adjusted linear functions determined by the uniaxial compression of the whole fruit in postharvest at 20 °C of three peach and three nectarine cultivars analyzed using a 20 mm plunger.

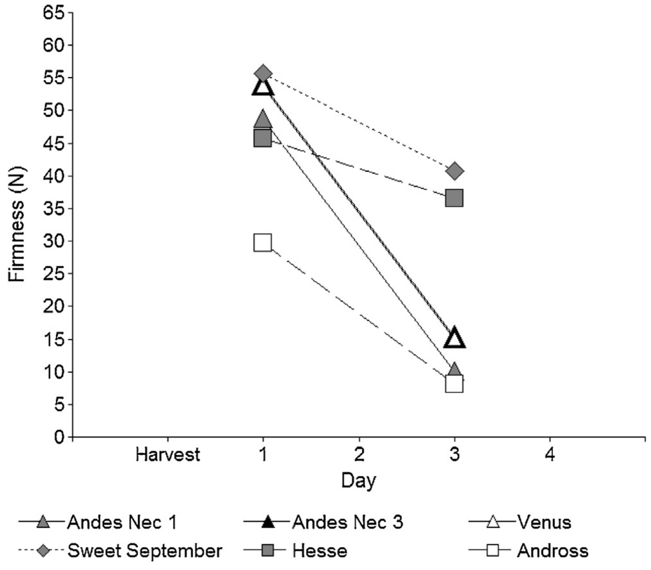

Fig. 5. Targeted modeling based on the sampling of fruit on the first and third days of the evolution of flesh firmness of three peach and three nectarine cultivars in postharvest at 20 °C using flesh penetration tests with a 7.9 mm plunger.

Table 5
Adjusted linear functions of the changes of the flesh firmness over time of three peach and three nectarine cultivars, analyzed using penetration tests. The modeling was targeted for sampling the first and third days in postharvest. The conditions of the trial were 20 °C and 90% RH.

Modeling					
			Function by cultivar	Flesh type[a]	
Firmness (Andes Nec 1)	=	65.66–19.38 Day	a[*]	MF	a
Firmness (Andes Nec 3)	=	70.91–19.38 Day	a	MF	
Firmness (Venus)	=	70.55–19.38 Day	a	MF	
Firmness (Sweet September)	=	65.66–7.48 Day	b	MF	
Firmness (Hesse)	=	52.81–4.61 Day	b	NMF	b
Firmness (Andross)	=	37.97–10.79 Day	c	NMF	

[*] Different letters indicate statistical differences as determined through mixed models and contrasts (p-value < 0.05).

[a] MF: melting flesh; NMF: non-melting flesh.

sense, and when we are focused on determining softening rates, it seems reasonable that we would not consider evaluating flesh firmness on the day of harvest, but rather, make our analysis 24 h later. Proceeding as described, the flesh will reach an even temperature, and, consequently, this will allow to build a more accurate and robust modeling process for peach flesh softening.

4. Conclusions

The traditional classification of peach flesh typology is insufficient to characterize the current wide range of cultivar assortment, and in which individuals with intermediate characteristics exist, located between *NMF* and *MF* softening patterns. The results of this study suggest that to characterize the softening patterns in peach, it is necessary only measure the maximum force on the first and third days of a cultivar's shelf life at 20 °C, as this phase is the most informative of the entire flesh-softening logistic curve. However, it is also important that the samples should be sufficiently homogeneous in terms of physiological age. This goal can be achieved by determining the absorbance of chlorophyll skin. This methodology would minimize the number of samples, resulting in more efficient phenotyping, and could thus generate clearer categories of peach softening patterns.

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