Eur. J. Hortic. Sci. *81*(4), 189–196 | ISSN 1611-4426 print, 1611-4434 online | http://dx.doi.org/10.17660/eJHS.2016/81.4.1 | © ISHS 2016

Original article

The relationship between instrumental tests and sensory determinations of peach and nectarine texture

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Summary

In this work, we evaluate the relationship between the rheological and the sensory texture of fresh peach and nectarine varieties. Two non-melting fleshed varieties (*NMF*), 'Andross' and 'Carson', and four melting fleshed nectarines (*MF*), 'Andes nec-1', 'Andes nec-2', 'Andes nec-3', and 'Venus', were harvested and kept in a ripening chamber for four days. A sensory-trained panel using the sensory texture descriptors 'hardness', 'crispness', 'crunchiness', 'melting', and 'juiciness' determined the sensory profile of each variety. Penetration tests, texture profile analysis (TPA), and mechanical acoustic analysis profile tests were performed using a TA-XT Plus texture meter. Principal component analysis defined the most influential rheological variables of each test. We also performed regression analysis by partial least squares (RPLS); as regressor variables we used the set of the rheological variables and the sensory attributes as dependent variables. The RPLS model explained 62% of the relationship between rheological and sensory variables. The strongest relationships were between 'hardness' and the TPA variables, and 'melting' and quantity of juice and the TPA variables. Finally, using a regression tree for the attributes 'hardness' and 'melting', we determined that the variable 'TPA hardness' was the most relevant to define both descriptors, thus classifying the varieties into two groups by obtaining a critical value for each attribute.

Keywords
sensory evaluation, rheology, texture meter, hardness, Texture Profile Analysis

Significance of this study

What is already known on this subject?
- In general terms, the change of texture of peaches and nectarines under normal ripening conditions has not been studied. From the rheological and sensory approaches, the maximum force, which is often determined by the fruit industry, measures solely the force just before the flesh collapses under the effect of the penetration of a 8-mm plunger. So the knowledge of the texture of peach and nectarine is limited to a single parameter, even if it is a quite complex phenotype.

What are the new findings?
- In this research, new rheological tests have been validated, which are feasible to be employed for analyzing the texture of peaches and nectarines, and relate it with the sensory perception of texture.

What is the expected impact on horticulture?
- These analysis procedures will improve the processes related with the phenotyping of the texture of peach and nectarine, and they will contribute to a better selection of new varieties and to attain a better characterization of the fresh fruit products in the production chain.

Introduction

Texture is a sensory attribute perceived through sight, hearing, and touch, and it is probably the most important sensory attribute linked to the structure of food (Ross, 2009; Szczesniak, 2002). In fresh fruit, texture is a key factor because, after appearance, it determines acceptability (Harker et al., 2010). Genetic control in flesh typology of peach is a well-known Mendelian trait (Bailey and French, 1941), and this trait segregates the pulps into two categories: melting flesh (*MF*), which behaves as a dominant trait, and non-melting flesh (*NMF*), which is expressed in its recessive form, always linked to the clingstone phenotype, and present in peach varieties destined for industrial processing. Further, it has been determined that the biggest difference between these two categories is in the presence/action of the enzyme endo-polygalacturonase (Callahan et al., 2004), present in the *MF* genotypes, which is the enzyme largely responsible for decoupling the structural components of the cell wall that form the flesh. This constitutional difference provokes that *MF* varieties show a rapid softening of the flesh in postharvest while the *NMF* varieties soften quite slowly. However, very few investigations have focused on the identification, perception, and quantification of the different textures of peach during regular ripening. Researchers have determined that peach sensory quality is mainly related to sweetness (Delgado et al., 2013; Iglesias and Echeverria, 2009), appearance, and flavor (Cano-Salazar et al., 2013a; Shinya et al., 2014), while texture has been mainly studied when physiopaties appear, induced during cold storage (Arana et al., 2007; Lurie and Crisosto, 2005; Cantin et al., 2010). Cold storage is a basic tool used to reduce postharvest fruit decay and maintain overall fruit quality, since reducing metabolism and respiration rates effectively slows ripening (Lurie and Crisosto, 2005). Recently, the peach texture has also been studied to determine the main factors related to its acceptance (Delgado et al., 2013). Seven texture attributes were defined, and among these, are 'hardness', 'crispness', and 'juiciness', as assessed by a trained panel (Delgado et al., 2013). Further, Cano-Salazar et al. (2013b) analyzed the descriptors 'crispness', 'easy to swallow', 'fibrousness', 'hardness', and 'juiciness' in fruit varieties stored in cold chambers under different environmental conditions. These analyses show that peach and nectarine texture is a relevant factor related to the general perception of quality.

As stimuli perception of the texture is mainly determined by mechanics (Bourne, 2002; Szczesniak, 2002), both sensory evaluation through trained panels and the evaluation of rheological variables by instruments, are the two main ways to study fresh fruit texture. The combination of both approaches can increase the analytical accuracy because sensory methods are more complex, subjective, and expensive, even if they provide the immediate and relevant information of human perception. On the other hand, instrumental methods are cheaper and more objective, but they do not always reflect the sensory traits of food (Ross, 2009; Chen and Opara, 2013). In general, research on fresh fruits has not integrated sensory evaluations and rheological measurements, so this bias might affect the understanding of the whole phenomenon of texture in fruit (Rolle et al., 2012).

Sensory evaluation is an irreplaceable method of analysis, even if it is laborious and expensive (Harker et al., 2002, 2010). There are no instruments that can reveal the mouth's complexity, sensitivity, and range of movements. The most relevant sensory attributes to describe texture are 'hardness' (or 'firmness') (Harker et al., 2010; Zdunek et al., 2011); 'crunchiness' and 'crispness', which are associated with the sound produced when biting and chewing (Harker et al., 2010; Fillion and Kilcast, 2002; Zdunek et al., 2010; Cano-Salazar et al., 2013b; Valente et al., 2011); 'melting', which is the ease of disintegrating the sample without chewing (Harker et al.; 2010, Valente et al., 2011; Bugaud et al., 2011); and 'juiciness' (Contador et al., 2011; Infante et al., 2009; Harker et al., 2010). Texture is mainly determined using instruments called 'texture meters', which provide precise measurements of force, time, distance, and deformation of food (Fiszman and Damasio, 2000; Rolle et al., 2012). Compression tests, such as texture profile analysis (TPA), and puncture tests with different probes are commonly used in fresh fruit (Madieta et al., 2011; Bourne, 2002). Responding to the need to explore the different textures of peach in more depth, the objective of this study is to relate the sensory attributes of texture to the rheological parameters to determine the instrumental parameter that is most effective for predicting sensory texture.

Materials and methods

Fruit sorting

We conducted our trial during the 2015 season. The fruit was harvested in an experimental orchard near Santiago, Chile (70°40'6.54''W, 33°48'14.85''S); it was harvested when physiologically ripe, in the pre-climacteric stage, and when the ground had reached a yellowish color (Contador et al., 2011; Zhang et al., 2010). The varieties and harvest dates of this trial were for the *NMF* varieties 'Andross' (January, 3) and 'Carson' (January, 20); and for the *MF* varieties 'Venus' (January, 20), 'Andes nec-1' (January, 27), 'Andes nec-3' (February, 3), and 'Andes nec-2' (February, 27). Immediately after harvesting, the fruit was transported to the lab. Due to that peaches and nectarines show high variability in terms of ripeness level even if they are harvested the same day, one subgroup of fruits with homogeneous ripeness was segregated by means of the chlorophyll absorbance index (I_{AD}), measured on both cheeks of each fruit with a Da-meter device (Sinteleia, Bologna, Italy). Because the *NMF* varieties soften more slowly than the *MF* varieties (Lester et al., 1994; Haji et al., 2005), they were also subjected to the same conditions for ripening as a way to standardize the conditions of the experiment. The fruit was transferred to a ripening chamber at 20°C and 90% RH for four days, which is when the flesh reached a firmness level adequate for consumption (approx. 10–20N). For the evaluations, each fruit was divided into two halves: one half was used for instrumental analysis and the other half for sensory evaluation.

Sensory analysis

Thirteen adults (eight males and five females) were recruited and trained to perform the descriptive analysis of the peach texture. We followed the methodology proposed by Contador et al. (2014), and the general protocols were those set out by ISO 8586-1 (ISO, 1993). The training process was conducted in 16 sessions of approximately 1.5 hours each. In the qualitative phase, open discussions took place regarding those descriptors that best define the texture of peaches. The defined sensory texture attributes were 'crispness', 'hardness', 'crunchiness', 'juiciness', and 'melting' (Table 1). Then, in the quantitative phase, the participants were taught to quantify the five textural descriptors defined in the previous stage. Evaluations were conducted in individual booths under standard and controlled light and temperature conditions. The pieces of fruit were cut into slices with skin a few minutes before each sensory evaluation. Samples were presented to each judge in white porcelain dishes marked with three-digit codes that matched numbers on an answer sheet. The attributes were evaluated on a 0 to 15 unstructured scale, with the value 0 corresponding to 'extremely low', and the value 15 to 'extremely high'.

Table 1. Sensory descriptors and technique used to describe the texture of the peach and nectarine flesh, in the order we requested the sensory evaluation panel to assess them.

Descriptor	Definition	Evaluation technique
Crispness	Unique, strong, clean, and sharp sound produced in the first bite with incisors and open lips.	Place the sample between the incisors and penetrate. Evaluate the intensity of the sound produced at the first bite.
Hardness	Force required for compressing the sample between the molars.	Place the sample between the molars and evaluate the force needed to compress until the molars come together.
Crunchiness	Multiple and deep sounds perceived as a series of events, evaluated with the molars and closed lips.	Place the sample between the molars and chew three times and evaluate the intensity of the sound produced.
Juiciness	Amount of liquid released during mastication.	Place the sample between the molar, chew three times and assess the amount of juice released.
Melting	Ease with which the pulp disintegrates under slight pressure between the tongue and palate.	Place the sample between the tongue and palate and apply a slight pressure.

Table 2. Rheological variables definitions and probe used in the tests executed with a TA-XT Plus (Stable Micro Systems, Surrey, U.K.) texture meter, used to characterize the flesh of fresh peach and nectarine.

Probe	Variables	Definition
Puncture 2 mm	Maximum force (N)	Maximum force registered
	Total area (N*mm)	Area under the curve between the initial force and the final force
	Final force (N)	Force registered at the end of the plunger run
Puncture 7.9 mm	Total area (N*mm)	Area under the curve between the initial force and the final force
	Number of peaks	Number of positive peaks registered from the maximum force until final force
Texture Profile Analysis (TPA)	TPA hardness (N)	Maximum force registered in the first compression cycle
	TPA resiliency (%)	Capacity to recover the shape of the sample after the first compression
	TPA chewiness	Hardness * elasticity * cohesiveness
Acoustic emission	Number of acoustic peaks	Number of positive peaks above 10 dB
	Minimum acoustic peaks (dB)	Minimum value of acoustic peaks registered

Instrumental analysis

Half of a fruit was designated for rheological testing with a TA-XT Plus (Stable Micro Systems, Surrey, U.K.) texture meter, applied to the equatorial area of the fruit. First, two puncture tests were performed with 2.0 (P2) and 7.9 (P7.9) mm diameter probes; the latter was performed after removing the skin with a scalpel. The plungers penetrated 10 mm at a steady speed of 5 mm s^{-1}. In the second trial, the Texture Profile Analysis (TPA) was performed, so a flesh cylinder, 10-mm wide and 10-mm high, was extracted. The skinless flesh cylinder underwent a double compression test with a 75-mm diameter plunger (P75). A second skinless flesh cylinder, 20-mm wide and 10-mm high, was subjected to a penetration test with a 5-mm depth with a 4-mm diameter plunger (P4). During this test, we recorded the acoustic signal produced by the penetration of the plunger. For recording the acoustic signal, an *Acoustic Envelope Detector* (AED) was used, set with a *Gain* 0 and 1 KHz *Envelope Corner Frequency*. The amount of juice was determined via the absorption of juice through common absorbent paper (Infante et al., 2009), allowing us to obtain the percentage of juice (w/w) released by each sample.

Data analysis

We applied a completely random design, in which varieties corresponded to treatments of 14 repetitions each, and each fruit was the experimental unit. To characterize the ripeness levels at harvest, an analysis of variance (ANOVA) was performed, based on the variable maximum force (N), as measured by the penetration test with the 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 Fisher's least significant difference (LSD) test (5%). Additionally, principal component analysis (PCA) was performed to determine the rheological variables with greater weight for each test. The variables with greater weight in the first component are detailed in Table 2.

Subsequently, we conducted regression analysis by partial least squares (RPLS) (Abdi, 2003), in which the set of rheological variables resulting from the PCA were the regressor variables, and the sensory attributes were the dependent variables. In addition, the varieties were used as classification criteria. RPLS is a multivariate statistical method that generalizes and combines PCA and linear regression. It has been described as a modeling procedure commonly used to correlate sensory attributes and instrument measurements (Vilanova et al., 2013; Valente et al., 2011). The results are described by a triplot chart, and the interpretation was made according to the relative positions of the points corresponding to the classification predictor variables and the response variables (Balzarini et al., 2008).

Of those sensory variables that showed greater weight in the first axis, or a latent variable of the RPLS ('hardness' and 'melting'), a tree regression procedure was carried out to find those rheological variables with higher influence on the characterization of the sensory attributes, allowing them to be categorized into groups. Each category was characterized by an adjusted ANOVA using a general linear model. Means were separated using the LSD Fisher test (5%). For all analyses, we used the statistical program InfoStat version 2014 (Grupo InfoStat, Córdoba, Argentina).

Results and discussion

Fruit ripeness characterization

The parameter flesh firmness is commonly used for determining the ripeness level in peach and nectarine along the production chain. The most frequent way to evaluate firmness is by determining the maximum force by penetrating in different areas of the fruit once the skin has been removed, usually by a portable instrument – a penetrometer – equipped with a cylindrical 7.9-mm diameter plunger (Infante et al., 2008; Valero et al., 2007). According to the maximum force scores, the day on which the assessments were initiated, i.e., after a ripening phase at 20°C and 90% RH for four days, the varieties showed maximum force values close to the range adequate for consumption (10–20N) (Table 3). The 'Andes nec-2' variety was firmer than the rest, and the 'Andes nec-1' variety was of the least firmness.

Table 3. Maximum force measured with a 7.9-mm diameter plunger; measured in peach and nectarine varieties after a four-day period at 20°C and 90% RH.

Variety	Maximum force (N)	
Andes nec-2	23.3	a*
Venus	12.7	b
Carson	12.6	b
Andes nec-3	9.3	bc
Andross	9.0	bc
Andes nec-1	8.7	c

* Different letters in the same column indicate significant differences at 5%.

The relationship between texture sensory and instrumental variables

The model explains 62% of the relationship between rheological and sensorial variables (Figure 1). The summary of the scores reached by all the variables for each variety is shown in Table 4. The sensory variable of 'hardness' is mainly explained by the variable 'TPA hardness', as well as 'TPA chewiness' and 'TPA resiliency'. A greater 'TPA hardness', which corresponds to the sample's resistance to the first compression, is associated with high sensory hardness, which is estimated by compressing the sample using molar teeth (Tables 1–2). In turn, a harder flesh requires a greater number of chewings, which is reflected in the instrumental variable 'TPA chewiness', which emulates the chewing action of a consumer.

The variable 'melting' could be positively related to the percentage of juice, which is calculated by the weight difference. It is also negatively related to the TPA variables, mainly by 'TPA hardness'. Calculating the percentage of juice requires that the flesh sample pass through two metal rollers that squeeze it, separating the juice from the solid tissue (Infante et al., 2009). This resembles the sensory technique employed for evaluating 'melting', during which pressure is applied to the sample when between the tongue and the palate (Table 1). Samples that showed high values of 'TPA hardness', 'TPA resilience', and 'TPA chewiness', showed low scores for 'melting'.

With the variables 'hardness' and 'melting' in opposite positions (Figure 1), these sensory descriptors are inversely related, as has also been reported in mango flesh (Valente et al., 2011). The 'Andes nec-3' variety was defined as the most melting (Figure 1). Additionally, the sensory terms 'hardness' and 'melting' are descriptors that have the greatest capacity to segregate varieties, given the projection of this attribute on the main axis (Figure 1). These results are interesting because peach flesh is classified traditionally in terms of texture as either *MF* or *NMF*. The results show that although 'Carson' and 'Andross' (both *NMF*) and the *MF* 'Andes nec-3' belong to different typologies, from the sensory point of view, they contrast in texture. Moreover, the nectarines 'Andes nec-2' and 'Venus', both classified in the *MF* typology, are much harder and crunchier.

'Crispness' is defined mainly by the variable 'number of acoustic peaks', and also variables of the puncture tests ('P2 final force', 'P7.9 total area', 'P2 total area', and 'P2 maximum force'). The descriptor 'crunchiness' is related to the TPA variables ('TPA hardness', 'TPA chewiness', and 'TPA resilience'), as well as with puncture test variables ('P2 final force', 'P7.9 total area', 'P2 total area', 'P2 maximum force'). It was observed that the higher the values of these variables, the greater the value of 'crunchiness' is observed. 'Venus' nectarine showed the highest values on puncture tests. 'Crunchiness' and 'crispness' variables are associated with the fracture properties of food, as manifested in non-deformable materials, and which, therefore, break relatively easily (Luyten et al., 2004; Vickers, 1982; Van Vliet and Primo-Martin, 2011). This may be considered contrary to an elastic texture that is highly deformable, and not easily broken. The descriptor 'juiciness' is negatively related to TPA variables ('TPA hardness', 'TPA chewiness', and 'TPA resilience'), i.e., when chewed, a peach with harder flesh would be associated with a lower release of juice. Studies demonstrate that the natural flesh softening during maturation show a tight relationship between juicier flesh and tender flesh (Jaeger et al., 2003; Harker et al., 2010).

Variety segregation by 'hardness' and 'melting'

Whereas the sensory attributes 'hardness' and 'melting' were those that exercised the greater weight on the main axis of the RPLS analysis (Figure 1), we performed a 'tree' regression to determine the rheological variable with greater weight, thus allowing the attributes of 'hardness' and 'melting' to be categorized through a critical value (Figure 2). Such critical values allow categorizing peach using the results of the instrumental tests, and they describe how these categories are perceived from the sensory point of view. The results establish that the variable 'TPA hardness' exerts the greatest influence, categorizing both sensory descriptors into two groups. The critical value of 'TPA hardness' to categorize the sensory 'hardness' reaches a threshold at 4,687 N (Figure 2).

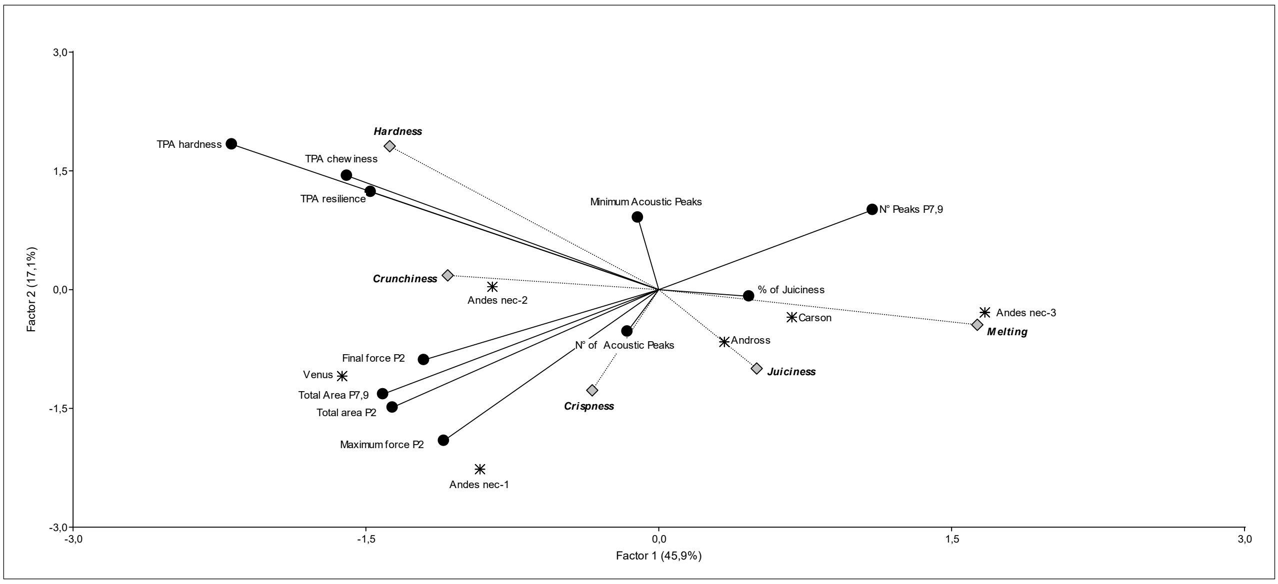

Figure 1. Peach and nectarine texture explained by a partial least squares Regression (RPLS), in which the regressor variables are shown with dots, the dependent variables of the sensory analysis with the rhombuses, and the peach varieties with the asterisks.

The fruit that showed instrumental test values greater than 4,687 N reached, on average, 6.2 sensory 'hardness' (on a 0 to 15 scale, where 0 = 'extremely soft', and 15 = 'extremely hard'), while those that showed a 'TPA hardness' less than this critical value achieved, on average, a sensory score of 2.8 'hardness' (i.e., very soft fruit), confirming the direct relationship between both types of hardness measurements.

To categorize 'melting,' the critical value of 'TPA hardness' that separates the group is 3,535 N (Figure 2). On the contrary, the relationship between 'TPA hardness' and 'melting' is inverted, which was evident, as both vectors are oriented in opposite directions (Figure 1). When hardness is determined instrumentally as less than 3,535 N, the fruits reach a sensory 'melting' score of 8.9 (on a 0 to 15 scale, where 0 = 'no melting', and 15 = 'extremely melting'); when the 'TPA hardness' is higher, the pulp is perceived as low melting, reaching a value of 4.1. Other studies have reported the importance of the first compression hardness test of TPA ('TPA hardness') as a variable that correlates directly with the sensory perceived firmness of grape berries (Le Moigne et al., 2008), and on other foods (Loredo and Guerrero, 2011).

Texture is a quality attribute that is multi-parametric in nature, i.e., it is evaluated by considering a set of descriptors that are related to one another (Bourne, 2002; Szczesniak, 2002). After appearance, texture is the attribute that most determines the acceptability of fresh fruit. For this reason, the fruit industry should make texture analysis a primary objective (Redgwell and Fischer, 2002). In peach and nectarine, texture is even more relevant because there is a wide range of new varieties (Reig et al., 2013), and the quality of the product determines its success or failure on the market. Even though flesh firmness is a component of texture, which is commonly used along the entire production chain – as a harvest index (Infante, 2012), to monitor maturity during postharvest (Zhang et al., 2010), and as a parameter of determining consumer acceptability (Delgado et al., 2013) – texture has not yet been studied in depth. It is necessary to study the behavior of the peach flesh as its specific sensory attributes evolve, quantifying its rheological parameters and understanding the interaction between the sensory attributes and the instrumental parameters.

A first step is to explore the attributes that define texture from the sensory point of view, and then to define the most suitable instrument tests to assess them. Most research that addresses peach texture has focused on determining the effects of cold storage and its relationship to pulps that develop abnormal textures during that period (Shinya et al., 2014; Lurie and Crisosto, 2005; Arana et al., 2007; Cano-Salazar et

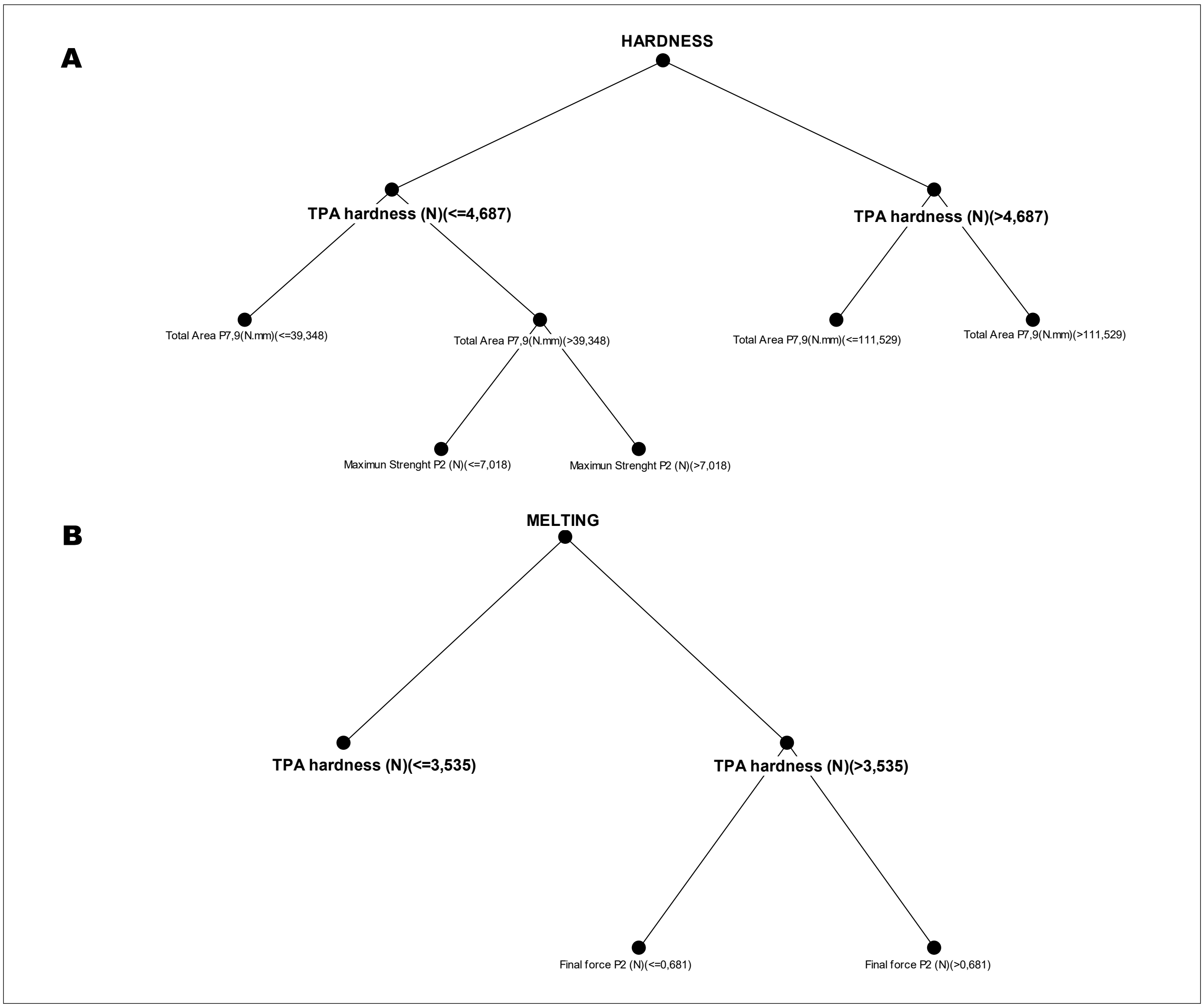

Figure 2. Tree regression analysis for Hardness (A) and Melting (B) sensory attributes in peach and nectarine varieties during postharvest.

al., 2013b). Recent studies indicate that texture is a fundamental attribute of quality, even if most studies have been limited to using penetration tests to determine it (Cano-Salazar et al., 2013b, Delgado et al., 2013). These studies have measured only 'firmness' through penetration with 7.9-mm plungers (Infante et al., 2008), and although this test is useful in determining the state of ripeness, it is not adequate for determining the texture or quality. In fact, two fruit may have similar firmness levels, but completely different textures (Redgwell and Fischer, 2002; Crisosto, 1994). This observation is also demonstrated in varieties belonging to the same textural group, for example, when 'Andes nec-1' and 'Andes nec-3' – both *MF*, and having similar firmness (Table 3) – show very different textures (Figure 1). For this reason, 'TPA hardness' is a more relevant measure than the penetration test with the 7.9-mm plunger because such analysis is based on imitating the jaw movements (Bourne, 2002), and because it has presented high correlations with sensory assessments with different foods (Rosenthal, 2010), including fresh fruits (Guine et al., 2011; Cho et al., 2010).

Both the perception of sound produced when biting and eating a fresh fruit and the sound produced when it is penetrated with a probe are also important in this work. Initially, it would seem that in terms of its texture, peach has an uncomplicated flesh, as it mainly melts as it matures and shows almost no fracture phenomena, both of which are more associated with the descriptors 'crunchiness' and 'crispness', which are characteristic of other fruit species, e.g., apple (Harker et al., 2002, 2010; Costa et al., 2012) or nuts (Civille et al., 2010; Contador et al., 2015). However, it should be noted that although the phenomena of fracture, measured as sound emissions showing low entity in peach, there are new peach varieties on the market for which these phenomena would be relevant, making attributes like 'crispness' and 'crunchiness' more important, thus creating the need to explore rheological tests that address this topic in peach. This is the case, for example, of the phenotypes known as 'stony hard', corresponding to a mutant peach that does not produce ethylene, and with flesh that is similar to apple (Haji et al., 2005). There are also some recently described peaches that have 'slow melting flesh', which, therefore, maintain crispy and firm flesh over a longer postharvest period (Ghiani et al., 2011b, 2011a). Other researchers have related the number of acoustic events with sensory perception, proving the effectiveness of the acoustic test as a predictive tool of texture (Salvador et al., 2009; Costa et al., 2011). In peach, only two works report the evaluation of flesh 'crunchiness' in different varieties (Cano-Salazar et al., 2013b; Arana et al., 2007), but there are no reports on the relationship between that descriptor and a known instrumental parameter. The results of this study highlight the importance of new rheological tests that delve into the development and perception of texture in peach and nectarine.

Conclusions

In order to define and study the texture features of peach and nectarine, it is first necessary to define the sensory attributes that characterize them, to explore the most appropriate instruments for measuring them, and, finally, to establish the main relationships between instrumental and sensory analysis. The 'hardness' and 'melting' sensory descriptors showed higher weights in the analysis, and, therefore, they are the best choice for classifying perceived sensory texture. In addition, these descriptors do not differentiate varieties the same way as the traditional classification *MF*/*MFN*, thus highlighting the complexity of the sensory perception of texture in this type of fruit. Although the puncture test with the 7.9-mm plunger is the instrument test used most often in peach, this research shows the variable 'TPA hardness' as being more closely related to the sensory attributes of 'hardness' and 'melting'. The determination of an instrumental variable's critical value allows us to classify and describe peach from a sensory point of view, showing that this instrumental parameter is a valuable one that should be explored further, for example, with a greater number of varieties. While the acoustic emission technique was not as powerful as the TPA test, we did find an important relationship with the sensory attribute of 'crunchiness'. This demonstrates the feasibility of including both instrument tests and sensory acoustic analysis in future peach and nectarine texture studies.

Acknowledgments

This work was made possible by funding provided to FONDECYT Project #1130198, as well as funding provided by CONICYT through the scholarship 'Doctorado Nacional 2010', awarded to LC (Doctorado en Ciencias Silvoagropecuarias y Veterinarias, Universidad de Chile).

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Received: Apr. 8, 2016
Accepted: Jul. 5, 2016

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