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Water spectral pattern as a marker for studying apple sensory texture

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Abstract: Aquaphotomics is a scientific discipline which investigates the water-light interactions in biological systems by using NIR spectroscopy and multivariate analysis to relate water absorption patterns to bio-functionalities. This work aimed at evaluating the feasibility of Aquaphotomics to study apple fruit sensory texture. 'Braeburn', 'Gala' and 'Kanzi®' apples were analyzed by a MicroNIR spectrometer and for mechanical, structural and texture sensory characteristics. Cluster analysis on sensory texture attributes showed four different profiles for each cultivar having different water spectral patterns (WASP). On average, the WASP of mealy apples showed the highest absorbance values at 1364, 1372 and 1382 nm and the lowest in the 1438-1492 nm range suggesting a preponderance of water structures with weak-hydrogen bonds; the opposite was found in crispy and juicy apples indicating the presence of more organized water structures with medium-strong hydrogen bonds. This WASP difference could be due to a different softening rate: apples clustered as firm/crispy/juicy had the highest firmness and the lowest intercellular spaces, while mealy apples had low firmness and high intercellular spaces indicating a more advanced softening. The chemical changes due to the pectin hydrolyzation could affect the water structures. The Aquaphotomics approach could be a useful tool for studying the sensory texture of fruits as water structures actually change in apples with different textural characteristics whatever the cultivars.

1. Introduction

The preferences of apple consumers are generally based on a combination of texture and flavor (Harker *et al.*, 2003). Textural properties can be considered as the main factors responsible for fruit freshness, having crunchiness and juiciness a positive effect and mealiness a negative effect on consumer choice (Péneau *et al.*, 2006). Texture consists of a number of different properties perceived by human senses and depends on mechanical and structural characteristics of the fruit pulp. Texture changes with fruit softening due to degradation and modifications of the cell wall and middle lamella structures (Gwanpua *et al.*, 2014). During softening, pectins undergo extensive structural modifications so that cell wall polymers are less bound together and become highly hydrated (Goulao and Oliveira, 2008). The hydrolysis of pectin requires water as substrate and occurs in the intercellular water (De Smedt *et al.*, 2002). All these changes influence the organization of water molecules (free water, dimers, trimers, solution shells) in the fruit tissue depending on the strength of hydrogen bonding, and produce changes in the water absorbance pattern. Thus, when an aqueous system, such as apple fruit, is measured by near infrared (NIR) spectroscopy, the light absorbance reflects the water vibrations and the contribution (concentration and structure) of the other molecules interacting with water. Tsenkova (2009) proposed the Aquaphotomics approach to relate water absorption patterns to bio-functionalities. In a series of experiments, it was found that Vis-NIR spectra acquired in living systems under various perturbations (temperature, ion concentrations, oxidative stress, illumination, disease, damage, etc) are characterized by twelve common water bands repeatedly occurring in different combinations in the spectral regression or classification models predicting the investigated perturbations. These water absorption bands (6-20 nm width, each) called Water Matrix Coordinates (WAMACs) corresponded to 1344 (C1), 1364 (C2), 1372 (C3), 1382 (C4), 1398 (C5), 1410 (C6), 1438 (C7), 1444 (C8), 1464 (C9), 1474 (C10), 1492 (C11) and 1518 (C12) nm (Tsenkova, 2009). The variation of WAMACs defines the water spectral pattern (WASP) which can be visualized by star-charts called 'aquagrams' (Tsenkova, 2010). Changes in water absorbance pattern have been used as biomarkers to monitor water quality (Kovacs *et al.*, 2016), to reveal the presence of bioactive compounds from propolis in smart packaging materials (Barzaghi *et al.*, 2017), to detect honey adulteration with high fructose corn syrup (Bázár *et al.*, 2016), to distinguish the effects of various coatings on the quality of different types of cheese and of winter melon (Cattaneo *et al.*, 2016). Aquaphotomics has also been shown to be a useful tool for discriminating fresh and stored apples and for distinguishing apples stored under different atmospheres. Differences between fresh and stored apples involved C5, C7, C9 and C12 water matrix coordinates, while differences between apples stored in normal and in controlled atmosphere activate the C8 and C10 WAMACs (Barzaghi *et al.*, 2014). Significant relationships were found between Vis/NIR wavelengths and some sensory attributes of apples, involving water absorption bands. Absorption measured at 720, 1440, and 2338 nm was positively correlated and at 1940 nm negatively related to mealiness, while the opposite was found for crunchiness and chewiness. The absorbance in the 630-700 nm range and at 1940 nm was positively correlated with juiciness, while negative correlations were found at 940 and 1450 nm (Mehinagic *et al.*, 2004).

The aim of this work was to evaluate the feasibility of Aquaphotomics to study the texture sensory profiles of apple fruit belonging to three cultivars having different texture characteristics.

2. Materials and Methods

Fruit

'Braeburn' apples (808 fruits) picked in 2014, 'Gala' apples (270 fruits) picked in 2015 and 'Kanzi®' apples picked in 2014 (540 fruits) and in 2015 (270 fruits) at the experimental orchard of Laimburg (BZ) were stored for 4 ('Gala') or 6 months ('Braeburn' and 'Kanzi®') under controlled atmosphere ('Gala': 1% O_2, 1.5% CO_2; 'Braeburn': 1.8% O_2, 1.3% CO_2; 'Kanzi®': 1% O_2, 1.5% CO_2) and analyzed after 1 and 7 days of shelf life at 20°C ('Kanzi®'2014) or after 1, 7 and 14 days at 20°C ('Braeburn', 'Gala', and 'Kanzi®'2015).

All fruits of the experiment were individually measured by a MicroNIR spectrometer and individually analyzed for texture sensory properties (firmness, crispness, mealiness, juiciness), mechanical characteristics and Relative Internal Space Volume (RISV).

Sensory analysis

Sensory analysis was carried out separately per cultivar according to Eccher Zerbini *et al*. (1999) and Rizzolo *et al*. (2010). Each sensory session corresponded to a time of analysis, that was 9 sampling times for 'Braeburn', 'Gala' and 'Kanzi®'2015 apples and 6 sampling times for 'Kanzi®'2014 apples. As for 'Braeburn', 'Gala' and 'Kanzi®'2015 apples, in each session 9 fruits were presented to each panelist, while for 'Kanzi®'2014 apples 3 fruits per panelist were used. When 9 fruits were presented to each panelist, two sensory sessions were scheduled, the first with 5 fruits and the second with 4 fruits, at 10:30 and at 12:00 AM, respectively. A total of 810 ('Braeburn'), 540 ('Kanzi®' 2014) and 270 ('Gala', 'Kanzi®' 2015) apples were tested. The sensory tests were performed by a panel of ten short-term-trained judges from Research Centre for Engineering and Agro-Food Processing of the Council for agricultural research and economics (CREA-IT, Milano), who had participated in prior studies on sensory evaluation of apples, in a sensory lab equipped with six computerized individual booths under white artificial lighting at room temperature (20°C). The FIZZ-Network 2.47B (Biosystemes, Couternon, France) software was used for test implementation and results collection.

Fruits (one peeled slice/apple) were presented to the judges at most 1 h after the cut to avoid the browning process, coded with three-digit random numbers and placed on a white flat-bottomed dish in randomized order for each assessor. At the beginning of each session, a peeled slice of a fruit not included in the experimental plan was tasted to eliminate the first tasting effect. Drinking water was provided as a palate cleaner between samples. As the quality of consumer perception is influenced by extrinsic attributes (e.g., price, dimension, size, origin, ripening stage), none of these attributes were mentioned to the judges (Taiti *et al.*, 2017). Each sample was evaluated for the intensity of four sensory attributes related to fruit structure: firm, crispy, mealy and juicy. Evaluation was based on a form with a continuous open linear scale, consisting of 40 characters, each panelist rated the intensity of each attribute on the open linear scale anchored to 0 (no presence) and 120 (maximum intensity). Definitions of the sensory attributes are as follows: firm, the resistance to mastication perceived at the first and successive bites; crispy, the textural property perceived at the first bite when the fruit yields suddenly with a characteristic sound; mealy, the textural property consisting of the presence of lumps formed during mastication; juicy, the textural property giving the sensation of progressive increase in the free fluids in the oral cavity during mastication (Eccher Zerbini *et al.,* 1999).

Prior to statistical analyses, the rating scores of each attribute were standardized by panelist in order to remove the variability due to panelists using different parts of the scale according to Bianchi *et al.* (2009).

Mechanical properties and relative internal space volume

Individual fruit were analyzed for flesh firmness, compression and intercellular spaces (RISV). Flesh firmness and compression were measured on two opposite sides of each fruit (the blush side and the opposite one) in the equatorial region and data were averaged per fruit. Firmness was measured with an 11 mm diameter cylindrical plunger mounted on a TA-XT plus Texture Analyzer (Stable Micro Systems, Godalming, UK) at the cross-head speed of 3.33 mm/s to a depth of 8 mm.

Mechanical properties were also nondestructively assessed by a uniaxial compression test. In this test, each apple was compressed between two steel parallel plates to a fixed deformation of 1 mm at a speed of 25 mm/min on an Instron Universal Testing Machine and the modulus of deformability (Ed) was computed according to Eccher Zerbini (1981):

$$\frac{Ed}{1-\mu^2} = \frac{F}{(d_L/2)^{3/2}\, D^{1/2}}$$

where F is the force at 1 mm of compression (N), d_L is the total deformation (mm), D is the fruit diameter (mm); μ is the Poisson's ratio. As Poisson's ratio was not measured in this work, it was fixed at a value equal to 0.3 (Ahmadi *et al.*, 2016).

Relative Internal Space Volume (RISV) was computed according to RISV=100×[1−(d_f−d_j)] where d_f is the density of the fruit (i.e. ratio fruit mass to fruit volume under water), and d_j is the density of the fruit juice (Baumann and Henze, 1983).

NIR analysis

NIR spectra were acquired on each intact fruit in reflectance mode using a diode array spectrometer (MicroNIR 1700 VIAVI, Diessechem Instrument, Italy) over the 900 to 1670 nm range (50 scans, 128 reading points) on two opposite sides of each fruit (the blush side and the opposite one) in the equatorial region and data were averaged per fruit. The spectra were pretreated using multiplicative scatter correction (MSC) to remove scatter effects, followed by Savitsky-Golay second derivative (15 points, second-order polynomial) after converting the spectra from reflectance to absorbance (The Unscrambler Software, ver. 10.0.1, CAMO Process AS, Norway). Aquagrams were built using the 12 characteristic water absorption wavelengths (1344, 1364, 1372, 1382, 1398, 1410, 1438, 1444, 1464, 1474, 1492 and 1518 nm) within each cluster sensory profile of each cultivar (see *Sensory analysis* paragraph). The values of the aquagrams (*Aq*) were obtained according to:

$$Aq_\lambda = \frac{A_\lambda - \mu_\lambda}{\sigma_\lambda}$$

where A_λ is absorbance, μ_λ is the mean value of all spectra and σ_λ is the standard deviation of all spectra at wavelength λ (Cattaneo *et al*., 2016). Aquagrams were calculated using MS Excel 2010 (Microsoft, USA). The aquagram displays normalized absorbance values from different sample groups at several water bands on the axes originating from the center of the graph. Absorbance values at the WAMACs were placed on the respective radial axes.

Statistical analysis

RISV, mechanical and texture sensory data were submitted to analysis of variance (ANOVA) considering apple cultivar as factor and means were compared by Bonferroni's test at $P \leq 0.05$.

Data of texture sensory attributes were also analyzed by using an agglomerative hierarchical clustering of observations. Ward's clustering method and squared Euclidean distance were applied to create four data sets having distinctive sensory texture profiles. To form the clusters, the procedure begins with each observation in a separate group and then combines the two observations which are closer together to form a new group. After re-computing the distance between groups, the two groups then closest together are combined. This process is repeated until only the *n* fixed groups remained. As cultivars had different texture characteristics, Cluster Analysis on sensory texture attributes was carried out within each cultivar and year. Data of firmness, compression and RISV were submitted to ANOVA considering sensory cluster as factor and means were compared by Bonferroni's test at $P \leq 0.05$.

All the statistical analyses were performed using Statgraphics version 7 (Manugistic Inc., Rockville, MD, USA) software package.

3. Results

Mechanical and sensory analyses showed that the three cultivars had different texture characteristics (Tables 1 and 2). 'Braeburn' apples had very low firmness, intermediate RISV and the highest Ed, along with the lowest scores for firm, juicy and crispy and the highest scores for mealy. 'Gala' apples had the highest RISV, the lowest Ed and firmness, showing intermediate scores for juicy, crispy and mealy . 'Kanzi®' fruit had the highest firmness, the lowest RISV, intermediate Ed and were perceived as the most firm, juicy and crispy and the least mealy; Kanzi®'2015 showed lower firmness and Ed, higher RISV and higher scores for mealy than 'Kanzi®'2014.

Table 1 - Mechanical properties and intercellular spaces (RISV) of 'Braeburn', 'Gala' and ' Kanzi®' apples

Cultivar	Firmness (N)	Ed (N/mm^2)	RISV (%)
'Braeburn'	56.2 bc	10.5 a	17.6 b
'Gala'	55.2 c	6.5 d	19.6 a
'Kanzi®'2014	61.5 a	9.8 b	15.0 d
'Kanzi®'2015	57.6 b	7.5 c	15.9 c

Means in the same column followed by different letters are statistically different at P≤0.05 (Bonferroni's test).

Table 2 - Mean scores of texture sensory attributes of 'Braeburn', 'Gala' and 'Kanzi®' apples

Cultivar	Firm	Juicy	Mealy	Crispy
'Braeburn'	47.0 b	40.9 c	46.2 a	33.3 c
'Gala'	55.0 a	50.0 b	29.0 b	40.0 b
'Kanzi®'2014	57.2 a	62.0 a	18.1 d	53.4 a
'Kanzi®'2015	59.5 a	59.4 a	24.5 c	51.5 a

Means in the same column followed by different letters are statistically different at P≤0.05 (Bonferroni's test).

From an exploratory analysis carried out by dividing the standard score of each texture sensory attribute into five arbitrary classes according to: very low (<20), low (21-39), medium (40-59), high (60-80) and very high (>80) intensity of the attribute, and by pairing the classes of all attributes for every fruit, it was found that the minimum number of combinations of intensity classes between attributes was four. So clustering analysis was applied with the aim of creating the four data sets having distinctive texture sensory profiles, according to the descriptions and centroid values reported in Table 3. Profile W1 corresponded to a very firm, juicy and crispy texture for 'Kanzi®' apples, and to a very firm/juicy texture with a medium crispness for 'Braeburn' and 'Gala'. Profile W2 of 'Kanzi®' apples was very similar to profile W1 of 'Braeburn' and 'Gala' apples, while that of 'Gala' and 'Braeburn' was characterized by a medium juicy and crispy texture, with low scores for mealiness in 'Braeburn' fruit. Profile W3 of 'Kanzi®' apples showed a soft (2014) or a medium texture (2015) without mealiness, while that of 'Gala' and 'Braeburn' was quite firm with low ('Gala') or medium scores ('Braeburn') for mealiness. Profile W4 was characterized by a soft, dry and mealy texture with high scores for mealiness in 'Braeburn' and 'Kanzi®'2014. Considering the cluster distribution in relation to storage time and shelf life period, profile W1 was found in 65% of fruit at harvest and at stor-

Table 3 - Texture sensory profiles of the four clusters within each cultivar

Cluster number and texture sensory profile	firm	juicy	mealy	crispy	% obs
'Braeburn'					
W1 - very firm/juicy, medium crispy, not mealy	66.0	59.8	21.2	52.3	26.7
W2 - medium firm/juicy, quite crispy/mealy	52.4	40.7	35.4	37.3	23.9
W3 - quite firm/juicy/crispy, mealy	38.5	36.6	55.5	24.1	29.0
W4 - very soft, not juicy, not crispy, very mealy	28.0	22.4	78.2	16.9	20.4
'Gala'					
W1 - very firm/juicy, medium crispy, not mealy	75.5	67.9	19.2	58.6	31.9
W2 - very firm, medium juicy/crispy, not mealy	65.1	46.4	24.5	45.0	20.0
W3 - medium firm/juicy, quite crispy/mealy	43.4	44.4	32.5	30.6	30.4
W4 - very soft, not juicy, not crispy, mealy	26.7	31.6	45.7	17.1	17.8
'Kanzi®'2014					
W1 - very firm/juicy/crispy, not mealy	75.5	85.3	7.0	82.7	32.3
W2 - very firm/juicy, medium crispy, not mealy	61.6	64.5	17.0	54.7	34.8
W3 - low firm/juicy/crispy, not mealy	34.1	35.9	16.8	24.1	25.1
W4 - very soft, not juicy, not crispy, very mealy	35.5	37.8	73.8	20.8	7.8
'Kanzi®'2015					
W1 - very firm/juicy/crispy, not mealy	80.2	78.5	15.2	74.2	23.3
W2 - very firm/juicy, medium crispy, not mealy	67.2	61.7	20.0	55.6	36.7
W3 - medium firm/juicy/crispy, not mealy	51.9	48.9	25.7	41.7	20.0
W4 - very soft, not juicy, not crispy, mealy	28.8	43.4	42.3	27.5	20.0

For each cluster are reported: the description of the texture sensory profile, the values of centroids for each descriptor and the percentage of observations (% obs) grouped in the cluster.

age removal in 'Braeburn' and in 'Gala', and in 84% of 'Kanzi®' apples; profile W2 of the three cultivars and profile W3 of 'Kanzi®'2014 were equally distributed among the storage times and the days of shelf life; profile W3 was found in 75% of 'Kanzi®'2015, 'Braeburn' and 'Gala' apples kept at 20°C; profile W4 was characteristic of fruit held for 14 days at 20°C and was found in 75% of 'Kanzi®'2015, 80% of 'Gala' and 90% of 'Braeburn' apples. Each cluster showed different mechanical properties and intercellular spaces (Fig. 1). Firmness significantly decreased from W1 to W2, W3 and W4 profiles in 'Braeburn' and in 'Kanzi®' 2014 and 2015 apples, while in 'Gala' had the same values in W1 and W2 profiles and decreased in W3 and W4, where showed the lowest values. RISV significantly increased from W1 to W2, W3 and W4 profiles in Braeburn and in 'Kanzi®' 2015, while in 'Gala' had the same values in W1 and W2 and increased in W4; in 'Kanzi®' 2014, RISV showed the lowest values in W1 and the highest in W3 and W4. Ed distinguished only profile W1 from the other ones, except for 'Kanzi®'2014 (Fig. 1).

NIR spectra of the three cultivars (Fig. 2) show high variability in the 1344-1518 nm range corresponding to the first water overtone considered in the aquaphotomics approach.

Aquagrams showed a different water organization according to the different texture sensory profiles and to the cultivars (Fig. 3). 'Braeburn' apples

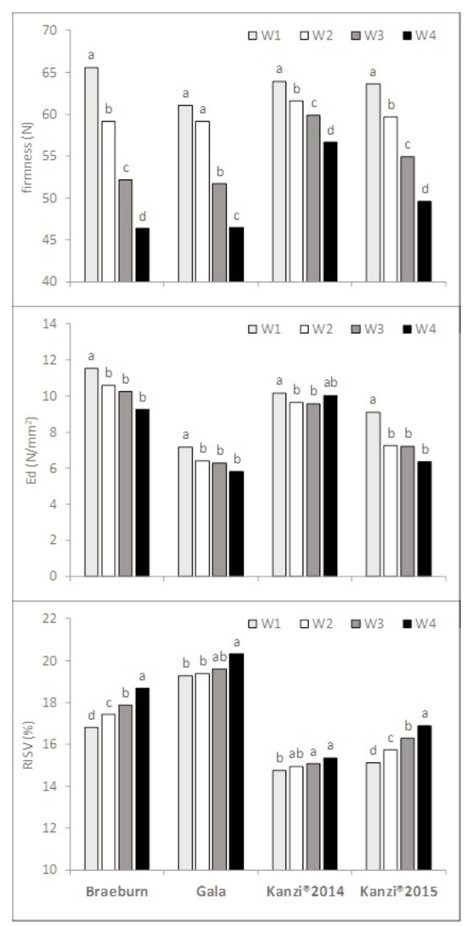

Fig. 1 - Mechanical properties and intercellular spaces of 'Braeburn', 'Gala' and 'Kanzi®' apples according to the texture sensory profiles described in Table 3.

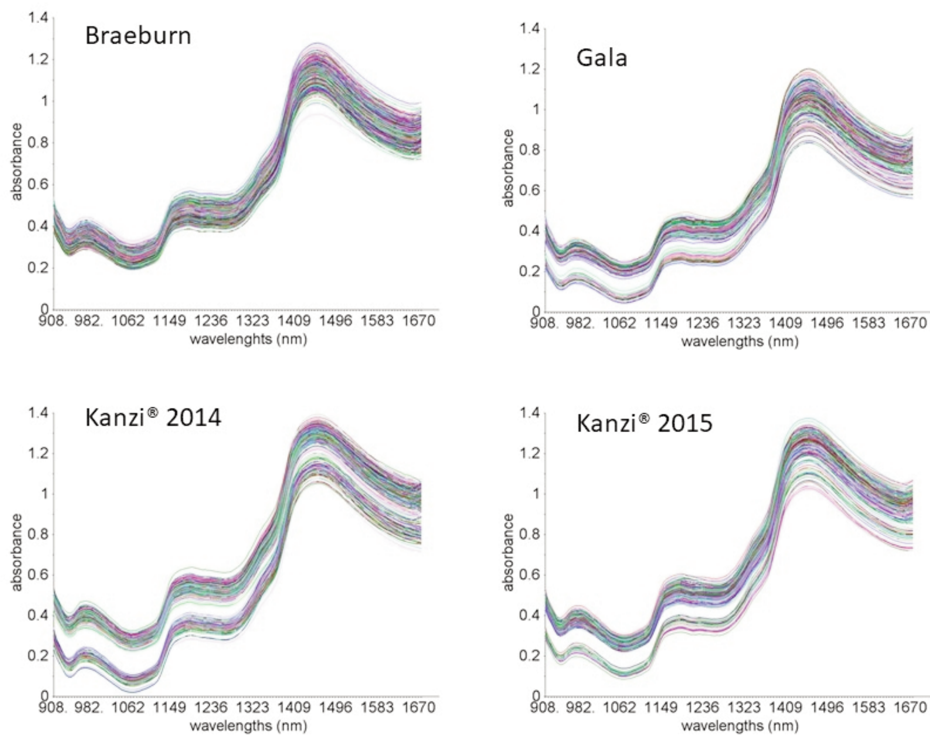

Fig. 2 - Raw NIR spectra of 'Braeburn', 'Gala' and 'Kanzi®' apples.

showed the greatest changes in the water absorbance pattern (WASP) according to the different texture profiles, while 'Kanzi®'2014 the lowest ones. Mealy apples belonging to 'Braeburn' W3 and W4, 'Gala' W4 and 'Kanzi®'2015 W4 profiles were associated to very high values in the 1364-1382 nm range and to very low values in the 1410-1518 nm range, while mealy apples belonging to 'Kanzi®'2014 W4 profile showed a balanced distribution of water structures with highest absorbance at 1344 nm and

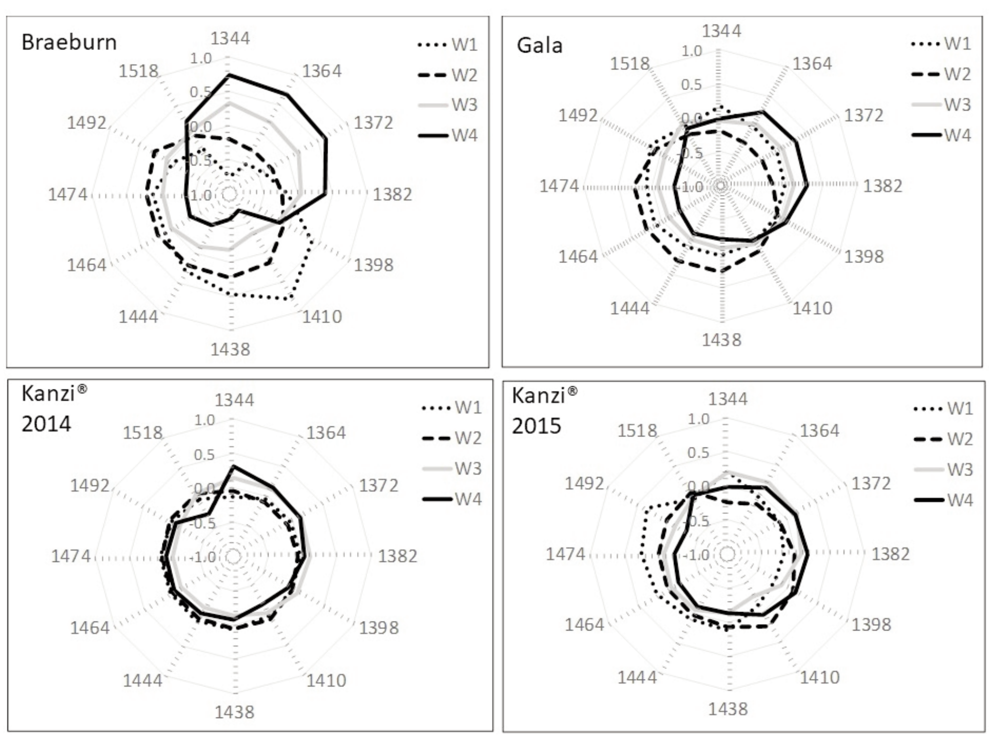

Fig. 3 - Aquagrams of 'Braeburn', 'Gala' and 'Kanzi®' apples according to the texture sensory profiles described in Table 3.

lowest absorbance values at 1518 nm. In low mealy apples ('Braeburn' W2, 'Gala' W3) the absorbance decreased at 1344-1382 nm while increased at 1410-1492 nm comparing to mealy apples of the same cultivars. In contrast, the WASP of firm, juicy and crispy sensory profiles ('Kanzi®'2014 and 2015 W1 and W2, 'Gala' W1) were characterized by a regular distribution of the different water structures with lower absorbance values at 1344-1382 nm and higher absorbance values at 1410-1492 nm comparing to the other textural profiles, while the WASP of 'Braeburn' W1 showed the lowest values at 1344 and at 1364 nm and the highest in 1398-1438 nm range.

4. Discussion and Conclusions

'Braeburn', 'Gala' and 'Kanzi®' apples showed different texture characteristics and were clustered according to the texture sensory attributes in four different profiles, ranging from a very firm/crispy/juicy texture to a mealy or a very mealy one. Each texture profile was characterized by specific mechanical and structural properties and showed different water spectral patterns. The differences in the textural profiles could be due to different softening rates occurring in apples. It is well known that fruit softening involves degradation and modifications of the cell wall and middle lamella structures, loss of turgor pressure, starch degradation and modification in the symplast/apoplast relations affecting the textural characteristics of the pulp: when cell-to-cell adhesion is weaker than the individual cell walls, cell separation occurs and the intact cells are responsible for the mealy texture, while when the individual cell walls are weaker than cell-to-cell adhesion, cell wall breakage occurs and the cellular content is released producing a juicy texture (Goulao and Oliveira, 2008; Harker *et al*., 2002; Vanoli *et al*., 2009). In this work it was found that apples clustered as very firm and firm had the highest firmness and the lowest RISV, whereas apples clustered as mealy showed low firmness and high RISV indicating a more advanced softening as reported by Ting *et al.* (2013), Vanoli *et al.* (2011) and Rizzolo *et al.* (2016). Moreover, mealy texture mainly belongs to apples held at 20°C for 7-14 days, that are fruits in which the softening process has already occurred, while the firm texture was typical of apples just picked or at storage removal, when softening was only at the beginning and apples had a rigid cellular structure with intact and adherent cell walls.

The chemical changes occurring with softening also affected the water structures with a similar pattern for the three cultivars and for the two seasons in 'Kanzi®' apples. The WASP of mealy apples were characterized by a preponderance of water structure with weak-hydrogen bonds (1364-1382 nm), whereas the WASP of crispy and juicy apples indicated the presence of more organized water structures (dimers, trimers) with medium-strong hydrogen bonds (1410-1492 nm). Peirs *et al*. (2005) found that when cell walls deteriorate, water molecules may relocate into the intercellular spaces, and this phenomenon changes the refractive indices at the cell walls. Møller *et al*. (2013) reported that apples treated with 1-MCP clearly differed in water state and dynamics compared to untreated fruit, as water in the cytoplasm and extra-cellular compartments and water in the vacuole were less restricted in treated apples, suggesting that a high firmness is associated with a low amount of vacuole water. The involvement of the first overtone of the OH vibration and pectin metabolism was observed by Boeriu *et al.* (1998) who found that the absorptions in the 1440-1445 nm range varied with the percentage of the degree of pectin esterification in green beans. Similarly, Sirisomboon *et al.* (2007) found a correlation between the absorption in the 1418-1464 nm range and the oxalate soluble pectin fractions in the AIS of intact pears and between the absorptions at 1368 and at 1452 nm and the total pectin content in pear juice. Barzaghi *et al.* (2014) observed that in stored apples the organization of water molecules involved more hydrogen-bonded water than in fresh fruit. This is contrary to our results; this difference could be due to the fact that in this work intact fruits were measured while Barzaghi *et al.* (2014) measured apple slices.

In conclusion, the aquaphotomics approach could be a useful tool and the water spectral pattern could be a marker for studying the texture sensory profiles in apple fruit as water structures actually change along with texture characteristics whatever the cultivar. Furthermore, being aquaphotomics based on NIR spectroscopy it could be possible to discriminate apples with different texture sensory properties in a nondestructive way. However, further studies are needed to better understand the relationships between the water spectral pattern and pectin metabolism and the different water structure organizations and the sensory profiles.

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