Development of a smartphone APP for assessment of chilling injuries in zucchini

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Abstract

Depending on their type and variety, horticultural products are injured by cold when this follows a period of exposure to a range of temperatures above their freezing point. These lesions are a result of tissues having been weakened rendering them unable to conduct normal metabolic processes. Cold stress produces physiological and biochemical alterations and cellular dysfunctions in response to cooling that negatively affect their quality and frequently result in products unsuitable for sale. Currently, evaluation of cold damage is usually carried out qualitatively by experts. This is not particularly accurate and, above all, depends on the observer and is therefore subjective. This study developed an application for a smartphone that automates this process and applied it to one of the most sensitive vegetables, zucchini or courgette. The results provide qualitative values and avoid all subjectivity. To demonstrate their validity, the results were compared to those of the standard method. They were also compared among distinct varieties allowing thresholds of hue and saturation settings to be adapted to these different varieties. These settings allow users to calculate damage in a more reliable manner depending on the specific variety analysed. This application allows for new perspectives in the field of evaluation of cold damage to fruits and vegetables in general and in particular zucchini.

Keywords: Chilling injury; Image processing; Smartphone; Zucchini; Courgette; Marrow

1. Introduction

Cold storage of fruits and vegetables is the primary tool to maintain the quality of harvested horticultural crops over time (Aghdam and Bodbodak, 2014, Belay et al., 2018). A low temperature reduces the rate of many metabolic processes responsible for the deterioration and loss of quality for vegetables and fruits (Bentini et al., 2009,Rao, 2015). However, low non-freezing storage temperatures can shorten post-harvest life and cause damage leading to loss of quality and preferred appearance. For certain horticultural products, being subject to low temperatures or a long period of cold storage implies that they are damaged owing to their susceptibility to a syndrome known as chilling injury (Sevillano et al., 2009).

Chilling injury refers to a syndrome that involves the expression of a series of physiological events that result in a series of characteristic and recognisable symptoms that are difficult to accurately assess. The exact type and extent of the syndrome varies with the species, cultivar, cold storage conditions, and other factors including cropping conditions; thus, it is difficult to identify a single definition that encompasses all damage and all products. Some of these changes occur at the cellular level such as changes in membrane structure, plasmolysis of cells, and increased rate of leakage (Fernández-Trujillo and Martínez, 2012, Kratsch and Wise, 2000). ther changes imply an altered metabolism such as abnormal increases in ethylene production, high levels of compounds resulting from anaerobic respiration, and other abnormal metabolites (Megías et al., 2015). As result of oxidative damage that is an early response to cold exposure, some of these result in an accumulation of reactive oxygen species as malonyl dialdehyde (Megías et al., 2016). Damage to the fruit surface is among the most notable changes affecting the external appearance of the fruit. This external damage includes pitting, large sunken areas, discolouration, translucent water-soaked spots, and water-soaked areas and deep wounds that can reach sub-epidermic tissues (Fernández-Trujillo and Martínez, 2012). These macroscopic changes are commonly used to assess the extent of damage. However, evaluation is completed visually, with consequent subjectivity that implies errors in the assessment. In nearly all post-harvest studies where cold damage must be assessed, it is evaluated by estimating the affected fruit surface subject to pitting (Simón et al., 2012; Fernández-Trujillo and Martínez, 2012; Megías et al., 2015). The study of cold damage is important to valorise the species of fruits and vegetables that are the most resistant to damage, which has commercial implications. Since it depends on the value of the product that this can be stored (alone or jointly with others) while maintaining a temperature of commitment between the ranges of all varieties stored (Table 1) and always above the freezing temperature. Moreover, knowledge of temperature limits allows transport costs to be saved since the most expensive and most cold-resistant products can share cooler storage temperature areas with other less valuable products. Tomatoes, for example, need storage temperatures lower than those of zucchini and tomatoes are more expensive than zucchinis. Thus joint transport of these two products, it is usually prioritised towards the temperature conditions of the tomato, but all have a requirement to arrive at their destination with optimal quality for sale and consumption.

Tabla 1. Chilling injury of fruits and vegetable with temperature (Gross, Wang, & Saltveit, 2009) (Cantwell, 2001).

Fruit and Vegetable	**Highest Freezing Temp. (°C)**	**Critical Temp. (°C)**	**Range for optimum storage conditions (°C)**	**Chilling injury**
Asparagus	−0.6	−0.5	0 to 2	Loss of sheen and glossiness and greying of tips are among the symptoms.
Avocados	−0.9 to −0.6 according to variety	4.5 to 13	5 to 12	Greyish-brown discolouration of flesh is an internal chilling injury. Irregular patches blackening the skin (<3 °C) are considered as external chilling injury.
Bananas	−0.8	11.5 to 13	13 to 14	Cultivar, maturity, condition of the fruit, temperature, and duration of exposure. Affect the chilling injury for <13 °C. Subepidermal discolouration seen as brown or black streaks in a longitudinal cut, a dull or greyish (smokey) hue on ripe fruit, and failure to ripen are among the symptoms.
Beans	−0.6 to −0.7 according to variety	7	5 to 7.5	The entire bean is discoloured (opaque) at <5 °C. Pitting on the surface and increased water loss are fewer common symptoms. At 5–7.5 °C, there is apparition of some discrete rusty brown spots.
Carrot	−1.4	0	0 to 1	No sensitivity of chilling and necessity of storage in as much possible of cold without freezing.
Cucumbers	−0.5	7	10 to 12.5	Duration of exposure, temperature, cultivar, growing conditions, and storage environment affect sensitivity. Chilling injury are pitting, water-soaked spots, decay.
Eggplant	−0.8	7	10 to 12	Storage for 6–8 days at 5 °C cause chilling injury. External symptoms such as surface pitting and scald take place.
Kiwifruit	−0.9	0	0	Symptoms such as apparition of a ring or zone of granular, water-soaked tissue in the outer pericarp at the style end of the fruit accompany chilling of kiwifruits at

Fruit and Vegetable	**Highest Freezing Temp. (°C)**	**Critical Temp. (°C)**	**Range for optimum storage conditions (°C)**	**Chilling injury**
				temperatures near 0 °C. Development of diffuse pitting and a dark and scald like appearance on the skin are other symptoms. Curing alleviated symptoms of chilling Injury.
Onion - Mature bulbs, dry - Green onions	 -0.8 -0.9		0	Freezing injury is caused at storage at <−4 °C.
Pepper - Green - Red	-0.7		 8 to 10 5 to 7	Surface pitting, water-soaked areas, decay, and discolouration of the seed cavity are among the symptoms.
Potatoes - early crop - late crop	−0.8	3	 10 to 15 4 to 8	At 1–2 °C, there is a possibility of internal mahogany browning. At 3–4 °C, there is typically an increase of reducing sugar levels that are not reversible with reconditioning. Mahogany browning, sweetening.
Tomatoes - Ripe - Mature - green	−0.5	 8–10 10–13	 19 to 21 13 to 21	Chilling injury including pitting, non-uniform ripening, and storage decays are visual symptoms. With ripeness appear water soaking and softening, decay, poor colour and Alternaria rot.
Zucchini		0	5 to 10	At 0 °C, development of water-soaked areas in sliced zucchini (chilling injury). At 5–10 °C to 50 °F, there is brown discolouration which increases with storage duration

Recently, there has been widespread transportation of fruits and vegetables throughout the world and with the increase in globalisation, fresh edible products of limited duration are being exported and consumers appreciate both the quality and the price.

The worldwide production of zucchini has recently increased. It increased by 9 % in 5 years from 2012 to 2017, stabilising in 2018 at 27 million tonnes. China is the world's largest producer, but China it does not export fresh zucchini, as northern China supplies the zucchini market in summer, while in winter supply comes from the south of the country. Spain and Italy produce 66 % of the zucchini in the whole of the European Union (EU), almost all produced in greenhouses (Márquez et al., 2011). For Spain, annual production is estimated at 530 million kg, the major part of which is for export using cold stores. The recommendations of temperature for zucchini range between 5 and 10 °C (Gross et al., 2009); however, developed tests showed that a temperature of 8

°C can cause considerable damage depending on the variety (Megías et al., 2016). Zucchini chilling injury assessment generally is performed using a visual ranking from 0 to 5, where the scale increases as the severity of the damage increases. Thus, zero denotes no pitting, whilst 5 (or the highest value in the scale) denotes a level of damage so severe that the fruit has already lost all its commercial value. In general, most studies use a scale for the damaged surface. This scale is typically applied to several fruits and an average is calculated, thus obtaining a chilling injury assessment for a lot (Crisosto, Valero, & Slaughter, 2007). Although this method is widespread, it presents serious limitations because it depends on the ability of the observer, and therefore it is subject to an inevitable bias.

Potential injury imposes a limit on minimum temperatures and duration of cold storage. While considerable information regarding the symptom development and prevention, most sensitive commodities, and influence of genetic conditions is available, a rapid, accurate, and objective assessment method is at present not available. Moreover, the available realised studies are also based on visual valuations of an expert and accordingly are also subjective and observer dependent.

The use of artificial visual systems has proved its validity as technical smart support for the detection of damage in fruits and vegetables post-harvest (Sun, Tang, He, Zou, & Xiong, 2016). Images are an important source of data and information in agriculture science as they provide technical support in different applications such as the inspection of quality in form, size (Clement et al., 2012, Clement et al., 2013), and state of maturity (Mhaski, Chopade, & Dale, 2015). Damage and defects occurring during the transportation and storage of agricultural products have been detected as well (Wang, Tian, Li, & Li, 2014). Some attempts have been made to avoid subjectivity in chilling injury assessment using image analysis, but thus far, these methods are either slow or too complex and also require equipment not readily available. Simón et al. (2012) showed that an image processing software for calculating the percentage of damage in surface of vegetable was a useful and precise tool for chilling injury assessment (WinDias - http://www.plant-image-analysis.org/software/windias), but it requires previously damaged areas to be marked on the image and this takes considerable time. At present, hyperspectral imaging has been widely researched in food and agricultural products as a tool for quality evaluation or defect and disease detection. El-Masry, Wang, and Vigneault (2009) successfully distinguished between chilling-injured apples and normal apples using hyperspectral imaging and neural networks, though this is a complex methodology. In addition, a relevant study demonstrated the potential of a hyperspectral imaging technique combined with feature selection methods and supervised classification algorithms for online detection of chilling injury in cucumbers (Cen, Lu, Zhu, & Mendoza, 2016).

The use of image processing on mobile devices has proven useful in many agricultural related applications (Aquino et al., 2017, Aquino et al., 2018, Cubero et al., 2018, Massah and Vakilian, 2019). However, a method that is inexpensive and readily implemented is needed for the problem under consideration here because to date classification has been completed only by an expert. Therefore, the objective of the work reported here was to develop an application embedded in a mobile Android smartphone to determine the extent of cold-chilling injuries in zucchini to limit the subjectivity of the method based on visual valuation.

2. Methodology

To automate the study of injuries on the surfaces of fruits and vegetables, it was necessary to undertake an image acquisition process, largely customised, through a charge-coupled device (CCD) sensor such as that typically installed in commercial smartphones. Figure 1 shows a block diagram of the methodology, and Fig. 2 shows this customised and optimised procedure to analyse a specific variety with images.

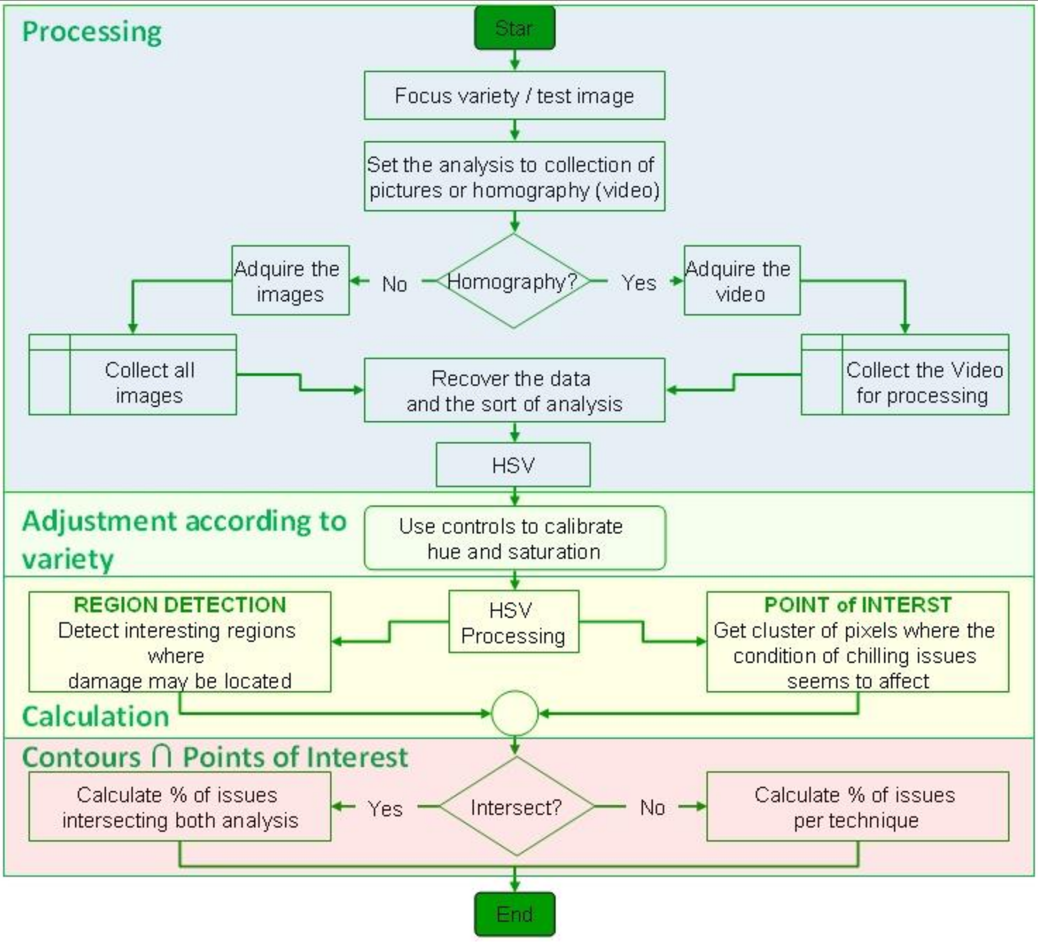

Fig. 1. Block diagram of the methodology to calculate damages.

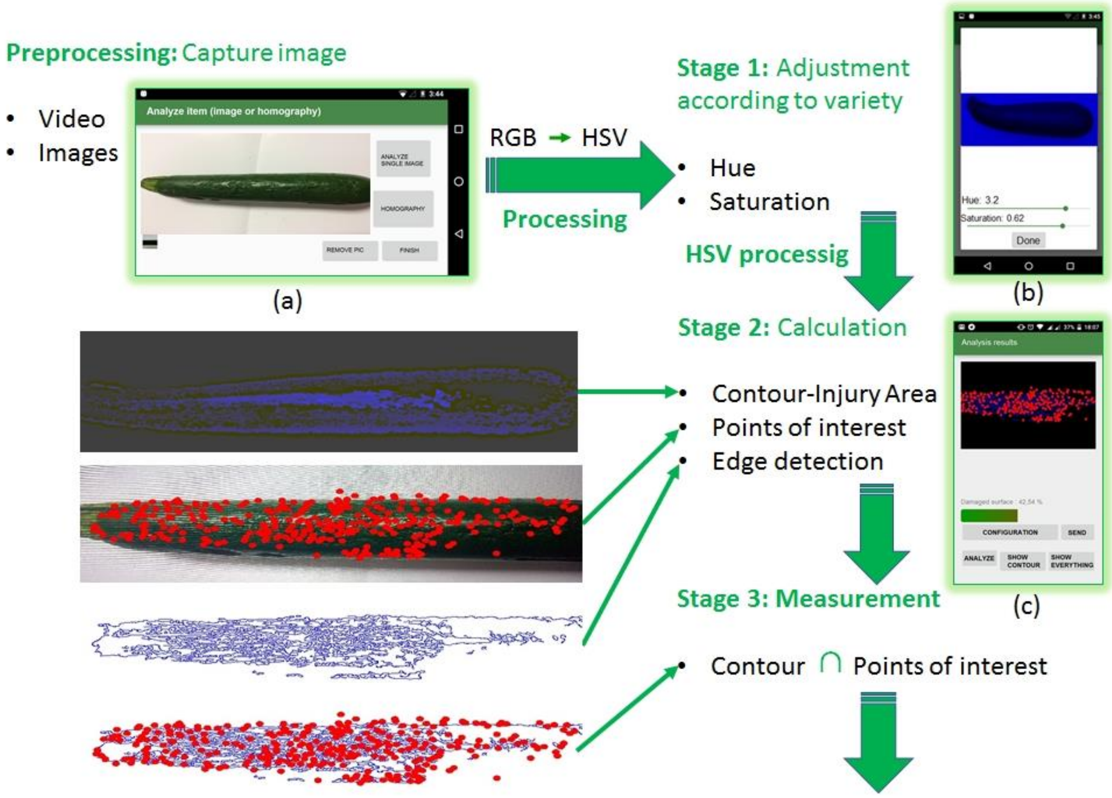

Fig. 2. Methodology for calculating chilling injuries.

The smartphone used in this work was a Samsung Galaxy S5 G900F. The built-in camera was a 16 MP (f/2.2, 31 mm, 1/2.6″, 1,12 μmm). When used to extract frames from the video capture, the resolution used was 720 p at 30 fps. The format of the stored images was PNG and the format of the video was mp4. Decoding the frame into a Java ByteBuffer with the Open GL command glReadPixels took less than 8 ms on the smartphone.

2.1. Stage 1: adjustment according to variety

Depending on the zucchini variety an adjustment of the hue and saturation parameters is needed (see Fig. 2). A threshold is selected to highlight the parts of the image that are required for the following analysis, that used for apple sorting (Mizushima & Lu, 2013) or in vegetation detection in herbaceous crops (Torres-Sánchez, López-Granados, & Peña, 2015). Fig. 2(b) shows the interface of the APP with an example of a selection of hue and saturation of the analysed samples. As the green hue depends on the specific variety of zucchini, this selection allows one to adapt the application to a specific variety. Once the threshold is obtained, the image is ready for analysis.

2.2. Stage 2: calculation

The calculation stage determines the area damaged by chilling injuries, see Fig. 2(c). This allows to calculate the percentage of injury directly. The analysis is completed by means of points of interest (grouping of pixels with potential injuries) and by detecting regions of damage.

2.2.1. Analysis of edges

This part of the computation is based on the Canny edge detection algorithm (McIlhagga, 2011) to define areas of interest that may point to damaged surfaces (Fig. 3). According to the Xin, Ke, and Xiaoguang (2012) efficient edge detection operations can be achieved by means of the following:

a. Noise reduction. Edge detection in images is susceptible to noise thus Gaussian filters are used.
b. Identifying the intensity gradient of the image. A filter with a Sobel kernel is applied to the image obtained from the previous stage.

c. Non-maximal suppression. Once the gradient of the magnitude and direction is obtained, an exploration of the whole image is necessary to eliminate non-desired pixels that do not correspond to the edge. The procedure is checked pixel-by-pixel to obtain a local maximum in its neighbourhood in the direction of the gradient. Following this process, a binary image with 'thin edges' is obtained.

d. Hysteresis thresholding. This step allows for the identification of pixels that corresponded to edges and those that do not.

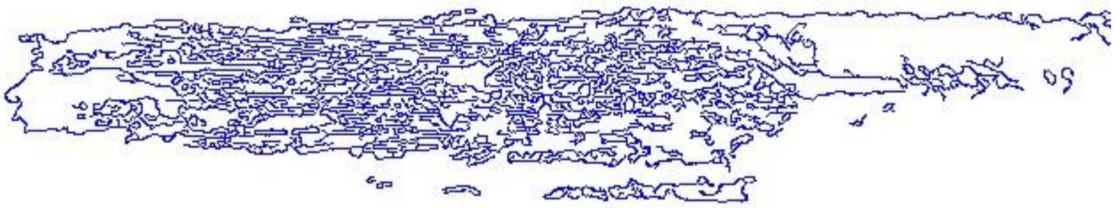

Fig. 3. Analysis of the region of interest on which the analysis of points of interest is later applied.

2.2.2. Points of interest analysis

The points of interest analysis is based on the Hessian Affine algorithm (Mikolajczyk & Schmid, 2004). The points marked in the image are visible to the user as shown in Fig. 4 (each spotted area corresponds to five pixels, a decision based on previous experience). With the resulting points, the percentage of the damaged surface is calculated. Figure 4 shows the points marked as of interest from the analysed surface of a zucchini. The points marked in red spots denote injury; the region detection described in the previous section avoids computation of possible spots that fall out of bounds.

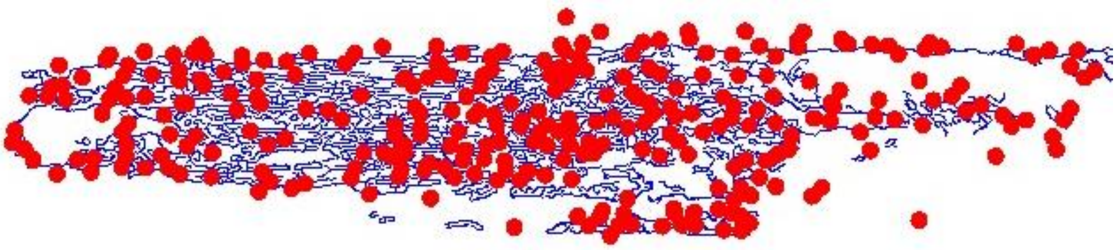

Fig. 4. Image obtained after calculating the points of interest and area of the example image.

2.2.3. Stage 3: measurement

Finally, a combined processing is applied where the previous analysis was now treated jointly to obtain a resulting output such as that shown in Fig. 4. The percentage of damage on a zucchini is calculated computing the number of pixels spotted in red and that are located inside the region of interest marked by the edge detector.

The analysis of the damages from which suffer the Zucchini is calculated for the total area as shown in Fig. 8. The damaged regions are localised using the edge detection algorithms for the processed image (to eliminate the background part which does not correspond to Zucchini). The image is analysed and the pixels with other than black colour are obtained knowing that the total pixels contained in the Zucchini are counted without including the obtained points. When the points of interest (red points in Fig. 7) are obtained, the 5 pixels of known radius (5) and area (PI × 52) form a circle for which the area can be calculated. When the regions injured by cold, a counting analysis of injuries per colour is applied within these regions (where red points appear). The total area obtained is calculated and the percentage of the area holding the circles. Fig. 5 shows a block diagram of the methodology to calculate the percentage of injuries as summary.

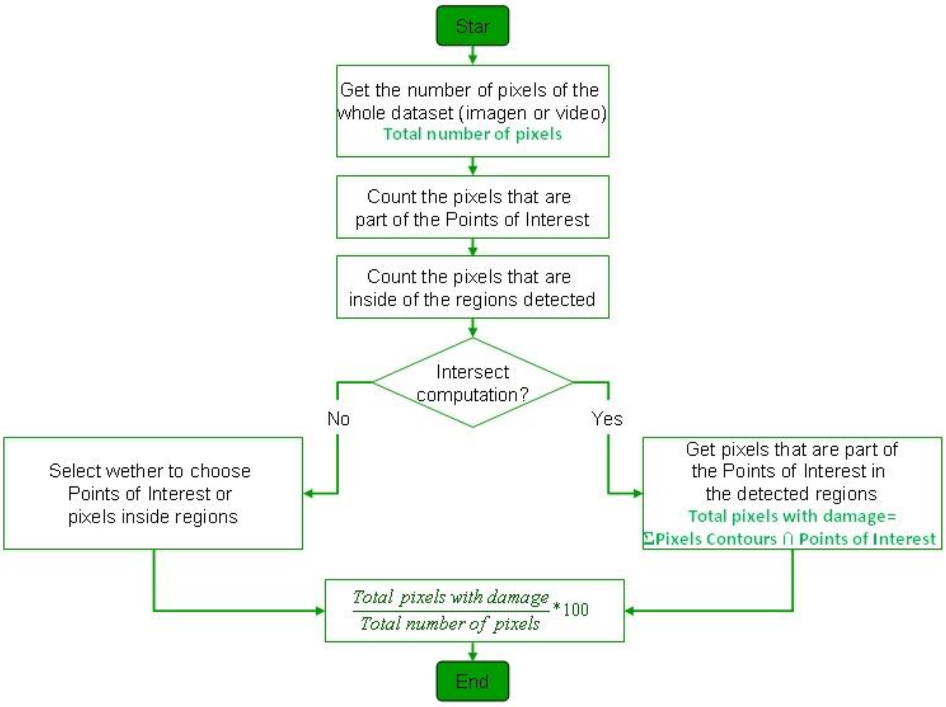

Fig. 5. Block diagram of the methodology to calculate the percentage of injuries.

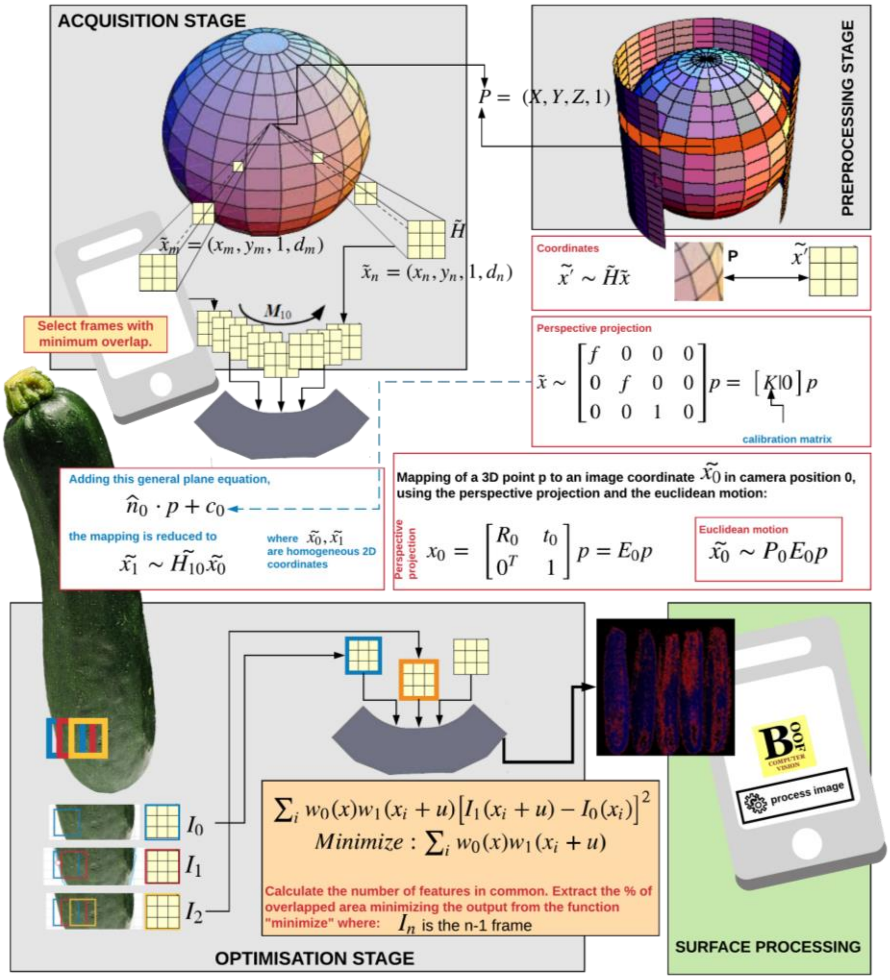

Fig. 6. Android APP architecture.

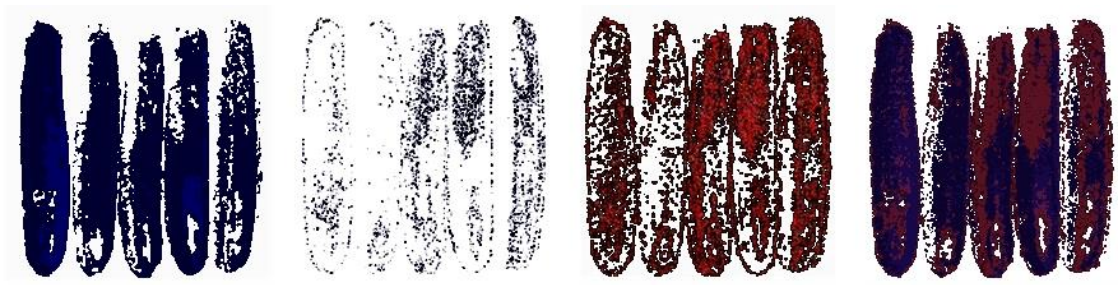

Fig. 7. Images of the reconstruction of the surface surrounding the zucchini.

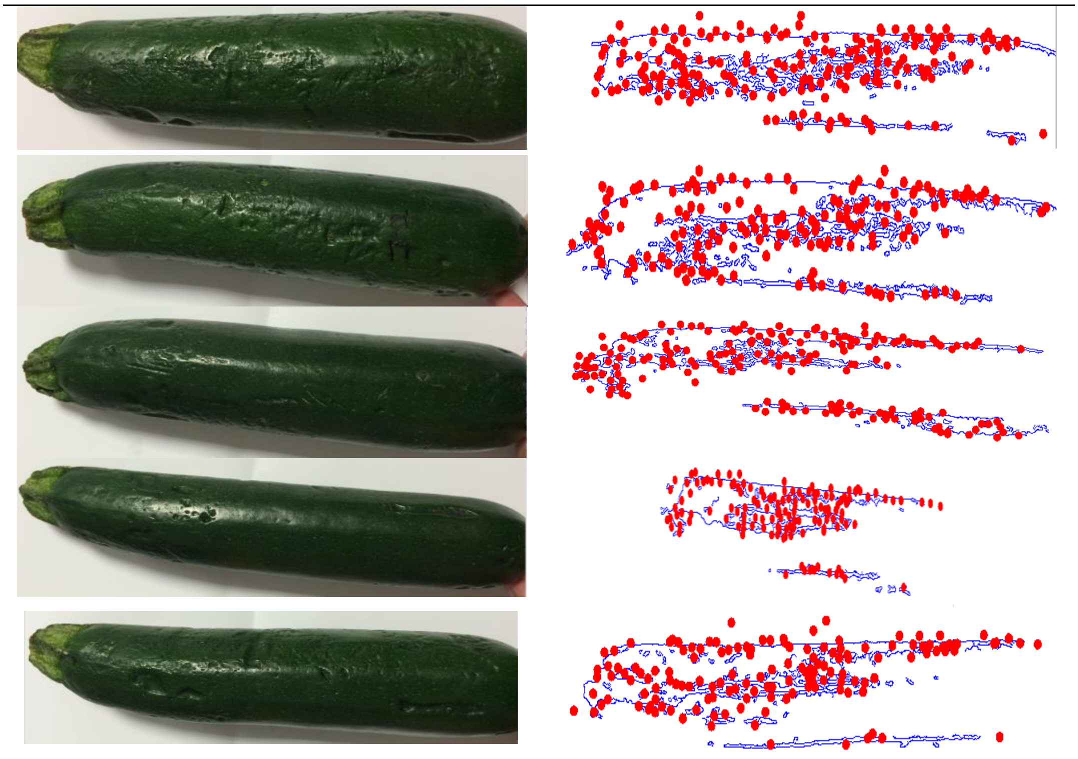

Fig. 8. Graphical result examples obtained from the APP (all the photos correspond to the same sample-Sinatra 1).

2.2.4. Homography

The computation of damage is completed using a set of stacked pictures or a sequence of frames (if the video function is selected to acquire the data). The application composes the homography or stitch and analyses it. Brown and Lowe (2007) described image stitching or homography as a problem that has been solved in different manners. Image stitching is defined as the task of combining overlapping images to form a single large image (Wang, Reimeier, & Wolter, 2016). Image stitching methods are divided mainly into two types; a direct method that uses image data and minimises pixel-to-pixel similarities or a feature-based method that attempts to match extracted features from the images. For the feature-based method, the overlapping parts can be automatically detected from the correlation of images. The next section describes how homography was efficiently implemented in the smartphone APP.

The transformation of the homography is based on the following equations:

$$X = \frac{ax + by + c}{gx + hy + 1} \qquad Y = \frac{dx + ey + f}{gx + hy + 1} \qquad (1)$$

Where X and Y are the coordinates to be calculated in the second system of references, given the coordinated x,y of the first system of references in function of 8 transformation parameters a, b, c, d, e, f, g, h. Moreover, having these 8 unknowns, 4 points are required on minimum for each system. Equation (1) is transformed to a matrix (Eq. (2)) allowing the calculation of all the transformation parameters:

$$\begin{vmatrix} x1\ y1\ 1\ 0\ 0\ 0 - x1X1 - y1X1 \\ x2\ y2\ 1\ 0\ 0\ 0 - x2X2 - y2X2 \\ x3\ y3\ 1\ 0\ 0\ 0 - x3X3 - y3X3 \\ x4\ y4\ 1\ 0\ 0\ 0 - x4X4 - y4X4 \\ 0\ 0\ 0\ x1\ y1\ 1 - x1X1 - y1X1 \\ 0\ 0\ 0\ x2\ y2\ 1 - x2X2 - y2X2 \\ 0\ 0\ 0\ x3\ y3\ 1 - x3X3 - y3X3 \\ 0\ 0\ 0\ x4\ y4\ 1 - x4X4 - y4X4 \end{vmatrix} \bullet \begin{vmatrix} a \\ b \\ c \\ d \\ e \\ f \\ g \\ h \end{vmatrix} = \begin{vmatrix} X1 \\ X2 \\ X3 \\ X4 \\ Y1 \\ Y2 \\ Y3 \\ Y4 \end{vmatrix} \qquad (8)$$

Where :

a: the fix scale factor in the direction X with Y scales without changes.

b: the factor of scale in direction X proportional to Y in distance from the origin.

c: the translation of the origen in the direction of X.

d: the scale factor in direction Y proporcionally to X in distance from the origin.

e: the fix scale factor in the direction Y with scale X without changes.

f: the translation of the origin in the direction of Y.

g: correponds to the proportional factors of scale X and Y in fucntion of X.

h: correponds to the proportional factors of scale X and Y in fucntion of Y.

Once calculated, these 8 parameters can be easily used to transform any point from the first system to the second one.

3. Android application architecture

Computation of damage is completed using a set of stacked pictures or a sequence of frames (again, if the function video is selected to acquire the data). Image stitching is defined as the task of combining overlapping images to form a single large image (Wang et al., 2016). Image stitching methods are divided mainly into two types; a direct method that uses image data and minimises pixel-to-pixel similarities or a feature-based method that attempts to match extracted features from the images. For the feature-based method, the overlapping parts can be automatically detected from the correlation of images. This section describes how the method was implemented in the smartphone application (APP) and is devoted to a complete description of the video acquisition step. A schematic diagram of the process whereby the smartphone obtains a stream of images from a CCD sensor is shown in Fig. 6. Images are saved one by one as the user operates the camera function. All the images are then linked using the well-known technique homography or image stitch (Szeliski, 2006) (Mikolajczyk & Schmid, 2002). The number of images depends on the speed of the rotation and the time of the exposure. The set of images linked using the stitch is the final PNG image used to calculate the damages. The images are moved to the HSV colour space with light independency.

The smartphone only applies a stitch to a pair of images with a minimum number of common points, so the overlapped parts are avoided. The interesting points of the images are tagged using the algorithm of Mikolajczyk and Schmid (2002). E.g. If two images have a lot of common key points mean that have a lot of surface in common, so the images will be discarded by the algorithm. In a sequence of PNG images, only when the common key points are reduced the images are selected by the algorithm, and then they will be linked using those key points as reference to "link" the surfaces. Therefore, the number of images is not considered by the APP. As Fig. 6 shows, each point of the surface of the zucchini can be observed from different perspectives as it is rotated (see the perspective projection matrix in Fig. 6 that shows how the coordinates can be interpolated). To solve the issue that the cameras used do not provide information regarding the depth coordinates of the pixels under study, equations had to be modified using planar scenes.

The processing, therefore, works on a given frame F0(x) and it is our aim to find where the F1(x) pixels of interest are located and calculate how closely related are these two frames. From this we can calculate the overlap area (as expressed in Fig. 6). This formula provides the overlapping between two images. In this case, instead of processing the whole group of images with the stitch procedure, the APP selects the two images that are related sharing the minimum overlap area.

Once the images that provide larger areas of overlap are discarded, the BoofCV library (http://boofcv.org/) was used, specifically its implementation to obtain the composed image (https://github.com/lessthanoptimal/BoofCV). The APP is used to compose the image (Fig. 6) and a render script was used (Guihot et al., 2012) using a high-performance library for Android to move computation to the GPU of the device. For the APP, the BoofCV code was modified to send the images to the GPU. Once the stitch is composed, the APP has the completed image and it can be processed. This surface is processed with the cited algorithms (border detection and points of interest) which calculate the number of pixels inside of damaged areas due to cold. Then the total number of pixels of the surface is computed and the damaged ratio is calculated. Figure 6 shows the image composition ready to be analysed for computing chilling injuries. As depth values cannot be sensed due to the nature of the camera used, the calibration matrix K is created using f as the focal distance, the z-buffer is ignored. In Fig. 2 can be seen the calibration matrix K where the z-buffer is ignored.

As shown in Fig. 7, there are different steps in the homography procedure. The first (on the left) is the pre-processed image to eliminate the background that is not zucchini, as the homography combines all the images including the background. The second consists of the analysis of region detections, and the third is colour characterisation. The final step is that upon which the analysis is applied (it combines all the techniques). Figure 8 shows the process of several partial views of the same zucchini and their partial analysis.

4. Data

The developed methodology was applied to four varieties of zucchini (*Sinatra*, *Atlantis*, Musa, and *Natura*) and for 16 to 20 samples of each according to the variety. Thus, 10 fruits are analysed after 7 day-period and another 10 fruits after 14 day-period. When 16 fruits in a variety are shown instead of 20, it is because 4 fruits were discarded in the 14-day period because they had such a high damage that they are not worth evaluating. These varieties are shown in Fig. 9. The analysis process was completed twice on the samples, after 7 d and after 14 d, but always while maintaining a temperature of 9 °C.

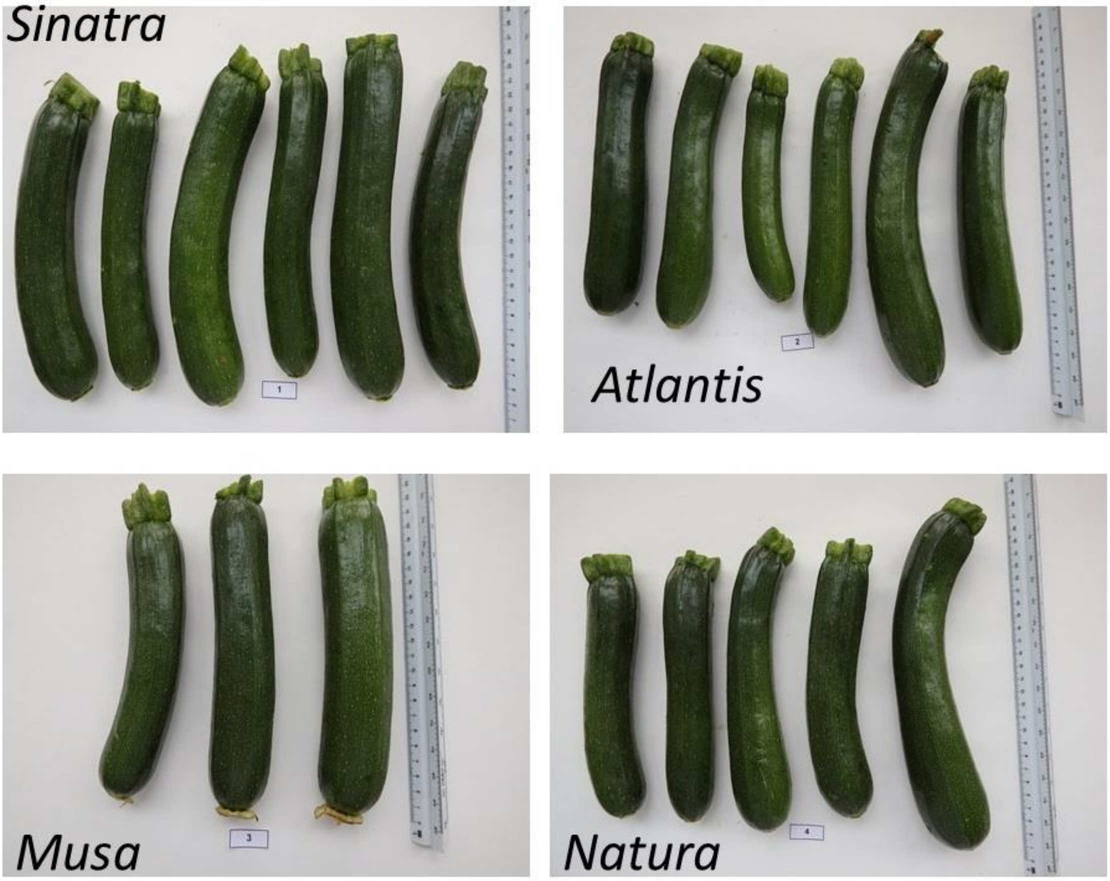

Fig. 9. Varieties of zucchini used.

5. Results and discussion

In general, most studies use a scale as described in Table 2, the standard method, where 0 denotes 0 % of the surface area is pitted or damaged and 5 denotes that more than 50 % of the surface is damaged. This scale is typically applied to several fruits and the average is calculated, thus, obtaining the chilling injury assessment of a lot (Crisosto et al., 2007).

Table 2. Scale for chilling injury evaluation in vegetables (standard method).

Scale	Classification	Surface damaged (%)
0	No pitting or damage	0
1	Very slight	1-5
2	Slight	6-15
3	Moderate	16-25
4	Severe	26-50
5	Very severe	> 50

Our samples were assessed using a visual revision of an expert (standard method, previously described) and the APP. Figure 10 shows both results at 7 d for the 40 samples. Figure 11 shows both results at 14 d for 29 samples (the others were lost, as the damage was too severe for evaluation). Table 3 presents only the differences in the classification of the samples according to the method used.

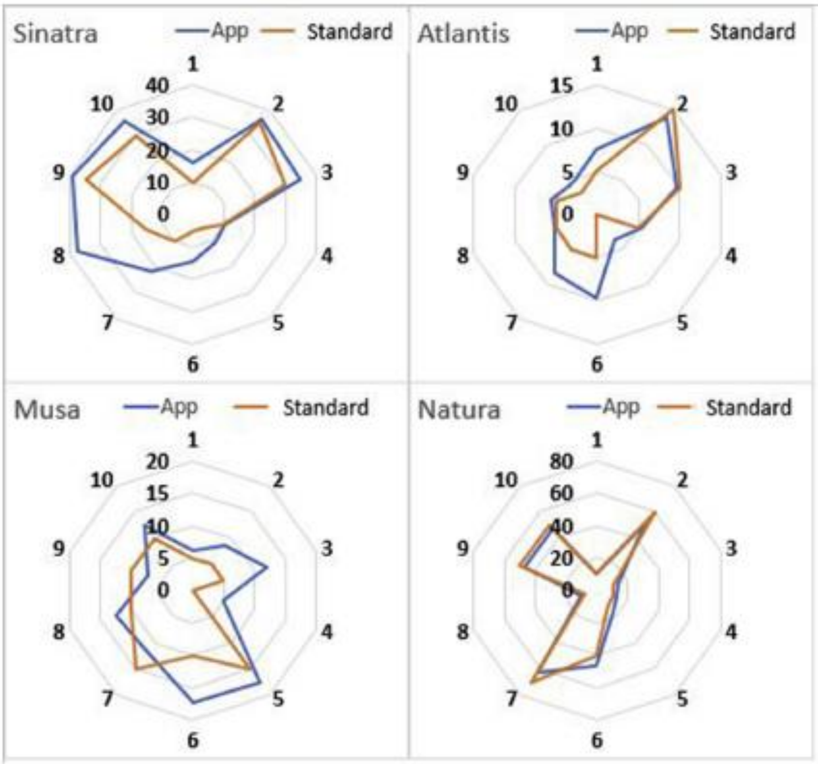

Fig. 10. Comparison between the APP and classical method of the injury percentage after 7 days of conservation in cold (% of chilling injury).

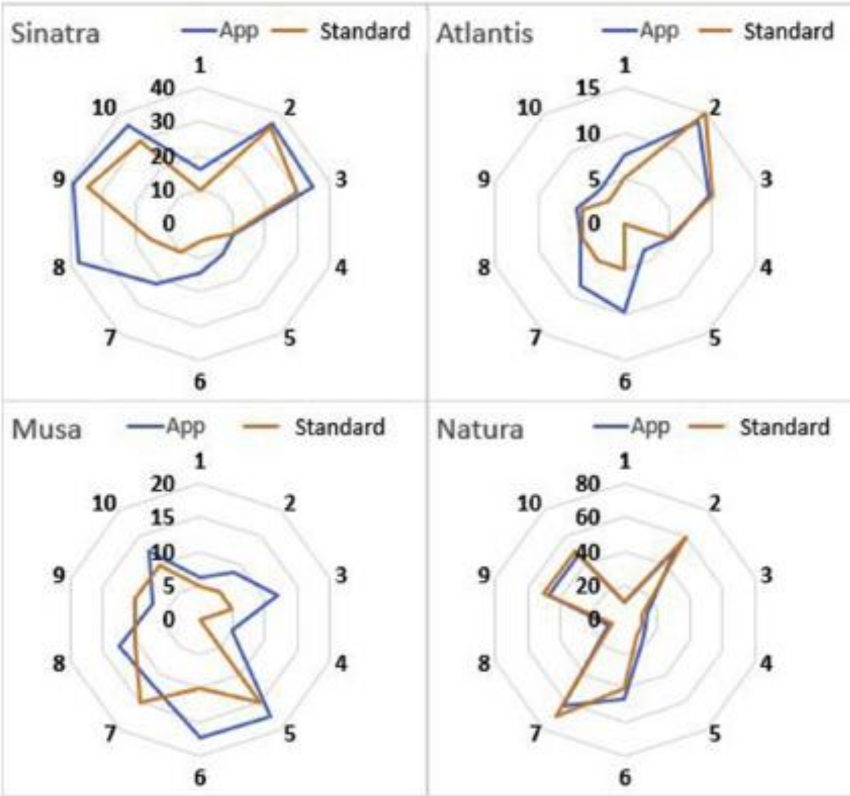

Fig. 11. Comparison between the APP and standard method of the injury percentage at 14 d of storage in cold (% of chilling injury).

Table 3. Results of the samples with differences in classification.

Sample	day	APP	Classification	Classical	Classification
Sinatra-1	7	15.78	Moderate	10	Slight
Sinatra-7	7	21.7	Moderate	10	Slight
Sinatra-8	7	37.41	Severe	15	Slight
Musa-5	7	17.41	Moderate	15	Slight
Musa-6	7	17.30	Moderate	10	Slight
Natura-2	7	50.91	Severe	60	Very Severe
Natura-5	7	16.13	Moderate	12	Slight
Sinatra-14	14	50.73	Very Severe	45	Severe
Atlantis-12	14	16.09	Moderate	15	Slight
Atlantis-13	14	20.93	Moderate	10	Slight
Atlantis-14	14	16.75	Moderate	10	Slight
Atlantis-15	14	48.54	Severe	50	Very Severe
Musa-15	14	56.30	Very Severe	50	Severe
Musa-16	14	18.43	Moderate	15	Slight
Natura-12	14	65.20	Very Severe	35	Severe
Natura-13	14	74.74	Very Severe	40	Severe

It can be seen that, in general, the standard qualitative method underestimates the percentage of injuries and has significant variability. However, the quantitative developed method values are not considerably different from those of the standard method, confirming its validity, with a major difference of 7% in one of the studied varieties (see Table 4). It is interesting to note that although the values are not that different, in six of the 40 cases there is a change to a category of a more severe state, and in one to a less severe state, following the classification of injuries by the rules presented in Table 2. For three of the varieties of zucchini, only when the injuries are severe is the classification maintained. In fact, for the classification presented in Table 2, it can be seen that the expert never classified as moderate any zucchini after 7 days of conservation. If the data are analysed by variety, it is observed that two varieties, Sinatra and Atlantis, would have significant changes in their results according to the APP or the standard method, Table 4. So, for an overall assessment of varieties, e.g. for genetic studies, the chilling injury of this two varieties assessed would turn out to be different.

Table 4. Summary of commercial evaluation of four varieties of zucchini at 9º C and stored up to 7 days: APP vs. classical method.

7 days	***Sinatra***		***Atlantis***		***Natura***		***Musa***	
	APP	Classical	APP	Classical	APP	Classical	APP	Classical
Mean	25.67	18.50	7.37	5.80	11.04	8.50	31.69	32.00
Difference	7.17		1.57		2.54		-0.31	
Classification	Severe	Moderate	Slight	Very slight	Slight	Slight	Severe	Severe
SD	12.02	12.48	3.12	4.05	4.33	4.74	20.60	24.42

When the evaluation of chilling injuries was completed after 14 d, as seen in Table 5 and Fig. 11, it was found that the APP in general provided results that were more severe than those of the standard method, as occurred in eight of the 29 evaluations, and only one of less severity, although with a very slight variation. As shown in the classification of Table 4, they do not affect the evaluation of commercial quality, the range of the category being so wide it is retained in all the cases.

Table 5. Summary of the commercial evaluation of four varieties of zucchini at 9º C and stored up to 14 days: APP vs classical method.

14 days	**Sinatra**		**Atlantis**		**Natura**		**Musa**	
	APP	Classical	APP	Classical	APP	Classical	APP	Classical
Mean	23.53	21.80	24.53	20.83	23.24	19.17	62.87	59.29
Difference	1.73		3.69		4.07		3.58	

Classification	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Severe	Severe
SD	22.68	23.97	14.27	16.25	18.13	16.56	12.24	18.35

Finally, to compare the results of the APP and the standard method, all samples were statistically analysed together, without considering variety. After 7 d, in the case of the samples observed using the APP, the risk to reject the null hypothesis H0 (the sample follows a normal distribution) while it is true is less than 0.58%, and for the standard method, the risk to reject the null hypothesis H0 while it is true is less than 0.09%. First, it was observed that neither the observations of the APP nor those of the standard method follow a normal distribution. Similar results were obtained at 14 d, and with all data together.

Moreover, the observations were analysed using the Wilcoxon signed-rank test to determine if they follow the same statistical distribution, because the normal distribution of samples cannot be assumed. As the computed p-value is less than the significance level $\alpha = 0.05$, one should reject the null hypothesis H0 (the two samples follow the same distribution) and accept the alternative hypothesis Ha (the distributions of the two samples are different). These same results were obtained for the results after 14 d, and for a joint analysis of all the data, after 7 and 14 d.

If the histograms of the samples are represented at 7 d as seen Fig. 12, it was observed that in general there is a higher concentration of data in the less severe values using the standard method. If the data are examined at 14 d, as can be seen in Fig. 13, the same pattern is observed. In short, the standard method has more data frequency at less severe values.

This, in our opinion, may be a sign of the observer's subjectivity using the standard method. For example, when observing zucchini with few cold injuries, it is possible that it tends to present a less damaged surface than actually exists, or that even as the expert knows that it is evaluating the same variety, it is possible to be influenced by the result of the previous evaluation, i.e. comparing the observation of that sample with the result of the previous sample without intending to do so.

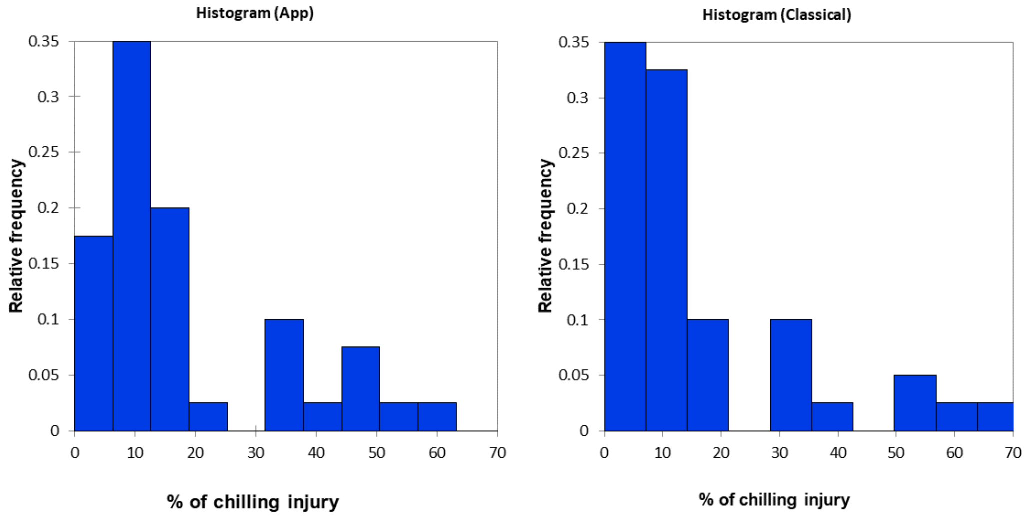

Fig. 12. Histograms of data observed using the APP and classical methods at 7 days.

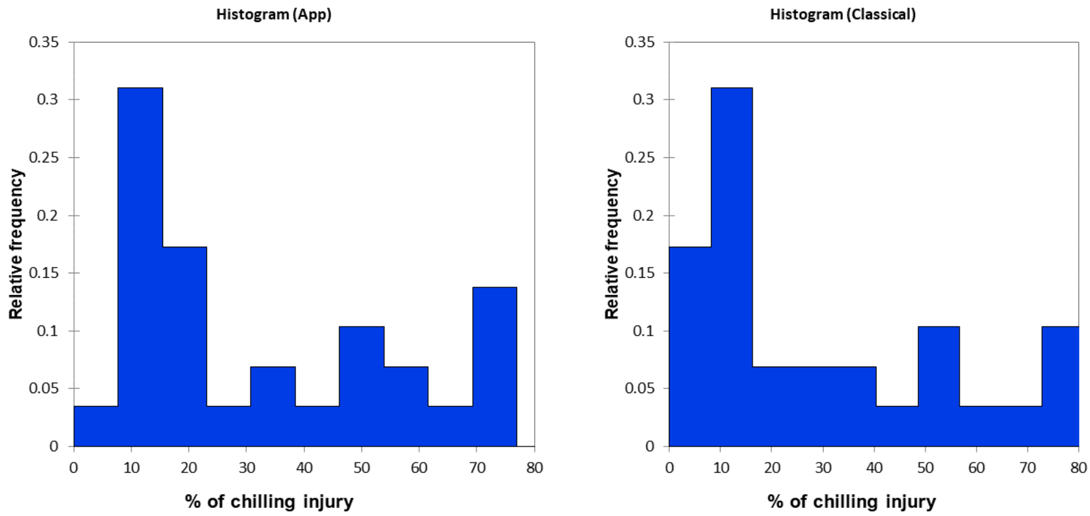

Fig. 13. Histograms of data observed using the APP and classical methods at 14 days.

6. Conclusion

Cold injury must be studied specifically for each product to ensure a condition suitable for its market. In particular, zucchini is considered quite sensitive. Thus far, the method validated and accepted for assessment has been visual evaluation by an expert who classifies the cold-injured surface according to a table of qualitative values, specifically six categories, ranging from no pitting or damage (0%) to very severe (>50%) damage. An APP developed for a smartphone has been shown to be adequate for this assessment as its results vary only slightly in absolute terms from those using the standard method. Showing total objectivity, it can adjust the tonality to a specific variety of zucchini which allows for better adaptation to a specific product. Above all, it is an objective method. The APP was developed to serve as a quantitative method and has proven to be very important in the assessment of chilling injury during the early stages, within 7 d, where small variations can influence treatments in the case of marketing or dismissal of varieties in studies of plant breeding. Use of the APP via a smartphone allows for easy application at low cost, and for standardisation of evaluation of a product without relying on a particular expert, as it can compare the results of one variety in different geographic areas. In summary, the developed APP allows one to evaluate very accurately the surface affected by chilling injuries, and therefore, can be used either in breeding or selection studies.

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