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# Accumulation and distribution of potassium and its association with water balance in the skin of Cardinal table grapes during storage

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ABSTRACT

Although potassium participates in distinct mechanisms that influence grape growth and development, including osmoregulation, little is known about the association between water and potassium in grape during storage at low temperature. We analyzed the relationship between potassium and the bound water fraction in the skin of early-harvested Cardinal table grapes (*Vitis vinifera* L.) from two different harvest years, both of which were stored at 0 °C for 3 days in air (20% $O_2$ + 0.03% $CO_2$) or in air + $CO_2$ (20% $O_2$ + 20% $CO_2$). The relative $K^+$ content and distribution in the skin cells was determined by energy dispersive X-ray microanalysis, revealing a non-uniform accumulation of $K^+$ in grape skin cells. Storage at 0 °C in air causes a significant decrease in bound water levels and greater soluble-water $K^+$ accumulation, irrespective of the harvest year. Furthermore, low temperature-scanning electron microscopy images revealed that the epidermal and the first hypodermal layers of the cells were compressed in the skin of fruit stored in air. However, when exposed to air plus 20% $CO_2$, there was no decrease in the bound water content or in the associated $K^+$ accumulation, nor were the outer skin cells compressed.



## 1. Introduction

Table grapes are very sensitive to fungal decay and water loss, both causing substantial postharvest losses. Susceptibility to disease and water loss is particularly dependent on the cuticular barrier and the condition of the underlying epidermal cells in the skin (Boyer et al., 1997; Comménil et al., 1997). The central vacuole of grape berry cells plays an important role in maintaining their volume, and in controlling the gradients of vacuolar ion concentrations that are essential for acid and sugar balance in the berry. Potassium is perhaps the most important ion in grapes, playing an important role in controlling the vacuolar ion concentration.

The post-harvest quality of table grapes can be enhanced by short-term high $CO_2$ treatments during low temperature storage (Retamales et al., 2003; Sanchez-Ballesta et al., 2006). The effectiveness of high $CO_2$ treatment is influenced by the stage of ripeness (Romero et al., 2009) and it also varies according to the temperature at which the commodity can be stored without producing damage (Ahumada et al., 1996; Prange and Lidster, 1992). In grapes, sugar accumulation in the flesh and anthocyanin accumulation in the skin have traditionally been studied to discriminate the harvest maturity. Berry development and ripening also affect the concentration of potassium and its transport by channels (Pratelli et al., 2002), transporters (Davies et al., 2006) and cation/proton antiporters (Hanana et al., 2007). In tomato fruit, changes in pH and $K^+$ levels have been described in the apoplastic fluid during ripening (Almeida and Huber, 1999, 2007). However, little is known about the relationship between potassium and water fractions during post-harvest storage of table grapes at low temperature.

Different stresses are known to alter a plant's water status (Karen et al., 1992; Ashraf and Foolad, 2007), and bound water is the fraction that probably plays the most important role in tolerance to abiotic stress given that it is responsible for maintaining the structural integrity and cell wall extensibility of living tissues (Singh et al., 2006). In harvested fruits, the ability to cope with changes in internal water content by varying the water fractions seems to be fundamental to maintain quality during storage. We previously demonstrated the benefits of short-term exposure to

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high concentrations of $CO_2$ on water status, highlighting the general increase produced in the bound water fraction in the different cluster tissues, and a decrease in the drip loss during freeze-thaw cycles (Goñi et al., 2011). Indeed, among the natural osmoprotective mechanisms induced when high $CO_2$ treatment is associated with low temperature storage, grapes accumulates water-soluble fructo-oligosaccharides (Blanch et al., 2011).

Besides organic solutes, ions also play key roles in the osmoregulation of cells, and they are involved in a range of other important biological phenomena (Salt, 2004). Specifically, the strong correlation between potassium and sugar in the skin and flesh of grape berries, respectively, suggests that potassium acts as an osmoticum in skin cells, as sugars does in the flesh. Indeed, potassium may play different roles depending on the stage of berry development, and as well as contributing to the charge balance it may also be involved in sugar transport (Lang, 1983). However, excess potassium in berries at harvest has negative effects, reducing wine quality by increasing the pH value, particularly for red wines. Considering that we are working with detached grapes and the mechanism for potassium loading is disrupted, it is important to understand the functional implications of the subcellular localization and the dynamics of water-soluble potassium. Moreover, the effect of this ion on low temperature storage and the response to high $CO_2$ levels should also be defined.

The aim of this study was to investigate whether potassium accumulation is associated with changes in water balance caused by storage of table grapes at low temperature (0 °C) in air (20% $O_2$ + 0.03% $CO_2$). Moreover, we studied whether these changes are dependent on the harvest date and if they are preserved by storage in air plus high $CO_2$ (20% $O_2$ + 20% $CO_2$). Accordingly, changes in the different water fractions were assessed by differential scanning calorimeter (DSC). Moreover, single-cell measurements of $K^+$ by energy dispersive X-ray microanalysis (EDX) of the different types of skin cells were accompanied by ultrastructural analysis of skin tissues by low temperature-scanning electron microscopy (LT-SEM). In addition, the mineral content was evaluated using inductively coupled plasma optical emission spectrometer (ICP–OES).

## 2. Materials and methods

### 2.1. Plant material

Table grapes (*Vitis vinifera* L. cv. Cardinal) were sampled from field-grown vines cultivated in Camas (Sevilla, Spain). Clusters were collected randomly at the beginning of the commercial harvest on two different years. In the first harvest, (G1) grapes were picked with 11.9 °Brix and in the second, (G2) grapes were picked with 12.8 °Brix. At each harvest (G1 and G2), field-packaged bunches were transported to the laboratory, and those free from physical and pathological defects were randomly divided into two lots, storing both at 0 ± 0.5 °C and 95% relative humidity (RH) in two sealed neoprene 1 $m^3$ containers. Ten plastic boxes containing about 3 kg of table grapes per box were stored in each container. One lot was stored in air for 3 days (air) and the other lot in air with 20% $CO_2$ (air + $CO_2$) for 3 days. At the end of this 3-day period, five clusters from each treatment were sampled, and 45 berries were randomly removed and distributed among the three replicates of 15 berries each. For (G1) and (G2), quality parameters were analyzed in three replicates samples containing five-berries. Furthermore, three replicates of one-berry samples were immediately frozen in liquid nitrogen and the skin's micro-structure was analyzed. Finally, another three replicates of nine-berries were deseeded and peeled, and the skin and flesh was ground into a fine power, and stored at −80 °C.

### 2.2. Unfreezable water fraction content determination by differential scanning calorimetry

A method based on the heat of fusion was used to calculate the amount of unfreezable water (UFW) or bound water, expressed in g per g of dry weight. It was assumed that the heat of fusion of freezable water in the tissue studied was equal to the heat of fusion of pure water at 0 °C. This analysis was performed using a DSC822e Mettler-Toledo differential scanning calorimeter (Mettler-Toledo Inc., Columbus, OH, USA) equipped with a liquid nitrogen cooling accessory (as described by Goñi et al., 2007), and calibrated using indium, *n*-octane and pure water. Frozen pulverized tissue was placed in 100 μL coated aluminum pans, which were immediately hermetically sealed and weighed. Samples were cooled from 25 °C to −80 °C at a rate of 10 °C/min, left at −80 °C for 5 min and then warmed to 25 °C at 10 °C/min. The total water content (%) in skin tissues was determined by heating to 65 °C until the skin tissue reaches a minimum constant weight. All the results are the means of at least three measurements.

### 2.3. Cellular distribution of $K^+$ in the skin of table grapes at harvest

LT-SEM studies were performed using a Zeiss DSN-960 electron scanning microscope equipped with a cold stage (Cryostrans CT-1500, Oxford Instruments). Frozen tissue sections were cryofractured at −180 °C, etched at −90 °C, gold-coated and subsequently transferred to the microscope where they were analyzed at −150 to −160 °C. Samples were observed with both secondary and retro-dispersed electrons, and the best images were selected in each case. Through LT-SEM, the components of the samples, including water, are physically stabilized by freezing in situ. A combination of scanning electron microscopy and energy dispersive X-ray microanalysis (SEM–EDX) was used on frozen skin sections to measure the $K^+$ content in the epidermal and hypodermal skin cells. Freshly harvested fully-flattened skin was analyzed by EDX using the method described previously (Leidi et al., 2010), and in the same cryopreparation chamber (CT1500; Oxford Instruments) attached to the SEM (DSM 960; Zeiss). The SEM was fitted with an ATW detector that interfaced with a Link ISIS analyzer. Measurements were taken after focusing on epidermal cells from the top and continuing to penetrate through to the deepest hypodermal layers of the skin tissues.

### 2.4. Water-soluble $Ca^{2+}$, $Mg^{2+}$, $K^+$, $Na^+$ and P content.

A sample of frozen fruit (1 g) was homogenized for 5 min in 10 mL of ultra-pure water (for $Na^+$ and $K^+$) or 10 mL of ultra-pure water slightly acidified with 5 mM hydrochloric acid (for $Ca^{2+}$ and $Mg^{2+}$), and this homogenate was analyzed as described previously (Blanch et al., 2012). Samples were centrifuged at 2000 × *g* for 20 min, after which the levels of soluble ions were determined in the supernatants. The samples were digested with $HNO_3$ and $H_2O_2$ in a Microwave Digestion Labstation (Milestone, mod. Ethos 1: Milestone, Shelton, CT—USA) and the digested samples were then diluted with ultrapure deionized water. The mineral content was evaluated on an Optima 4300 DV ICP–OES (inductively coupled plasma optical emission spectroscope: Perkin-Elmer, Norwalk, CT, USA). The data represent the means of three replicates, with two different measurements taken from each. The ICP–MS values obtained were used to calculate the ion content (mg/100 g FW) based on the mass and dilution of each sample.