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Distribution and timing of cell damage associated with olive fruit bruising and its use in analyzing susceptibility

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ABSTRACT

Bruising, damage caused by impact on the fruit during harvesting, is a major limiting factor in the production of table olives and currently restricts the applicability of mechanical harvesting. Bruising is characterized by darkening of the olive fruit surface and softening of the mesocarp or pulp. It decreases final product quality and may cause consumer rejection. This paper describes and quantifies anatomical changes in the olive mesocarp (pulp) related to bruising in fresh olive fruits after an induced impact. The study assessed two harvested table olive cultivars differing in bruise susceptibility (‘Manzanilla de Sevilla’ and ‘Hojiblanca’) at two different times (4 and 24 h after induced impact). Qualitative observations of tissue bruising prior and after histological analysis revealed changes in the damaged mesocarp including ruptured cells, loss of cell wall thickness, and discoloration in the damaged areas. These changes appeared greater in ‘Manzanilla de Sevilla’ than ‘Hojiblanca’, and more evident 24 h after the impact. Bruising damage was quantified using eleven parameters related to the area of the damaged zone, and incidence and position of tissue ruptures. Nine of these parameters changed significantly with time, and significant differences were observed between the two cultivars studied in seven of the parameters. Three of these, the total damaged area (*TDA*) measured in mesocarp portions prior to histological procedure, the number of tissue ruptures in the mesocarp intersected by circumferential *arc2* (located at one-fourth of the pulp thickness from the fruit exterior; *Brk-arc2)*, and the distance from the fruit exterior) to the first found tissue rupture (*D-min*)), measured in histological preparations, were the most discriminating and easy to assess parameters, and are thus recommended for the evaluation of susceptibility to bruising among harvested table olive cultivars.

1. Introduction

Worldwide table olive (*Olea europaea* L.) annual production is about 2.2 million tons (IOOC, 2012). Table olives are one of the most important agricultural products of Andalusia (southern Spain) and a traditional food of the Mediterranean diet. Table olives have numerous nutritional properties, based on their phenolic compounds, monounsaturated fatty acid, sugars, fiber, vitamin E and triterpenic acids (Rallo et al., 2011). Only three varieties (‘Manzanilla de Sevilla’, ‘Hojiblanca’ y ‘Gordal Sevillana’) comprise almost 89% of the total cultivated area in Andalusia (CAP, 2010).

Table olives for “green processing” have been traditionally harvested by hand in spite of the high cost of this operation. Nowadays this cost has become economically unbearable, so the main table olive producing countries are developing mechanical harvesting methods (Ferguson et al., 2010). Unlike the case of olive oil production, however, mechanical harvesting is not being extended to table olive plantations (Ferguson, 2006), as the high percentage of fruit damaged by impacts that take place during mechanical harvesting (Humanes et al., 1977) is a major constraint for the mechanization of these orchards (Jiménez-Jiménez et al., 2013b). Meeting the demand for high quality products is at present one of the most important goals of the agri-food industry. The presence of damage in the skin or the flesh of the olive fruit substantially decreases commercial fruit value and causes consumer rejection. Fruit bruising is considered to be the most decisive factor in determining the external quality of the processed olive (Rejano, 1999; Riquelme et al., 2008) and one of the most important aspects to resolve in order to successfully establish mechanical harvesting in the sector.

The fruit of the olive is a drupe composed of three major tissues: the epicarp or exocarp (a thin external protective layer), a fleshy mesocarp (the pulp), and an inedible stony endocarp (the stone or

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pit) (King, 1938). The mesocarp is the part of principal economic interest, and represents the edible portion of the table olive (Rapoport, 2008). The exocarp and external mesocarp layers constitute the external region (Hammami and Rapoport, 2012) where the bruising impact is received, although discoloration of tissue below the bruise has been noted to extend centripetally into the mesocarp (Jiménez-Jiménez et al., 2013b), even reaching as far as the pit (Jiménez et al., 2011). However, the response of these tissues to bruising and their possible role in bruise susceptibility or resistance in table olives has been little studied, particularly at the histological level. In general the bruising of fresh produce is described as a "type of subcutaneous tissue failure without rupture of the skin" (Opara and Pathare, 2014), although in some cases microscopic studies have revealed small cracks, such as in the exocarp tissue of bruised peach and pear fruit (Crisosto et al., 1993). For the olive fruit, however, the occurrence of epidermal ruptures has not been reported, nor have specific histological observations of the epidermis been carried out.

In bruised olive fruit, dark spots appear on the fruit exterior following damage caused by impacts during picking, most notably in mechanical harvesting (Castro-García et al., 2009). This phenomenon appears to be similar to that described for many other fruits and fresh horticultural produce (Opara and Pathare, 2014). Usually these surface blemishes stand out against the rest of the olive's green colour and remain even after the complete fermentation process (Segovia-Bravo et al., 2011). As time progresses, and depending on the impact severity, the affected zone darkens and may spread throughout the mesocarp, even reaching the endocarp (Jiménez et al., 2011). Nevertheless, most studies of table olive bruising, and even commercial regulations (International Olive Council Trade Standard for Table Olives (IOOC, 2004)), are limited to an assessment of the external visual damage of the fruit. The discoloration and browning in the damaged areas of the olive fruit is produced by different polyphenols and enzymes, particularly polyphenol oxidase (PPO) (Segovia-Bravo et al., 2007, 2009). However, although those authors mention that the browning may be restricted to the fruit surface or extend within the pulp, depending on the bruise intensity, their biochemical analyses use extracts of the total fruit pulp and provide little information regarding the spatial extension of browning within the fruit.

Several techniques have been used to quantify the amount of external bruise damage in table olives, including manual measurement of the damaged area (Saracoglu et al., 2011; Jiménez-Jiménez et al., 2013a; Morales-Sillero et al., 2014) and the use of image analysis software to quantify the size of the bruises (Jiménez-Jiménez et al., 2013b). Bruise assessment is commonly based on visual rating. Visual scales have been based on different categories according to the severity of the damage (Jiménez et al., 2011; Jiménez-Jiménez et al., 2013b; Morales-Sillero et al., 2014). However, it is difficult to compare different evaluation of bruise intensity, given that there is no consensus on the categories nor terminology used in description. Non-destructive techniques such as NIRS (Near infrared spectroscopy) (Jiménez-Jiménez et al., 2012), colorimetry (Glozer et al., 2008) and spectrophotometry (Segovia-Bravo et al., 2007, 2011) have been also explored to evaluate bruising damage in table olive. With the exception of a preliminary study by this group (Jiménez et al., 2011), however, there have been no attempts to study or quantify table olive bruising extending within the fruit pulp.

Bruising may be effectively prevented if the factors responsible for its development are known. Bruise susceptibility can depend on multiple factors (Studman, 1997) as has been reported, for example, for apple (Grotte et al., 2000; Bollen, 2005) and for pears (Berardinelli et al., 2005). Both external factors such as fruit size, shape, firmness and internal factors such as cell wall strength, elasticity, cell shape and internal structure (Studman, 1997; Van linden et al., 2006) affect the severity of bruising. It has also been observed that, within a produce type, cultivar differences contribute most to bruising susceptibility differences, although it is not known which traits are responsible (Opara and Pathare, 2014). Among the three main Spanish table olive cultivars, 'Manzanilla de Sevilla', 'Hojiblanca' and 'Gordal Sevillana', the first one is the most susceptible to bruising (Jiménez-Jiménez et al., 2013a), but while a few possible factors such as cuticle thickness (Hammami and Rapoport, 2012), fruit firmness and flesh-to-pit ratio (Rejano, 1999) have been suggested to explain those cultivar differences, no specifically oriented studies have been carried out. In summary, there is very little information about how fruit bruising affects table olives at the cellular level, and particularly within the mesocarp, the edible fraction of the fruit. Standardized quantitative methods are also needed to evaluate bruising susceptibility in table olive cultivars.

The goals of this study were first, to describe and quantify structural changes produced in the olive fruit as a consequence of bruising, and second, to analyze those changes in two cultivars with different bruising susceptibility ('Manzanilla de Sevilla' and 'Hojiblanca') and at different times after the impact (4 and 24 h). The structural features observed included the spatial extension of browning within the fruit mesocarp and, using histological preparations, the cellular characteristics of the mesocarp and exocarp. Damage was quantified by determining its extent and location with respect to impact position and mesocarp depth.

2. Material and methods

2.1. Plant material

The two most important table olive varieties internationally, 'Manzanilla de Sevilla' and 'Hojiblanca' (Rejano et al., 2010) were

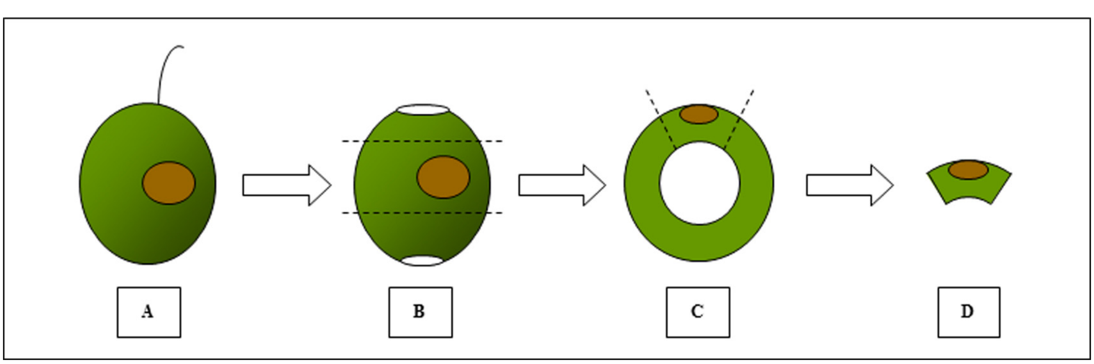

Fig. 1. Diagram of how sample segments were taken from bruised olives. (A) Olive fruit with bruised area on the surface. (B) Pitted olive from which a 4–5 mm central transverse slice containing the bruise is obtained. (C) A portion of the transverse slice is cut with the bruise in its center. (D) The piece used for histological processing. Dark oval = external bruise.