BRUISE DAMAGE SUSCEPTIBILITY OF POMEGRANATES AND IMPACTS ON FRUIT QUALITY

By

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DECLARATION

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own original work, that I am the sole author thereof (unless to the extent explicitly otherwise stated), and that I have not previously in its entirety or in part submitted itat any other university for obtaining any qualification.

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SUMMARY

The consumption of pomegranate (*Punica granatum*, L) fruit is attributed to its health and nutritional benefits, which are linked with reported high antioxidant capacity, anti-mutagenic, anti-inflammatory, anti-atherosclerotic and anti-hypertension activities. Postharvest handling of pomegranate fruit takes a couple of weeks (5 – 8) and includes a series of operations from harvest to export (i.e. harvesting, sorting, packing/repacking and transportation). In the course of these operations, there are various situations where pomegranate fruit are subjected to multiple modest drop impacts that predispose the fruit to varying levels of excessive external forces resulting in bruise damage. Impacts may occur as the result of sudden fall of fruit onto other fruit, parts of the tree, harvesting bucket and bin, or any other uncushioned surfaces in the course of loading and offloading. The presence of a bruise on pomegranate fruit causes produce quality deterioration that contributes to downgrading, rejection of produce and ultimately, to postharvest losses. It is therefore important to understand the mechanism of bruising and how to minimise it. The overall aim of this research was to investigate the bruise damage susceptibility of selected pomegranate fruit cultivars, to ascertain the effects of bruising and storage duration on fruit quality attributes and finally, to explore the feasibility of non-destructive measurements to detect and characterise bruise damage.

The studies reported in Chapter 4 investigated the susceptibility of three pomegranate fruit cultivars (‘Acco’, ‘Herskawitz’ and ‘Wonderful’) to impact bruising. The impact threshold required to bruise pomegranate fruit was investigated for each cultivar with a view to identify the cultivar that is most susceptible to bruising. The probability of bruise occurrence (PBO) was determined from the population of selected fruit impacted at minimal drop heights (0.10, 0.15, 0.20 m). At the drop impact of 0.10 m, results showed that ‘Wonderful’ had the lowest impact threshold, with a PBO value of 0.44 and an impact energy of 371.87 mJ, whereas neither ‘Acco’ nor ‘Herskawitz’ showed any signs of bruising. At the drop impact height of 0.15 m the highest bruise occurrence was seen in ‘Wonderful’ (PBO = 1; 692.98 mJ), followed by ‘Acco’ (PBO = 0.75; 406.26 mJ) and ‘Herskawitz’ (PBO = 0.5; 511.57 mJ). These results showed that ‘Wonderful’ fruit had a higher susceptibility to bruising compared to the other investigated cultivars, and therefore needs to be handled with extra care during harvest and postharvest handling. Furthermore, the study investigated the effect of cold (5 ºC) and ambient (20 ºC) storage temperatures on bruise damage susceptibility. Fruit were dropped at higher drop impact levels (0.2, 0.4 and 0.6 m), stored for a period of 10 d at either 5 ºC or 20 ºC, during which the physiological responses including weight loss and respiration rate were evaluated. Bruise size were determined in terms of

bruise volume (BV) and bruise area (BA), while bruise susceptibility was calculated as the BV per unit of impact energy. The results revealed that bruise size and bruise susceptibility at higher drop heights (0.2, 0.4 and 0.6 m) were cultivar dependent and in the order of 'Wonderful' > 'Herskawitz' > 'Acco'. The bruise size of cold (5 ºC) conditioned pomegranate fruit was significantly higher than that of fruit conditioned at an ambient (20 ºC) temperature. Further results showed that drop impact bruising had a larger effect on the fruit physiological response (respiration rate and weight loss) for bruised fruit in comparison to non-bruised fruit. Fruit impacted at higher drop impact levels (0.4 or 0.6 m) exhibited two to three-fold higher respiration rate than fruit bruised at a lower impact level (0.2 m) or non-bruised fruit. Respiration rate and weight loss increased with prolonged storage duration and at an ambient temperature, both in bruised and non-bruised fruit.

Further study to evaluate the feasibility of X-ray micro-computed tomography (X-ray μCT) in detection and characterization of bruise damage on pomegranate fruit is reported in Chapter 5. Pomegranate fruit bruised by dropping at 0.6 m was scanned with X-ray μCT. The results showed that two-dimensional CT images of fruit scanned at 0 h (immediately after drop impact), 48 h, 3 d and 5 d after impact bruising showed no evidence of bruise damage. Changes in bruise-damaged tissue as characterised by a darker appearance were observed in pomegranate fruit scanned after 7 d of impact bruising. Furthermore, visual assessment of two-dimensional X-ray μCT images were buttressed by the results of quantitative μCT data analysis. The latter demonstrated that bruised pomegranate fruit can be visualised and differentiated from 7 d after impact bruising with lower grey values (18000 - 30000) compared with non-bruised fruit (26000 - 34000). The image analysis and quantitative μCT data obtained in this study confirmed that X-ray μCT is not a suitable non-destructive method to detect and characterise fresh bruises (immediately bruised) on pomegranate fruit. Studies to explore alternative non-invasive techniques, such as a hyperspectral imaging system for early detection of fresh bruises on pomegranate fruit, are warranted.

Chapter 6 focused on evaluating the physical, biochemical and microstructural changes of impact-bruise damaged pomegranate fruit. The results showed that there were significant changes in colour (browning), peel electrolyte leakage (PEL), polyphenol oxidase (PPO) enzyme activity and accumulation of reaction oxygen species (ROS) measured in pomegranate fruit peel with increasing drop impact bruising. The combination of time and temperature (in which fruit was incubated) significantly ($p < 0.05$) contributed to changes in PEL, PPO enzyme activity and fruit browning. Cellular microstructural differences between control and bruised fruit tissues were visible in scanning electron microscope images after 4 and 48 h of drop impact. These findings provided evidence that the loss of membrane integrity of pomegranate fruit skin cells are caused by impact bruising.

Chapter 7 covered the study on bruise damage of pomegranate during long-term cold storage, focusing on susceptibility to bruising and changes in textural properties of fruit. Fruit from cold (5 ºC) storage were impact bruised from different drop heights (0.2, 0.4 and 0.6 m). The bruise volume and bruise area of pomegranate fruit increased with increasing drop impact heights and storage duration for the first two months of storage, and then decreased in the last month of storage. Similarly, the results of textural properties showed that increase both in puncture resistance, cutting and compression strength were dependent on impact bruising and storage duration. These results have demonstrated that bruise damage would result in significant changes in fruit textural attributes with concomitant low consumer appeal.

Studies in Chapter 8 investigated the effects of bruising and long-term cold (5 ºC) storage on the physiological response, physico-chemical quality attributes, textural properties and antioxidant content of pomegranate fruit. Respiration rate and weight loss of whole fruit were both influenced by increasing drop impact bruising and storage duration. Furthermore, there were increases in chemical quality attributes (total soluble solids, titratable acidity, Brix-to-acid ratio and BrimA), and antioxidant content of bruised pomegranate fruit during long-term storage. This was partly attributed to the concentration effect due to an increased moisture loss from bruise damaged fruit. Results on changes in aril colour and texture were dependent on both bruising and storage duration ($p < 0.05$).

Overall, this research represents a pilot study aimed at providing scientific insights to broaden the understanding of pomegranate fruit susceptibility to bruising during postharvest handling and its impacts on fruit quality. The findings in this dissertation have established that bruise susceptibility of pomegranate fruit is dependent on the level of drop impact, cultivar, storage temperature and duration. Furthermore, this study showed that bruising, storage conditions and duration play a crucial role on physiological responses (i.e. respiration rate and weight loss), textural properties and chemical quality attributes of the fruit. From a practical point of view, the study has revealed that, bruise damage affects the sensory appeal of pomegranate fruit during storage, which could result in downgrading of fruit market value or complete fruit loss.

OPSOMMING

Die verbruik van granate (*Punica granatum*, L) word toegeskryf aan gesondheids- en voedingsvoordele wat verband hou met berigte hoë antioksidant kapasiteit, anti-mutageniese, anti-inflammatoriese, anti-aterosklerotiese en anti-hipertensie aktiwiteite. Na-oes hantering van granate duur 5 – 8 weke en sluit 'n reeks praktyke in van oes tot verskeping (o.a. oes, sortering, verpakking/herverpakking en vervoer). Gedurende hierdie praktyke is granate onderhewig aan vele gematigde val-impakte wat ‘n reeks eksterne kragte op die vrug uitoefen en kneusing veroorsaak. Hierdie impakte gebeur wanneer vrugte skielik op ander vrugte, dele van die boom, in die oes-krat en pallet, of enige ander onbedekte oppervlakke tydens die laai en aflaai proses val. Kneusing verlaag die gehalte van granaat vrugte, wat bydra tot afgradering, verwerping en uiteindelik na na-oesverliese. Dit is dus belangrik om die meganisme van kneusing te verstaan en hoe om dit te verminder. Die oorhoofse doelwit van hierdie navorsing was om die kneusingsvatbaarheid van gekeurde granaat kultivars te ondersoek, om die effek van kneusing en bergingsduur op kwaliteitskenmerke van die vrugte te bepaal, en ten slotte om die uitvoerbaarheid van nie-vernietigende metings te ondersoek om kneusingskade vroegtydig te identifiseer en te kenmerk.

Die studie in Hoofstuk 4 het die vatbaarheid van drie granaat kultivars (‘Acco’, ‘Herskawitz’ en ‘Wonderful’) vir kneus-impak ondersoek. Die impak-drempel wat nodig is om granate te kneus, is ondersoek vir elke kultivar met die oog op die identifisering van die kultivar wat die mees vatbaarste is vir kneusing. Die waarskynlikheid van kneusing (PBO) is vasgestel vanuit die populasie van geselekteerde vrugte wat by ‘n minimale valhoogte (0.10, 0.15, 0.20 m) geaffekteer is. By die valhoogte van 0.10 m het 'Wonderful' die laagste impak-drempel gehad, met 'n PBO-waarde van 0.44 en 'n impak-energie waarde van 371.87 mJ, terwyl 'Acco' en 'Herskawitz' geen kneusings vertoon het nie. By die val-impak hoogte van 0.15 m is die hoogste kneus-waarskynlikheid in ‘Wonderful’ (PBO = 1; 692.98 mJ) gevind, gevolg deur 'Acco' (PBO = 0.75; 406.26 mJ) en ‘Herskawitz’ (PBO = 0.5; 511.57 mJ). Hierdie resultate het getoon dat ‘Wonderful’ granate ‘n hoër vatbaarheid vir kneusing gehad het in vergelyking met ‘Acco’ en ‘Herskawitz’. Daarom moet ‘Wonderful’ granate met ekstra sorg hanteer word tydens oes- en na-oes hanteringspraktyke. Verder het die studie die effek van koue (5 ºC) en omringende (20 ºC) bergingstemperature op kneusingsvatbaarheid bestudeer. Vrugte is by hoër val-impakhoogtes (0.2, 0.4 en 0.6 m) laat val, gestoor vir ‘n periode van 10 d by 5 ºC of 20 ºC, waartydens die fisiologiese reaksie, insluitend gewigsverlies en respirasietempo, geëvalueer is. Kneusgrootte is vasgestel in terme van kneusvolume (BV) en kneusoppervlakte (BA), terwyl kneusingsvatbaarheid bereken is as die BV per eenheid impak-energie. Die resultate het getoon dat die kneusgrootte en die

kneusingsvatbaarheid van granate by hoër valhoogtes (0.2, 0.4 en 0.6 m) van die kultivar afhang in die volgorde: 'Wonderful' > 'Herskawitz' > 'Acco'. Die kneusgrootte van verkoelde (5 ºC) granate was aansienlik hoër as dié wat by 'n omringende temperatuur (20 ºC) geberg was. Verdere resultate het getoon dat val-impak kneusing 'n groter effek op die fisiologiese reaksie (respirasietempo en gewigsverlies) van gekneusde vrugte in vergelyking met nie-gekneusde vrugte gehad het. Vrugte wat geraak is by hoër val-impakhoogtes (0.4 of 0.6 m) het twee tot drie keer die respirasietempo getoon van vrugte wat teen 'n laer impakhoogte (0.2 m) of glad nie gekneus was nie. Respirasietempo en gewigsverlies het toegeneem met bergingsduur en by omringde temperatuur, in beide gekneusde en nie-gekneusde vrugte.

Hoofstuk 5 evalueer die uitvoerbaarheid van mikrofokus X-straal rekenaartomografie (X-straal μCT) in die opsporing en karakterisering van kneusingskade in granate. Granate is gekneus deur die vrugte teen 'n valhoogte van 0.6 m te laat val, voor dit met X-straal μCT geskandeer is. Die tweedimensionele CT-beelde kon nie enige kneusingskade vind in vrugte wat geskandeer is by 0 h (direk na die val-impak), 48 h, 3 d en 5 d. Veranderinge in die beskadigde weefsel van geskandeerde granate kon slegs 7 d na die kneus-impak gekenmerk word deur 'n donkerder area. Die visuele beoordeling van tweedimensionele X-straal μCT beelde is verder versterk deur kwantitatiewe μCT data-analise. Laasgenoemde het getoon dat gekneusde granate gevisualiseer en gedifferensieer kan word vanaf 7 d na kneus-impak met laer grys waardes (18000 - 30000) in vergelyking met nie-gekneusde vrugte (26000 - 34000). Die beeldanalise en kwantitatiewe μCT-data wat in hierdie studie verkry is, het bevestig dat X-straal μCT nie 'n geskikte nie-vernietigende metode is om vars kneusing (wat onmiddellik gekneus is) in granate op te spoor en te karakteriseer nie. Studies om alternatiewe nie-indringende tegnieke te ondersoek, soos 'n hiperspektrale beelding stelsel, vir vroeë opsporing van vars kneusing in granate is geregverdig.

Hoofstuk 6 het gefokus op die evaluering van die fisiese, biochemiese en mikrostruktuur veranderinge van kneus-impak beskadigde granate. Die resultate het getoon dat daar met 'n toenemende val impak kneusing aansienlike veranderinge in kleur (verbruining) skil-elektroliet lekkasie (PEL), polifenool oksidase (PPO) ensiem aktiwiteit en ophoping van reaktiewe-suurstofspesies (ROS) in die granaatskil was. Die kombinasie van tyd en temperatuur (waarby die vrugte geïnkubeer was) het beduidend ($p < 0.05$) bygedra tot veranderinge in PEL, PPO ensiem aktiwiteit en verbruining. Sellulêre mikrostruktuur verskille tussen die kontrole en gekneusde vrugteweefsels was na 4 en 48 uur van die val-impak sigbaar in skanderingselektronmikroskoop beelde. Hierdie bevindinge lewer bewyse dat die verlies van sellulêre membraan-integriteit in granaatskille veroorsaak word deur kneus-impak.

Hoofstuk 7 bestudeer die skade aan granate tydens langtermyn verkoeling, met die klem op vatbaarheid van kneusing en veranderinge in tekstuureienskappe van die vrugte. Vrugte wat by koue (5 ºC) temperatuur geberg is, is by verskillende valhoogtes (0.2, 0.4 en 0.6 m) laat val. Die kneusvolume en kneusoppervlakte van granate het toegeneem met toenemende val-impak hoogte en bergingsduur vir die eerste twee maande, waarna dit afgeneem het in die laaste maand van opberging. Die tekstuureienskappe resultate toon verder, dat die verhoging in beide die punthoudingsweerstand en die sny- en druksterkte, afhangend is van die kneus-impak en bergingsduur. Hierdie resultate dui aan dat kneusingskade tot beduidende veranderinge in tekstuureienskappe van granate lei, wat moontlik met 'n lae verbruikersappèlle gepaardgaan.

Hoofstuk 8 ondersoek die effekte van kneusing en langtermyn koue (5 ºC) berging op die fisiologiese reaksie, fisiese-chemiese kwaliteitseienskappe, teksturele eienskappe en antio-oksidant-inhoud van granate. Respirasietempo en gewigsverlies van heel vrugte is albei beïnvloed deur toenemende val-impak kneusing en bergingsduur. Verder was daar 'n toename in chemiese kwaliteitseienskappe (totale oplosbare vastestowwe, titreerbare sure, Brix-tot-suur verhouding en BrimA), en anti-oksidant-inhoud van gekneusde granate tydens langtermyn opberging. Dit is deels toegeskryf aan die konsentrasie-effek as gevolg van 'n verhoogde vogverlies van kneus beskadigde vrugte. Die veranderinge in ariel kleur en tekstuur het van beide kneusing en bergingsduur ($p < 0.05$) afgehang.

Oor die algemeen verteenwoordig hierdie navorsing 'n loodsstudie wat daarop gemik is om wetenskaplike insigte te verskaf om die begrip van kneusingsvatbaarheid in granate tydens na-oes hantering, sowel as die impak daarvan op vrug kwaliteit te verbreed. Die bevindinge in hierdie proefskrif het vasgestel dat vatbaarheid vir kneusingskade in granate afhangend is van val-impak hoogte, kultivar, bergingstemperatuur en -duur. Verder het hierdie studie getoon dat kneusing, bergingstoestande en -duur 'n deurslaggewende rol op fisiologiese reaksie (o.a. respirasie tempo en gewigsverlies), tekstuureienskappe en chemiese kwaliteitseienskappe van die vrug speel. Uit 'n praktiese oogpunt het die studie gewys dat kneusingskade die sintuiglike aanloklikheid van granate tydens berging beïnvloed, wat verder tot die afgradering van vrug markwaarde of algehele vrugte verlies kan lei.

LIST OF PUBLICATIONS, SUBMITTED MANUSCRIPTS, CONFERENCE PROCEEDINGS AND PRESENTATIONS

Publications/ submitted articles:

1. **Hussein, Z.,** Fawole, O.A. & Opara, U.L. (2018). Preharvest factors influencing bruise damage of fresh fruits – a review. *Scientia Horticulturae*, **229**, 45–58.

2. **Hussein, Z.,** Fawole, O.A. & Opara, U.L. (2019). Bruise damage susceptibility of pomegranates (*Punica granatum*, L) and impact on fruit physiological response during short term storage. *Scientia Horticulturae,* 246, 664–674.

3. **Hussein, Z.,** Fawole, O.A. & Opara, U.L. Harvest and postharvest factors influencing bruise damage of fresh fruits – a review. (Submittedin November 2018). *Horticultural Plant Journal.*

4. **Hussein, Z.,** Fawole, O.A. & Opara, U.L. Determination of physical, biochemical and microstructural changes in impact-bruise damaged pomegranate fruit. (Submitted in November 2018). *Journal of Food Measurement and Characterization*.

5. **Hussein, Z.,** Fawole, O.A. & Opara, U.L**.** Effects of bruise damage and storage duration on physiological response, physico-chemical changes and antioxidant content of pomegranate fruit (cv. Wonderful). (Submitted in November 2018). *LWT - Food Science and Technology.*

Conference proceedings and presentations

1. **Hussein, Z.,** Fawole, O.A. & Opara, U.L. (2016). Reducing susceptibility of fresh produce to physical damage during postharvest handling: The case of pomegranate fruit: 5thAfrican Higher Education week and RUFORUM Biennial Conference. Cape Town, South Africa, 17-21 October 2016.

2. **Hussein, Z.,** Fawole, O.A. & Opara, U.L. (2017). Analysis of cellular microstructural changes, enzyme activity and reaction oxygen species associated with pomegranate fruit bruising. VII International Conference on Managing Quality Chains (MQUIC2017). International Society for Horticultural Science,Stellenbosch, South Africa, 4-7 September 2017

3. **Hussein, Z.,** Fawole, O.A, du Plessis, A. & Opara, U.L. (2018). Application of X-ray micro-computed tomography to detect bruise damage of pomegranate (cv. Wonderful) fruit. 6thAfrican Higher Education week and RUFORUM Biennial Conference, Nairobi, Kenya, 22-26 October 2018.

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PREFACE

This dissertation is composed of a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable. The language and style used in this dissertation are in accordance with the requirements of the International Journal of Food Science and Technology, as prescribed by the Department of Food Science, Stellenbosch University, to which the following chapter(s) was/were submitted for publication.

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CHAPTER 1

GENERAL INTRODUCTION

General introduction

1. Background

Commercial farming and consumption of pomegranate (*Punica granatum* L.) fruit have gained momentum in recent years due to increasing consumers' awareness of its multi-functionality and abundant nutritional benefits in the human diet (Fawole *et al*., 2012). The consumption of pomegranate fruit has been associated with reduced incidence of several diseases such as cancer, cardiovascular disease and diabetes (Fawole & Opara, 2013; Caleb *et al*., 2015). These and several other reported health and nutritional benefits that are linked with the consumption of pomegranate fruit and by products have been attributed to the fruit's reported high antioxidant capacity and anti-mutagenic, anti-inflammatory, anti-hypertension and anti-atherosclerotic, and anti-hypertension activities (Viuda-Martos *et al*., 2010; Fawole & Opara, 2013).

Commercial farming of pomegranate fruit has increased to over 3.5 million tons of global commercial production the last decade (Pomegranate Association of South Africa, 2017). South Africa has emerged as a new commercial producer of pomegranates, competing with a few countries in the Southern Hemisphere such as Chile Australia, Peru and Argentina (Holland & Bar-Ya'akov, 2008). Production of pomegranate fruit in South Africa is over 8 000 tons produced in the total production area of over 1000 ha (Hortgro, 2017; POMASA, 2017). Limited storage duration of pomegranate fruit (4 - 5 months) and the rotation in seasonal production existing between producing countries in the Northern (Iran, India, USA, Turkey, Israel and Spain and Southern hemisphere have created an opportunity window for South Africa to export to countries in the North during the counter season (Brodie, 2009; Pomegranate Association of South Africa, 2013). The existing window for export, coupled with increasing global demand for fresh pomegranate fruit, has consequently spurred large scale production and exports (Pomegranate Association of South Africa, 2013). However, in order to satisfy the demand for good quality fruit for local and international market, the South African pomegranate industry needs an improved and efficient pre and postharvest systems.

South African pomegranate fruit industry is expanding annually, with an expected growth in production of over 200 % by 2019 (Hortgro, 2017). However, the prospects for a competitive South African pomegranate industry for export market is plagued with postharvest losses due to apparent fruit sensitivity to various physiological disorders and damage to the husk such as sunburn, cracking, husk scalding, chilling injury and mechanical damage. There are several studies reported on various aspects of pomegranate fruit disorders such as chilling injury (Elyatem & Kader, 1984; Kader *et al*., 1984; Artés *et al*., 2000); husk scald (Ben-Arie & Or, 1986; Defilippi *et al*., 2006) and decay (Nerya *et al*., 2006; D'Aquino *et al*., 2010). Comperatively, little information on the mechanical damage of pomegranate fruit is available. Mechanical damage can be in the form of plastic deformation, superficial rupture and/ or destruction of fruit tissue (Montero, 2009; Opara & Pathare, 2014), and includes bruising, cuts, crushing or rupturing of produce (Polat et al., 2012). Bruising is the most common type of mechanical damage, a type of subcutaneous tissue failure without rupture of the skin of fresh produce where the discolouration of injured tissue indicates the presence of a damaged spot (Opara & Pathare, 2014). The physical evidence of bruising is a result of cell breakage from stress and distortion of individual cells leading to cell wall extension (Ruiz-Altisent & Moreda, 2011).

Like any other fresh fruit, pomegranate is subjected to mechanical damages during postharvest handling due to the action of mechanical operations involved both in harvesting and postharvest handling (Shafie *et al*., 2015; Shafie *at al*., 2017). Overall, fresh fruits such as pomegranates are subjected to a number of operations during harvest and immediately after harvest that vary between commodities but combining similar individual treatments. These operations follow a complex route from the fruit tree in an orchard to the shelves of supermarket that comprises of various stages and processes such as harvesting, packing, sorting, storage and transport (Kafashan *et al*., 2008; Lewis *et al*., 2008; Eissa*et al*., 2013). These processes involve numerous mechanical operations that predispose the fruit to the varying levels of static and dynamic forces causing mechanical damage (Stropek & Gołacki, 2015; Shafie *et al*., 2015). Damages to fruit may occur mainly due to sudden drops during the course of loading and offloading, where packages of fruits or individual fruits are thrown from certain heights onto other surfaces (Shafie *et al*., 2015; 2017). Tabale 1 illustrates various loading situations and associated potential drop heights that predispose pomegranate fruit to multiple modest impacts resulting to bruise damage during harvest and postharvest handling of pomegranate fruit.

Table 1 Potential dynamic pomegranate loading situations and associated drop heights

Lodaing situation	Process stage	Potental drop height
Ochard	Picking bucket	0.6 m
Packhouse	Bulk bin	0.6 – 1 m
	Repack	0.05 – 0.15 m
Distributor	Sorting	0.05 – 1.15 m
Retailer	Putting on dispay	0.05 – 0.3 m

Adopted from Shafie *et al.*, 2017.

Bruising is an important limiting factor in producing quality fruits due to its impacts on produce quality deterioration and subsequent economic losses (Polat *et al.*, 2012; Opara & Pathare, 2014; Stropek & Gołacki, 2015). Pomegranate fruit bruising represents a potential threat to quality as well as the significant reduction in the overall value as it contributes to losses both in fruit quality and revenue. In addition to chemical constituents such as sugar content, acidity and flavour, pomegranate fruit acceptability by consumers and processors depends on a combination of other several external quality attributes, including physical appearance (colour and size) as well as the absence of physical defects such as cracks, rot or any sort of damage (Al-Said *et al.*, 2009; POMASA, 2013). Bruising affects the external quality, which is considered of paramount importance in the marketing and sale of fruits which is often associated with desirable internal quality characteristics (Brosnan & Sun, 2004; Al-Said *et al.*, 2009; Magwaza *et al.*, 2012). Pomegranate fruit appearance characterised by size, shape, colourand absence of blemishes influences the consumers' perceptions, and therefore determines the level of acceptability prior to purchase (POMASA, 2013).

The signs of bruising on pomegranates may not be apparent on damaged fruit until at a later stage in the handling chain (Shewfelt, 1986; Hussein *et al.*, 2018). Given the effects of bruising on changes in physiological processes such as respiration and transpiration, bruised fruit within the consignment could be susceptible to weight loss, senescence, microbial decay as well as loss of nutritional value along the cold chain (Elshiekh & Abu-Goukh, 2008; Li *et al.*, 2011). In addition, the presence of decayed pomegranates within the consignment affect healthy, undamaged fruit, and/ or contaminate the whole batch of fruit and hence compromise the quality of exported fruit (Opara *et al.*, 2007; Prusky, 2011; Lü & Tang, 2012). This results into economic losses from down-grading of exported fruit (with latent or hiden bruises that develop to other quality deterioration problems such as decay) in the international markets. Therefore, for South African pomegranate industry to maintain the quality of pomegranate fruit for the international market and preventing additional economic losses due to damage, there is the need for searching of accurate and cost-effective destructive and non-destructive assessment methods both for the field and laboratory measurement. Currently, the pomegranate fruit losses between harvest and export amount to 40 – 50 % of total harvested fruit (Hortigro, 2017). This could also highlights that bruise damage could be contributing significantly to such losses.

Bruise damage is a limiting factor for successful mechanisation and automation of harvesting, postharvest handling operations (Opara *et al*., 2007; Polat *et al*., 2012). Hence, postharvest management practices could offer potential opportunities to reduce the incidence of bruising including the optimisation of farm management practices, improved handling techniques and produce conditioning (Opara, 2007). To achieve this, the knowledge of factors influencing bruising during postharvest handling of fresh fruit is paramount to both growers and postharvest value chain in order to reduce bruise damage and its associated losses. However, a limited information on the bruise damage susceptibility and potential impact on pomegranate fruit quality is available. A few research studies have investigated the effects of pomegranate fruit temperature, storage time and fruit impact region on bruise susceptibility (Shafie *et al*., 2015; 2017). However, given that cultivar differences account for most of the differences in bruise susceptibility among various fruit (Opara *et al*., 1997; Li *et al*., 2010), previous results on specific pomegranate fruit cultivar cannot be extrapolated. Furthermore, to the best of our knowledge, there is no study that has been conducted to ascertain the influence of bruising on the overall quality of pomegranate fruit. Additionally, there is a dearth of scientific knowledge on the pomegranate physical, physiological and biochemical changes induced by fruit bruising fruit underlying the postharvest fruit quality. Hence, the awareness of pomegranate fruit susceptibility to bruising and its impact on overall fruit quality is paramount for developing a science-based tool to assist in the application of appropriate handling of fruit during harvesting and postharvest handling.

Much has been reported on various aspects of bruising for many other fruits such as apples, peach, tomatoes, pears, citrus fruits, banana etc. Nonetheless, very limited data is available on bruising of pomegranates, in part due to the complex structure of the fruit characterized by thick rind. Pomegranate fruit internal structure is different from other fruit, hence making it difficult to detect, measure and characterise the bruise damage. It is still unknown to what extent bruise susceptibility of pomegranate is dependent on cultivar, fruit properties, storage condition or duration. The minimum impact energy level (impact threshold) which could result in bruise damage of pomegranate fruit during handling is still unknown. In addition, the accurate method to quantify pomegranate bruising (destructively and non-destructively) is also lacking. Given the highlighted nutritional and economic losses due to bruise damage and potential gains in developing measures to reduce the problem, it is important to generate an in-depth understanding of bruise occurrence and bruise susceptibility of pomegranate fruit, as well as the influence of biotic and abiotic factors such as cultivar, storage temperature and storage duration on bruisingthat will lead to the development of postharvest management tools.

2. Research aim and objectives

The overall aim of this research was to investigate the bruise damage susceptibility of selected pomegranate fruit cultivars, to ascertain the effects of bruising and storage duration on fruit quality attributes and explore the feasibility of non-destructive measurements to detect and characterise bruise damage.

The research aim was accomplished through the following specific objectives;

i. Studying the bruise susceptibility and bruise threshold of three pomegranate fruit cultivars (Acco, Herskawitz and Wonderful).

ii. Exploring the application of non-destructive X-ray micro computed tomography in detection and characterization of pomegranate fruit bruise damage

iii. Evaluating the bruise damage of pomegranate during long-term cold storage: susceptibility to bruising and changes in textural properties of fruit.

iv. Analysing the physical, biochemical and microstructural changes in impact bruise damaged pomegranate fruit cv. Wonderful.

v. Investigating the effect of bruising and storage duration on physiological response, physico-chemical changes and antioxidant properties of pomegranate (cv. Wonderful) fruit.

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CHAPTER 2

PREHARVEST FACTORS INFLUENCING BRUISE DAMAGE OF FRESH FRUITS - A REVIEW*

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Preharvest factors influencing bruise damage of fresh fruits: A review

Abstract

Bruise damage of fresh fruit is a major problem in the horticultural industry, potentially occurring during preharvest, harvest and at all stages of postharvest handling chain. This damage can cause considerable postharvest and economic losses, reduce produce quality and result in serious food safety concerns. Understanding the factors influencing susceptibility or resistance of produce to bruising is important in developing strategies for reducing the problem. This review discusses main preharvest factors that could be manipulated by producers prior to harvest in attempts to reduce bruise damage of fresh fruits during postharvest handling. These factors include: (1) genetic (species/genotype); (2) climatic and environmental; (3) seasonal variation; (4) orchard management practices; and (5) effect of fruit properties. A critical discussion of these factors and their relative influence on bruise susceptibility of fresh fruits is presented. Among other factors, orchard management practices such as irrigation and fertilization could be an important strategy to manipulate fruit mechanical strength to enhance resistance to bruising. Future research directions are discussed.

1. Introduction

Fruits play an important role as essential part of human diets, providing essential macro and micronutrients, vitamins, dietary fibres and phytochemicals to the world's population (Li &Thomas, 2014; Hussein *et al*., 2015). The close association between the consumption of fresh fruits with many nutritional and health benefits has made produce highly recommended as health diet to fight against sedentary life style and degenerative diseases such as cancer, high blood pressure, cardiovascular diseases and ageing (Viuda-Martos *et al*., 2010; Fawole *et al*., 2012a,b; Mphahlele *et al*., 2016). Hence, the perceptions of health benefits coupled with a change in consumers' life style and increase in consciousness of healthy diet have heightened the global demand for fresh fruits and vegetables (Li *et al*., 2011; Li & Thomas, 2014). In the quest to satisfy this demand, the rapid expansion of mechanized horticulture industry to multiple digit growth has been evident (Montanez *et al*., 2010; Siddiqui *et al*., 2011). Hence, large-scale mechanization which involves large-scale planting and mechanical handling (e.g. harvesting, packaging and transport) of fruits has been necessary (Li *et al*., 2011).

Fruits have high potential to be mechanically damaged during their developmental stages and/or before harvest (Kays, 1999; Lurie, 2009). Generally, the chances that fruit can be damaged while still on the tree are quite substantial, and this can happen from a variety of sources. Several ways in which fruit can be mechanically damaged whilst on tree include (i) forceful contact of fruit with other fruit or parts of the tree such as branches during growth which may cause abrasion, puncture and bruising, (ii) predation by slugs, insects, birds and mammals can also puncture the skin and consume a portion of the tissue, and (iii) effect of weather, such as wind and hail that can aggravate damage caused by contact of fruit with other parts of the tree, causing mechanical injury such as bruising, cleavage, slip and buckling (Kays, 1999; Van Zeebroeck *et al*., 2007a). For instance, Kumar *et al*. (2016) reported an average preharvest loss of up to 30.4% in litchi fruit during sorting at harvest, which mainly comprised losses due to sunburn, cracking, bruising anthracnose, and fruit borer infestation, among others.

The most common type of mechanical damage to fruits is bruising, commonly occurring during harvesting, handling and transport (Ahmadi *et al*., 2010; Tabatabaekoloor, 2013). Bruise damage is a type of subcutaneous tissue failure without rupture of the skin of fresh produce resulting from the action of excessive external force on fruit surface during the impact, compression or vibration against a rigid body or fruit against fruit which result in cell breakage (Kitthawee *et al*., 2011; Li & Thomas, 2014; Opara & Pathare, 2014; Stropek &Gołacki, 2015). The physical evidence of bruising onto a produce is usually indicated by discolouration of injured tissue which marks the damaged spot (Blahovec & Paprštein, 2005; Opara &Pathare, 2014).

Mechanical impact or compression (due to loading) onto a biological produce provokes mechanical stress that induces cell wall and membrane rupture and hence bruising (Ahmadi, 2012). Bruise susceptibility (BS) is the measure of produce response to external loading (Opara & Pathare, 2014; Van Linden *et al*., 2006; Van Zeebroeck *et al*., 2007b; Ahmadi, 2012). Hence, the extent of dynamic or static loading onto a produce is considered the most important bruise factor, usually expressed in terms of loading or absorbed energy (Blahovec &Zidova, 2004; Blahovec, 2006). The former comprises all impacts likely to occur during harvesting and handling operations such as fruit dropping into the picking buckets or during sorting, or a vibration, mainly occurring during transportation (Komarnicki *et al*., 2016). On the other hand, static or compression loading can occur during harvesting, transportation or storage when poorly designed bins are overfilled and stacked such that the produce in the lower bins support the weight, which possibly causes damage (Thompson, 2003; Lewis *et al*., 2007; Komarnicki *et al*., 2016).

In agreement with the above hypothesis, several authors have stated that irrespective of differences both in preharvest and postharvest factors,the amount of mechanical energy applied and absorbed by produce during impact, compression or vibration is a major deciding factor on the severity of damage that occurs (Blahovec & Paprštein, 2005; Opara, 2007; Zarifneshat *et al*., 2010). Hence, this clearly shows that impacts, compressions or vibrations on produce during mechanical handling should be avoided to prevent damage (Li & Thomas, 2014). Nonetheless, while the mechanical force in contact with produce has been identified as obvious factor affecting bruising, this phenomenon is dependent on a number of other factors relating to physiological and biochemical properties of the produce on one hand, and growing environmental conditions on the other hand (Van Linden *et al*., 2006; Strehmel *et al*., 2010; Ahmadi, 2012).

Bruising of produce at the preharvest stage is uncommon and usually not easily controlled (Van Zeebroeck*et al*., 2007a). Traditionally, produce that is physically damaged before or at harvest or those with various defects are usually discarded either on the field or in the packhouse (Knee & Miller, 2002). This further exacerbates the problem as it virtually becomes difficult to quantify losses due to such damages. However, it remains pertinent to understand distinctively the difference between preharvest and postharvest mechanical damages. This could be helpful as a tool to reduce harvest losses resulting from such damages, and possible measures to alleviate the problem.

Previous research has indicated that among other factors, the agricultural production practices greatly affect the overall quality of fresh produce at harvest, after harvest and even during shelf life storage (Prusky, 2011). This could imply that, to a large extent, the quality of fresh produce depends on various factors prevailing during their growth, mainly including climate, seasonal variation and orchardmanagement practices (Opara, 2007; Tahir *et al*., 2007; Prusky, 2011). In view of that, limited studies have been conducted to ascertain the effects of preharvest factors on bruise damage of fresh fruit using different simulated impact and compression loadings.

Over the past decades, manipulation of preharvest factors had largely been the unexplored option for orchardists to reduce bruise damage, in spite of the presence of a considerable number of preharvest factors that could potentially influence the susceptibility of many horticultural produce to bruise (Shewfelt & Prussia, 1993; Mowatt, 1997). Figure 1 conceptualizes the effects of preharvest factors on the susceptibility of fruits to bruising. Instead, most efforts to reduce bruisedamage by orchardists or packhouse operators revolved around improving handling techniques from harvest, through postharvest handling activities to final retail points (Shewfelt, 1986; Mowatt, 1997). Nonetheless, there are several preharvest factors that could be manipulated quite easily by both orchardists and packhouse operators in a quest to reduce bruising of fresh fruit produce (Fig. 1). While research has put much attention on the postharvest factors that potentially influence bruising, little is known about the preharvest factors affecting bruising of horticultural fruits. This current review presents the discussion of previous research that explored various preharvest factors and their relative influence on bruise susceptibility of various fresh fruits.

2. Fruit bruising: causes and effects on fruit quality

Application of impact or compression forces directly to the surface of fruit can cause external (surface) and/or internal bruising (Li & Thomas, 2014; Opara & Pathare, 2014). External bruising is usually described by the presence of any defect(s) such as skin rupture and/or manifestation of browning in the exocarp surface of a fruit (Li & Thomas, 2014). On the other hand, internal bruising involves either damage of fruit tissues beneath the exocarp or tissues not in contact with the exocarp (Vursavus & Ozguven, 2004; Li & Thomas, 2014).External bruising of fruit is visible and therefore can be quantified eitheras diameter by assuming the circular shape of the visible bruisedamage (Vursavus & Ozguven, 2004) or as an area that assumescircular or elliptical shape of the bruise (Pang *et al*., 1996; Bollen,2002). Fruit defects due to external bruising might be eliminated during sorting and grading or processing, hence leading to rejection and price adjustment requests by buyers and receivers in both domestic and export markets (Grant & Thompson, 1997).

The formation of external bruising is associated mainly with the breakage of cell structures and the failure of membranes (Lee *et al*., 2005; Rinaldo *et al*., 2010). Damage of cells and fruit tissue initiates the contact between polyphenol oxidase (PPO) and peroxidase (POD) cytoplasmic oxidizing enzymes and phenolic contents originally stored in the vacuole (Billaud *et al*., 2004; Jiménez *et al*., 2011). In the presence of oxygen, the enzymatic oxidation in the damaged cells transforms phenolic substances into quinones, which polymerize to form dark/ brown pigments on the damaged part of the fruit (Lee *et al*., 2005; Franck *et al*., 2007; Holderbaum *et al*., 2010). The formation of brown pigment on the surface of the damaged region provides the external sign of an impact or compression bruising (Van Linden & De Baerdemaeker, 2005; Opara & Pathare, 2014). The difference in the concentration of phenolic contents and the activity of oxidizing enzymes between the fruit exocarp, mesocarp and endocarp tissues makes the browning inhomogeneous (Rinaldo *et al*., 2010; Li & Thomas, 2014). For instance, in fruits such as litchi, the phenolic content and oxidizing enzymes (PPO and POD) activity are higher in external tissues, and hence the browning predominantly occurs on the external surface of the fruit's bruise damaged region. On the contrary, other fruits such pear, tomato and longan, the phenolic content and PPO and POD activity are lower in the external tissues and therefore browning occurs internally (Casado-Vela *et al*., 2005; Quevedo *et al*., 2009).

Unlike external bruising, the internal bruising on fruit is characterisedby hidden damage and hence easily overlooked and difficult to measure. Shewfelt (1986) described internal bruising as 'latent damage'suggesting that damage is usually incurred at one step in a postharvest system but not apparent until a later step in the handling chain. Internal bruising is traditionally estimated or measured by assuming a non-visible shape of an internal damage (Li & Thomas, 2014). The shape for an internal bruising is either assumed as spherical (Ahmadi *et al*., 2010; Ahmadi, 2012), an elliptical cone (Bollen, 2002; Shafie *et al*., 2015), or an ellipsoidal shape (Lu & Tang, 2012; Kitthawee *et al*., 2011). Measurement of such dimensions as diameter, width and depth of the bruised tissues using digital callipers is usually followed by calculation of the bruise volume (BV) or bruise area (BA) of an internal bruising (Ahmadi *et al*., 2010; Kitthawee et al., 2011; Shafie*et al*., 2015).

The symptoms of internal bruising can develop into more severe external blemishes over time; the changes that are usually accompanied by a number of serious quality hazards (Lee, 2005). Overall, the onset of either external or internal bruising could hasten the deterioration of fruit while detracting fruit from the cosmetic appearance and saleability (Grant &Thompson, 1997; Brosnan & Sun, 2004). Internal bruising critically affects the quality attributes of fresh fruits such as a firmness, sugar content and acid content (Montero *et al*., 2009; Alfatni *et al*., 2013). Researchers have demonstrated that the presence of bruise damage on freshly harvested produce significantly affects some physiological processes such as respiration and moisture loss through injured skin (Aktas *et al*., 2008; Elshiekh & Abu-

Goukh, 2008; Kumar *et al*., 2016). In particular, change in metabolic processes such as ethylene production, relative electrical conductivity, respiration and transpiration usually leads to produce a mass loss, senescence and spoilage as well as loss of nutritional value (Elshiekh & Abu-Goukh, 2008; Li *et al*., 2011).

Furthermore, bruise damage of fresh fruit is not limited to visual aspects but may also accelerate other biological processes such asmicrobial spoilage (Moretti *et al*., 1998; Prusky, 2011; Eissa *et al*., 2013). Fruit bruising aggravates the risk microbial contamination (Blasco *et al*., 2003; Van Zeebroeck *et al*., 2007b; Prusky, 2011), hence providing potential causes for fruit quality losses and lower shelf-life. Postharvest rots and decay are more prevalent in bruised or otherwise mechanically damaged fruits and vegetables than in undamaged produce (Wilson *et al*., 1995). Decay pathogens such as bacteria and fungi easily enter through dead or wounded tissues before contaminating the rest of the fruit or vegetables, resulting in significant losses during storage and long-distance transportation (Blasco *et al*., 2003; Van Zeebroeck *et al*., 2007c; Pholpho *et al*., 2011). This buttresses thefindings reported by Wilson *et al*. (1995), where bruised plums had 25 % decay severity in comparison to 1.3 % decay severity observed for unbruised prunes during storage. This could suggest that the problem of postharvest loss due to decay and subsequent economic losses could be reduced by applying proper measures to reduced bruise damages bothat prior to harvest, at harvest and during subsequent postharvest handling.

3. The economic importance of bruise damage to fresh fruit – an overview

The fruit industry suffers considerable economic losses annually due to bruising and other physical injuries of fruits occurring before and after harvest (Montero *et al*., 2009; Ghaffari *et al*., 2015). Bruise damage occurring between the point of harvest and consumption contributes the most to the decrease of fruit quality, reduce the market value and ultimately leading to significant reductions in potential revenue (Yurtlu & Erdogan, 2005; Ahmadi *et al*., 2010; Saracoglu *et al*., 2011). Extensive research has revealed a high incidence of fruit bruising during harvesting and grading. Impacts, vibration and compression during harvesting, transport and handling cause bruise damage to fruit (Eissa *et al*., 2008). The consequence of these damages is low grade and low quality fruits, hence less income to both growers and packers (Timm *et al*., 1996; Eissa *et al*., 2008). A recent study by Jiang et *al*. (2016) reported that fruit bruising causes around 10 % of total economic losses of the blueberry industry in the U.S. every year. In 2006, losses in fresh fruit due to bruising rose by

17 % during transport and distribution in Japan (Ministry of Agriculture, Forestry and Fisheries, 2008).

Reports suggest that bruising potentially limits the production of quality fruit and hence contributing to postharvest losses of fresh fruit (Van Zeebroeck *et al*., 2007a; Polat *et al*., 2012; Opara & Pathare, 2014). Timm *et al*. (1989) reported as high as 81 % of bruised apples during harvesting, 93 % after transporting, and 91–95 % caused by bagging, all using manual harvesting systems. Valenciano Garcia (1990) examined apple and pear fruit samples at retail stores and revealed that bruise damage was responsible for 50 % of the total damage observed, out of which 10–25 % of the observed total class-rejection damage was due to bruises in pears alone. Similarly, Kampp and Nissen (1990) conducted research in the Danish market to examine fruit samples that meet the European Community (EC) quality standards at the retail level. Results showed that more than 20 % of strawberries; 20 % of peaches and nectarines had pressure or impact damages; and about 95 % of the apples did not comply with the EC standards forbruises due to either pressure or impact bruising.

In a recent study, Kumar *et al*. (2016) assessed postharvest losses of litchi at various stages of supply chain in India and observed that the mean loss due to mechanical damages (bruised and compressed fruit) at the wholesale level in the market was between 15.8 and 12.4% of the total losses recorded during the 2012 and 2013 seasons, respectively. Overall, this magnitude of losses suggests that attempts to reduce in the fruit industry could provide an annual payback of millions of dollars (Baritelle & Hyde, 2001). Overall, the discussion presented under this section has enlightened that a higher degree of fruit bruising occurs at harvest and during postharvest handling (especially grading, packing, and transportation/n distribution). However, during the fruit developing on the plant, a widerange of factors can modulate the way fruit respond to various mechanicalloading conditions at harvest and or during postharvesthandling. Therefore, an attempt to reduce the problem of fruit bruisingand associated economic losses after harvest, an alternative strategywould be for orchardists to identify and control preharvest factors thatcan influence the fruit susceptibility to bruise. Control of these factorscan potentially influence the change in composition and structure andmodify the mechanical strength of fruit, and consequently, that could possibly influence the fruit susceptibility to bruise (Mowatt, 1997; Tahir *et al*., 2007).

Studies have confirmed that there is a scope to manipulate preharvest factors to reduce bruising incidence. Several research have shown that control such factors like cultivar and/or rootstock (Menesatti *et al*., 2001; Tahir, 2006; Stropek & Gołacki, 2013; Jiménez *et al*.,

2016) and climatic conditions during growing season (Mowatt, 1997; Tahir *et al*., 2009; Lv *et al*., 2016) have the potential to reduce bruise damage severity and incidences. In addition, better developed orchard management practices such as fertilizer applications (Pasini *et al*., 2004; Opara, 2007), mulching (Tahir *et al*., 2005; Eckhoff *et al*., 2009), pruning/canopy management (Mowatt, 1997; Tahir *et al*., 2007) and foliar sprays and irrigation/fertigation (Garcia *et al*., 1995; Opara, 2007; Tahir *et al*., 2007; Eckhoff *et al*., 2009) offer potential gains not only in improved fruit yield, fruit maturity and quality, but also in modulating the fruit resistance to mechanical bruising (Mowatt, 1997; Tahir *et al*., 2005, 2007; Opara, 2007). Particularly, these authors have established that the relationships between the investigated preharvest factors and the fruit susceptibility to mechanical bruising clearly offer a suitable approach to reduce the high incidence of postharvest and economic losses of horticultural commodities resulting from bruise damage.

4. Preharvest factors influencing bruising

4.1. Genetic factors

Genotype (cultivar and/or rootstock) has an important role in bruise susceptibilities of many fruits (Li *et al*., 2010; Buccheri and Cantwell, 2014; Lv *et al*., 2016). In their recent review, Opara & Pathare (2014) stated that cultivar differences account for most of the differences in bruise susceptibility among various fruits. The previous review by Kays (1999) has noted that fruit cultivar and/or genetics could influence the produce susceptibility to mechanical bruising, in addition to other produce physical characteristics such as appearance, shape, size andcolour. There is a general agreement that bruise susceptibility is a function of one or a combination of several physico-mechanical properties of fruit. Such properties include firmness and turgidity (Garcia *et al*., 1995; Opara *et al*., 1997; Opara *et al*., 2007; Tabatabaekoloor, 2013), peel hardness, peel thickness, and water content (Opara *et al*., 1997; Studman et al., 1997; Van Linden *et al*., 2006; Bugaud *et al*., 2014). These parameters affect the mechanical properties during impact or compression of fruits, such as deformation energy, bioyield point force, toughness, rupture force and energy absorbed by the fruit and vegetable upon rupture (Ozturk *et al*., 2010; Ekrami-Rad *et al*., 2011; Polat *et al*., 2012). Hence, this could suggest that bruise susceptibility may also differ between cultivars of same fruit based on the differences in the aforementioned physico-mechanical properties among cultivars.

Studies in pome fruits described the hysteresis loss and modulus of elasticity as important characteristic descriptors used to measure the bruise susceptibility among pear and apple fruit cultivars (Blahovec & Paprštein, 2005; Van Zeebroeck *et al*., 2007b). Hysteresis loss defines energy dissipated due to the internal friction and/or cellular structure destruction (Ciupak & Gładyszewska, 2011), resulting from the mechanical vulnerability of fresh produce such as cracking, puncture or bruising. Accordingly, these parameters could be different from one cultivar of the same species to the other (Blahovec & Paprštein, 2005; Ciupak & Gładyszewska, 2011; Param & Zoffoli, 2016). Blahovec *et al*. (2003) stated that the higher the hysteresis loss suffered by produce due to mechanical stress, the less susceptible is the tested fruit cultivar to bruising. In contrast, as modulus of elasticity correlates negatively with hysteresis loss (Blahovec & Paprštein, 2005), an opposite association between modulus of elasticity and susceptibility to bruising is expected, such that the increase in values of modulus of elasticity resulted in high bruising susceptibility of the tested cultivar (Van Zeebroeck *et al*., 2007b). Research by Ozturk *et al*. (2010) identified wide differences among the apple cultivars 'Granny Smith', 'Golden Delicious' and 'Starking Delicious' in terms of rupture force, toughness and absorbed energy during compression that could also affect their susceptibility to bruising.

In support of aforementioned observations, the measurement of impact related to bruising in apples recently reported by Stropek & Gołacki (2015) revealed that at each impact velocity, 'Florina' hard cultivar had lower values of permanent deformation than the 'Freedom' soft cultivar. Overall, the differences in mechanical properties could be due to differences in response to loading and absorbed energies among apple cultivars that could subsequently affect the fruit susceptibility to bruising. This conclusion is strengthened by Montevecchi *et al*. (2012) who reported that both the firmness and compressibility of peach fruit were cultivar dependent. Chen *et al*. (1987) have previously observed that the impact of a given energy level will result in higher maximum stress in firmer (high modulus of elasticity) fruit than a soft one. In their research to determine the impact and compression damage of Asian pears, Chen *et al*. (1987) concluded that the cause of tissue failure and subsequently large bruise depth in firmer 'Chojuro' pears fruit cultivar was mainly due to excessive stresses in the fruit, in comparison to moderate stresses received by soft fruits cultivars of 'Twenties Century', 'Ya Li' and 'Tsu Li'. This was in contrast to Param and Zoffoli (2016) for other types of fruits. The authors reported the lowest bruising values (expressed as bruise damage indices of an arbitrary 5-point scale, 0–4) in sweet cherries

'Bing' (0.81) and 'Regina' (1.57) and the values were closely correlated to the fruit's highest values of modulus of elasticity of 2.59 and 2.67 MPa, respectively.

Fruit structural traits such as cuticle thickness, fruit size and firmness have also been suggested as cultivar dependent factors influencing differences in bruise susceptibility of many fruits such as apples, olive, peach and nectarine (Maness *et al*., 1992; Menesatti *et al*., 2001; Hammami & Rapoport, 2012; Stropek & Gołacki, 2013; Jiménez *et al*., 2016). Research findings have revealed that tomato bruising is dependent on cultivars and associated differences in structure and composition of fruit (Van Linden *et al*., 2006; Li *et al*., 2010; Buccheri & Cantwell, 2014). Earlier observation by Maness *et al*. (1992) indicated that fruit firmness influenced peak impact force by altering the tissue's response to impact. Accordingly, the adjacent cells of a less firm fruit are inclined to slide more easily with respect to each other, hence providing a cushioning effect on the fruit during impact with a net result of increased contact time with the impacted surface.

In a study comparing bruise response of seven drupe cultivars (5 peaches and 2 nectarines) to impact bruising, it was observed that differences in fruit responses were attributed to the observed difference in fruit firmness between cultivars (Menesatti *et al*., 2001). The comparison made between the two fruits of the same species identified nectarines of both cultivars studied (Weinbergerand Nectaross) as more resistant to impact bruising than all peach fruit cultivars (Maycrest, Domiziana, Roza, Flaminia and Emilia). Furthermore, the authors also observed the differences in bruise susceptibility between peach cultivars. It was clearly indicated that the difference in fruit firmness was significant among five cultivars of peaches investigated, an observation which underlined a well-known effect of firmness on bruising susceptibility (Menesatti *et al*., 2001). Likewise, lower susceptibility to impact bruising in table olive fruit of 'Hojiblanca' cultivar compared to that of other cultivars 'Manzanilla' and'Gordal Sevillana' both at high and medium impact energy levels was also in part attributed to its greater fruit firmness (Jiménez-Jiménez *et al*., 2013).

Consistent with earlier reports, Stropek and Gołacki (2013) assessed the effects of impact velocity on bruising of three apple cultivars('Rubin', 'Rajka' and 'Freedom') and revealed that, for almost all impact velocities 'soft' 'Freedom' apple cultivar had the lowest BS measured as bruise depth whereas the 'hard' apple 'Rajka' cultivar had the highest. Further results revealed that the later was characterized by the highest firmness (hard flesh), and thus the largest bruise depth, whereas the former showed the opposite. In contrast, measurements of bruise surface area of tested apple cultivars showed that 'Rajka' apples had the lowest value, while soft 'Freedom' apples had the highest for the same value of impact velocity (Stropek & Gołacki, 2013). The response of avocado fruit cultivars to vibration bruising was found to be dependent on the fruit flesh firmness (Arpaia *et al*., 1987). At flesh firmness level less than 11.3 kgf, 'Fuerte' cultivar fruit exhibited greater internal injury from 20 min vibration (vibration injury was produced by accelerating avocados at 1.1–1.2 × g for 20 min) than 'Hass' or 'Pinkerton'cultivars, whereas at flesh firmness levels of 0.45 kgf, all fruit exhibited severe injury after 20 min vibration. Furthermore, Arpaia *et al*. (1987) reported little or no external evidence of bruise damage in avocado (Fuerte, Hassand Pinkerton) fruit of high flesh firmness (> 6.8 kgf) injured by impaction at 23, 46 or 92 cm drop heights. Overall, their results highlight that despite genetic differences in avocados, the effect of fresh firmness could potentially surpass the effect of cultivar difference.Fruit size differences may also explain some differences between cultivars susceptibility to bruising. Maness *et al*. (1992) assessed the variables related to bruise resistance for four peach fruit cultivars by impact test and revealed that irrespective of ripeness stage, peach of'Ranger' cultivar which was noticeably smaller appeared to absorb less total energy. At similar ripeness stage, impact energy absorbed by 'Ranger' was in the range of $35 - 130 \times 10^3$ J for the lowest and highest impact, respectively, comparatively lower than energy absorbed by other three cultivars, 'Topaz', 'Glohaven', and 'Elberta', which ranged from 44 to 190×10^3 J. The above findings is strengthenby Ericsson and Tahir (1996) who also noted large sized apple fruit'Aroma' were more prone to bruising with an increment of about 5–40 %more bruise weight percentage for various drop heights than smallersized 'Ingrid Marie' (10–20%), and 'Cox's Orange Pippin' (4–13%). Theauthors attributed this bruise susceptibility difference to higher impactenergy during dropping in larger fruit than smaller fruit.

A few researchers have obtained conflicting results on the influence of firmness on bruise susceptibility. According to Tabatabaekoloor (2013), selected engineering properties and bruise susceptibility of peach cultivars, 'Elberta' and 'Spring Time' had a significant difference in firmness and compressibility. Nonetheless, despite the slight differences in impact energy due to difference in fruit mass between cultivars, the measured BA and BV were not significantly affected by cultivar at all impact levels (0.051J, 0.103J and 0.155J for 'Spring Time', and 0.065J, 0.129J and 0.194J for 'Elberta' and impact surfaces, fruit, steel and rubber. Ericsson and Tahir (1996) noted no relationship between fruit firmness and bruise susceptibility of three apple cultivars, 'Aroma', 'Cox's Orange Pippin' and 'Ingrid Marie'.

Similarly, Bollen (2005) investigated the effect of cultivar on bruising of 'Royal Gala', 'Braeburn', 'Granny Smith', and 'Splendour' cultivars of apple fruit to establish the main influencing factors among many others on the susceptibility to bruising. Using logistic susceptibility analysis, results showed that for a low level of bruises (> 1 cm^2) which was described as the commercial grading threshold, 'Splendour' cultivar was the most susceptible (0.03 J), followed by 'Granny Smith' (0.04 J). The bruise susceptibility difference was slight but significant when compared with the least susceptible cultivars 'Braeburn' and 'Royal Gala' which had mean values of bruise energy (~0.06 J).

The difference in bruise damage response between fruit cultivars is also being ascribed in part to specific cultivar differences in levels of phenolic substrates, phenolic enzyme activity or both (Arpaia *et al*., 1987; Valentines *et al*., 2005; Rinaldo *et al*., 2010). Imeh and Khokhar (2002) emphasized that various apple cultivars have the same polyphenol oxidase activity, substrate specificity and same pH optimum but differ in their content of polyphenols. Rinaldo *et al*. (2010) suggested that differences in bruise susceptibility among cultivars of banana could potentially depend on differences in enzyme activity linked to polyphenol metabolism including both phenylpropanoid synthesis and oxidation of polyphenols. Similarly, an early research by Golan *et al*. (1977) measured substantial differences in the phenolic activity of the avocados 'Fuerte' and 'Lerman' cultivars. Ericsson and Tahir (1996) investigated the bruise susceptibility of apple cultivars 'Aroma', 'Cox's Orange Pippin' and 'Ingrid Marie' grown in Sweden during two successiveseasons. Results of bruise measurement in bruise diameter and BA following drop impact at selected drop heights showed that 'Cox's Orange Pippin' fruit was the more resistive against bruising whereas'Aroma' showed the highest bruise susceptibility of the three cultivars. Moreover, Ericson (2004) reported that apples of 'Aroma' cultivar had ahigher content of polyphenols than 'Ingrid Marie' cultivar.

In addition to differences attributed to fruits' structure, several types of researchin bruising of drupe and pome fruits have reported differences in bruise susceptibility between cultivars, which further buttressed the role played by endogenous phenolic substrates in bruising. For instance, Lv *et al*. (2016) established that irrespective of the harvest season, apple fruit of 'Aroma' cultivar had a larger BA at harvest time compared to 'Discovery' and 'Gloster' cultivars, while 'Discovery' showed higher sensitivity to bruising than 'Gloster'. Similar observations were reported in nectarine fruit by Bollen *et al*. (2001), where cultivar 'Fantasia' was more susceptible to bruise damage than 'Firebrite' fruit by 68 % from the same harvest season and all subjected to same bruising conditions. Further findings by Lv *et al*. (2016) showed the decrease in bruise diameter in 'Aroma' cultivar during cold storage of apple fruit, whereas no differences were found for 'Discovery' and 'Gloster' cultivars at the same levels of impact. Tahir (2006) revealed that irrespective of postharvest treatment, apples of cultivar 'Aroma' had the highest bruise weight percentage (2 %) (i.e., percentage of bruise weight to fruit weight) susceptibility than cultivars 'Cox's Orange Pippin' (1.5 %), and 'Ingrid Marie' (1.75 %).

Physico-chemical and rheological properties of fruits influence bruise susceptibility and have been reported to depend on the fruit cultivar (Rinaldo *et al*., 2010; Bugaud *et al*., 2014; Param & Zoffoli, 2016). It is hypothesized that the first step to bruising is initiated by a decrease in peel resistance to mechanical damage due to loss of cell and membrane integrity (Bugaud *et al*., 2014; Jiménez *et al*., 2016). Jiménez *et al*. (2016) investigated the impact bruise susceptibility of two table olive fruit cultivars, 'Manzanilla de Sevilla' and 'Hojiblanca' and noted that the cell damage in the skin (cell rupture, loss of cell wall thickness) is associated with the bruising effect. Through quantitative measurement of parameters related to such damage in olive fruit cells (i.e., the area of the damaged zone, number and position of the damaged cells in the mesocarp) the authors proposed that the total damaged area (TDA) and number of tissue ruptures provided a better estimate of susceptibility to bruising among cultivars of table olives. They hence concluded that TDA in 'Manzanilla de Sevilla' was significantly larger (20.91 mm^2) than 'Hojiblanca' (16.58 mm^2).

In agreement with the previous arguments, Bugaud *et al*. (2014) attributed the difference in bruisesusceptibility between banana cultivars 'French Corne', 'Grande Naine' and 'Flhorban925'to their differences in the rheological properties, mainly the membrane permeability. At the same impact condition, it was observed that 'French Corne' bananas were susceptible to bruising (20 mJ). The difference in bruise susceptibility was attributed to the differences in membrane permeability between bananas cultivars studied such that the most bruise-prone (cultivar 'French Corne') displayed the highest membrane permeability during ripening (Bugaud *et al*., 2014). Interestingly, with the exception of peel electrolyte leakage which appeared to be the best physico-chemical indicator, the rest of the parameters (fruit peel hardness, peel thickness, and water content) could not sufficiently serve asdistinguishing indicators for bruise susceptibility between investigated cultivars of bananas. Differences in rheological properties between banana cultivars, specifically those relating to cell wall strength could help in better understanding of existing differences in genotypes with respect to their susceptibility to bruising (Bugaud *et al*., 2014; Param & Zoffoli, 2016). A recent study by Param and Zoffoli (2016) revealed that structural and rheological properties of the mesocarp and epidermis of six sweet cherry fruit cultivars of 'Bing', 'Lapins', 'Regina', 'Santina', 'Sweetheart' and 'Van' correlated with their mechanical damage sensitivity. In addition, the authors noted the differences in terms of force necessary to cause cell rupture, as marked by the bioyield point among different cultivars. Table 1 summarizes the cultivar/genetic differences on bruise susceptibility for various fruit crops.

4.2. Climatic factors

Prevailing climatic conditions during growing season such as sun exposure (light), temperature and rainfall or water availability are known to influence the bruise susceptibility of fresh fruit, although there is limited information in the literature, with very few reports focusing on apple fruit as shown in Table 2. Prior to harvest, long and intensive sunlight and its associated high temperature have a considerable impact on fruit sensitivity to bruising due to a number of causes as previously cited by several researchers. Woolf and Ferguson (2000) reported that sun-exposed sides of avocados showed greater flesh firmness that potentially affected bruise susceptibility of fruit. In addition to enhanced water loss and increased firmness of fruit, sun exposure of fruit is attributed to the formation of thicker and more numerous wax clumps that contribute to cushioning and hence less bruise susceptibility on the sun-exposed side as opposed to the shaded side of the fruit (Tahir *et al*., 2009). Tahir *et al*. (2009) observed sun exposure within the tree canopy affected the sensitivity of four apple fruit cultivars 'Aroma', Ingrid Marie' and two red cultivars 'Amorosa' and 'Karin Schneider' to bruising. 'Aroma' and 'Ingrid Marie' cultivars were more sensitive (larger BV) to bruising than the respective red 'Amorosa' cultivar (by 15% on the shaded side and 12% on the sun exposedside) and 'Karin Schneider' cultivar (by 14% on the shaded sideand 10% on the sun-exposed side).

Similarly, Lv *et al*. (2016) investigated the effect of sun exposure among other aspects, on bruising of three apple cultivars, 'Discovery', 'Aroma and 'Gloster' in two successive seasons. The authors observed that bruised area on the sun-exposed side of 'Aroma' apples measured as bruise diameter was smaller (25.9 mm) than on the shaded side (27.1 mm), irrespective of the harvest season. However, 'Gloster' apples had the bruised area of sun-exposed side smaller (19.4 mm) than their respective shaded side (19.8 mm) for only the first harvest season. Further results revealed that the bruised area of 'Discovery' apples did not change between season for both sun-exposed and shaded sides. This highlights the need for creating an adequate canopy for the trees via training system and/or pruning to enable equal and adequate distribution of sun-exposure to a growing fruit.

Mowatt (1997) elaborated that prior to harvest, transpirational water losses from the tree leaves can exceed xylem inflows during warm days, and this phenomenon forces the tree to draw water from the fruit. A previous investigation has found that fruit can lose as much as 1% of their early-morning weight by mid-day and proposed that this weight loss would likely cause reductions in turgor. While water deficits early during fruit growth cause reductions in osmotic potential and hence maintenance of turgor, late season water deficits do not induce the same fruit osmotic adjustments (Mills *et al*., 1996). It is presumed that this reduction in fruit turgor leads to a consequential reduction in tissue sensitivity to bruise damage. In view of this, orchardists have often noted that fruits are most susceptible to bruising if harvested early in the morning which could be explained by low fruit temperature and/or higher water content of fruit at that time (Zhang *et al*., 1992; Mowatt, 1997). Similarly, in the study reported by Banks and Joseph (1991), it was revealed that bananas of the cultivar'Robusta' harvested in the early morning had an 18% greater compression bruise threshold than those harvested later in the day. This concurs with the previous findings that have reported that turgor of fruit in the field declines from early morning to midday (Banks 1990; Mowatt, 1997), which result from temperature changes within the fruit cortical tissue. Mowatt (1997) found that 'Golden Delicious' apples harvested later in the day were less susceptible to bruise damage (7.3%) than those harvested early in the morning. The author attributed less sensitivity of fruit to bruising during the day to the elevated temperatures and reduced water status.

Temperature could be the second most influencing factor associated with day-time effects on bruise susceptibility after water status (Mowatt, 1997), and the two factors are inter-related. Temperature influences the physico-mechanical properties of the fruit and its bruise susceptibility (Hertog *et al*., 2004; Ahmadi *et al*., 2010). However, depending on the type of fruit and its physiological status, the relative contributions of temperature and the mechanical strength of the cell wall might vary from fruit to fruit (Ahmadi *et al*., 2010). It is on that basis that reports of temperature effects on bruising are quite inconsistent. Nonetheless, most of the reports available have focussed on the postharvest and storage effects of the fruit temperature on bruise susceptibility (Garcia *et al*., 1995; Mowatt, 1997;

Baritelle & Hyde, 2001; Ahmadi, 2012; Ahmadi *et al*., 2014). Possibly, this could be due to difficulties in determining the influence of climatic conditions especially temperature and water status on fruit bruising prior to harvest (Mowatt, 1997).

Prior to harvest, fruit is usually exposed to considerable fluctuations in temperature from low (cold) night-time temperatures to high daytime temperatures. The role of water status for fruit firmness is revealed through the reversible physical effect that temperature has on firmness as previously reported in apple (Johnston *et al*., 2001) and kiwifruit (Jeffery &Banks, 1994). The increase and decrease in fruit temperature cause the water inside the fruit to expand and contract in volume, a phenomenon comparable to that of turgor. As a result of increased cell tension due to increased turgor or temperature, both the stiffness and elastic modulus of fruit tissue will increase (Johnston *et al*., 2001; Hertog *et al*., 2004). As stated earlier, the elastic modulus decreases with increasing fruit temperature, and the former is positively related to bruise damage (Blahovec & Paprštein, 2005; Ahmadi *et al*., 2010). Nonetheless, fruit temperature might also have the opposite effect on tissue stiffness and/or fruit firmness. The decrease in fruit temperature will tend to increase the viscosity of the cell walls, an effect that might cause the cell walls to become more brittle and stiff while reducing the cell wall strength (Hertog *et al*., 2004; Ahmadi *et al*., 2010).

4.3. Seasonal variation

Variation between growing seasons in fruit characteristics is possibly attributable to the interaction of numerous factors, such as climatic conditions, the maturity of plantings and several management changes by orchardists (Mowatt, 1997, Opara, 2007). Consequently, these factors, alone or in combination influence the cell number, size, contents and strength that subsequently affect the produce ability to absorb impact energy with or without damage (Mowatt, 1997; Mitsuhashi-Gonzalez *et al*., 2010). There is limited information on the effects of growing season on bruising of fruit. Nonetheless, a few available reports predominantly on apples have shown that variation in growing season has the potential to influence the fruit susceptibility to bruise as presented in Table 3.

Bollen *et al*. (2001) observed that 'Braeburn' and 'Granny Smith' apples from the previous season was more susceptible to bruising thaneither 'Royal Gala' or 'Braeburn' apple cultivars from the succeedingharvest season. The authors identified the difference in fruit maturity, in addition to the between-season variation of harvested fruits as mainfactors that potentially influenced bruise susceptibility among studied cultivars of apples. A later study by Bollen (2005) investigated major factors causing variation in bruise susceptibility of apples grown in New Zealand and reported a huge difference in bruise susceptibility between growing seasons for three apple cultivars, 'Granny Smith', 'Royal Gala' and 'Braeburn'. The impact energy required to produce the same bruising levels in different seasons differed by up to 35% for 'Granny Smith' over 3 years, 48 % for the 2 years of 'Royal Gala', and 20 % for the 2 years of 'Braeburn'. Consistently, Lv *et al*. (2016) observed the larger BA in 'Gloster' apples in the later season than the previous season both at harvest and after cold storage, whereas the BA of 'Aroma' apples was only larger after storage. Further results from the study by Lv *et al*. (2016) revealed that while 'Discovery' had higher bruise depth (21.4 mm) than 'Gloster' (19.4) in the first season, the succeeding season revealed higher bruise depth (22.7 mm) for'Gloster, than the previous season (19.4 mm).

In a study that aimed at investigating factors influencing the bruising susceptibility of apples, Johnson and Dover (1990) tested'Bramley's Seedling' cultivars during six years in 24 commercial orchards. Results from their study reported a 23 % difference in mean BS between-season at both maximum and minimum standard impact bruise diameters. Similarly, working with New Zealand grown 'Granny Smith' apples, Mowatt (1997) generated bruise (measured as bruise diameter i.e., the percentage of bruise diameter to fruit diameter, PBD) using an instrumented pendulum to produce standard impact and observed 2.8 % difference in mean bruise diameters in between-season during the two-year study.

Mitsuhashi-Gonzalez *et al*. (2010) reported that the degree to which bruising occurred in 'Golden Delicious' apple cultivar at both green and white peel stages was seasonal dependent. The authors revealed the variation of bruise volume with peel colour significant, especially at the green and white peel stages. Their results showed that bruise susceptibility of apples harvested at the green (500 mm3) and white colour (1100 mm3) in the first season was less than apples harvested at respective colour stages by 77.3 % and 64.5 %, respectively in the succeeding season. This difference among seasons was ascribed to the environmentally-induced differences in tissue characteristics since cell division and cell number influence fruit tissue characteristics. It could also be argued that alternating cropping pattern, which occurs often in apples, may have contributed to the difference among seasons.

4.4. Orchard management practices

Several studies have identified activities such as irrigation, cultivation practices, use of fertilizers and other orchard management practices influence several quality attributes of fresh produce (Opara, 2007; Eckhoff *et al*., 2009; Prusky, 2011). Overall, such practices influence the fruit characteristics such as mass, maturity, tissue structure and composition, which consequently affect the sensitivity of fruit tissue to bruising (Mowatt, 1997; Opara, 2007; Prusky, 2011). The information available in the literature on various orchard management practices and their impact on bruise susceptibility are mostly covering various cultivars of apple fruit as presented in Table 4.

Optimal fertilization plays a key function in obtaining high yield and quality of crops (Rezk *et al*., 2005), but could also impact both the mechanical strength and physico-mechanical properties of fruit tissues during growth and development (Opara *et al*., 1997). Pasini *et al*. (2004) investigated the effect of different fertilization systems (conventional fertilization or soil surface application and fertigation or application of mineral hydro-soluble compound fertilizers) on the susceptibility to bruising at low and high impact level for the two apple fruit cultivars, 'Fuji' and 'Gala'. Overall results revealed that fertilisation systems influenced bruise damage resistance of 'Fuji' and 'Gala' apples. The authors reported that conventional fertilization using nitrogen N (8 gm^{-2}), phosphorus P (2.5 gm^{-2}), potassium K (10 gm^{-2}) and magnesium Mg (0.1 gm^{-2}) induced high susceptibility to darkening and fracturing of the apples. In contrast, fertigation using similar hydrosoluble mineral compound fertilizers at a reduced rate with and without K caused the lowest susceptibility to bruise damage in the two-short stored apple cultivars while highest damages were observed in the 'Fuji' apples stored for a long period.

Similarly, Tahir *et al*. (2007) observed a positive correlation between bruise occurrence and N, K, and K/Ca content of 'Aroma' apple cultivar. The effect of fertigation using minerals influenced the susceptibility of apple fruit to bruising (high bruise diameter percentage, BDP) and fruit coloration (lower hue, h°). Further findings revealed that increasing N fertigation to moderate or excess levels increased fruit N and K content, while decreasing Ca content and maintaining the content of Mg, hence enhancing higher bruise susceptibility of apple fruit. Eckhoff *et al*. (2009) investigated the influence of foliar fertilization (application of liquid fertilizer with Ca, Mn or Zn directly to plant leaves) and storage conditions on the bruise sensitivity of 'Braeburn' and 'Jonagold' apple cultivars. Their results revealed that foliar fertilization had no effect on the bruise susceptibility of both cultivars.

Tahir *et al*. (2005) studied the effects of different ground cover material systems (GCMS) on 'Aroma' apple fruit maturity, quality, and fruit resistance to bruising and decay.

From their study, it was deduced that application of GCMSs generally improved fruit resistance to bruising, such that fruit from trees with mechanical cultivation havingmore sensitivity than fruit from orchard managed with GCMSs or herbicides. This contrasted with results found in conventional mulching methods. Soil mulching with aluminium or bark lowered bruise occurrence by 15 % to 20 % in comparison with conventional mulching (mechanical cultivation or use of chemical herbicides). Additionally, soil covered with a black plastic (polypropylene) sheet slightly improved the fruit resistance to bruising with visible effect manifesting only for some growing seasons.

In another study, Tahir *et al*. (2007) established a good relationship between pruning time of apple trees of ‘Aroma’ cultivar and some fruit quality parameters including fruit flavour, decay and resistance to bruising. Results from their study revealed that summer pruning decreased storage decay by 60 % while improving fruit resistance to bruising, in comparison with other pruning dates of winter that decreased BDP by 15 % only. Furthermore, little changes in firmness and flavour quality observed in summer pruned fruit, while winter or early autumn pruned ones reduced bruise resistance (BDP) further below 15 %. With respect to fertigation application, it was found that fruit from trees fertigated with N in any level had decreased resistance to bruising. Excess N fertigation had even more negative effect as it increased BDP by 50 %, while moderate fertigation increased it only by 16 %.

Water status of harvested fruit is an important factor influencing its susceptibility both to mechanical damage and decay during storage (Benkeblia & Tennant, 2011). Prior to harvest, water status of fruit potentially affects the water stress that may lead to soft or dehydrated fruit as the result of a change in turgidity (Banks & Joseph, 1991; Opara *et al*., 2007). The produce water status may be influenced by irrigation, rainfall and/or leaf transpiration rates whilst fruit are still on the tree (Mowatt, 1997). The influences of irrigation (frequent versus none), crop load (high versus low), and fertilization (1 % foliar urea sprays versus none) on bruise sensitivity of mature ‘Gala’ apple fruit picked on three harvest dates was evaluated by Opara (2007). From this study, the author showed that orchard management practices generally affected bruise size, both the bruise diameter, bruise depth and bruise volume (BV), such that the two parameters increased consistently with frequent irrigation in both early and late harvested fruit by 6.35, 1.54 and 4.18 %, respectively. On the other hand, the author found that the effect of crop load and nitrogen fertilization was either marginal or insignificant and inconsistent across the three harvest dates. Further results highlighted that two indices used to describe bruise sensitivity, the general bruise susceptibility index (GBS, $mm^3\ J^{-1}$), and specific bruise susceptibility (SBS, $mm^3\ J^{-1}\ g^{-1}$) varied significantly between the effects of orchard management practices. While GBS was influenced by frequent irrigation at each harvest date, the latter was affected by the influence of crop load at each harvest date with an average decrease in SBS of over 9 % in fruit from low crop load trees.

Opara *et al.* (1997) explained that 'Gala' apple fruits from non-irrigated trees were firmer than those from frequently irrigated trees, a fact that was ascribed to the alteration of tissue cellular structures. In this regard, Opara *et al*. (1997) suggested the alteration of cultural practices such as frequent irrigation could be an important strategy to manipulate fruit mechanical strength (such as skin bursting) and the concomitant reduction of fruit firmness. Accordingly, the two Physico-mechanical attributes are mentioned to influence the fruit sensitivity to bruising (Herppich *et al*., 2005). In addition, Opara *et al*. (1997) highlighted that regulated application of irrigation water could also be the influential factor for fruit fresh firmness without significantly altering skin strength, a critical factor affecting resistance to physical damage during postharvest handling. It has previously been reported that turgor significantly affects the failure properties of biological tissue (Mohsenin, 1986). However, there have been different views of how the fruit turgor affects the plant tissue's sensitivity to bruising. Baritelle and Hyde (2001) highlighted that reducing relative turgor through slight dehydration can reduce tissue stiffness which can, in turn, make a sort of 'self-cushioning', an effect that reduces sensitivity to bruising. In contrast, Hiller and Jeronimidis (1996) indicated that high turgor may result in tight packing of cells of produce thus increasing the mechanical resistance to bruising or cuts. This was buttressed by Garcia *et al*. (1995), who studied the effects of irrigation schedules on the firmness of apple cultivars 'Golden Supreme' and 'Golden Delicious' fruits. According to the authors, watered trees produced firmer fruit than non-irrigated trees, with firmer fruit more susceptible to bruising. The author concluded that both turgidity and firmness influenced bruise susceptibility independently, however, their effects were combined during ripening.

Similarly, Mowatt (1997) noticed that reduced irrigation of apple 'Braeburn' trees produced fruit that were less susceptible to bruising by 6 % than fully irrigated trees. Based on this conclusion, it was presumed that structural attributes of the fruit that are associated with reduced susceptibility of fruit to bruising are influenced more by long-term water stresses, rather than the fruit water status (Mowatt, 1997).

Tree training system is one of the useful techniques in many fruit crops for improving both yield and quality of fruit. Tree shape determines the exposure of leaf and fruit parts exposed to incoming radiation, and therefore pruning is practiced to enhance the quantity of sunlight intercepted by trees (Ahmad *et al*., 2006). Tahir *et al*. (2007) investigated the effect of pruning systems on 'Aroma' tree yield and fruit quality, storability and fruit resistance to bruising. In their results, it was found that fruit from summer pruned trees showed higher resistance to bruising (3.5 bruise diameter percentage) than fruit from winter, spring or winter and summer pruning which had average bruise diameter percentage of 5.2. According to Tahir (2006), sunny side of 'Aroma' fruit contains more wax than shadow side. Accumulation of more wax on the sunny side of the fruit might provide some cushions, partly decreasing the impact energy.

4.5. Effect of fruit properties

Fruit of same or different cultivars may also have different susceptibilities to bruising due to differences in fruit properties (Blahovec *et al*., 2003; Ahmadi, 2012). Fruit properties such as fruit size, mass and shape or morphology play a major role in bruise susceptibility during mechanical impact (Maness *et al*., 1992; Crisosto *et al*., 1999; Blahovec *et al*., 2003; Blahovec & Paprštein, 2005). Fruit size has been reported to affect bruise sensitivity of apples, which was explained by the difference in tissue strength between smaller and larger fruit (Van Zeebroeck *et al*., 2007b). Johnson and Dover (1990) revealed the difference in a tissue structure between small and large apples and explained that large apples have larger cells with thinner cell walls. The latter are presumed to be weaker tissue that can easily develop bruise damage upon impact (Van Zeebroeck *et al*., 2007b). Similarly, Maness *et al*. (1992) noted that a noticeably smaller 'Ranger' cultivar of peach fruit absorbed less total energy and, and hence had less bruise damage (BV) upon impact than the other three cultivars, 'Topaz', 'Glohaven' and 'Elberta'. Small peaches were found to be more susceptible to bruise damage than large peaches at low impact, while the difference was very small at high impact (Ahmadi *et al*., 2010).

Fruit indices previously studied such as the ratio of fruit length to the maximum fruit diameter have also been reported to influence bruise susceptibility in pear fruit cultivars

(Blahovec *et al*., 2003; Blahovec & Paprštein, 2005). It has been suggested that a fruit cultivar with higher fruit index may be more susceptible to bruising than the same fruit of different cultivar with lower fruit index. A study in pear fruit cultivars reported by Blahovec and Paprštein (2005) on the role of fruit shape among other factors affecting bruising in 22 pear varieties concluded that more elongated fruit were more susceptible to bruising. In a similar study conducted by Blahovec *et al*. (2003) it was revealed that among the 16 tested cultivars of pear fruit, cultivars 'Electra', 'Konference,'Lada' and 'Vilu' which had higher indices (ratio of fruit length to the maximum fruit diameter) were found to be more susceptible to bruising compared to other cultivars whose indices were close to 1, such as 'Astra', 'Vonka', 'Delta', 'Dicolor' and others to mention but a fewfruits of the same or different cultivars with higher mass could sustain higher impact forces after free falls (Blahovec *et al*., 2003; Hertog *et al*., 2004; Van Zeebroeck *et al*., 2007b). Blahovec et al. (2003) introduced an important mass parameter, the ratio of fruit mass to the maximum fruit diameter as the measure of loading the surface area during impact after fruit free falling. In their study to determine the sensitivity of 16 pear fruit cultivars to bruising, Blahovec *et al*. (2003) observed the highest values of this fruit bruise index in 'Electra' and 'Vilu' pears, which were the most bruise susceptible cultivars of all. In view of that, Blahovec *et al*. (2003) established that the fruit mass to maximum fruit diameter ratio is a good measure of the loading stress rather than loading local force that can be expressed by fruit mass. Further, higher pit to fruit index (i.e., fruit with more elongated pits and radius of curvature ratios) had lower bruise volumes as previously indicated in table olives by Jiménez-Jiménez *et al*. (2013). The authors' findings further indicated the important role played by fruit pits in bruising. Jiménez-Jiménez *et al*. (2013) demonstrated that olive fruit of'Hojiblanca' cultivar which had lower contact time and the less flesh-to-stone ratio (i.e., fruit mass to stone mass ratio) that was found to be most resistant to impact damage than cultivars 'Gordal Sevillana' and 'Manzanilla' with higher flesh-to-pit ratios.

Physico-morphological properties measured on each impacted fruit in four fruit species of drupe (peach and apricot) and pomes (apple and pear) fruits were used to develop the drop damage index of fruit resistance to bruising in a study reported by Menesatti and Paglia (2001). Findings from their study revealed that apple fruit had the highest bruise susceptibility of all fruits tested under the same loading conditions, as indicated by the highest mean value of bruise size (bruise depth), followed by pear, peach and apricot. The difference in bruising between the fruits reported was attributed to cultivar differences in the fruits' physico-morphological variables such as fruit mass, volume and the fruit equatorial diameters (i.e., the radius of curvature). Aliasgarian *et al*. (2013) investigated the influence of physical properties (linear dimensions i.e., length and diameter, volume, geometric mean diameter, and fruit mass and sphericity) of strawberry fruit on bruising, and established the relationship between these properties and mechanical damage of the fruit. Their findings identified 'Gaviota' as more susceptible to bruise damage than 'Selva', cultivar and presumed the fruit shape as the most effective factor that caused their difference in susceptibility to damages while their differences in masses and volumes were insignificant.

The radius of curvature is another property of huge influence on bruise sensitivity of various fruits (Van Zeebroeck *et al*., 2007b,c; Zarifneshat *et al*., 2010; Shafie *et al*., 2015), and is related to the fruit shape and morphology. The effect of the radius of curvature on absorbed energy for kiwifruit, apple, apricot, peach and tomato are well documented in literature. Van Zeebroeck *et al*. (2007b) concluded that the radius of curvature has a double effect on the bruise damage sensitivity: (i) a smaller fruit's radius of curvature increases the peak stress but decreases the contact area during impact. This could suggest that for the fruit to suffer more bruise damage at high impact it is more important to have sufficient tissue in contact (i.e., large radius of curvature) rather than to induce a high peak stress, and (ii) at lower impact, the fruit could suffer more bruise damage if it is induced by higher peak stress rather than its larger contact area during impact, which is in contrast to the former suggestion (Van Zeebroeck et al., 2007b; Zarifneshat *et al*., 2010). Similarly, fruit's radius of curvature was presented in apples by Zarifneshat *et al*. (2010), who demonstrated that apples with a low radius of curvature had higher bruise volumes than those with a higher radius of curvature (at the contact area).

Furthermore, Van Zeebroeck *et al*. (2007c) reported that tomatoes with a small radius of curvature impacted at room temperature (20 °C) absorbed more energy compared to those with a larger radius of curvature. However, the authors noticed the temperature (of fruit) dependence of the effect of the radius of curvature on the absorbed energy at the time of impact. In line with this, Van Zeebroeck *et al*. (2007c) revealed that tomatoes impacted at 15 °C, had quite the opposite effect, such that less energy was absorbed by tomatoes with a lower radius of curvature and more energy was absorbed as the radius of curvature increases (i.e., as fruit becomes flat in shape). It was presumed that the opposite effect of the radius of curvature for tomatoes at two different temperatures was attributed to the consequent difference in acoustic stiffness (which is positively correlated with firmness for tomatoes). According to the authors, the relationship between the two parameters is in such a way that at a high temperature of tomatoes (20 °C) there is higher firmness failure stress, and therefore the small radius of curvature gives rise to higher peak stresses necessary to overcome the high failure stress. In contrast, tomatoes at 15 °C have a lower failure stress, with the consequence that the higher contact area of tomatoes with a higher radius of curvature becomes more important than the lower peak stress compared tomatoes of the lower radius of curvature (Van Zeebroeck *et al*., 2007c).

Ahmadi *et al*. (2010) worked on peach fruit of 'Haj Kazemi' cultivar and observed that peaches with small (30 mm) radius of curvature had larger BV than those with a larger (50 mm) radius of curvature both at higher and lower impacts. At lower impact (18.4 N), the BV was 490 and 585 mm^3 for the smallest and largest curvature peaches, respectively. Likewise, the effect of the fruit radius of curvature followed the similar trend at the highest impact level (56.7 N), where BV was 2345 and 2460 mm^3 for small and large curvature peaches, respectively.Results reported in pomegranate fruit by Shafie *et al*. (2015) indicated that irrespective of impact level and fruit temperature, the fruit calyx shoulder was the most susceptible region to bruising due to minimum values of peel thickness and radius of curvature. Conversely, the stem shoulder region was the most resistant to bruising despite its intermediate values of peel thickness and radius of curvature. According to their observation, the fruit part with a smaller radius of curvature had a reduced impact surface area and hence, a reduction in the bruise size as suggested in several other previous studies.

Radius of curvature was found to affect negatively the energy absorbed by during an impact test of apricot (cv. Ziaolmolki) (Ahmadi *et al*., 2014). At the high impact force (55 N), energy absorbed by apricot fruit with a radius of curvature of 38 mm was 38 % less than the one having a curvature radius of 24 mm. The authors revealed that at the low impact force (20 N), the energy absorbed by fruit having a radius of curvature of 24 mm was 10 % higher than that for an apricot with a curvature radius of 38 mm, resulting in an increase in bruise susceptibility. Similar results were reported in kiwifruit (cv. Hayward) by Ahmadi (2012) who observed more absorbed energy with a low radius of curvature. According to their results, kiwifruit with a small radius of curvature had more bruising than those with a larger radius of curvature.

Acoustic stiffness is another fruit property that indirectly affects bruising through its influence on the fruit's contact time with the impact surface during impact (Van Linden *et al*., 2006). According to Van Linden *et al*. (2006) and Van Zeebroeck *et al*. (2007c), fruit with higher stiffness will have shorter contact time with the impact surface, which could reduce the risk to develop bruising in tomatoes. It has been earlier stated that stiffness is a complex texture characteristic which varies with fruit ripening (Van Linden *et al*., 2006; Van Zeebroeck *et al*., 2007c; Zarifneshat *et al*., 2010). As previously described in tomatoes by Duprat et al. (1997), and later by Van Zeebroeck *et al*. (2007c), there is a positive correlation between firmness and acoustic stiffness which indicates that the former decreases with increasing ripeness. Overall, the acoustic stiffness is largely a measure of the mechanical stiffness of the tissue that is based on the cell wall mechanical strength and cell wall turgidity (Hertog *et al*., 2004). Results reported in peaches indicated that the BV decreased with the increase of acoustic stiffness (Ahmadi *et al*., 2010). The BV for peaches with smaller acoustic stiffness (19 s^{-2} $kg^{2/3}$) was up to 43% higher than stiffer peaches (25 $s^{-2}kg^{2/3}$) at low impacts (18.4 N) and up to 6% higher at highimpacts (56.7 N).

In another similar study conducted on kiwifruit by Ahmadi (2012), it was revealed that kiwifruit with lower acoustic stiffness showed more bruise damage as indicated by more absorbed energy during impact than fruit with higher acoustic stiffness. At the low and high impact levels, the difference in absorbed energy between soft and stiffer fruit was 3.4 and 7 mJ, respectively. Nonetheless, there have been conflicting reports on the of the effect acoustic stiffness between apples and tomatoes. In contrast to earlier reports in tomatoes, Van Zeebroeck *et al*. (2007b) explained that higher acoustic stiffness leads to more bruise damage in ‘Jonagold’ apple cultivar. However, like the case for tomatoes, it has been stated that higher acoustic stiffness leads to higher peak stress during impact (Van Zeebroeck *et al*., 2007c). Van Zeebroeck *et al*. (2007b) explained the apparent contradiction by the failure stress, based on the fact that stiffer unripe tomatoes do suffer higher peak stress which does not necessarily lead to more bruise damage (or absorbed energy) because the failure stress is higher for the unripe tomatoes as well. The effects of fruit properties on the bruise damage susceptibility for a range of fruits are summarized in Table 5.

5. Conclusion and future prospects

A diverse range of factors influencing bruise damage of fresh fruit prior to harvest has been reviewed. Major factors of huge influence to bruise susceptibility of most produce include genetic or cultivar differences, climatic factors, growing season, fruit properties and most of the orchard management practices usually applied in an attempt to increase productivity. This review has enlightened main preharvest factors that could be manipulated by orchardists to reduce bruise damage of produce after harvest and or during subsequent handling. Practices such as alteration and/ or proper management of orchard management practices such as irrigation and fertilization could be an important preharvest strategy to manipulate fruit mechanical strength and or improve the produce resistance to bruising. It was also shown in this review that genetic differences influence the cultivars of the same fruit to respond differently to the same loading conditions. This provides a useful guideline especially when introducing new cultivars for commercialization, and informs selection of cultivars which are less prone to bruising. It could also encourage the development of optimum handling and care for cultivars which are more prone to bruising.

Overall, raising awareness of the effects of various preharvest factors on the produce susceptibility to bruising presented in this review enlightens the extent to which orchardists can go in a quest to reduce losses due to bruise damage. This review has revealed that most of the orchard management practices once applied correctly can offer a lot of other positive benefits toproduce quality attributes besides their potential to reducing susceptibility to bruising. Therefore, the current study permits further exploration of various orchard management practices and their relative influences on bruise damage susceptibility and other quality attributes. This could be used for developing a valuable evidence-based tool for orchardists to improve quality production of various fruit. Overall, the choice of fertilization formulas, irrigation schedules and pruning routines based on specific plant requirement could be an important tool to increase fruit yield, enhance quality and reduce susceptibility to mechanical damage. Therefore, well balanced and integrated fertilization of different nutrients is warranted to improve the quality of fruit at harvest.

Despite the existence of a wide range of literature on bruising of fresh fruits, more focus has been on soft fruits, mainly apples, peaches, tomatoes and the like. Limited information is available on studies of bruise susceptibility of hard and/or thick ‘rinded’ fruits such as pomegranates, coconuts, lemons and vegetables. Future research should, therefore, focus on how the bruising of these fruits could be influenced by the prevailing preharvest factors. Furthermore, the influence of such factors as climatic conditions, growing season and orchard management practices have not been explored for many varieties of fruits other than apple.

Based on the discussion in this review, further research is required to precisely evaluate the relationships between preharvest factors reviewed (especially those not widely explored) and their relative influence on bruise damage susceptibility for several other fruit varieties. Overall, further research in this area will provide a better understanding of factors with the greatest effect on fruit bruising, so that both fruit quality and bruise damages can be managed better to meet consumers' expectations.

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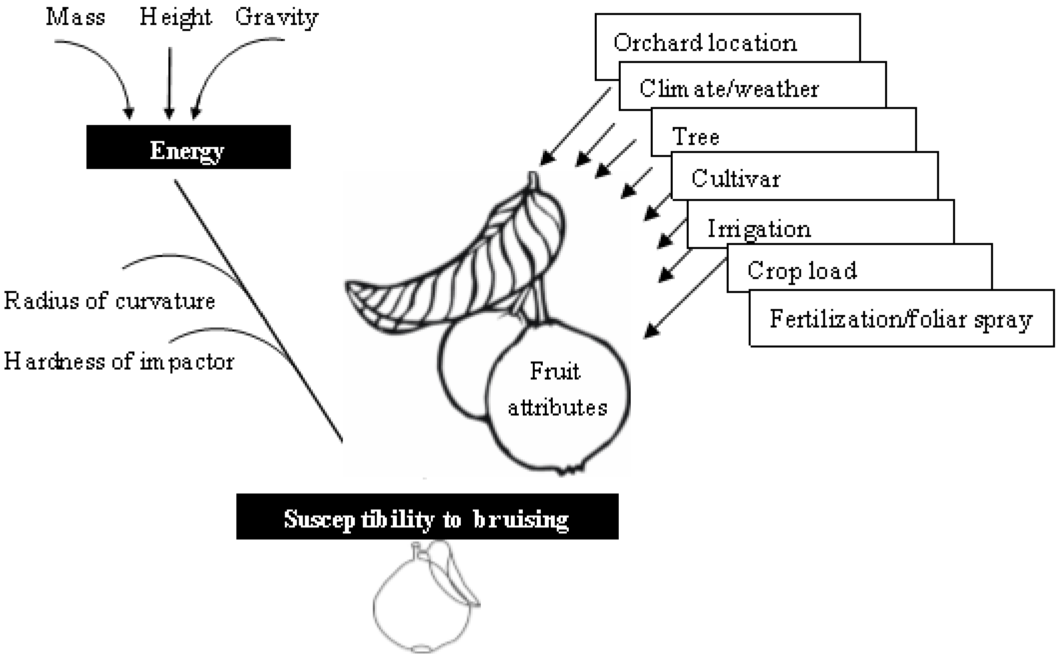

Fig. 1 A model conceptualizing the influences of preharvest factors on susceptibility of fruits to bruising (Modified from Mowatt, 1997).

Table 2 Genetic and cultivars differences in bruise susceptibility of selected fruits

Fruit (s)	Impact energy (Ei)*	Key finding	References
Apple	0.03 J, 0.04 J, ~0.06 J	Low level of bruises (>1 cm^2) was produced by the lower impact energies (0.03J and 0.04 J) in Splendour' and 'Granny Smith' cultivars, respectively 'Braeburn' and 'Royal Gala' produced same bruise level at impact (~0.06 J)	Bollen, 2005
	Aroma: - Small fruit (0.06 – 0.35 J) -Large fruit: (Ei >0.41J) COP and Ingrid Marie: -Small fruit (0.0 4 – 0.26 J) -Large fruit: (Ei >0.26 J)	Drop impact at 10, 15, 25 and 30 cm caused lower bruise diameter and BA in 'Cox's Orange Pippin' than 'Aroma'	Ericsson and Tahir, 1996
	Aroma: ~0.23J COP: ~0.21J Ingrid Marie: ~0.18J	Highest percentage of bruise weight was observed in 'Aroma' (2%), followed by 'Ingrid Marie' (1.75%) while 'Orange Pippin' had the lowest (1.5%)	Tahir, 2006.
	Ei: 0.125- 240.34 mJ Corresponding impact velocities: (0.125 – 1.6 ms $^{-1}$)	'Freedom' had the lowest bruise depth (1.5 - 6.7mm); 'Rajka'had highest bruise depth (1.6 - 7.8 mm) for almost each value of impact energy and velocity Bruise surfacearea was lowest in hard'Rajka' apples and highest in soft 'Freedom' for the same value of impact energy and velocity	Stropek and Golacki, 2013

*Impact energy presented (where not specified in paper) is estimated from drop height and average fruit mass or of an impactor

Table 1 Continued.

Fruit (s)	Impact energy*	Key finding	References
Apple	-	'Aroma' had a larger bruise diameter (25.9 mm) than 'Discovery' (21.4 mm) and 'Gloster' (19.4 mm)	Lv et *al*., 2016.
Peach	Maycrest: (0.37 – 0.64 J), Domiziana: (0.58 – 1.0 J), Roza: (0.73 – 1.25 J), Emilia: (0.81 – 1.38 J), Flaminia: (0.83– 1.42 J)	Bruise damage was in the order of Roza (10.4 mm)>Domiziana (8.4 mm) > Maycrest (7.6 mm) > Emilia and Flaminia (5.9 mm)	Menesatti *et al*., 2001
Avocado	Impaction: Ei (0.18 – 0.74 J) Vibration: (1.1 – 1.2 × g for 20 min)	Fruit flesh firmness <11.3 kgf: vibration caused higher BV in 'Fuerte' than 'Hass' or 'Pinkerton' Flesh firmness 6.8 – 11.3 kgf:both cultivars had smaller BVs (av. 0.25 cm^3)	Arpaia *et al*., 1987
Table olive	0.046 J	Olive of 'Manzanilla' cultivar had higher bruise damage (measured as total damaged area, 20.91 mm^2) than 'Hojiblanca' cultivar (16.58 mm^2)	Jiménez *et al.,* 2016
Table olive	High Ei: 56 ± 8 mJ MediumEi: 26 ± 3 mJ Low Ei: 13 ± 1 mJ	High impact: higher BV (235.0 mm^3) and BA (77.9 mm^2) in 'Manzanilla', like 'Gordal Sevillana' and twofold the bruise size in 'Hojiblanca' Medium impact: higher BV (154.6 mm^3) and BA (52.0 mm^2) in 'Manzanilla', similar to 'Gordal Sevillana' and twofold the bruise size in 'Hojiblanca'	Jiménez-Jiménez *et al*., 2013

*Impact energy presented (where not specified in paper) is estimated from drop height and average fruit mass or of an impactor

Table 1 Continued.

Fruit	Impact energy*	Key finding	References
Nectarine	0.47 – 0. 79 J)	'Fantasia'nectarine fruit wasmore susceptibleto impact damage (0.47J) than 'Firebrite' fruit (0. 79 J)	Bollen *et al*., 2001
Peach	Spring time: (0.051 -0.15J), Elberta: (0.065 - 0.194 J)	No difference in measured bruising (BA and BV) among peach cultivars at all impact levels and impact surfaces	Tabatabaekoloor , 2013
Peach	Ranger: (35 – 130 J $\times 10^3$), Topaz: (49 – 190J $\times 10^3$), Glohaven: (44 – 170J $\times 10^3$), Elberta: (49 – 170J $\times 10^3$)	'Ranger' absorbed less impact energy (less bruise damage av.BV = 0.04 cm^3) than 'Topaz', 'Glohaven' and 'Elberta' (av.BV = 0.06 – 2.74 cm^3)	Maness *et al*., 1992
Pear	0.03 – 0.04 J	'Chojuro' was more resistant to impact bruising (10 mm, bruise depth) than pears of the other three cultivars (bruise depth > 10mm)	Chen *et al*., 1987
Nectarine	Nectaross: (0.7 – 1.2 J), Weinberger: (0.43 – 0.74 J)	'Nectaross' was more resistant to bruising (bruise damage, 2.6 mm) than 'Weinberger' nectarines (6.5 mm, bruise damage)	Menesatti *et al*., 2001
Banana	20 – 200 mJ	'French Corne' banana was susceptible to bruising at lower impact (20 mJ) Bruising in 'Grande Naine' and 'Flhorban925' bananas was observed at higher impact (above 20 mJ)	Bugaud *et al*., 2014

*Impact energy presented (where not specified in paper) is estimated from drop height and average fruit mass or mass of an impactor

Table 1 Continued.

Fruit	Impact energy*	Key finding	References
Nectarine, Peach	Nectarine: (0.37 – 1.42 J), Peach: (0.43 – 1.2 J)	Nectarines of all cultivars had higher drop damage index (av. DDI = 222 mm) (more resistant to bruising) than peaches (av. DDI = 114 mm)	Menesatti *et al*., 2001
Peach, Apricot, Apple, Pear	Apple: (0.43 – 0.66 J), Apricot: (0.22 – 0.3 J), Peach: (0.49 – 0.82 J), Pear: (0.82 – 0.93 J)	Apple had the highest bruise depth (20.56 mm), followed by pear (10.26 mm). Lowest bruise depth in apricot (6.54mm) and Peach (6.86 mm) fruits	Menesatti and Paglia, 2001

*Impact energy presented (where not specified in paper) is estimated from drop height and average fruit mass or mass of an impactor

Table 3 Influence of growing seasons on bruise damage susceptibility of apple fruit

Number of growing season	Key finding	References
2	No difference in measured bruise volume for both apple cultivars from the two successive seasons	Klein, 1987
5	23% difference in mean bruise diameter at both maximum and minimum standard impact bruise diameters	Johnson & Dover, 1990
2	Variation in mean bruise diameter ranged between 0 – 3% for 'Granny Smith' and 'Royal Gala'	Mowatt, 1997.
2	'Braeburn' and 'Granny Smith' from the first season was 0.015 and 0.01 J less impact energies, respectively compared to 'Royal Gala' and 'Granny Smith' of the succeeding harvest season	Bollen*et al*., 2001
5	Difference in impact energy betweenseasons differed by up to 35% for 'GrannySmith' over 3 years, 48% for the 2 years of 'RoyalGala', and 20% for the 2 years of 'Braeburn'	Bollen, 2005
2	'Discovery' showed higher sensitivity (5% larger bruise diameter) than 'Gloster' in the first season compared to the second season (6.6% less bruise diameter than 'Gloster') 'Gloster' harvested in the second season 14.5% larger bruise depth than that from previous season	Lv *et al*., 2016
2	Fruit harvested at green and white peel stages in the first season was less susceptible to bruising compared to fruit from succeeding season by 77.3 and 64.5%, respectively	Mitsuhashi-Gonzalez *et al*., 2010

Table 4 Influence of orchard management practices on bruise damage susceptibility of apple fruit

Cultural practice	**Key finding**	**References**
Irrigation	Fruit from trees irrigated a few weeks before harvest had higher firmness than fruit from non-irrigated trees but did not affect the susceptibility of fruit to bruising	Garcia *et al*., 1995
Conventional fertilization versus fertigation with N, P, K and Mn.	Fertigation at reduced rate without K caused the highest probability of bruise damage, about 30% higher than conventional fertilization (with or without leaf treatments) or the fertigation at full rate, both in 'Fuji' and 'Gala' apples	Pasini*et al*., 2004
Fertigation with N, K and Ca/K minerals	Fertigation with nitrogen decreased fruit resistance to bruising by up to 50%	Tahir *et al*., 2007
Foliar fertilization with Ca, Mn or Zn.	Foliar fertilization did not affect bruisesusceptibility both in 'Braeburn' and 'Jonagold' apple	Eckhoff *et al*., 2009
Groundcover materials	Fruit from soil covered with ground cover materials had improved resistance to bruising than chemically cultivated fruit	Eckhoff *et al*., 2009
Soil mulching with aluminium	Soil mulching lowered bruise occurrence by 15 to 20% in comparison with conventional (chemical) mulching	Tahir *et al*., 2005
Pruning	Timely pruning (July) reduced bruising (BDP) in fruit by 15% lower than late (August) pruned trees	Tahir *et al*., 2007
Irrigation and fertilization with organic nitrogen	'Gala' apples from frequent irrigated trees had 6.35% higher bruise diameter, 1.54% higher bruise depth and 4.18% higher bruise volume than fruit from non-irrigated trees. Bruise sizes (bruise diameter, depth and bruise volume) were not significantly affected by fertilization with nitrogen	Opara, 2007
Crop load	Effect of crop load on bruise sizes (bruise diameter, depth and bruise volume) was insignificant Specific bruise susceptibility of fruit from low crop load decreased by 9% in comparison to high crop load	Opara, 2007

Table 5 Influence of fruit properties on bruise damage susceptibility

Fruit	Fruit property	Key finding	References
Strawberry	Sphericity (%)	Smaller fruit sphericity (0.89%) influenced higher damage index in 'Gaviota'(0.53) than 'Selva' cultivar (0.45)	Aliasgarian *et al.*, 2013
Table Olive	Flesh-to-stone ratio	Lower flesh-to-stone ratio (5.1) in 'Hojiblanca' influenced more resistance to impact damage compared to higher ratios of 6.7 and 6.9 in 'Manzanilla' and 'Gordal Sevillana' olive cultivars, respectively	Jiménez-Jiménez *et al.*, 2013
Pear	Fruit index (Fruit length to max.fruit diameter ratio)	Cultivars 'Electra', 'Konference', 'Lada' and 'Vilu' of higher fruit indices (1.50 – 1.58) were more susceptible to bruising than the rest of cultivarswhose indices were close to 1	Blahovec *et al*, 2003
Tomato	Radius of curvature (mm)	At 15 ºC: tomato of 'Tradiro' cultivar with a higherradius of culture absorbed higher impact energy than tomatoes at 20 ºC; hence suggesting a lower bruise susceptibility of tomatoesat20 ºC.	Van Zeebroeck *et al*., 2007c
Apple		Smaller radius of curvature (65mm) of 'Jonagold' apple developed up to 50% more BV for small impacts compared to apples of higher radius of curvature (90mm) and up to 9% less BV for high impacts (0.18 J).	Van Zeebroeck *et al*., 2007b
Apple		At the low impact force (25.8 N) the bruise volume of 'Golden Delicious' apple fruit with a smaller (34 mm) radius of curvature was 54% higher than the one having a larger (46 mm) radius of curvature At the high impact force (101.5 N) the bruise volume of the apple fruit with a smaller (34 mm) radius of curvature was 27% higher than the apple with larger (46 mm) radius of curvature radius	Zarifneshat *et al.*, 2010
Pomegranate fruit		Bruise volume was highest at the calyx (5200 mm3 and 8200 mm3) and cheek (5000 mm3 and 7900 mm3) for medium and high impacts corresponding to higher radius of curvatures, 51.5 and 59.5 mm respectively	Shafie *et al.*, 2015

Table 5 Continued

Fruit	Fruit property	Key finding	References
Apricot	Radius of curvature (mm)	At the high impact energy (0.17 J), the energy absorbed by apricot of 'Ziaolmolki' cultivar with a smaller radius of curvature (24 mm) was 38 % more than the fruit with a larger radius of curvature (38 mm) At the low impact energy (0.029 J), the energy absorbed by apricot fruit with a smaller radius of curvature was 7 % higher than that for an apricot with a larger curvature radius.	Ahmadi *et al.*, 2014
Peach		BV of a peach with 30 mm curvature radius was 19% higher than the fruit having a curvature radius of 50 mm at the lowest impact (18.4 N) Bruise volume of the peach fruit with a curvature radius of 30 mm was 5% higher than the peach with a curvature radius of 50 mm at the highest impact (56.7 N)	Ahmadi *et al.*, 2010
Peach, apricot apple and pear		Highest bruise depth in apple (20.56 mm), and pear (10.26 mm) The lowest bruise damage was found in peach (6.86 mm) and apricot (6.54 mm) corresponding to radius of curvature of 71.5, 72.1, 70.3 and 46.9 mm, respectively	Menesatti & Paglia, 2001
Tomato	Acoustic stiffness (sec^{-2} $kg^{2/3}$)	Lower acoustic stiffness (more ripened) tomatoes absorbed higher impact energy Difference in absorbed energy between ripe and unripe tomatoes ranged from 40 to 12% for lower and high impacts, respectively	Van Zeebroeck *et al.*, 2007c
Apple		Higher acoustic stiffness (39 sec^{-2} $kg^{2/3}$)increased the BV of apple (~42% higher than one having 25 sec^{-2} $kg^{2/3}$) at low impacts levels (25.8 N), and up to 21% higher at high impact levels (101.5 N)	Zarifneshat *et al.*, 2010
Kiwifruit		The difference in energy absorption at impact between two extremes of kiwifruit radius of curvature (21.8 and 34.3 mm) was 83% and 20%, at the low impact (19.2 N) and high impact (70.6 N), respectively	Ahmadi, 2012

Table 5 Continued.

Fruit	Fruit property	Key finding	References
Peach	Acoustic stiffness (sec^{-2} $kg^{2/3}$)	'Haj Kazemi' peach of small acoustic stiffness1(9 s-2 kg2/3) had up to 45% higher bruise volume stiffer peaches (25 s-2kg2/3) at the low impact energy (0.01 J) and up to 5% higher at the high impact (0.09 J)	Ahmadi *et al*., 2010
Kiwifruit		Energy absorbed by soft 'Hayward' fruit (acoustic stiffness of 16.7 s-2kg2/3) was up to 58% higher than that of stiffer fruit (31.4 s-2kg2/3) at low impacts (19.2 N) and up to 6% higher at high impacts (70.6 N)	Ahmadi, 2012

CHAPTER 3

HARVEST AND POSTHARVEST FACTORS AFFECTING BRUISE DAMAGE OF FRESH FRUITS – A REVIEW

Harvest and postharvest factors affecting bruise damage of fresh fruits – A review

Abstract

Fresh fruit and vegetables are susceptible to bruising, a common type of mechanical damage duringharvest and at all stages of postharvest handling, especially during packhouse operations, transport, andstorage. Severe bruise damage during mechanical handling can result in postharvest losses and a substantialreduction in quality of fresh produce due to excessive weight loss, senescence, spoilage and high risk of microbial decay. In the quest of developing and adoption of strategies to reduce this problem, it is of utmost importance to understandmajor factors influencing bruise susceptibility of fresh produce at these stages. This review presents a critical discussion of factors affecting bruising during harvest and postharvest handling of fresh fruits. Excessive compression forces during harvesting by handpicking or machines, and a series of impacts during harvesting, transport and packhouse operations can cause severe bruise damage. The review has further revealed that bruising is dependenton a number of other factors such as produce maturity, ripening, harvest time (during the day or season) and time-lapse after harvest. It is pertinent to understand that the susceptibility of fruits to bruising partly depends on how these factors alter the produce physiological and biochemical properties, and so do the environmental conditions such as temperature, humidity, and several other postharvest treatments. Hence, the successful application of proper harvesting techniques by use of trained personnel and proper harvesting equipment isessential to reduce both the incidence and severity of bruising. Furthermore, the need for careful selection of postharvest handling temperature and other treatments alone or in combination can possibly increase the resistance of fresh produce to bruise damage.

1. Introduction

Fruit and vegetables offer a wide source of micronutrients, fibres, vitamins and a remarkable source of phytochemicals and antioxidants (Allende *et al*., 2004; Rico *et al*.,2007; Hussein *et al*., 2015). In particular, phytochemicals such as anthocyanins, carotenoids, polyphenols and flavonoids make fruit and vegetables essential components of many daily human diets (Opara & Al-Ani, 2010; Li & Thomas, 2014; Hussein *et al*., 2015). It is in this regard that to date, the consumption of fruit and vegetables is highly recommended as health diet due to its association with such numerous nutritional and health benefits, including fighting against sedentary life style and degenerative diseases such as cancer, cardiovascular diseases and ageing (Rico *et al*., 2007; Ramos *et al*., 2013; Hussein *et al*., 2015). As the results of health and nutritional benefits reported from many horticultural produce, the trend inconsumers' lifestyle has changedtowards increase in consciousness to health diets leading to high demand forhealthy, fresh-like and ready-to-eat fruits and vegetables (Rico *et al*., 2007; Caleb *et al*.,2013; Ramos *et al*., 2013).

In recent decades, there has been a rapid expansion of fresh horticultural produce industry (Allende *et al*., 2004; Montanez *et al*., 2010; Siddiqui *et al*., 2011). The increasing demand for fresh fruit and vegetables by many consumers worldwide has sparked the need for a large-scale mechanisation in both harvesting and postharvest handling operations (Li & Thomas, 2014; Stropek & Gołacki, 2015). Unfortunately, the harvest and postharvest activities such as producehandling, sorting, grading, packing, transportation, and distributioninvolve numerous mechanical operations, which predispose horticultural produce to the action of static and dynamic forces causing mechanical damage (Opara, 2007; Montero *et al*., 2009). Mechanical damage is the plastic deformation, superficial rupture and/or destruction of vegetable tissues (Montero, 2009), and is inclusive of bruising, crushing, or rupturing of either vegetables or fruits tissues (Polat *et al*., 2012). Mechanical damage generally causes produce quality deterioration and subsequent economic losses (Stropek & Gołacki, 2015).

Bruising is the most common type of mechanical damagetofruit which can occur during harvesting, handling and transport (Ahmadi *et al*., 2010; Tabatabaekoloor, 2013). A bruise is a type of subcutaneous tissue failure without rupture of the skin of fresh produce where the discolouration of injured tissue indicates the presence of a damaged spot (Blahovec&Paprštein, 2005; Opara & Pathare, 2014).Bruising results from the action of excessive external force on fruit surface during the impact against a rigid body or fruit against fruit (Kitthawee *et al*., 2011; Li & Thomas, 2014; Stropek & Gołacki, 2015). The physical evidence of bruising is a result of cell breakage(Schoorl & Holt, 1983), which results from stress and distortion of individual cells leading to cell wall extension (Ruiz-Altisent & Moreda, 2011).The breakage of cell membranesleads to cytoplasmicenzymes release into the intercellular spaces and react with vacuolar contents (Mitsuhashi-Gonzalez *et al*., 2010). Bruise damage could result from the excessive impact, compression or vibrational movements of the fruit againstother fruit, parts of the treesor containers when fruit fall during harvest or when emptied into large bins during picking (Ruiz Altisent, 1991; Van Zeebroeck *et al*., 2007b; Kitthawee *et al*., 2011).

The impacts of bruising on produce quality and its economic importance in the horticultural industry are well known and documented. There are several studies on various aspects of bruising for different fresh produce sustained during harvesting and after harvest. Major focus of these researches has been in on evaluation of the bruise damage susceptibilities in relation to the fruit physical, mechanical and/ or engineering properties (Ahmadi*et al*., 2010; Kitthawee, *et al*., 2011; Ahmadi*et al*., 2012; Tabatabaekoloor, 2013; Abedi&Ahmadi, 2014; Shafie*et al*., 2015). There are dozens of other published works that have exploredthe effects of a number of physical quantities relating toproduce and/ or the magnitude of impact or compression energy on bruise susceptibility, occurrence and bruise severity (Kitthawee *et al*., 2011; Boydas *et al*., 2014; Ghaffari *et al*., 2015). Fruits of major research interest have been soft rinded fruit such as apples (Ericson, 2004; Tahir, 2006; Ozturk*et al*., 2010; Stropek&Golacki, 2013;Lv *et al*., 2016), peaches (Hung &Prussia, 1989; Martínez-Romero *et al*., 2000; Ahmadi *et al*., 2010; Tabatabaekoloor, 2013) and kiwifruit (Crisosto*et al*., 1999; Ahmadi, 2012). Others includenectarine fruit (Bollen *et al*., 2001; Polat *et al*., 2012), cherries (Blahovec, 1999), pears (Garcia *et al*., 1995; Blahovec *etal*., 2002; Kabas, 2010; Komarnicki*et al*., 2016), and tomatoes (Van Linden*et al*., 2006a, 2006b; Li*et al*., 2010; Buccheri & Cantwell, 2014).

Previous findings have related the bruise damage susceptibility of fresh produce to factors such asphysiological and biological makeup of the fruits, maturity, variations in cell wall thickness, cell strength and elasticity,texture and/ or firmness, cell packingarrangement and fruit turgidity (Mohsenin, 1986; Schulte*et al*.1992; Van Linden*et al*., 2006b; Strehmel*et al*., 2010; Ahmad, 2012). However, the magnitude of bruise damage and consequent postharvest losses of fresh produce sustained after harvest could be dependent on a number of other factors relating to harvest, postharvest handling, and environmental storage conditions as well as to various postharvest treatments. Therefore, it is appropriate to understand that any change in one or a combination of these factors could potentially modulate biological and/ or physiological makeup of produce and subsequent changes to its susceptibility to bruise damage.

During harvest and immediately after harvest, fruit are subjected to anumber of operations varying between commodities (Ruiz-Altisent, 1991; Lewis *et al*., 2008). These operations follow a complex route from the fruit tree inan orchard to the shelves of supermarket (Kafashan *et al*., 2008; Lewis *et al*., 2008; Eissa et al., 2013), and comprises of variousstages and processes of which fruit undergoes,including harvesting, packing, sorting, storage and transport (Fig. 1).Handling operations and processes mentioned above predispose fruit into varying levels of loading (static or dynamic) conditions that cause various forms of mechanical damage including bruising (Ahmadi, 2012; Eissa*et al*., 2013).

Despite the significant amount of research carried out to address various aspects of bruising of fresh fruit, there is lack of extensive review on the subject of harvest and postharvest factors that could be of paramount influence to bruise damage susceptibility. For instance, Opara and Pathare (2014) reviewed the measurements and analysis of bruise damage, with a major focus on the recent technologicaldevelopments in bruise measurement, detection, and analysisof fresh horticultural produce. Li*et al*. (2014) reviewed the quantitative evaluation of mechanicaldamage to fresh fruit, with focus on the sources of fruit damage during mechanical handling, the mechanisms, quantitative assessments to characterise surface and internal mechanical damage and its predictive models. Hence, this chapter presents an extensive review of the harvest and postharvest factors that affect the bruise damage susceptibility of various fresh fruit. Distinct attention has been accorded to the identification and discussion of these factors, and their relative influence on bruising and bruise damage characteristic for various fruits.

2. Harvesting factors

2.1. Harvest methods

The cost of fruit harvesting can range between 20 – 40 % of the total on-farm variable production costs and is largely contributed by manual labour (Sargent *et al*., 2013; Mika, 2015; He *et al*., 2017). In addition to the increasing labour costs and uncertainty in manual labour availability, the problem of fruit bruising during harvest adds to the equation an important factor to put into consideration when deciding on which harvest method to be employed (Hu *et al*., 2017). Harvesting is the crucial stage where fruit are prone to bruise damage due to various causes such as method of harvesting and other associated activities and overall harvesting management (Thomson, 2003; Toivonen *et al*., 2007; Lurie, 2009). Bruising in most fruits starts in the field, and is by high chance induced by compression and impact stress during harvest, field packing and subsequent handling operations (Ferreira *et al*., 2008). Furthermore, the substantial amount of bruising at harvest also result from such processes as dumping of fruit from picking bags and overfilling of the bins in the orchard (Kupferman, 2006). A report by Kupferman (2006) suggested that apple bruising could reach as high as 35 % during harvesting and transport alone. This emphasises the magnitude of bruise damage problem caused by harvest practices. Hence, the need for use of trained workers, adequate harvesting facilities and techniques coupled with gentle fruit handling procedures during manual harvesting and hauling could be an essential requirement to reduce bruise incidence during harvest.

Manual fruit harvesting operations are traditionally carried out by handusing thumb and fingers, secateurs or clippers (Dhatt & Mahajan, 2007) (Fig. 2). Fruits such as grapes, strawberries, blueberries, apples, cherries prunes, peaches and blueberries have soft outer skins that are highly prone to mechanical damage such as bruising (Stow *et al*., 2004; Aliasgarian*et al*., 2013: Xu *et al*., 2015, Hu *et al*., 2017). Fruits that are destined for processing could suitably be harvested by any means, manual or mechanical since harvest bruise damages might not significantly affect the quality of final processed products and often times the produceis processed quicker (Aliasgarian*et al*., 2013; Xu *et al*., 2015; Hu *et al*., 2017). However, this may not be true for all fruits; for instance, bruising in olives has a high potential to reduce the quality of the final processed products (Jimenez *et al*., 2011; Morales-Sillero *et al*., 2014). For fruits destined for fresh market, harvesting practices should cause as little bruise damage to produce as possible, and hence manual harvesting by hand picking is preferred over mechanical harvesting machines (Aliasgarian*et al*., 2013).

Harvesting by hand picking can cause compression damage when grasp forces surrounding the fruit exceed a threshold for tissue failure (Li *et al*., 2014).Knee and Miller (2002) stated that if the fruit does not detach easily from the plant it has a high chance of being injuredby the hand forces during pulling and bending movement in an attempt to concentratethe force on the produce pedicel. Hence, unless proper means of picking are adopted, it is likely that fruits can receive maximum bruising during harvesting by hand picking (Knee & Miller, 2002). For instance, Li *et al*. (2016) showed that manually harvested (hand-picked) apples did not show any detectable bruising damage in comparison to robotic picked ones that resulted in some degree of bruise damage. This was explained by theminimal grasping force required during manual picking that resulted in no picking-induced bruising damage of apple fruit (Li *et al*., 2016).On the other hand, Yu *et al*. (2014) reported the bruising incidence of handpicked blueberries were close to 2 % for all studied cultivars just after fruit harvest. According to the authors, bruising incidence was due to initial bruise damages likely sustained during thepicking and handling process of berries in the orchard. It has been suggested that forceful detachment of fruit from trees using thumbs and fingers and dropping of harvested fruit into harvesting buckets (plastic pail) or uncushioned surface is seemingly equivalent to multiple modest drop impacts that can cause bruising (Yu *et al*., 2014). Likewise, in the results reported by Brown *et al*. (1996), it was also shown that an average of 23 % of the handpicked northern highbush blueberries sustained some internal bruise damage just after a week in storage.

Mechanical harvesting are developed to address the challenge of reliance on manual harvesting due to its potential to reduce harvest cost and postharvest losses resulting from bruise damages (He *et al*., 2017). Additionally, mechanical harvesting methods are employed as the means to speed up harvest and field handling operations (Thompson, 2003). Recently, the increasing labour shortages and labour costs have significantly contributed to the critical needs for technological innovations, developing and the use of mechanical techniques for harvesting (Li *et al*., 2016; Fu *et al*., 2017). However, despite the advantages of fast operations and mass harvest, use of mechanized harvesting techniques contribute much to the additional wounding to fruit due to mechanical forces involved. In addition to mechanical forces involved, mass harvest using shake-and-catch systems can potentially cause bruising during fruit catching and collecting operations and while falling through the tree to uncushioned surface (Toivonen *et al*., 2007; Yahia, 2011; Fu *et al*., 2017).

Robotic fruit harvesting is one of the mechanical harvesting technique that for some reasons has not found its full utilization in the industry so far but has the potential to reduce the labour costs for manual harvesting (Sarig *et al*., 2012; Li *et al*., 2016). Automated robotic fruit picking machines are designed to use a system that emulates the human picker for decision making and picking (Sarig, 2012; Li *et al*., 2016). However, studies have shown that excessive grasping force during robotic picking can potentially induce bruise damage on fruit during picking (Van Zeebroeck *et al*., 2006; Mika *et al*., 2015; Li *et al*., 2016). Bruise damage during robotic picking could also be linked to the impact level caused by static or dynamic grasping force, and the picking pattern (Van Zeebroeck *et al*., 2006). Li *et al*. (2016) found that the use of a three-finger gripper of robotic fruit picking machine resulted in higher percentages of picking-induced fruit bruising than manual/hand harvesting due to the higher grasping pressure required by the former to detach apples from the tree. Furthermore, the authors observed that the increasing percentages of bruised apple fruit and/ or fruit with more than one bruise spots was attributed to the increase in both grasping force and grasping pressure of the robotic picking machine in comparion to hand harvesting method.

There are other mechanical harvesting techniques also used for commercial harvest of various types of fruit that have also been linked to fruit bruising during harvest. Mika *et al*. (2015) found that mechanical harvesting using straddle fruit harvester resulted in an overall higher percentage of bruise damaged plum and prune fruits than handpicked fruit, although the quantity of mechanically damaged fruit varied among cultivars. Similarly, earlier findings by Brown *et al*. (1996) showed that ~32 % of harvested blueberries were bruised during the harvesting process using commercial mechanical harvester. However, the shake-and-catch harvesting system performed better in terms of fruit removal efficiency and quality during the mechanical harvest of apple cultivars Jazz, Pacific Rose and Pink Lady, resulting in only 1, 2 and 5 % of bruise damaged fruit and for respective cultivars. In contrast, the same harvesting system resulted in higher percentages of downgraded apple fruit with puncture or cuts, about 4, 5 and 9 % for Pacific Rose, 'Pink Lady' and 'Jazz', respectively.

The incidence and severity of fruit bruising during harvest could also depend on other factors that are directly linked to the harvesting method employed, such as the type of fruit and fruit cultivars. For instance, handpicked 'Manzanilla de Sevilla' olives had ~50% bruise incidence and just 9 % for 'Manzanilla Cacerena' olives; most of which were classified as slight damage for both cultivars. On contrary, 100 % of mechanically harvested 'Manzanilla de Sevilla' olives were bruised whereas 91 % of bruise incidence was observed for 'Manzanilla Cacerena' olives when harvested with the straddle mechanical harvester, with the severe bruise and slight bruise damage for the former and the later, respectively. Similarly, Zipori *et al*. (2014) compared manual harvesting against the mechanical trunk shaker harvesterand observed significant variations in bruise damage incidence between harvest methods. In addition to the type of fruit or cultivar, the choice of harvesting method could also be determined by factors relating to the harvesting efficiency (capacity to detach fruit from the tree and time required to do so), and the quantity of damaged fruit as well as the severity of the damage. Low efficiency of fruit removal from the tree has been related to the fruit retention force, which could also depend on the type of fruit or cultivar that may have significant effect on bruise damage susceptibility of detaching fruit (Morales-Sillero *et al*., 2014). Hence, the choice the efficient harvesting method may potentially compromise the desired quality of fruit harvest. This highlights the need for careful selection of harvesting method/ technique based the desired quality of fruit at harvest and the final destination of the harvest while taking into account the type of fruit or cultivar. Table 1 summaries some studies reported various harvest methods and the influence of each fruit bruising during harvest.

2.2. Harvest time

Time of harvest during the day affects the susceptibility of fresh fruit to bruising (Table 2). Overall, harvesting during the hotter part of the day results in faster senescence, shriveling and wilting of fruits, which could in part contribute into bruise damage susceptibility of fruit at harvest (Garcia *et al*., 1995; Abbott *et al*., 2009). Banks and Joseph (1991) revealed that banana fruit harvested in the early morning hours had a higherthreshold for compression bruising than those harvested later in the day. According to the authors, observed differences in compression energy between late and early harvested bananas were attributed to the change in turgidity of banana fruit over time during theday. This has led to the conclusionthat maximizing fruit turgor could be an importantfactor in elevating the threshold at which bananas begin to sustain compressionbruising. These findings concurred with an earlier report by Banks (1990), which established that turgor of fruit on banana plants in the field declines from early morning to midday, as indicatedby the decrease in latex released from developing fruit tips following flower removalat the later time of day.

Abbott *et al*. (2009) examined the effect of harvest time on bruise susceptibility of ‘Cripps Pink’ and ‘Granny Smith’ apples. Among the three studied harvest times (morning, midday and the late afternoon), fruit harvested later in the day were less susceptible to bruising than those harvested in the morning hours. The authors suggested that the observed reduction in bruise susceptibility as the day progressed could be due to reductions in cell turgidity. This is in agreement with several other studies that have shown the influence of tissues’ cell turgor on susceptibility to bruising of apples (Garcia *et al*., 1995; Opara, 2007). Furthermore, Abbott *et al*. (2009) concluded that regardless of fruit cultivars, fruit are more likely to suffer larger bruise damage during morning hours than in the late afternoon if fruit of the same species are subjected to the same impact energy. Hence it would pertinent to suggest that that fruit harvested later during day-time would be less prone to bruise damage as the result of reduced turgor, elevated temperatures or a combination of both of these effects. On the contrary, citrus bruising manifests as oleocellosis which is highly depend on fruit turgidity and time of the day during harvest (Ortiz *et al*., 2011).

2.3. Seasonal variation at harvest

Reports on the effect of harvest time during the season (late or early) are evenly split with some studies showing an increase in bruise damage with a late harvest, and others reporting a decrease in bruise damage susceptibility (Table 2). Several studies have found that fruit harvested at the end of the commercialharvest period (late harvest) are more susceptible to bruising than those harvested at the beginning (early harvest) of the season (Garcia*et al*., 1995; Gunes *et al*., 2002; Opara, 2007). Garcia*et al*. (1995) reported that early-picked ‘Golden Delicious’ and ‘Golden Supreme’ apples and pears were less susceptible to bruising than those harvested at a later stage of development. These results were similar to the effect of harvest dates on the bruise occurrence in three apple cultivars ‘Aroma’, ‘Cox’s Orange Pippin’ and ‘Ingrid Marie’ reported byEricsson and Tahir (1996). These authors found that the bruise susceptibility measured as bruise weight percentage (BWP) increased by delayed harvest in ‘Aroma’ and ‘Ingrid Marie’. The authors also concluded that less sensitivity to bruising in late-harvested fruit could be attributed to higher pulp firmness as opposed to less mature fruits, as previously reported by Garcia *et al*. (1995).

Similarly, Bollen *et al*. (2001) reported thatlate harvested 'Braeburn' and 'Granny Smith' apple fruit from the first season were more susceptibleto damage than early harvested ones. Their results showed that 'Braeburn' and 'Granny Smith' apples harvest on season 1 absorbed less impact energy than those harvested during season 2. However, during the same period (season 1 and 2), the authors could not find any significant difference in bruise susceptibility between early and lateharvested 'Royal Gala' and 'Granny Smith' apples. Consistently with that, Gunes *et al*. (2002) reported higher losses of cranberry fruit in the later harvest lot than early harvest fruit lot of two cultivars, 'Pilgrim' and 'Stevens'. It was presumed that the later harvested cranberry fruit were riper and hence more prone to bruising during harvesting and handling compared to early harvested berries. In buttress of the earlier statement, Studman (1997) highlighted that the ripening process is associated with a loss in cell wallstrength. In that regard, the cells of a riper fruit stand less to withstand external loading that subsequently increases the bruise damage susceptibility of fruit (Studman, 1997; Van linden*et al*., 2006b). Bruise damage in 'Jonagold' apples increased from late to early harvest as reported by Van Zeebroeck (2006). According to the authors, early harvested apples were more susceptible to bruise damageduring transport in comparison to late or optimal harvested apples.

Eckhoff *et al*. (2009) investigated the influence of early, mid and late harvest time on the susceptibility of 'Braeburn', 'Jonagold' and 'Golden Delicious' apples to bruise damage measured as pressure sores.The compression tests conducted by computerized penetrometer to generate pressure points at 30, 40 and 50 N showed that the influence of harvest time was significant, such that that the subsequent (late) harvesting lead to an increased expression of pressure points. The later the harvest took place, the stronger was the expression of the pressure points generated.This effect was attributed to the influence of fruit ripening on the bruising sensitivity, which has previously been reported in apples (Bollen, 2005; Tahir, 2006; Opara, 2007).

Similarly, Opara (2007) reported that bruise susceptibility of mature apple fruit cv. 'Gala' picked on three harvest dates, early, mid (11days later), and late harvest (21 days later) differed significantly. The bruise volume increased between early and mid-season fruit, followed by a decrease in late-harvested fruit. Similarly, the author found that the increase in bruise diameter (mm) followed the same trend as observed in bruise volume but no statisticaldifferencewas observed among the harvest dates. Furthermore, the author found that bruise susceptibility increased between early and mid-season harvested fruit while that of late seasonharvest there was a declined. According to Opara (2007), theincrease in bruise susceptibility of 'Gala' appleswas ascribed tothe differences in fruit size that could be affected by advancing maturityand delaying in harvesting. Overall, the observed differences in bruise damage susceptibility in 'Gala' apples between harvest dates were attributed to the differences in fruit's physico-textural attributes at harvest. Data revealed that early harvested apple fruit were firmer and had higher skin strength by 23.6 % and21.4 %, respectively in comparison to late harvested fruit (Opara, 2007). This demonstrates the need for harvesting on the right time and right maturity stage.

2.4. Time after harvest

The understanding of the effects of time-lapse after harvest on bruising susceptibility of fresh fruit is crucial as a useful tool for fruit packers to provide guidance for the right time for fruit packaging and/ or storage. The prolonged time between harvest and transportation of fruit for further operations leaves the produce with field heat for a longer time, which subsequently leads to faster senescence and reduced fruit turgidity (Mowatt, 1997; Bollen, 2005).Martinez- Romero*et al*. (2004) identified that the number of days elapsed between harvest and onset of the observed mechanical damagescould be an effective factor impacting the fruit bruising. Furthermore, increase in the time elapsed (days) after harvestis attributed to the decline in fruit turgidity of young tissue which consequently improves their resistance to bruise damage. Research in 'Braeburn' apples shows that fruit damaged 24 h after harvest was less susceptible to bruising than those damaged within 10 min of harvest (Bollen, 2005). It has been suggested that increased water loss from fruit after harvest results in a loss of turgor, which can potentially reduce the susceptibility of fresh fruit to bruising (Zhang *et al*., 1992; Bollen, 2005). Similarly, Mowatt (1997) used a small commercial grader to simulate bruise damage occurring under normal grading operations and found a substantial difference in average bruise area per fruit drop between harvest and subsequent storage days.

3. Maturity and ripening at harvest

Maturity stage is one of the most important factors influencing bruise damage susceptibilityfor many fruits (García *et al*., 1995; Martínez-Romero *et al*., 2004; Lee, 2005). Previous studies have revealed that mature fruit is more susceptibleto bruise damage than immature fruit (García *et al*., 1995; Martínez-Romero *et al*., 2004; Van Zeebroeck *et al*., 2007c; Canete *et al*., 2015). Kader (1983) reviewed that fruit maturity is one factor singled out as an important parameter with a huge effect in the force required for fruit removal from the plant during picking. Similarly, fruit maturity determines the fruit mechanical properties, physiological processes, and so the relative susceptibility to mechanical damage. As the fruit nears maturity, it undergoes through a series of physiological changes, which conceivably affect susceptibility to bruise damage (Bollen, 2005). Furthermore, maturity at harvest stage potentially influences produce's susceptibility to water loss and mechanical damage (Van lindenet *al*., 2006b; Canete *et al*., 2015). Recent, Canete *et al*. (2015) reported that the stage of maturation at harvest affects the fruit response to mechanical stress in fleshy harvested fruit. In their work, the authors revealed an increase in the bruise area (mm^2) and bruise volume (mm^3) of 'Algerie' loquat fruit with increased maturation stage. Similarly, Hung and Prussia (1989) observed higher bruise susceptibility (mm^3J^{-1}) and lager bruise volume in mature 'Red Globe' peaches than immature peaches.

Fruit firmness has been useful as a criterion for sorting the fruit into a different level of maturity or separating overripe and damaged fruits from good ones (Wang *et al*., 2006; Tabatabaekoloor, 2013). Garcia *et al*. (1995) established a good relationship between fruit firmness, turgor, ripening process and bruise susceptibility through modelling. Proposed models suggested that susceptibility to bruising is affected by fruit turgidity and changes in firmness occurring during ripening. Working with apples, Garcia *et al*. (1995) observed the decrease in bruise damage with declining fruit turgor, while the opposite was true for the fruit firmness. This lead to the conclusion that, the effect of harvest date on bruise damage of fresh applesdepends on change in turgor and/orfirmness that primarily dominates the ripening process. In concurrent with this, Hydeet *al*. (2001b) revealed that adjusting turgor by a slightreduction in hydration (equivalent to 2 - 3 %massloss) of apple fruit could reduce enough fruit turgor to double the bruise threshold. Hertog *et al*. (2004) later found that the cellturgor also contributes tofirmness of producebesides the structural cell wall components.

Bugaudet *al*. (2014) indicated that during ripening of studied banana cultivars, the peelelectrolyte leakage, and to a lesser extent peel hardness were the main parameters closely related to the observed differencesin bruise damage susceptibility. The peel electrolyte leakage reflects membrane permeability (Saltveit,2002), such that as ripening progresses, banana peel tissues lose their cohesiondue to solubilization of the cell wall (Kojima *et al*., 1994)and thus losing membrane integrity. Consequently, stress due to impact damage overcomes the cell wall strength, causing the breakdown. According to Bugaudet *al*. (2014), the membrane permeability of 'Grande Naine'and 'Flhorban925' bananas that showed higher resistance to bruising was below 27 % irrespective of the ripening stage while the most bruise-prone 'French Corne' bananas exhibited the highest membrane permeability during ripening.This highlights that fruit maturity and ripening process is associated with several physico-chemical parameters that could be linked to variations in bruise susceptibility among fruit cultivars.

The effects of fruit ripening on bruise damage susceptibility have been reported in other fruits including apples (Bollen *et al*., 2005), tomato (Van Zeebroeck *et al*., 2007a) and loquat (Canete*et al*., 2015). The degree of fruit ripening affected the incidenceof bruising in cv. Algerie loquat fruit (Canete*et al*., 2015). They found ~56 % increase in bruise area an initial maturation stage to the final ripening stage. It was further concluded that ripe loquat fruit are highly susceptible to bruise damage in comparison to unripe mature fruit, whereas less maturefruit have lower bruise incidence compared to mature ones (Canete*et al*., 2015). Similarly, higher bruise damage was observed with advanced ripeness of tomato (Van Linden& De Baerdemaeker, 2005). Working with tomatoes, Vanlindenand De Baerdemaeker (2005) highlighted that the mechanismof bruising is a combination of physical injury and thesubsequent breakdown of the cell wall components by theaction of cell wall-related proteins. Furthermore, Van Linden *et al*. (2006b) described the fruit texture and the fruit susceptibility to bruising as two parameters that change throughout the fruit development and ripening process.Overall, maturation and ripening stages and their respective relative influence in bruise susceptibility of fruit need to be fully comprehended prior to harvest in order to avoid bruising while maintaining the fruit quality attributes. For instance, some fruit such as peach and loquat may fail to ripen properly or may ripe abnormally if harvested too soon (immature) (Crisosto & Valero, 2008; Canete *et al*., 2015). Consequently, this may affect the fruit marketability due to impaired physico-chemical quality attributes such as colour, texture, sugar and acidity. This indicates the need for identifying the best harvest stage that meets fruit quality attributes at harvest without compromising resistance to their mechanical properties in relation to their susceptibility to bruising at harvest.

4. Effects of pre-cooling

Pre-cooling after harvest could potentially affect the way in which fruit respond to impact or compression, as shown in Table 3. Ferreira *et al*. (2009) studied the effect of cooling methods on fruit response to impact and compression bruising. Their results indicated that both the cooling effect and cooling method used could lead to varying responses to both compression and impact damage of fruit. For instance, ‘SweetCharlie’ strawberries forced-aircooled to 1 ºC had larger bruise damage (measured as bruise volume, mm^3) than those held at 20 ºC both subjected to the same drop impact (38 cm). In comparison, hydro-cooled fruit had larger damage at20 ºC than those held at 1 ºC. Further results revealed that strawberries that were forced-air cooled to 1 ºC, had larger bruise volumecompared to berries hydro-cooled to 20 ºC. These findings suggest that immediate cooling after harvest could be a potential approach to improve fruit resistance to bruise damage. Toivonen*et al*. (2007) suggested rapid air-cooling after harvest could also be a useful strategy to ensure complete recovery from apple bruising for none severe harvest-induced bruise damages.

The effect of pre-cooling using forced-air on the fruit firmness and bruise damage of plums was studied by Martinez-Romero*et al*. (2003).The study revealed that fruit firmness was 39.4 % higher in pre-cooledbruise damaged fruit in comparison to fruit damaged before the pre-coolingprocess. It is conceivable that improved fruit firmness influenced the mechanical strength of plums, as shown by reduced bruise damage and prolonged fruit shelf life in pre-cooled damaged fruit. These results strengthen the need for pre-cooling of fruit immediately after harvest to reduce the incidenceof bruising during handling andtransport and improve postharvest storage and shelf life. Tahir (2006) reported a positive relationship between the decrease in bruise susceptibility of apple cultivars and pre-cooling treatment. Pre-cooling with air reduced the bruise damage (measured as bruise area, mm^2) of ‘Aroma’ apples by 25 % and ‘Ingrid Marie’ by 15 % in comparison to untreated fruit, with no significant effect observed in ‘Cox’s Orange Pippin’ apples. The effect of pre-cooling with air on decreasing bruise damage susceptibility of fruit relies in part on creating a vapour gradient between the interstitial air spaces in the fruit cortex and the atmosphere around the fruit that enhance increased water loss from the fruit (Klein, 1987). Subsequently, water loss from fruit tissue causes the decrease in turgidity resulting to improved fruit resistance to bruising (Tahir, 2006).

5. Postharvest environmental storage conditions

5.1. Effects of temperature

Temperature is one of the major post-climacteric factorsthat influencesbruising of various fruit (Hyde *et al*., 1997; DeMartino *et al*., 2002; Van Zeebroeck*et al*., 2006). Various scientific evidence suggests that temperature influences fruit bruising. Temperature of the fruit flesh affects tissue flexibility, and hence equally affecting susceptibility to bruising

(Bajema *et al*., 1998; Studman, 1997; Hertog *et al*., 2004). When there is temperature fluctuation in the surrounding environment, water volume inside the fruit to expand and contract, resulting in an effect comparable to that of turgor (Hertog *et al*., 2004). Lee (2005) stated that temperature influences the tissue resistance to bruising by affecting cell hydration that leads to increased turgor. Subsequently, an increase in fruit turgor potentially increases both the stiffness and elastic modulus of the tissue resulting from an increased cell tension. An increase in the cell internal pressure tends to reduce the additional force needed to fail the preloaded tissue (Bajema *et al*., 1998), which means the increase in tissue susceptibility damage. Concurrently, Studman (1997) indicated that the cell wall of fresh produce become less flexible at low temperature, which may contribute to higher susceptibility to bruising. There is another suggestion that lowtemperature affects a lag in metabolic activity and a change infruit texture, and concluded that the final effect of temperature on bruisesusceptibility could depend on thebalance between the aforementioned processes (Van Linden *et al*., 2006b).

Hertog *et al*. (2004) hypothesized that temperature drop causes an increase in viscosity of the cell walls and reduction in the cell wall strength resulting to increased brittleness of weak cell walls and increased tissue stiffness. The increase in failure stress of potato tissue with an increased temperature that was attributed to changes in cell wall viscosity (Bajema *et al*., 1998b). Baritelle and Hyde (2001) revealed similar observation in pears that an increase in handling temperature increased tissue failure strain. Chun and Huber (1998) worked on tomatoes and found that cold temperature reduces the bruise damage susceptibility. Accordingly, metabolic activity, notably softening rate of fresh fruit increases with storage temperature, and sodoes polygalacturonase activity, an enzyme that isresponsible for increased bruise damage susceptibility in higher concentrations (Chiesa *et al*., 1998; Chun & Huber, 1998).

Based on the discussion above on various means in which temperature influences the bruise damage susceptibility in fruit, frequently researchers have reported conflicting results. Some works have established the dependency of fruit susceptibility to bruise damage on fruit temperature or temperature of handling environment (Hyde *et al*., 1997; Bollen, 2005). Stow *et al*. (2004) noted that at the same impact level, 'Colney' sweet cherries at 0 °C had higher impact bruise damage than those maintained at 5 °C. Bugaud *et al*. (2014) found that reduction in storage temperature from 18 ºC during ripening to 13 ºC tomimic the real storage conditions of commercial ripeners reduced susceptibilityto impact bruising in bananas. The drop in temperature delayed maturity of bananas by two days, which was indicated by the rate of ripening, the pulpfirmness, and soluble solids. The authors attributed the delay in banana maturityprior to peel softening at a lower temperature to the observed decrease in bruise damage susceptibility of banana.

Study of the effect of pulp temperature on susceptibility to bruising of three strawberry fruit cultivars'Chandler', 'Oso Grande' and'Sweet Charlie' was reported by Ferreira*et al.* (2009). Their findings highlighted the incidence and severity of strawberries bruising as temperature dependent. During compression test, the measured bruise size (mm^3) of strawberries decreased with declining temperature of the fruit pulp, with highest values of bruise volume being significantly higher at 30 °C compared to that of cold pulp (1 °C). Similarly, Ferreira*et al*. (2009) showed the bruise size due to drop impact of strawberry fruit increased with increase in drop height and decreasing temperature of the fruit pulp. These observations are in agreement with earlier findings in bananas, pears and some stonefruit that fresh fruit have a different response to injuries when subjected to the differenttype of forces at low temperatures (Banks & Joseph, 1991; Hyde *et al*., 2001a). The temperature of sweet cherries fruit flesh at the time of impact affected the bruising incidence of four cultivars, 'Bing', 'Brooks', 'Tulare', and 'King' Crisosto *et al*. (1993). The authors observed that sweet cherries handled at a temperature < 10 ºC had a higher internal and external bruise damage whereas less percentage of cherries damaged was noticed for fruit handled at a temperature > 10 ºC. Overall, the understanding the fruit response on compression or impact loading at different temperatures could be used as a strategy to minimise the incidence and severity of fruit bruising during harvest and postharvest handling. The guidelines on the appropriate time of the day suitable for fruit harvesting could also rely on such information. For instance, fruit that are sensitive to bruising at higher temperature should be harvested early in the day when fruit pulp temperatures are lowest.

There are a few reports that the temperature of the fruit pulp/ flesh or of storage environment does not affect bruise damage susceptibility of fruit. Jung and Watkins (2009) reported that the bruise size in impact damaged 'Empire', 'Fuji' and 'Golden Delicious' apples were not affected by fruit temperature at the time of bruising. Similar results were confirmed in New Zealand apples, where a few studies conducted have shown that fruit temperature at the time of impact has no significant influence on the bruise damage susceptibility of fruit (Klein, 1987; Mowatt, 1997; Bollen, 2005). Bollen (2005) could not find any significant difference in bruising between 'Braeburn' apples dropped at 8 °C and those at 26 °C.

5.2. Effects of humidity

Limited data are available on the effects of storage humidity on bruise damage of fresh fruit. However, a few earlier published works have revealed that humidity during storage potentially affects the bruising during storage. Garcia *at al*. (1995) reported that there was a difference in bruising susceptibility (mm^3J^{-1}) between 'Golden Supreme' and 'Golden Delicious' apples stored in dry and those at humid air. They further revealed that apples from both studied cultivars stored at room temperature and dry condition (20 – 25 ºC; 35 – 49 % RH) for 16 h had reduced bruise size (mm^3) compared to those stored at higher humidity (100 % RH), for 'Golden Supreme' and 'Golden Delicious', respectively. Likewise, Akkaravessapong*et al*. (1992) investigated the effects ofhumidity, low (~50 %), medium (~70 %) and high (~90 %) on the bruise susceptibility of bananas cv. Williams during subsequent storage and ripening. Storage humidity did not influence the susceptibility of bananas to bruise. However, the authors observed the difference in colour of bruised areas of bananas at low or medium humidity stores (black) and those stored in high humidity (light brown). The observed colour change was attributed to desiccation of the damaged surface tissues, presumably due to the higherrelative loss of water from the fruit in low or medium humidity stored bananas (Akkaravessapong *et al*., 1992). These observations were confirmed by Wills *et al*. (1989) who established that low humidity storage worsens the appearance of the damagedsurface of fresh produce by making the bruise more pronounced.

Banks and Joseph (1991) examined the effects of time and humidity level on the compression bruising threshold and weight loss of harvested bananas. The results showed the drop in compression bruising threshold of banana with time after harvest at low humidity treatment (75 % RH). These results were similar for bananas held at higher humidity (92 % RH), where the bruise threshold declined within 48 h of harvest. Even so, the authors' results clearly indicated that at higher humidity, the decline in bruise threshold was delayed forabout 24 h, an effect that confirmed the time effect was largely due to water loss. Furthermore, the pattern of weight loss results of bananas suggested that the initial increment of total weight loss (1.62 %) at lower humidity (75 %) was crucial in determining the fruit's threshold for compression bruising. The summary of the effects of temperature and humidity on bruise damage susceptibility of various fruit is presented in Table 4.

6. Effects of storage duration

Several studies have identified the length of storage as an essential factor influencing susceptibility to bruise for many type fruit. Generally, stored fruit are less susceptible to bruising than freshly harvested fruit (Klein, 1987; Pang, 1993; Garcia *et al*., 1995). Klein (1987) studied the effects of harvest date and length of time in storage of New Zealand 'Gala' and 'Granny Smith' apples. The results showed an increase in bruising with the lateness of harvest and decreased over storage time. Similarly, Pang (1993) working with apples Cv. 'Jonathan' and 'Delicious' reported an increase in bruise size with advancing preharvest maturity while decreasing with an increase in storage time. Vursavus and Ozguven (2003) reported that immediately after harvest, peaches fruit exhibited superior strength properties measured as bio-rupture forces, modulus of elasticity and shear stress before rapid softening observed after 14 d of storage.

Hung and Prussia (1989) reported changes in both bruise volume (BV, mm^3) and bruise susceptibility (BS, mm^3J^{-1}) of peaches were not significant during storage. However, the authors noticed changes in BS after 14 d. Garcia *et al*. (1995) noted that apple fruit cultivars 'Golden Delicious' and 'Golden Supreme' at harvest were more susceptible to bruising than after storage. In the same study, similar results were found true for pears 'Blanquilla' and 'Conference' cultivars. Decrease in fruit turgidity during storage has been attributed to the decrease in susceptibility to bruising (Klein, 1987; Garcia *et al*., 1995). Furthermore, it has been established that, at a given impact energy, turgid fruit have lower deformation than flaccid fruit (Garcia *et al*., 1995). This has been attributed to higher absorption on mechanical stresses in turgid fruit that result in more susceptibility to bruising (Timm *et al*., 1998). Similarly, Pasini *et al*. (2004) stated that long storage duration of fruit could potentially increase the resistance to mechanical impact. Yurtlu and Erdogan (2005) worked on two cultivars of pears (Williams and Ankara) and apple (Starkspur Golden Delicious and Starking). The authors found that BV (mm^3) and BS (mm^3J^{-1}) of both apple fruit cultivars dropped from 10, 15 and 20 cm heights onto a metal surface tended to decrease as the time in cold storage increased, except for cv. Ankara whose bruise size increased. The authors attributed this propensity for both apple cultivars and 'Williams' pear to the increase in fruit skin resistance and changing texture during storage that could result in decreasing the energy absorbed during impact.

There are a few reports that have revealed that storage duration can increase the sensitivity of fruit to bruising. Lippert and Blanke (2004) observed that longer cold (2 ºC) storage of mechanically harvested European plums cv. Hauszwetsche induced fruit softening and bruising. Similarly, Brusewitz and Bartsch (1989) reported that bruise damage of apples increased with increasing storage period. Gołacki *et al*. (2009) observed higher values of bruise resistance coefficient (BRC) for fresh apples dropped within studied range of damaging heights and less for 4-week- stored apples. Results in BRC between two sets apples lead the authors into conclusion that fresh apples suffer less interior damages under the identical impact conditions compared to stored apples, hence confirmed that apples after storage exhibited lower bruise resistance than the fresh ones.

A study in pomegranate fruit examined the effect of storage (120 d) on bruising (Shafie*et al*., 2015). Their results showed that storage time of fruit impacted at cheek position at a high impact energy (1390 mJ) increased slightly the bruise size by ~5 % after 120 d of storage. A similar trend of increase in BV was also observed for fruit impacted at calyx position using same impact energy level. According to the authors, the observed changes in bruising of pomegranate fruit was attributed to physiological and structural changes of fruit during cold storage, usually loss of cell-wall integrity due to the breakdown of pectin substances, leading toan increase in soluble pectin and a decrease in fruit firmness (Mirdehghan*et al*., 2006; Ekrami-Rad*et al*., 2011). Overall, the influence of storage duration on fruit bruising could rely on the storage conditions such as temperature, humidity as well as the atmosphere surrounding the stored fruit, which greatly influences physiological changes of the fruit the course of storage.

7. Effects of controlled atmosphere storage

Application of controlled atmosphere storage (CAS), in combination with appropriate temperature control, has been a common practice for maintaining quality and extending shelf-life of fresh of fresh produce (Hussein *et al*., 2015). Stable storage conditions such as gas composition, temperature, and humidity are a major requirement for effective CAS (McMillin, 2008). Hence, the effectiveness of CAS in maintainingfruit quality could be achieved through the regulation of humidity, in addition to air (oxygen, O_2, and carbon dioxide, CO_2) concentrations. Hence, changes in other attributes such as physico-mechanical properties of fruit during storage in CAS could also rely on the aforementioned conditions. Prange *et al*. (2001) studied the effect of low-humidity CAS (gas composition: 4.5 % CO_2 +

2.5 % O_2) on compression bruising of apple cv. McIntosh. The authors observed the decrease in visible bruising on both green and red side of the 'McIntosh' apples when compressed with a force of 90 N. It was further concluded that low-humidity CAS coupled with increased handling temperature (10 ºC) contributed towards greater fruit moisture loss observed, though the treatment had a huge potential in reducing compression bruising.

The effect ofpostharvest heating on bruise susceptibility of normal atmospheric air- or controlled atmosphere storage oftwo apple cultivars 'Aroma' and 'Ingrid Marie' was investigated by Tahir *et al*. (2009). Heat treatment (at 40 ºC and 80 % RH for 24 h) and CAS (gas composition: 2.0 kPa O_2 + 2.0 kPa CO_2 and 90 % RH), eitheralone or in combination, decreased the bruise damage (lower BV) in both cultivars. The combined effects increased the positive effectof each individual treatment on bruise susceptibilityof the two cultivars. Heat treatment results in the cushioning effect that decreases the impact pressure by melting skin wax and induce structural changes of the fruit (Roy *et al*., 1994). Additionally, according to Tahir *et al*. (2009), the combined treatment effectfurther maintainedbetter firmness of both apple cultivars in comparison to the control, non-heated or normal air stored fruit. Hence the improved resistance to bruising (lower bruise volume) in treated and controlled atmosphere (CA) stored apples was attributed to the improved firmness that might have increased the ability of fruit to withstand impactinjury (Tahir *et al*., 2009; Li*et al*., 2016).

Eckhoff *et al*. (2009) studied the effect of storage method on the bruise sensitivity of apple cultivars 'Braeburn', 'Jonagold' and 'Golden Delicious'. The authors reported neither of the storage conditions investigated, CA or ultra-low oxygen (ULO) storage at 2 ºC influenced the bruise sensitivity of 'Braeburn' and 'Jonagold' apples as opposed to normal atmosphere. These results were ascribed to the positive influence of the CAS/ ULO storage in reducing the degradation of the pulp strength, which subsequently maintains the resistance of the fruit towards the development of pressure sores. In contrast, normal atmosphere storage lowered the relative humidity a condition that influenced water loss from the stored fruit (Eckhoff *et al*., 2009). Increased water loss during storage reduces the fruit sensitivity to bruising as indicated by formation of less of pressure sores (Prange and Delong, 1998; Kupferman, 2006). However, Tahir (2006) found the opposite results of the effect of ULO on bruising of 'Aroma' apples. According to the author, apples in ULO storage had the improved resistance to bruise damage of incomparison to normalatmosphere stored fruit. This effect was attributed to delayed softening (Johnston et al., 2003) or to a decrease in phenolicacid concentration (Van der Sluis et al., 2003) associated with ULO storage. In this regard, a close relationship between bruising and/ or browning susceptibility ofinvestigated apple cultivars to their endogenous phenolic substrates (Phenolic compounds and polyphenoloxidase activity) shows that higher the total phenol contents of cultivar, the higher the susceptibility to bruising and/ or browning (Milani and Hamedi, 2005; Valentines et al., 2005; Tahir, 2006).

Application of rapid CAS (21 % O_2 + 30 % CO_2) reduced the cranberry fruit losses due to bruise damage, physiological or fungal breakdown (Gunes *et al*., 2002). The authors observed fewer incidences of bruising in 'Stevens' CAS stored cranberries after 2 months storage, in comparison to the high level of bruise incidences observed in normal atmospheric air storage fruit. On the other hand, super-atmospheric O_2 in combination with high CO_2 levels resulted in greater losses of cranberries possibly due to its effect on the physiological breakdown of the fruit tissue. As mentioned earlier, better storability of cranberries in the rapid CA storage conditions is attributed to the influence of the later on improving fruit firmness. Firmer fruit are more resistant to bruise damage under normal harvesting and handling conditions (Canete *et al*., 2015; Li *et al*., 2016). Table 5 present the summary of application controlled atmosphere and relative influence on fruit bruising during storage.

8. Effects of chemical treatment

Application of exogenous polyamines such as putrescine and spermidine as postharvest chemical treatment play important physiological functions including delaying fruit senescence, improving firmness and bruise resistance while prolonging fruit storage (Valero *et al*., 1998; Martínez-Romero *et al*., 2000). A study by Martínez-Romero *et al*. (2000) reported the deformation (bruising) caused by 50 N compression was significantly lower in putrescine- treated and calcium-treated lemon fruit than in control (untreated) fruit. It was found that the application of exogenous chemicals affected the lemon firmness. Furthermore, after 21 d of storage, relatively lower decline in initial firmness was observed in putrescine- and calcium-treated lemon fruit in comparison to 27 % reported in control (untreated) fruit. Higher firmness level in putrescine treated lemon was contributed by an additional effect of exogenous putrescine in the inhibition of the action of enzymes involved in softening of peel (Kramer *et al*., 1989). Hence, compression of chemical-treated fruit with 50 N force resulted in less deformation than that observed for control (untreated) lemon using the same compression force.

Martínez-Romero*et al*. (2002) reported the putrescinetreatedapricotsshowed lesssusceptibility to compression damage as indicated by lower bruise volume (BV, mm^3) and bruise area (BA, mm^2) and percentage of fruit deformation compared to untreated ones. After 4 d storage, treated fruit (at 1 mM) had lower BA and BV than untreated apricots. They attributed the reduced fruit sensitivity to impact bruising to higher firmness and lower tissue disruption. Martínez-Romero*et al*. (2002) reported similar findings on apricot fruit treatedwith putrescine (at 1 mM), where the later maintained higherpeel resistance and wholefruit firmness than untreated fruit. The capacity of putrescine tobind pectic substances at the cell wall level(Abbott & Conway, 1989) coupled with the inhibitionof enzyme activity that degradespectic acids (Kramer *et al*., 1989) contributes to the effective function of putrescine in increasing fruit resistance to bruising. In agreement with the results foundin apricots, Martínez-Romero *et al*. (2000) reported the treatment of peaches with putrescine (1 mM) or gibberellic acid (GA_3) (100 mg L^{-1}) was found effective in modifying the fruit susceptibility to mechanical damage. According to their results, treated peaches compressed by 25 N had lower bruise volume and percentage of fruit deformation in putrescine and GA_3 treated peach fruit than untreated fruit.

Li *et al*. (2016) demonstrated thattreatment with 10 ppm of 1-Methylcyclopropene (1-MCP) prior to impact damage reduced the bruise susceptibility of ‘Yali’ pears. According to the authors, the observed lower BV was attributed to improved firmness of 1-MCP treated pears. The role of 1-MCP in improving fruit firmness has also been previously reported in other several produce such as different cultivars of plum fruit (Menniti*et al*., 2004; Khan *et al*., 2009), and pears (Li*et al*., 2016). In view of that, treatment with 1-MCP improves fruit texture, one among other essential rheological parameters that contribute tobruise damage susceptibility (Ahmadi, 2012; Canete *et al*., 2015). Similarly, application of 1-MCP after manual or mechanical harvest of European plums cv. Hauszwetsche improved the fruit quality by retarding bruising in 2 – 3 weeks of cold storage (Lippert & Blanke, 2004). Reported results indicated that manually harvested plums without 1-MCP treatment lower bruise incidence in the first 4 weeks after harvest. On contrary, bruising in untreated plums dropped at 1 - 2 m to simulate mechanical harvest increased exponentially, reaching 50 % incidences. However, treatment with 0.5µL/L of 1-MCP before the mechanical harvest of plums increased the percentage of bruising incidence, while the similar application (mechanical harvest) after the 1-MCP treatment had no effect on bruising of plums.

Jung and Watkins (2009) reported that treatment with 1-MCP reduced slightly the bruising effect of apple cv. Empireand Golden Delicious.Further results indicated that no changes inBV of 1-MCP treated ‘Fuji’ apples was observed while bruise depth (mm) of 1-MCPtreated ‘Golden Delicious’ apples was lower than that of untreated fruit. In conclusion, the authors attributed the lack of changes in measured BV and BD of cold (0.5°C) 1- MCP-treated apples to the possible effect of temperature on the response of 1-MCP.

In conclusion, the discussion under this section highlights the benefits of the timely use of 1-MCP in reducing or preventing bruise incidence and, potentially its application on the tree prior to harvest. In addition,treatment of bruised fruit with 1-MCP has also been proved beneficial in preventing thebruise response such as browning, also known as bruiserecovery in apples (Toivonen *et al*., 2007), and pears (Li *et al*., 2016). Hence, a postharvest chemical treatment that modifies the fruit texture could be a useful tool for reducing fruit susceptibility to bruising by improving its resistance to bruising. The summary of chemical treatments and response to bruise damage susceptibility of various fruit is presented in Table 6.

10. Effects of packaging

Type of packaging and arrangement of produce inside the package alone or in combination could influence the bruising and bruise damage characteristics for fresh produce. In the course ofloading, offloading orduring handling, packages containing fresh produce are at times thrown from certain heights on to other surfaces, an attempt that could resultin impact bruising (Idah*et al*., 2007; Jarimopas *et al*., 2007). Impact bruise damageof produce insidethe package could also result from vibrational movement during transportation of packed fruit in trucks (Fadiji *et al*., 2016a, b). Bruise damage of fruit inside the package is due to the energytransformation as some of the kineticenergy of drops absorbed by produce leads to bruising (Jarimopas *et al*., 2007; Zarifneshat *et al*.,2010). Hence, bruise damage of box-packaged fresh produce by compression represents one of the damaging causes during transportation.

Aliasgarian*et al*. (2013) designed an experiment tostudy the mechanical damage phenomena in strawberry during the harvest and postharvest operations as influencedbyfruit variety, fruit position inthe box as well as box position on the truck. They reported that bruise damage of berries inflicted during transport did not differ between the top and middle layers within the boxes, whereas those in the bottom layers differed significantly from the two other layers. This was attributed to the creep due to compression that might have occurred to the bottom layered fruits. Kumar *et al*. (2016) reported similar findings with litchi fruit packaging, where they assessed losses during long distance transportation. It was found that corrugated fibreboard box (CFB) packaging was more effective in reducing both mechanical (bruised and compressed) and pathological (fruit decay) losses, as opposed to conventional wooden boxes. Similarly, the effects of CFB packaging on bruising of 'Fuji' apples were evaluated by Lu *et al*. (2010). Two commercial CFB packages, the single-wall and double-wall corrugated boxes filled with apples were compared by subjecting to dropping impact loads. The results showed that the percentage of damaged apples was less in the double-wall corrugated fiberboardbox than that in the single-wall corrugated fiberboardbox. This was explained by the ability of double-wall corrugated fiberboardbox to absorb more impact energy, and hence less energy left for the apple resulting in fewer bruises on the apples, as opposed by the single-wallcorrugated fiberboard box.

Fadiji*et al*. (2016a) evaluatedthe susceptibility of apple cv. Golden Delicious packed intwo commercial ventilated corrugated paperboard (VCP) packages, MK4 and MK6 to vibrational bruise damage induced by simulated transport system using an electro-dynamic shaker to excite vibrations.Inallvibration frequencies tested, the bruisedamage susceptibility was highest for MK6 packed fruit, measured as bruise area (BA, 661.10 mm^2) and lowest BA (571.92 mm^2) in MK4 packed fruit. The difference in bruise damage size of fruit between packages was explained in part by differences in transmissibility between the MK6 and MK4 package design. According to the authors, MK6 packagetransmitted more vibration and hence more damage to the fruit packed inside due to the lower length-to-height ratio (1.45); in comparison to its counterpart ratio of 1.86. Furthermore, the lesslength-to-height ratio of MK4 allowed lesser apple -to-apple impact during vibrationthan the fruit inside the MK6 package (Fadiji *et al*., 2016a).

Another research assessed the impact bruise damage of 'Golden Delicious' apples inside the two types of ventilated corrugated paperboard packagedesigns, Bushel MK4 and Econo) Fadiji *et al*., 2016b). Irrespective of drop height, fruit placed in the MK4 package in layers (with plastic trays) experienced less bruising than bulk packed fruit inEcono package (inside polyethylene plasticbags without trays). They estimated 50 % higher bruise incidence and 66 % higher BS (mm^3J^{-1}) in bulk packaged fruit than on those packed in the layeredpackage. Despite the lower impact energy exerted on the Econo package with bulk fruit arrangement and comparatively higher energy measured in MK4 package with the tray arrangement, the BS was higher for fruit in the former package than the latter. They found that lower bruise damage corresponds to the effectiveness of trays inside the package to absorb more of the exerted impact energy than theenergy transferred to the fruit. These findings highlight that both the package design and fruit arrangement within the package potentially influence the incidence and severity of bruise damage.

Evaluation of protective performance of various shipping packages (corrugated fiber boxes, reusable plastic crates, and foam nets) based on the measurement of bruise damage inflicted to packed mangoes during simulated shipping test was reported by Chonhenchob and Singh (2003). The results indicated that the percentageofbruise damage during shipping was reduced with the use of foam net cushions of individual fruit in comparison to crates and box-packed bare fruit. In addition, the performance between of crates which diffed in layout configuration and corrugated fiber box packages were further compared and revealed that mangoes in a ten fruit-per-layer and five fruit-per-layer configurations crates showed less percentage bruising as compared to nestable reusable and straight-walledplastic containers. Reduction in bruise damage was 50% higher in the crate-packed mangoes in comparison to the latter containers whose reduction was in the range of 4.87–21.24%. These results were attributed to the inner stacking and configuration layout ofmangoes in these containers. The conclusion from this study was made that choice of container system and packing configurationin the container can greatly affect thebruising of fruitsduring shipping and handling.

Application various packaging materials to wrap individual fruit to provide cushioning in the adverse distribution environment effects is being used as a strategy for reducing both the incidence and bruise damage severity. It has been suggested that good interior packaging should be characterised by practical ability to treat a fruit as separate units, avoids fruit-to-fruit contact, and above all capable of absorbing the impact energy (Jarimopas *et al*., 2007). Jarimopas *et al*. (2007) investigated the impact bruise damage characteristics of two cushioning materials, foam net and corrugated board wrapped to individual apple cv. Fuji. The results presented showed that irrespective of the cushioning materials used, small values ofBV (mm^3) were measured in cushioned apples of lower lines in the package, in comparison to BV for bare or uncushioned fruit. The low impact bruising for cushioned apples was due to absorption of little amount impact energy absorbed by fruit while the cushioning material absorbed the larger fraction of the remaining energy. Another research by Jarimopas *et al*. (2002) studied the suitability of paper as the internal lining surface of plastic and bamboo fruit containers for protecting fresh fruit from bamboo cuts and moisture loss during transport and found it as a poor cushioning material against impact damage. Elsewhere, wrapping of apples with dry banana string made-netting provided suitable cushioning against impact energy of 1.1 J (Jarimopas *et al*., 2004).

Chonhenchob and Singh (2005) evaluated the efficiency of two cushioning systems, the plastic foam nets, and paper-wrap materials in terms of physical protection among other quality parameters of papaya fruit cv. Khagdum and cv. Solo packed in shipping containers during postharvest, quality maintenance, and marketing by performing actual shipment and vibration tests. Results from their study showed that papayas both wrapped with foam nets and paper-based materials had the lowest percentage of bruise damage for both cultivars studied in comparison to uncushioned fruit. Hence, the conclusion was made that, in order to maintain the quality of papayas throughout the handling and distribution system, a single or double layer placement of papayas inside packages coupled with cushioning is recommended.

Another research examined the parameters essential for apple packaging processes by exposing them to random excitation and evaluated effecting of individual apple cushioning on vibrational bruise damage (Eissa *et al*. (2013). Foam-net and paper-wrap efficaciously reduced bruise volume per fruit compared to bruise incidence observed in uncushioned apples. Further results singled out the foam-net cushioning materials as more effective in reducing bruise damage to individual apples than paper-wrap materials or uncushioned apples. The performance of cushioning materials was attributed to their ability to reduce vibration levels during transport beside their sole function of being ‘separating layer’ between each fruit within a package (Eissa *et al*., 2013). It is presumed that cushioning material on packed fruit moves the natural frequencies of the fruit out of range of that the generated by transport vehicle resulting in reduced resonant vibration and vibrational bruise damage.

Overall, the strategies to reducing bruise damage incidence and severity during transportation of packed fresh fruit could revolve around designing of new packaging systems aimed at better protective performance.Hence, the use of fruit handling materials with improved cushioning features in order to reduce the susceptibilityof packaged fruits to bruising during handling and transport is paramount. Other factors such as appropriate stalking, avoiding overfilling in the package and proper handling of packages during loading and off-loading could also contribute to the same.

11. Static versus dynamic loading

As stated earlier, fruit bruising start in the field andmore likely is induced by either dynamic (impact or vibration) or static (compression) stress during harvest, transport, field packing, and subsequent handling operations. Hence, the mechanical energy applied to or absorbed by produce during impact or compression is a major deciding factor on the occurrence and severity of bruise damage (Blahovec & Paprstein, 2005; Opara, 2007; Zarifneshat *et al*., 2010). Dynamic loading islikely to occur during harvesting in such incidences as fruit dropping into the picking buckets, during sorting and packing or vibration movements, mainly occurring during transportation (Li *et al*., 2014; Komarnicki*et al*., 2016). Likewise, after harvest produce are occasionally subjected to static loading conditions in the field, during transportation or storage , especially when poorly designed bins are overfilled and stalked such that the produce in the lower bins supports the weight, which possibly causes damage (Thompson, 2003; Lewis*et al*., 2007; 2008; Li *et al*., 2014; Komarnicki*et al*., 2016).

Banks *et al*. (1991) suggested that the effects of tissue injuries due to compressive and impacting injuries might differ significantly. Mechanical damage caused by impact and compression are related to the conformation of the fruit' cell wall (Ferreira *et al*., 2008). Dynamic loading leads to the failure of theintercellular bonds or actual cleavage of the producecells, whereas compressionunder constant loading affects the viscoelastic cell wall, causing cell bursting under high stress. Eventually, compression tends to straighten out the sinuous microfibrils of fruit tissue followed by a slip up relative to eachother whereas impact causes the microfibrils to straighten out and separate abruptly due to breakage (Holt & Schoorl, 1976; Holt & Schoorl, 1982). However, as shown in Table 7, under normal harvesting and postharvest handling practices, fruit are more exposed to dynamic loading than static, as the former is higher in incidence and magnitude (Mohsenin, 1986; Kupferman, 2006).

With respect to bruise severity, studies have reported conflicting results between compression and impact bruising. Holt and Schoorl (1976) suggested that more energy is dissipated in the breaking of microfibrils of the stressed tissues during an impact stress, and hence resultant bruise severity isless than it is under a compressive loading. Ferreira *et al.* (2008) simulated conditions encountered during commercial handling of strawberry fruits cv. Chandler, Oso Grande, and Sweet Charlie by subjecting individual fruit to impact or compression forces with similar energy to determine the sensitivity to bruising. Irrespective of cultivar differences, strawberries subjected to impact had lower bruise volumethan compressed fruit, although it was also found that cultivar such as ‘Sweet Charlie’ was more susceptible to compression bruise damage than others.

12. Effects of impact surface

The size and shape of the bruise are influenced by the impact surface among others (Altisent, 1991; Kupferman, 2006; Xu*et al.*, 2015). Different materials have been used as cushioning to reduce the incidence and severity of bruising in both harvest and postharvest handling systems (Armstrong*et al.*, 1995). Materials for cushioning are being used to provide effective energy absorption and dissipation, unlike hard surface that can create the critical stress/strain level in the plant tissue that will initiate bruising (Armstrong*et al.*, 1995; Ortiz *et al.*, 2011). In view of this, Kupferman (2006) revealed that picking into cushioned buckets could reduce bruising at harvest compared to picking into a soft-sided bag or uncushioned bucket. A case study by Kupferman (2006) further stated that rough filling of an uncushioned bin resulted in 89 % bruised apple fruits.

Impact surfaces differ in their ability to absorb the energy generated during fruit impact (Jarimopas *et al.*, 2007; Ortiz *et al.*, 2011). Ortiz *et al.* (2011) investigated the shock absorbing capacity of different impact surfaces (concrete floor, elevated canvases and concrete floor covered with shock absorber canvases) during simulated mechanical harvesting of Mandarins (cv. Orogrande and Clemenules), orange (cv. Navel Lane Late) and lemon (cv. Fino). The authors revealed that bruisingof citrus fruit during harvest depends on the impact surface, among other factors. Idah *et al.* (2007) assessed the impact damage of fresh tomato fruit by dropping from different heights onto different impact surfaces. The results showed that irrespective of drop height, impact bruise damage measured both as bruisediameter and bruise area was highly influenced by the impact surfaces, with greatest bruise damage measured on fruits dropped onto metal surface, followed by wood and plastic whereas foam surface inflicted the least impact damage.

Fu *et al*. (2017) studied the impact bruising of 'Jazz' apples by comparing three types of cushioning materials (polyurethane foams, 1, 2 and 3) with firmness ratings of 2.1, 4.8, and 9.7 to 11 kPa, respectively of 12.7 mm thick each to cover an aluminum plate impact surface. They found an increase in non-bruising impact level to 95, 160, and 160 N, for form 1, 2 and 3, respectively, in comparison to 22 N for fruit impacted in a bare aluminum plate. It was concluded use concluded that use of cushioning forms 2 and 3 provided sufficient cushioning for apples due to the relatively higher non-bruising level of impact (160, and 160 N) tolerated by fruit at impact showed that compared to using the bare aluminum plate or foam 1. Hong *et al*. (2018) determined the effect of load conditions on the mechanical damage of citrus and the protection performance of a different material for citrus. Their evaluation of damage degree of citrus revealed that corrugated paper had the best performance in reducing compression damage (damage degree = 0.4%), followed by plywood (damage degree = 4.7%). With respect to drop impact experiment, the use expanded polystyrene had the lowest damage degree (6.46 %), while high-density polyethylene had the best effect on reducing damage (damage degree = 4.33 %) due vibration forces.

13. Conclusion and future prospects

This comprehensive literature has reviewed a number of factors influencing the incidence and severity of bruising in fruits and vegetables at harvest and all along the postharvest handling chain, especially during packhouse operations, transport and storage. Incidences of bruise damage of various fresh produce during mechanical harvest using machines or by careless handpicking has been widely reported. Hence, the use of appropriate harvesting equipment and trained personnel coupled with careful handling of harvesting produce could reduce the incidences and severity to bruising.

It has further been established that, among other postharvest factors, temperature is the major post-climacteric factor of huge influencing to the susceptibility of various produce to bruising. Consistent contrasting results reported on the temperature effect on bruise susceptibility for various produce. Overall, the influence of temperature on bruising of many fresh horticultural produce is paramount. Appropriate and consistent temperature control could be one among other operating strategies across all handling operations in an attempt to reduce incidences and severity of bruise damage. Furthermore, the review has also shown the use of postharvest treatments alone or in combination with other storage methods such as CAS provides promising results in minimizing the sensitivity of fresh produce. A number of chemical treatments such as exogenous application putrescine, spermidine and 1-Methylcyclopropene have been proved effective in improving resistance of various produce to bruising. Careful selection of appropriate postharvest treatment alone or in combination to obtain the dual effects in improving the resistance to bruising should be a prerequisite.

Given the increasing demand of fresh horticultural produce in the global market and expanding use of mechanised techniques in both harvesting and postharvest handling operations, future research direction must target towards the exploration of how bruising is influenced by these emerging techniques at each stage and specific produce. Study of various postharvest treatments and their relative influence on reducing or increasing bruise damage susceptibility is also paramount. This could provide the horticultural industry with a science based-tool to help in adjusting operating conditions including changing the design of harvesting machines and plant architecturein an attempt to reduce bruising incidences.

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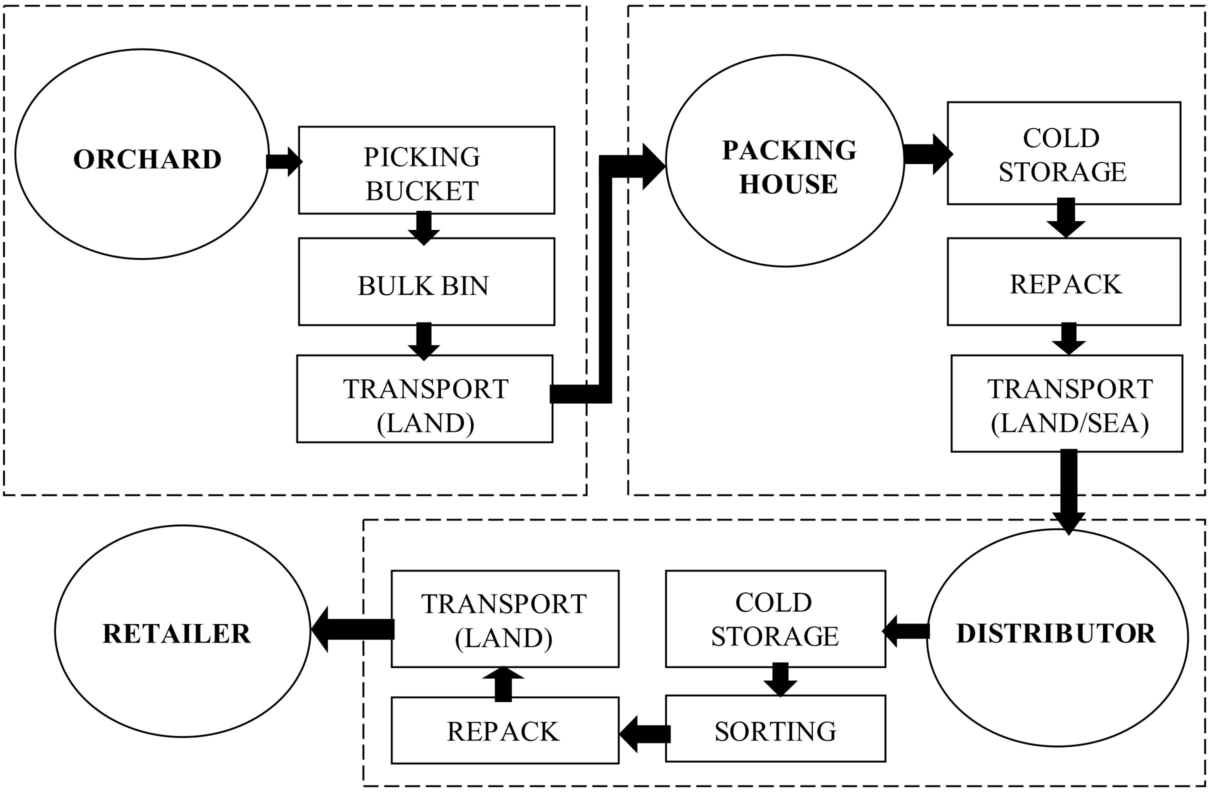

Fig. 1 Postharvest chain of fresh fruit from the orchard to consumer retail stores (modified from Lewis *et al*. 2008).

Fig. 2 Commonly used manual fruit harvesting and harvesting tools (Adopted from Dhatt and Mahajan, 2007)

Table 1 Influence of harvest methods on bruising of various fresh fruit cultivars.

Fruit (cultivar)	Harvest methods	Major conclusion	References
Blueberry (cv. King)	Commercial mechanical harvester	78 % of mechanically harvested blueberries had severe bruise damage	Brown *et al*., 1996
Blueberry (cv. King)	Hand picking	23% of hand harvested blueberries had detectable bruise damage	Brown *et al*.,1996
Apple (cv. Jazz, Pacific Rose, PinkLady)	Shake-and-catch harvesting system	~8 % of all three tested apple cultivars were bruised with bruising diameter between 6.4 and 19.0 mm	He *et al*., 20 17
Apple (cv. PinkLady)	Robotic picking using three-finger gripper	46.7 and 60 % of bruised 'PinkLady' apples picked using 14.47, 15.87 mean grasping force, and 0.28 and 0.29 MPa mean grasping pressure, respectively.	Li *et al*., 2016
Table olives (cv. Hojiblanca and Manzanilla)	Trunk shaking harvester versus manual picking	Manual picking of 'Hojiblanca' and 'Manzanilla' olives resulted in ~17.5 and 50.8 %, respectively of severely bruise-damaged olives. Harvesting by mechanical trunk shaker caused 61.9 and 77 % of bruise damage in 'Hojiblanca' and 'Manzanilla' olives, respectively.	Zipori *et al*., 2014.
Prune (cv. Sweet Prune)	Straddle harvester	< 10% of mechanically harvested prunes had signs of bruising	Mika *et al*., 2015
Table olive (cv. Manzanilla de Sevilla and Manzanilla Cacerenaz)	Grape straddle harvester	Mechanically harvested 'Manzanilla de Sevilla' olives had 100 % bruise incidence and 91 % bruise incidence for 'Manzanilla Cacerena' olives.	Morales-Sillero *et al*., 2014.

Table 1 Continued.

Fruit (cultivar)	Harvest methods	Major conclusion	References
Plums (cv.Cacanska Lepotica, Jojo and Valjevka)	Straddle mechanical harvester	~18% of the plums harvested mechanically showed some bruise damage	Mika *et al*., 2015.
Table olive (cv. Manzanilla de Sevilla and Manzanilla Cacerenaz)	Hand picking	Handpicked olives had ~50% of bruise damage for 'Manzanilla de Sevilla' and 9 % for 'Manzanilla Cacerena'	Morales-Sillero *et al*., 2014.
Apple (cv.PinkLady)	Manual (hand) picking	Average GF (5.05 N) and GP (0.24 MPa) exerted on fruit by grasping fingers did not cause any detectable bruise damage	Li *et al*., 2016

GF = grasping force (N); GP = grasping force (N).

Table 2 Effects of harvest time during the day, harvest season and time after harvest on bruising and bruise susceptibility of various fresh fruit cultivars.

Fruit (cultivar)	Major conclusions	Reference
	Harvest time	
Banana	Morning hours harvest: Compression bruise threshold 17 % higher than late (day harvest) harvested banana	Banks & Joseph, 1991
Apple (cv. Granny Smith and Cripps Pink)	Late day harvested fruit: less bruised (BS = 4.03 and 3.09 mL/J for 'Granny Smith' and 'Cripps Pink', respectively)	Abbott *et al*., 2009
	Morning harvest: more bruised (BS = 4.55 and 3.44 mL/J for 'Granny Smith' and 'Cripps Pink', respectively)	
	Seasonal variation at harvest	
Apple (cv. Golden Delicious and Golden Supreme)	Early picked apples (cultivars studied) were less susceptible to bruising (lower bruise volume, mm^3) than later harvested ones	Garcia *et al*., 1995
Pears (cv. Blanquilla)	Early season picked pears were less susceptible to bruising (lower bruise volume, mm^3) than season late harvested ones	
Apple (cv. Braeburn, Jonagold and Golden Delicious)	Compression pressure sores in early season harvested apples increased from average of 1.5 to 2 kg cm^{-2} in mid-season and then declined to 1.7 kg cm^{-2} in late season harvest	Eckhoff *et al*., 2009
Apple (cv. Braeburn and Granny Smith)	early season harvest were less susceptible to bruising (0.015 and 0.010J less absorbed Ei for 'Braeburn' and 'Granny Smith' cultivars, respectively) than later season harvest	Bollen *et al*., 2001

Ei = impact energy (J); BS = bruise susceptibility (mL/J).

Table 2 Continued.

Fruit (cultivar)	Major conclusions	Reference
	Seasonal variation at harvest	
Apple (cv.Gala)	Bruise volume of early picked frequently irrigated apples increased by 1.3 % at mid-season harvest and by 9.3 % in late season harvest fruit	Opara *at al*.,1997
	BS of early picked frequently irrigated apples increased by 14.1 at mid-season harvest and by 11.8 % in late season harvest fruit	
	Time after harvest	
Apple (cv. Braeburn)	Energy absorbed (Ei) during impact of 'Braeburn' apples increased by 17 % after 24 h of fruit harvest	Bollen, 2005
Apple (cv. Royal Gala, Braeburn, Splendour and Golden Delicious)	BA of apples per fruit drop declined from 1.59 cm^2 per fruit at harvest to 1.28, 1.17 and 0.85 cm^2 after 1, 3 and 9 days, respectively	Mowatt, 1997

Ei = impact energy (J); BS = bruise susceptibility ($mm^3 J^{-1}$); BA = bruise area (cm^2).

Table 3 Fruit bruising susceptibility as affected by pre-cooling

Fruit	Cooling method	Main finding	References
Strawberries (cv. Sweet Charlie)	Forced-air cooling versus hydro-cooling	Fruit forced-air cooled to 1 ^{0}C had larger BV by 29% compared to fruit hydro-cooled to 20 ^{0}C 84% larger BV than fruit forced-air cooled to 20 ^{0}C, and 164% larger than fruit hydro-cooled to 1 ^{0}C.	Ferreira *et al*., 2009.
Plums	Forced-air cooling	Higher firmness (2.38 N/ mm) and thus low damage was observed in plums pre-cooled by forced air and bruised as opposed to pre bruise damaged fruit and cooled (1.44 N/ mm)	Martinez-Romero *et al*., 2003.
Apple cv.Cox's Orange Pippin, Aroma and Ingrid Marie	Forced-air cooling	Pre-cooling by forced air reduced the BA of 'Aroma' apples by 25% and 'Ingrid Marie' by 15% in comparison to BA of untreated apples. BA of treated and untreated Cox's Orange Pippin apples was not affected.	Tahir, 2006.

Ei = impact energy; BA = bruise area; BV= bruise volume.

Table 4 Effects of temperature of the fruit at the time of bruising, storage temperature after bruising and humidity on bruising of various fruit.

Factors	Fruit	Main finding	References
Temperature	Sweet Cherry	Lower fruit temperature (2.5 – 3.8 °C) increased sensitive of cherries to bruising than higher temperature (7 – 10 ºC). Temperature range of 7 – 10 ºC for packing line operations was recommended to avoid bruise damage of cherries during packing.	Zoffoli &Rodriguez, 2014
	Banana	Temperature drop from 18 ºC to 13 ºC reduced bruise susceptibility (higher impact energy in low temperature fruit and lower in high temperature handled fruit).	Bugaud *et al*., 2014
	Apple	Apples cv. Jonagold handled at 1 ºC were more damaged by vibrational transportation than apples at 20 ºC. The effect of temperature on apple bruising was more noticeable at high acceleration amplitudes (‘rough handling’).	Van Zeebroeck *et al*., 2007
	Apricot	Impact of apricots cv. San Castrese at low temperature (4 ºC) inhibited the appearance of bruise symptoms	DeMartino *et al*., 2002
	Apple	No difference in bruise susceptibility observed between 'Braeburn' apple fruit held at 8°C and 26°C during impact. At minimal impact energy levels (0 – 0.1J), fruit temperature was not found to be a major factor affecting bruise susceptibility.	Bollen, 2005

Table 4 continued

Factors	Fruit	Main finding	References
Temperature	Strawberry	Decrease in fruit pulp temperature from at 30 to 1 ºC decreased BV due to compression test for cultivar 'Chandler', 'Oso Grande' and'Sweet Charlie'.	Ferreira*et al*., 2009
		Forimpact tests, change in drop height (from 20 to 38 cm) had more severe impact to bruising than change in pulp temperature (1 – 24 ºC), with variation among cultivars.	
	Sweet cherries	Fruits handled at temperature between 0 and 10 ºC had higher bruise damage (> 50 % increase for internal and 30 – 40 % for external damage)	Crisosto *et al*., 1993
		Handling at temperature above 10 ºC resulted in 5 – 40 % of fruit with fruit damage	
	Sweet cherry	Increase in drop impact on fruit by 0.01 J resulted in 6.3 and 5.7 % increase in bruise size (mm^3) for cherries maintained at 0 C and 5°C, respectively	Stow *et al*., 2004
Humidity	Apples	'Golden Supreme' and 'Golden delicious' apples stored in low humidity (35 – 49 %) had 0.04 and 0.07% less BS values, respectively compared to high humidity (100 %) stored fruit.	Garcia *at al*., 1995
	Banana	Low (~50%), medium (~70%) and high (~90 %) humidity did not affect bruising of banana Cv. Williams during subsequent storage and ripening.	Akkaravessapong *et al*., 1992
	Banana	Low humidity (75%) reduced bruising threshold due to compression within 48 h of harvest while at higher humidity (92%) the decline in bruise threshold was delayed for about 24 h.	Banks & Joseph, 1991

Table 5 Impact of controlled atmosphere and ultra-low oxygen storage on bruising of various fresh fruit

Fruit	Main finding	References
Apple	Controlled atmosphere storage (2.0 kPa O_2 + 2.0 kPa CO_2 and 90 % RH) reduced the bruise susceptibility of 'Aroma' and 'Ingrid Marie' apples by23% and 35%, respectively.	Tahir *et al*., 2009
Apples	Neither controlled atmosphere nor ultra-low oxygen storage influenced the bruise sensitivity of 'Braeburn' and 'Jonagold' apples during cold (2 ºC) storage	Eckhoff *et al*., 2009
Apple	Low-humidity controlled atmosphere storage (4.5% CO_2 + 2.5% O_2) reduced compression bruising of apple cv. McIntosh to 15 % Normal atmospheric air storage resulted in 75 % bruise incidences due to compression forces	Prange *et al*., 2001
Cranberry	Rapid controlled atmosphere storage (21% O_2 + 30% CO_2) reduced losses in 'Stevens' cranberry fruit due to bruise damage by 15% after 2 months of storage	Gunes *et al*., 2002

Table 6 Fruit bruising as affected by exogenous polyamines chemical treatment

Fruit	Postharvest treatment	Main finding	References
Lemon	Treatment with 1 mM putrescine	Compression bruising in putrescine- treated lemon was lower (5.18%) than calcium-treated (5.27%) and control (untreated) lemons (5.63 %).	Martínez-Romero *et al*., 2000.
Apricot	Treatment with 1 mM putrescine	Fruit treated with 1 mM of putrescine had lower BA and BV (90 mm^2 and 240 mm^3) than untreated apricots (160 mm^2 and 510 mm^3), respectively	Martínez-Romero *et al*., 2002.
Peach	Treatment with 1 mM putrescine or gibberellic acid (GA_3) (100 mg L^{-1})	Treated peaches had lower BV in putrescine (229.90 mm^3) and GA_3 (299.23 mm^3) in comparison to non-treated fruits (378.36 mm^3).	Martínez-Romero *etal*., 2000
Pears cv. Yali	Treatment with 10 ppm of 1-MCP	Treatment with 1-MCP prior to impact bruising reduced the bruise susceptibility of pears. 1-MCP treated fruit were 14.3 % firmer than non-treated fruit.	Li *et al*., 2016.
Plums	Treatment with 0.5µL/L 1-MCP	Treatment with 1-MCP before the mechanical harvest of 'Hauszwetsche' plums increased bruising incidence, while the same application (mechanical harvest) after the 1-MCP treatment did not.	Lippert & Blanke, 2004.
Apples	Treatment with 1 µL/L 1-MCP	BV reduced by 7 % and 7.6 in 1-MCP treated 'Empire' and 'Golden Delicious' apples, respectively in comparison to 'Fuji' and untreated apples.	Jung & Watkins, 2009.

Ei = impact energy; BA = bruise area; BV= bruise volume. 1-MCP = 1-Methylcyclopropene

Table 7 Potential loading situations influencing bruise damage of fruit from harvest to final destination, and across the postharvest handling chain

Destination/Inception point	Process stage	Type of loading
Orchard	Harvest into:	
	-buckets	Dynamic
	-field-boxes, or	Dynamic
	-pallet boxes	Dynamic
	Transportation to packing-house	Dynamic/Static
Packing house	Dumping, dry or into water	Dynamic/Static
	Sorting	Dynamic
	Grading	Dynamic
	Repack	Dynamic
	Transportation to:	
	-wholesale markets	Dynamic
	-chain store distributors	
	-retail markets	
	-Shelf storage	
Distributor	Sorting (conveyors etc.)	Dynamic
Retailer	Putting on display	Dynamic/static

CHAPTER 4

INVESTIGATING BRUISE DAMAGE SUSCEPTIBILITY AND BRUISE THRESHOLD OF POMEGRANATE FRUIT CULTIVARS (ACCO, HERSKAWITZ AND WONDERFUL) *

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Investigating bruise damage susceptibility and bruise threshold of pomegranate fruit cultivars (Acco, Herskawitz and Wonderful)

Abstract

Bruise damage resulting from excessive impact and compression forces between the point of harvest and consumptionis a major quality problem in fresh fruit marketing. This study investigated the susceptibility of three pomegranate (*Punica granatum*, L.) fruit cultivars ('Acco', 'Herskawitz' and 'Wonderful') to drop impact bruising. Impact threshold required to bruise fruit was investigated by determining the probability of bruise occurrence (PBO) from the population of the fruit of three pomegranate cultivars impacted at minimal drop heights (0.1, 0.15, 0.2 m). The effect of temperature on bruise susceptibility and fruit physiological response was studied by impacting fruit equilibrated at 5 and 20 °C from three higher impacts (drop heights) levels above threshold (0.2, 0.4 and 0.6 m) followed by storage for 10 d to monitor fruit weight loss and respiration rate. Minimum drop impact level at which bruising was first observed and the associated PBOswere 0.1 m (PBO; 0.44), 0.15 m (PBO; 0.5) and 0.15 m (PBO; 0.75) for 'Wonderful', 'Herskawitz' and 'Acco', respectively. Practically, 'Wonderful' pomegranate fruit had the lowest impact threshold, with the highest value of PBO (0.44) and lowest impact energy (371.87 mJ). Bruise susceptibility at higher impact levels above threshold (measured in bruise volume and bruise area) was cultivar dependent; in the order of 'Wonderful'>'Herskawitz'>'Acco'. Fruit stored in cold (5 °C) condition had larger bruise size (bruise volume and bruise area) than those stored at ambient (20 °C) temperature. Weight loss and respiratory activity were significantly reduced both in non-bruised control and bruised fruit stored in cold (5 ºC) temperature. Conversely, at ambient storage, the highest respiration rate and percentage weight loss were recorded in bruised 'Herskawitz' and 'Acco' fruit, which increased with the level of impact bruising and storage temperature. These findings provide an evidence-based understanding of the bruise damage susceptibility of pomegranates and could be used to develop a postharvest handling tool for the investigated pomegranate cultivars.

1. Introduction

Production of pomegranate (*Punica granatum* L.) fruit has recently widely spread to many parts of the globe (Fawole *et al*., 2013; Hassan *et al*., 2012). The consumption of pomegranate fruit has remarkably increased due to its unique sensory and nutritional properties. The edible portion of the fruit (pomegranate arils) is high in antioxidant activity and health-promoting phytonutrients i.e. polyphenols, vitamins, polysaccharides and sugars, acids and some essential minerals (Opara *et al*., 2009; Shafie *et al*., 2015).

From orchard to the points of consumption, pomegranate fruit goes through several main processes, including harvesting by hand, sorting, packaging, storage, transportation, and retailing at stores (Shafie *et al*., 2017). During these processes, fruit are predisposed to either static or dynamic loadings that potentially cause mechanical damage (Kitthawee *et al*., 2011; Polat *et al*., 2012; Ahmadi, 2012). Bruising is the most common type of mechanical damage which results mainly from dynamic loading due to excessive impact and vibration (Opara & Pathare, 2014; Ahmadi *et al*., 2010; Polat *et al*., 2012). Bruise damage is the type of subcutaneous tissue failure which does not involve rupture of the skin of fresh produce (Blahovec & Paprštein, 2005; Opara & Pathare, 2014), and may occur when stress induced on the fruit surface exceeds the failure stress of the fruittissue (Ahmadi*et al*., 2014; Opara & Pathare, 2014). Impacts on fruit commonly occur during sudden fall of fruit onto other fruit, parts of the tree, storage bin or uncushioned surface of machine or grading equipment (Opara & Pathare, 2014). Bruise damage by impact can occur due to rough or improper handling, poorly designed equipment, improper packaging, or inadequate supervision during handling of the fruit (Polat *et al*., 2012).

Mechanical damage due to bruising all along the postharvest chain declines the market value of fruit (Acıcan *et al*., 2007). Bruise damage to fruit reducesproduce quality, causing considerable postharvest and economiclosses. Bruise-induced wounds in fruit trigger a higher rate of metabolism and increased moisture loss, and hence weight loss (Crisosto *et al*., 1994; Aktas *et al*., 2008). Decreased weight of the fruit and the unsightly shriveling lead to economic losses (Crisosto & Valero, 2008).As one of the important quality attributes in fruit production, the fruit weight does not only influence consumer preference but also the marketing of fresh fruit (Holland *et al*., 2009; Fawole & Opara, 2014).Studies on bruising in fruit such as plums (Martínez-Romero *et al*., 2003), lemons (Martínez-Romero *et al*.,1999) and blueberries (Sanford *et al*., 1991) showed that weight loss increased significantly with bruise intensity. Also, studies on bruised peach and pear showed that fruit respiration rate increased significantly with increasing degree of damage (Zhao, 2005). In particular, according to Scherrer-Montero *et al*. (2011), bruise damage increased the respiration rate of citrus species (tangerines, limes, and oranges) by 66 %. To date, there have been no detailed reported studies on the physiological response, particularly weight loss and respiration rate of pomegranate fruit affected by impact bruising.

Fruit bruisingcan be replicated in the laboratory using various test methods, usually designed to simulate different kinds of dynamic loading involved in real time harvesting and/ or fruit handling operations (Kuang, 1998; Held*et al*., 2014; Shafie *et al*., 2015). This study focused on the impact loading, as this is the most prevalent cause of bruising (Mohsenin, 1986; Kupferman, 2006), especially in pomegranate fruit. For this purpose, impact test involving dropping of fruit on rigid surface is the most commonly used technique, and has been used to study bruise damage susceptibility of various fruit such as apples (Lu *et al*., 2010; Stropek & Gołacki, 2015), papaya fruit (Godoy-Beltrame *et al*., 2015), citrus (Montero*et al*., 2009); peaches (Menesatti *et al*., 2001; Zhao, 2005), olive (Jiménez-Jiménez *et al*., 2013; Jiménez *et al*., 2016), banana (Bugaud *et al*., 2014) and pomegranates (Shafie *et al*., 2015; 2017). However, previous findings have shown that the internal structure of pomegranate fruit is different from that of pome or stone fruit and within pomegranate cultivars. This study aimed at investigating the bruise damage susceptibility of three commercially grown pomegranate fruit cultivars ('Acco', 'Herskawitz' and 'Wonderful'). Different impact levels and storage conditions were investigated. Effects of bruising on the postharvest physiology of pomegranate fruit cultivars were also evaluated.

2. Materials and methods

2.1. Fruit selection and pre-conditioning

Three pomegranate fruit cultivars ('Acco', 'Herskawitz' and 'Wonderful') were obtained at harvest maturity from a commercial orchard in the Western Cape Province, South Africa. Fruit were packed in a cardboard box and transported in a ventilated vehicle to the Postharvest Research Laboratory at Stellenbosch University, where fruit were sorted to ensure use of fruit free of any physical defects (such as cracking, sunburn and husk scald). The fruit were selected to obtain fairly uniformity size by weighing each individual fruit using a Mettler weighing balance (± 0.01 g). The mass of fruit used varied significantly among cultivars, ranging between 218–281, 330–365, and 319–453 g for ‘Acco’ and ‘Herskawitz’ and ‘Wonderful’, respectively. Prior to testing, fruit werepre-conditioned at 22 ± 5 ºC and 60 ± 5 % relative humidity (RH) for 24 h in the laboratory benches.

2.2. Fruit impact bruising and storage

Bruises were produced in pomegranate fruit by impact using laboratory fabricated equipment (Fig. 1).The first experiment investigated the minimum impact energy (impact threshold) enough to cause bruise damage, by dropping individual fruit at different drop heights (impact levels) (0.1, 0.15, and 0.2 m) against a rigid flat ceramic floor along with a graduated wooden ruler. Each pomegranate fruit (10 fruit per drop height) across all experiments was dropped twice fromthe same height onto two opposite sides, to allocate an impact at each of the two equidistant points on the cheek position of the fruit. The fruit was caught by hand after the first rebound to avoid multiple impacts. Following impact tests, fruit were incubated at ambient condition (19 – 22 ºC, 60 ± 5 % RH) for 48 h to allow bruise manifestation on damaged tissue. Prior to incubation, the impacted region of each fruit was marked using a marker after every impact in order to facilitate the bruise detection during measurement. In order to ascertain the correct impact position on the fruit, some white powdered chalk was spread on the impact surface. Data from this study were used to calculate the number of fruit that sustained visible and measurable bruise at a given impact intensity, and results have been presented as the probability of bruise occurrence (PBO) (Jarimopas *et al*., 2007) using equation (1):

$$\mathrm{PBO} = \frac{\mathrm{N_b}}{\mathrm{N_s}} \qquad (1)$$

where N_b is the number of fruit sustained visible and measurable bruise, and N_s is the number of replications of the same treatment.

The second set of experiments studied the effects of fruit temperature on bruise susceptibility of the three pomegranate fruit cultivars at higher drop impact levels. Two sets of selected fruit (60 fruit from each cultivar) were pre-conditioned at cold (5 ± 2 ºC, 90 ± 5 % RH) and ambient temperature (22 ± 5 ºC, 60 ± 5 % RH) for 24 h, followed by dropping each fruit from three drop heights (0.2, 0.4 and 0.6 m) onto a flat ceramic floor impact surface as described above. Ten pomegranate fruit were individually dropped twice per drop height for each set of temperature and humidity condition. Impact energy (Ei, mJ) absorbed by the dropped fruit for each drop height was calculated using equation (2).

$$\mathrm{Ei} = \mathrm{m_f} * \mathrm{g} * \mathrm{h} \qquad (2)$$

where $\mathrm{m_f}$ is the mass of each individual pomegranate fruit, g is the gravitational constant, and h is the drop height.

Measurement of bruise size was performed by slicing through the centre of the drop impact (marked) damaged region of each fruit. The bruise damage of the fruit sliced through the impact region was identified by the presence of visibly damaged tissues which were clearly distinguishable from other unbruised parts of the same fruit. Bruise depth (d), and major and minor axes, w_1 andw_2, respectively, of the assumed bruise elliptical shape (Fig. 1 C), were measured using a digital calliper (Mitutoyo, ± 0.02 mm accuracy). Results of bruise damage size were expressed as bruise volume (BV, mm^3) and bruise area (BA, m^2) (Equations 3 and 4). The ratio of bruise volume to the energy absorbed (E_i) during impact (bruise susceptibility, BS, mm^3mJ^{-1}) was also presented using equation 5.

$$\mathrm{BA} = \left(\frac{\pi}{4}\right) * \mathrm{w_1 w_2} \qquad (3)$$

$$\mathrm{BV} = \frac{\pi \mathrm{d}}{24}(3\mathrm{w_1 w_2} + 4\mathrm{d}^2) \qquad (4)$$

$$\mathrm{BS} = \frac{\mathrm{BV}}{\mathrm{Ei}} \qquad (5)$$

In order to reduce further possible effects of pomegranate fruit mass on measured bruise susceptibility, the bruise sensitivity index known as specific bruise susceptibility (SBS, mm^3 mJ^{-1} g^{-1}) was introduced and calculated using the equation 6 (Opara, 2007).

$$\mathrm{SBS} = \frac{\mathrm{BS}}{\mathrm{m_f}} \qquad (6)$$

where $\mathrm{m_f}$ is the mass of fresh fruit (g).

The third experiment investigated the effects of fruit bruising, temperature and storage duration on the physiological response (weight loss and respiration) to simulate fruit handling between harvesting and packhouse operations such sorting, grading and packaging. Pomegranate fruit of all studied cultivars (Acco, Herskawitz and Wonderful) were bruised by drop impact test at 0.2, 0.4 and 0.6 m drop heights using previously described procedures.

Fruit were divided into two sets and stored at cold (5 ± 2 ºC; 90 ± 5% RH) and ambient temperature (22 ± 5 ºC; 60 ± 5 % RH). Measurement of fruit weight loss and respiration during storage were performed as described below (sections 2.3 and 2.4).

2.3. Fruit physiological response

2.3.1. Respiration rate

Respiration rate of pomegranate fruit 'bruised' or 'non-bruised' was measured at intervals using the closed system method as previously described by Caleb *et al*. (2012). In triplicate, one bruised or non-bruised (control) fruit was placed inside 2 L air-tight glass jars (previously equilibrated to the temperature and RH of the experimental storage conditions) with lid containing a rubber septum. Glass jars were kept at 20 ºC on the laboratory bench or 5 ºC in a cold chamber. Oxygen (O_2) and carbon dioxide (CO_2) gas composition was monitored every hour by drawing a gas sample from glass jar headspace using O_2/CO_2 gas analyser (Checkmate 3, PBI Dansensor, Ringstead, Denmark) with an accuracy of 0.5 %. In every sampling day, jar lids were left slightly open overnight to avoid build-up of gases inside the respiration jars. The respiratory activity was calculated from the jar volume, the fruit mass and the time that the jars were closed. Respiration measurements weredone over five consecutive days and results are presented as mean ± S.E (mL $CO_2Kg^{-1}h^{-1}$) of fivedeterminations.

2.3.2. Weight loss

Cumulative change in weight of pomegranate fruit was determined for each set of 'bruised and 'non-bruised' fruit during 10 d of storage at 5 ºC and 20 ºC. The initial fresh weight of 20 randomly selected pomegranate fruit samples were taken after bruising. A set of non-bruised fruit was included in the test as a control. Bruised and non-bruised pomegranates were numbered and changes in fruit weight was monitored daily over 10 d of storage using an electronic scale (Mettler Toledo, Model ML3002E, Switzerland, 0.0001 g accuracy), and expressed as percentage weight loss (% CWL) calculated using the following equation;

$$\%\ WL = [(W_i - W_f) \div W_i] \times 100 \qquad (7)$$

where W_i is initial weight (g) of the fruit at the beginning of storage; and W_f is the weight (g) of the fruit at the time of sampling during storage.For each cultivar, percentage weightloss was calculated as the cumulative mean of five fruit per treatment.

2.4. Statistical analysis

Experimental data were subjected to factorial analysis of variance (ANOVA) at 95 % confidence interval using Statistical software (Statistica 13.0, StatSoft, USA). Main effects (drop heights, cultivars and temperature) and interaction effects were assessed using Pareto analysis at 95 % confidence interval. Post-hoc test (Duncan's Multiple Range Test, DMRT) was used to test for statistical significance such that observed differences at $p < 0.05$ were considered significant.

3. Results and discussion

3.1. Impact threshold for bruise damage

The number of fruit bruised at a given drop impact intensity (probability of bruise occurrence, PBO) from selected minimal impact (drop heights) levels (0.1, 0.15, and 0.2 m) varied among pomegranate cultivars (Table 1). Results revealed that at the lowest drop height (0.1 m), neither 'Acco' nor 'Herskawitz' fruit were bruised while 44 % of 'Wonderful' fruit (PBO = 0.44)was bruised. 'Acco' and 'Herskawitz' fruit dropped from 0.1 m height generated the lowest impact energies of 234.88 and 336.03 mJ, respectively. Lack of observable bruise damage on fruit dropped at 0.1 m suggests that the impact energy generated was below the bruise threshold of the two cultivars. The high number of bruised fruit observed in 'Wonderful' could be attributed to relatively higher impact energy (371.87 mJ) absorbed. These findings suggest that 'Wonderful' pomegranate could be susceptible to bruising when dropped below 0.1 m ($Ei \leq 371.87$ mJ). Similarly, significant differences in impact energies were observed among fruit cultivars with increased drop heights (DMRT, $p < 0.05$). Increasing drop height to 0.15 m resulted in BPO of 0.75 and 0.5, corresponding to the impact energies of 406.26 and 511.57 mJ, for 'Acco' and 'Herskawitz', respectively. Furthermore, at 0.15 m drop height (equivalent Ei = 692.98 mJ), 100 % (PBO = 1) of 'Wonderful' fruit were bruised (Table 1). Impact energies generated at 0.2 m drop height were the highest and resulted in 100 % bruise in all fruit of the investigated cultivars. The lowest fruit drop height required to cause bruise damage of 'Wonderful' pomegranate would be associated with cultivar differences in morphology and cuticular structures (Opara and Pathare, 2014). Practically, the lower drop height of 'Wonderful' fruit indicates that it is the most susceptible to bruise damage, and therefore needs to be handled with extra care during harvest and postharvest operations. However, careful handling of other investigated pomegranate fruit cultivars that will minimize impacts during handling is highly recommended to reduce bruise damage.

3.2. Bruise size - impact energy relationship

Table 2 presents the bruise size to impact energy linear relationship of pomegranate fruit for studied cultivars. Results showed that the effect of impact energy on bruise size was significant during the fruit impact ($p < 0.05$). Both the bruise volume (BV) and bruise area (BA) increased with the impact energy (Ei) for all cultivars and fruit temperature.According to the linear regression analysis and the obtained coefficients of determination (R^2) values, all the BV – Ei relationships were $R^2 \geq 0.87$. The R square valuesof 0.99 and 0.95 were observed in 'Herskawitz' at 5 and 20 ºC, respectively suggesting a good correlation between BV and Ei (Table 2). Similar relationships were also observed in BA – Ei equations, with the highest R^2 of 0.70 and 0.96 for 'Herskawitz' and 'Acco' at 5 and 20 ºC, respectively. This linear relationship between bruise sizes and Ei agreed with that described in mechanical bruising of young coconut (Kitthawee*et al*., 2011), and apples (Aboud, 2006; Acıcan *et al*., 2007). The relationship between bruise size and impact energy highlights the role played by impact level or drop height (i.e. impact energy increases with increasing the height) on increasing fruit bruising. The straight line relationships obtained by linear regression also enlighten that the bruise sizes of pomegranate fruit could be estimated given the impact energy at which fruit are exposed is known. Scattered plots for impact energy and bruise size (BV, BA) versus impact energy for the pomegranate fruit cultivars, 'Acco', 'Herskawitz' and 'Wonderful' at 5 and 20 ºC fruit temperatures are shown in Figure 3 and 4.

3.3. Bruise damage size and bruise susceptibility at higher impact levels

3.3.1. Effects of drop impact level on fruit bruising

The drop heights of pomegranate fruit significantly affected the impact energy generated during dropping ($p < 0.05$). Likewise, the impact energy absorbed during impact significantly affected both the bruise volume (BV, mm^3) and bruise area (BA, mm^2) of the investigated pomegranate cultivars (Fig. 3).Irrespective of fruit temperature, increasing the drop heights significantly elevated the bruise size of both studied pomegranate fruit cultivars. Fruit kept at ambient (20 ºC) temperature showed that increase in drop height from 0.2 to 0.4 m and 0.4 to 0.6 m elevated the BV by 53.2 % and 15.9 % for 'Acco' and by 57.3 % and 27.7 %, for 'Wonderful' fruit, respectively. Likewise, increase in BV with increasing drop heights for

'Herskawitz' fruit followed the same order of 'Acco' and 'Wonderful' fruit, with 62.2 % and 33.8 % increase observed for increasing drop height from 0.2 to 0.4 m and 0.4 to 0.6 m, respectively. The current results corroborated with findings previously reported on pomegranates by Shafie *et al*. (2015), in which the impact energy was revealed to be the main parameter determining the BV in pomegranate fruit bruising. A recent study by Shafie *et al*. (2017) also revealedthat theBV of pomegranate fruit was nearly proportional to drop height and impact energy for different impact surfaces.

Similarly, BA (mm^2) increased significantly from the lower impact level (0.2 m drop height) to the higher (0.6 m) drop height across all cultivars. On average, BA for 'Acco' fruit increased with increasing drop height. For instance, BA increased by 39.1 % after doubling the drop height from 0.2 to 0.4 m, and by 18.6 % when drop height was increased from 0.4 to 0.6 m, respectively.This was inconsistent with the results observed for 'Herskawitz' and 'Wonderful' fruit, in which BA increased with increasing impact level (Table 3). Shafie *et al*. (2017) reported similar bruising results in 'Shishe Cap-e-Ferdows', 'Rabab-e-Neiriz' and 'Malas-e-Saveh' Iranian grown pomegranate cultivars, where an increase in drop height of pomegranate fruit increased the BA significantly. Tabatabaekoloor (2013) suggested that as fruit drop from higher heights more potential energy is released which potentially accelerates the intensity of contact hence resulting in the increased bruised area.

Change in bruise susceptibility, the ratio of BV to the impact energy (BS, mm^3/mJ) with drop impact levels did not follow the trend of other parameters such as BV and BA. Change in BS with increasing drop impact levels was not consistent across all three cultivars and storage conditions. For instance, there was a 24 % increase in BS for cold conditioned 'Wonderful' fruit when drop height was raised from 0.2 to 0.4 and the decline of 6.7 % and 14.7 %, for respective 'Acco' and 'Herskawitz'. In contrast, 'Acco', 'Herskawitz' and 'Wonderful' fruit conditioned at ambient had respective 16.5 %, 24.8 % and 17.5 % significant increase in BS when drop height was raised from 0.2 to 0.4 m. Similarly, the increase in drop height from 0.4 to 0.6 m significantly increased the average BS of ambient conditioned 'Acco' fruit from 4.83 to 5.10 mm^3/mJ.Similar to BS, the results of bruise sensitivity presented as specific bruise susceptibility (SBS, $mm^3mJ^{-1}g^{-1}$) showed that increase in susceptibility to bruising with increasing drop height did not follow the trend of other measured bruise parameters. The exception was observed in ambient conditioned 'Herskawitz' fruit although the increase in SBS between fruit subjected to medium (0.4 m) and high (0.6 m) drop impact was not significant ($p > 0.05$). Overall, the SBS was highest for 'Acco' fruit under both temperature condition, followed by 'Herskawitz'. Hence, bruise sensitivity based on SBS values suggest that 'Acco' fruit could be the most sensitive cultivar to drop impact bruising.

3.3.2. Effects of fruit cultivar on bruising

Pomegranate fruit cultivar significantly influenced the bruise size and bruise susceptibilityof ($p < 0.05$). Comparison of data using Duncan's test showedthat 'Wonderful' fruit was the most bruise susceptible cultivar, characterised by the highest mean values of BVand BA, followed by 'Acco' and 'Herskawitz' (Table 3). Given that mass of fruit for each cultivar is in the order of 'Wonderful'> 'Herskawitz'> 'Acco', the observed impact energy in Figure 2 could have resulted from the higher mass for 'Wonderful' as opposed to 'Herskawitz' and 'Acco' fruit with relatively small mass. It is thus not surprising that there were significant differences ($p < 0.05$) for impact energy amongst the cultivars at each drop height, with the order being 'Wonderful'> 'Herskawitz'> 'Acco' (Figure 2). Our results corroborate with findings of several other studies previously reported in apples (Van Zeebroeck *et al*., 2007b; Ozturk*et al*., 2010); peaches (Tabatabaekoloor, 2013); tomatoes (Hertog*et al*., 2004; Buccheri&Cantwell, 2014), and 'Shishe Cap-e-Ferdows', 'Rabab-e-Neiriz' and 'Malas-e-Saveh' cultivars of pomegranate fruit (Shafie *et al*., 2017). Overall, these authors revealed a tight relationship between impact level and bruising of studied fruit.

Differences in susceptibility to bruising of fruit cultivars subjected to the same impact loading conditions have been associated with their differences in mechanical properties (Van Linden *et al*., 2006; Ghaffari*et al*., 2015). In addition, the natural variabilitythat is common in biological materials (even within the same batch) could be another important source of differences in susceptibility to bruising (Van Linden *et al*., 2006; Van Zeebroeck *et al*., 2007b, c; Ahmadi *et al*., 2014). Pomegranate fruit is featured by natural irregularities both in shape and peels between and within cultivars, which might affect the fruit properties such a radius of curvature, an important property that affects fruit-to-surface impact (Ekrami-Rad *et al*., 2011; Ghaffari*et al*., 2015). Hence, the observed disparities in bruising between the studied pomegranate fruit cultivars in the present study could also be attributed to differences in the cultivars' mechanical and physico-morphological attributes such as firmness, peel thickness and fruit's radius of curvature (i.e. the equatorial diameter) (Shafie *at al*., 2015; Hussein *et al*., 2018). Cultivar differences in firmness and turgidity could also contribute to differences in susceptibility to bruising (Ghaffari*et al*., 2015; Shafie *et al*., 2017).Previous report in fruit bruising indicated that the differences in BV observed in Iranian 'Shishe Cap-e-Ferdows', 'Rababe-Neiriz', and 'Malas-e-Saveh' pomegranate fruit cultivars were attributed to significant differences in fruit properties such as fruit firmness and peel thickness among cultivars. Based on the results these properties, Shafie *et al*. (2017) revealed that higher firmness and peel thickness reduced bruise damage of 'Malas-e-Saveh'.

Physico-morphological properties for different pomegranate cultivarsreported in previous studies have revealed that among the three cultivars in the current study, 'Acco 'fruit had the lowest dimensions of peel thickness, fruit sphericity, and radius of curvature while 'Wonderful' fruit had the highest (Fawole & Opara, 2014). In their results, the authors revealed that 'Wonderful' had 18.5 to 24.8 % and 27.5 to 42.5 % higher radius of curvature and peel thickness compared to 'Herskawitz' and 'Acco' fruit, respectively. As observed in 'Acco' and 'Herskawitz' fruit, a smaller radius of curvature is likely to result to less BV at high impact due to high impact pressure and reduced tissue contact area during impact. On the contrary, fruit with larger tissue in the contact areawith the counter face (i.e., large radius of curvature) will result in greater bruise depth and BV at high impact pressure (Van Zeebroeck *et al*., 2007a; Zarifneshat *et al*., 2010). The present study has identified 'Wonderful' as the fruit cultivar that expressed relatively higher bruise damage followed by 'Herskawitz'. The current results are buttressed by bruising results previously reported in 'Malas Saveh' pomegranate fruit by Shafie *et al*. (2015), who found that higher BV of cheek and calyx regions of the fruit corresponded to the higher radius of curvatures as opposed to the stem position of the fruit. However, it has been further established that the fruit could still suffer more bruise damage if it is induced by higher impact pressure rather than its larger contact area during impact (Van Zeebroeck *et al*., 2007a). This buttresses the conclusion that, irrespective of several other factors, the energy absorbed during impact is the determining parameter for bruise severity of fruit (Opara, 2007; Zarifneshat *et al*., 2010).

3.3.3. Effects of fruit temperature on impact energy absorbed

There were significant differences in impact energy absorbed between fruit cultivars and between drop impact levels in both temperature condition ($p < 0.05$).The effect of fruit temperature on the energy absorbed during drop impact was more pronounced in 'Herskawitz' fruit, in which the mean differences between cold and ambient conditioned fruit was 133.2, 305.4 and 464.4 mJ for low (0.2 m), medium (0.4 m) and high (0.6 m) drop heights, respectively (Fig. 2). Similarly, cold conditioning increased albeit not significant. The impact energy for 'Wonderful' fruit dropped at medium and high drop heights by the mean difference of 90.63 and 12.28 mJ, respectively, in comparison to ambient conditioned fruit. However, 'Acco' fruit showed the opposite response to energy absorption during impact, where cold conditioned fruit had 103.7 and 45.5 mJ lower impact energy than ambient conditioned fruit at low and medium drop heights, respectively. Overall, variation in energy absorbed at impact among cultivarsand temperature could be ascribed to the notable differences in external morphology characterised by large fruit variability in size and shape. In addition, the internal structure of pomegranate fruit featured by natural inhomogeneity greatly affects the impact load and subsequent energy absorbed during impact (Van Linden *et al*., 2006).

3.3.4. Effects of fruit temperature on fruit bruising

Irrespective of the drop impact levels, pomegranate fruit of both three cultivars at cold temperature had higher bruise size than fruit conditioned at ambient temperature. The effect of temperature was more pronounced in 'Acco' and 'Herskawitz' such that the difference in BV between cold and ambient conditioned fruit ranged between 12 – 26 % for 'Acco' and as high as 48 % for 'Herskawitz' fruit. On the other hand, the marked effect of fruit temperature on BV was also observed in 'Wonderful' fruit, with the difference ranging from 10 to 23 % between cold and ambient conditioned fruit.For instance, lowering the fruit temperature to 5 ºC increased the BVof 'Wonderful' fruit by 10.4, 23, and 14.3 % for low, medium and high drop impacts, respectively. Likewise, the influence of fruit temperature on BA was noticed in 'Acco' and 'Herskawitz' fruit although not in a similar trend to that of BV, in which cold fruit expressed relatively higher BA during drop impact compared to fruit conditioned at ambient temperature (Table 3).The effect of temperature on bruising could, in part, be due to higher impact energy absorbed by fruit conditioned under the cold temperature as shown in Figure 2, thus resulting in more bruising. These results are in agreement with Shafie *at al.* (2015), who reported that increasing temperature resulted in a decrease in bruise size for pomegranate 'Malas Saveh' fruit stored at 5, 15 and 25 ºC and dropped at three levels of impact levels.

The effect of temperature on BS (mm^3mJ^{-1}) followed a similar trend of other parameters (BV and BA) for 'Acco' fruit, where the difference between cold and ambient conditioned fruit ranged at 16 – 34 %. The differences in BS between cold and ambient fruit temperature was not significant ($p > 0.05$) for 'Herskawitz 'and 'Wonderful' at all three drop impact levels. With respect to SBS ($mm^3mJ^{-1}g^{-1}$), the cold and ambient conditioned fruit varied significantly ($p < 0.05$) for 'Acco' and 'Herskawitz' fruit. At low and medium drop impact, cold conditioned 'Acco' fruit had a 33 % higher value of SBS than fruit at ambient temperature. On the contrary, ambient conditioned 'Herskawitz' fruit had a 50 % higher SBS then cold conditioned fruit both at medium and high drop impacts bruising.

Overall, studies on the effects of varying temperatures on mechanical properties of fruit such as apple, kiwifruit and tomatoes (Hertog *et al*., 2004; Zarifneshat *et al*., 2010; Ahmadi, 2012) have suggested that temperature affects the cell wall viscosity and cell wall strength. High temperature affects stiffness through the activity of enzymes resulting in cell wall degradation, whereas low temperature causes increase in cell walls viscosity and reduction in the cell wall strength (Hertog *et al*., 2004). Consequently, weak cell walls become more brittle which may result in increased tissue stiffness while reducing the cell wall rigidity (Hertog *et al*., 2004). For instance, fruit stiffness (measured by the modulus of elasticity) is positively correlated with the fruit bruising of apples, which diminishes with increasing fruit temperature (Hertog *et al*., 2004; Van Zeebroeck *et al*., 2007b). It is thus logical to suggest that pomegranate fruit bruising could indeed be influenced by temperature change at the time of impact which could result in changes in fruitphysico-mechanical properties such as elasticity and viscosity). Nonetheless, an in-depth analysis is warranted to establish the influence of temperature on the mechanical properties of pomegranates and subsequent susceptibility to bruising.

The effects ofeach individual factor, impact level (drop height), temperature, cultivar and theirinteraction on bruise size (BA, BV) and bruise susceptibility (BS) of pomegranate fruit were explored using standardised Pareto charts (Fig. 5). The results showed thatthe effects of all the investigated factors were significant ($p < 0.05$), except for that of fruit temperature on BA (Fig. 5B). Drop height and cultivar showed a positive effect on BV and BA whereas temperature had a negative effect on BV and BS. The factor that showed the largest effect was drop height followed by cultivar and finally fruit temperature. The marked effect of cultivar on BS could be due to the influence of fruit mass (Fig. 5C). Accordingly, higher fruit mass contributes to high impact energy that determines the magnitude of BS.

3.5. Fruit physiological response

4.5.1. Fruit respiration rate

Cellular respiration is a metabolic process through which chemical energy required for vital internal reactions and other processesinvolving cellular synthesis and maintenance is produced (Scherrer-Montero *et al*., 2011). Results from this study showed that respirationrates of the three investigated pomegranate fruit cultivars were significantly ($p < 0.05$) dependent on duration, temperature and cultivar (Fig. 6 and 7). Overall, the respiration rate decreased with time of storage and temperature, both in bruised and non-bruised fruit. However, drop impact had a larger effect oncellular respiration for bruised fruitin comparison to non-bruised fruit. For example, the decrease in temperature from 20 to 5 ºC reduced the rate of CO_2 production (mL $CO_2 Kg^{-1}h^{-1}$) by up to 100 % across all cultivars (Fig. 6 and 7). The observed heightened rates of respiration at a higher temperature has also been reported for other pomegranate fruit cultivars such as Acco and Herskawitz, Bhagwaand Ruby (Caleb*et al*., 2012; Fawole *et al*., 2013). Similarly, Segovia-Bravo*et al*. (2011) studied the postharvest changes of intentionally bruised Manzanilla olives and revealed that regardless of fruit cultivar and bruise damage, the fruit respiration rates were lower at 8 ºC (reduced to one-fourth at 8 ºC) than at 25 ºC.

Mechanical damage such as bruising is known to influence respiration rates in fresh produce (Moretti *et al*., 1998; Agar and Mitcham, 2000; Scherrer-Montero *et al*., 2011). The effect of bruising on the respiration rates for all the investigated pomegranate fruit was significant ($p < 0.05$) at both storage temperatures. At the same temperature condition, bruised pomegranate fruit respired faster than non-bruised (control) fruit (Fig. 6A -C). Furthermore, pomegranate fruit impacted at higher drop impact levels (0.4 or 0.6 m) exhibited2 to 3- fold higher respiration rate than fruit bruised at lower impact level (0.2 m) or non-bruised fruit. This is in support of results reported by Zhao (2005), which stated that the respiration intensity of bruised peach, pear and apple fruit increased with the degree of damage to the fruit.

Fruit respiration rate decreased with prolonging post-bruising duration regardless of cultivar and storage temperature. These results agree with those reported for onions bulb (Herold *et al*., 1998), tomatoes (Moretti *et al*., 1998), citrus (Scherrer-Montero *et al*., 2011) and olives (Segovia-Bravo*et al*., 2011). According to Herold *et al*. (1998), a single or multiple impacts loading increased the respiration rate of bulb onions by 142 % after 19 d of storage. In another study, Scherrer-Montero*et al*. (2011) observed an increase in CO_2 production by 66.2, 51.8, 53.2, and25.7% in Tahiti limes, Murcott tangors, Valencia oranges, and Montenegrina tangerines, respectively, when dropped at 0.8 or 1 m onto a rigid surface.

3.5.2. Fruit weight loss

Cumulative weight loss (% CWL) of pomegranate fruit during 10 d of storage after bruising is shown in Figure 8 and 9. The current study revealed that at ambient (20 ºC) temperature, change in fruit weight wassignificantly ($p < 0.05$) affected by both bruising and bruiseintensity. After 10 d of storage at ambient condition, the highest % CWL was observed in fruit dropped at 0.4 and 0.6 m (Fig. 8 and 9). At the end of 10 d of storage, the average % CWL measured in 'Herskawitz', 'Wonderful' and 'Acco' fruit impacted at 0.4 or 0.6 m drop heights and kept at ambient were 17.27, 19.29 and 29.28 %, respectively (Fig. 8, A-C). Similarly, a lower % CWL was observed in low impact (0.2 m) bruised or non-bruised control fruit with no significant differences ($p > 0.05$) between the two for Acco and Wonderful cultivars. The trend of increase in weight loss with bruise severity was only observed in cold (5 ºC) stored 'Herskawitz' and 'Acco' fruit, in which the highest % CWL of 3.49 and 3.75 %, respectively, were measured for fruit bruised at high (0.6 m) impact level. The effect of drop impact bruising on weight loss was less pronounced in 'Wonderful' fruit in comparison to the other two cultivars of 'Acco' and 'Herskawitz'. Overall, the effect of low temperature storage reduced weight loss by up to 8-fold lower than ambient stored fruit. The most crucial observation is thatlow temperature storage significantly reduced the moisture loss of bruised pomegranate fruit for all three cultivars. This could be attributed to a reduced rate of the metabolic process at a low temperature (Scherrer-Montero *et al*., 2011; Fawole *et al*., 2013), even for bruised fruit. Weight loss in pomegranate fruit during storage is promoted by high porosity of its peel which enables free vapour movement, the property that has been ascribed to the fruit's high sensitivity to moisture loss (Elyatem & Kader, 1984; Ambaw *et al*., 2017).

Bruise damage increased the weight loss as revealed in the current study. Bruising results in modification of tissue permeability and the resulting small cracks connecting both the internal and external atmospheres permit the interchange of atmospheric gases, particularly water vapour (Martinez-Romero, 2003). Even though pomegranate fruit susceptibility to weight loss could also be affected by storage conditionssuch temperature, humidity, storage time and type of cultivar (Fawole *et al*., 2013), results from the current study has shown that bruise damage could also accelerate the physiological weight loss and fruit senescence during storage.

4. Conclusions

This study provides information about the bruise damage susceptibility of pomegranate cultivars at impact levels below and above the threshold. The study also established the impact threshold for bruising for each cultivar. Our findings revealed that impact energy is the main parameter in bruise damage potential of studied pomegranate fruit cultivars. The increase in drop impact level (or impact energy) increased the potential for bruise damage to occur on fruit. Therefore, the first step to reducing bruise damage incidence could be to minimise impacts during fruit harvesting and postharvest handling. Based on the bruise damage size (bruise volume and bruise area) which is the most commonly reported measure of the amount of bruise damage, 'Wonderful' fruit was the most susceptible to bruising that was attributed in part to higher fruit mass than ' Acco' and ' Herskawitz'. Therefore, 'Wonderful' fruit is identified as the cultivar that requires additional care during handling due to its critically lower bruise threshold. This finding is of high practical relevance because in practice pomegranate fruit naturally differs in mass that could be due to cultivar differences and/ or orchard management practices. However, the fruit sensitivity to bruising measured as bruise susceptibility and specific bruise susceptibility that considered the impact energy and fruit mass suggested that 'Acco' was the most sensitive cultivar to impact bruising. Fruit temperature also played a crucial role in bruise damage size. Overall, fruit stored in a cold (5 ºC) condition absorbed a higher amount of energy upon impact and hence developed more bruise damage. Again, this highlights the need for temperature management during handling, especially in sorting, grading and packing. The marked rise in the respiration rate and loss in fruit weight was observed as consequences of bruise damage in the investigated pomegranate fruit cultivars.Increase in these physiological responses was influenced by impact levels which had a crucial effect on bruise intensity, the fruit temperature, and post-bruising duration. The effects of bruising on the physiological responses were more pronounced in fruit stored at ambient temperature (20 ºC) than those stored in cold (5 ºC) temperature. Overall, this study has provided new evidence on the bruise damage susceptibility of three important commercial pomegranate cultivars to assist in better postharvest handling practices to reduce fruit losses due to mechanical damage.

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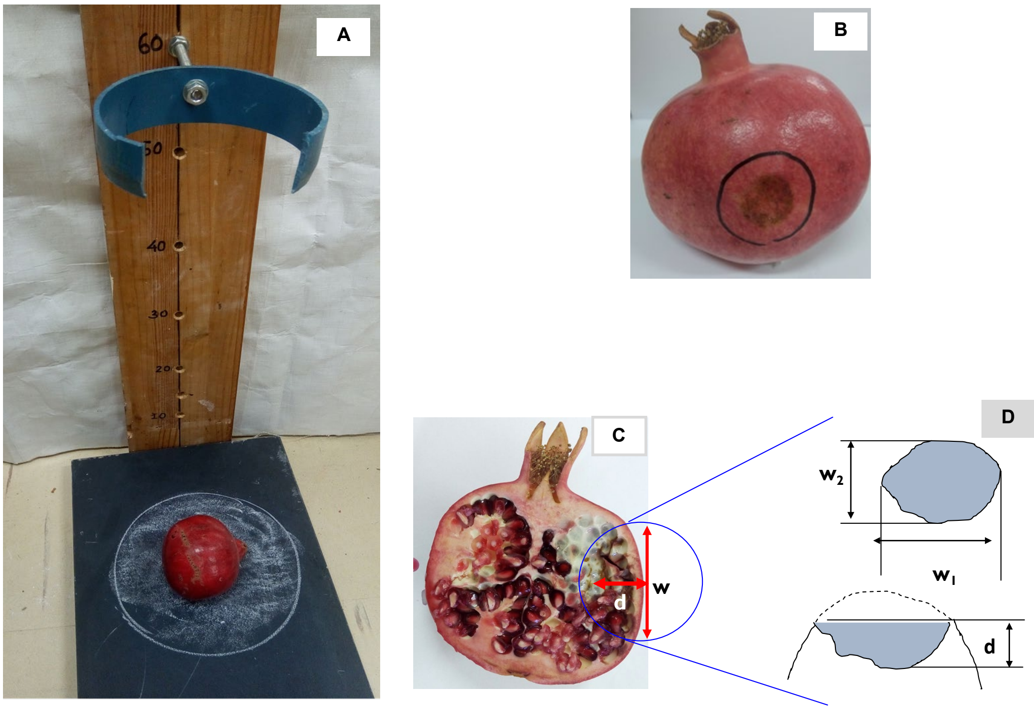

Fig. 1 Pictorial presentation of pomegranate fruit bruising by drop impact technique (A), bruised pomegranate fruit (B), sliced pomegranate fruit across the bruised region (C), hypothesized elliptical bruise shape and bruise dimensions (D): $\boldsymbol{w_1}$ and $\boldsymbol{w_2}$ are major axes (mm) of outer bruise damaged area of assumed elliptical surface shape, and d is bruise depth (mm) measured from peel surface.

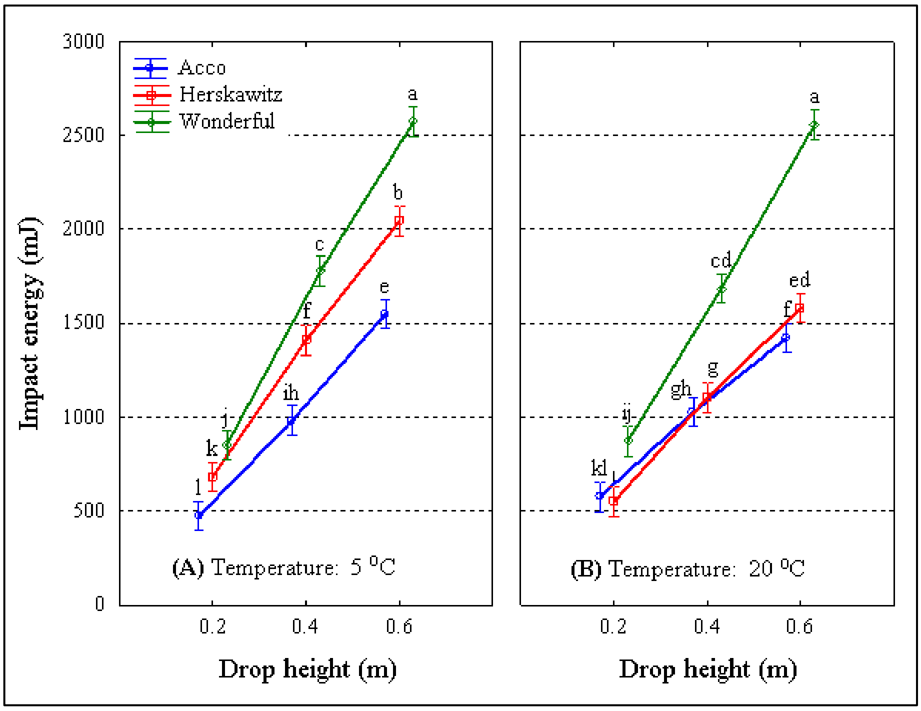

Fig. 2 Impact energy (mJ) absorbed during drop impact of cold (A) and ambient conditioned pomegranate fruit (B) of 'Acco', 'Herskawitz' and 'Wonderful' cultivars at higher drop impact levels above threshold.

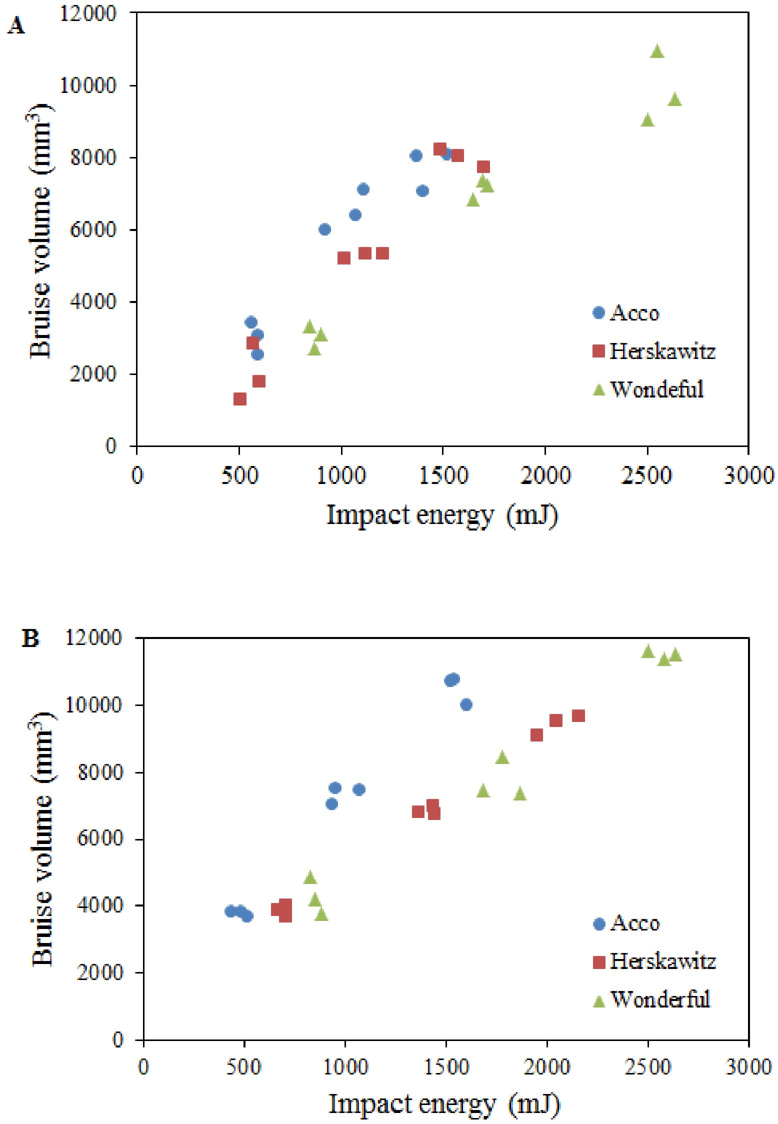

Fig. 3 Bruise volume versus impact energy relationship for 'Acco', 'Herskawitz' and 'Wonderful' pomegranate fruit conditioned at two temperature conditions, (A) ambient (20 ºC), and (B) cold (5 ºC) for 24 h and bruised by dropping at higher drop heights (0.2, 0.4 and 06 m) above threshold.

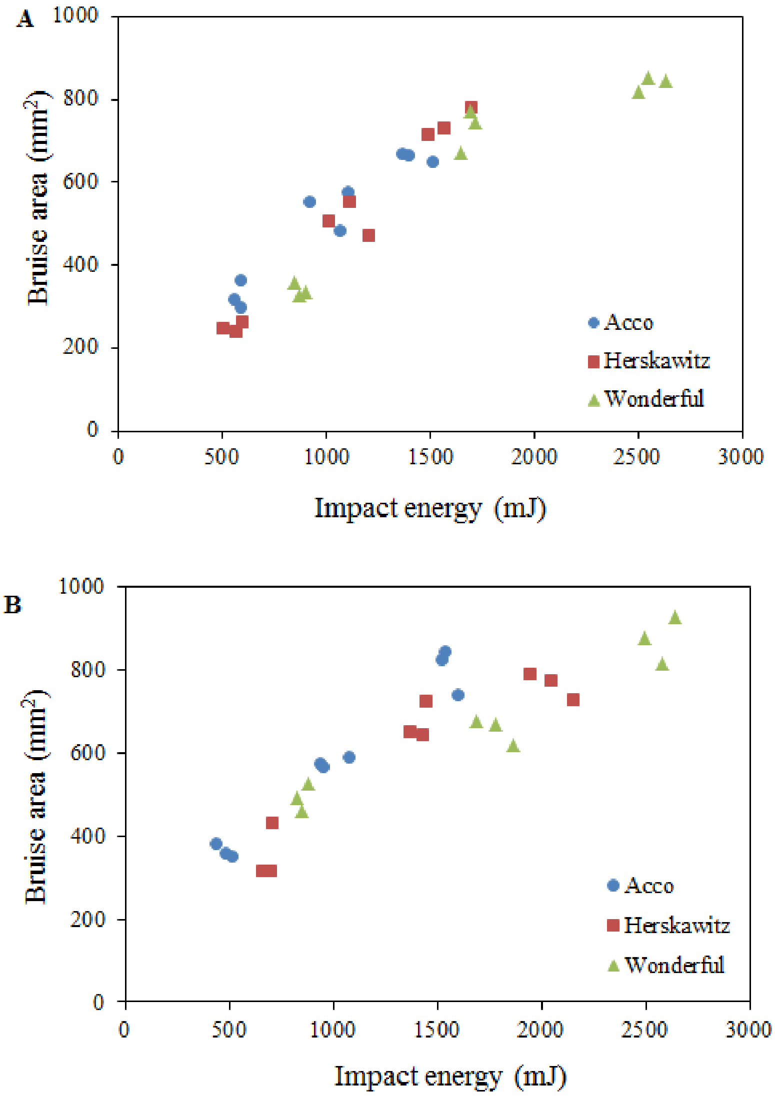

Fig. 4 Bruise area versus impact energy relationship for 'Acco', 'Herskawitz' and 'Wonderful' pomegranate fruit conditioned at two temperature conditions, (A) ambient (20 ºC) and (B) cold (5 ºC) (B) for 24 h and bruised by dropping at higher drop heights (0.2, 0.4 and 06 m) above threshold.

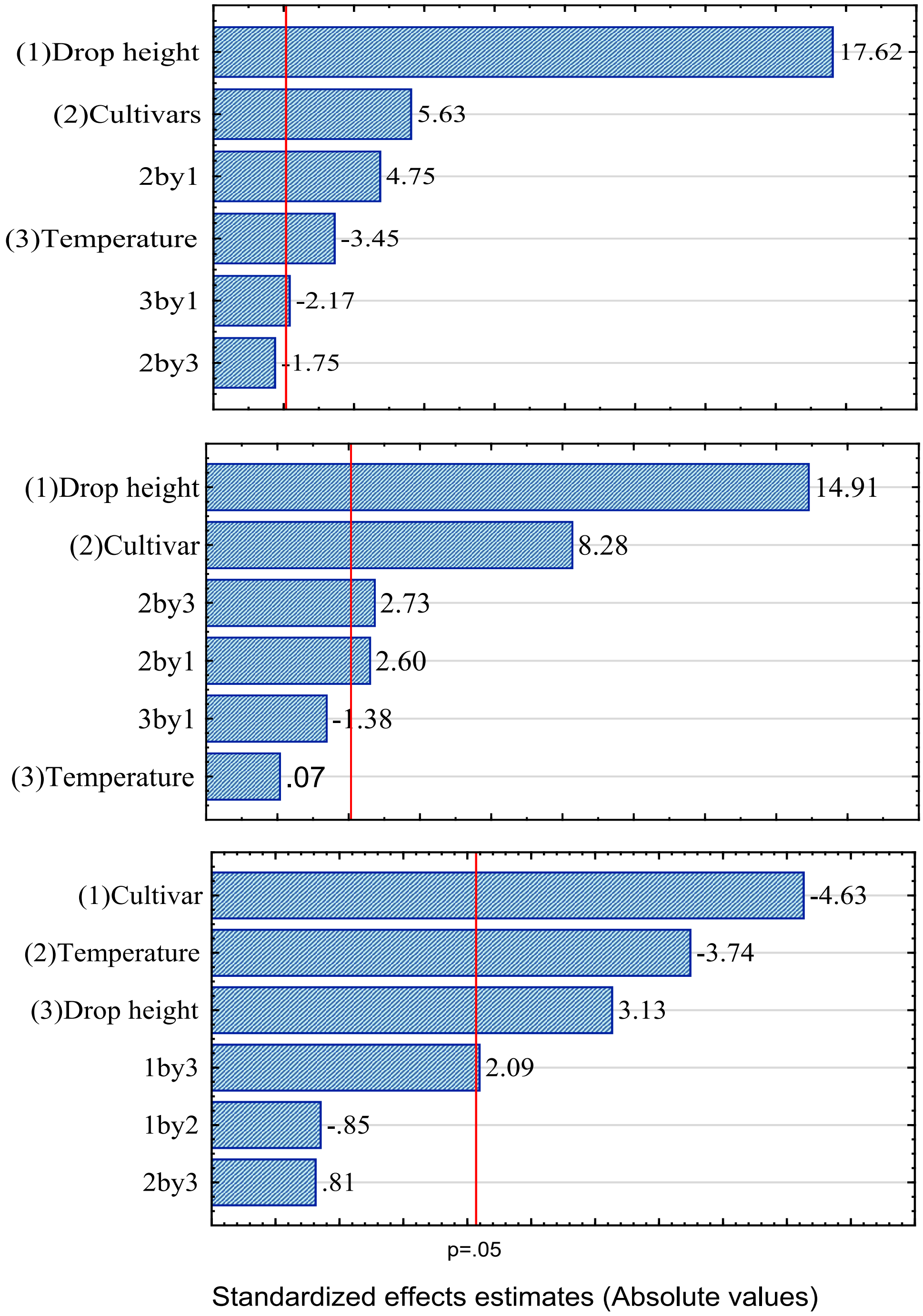

Fig. 5 Standardised Pareto charts showing the main effects (drop height, temperature, fruit cultivar) and their interaction on bruise size; bruise volume (A), bruise area (B); and bruise susceptibility (C).From the charts, the red vertical linecorresponds to the 95% confidence level such that allstandardised effects with bars passing over thisline are statistically significant (according to Duncan's multiple range test, $p<0.05$)

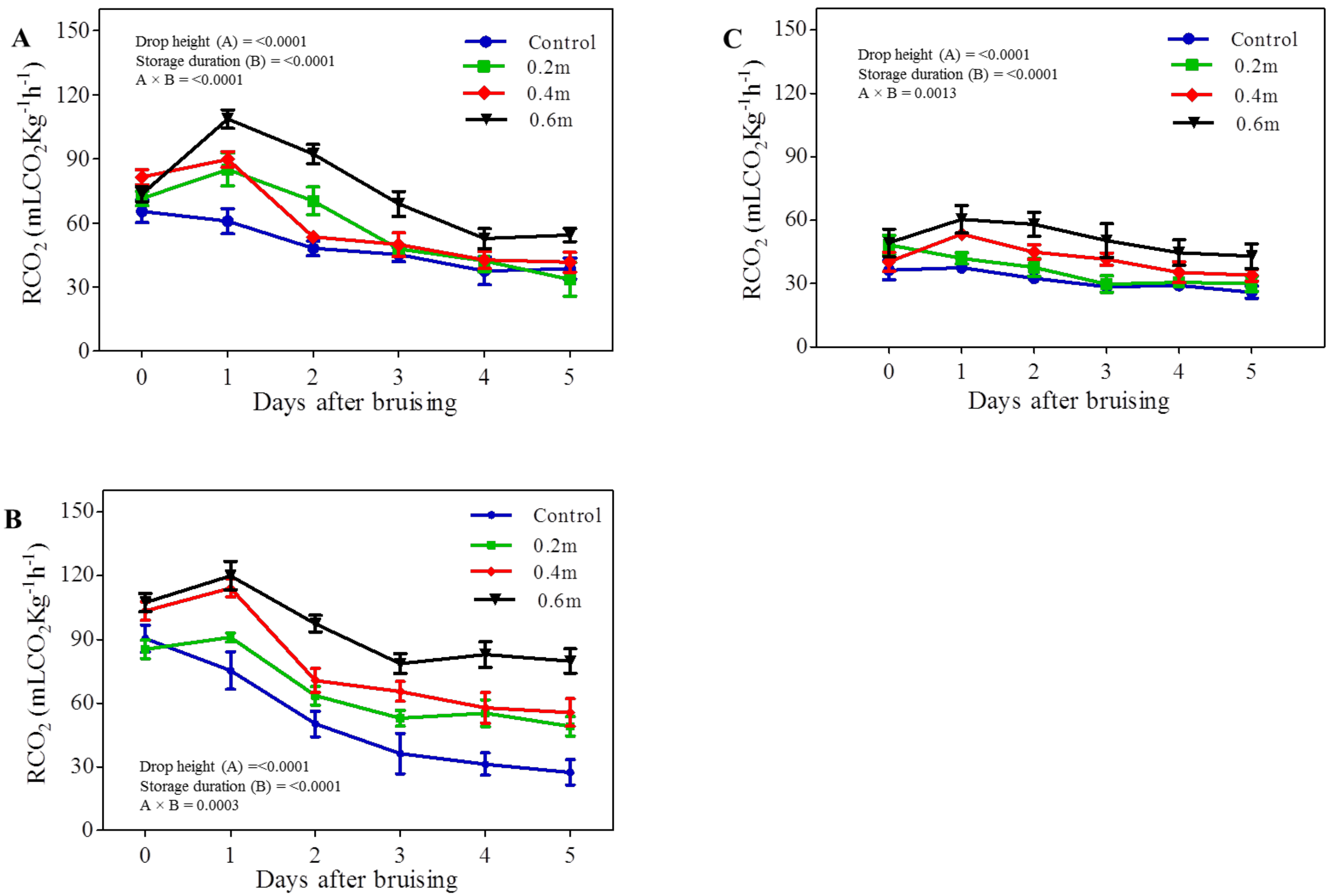

Fig. 6 Respiration rate (RCO_2) of bruised and non-bruised (control) pomegranate fruit cultivars, Acco (A), Herskawitz (B), and Wonderful (C) evaluated during5 d at ambient (20 ºC) temperature. Fruit were bruised by dropping at different drop impact levels onto a hard impact surface. Error bars indicate a 95 % confidence interval.

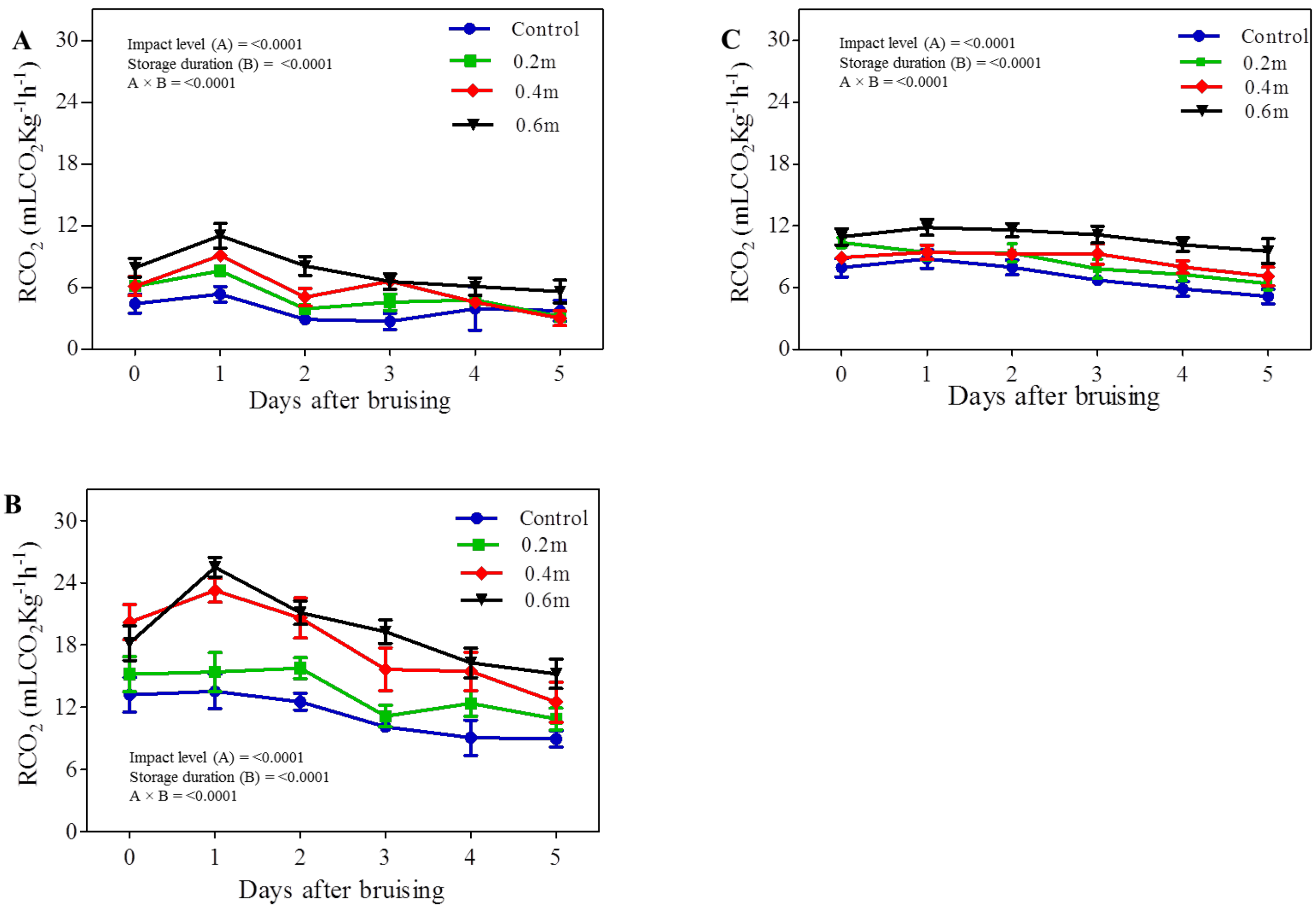

Fig. 7 Changes in respiration rate of bruised and non-bruised (control) pomegranate fruit cultivars; Acco (A), Herskawitz (B), and Wonderful (C) evaluated during5d in cold (5 ºC) storage. Fruit were bruised by dropping at different impact drop levels. Error bars indicate a 95 % confidence interval.

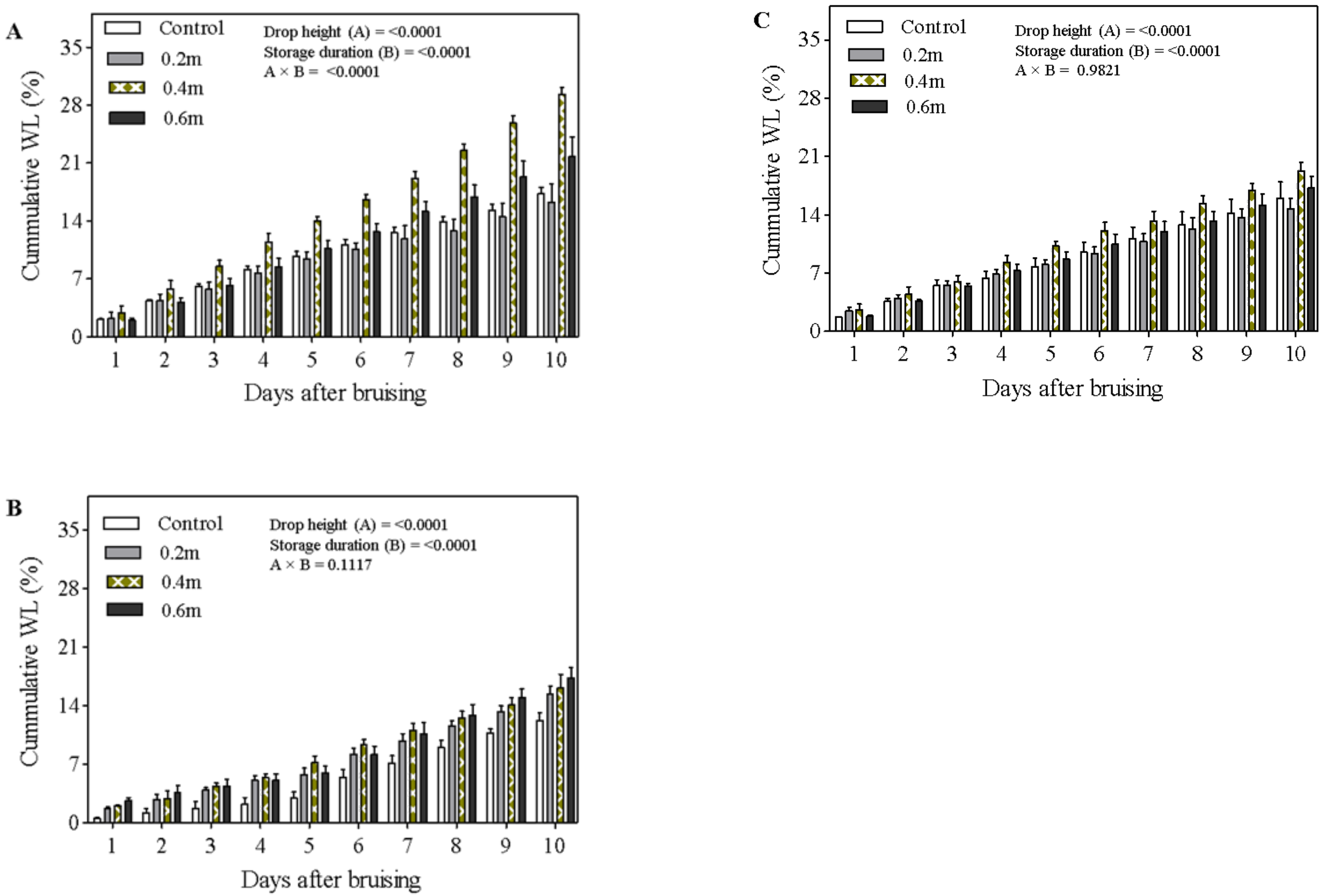

Fig. 8 Cumulative weight loss (WL) of pomegranate fruit cultivars; Acco (A), Herskawitz (B), and Wonderful (C) submitted to impact bruising at different drop impact levels and evaluated during10 d at ambient (20 ºC) temperature. Error bars indicate a 95 % confidence interval.

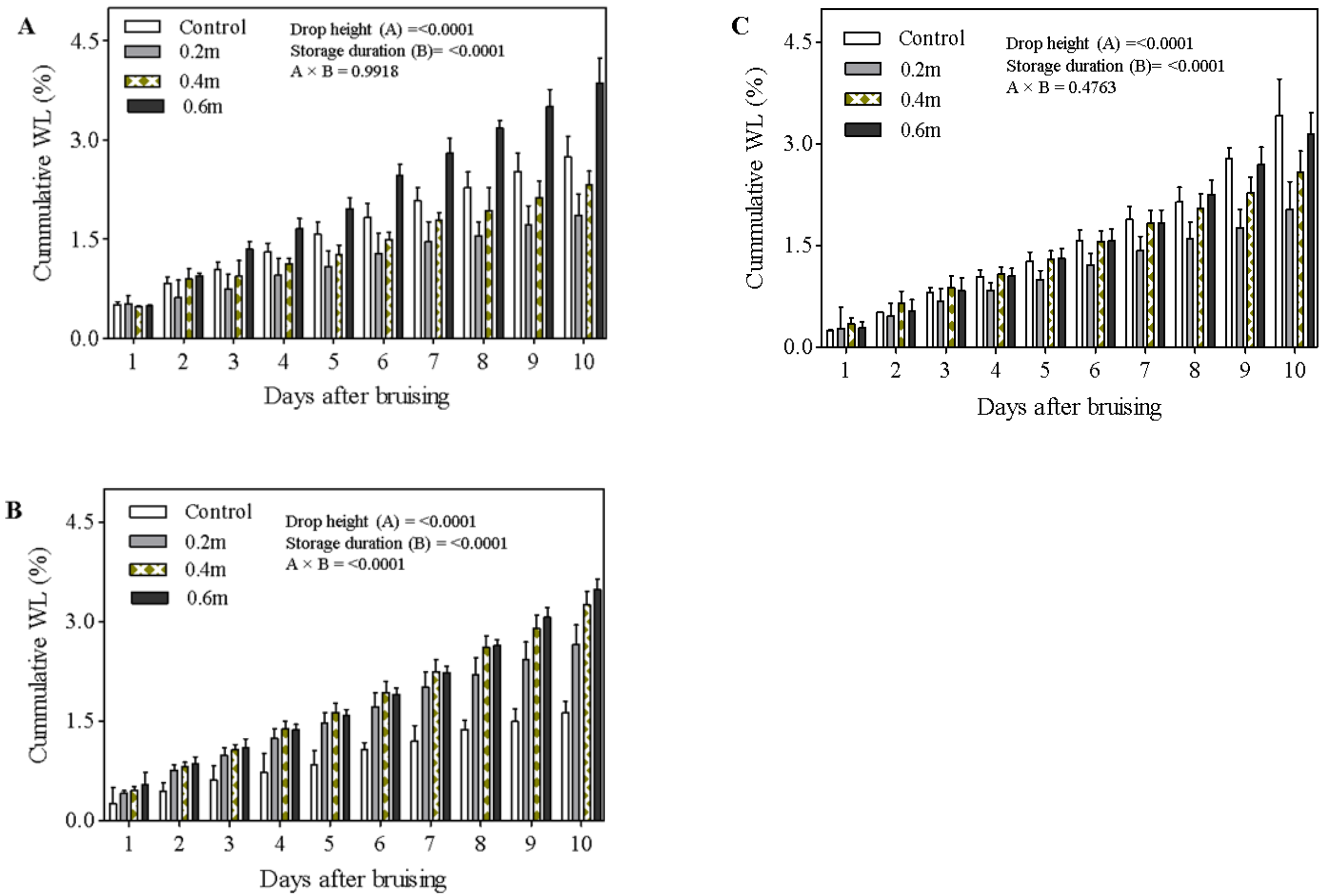

Fig. 9 Cumulative weight loss (WL) of pomegranate fruit cultivars; Acco (A), Herskawitz (B), and Wonderful (C) submitted to impact bruising at different drop impact levels and evaluated during 10 d in cold (5 ºC) storage. Error bars indicate a 95 % confidence interval.

Table1 Impact energy and probability of bruise occurrence at minimal drop heights for pomegranate fruit cultivars, 'Acco', 'Herskawitz' and 'Wonderful'

Drop height (m)	Pomegranate cultivar	Bruise damage impact threshold (mJ)	Probability of bruise occurrence
0.1	Acco	$> 234.88 \pm 11.98^b$	0
	Herskawitz	$> 336.03 \pm 10.38^a$	0
	Wonderful	$\leq 371.87 \pm 9.24^a$	0.44
0.15	Acco	$\leq 406.26 \pm 12.08^c$	0.75
	Herskawitz	$\leq 511.57 \pm 17.24^b$	0.5
	Wonderful	$< 692.98 \pm 22.95^a$	1
0.20	Acco	$< 515.38 \pm 21.35^b$	1
	Herskawitz	$< 838.25 \pm 14.90^a$	1
	Wonderful	$< 919.28 \pm 34.04^a$	1

Mean values are presented as mean ± SE in the same column of each drop height followed by a different superscript letter are significantly different according to Duncan's Multiple Range Test ($p < 0.05$).

Table 2 Fitted linear correlations for bruise size (bruise volume and bruise area) versus energy absorbed at drop impact

Temperature (°C)	Pomegranate cultivar	Linear relationship and coefficients of determination			
		Bruise volume (BV)	R^2	Bruise area (BA)	R^2
5	Acco	BV = 6.17 Ei – 1067.2	0.97	BA = 0.40 Ei + 180.3	0.96
	Herskawitz	BV = 4.07 Ei +1139.6	0.99	BA = 0.30 Ei + 185.8	0.87
	Wonderful	BV = 4.16 Ei + 649.7	0.97	BA = 0.22 Ei + 295.4	0.93
20	Acco	BV = 5.56 Ei + 163.14	0.91	BA = 0.38 Ei + 123.7	0.91
	Herskawitz	BV = 5.68 Ei – 101.36	0.95	BA = 0.47 Ei – 0.91	0.97
	Wonderful	BV = 4.03 Ei – 192.65	0.95	BA = 0.29 Ei + 136.4	0.88

From the linear equations; BV, BA, Ei and R^2 represent the bruise volume, bruise area, impact energy and coefficient of determination, respectively

Table 3 Effects of pomegranate fruit temperature and cultivar on bruising at high impact energy levels above threshold

Bruise parameter	Dh(m)	Pomegranate fruit cultivar and temperature					
		Cold (5 ºC)			Ambient (20 ºC)		
		Acco	Herskawitz	Wonderful	Acco	Herskawitz	Wonderful
Bruise area (BA) (mm^2)	0.2	364.71 ± 9.34^{h}	357.09 ± 38.26^{h}	493.09 ± 19.40^{h}	328.77 ± 19.92^{h}	251.99±16.66^{i}	340.51±18.81^{h}
	0.4	579.14 ± 7.15^{df}	674.71 ± 25.09^{gf}	654.58 ± 17.83^{c}	539.58 ± 327.67^{f}	513.75±22.86^{g}	729.65±29.80^{fe}
	0.6	804.87 ± 31.68^{b}	765.53 ± 18.29^{c}	872.83 ± 32.61^{a}	662.88 ± 35.98^{de}	744.20±19.50^{d}	837.76±10.65^{bc}
Bruise volume (BV) (mm^3) ×10^2	0.2	38.14 ± 157.69^{h}	39.13 ± 90.06^{h}	34.02 ± 135.27^{h}	30.55 ± 254.90^{h}	20.13 ±157.07^{i}	30.48 ± 185.14^{h}
	0.4	73.83 ± 157.69^{df}	68.81 ± 78.26^{f}	92.72 ± 255.38^{c}	65.24 ± 329.97^{f}	53.20 ± 52.07^{g}	71.46 ± 163.81^{fe}
	0.6	105.58 ± 240.01^{b}	94.75 ± 175.13^{c}	115.33 ± 162.88^{a}	77.64 ± 329.97^{de}	80.38 ± 150.20^{d}	98.80 ± 170.11^{bc}
Bruise susceptibility (BS) (mm^3mJ^{-1})	0.2	8.10 ± 0.48^{a}	5.72 ± 0.18$^{e\text{-}h}$	3.98 ± 0.33^{ij}	5.31 ± 0.52^{edf}	3.63 ± 0.77^{ijg}	3.49 ± 0.24^{j}
	0.4	7.54 ± 0.28^{ab}	4.88 ± 0.09^{if}	5.24 ± 0.43^{ijh}	6.36 ± 0.16^{c}	4.83 ± 0.20^{efg}	4.23 ± 0.06^{ij}
	0.6	6.81 ± 0.26^{cb}	4.63 ± 0.06^{if}	4.48 ± 0.08^{ij}	5.46 ± 0.24^{cd}	5.10 ± 0.29^{ed}	3.86 ± 0.22^{ij}
Specific bruise susceptibility (SBS) ($mm^3mJ^{-1}g^{-1}$)	0.2	0.03 ± 0.00^{a}	0.02 ± 0.00$^{e\text{-}h}$	0.01 ± 0.00^{ij}	0.02 ± 0.00^{edf}	0.01 ± 0.00^{ijg}	0.01 ± 0.00^{j}
	0.4	0.03 ± 0.00^{ab}	0.01 ± 0.00^{if}	0.01 ± 0.00^{ijh}	0.02 ± 0.00^{c}	0.02 ± 0.00^{efg}	0.01 ± 0.00^{ij}
	0.6	0.02 ± 0.00^{cb}	0.01 ± 0.00^{if}	0.01 ± 0.00^{ij}	0.02 ± 0.00^{cd}	0.02 ± 0.00^{ed}	0.01 ± 0.00^{ij}

Level of significance

Bruise parameter	Drop height (A)	Cultivars (B)	Temperature (C)	A×B	A × C	B × C	A × B × C
BA	**<0.0001**	**<0.0001**	**<0.0001**	0.1142	0.0967	0.0798	**<0.0001**
BV	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0212**	0.7321	**0.0034**
BS	**0.0261**	**<0.0001**	**<0.0001**	0.1075	**0.0032**	**0.0046**	**0.0313**
SBS	0.2713	**<0.0001**	**0.0015**	0.4413	**0.0010**	**<0.0001**	0.0728

All values are presented as mean ± standard error. Means presented in the same column with different letters indicate significant differences between drop heights, ($p < 0.05$); according to Duncan's multiple range test (DMRT). Means presented in the same row with different letters indicate significant differences between fruit cultivars and between temperature ($p < 0.05$), according to DMRT. All p-values in bold are statistically significant (DMRT, $p < 0.05$); Dh = drop height (m).

CHAPTER 5

APPLICATION OF X-RAY MICRO COMPUTED TOMOGRAPHY FOR DETECTION AND CHARACTERISATION OF BRUISE DAMAGE IN POMEGRANATE FRUIT *

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Application of X-ray micro computed tomography for detection and characterisation of bruise damage in pomegranate fruit

Abstract

The key to extending the storage life and maintaining the quality of fresh fruit could rely on early detection and separation of fruit with mechanical damage such as bruising. A commercial X-ray micro-computed tomography (X-ray μCT) system with a density calibration (1.27 to 2.17 g cm^{-3}) was used to detect and characterise bruise damage in pomegranate fruit (cv. Wonderful). Pomegranate fruit harvested at commercial maturity were dropped from 60 cm onto a flat ceramic surface to simulate inappropriate postharvest handling technique. Non-bruised (control) and bruised fruit were scanned at the following interval: 0 h (immediately after drop impact), 48 h, 3 d, 5 d, 7 d and 12 d after impact bruising. The X-ray radiation from the source operated at 245 kV voltage and 200 mA electron current produced optimal μCT (with an isotropic voxel size of 71.4 μm) that was used to generate two-dimensional (2D) radioscopic images. 2D images of X-ray μCT of pomegranate fruit scanned at 0 h, 48 h, 3 d and 5 d after impact bruising showed no evidence of bruise damage. Bruise damage manifestation was visualised by X-ray μCT after 7 d of impact bruising. In order to non-destructively differentiate between non-bruised and bruised fruit, the density, grey value frequency, total fruit volume and total internal void space were assessed for fruit scanned after 7 d. The results suggest that bruised fruit can be visualized and differentiated after 7 d of impact bruising based on its density (1.69 g cm-3) and grey level frequency (18000-30000). Overall, the application of X-ray μCT with an associated algorithm can be used to detect bruise damage in pomegranate fruit 7 d after impact.

1. Introduction

Pomegranate (*Punica granatum* L.) fruit undergoes several postharvest operations throughout the cold chain; ranging from harvest, sorting, packaging, storage and transportation. As a result of increasing use of mechanised operations at harvest and during postharvest handling, minor mechanical damages on fruits have become a very important problem to detect (Ergun, 2017). Preharvest factors, harvesting and postharvest handling operations predispose fruit to varying levels of dynamic forces that eventually lead to mechanical damage (Hussein *et al*., 2018). Bruising is the most common type of mechanical damage resulting from such forces (Opara & Pathare, 2014). Bruise damage is caused by the failure of subcutaneous cells when the loading pressure exceeds the failure stress of the fruit tissue (Diels *et al*., 2017).

Bruise damage is not always immediately visible after its occurrence, at least for most of the affected fruit (Van Linden *et al*., 2006). Unlike other types of fruit such as apples, tomatoes and banana, the presence of bruising on pomegranates is hardly detectable by flattening or softening. The peeled surface of bruised fruit may or may not be characterized by discoloration resulting from enzymatic reactions of polyphenol oxidase enzyme. Hence, since the damage on fruit is not apparent until a later stage in the handling chain, bruised fruit are easily overlooked and usually neglected during manual sorting and grading (Ergun, 2017). Consequently, symptoms of internal bruise damage become severe and potentially hasten the quality deterioration of affected fruit over time, leading to serious postharvest losses (Brosnan & Sun, 2004). Detection of bruise damage on fresh fruit is critical both to researchers and to the industry personnel in the quest for developing procedures to reduce consequent postharvest losses (Samim & Banks, 1993).

The key to extending the storage life and maintain the quality of fresh fruit could rely on early detection and separation of fruit affected by bruise damage and other quality defects. Application of accurate and cost-effective non-destructive assessment methods for field and laboratory measurement as well as in-line sorting and grading could be a viable option. X-ray micro-computed tomography (X-ray µCT) is one the non-invasive techniques that have been explored to characterise and detect several kinds of internal defects in agricultural produce (Donis-González *et al*., 2014; Magwaza & Opara, 2014; Arendse *et al*., 2016. Diels *et al*. (2017) successfully applied X-ray computed tomography to detect and quantify the bruise damage of different apple cultivars at a range of impact levels. Herremans *et al*. (2013) investigated the microstructural changes in vivo during the development of internal flesh browning of ‘Braeburn’ apples by means of X-ray micro-tomography and classified fruit tissue as healthy and disordered. Little is known about the potential of X-ray µCT to detect and classify bruises on fruit with thick and hard rind such as pomegranates (Arendse *et al*., 2018). The aim of this study was to evaluate the feasibility of X-ray µCT in detection and characterization of bruise damage on pomegranate fruit.

2. Materials and method

2.1. Fruit sampling and simulated impact bruising

Pomegranate fruit (*Punica granatum* L. cv. Wonderful) were handpicked at optimum maturity from a commercial orchard located in Porterville, Wellington area (33° 38' S, 19° 00' E) in the Western Cape Province, South Africa. Fruit were transported in a well-cushioned plastic creates to the postharvest research laboratory, Stellenbosch University. Pomegranate fruit for the micro-CT imaging were carefully sorted to ensure use of sound and healthy fruit (free from cracks, sunburn, husk scald or internal decay). Afterward, fruit were pre-conditioned at ambient condition (21 ± 3 ºC; 86 ± 5 % relative humidity) to minimise the effects of temperature on impact bruising. Pomegranate fruit were bruised by dropping from 60 cm drop height onto a flat ceramic surface. Drop impacts were controlled to ensure that fruit hit the impact surface only once on the cheek location along the fruit's equatorial region. Prior to scanning with X-ray µCT, fruit (those that were not scanned immediately after drop impact) were incubated at ambient temperature at (21 ± 3 ºC) and 86 ± 5 % relative humidity to allow for stabilization of bruises (Diels *et al*., 2017).

2.2. Polymeric material used for density calibration

The density of non-bruised and bruised pomegranate fruit were determined using a calibration function that was adopted as reported by du Plessis *et al*. (2013). Two calibration standards were used to extrapolate the unknown density; these included air (1.20 g cm^{-3} at 20 ºC) and a homogenous polymer called polytetrafluoroethylene (PTFE) (2.15 g cm^{-3}). The polymeric material was 10 millimetre (mm) in thickness and 25 mm in diameter. The density of these calibration standards ranged from 1.20 to 2.15 g cm^{-3}.

2.3. Fruit image acquisition by X-ray computed tomography

Image acquisition with micro-CT was performed at 0 h (immediately after drop impact), 48 h, 3 d, 5 d and7 d after drop impact. A total of 30 fruit (6 fruit for each time interval) were scanned. Non-bruised pomegranate fruit were scanned as a control to allow for a good comparison of results between bruised and non-bruised fruit. Images of bruised and control fruit were acquired in the Central Analytical Facility (CAF) at University of Stellenbosch, South Africa using a commercial X-ray computed tomography (CT) system (V|Tome|XL240, General Electric Sensing & Inspection Technologies GmbH, Phoenix, Wun-storf, Germany). Fruit scanning and quality optimization were achieved through several system pre-tested settings. The X-ray radiation from the source operated at 245 kV voltage and 200 mA electron current produced optimal µCT settings with an isotropic voxel size of 71.4 mm. The X-ray CT system was equipped with a copper filter (0.5 mm) to remove low energy X-rays and/ or prevent beam hardening. Scanning was performed for each individual pomegranate fruit sample mounted on a translation stage at a fixed physical distance of 210 mm from the X-ray source, and 600 mm from the detector with a scanning resolution of 70 microns was set. Image slices were acquired using a fully automated data acquisition system and saved onto a processing workstation, operated by system-supplied reconstruction software (Datos|x®2.1, General Electric Sensing & Inspection Technologies GmbH, Phoenix, Wunstorf, Germany). Throughout, X-ray µCT scanning phase pomegranate fruit were scanned concurrently with the polymeric disc to facilitate calibration and direct comparison of different scans. Total scanning time for each sample was approximately 1 h.

2.3. Image reconstruction, processing and analysis

In order to characterise bruising within pomegranate fruit, a series 16-bit greyscale tagged image files (Tiff) were imported and reconstructed into a three-dimensional (3-D) images. The reconstruction procedure of the 3-D object was accomplished by using Datos|x® 2.2 reconstruction software which contained filtered back algorithms. The grey values in each 2-D slice represent the attenuation in each pixel. Therefore, the obtained grey values would therefore depend on the densest object in the scan volume (PTFE polymeric disc with a density of 2.15 g cm^{-3}). Reconstruction volume graphics software (VG Studio Max 2.2, Germany) was then used to perform data processing and image analysis.

Data processing was performed by reducing random noise and smoothing the images. This was accomplished by application of Gaussian filtered method. Steps involved in the image processing of CT data are graphically represented in Figure 1 on a 2-D cross-sectional image of a stack of a single sample. The first step in image processing was filtering and smoothing of CT data using a procedure known as adaptive (5 x 5) Gaussian filtered method. A global image threshold was applied to the entire stack (6000 to 50000). This threshold was used to separate the fruit from the background (external air) and styrofoam from the stack by application of a surface determination procedure using an appropriate threshold of grey values (Fig. 1 a & b). Small objects originating from the surrounding Styrofoam were removed using an image morphological opening. Fruit and polymer disk were then separated from the background using an appropriate threshold of grey values. After the removal of the background and polymer, the image analysis was performed. Bruise damage in the impact region of the fruit was evident by the presence of a darker region (Fig. 1 c). In order to non-destructively characterise bruise within pomegranate fruit, the grey values, total fruit volume, and total internal void space were calculated using adaptive thresholding (Fig. 1 d). This was performed by application of advanced surface determination based on the region of interest (ROI). The later procedure evaluates the materials boundary reconstructs the component geometry more closely compared to the standard surface determination.

In order to non-destructively estimate the density, the average grey values of the polymeric disk, whole fruit and the background was determined. Each voxel related to a sample had an associated grey value dependent on the samples atomic weight and density. A linear function was used to obtain a calibration function (Guelpa *et al*., 2015).

$$\text{Density} = \text{m} \times \text{gv} \times \text{c} \qquad (1)$$

where m is the slope, gv is the grey value and c is the intercept.

2.4. Statistical analysis

One-way analysis of variance (ANOVA) was used to evaluate the difference in volume and density between non-bruised and bruised fruit. The difference between mean values of parameters was investigated using Duncan's Multiple Range Test.

3. Results and discussion

3.1. Visual assessment of bruise by X-ray µCT

Two-dimensional images of X-ray µCT of non-bruised and pomegranate fruit scanned immediately (0 h) after impact bruising showed no clear evidence of bruise damage (Fig. 2a and b). Similar observations were found for pomegranate fruit scanned 48 h, 3 d and 5 d after impact bruising (data not shown). However, changes in bruise-damaged tissue characterised by a darker appearance were observed in pomegranate fruit scanned after 7 d of impact bruising. Bruise damage in the impact region of the fruit was evident by the presence of a darker region, which was distinguishable from non-bruised fruit, which was characterised by a brighter appearance (Fig.1c). The dearth of evidence for the presence of bruise damage on 2D µCT images of pomegranate fruit scanned at 0 h (immediately) and 48 h, 3 d and 5 d after impact bruising could be due to lack of density change in bruise-damaged tissue. It has been established that the µCT images of fruit are characterised by the grey level of a pixel that depends on the density of the sample (Jiang *et al*., 2015), such that the lack of differences in density within the biological sample results in no changes in X-ray attenuation. We presume that the physical changes which occurred within the bruise-damaged tissue in 0 h to 5 d after the drop impact were not sufficient to affect the X-ray absorption of the fruit tissue.

4.2. Differentiating between non-bruised and bruised fruit

After data processing and image analysis, the densities of non-bruised and bruised fruit were calculated (Table 1). The results suggest that non-bruised fruit showed no significant differences in density to 0 h (immediately bruised), or fruit scanned 48 h, 3 d or 5 d after bruising. However, fruit bruised after 7 d showed a significantly ($p = 0.015$) higher density (1.99 ± 0.005 g cm^{-3}) compared to non-bruised fruit (1.67 ± 0.01 g cm^{-3}).

The results of total whole fruit volume and total internal void space for non-bruised and bruised fruit is shown in Table 1. The total fruit volume is the amount of space occupied by a 3-D object, while the total void space is the measure of all the voids within the fruit (Herremans *et al*., 2013). It was observed that there was a decrease in the total volume of the fruit immediately after bruising with no significant differences observed after 7 d of storage. Furthermore, the total internal void space decreased after impact and slightly increased after 7 d of storage. The decrease in void space immediately after impact may be due to rupture of arils resulting in void spaces filled with moisture and juice. On the other hand, the slight increase in total void space after 7 d of storage can be attributed to moisture loss and disintegrated aril tissue as a result of senescence, which is evident in the 2D images, and validated by manually cutting each fruit open and inspecting the impact bruised area.

The grey value distribution versus the frequency or the number of occurrence of pixels or voxels of a particular intensity for non-bruised and bruised fruit is presented in Fig. 2. The difference in peaks in the grey value histogram of the fruit corresponds to different phases associated with either bruised or non-bruised fruit. The difference between the non-bruised and bruise fruit were indicated by variances in the grey values of the X-ray images. The results suggest that bruised fruit can be distinguished after 7 d of impact bruising with lower grey values (18000-30000) compared with non-bruised fruit (26000-34000). Non-bruised and immediately bruised fruit showed similar grey values but different peak intensities (Fig. 3). The lower shift in the grey value end of the spectrum may be attributed to less volume of material as a result of bruising after 7 d storage period. It is known that bruising can cause a significant increase in weight loss in pomegranates as a result of physiological stress associated with impact compared to non-bruised fruit (Hussein *et al*., 2019). This observation can be confirmed at lower total volume and higher total void space recorded for fruit bruised after 7 d of storage, although there was no significant difference for total fruit volume and total void space between treatments.Overall, these observations were buttressed by visual assessment of 2D X-ray µ CT images (Figure 1 and 2), where the brighter regions of non-bruised tissue corresponded to a higher absorption of X-ray radiation (higher grey value) and the dark regions corresponded to a lower absorption of X-ray radiations (lower grey value).

4. Conclusion

This study has demonstrated the potential of X-ray µCT to detect bruise damage in fruit with hard rind such as pomegranate. However, X-ray µCT is limited by its inability to detect bruises at early stages of development i.e between 0 h to 5 d after impact bruising. Hence, improvement of X-ray CT to develop algorithms that could exploit the properties of fruit with hard rind to detect and segment bruises is a crucial requirement. In addition, the need for future studies to explore alternative non-invasive techniques such as hyperspectral imaging system for detection of fresh bruises on fruit with hard rind such as pomegranate is of utmost importance.

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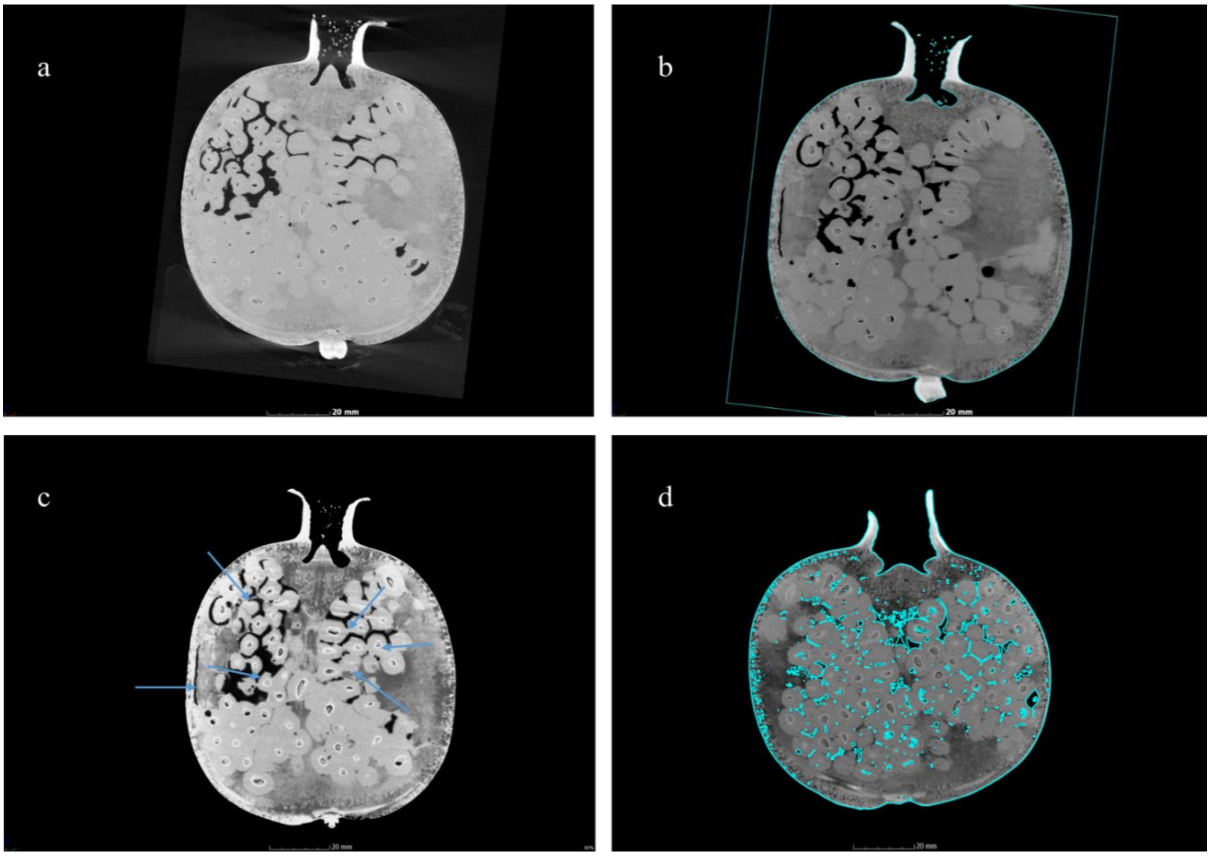

Fig. 1 Two-dimensional X-ray micro computed tomography images for a single pomegranate (cv. Wonderful) fruit at different post-bruising incubation time. A raw representative X-ray image slice of bruised fruit (a), background (external air and styrofoam) removed by applying of surface determination procedure (b), bruised area identified based on visual appearance on the impact region with arrows pointing in the direction of drop impact (c), Total fruit volume and total void spaces selected and segmented based on its region of interested (ROI) using adaptive threshold (d)

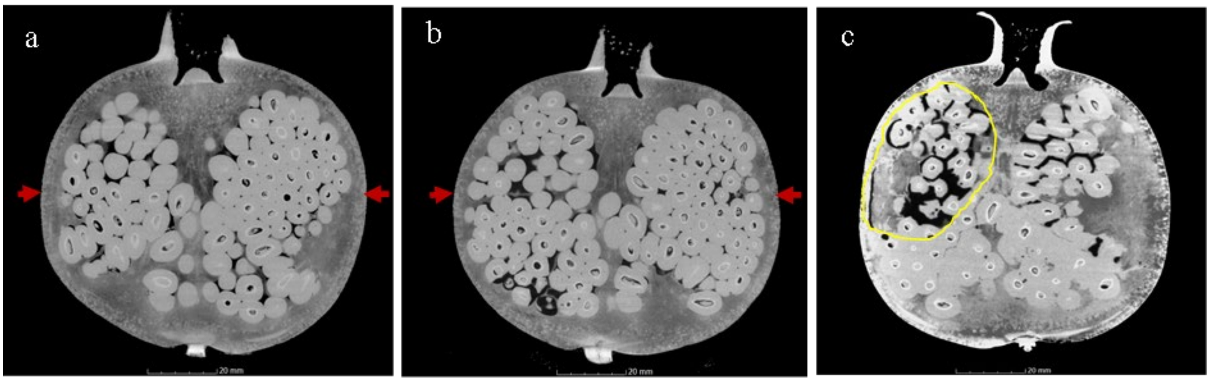

Fig. 2 Two-dimensional X-ray micro computed tomography images for a single pomegranate (cv. Wonderful) (a) non-bruised fruit (control) (b) 0 h (immediately) after bruising (c) 7 d bruising - bruised area identified (circled) based on visual appearance on the impact region. Arrows point the direction of drop impact.

Fig. 3 Grey value distribution histogram of non-bruised (blue), immediately after bruising (orange) and 7 d after bruising (grey) for pomegranate fruit (cv. Wonderful).

Table 1 Fruit density, total fruit volume and total internal void space of non-bruised and bruised pomegranate (cv. Wonderful) fruit

Treatments	Density (g cm^{-3})	Total fruit volume (mL)	Total void space (mL)
Non-bruised fruit	1.67 ± 0.01^b	331.86 ± 14.72^a	7.11 ± 0.72^a
0 h after bruising	1.68 ± 0.004^b	325.16 ± 15.27^a	5.05 ± 0.85^a
7 d after bruising	1.99 ± 0.005^a	315.81 ± 14.28^a	5.84 ± 1.55^a
p-value	0.015	0.45	0.15

Mean ± Standard deviation presented. Different letter(s) indicate significant difference ($p < 0.05$) according to Duncan's multiple range test.

CHAPTER 6

ANALYSIS OF PHYSICAL, BIOCHEMICAL AND MICROSTRUCTURAL CHANGES IN IMPACT BRUISE DAMAGED POMEGRANATE FRUIT

Analysis of physical, biochemical and microstructural changes in impact bruise damaged pomegranate fruit

Abstract

This study investigated the physical, biochemical and cellular microstructural changes of 'Wonderful' pomegranate fruit induced by impact bruising at cold (5 ºC) and room (20 ºC) temperature. Pomegranate fruit were bruised by dropping at various drop heights, from low (20 cm), medium (40 cm) to high (60 cm) impacts onto a rigid impact surface. Physico-chemical changes such as colour browning, peel electrolyte leakage and polyphenol oxidase (PPO) enzyme activity of bruised and non-dropped (control) fruit peels were measured.Reaction oxygen species (ROS) of fluorescent probe 2, 7-dichlorodihydrofluorescein diacetate (H_2DCF-DA) treated fruit peels were measured by confocal laser-scanning microscopy.Qualitative assessment of microstructures between control and bruised tissues of pomegranate fruit peels was performed using a scanning electron microscope (SEM). Micrographs of SEM showed cellular microstructural differences between control and bruised fruit tissues were visible after 4 and 48 h of drop impact. Medium and high impact-bruised fruit were characterized by high ROS production in comparison to control or low impact bruised fruit. Bruise damage to pomegranate fruit affected the membrane integrity of skin cells leading to increased electrolyte leakage (PEL) both at cold and ambient temperature. Bruising had more effect on increasing PPO activity than did the storage temperature and time. The activity of PPO enzyme was higher impact bruised fruit in comparison to control fruit. Browning score (BS) based on subjective visual assessment and the total colour difference (TCD) based on CIE *L*a*b**colour space both indicated highest values corresponding to medium and high drop impact bruising. Pearson's correlation showed strong to moderate relationship between PEL, PPO activity, BS, TCD and ROS. Increase in fluorescent units of ROS showed a strong significant correlation ($p < 0.05$; $r = 0.70$) with BS and moderately correlated with TCD ($p < 0.05$; $r = 0.67$) and PPO activity ($p < 0.05$; $r = 0.61$). This study has confirmed that pomegranate fruit bruising induces the physical and biochemical changes in addition to underlying cellular microstructural alterations.

1. Introduction

Mechanical damage on fruit has become a very important problem, mainly due to the increasing use of mechanised operations for harvesting and postharvest handling (Lee *et al*., 2005; De Martino *et al*., 2006). Bruising is the most common type of mechanical damage caused by sudden dropping of fruit due to improper handling and packaging, and poorly designed harvesting and packaging equipment (Zeebroeck *et al*., 2007; Opara & Pathare, 2014). Bruising on fruit is initiated by the breakage of cell membranes due to excessive impact or compression loading in contact with the fruit surface (Bugaud *et al*., 2014; Opara & Pathare, 2014). Loss of cell wall integrity and subsequent decrease in peel resistance to mechanical damage leads to bruising (Rinaldo *et al*., 2010). It is hypothesized that the oxidation of phenolic compounds aided by an oxidative enzyme, polyphenol oxidase (PPO) to o-quinones that polymerize to form browning pigments on damaged tissues of the fruit (Lee *et al*., 2005; Segovia-Bravo *et al*., 2009; Jiménez *et al*., 2016).

Enzymatic browning reaction and resulting discoloration can occur inside the cell following cell membrane damage or outside of ruptured cell following the release of cell contents into the intercellular spaces (Mitsuhashi-Gonzalez *et al*., 2010). Browning impairs visual quality of fruit and leads to an undesirable change in flavour and nutrition loss (Waliszewski *et al*., 2007; Ding & Ling, 2014). Bruising on fruit is indicated by the presence of brown spot on the surface of the fruit damaged region (Opara & Pathare, 2014). However, the browning intensity and time of bruise to evolve on the fruit surface differs among fruit and cultivars due to various factors such as total phenolic content, membrane and cell wall integrity, storage temperature as well as the activity of phenolic oxidizing enzymes (Rinaldo *et al*., 2010; Bugaud *et al*., 2014). Jiménez *et al*. (2011) revealed that brown bruises on olive fruit surface were visible soon after the impact and that browning intensified in 24 h of the observation period. In other fruit such as pomegranate, coconut and peach, discoloration of bruised tissue take between 24 to 48 h of incubation at ambient temperature to show noticeable browning on fruit surface (Kitthawee *et al*., 2011; Tabatabaekoloor, 2013; Shafie *et al*., 2015).

It is hypothesised that surface browning on the bruise-damaged region of the fruit could lag changes occurring inside the damaged inner tissues (Samim & Banks, 1993). However, commercial inspection and grading of good quality fruit rely on the appearance of external fruit surface over the traditional sectioning of internal bruises and measurement of bruise dimensions (Pang, 1993; Samim & Banks, 1993). Hence, in order to match the requirement of good visual fruit quality for fresh market, it is necessary to evaluate the timing of surface browning on bruise-damaged fruit. A recent investigation by Jiménez *et al*. (2016) compared the structural changes produced in the olive fruit cultivars 'Manzanilla de Sevilla' and 'Hojiblanca' bruised at different times after the impact (4 and 24 h). The authors reported that tissue ruptures in the impact-damaged zones of fruit were visualised both at 4 h and at 24 h after impact. However, it is not known for how long it would take for similar changes to occur on impact-bruise damaged pomegranate fruit.

Production of reaction oxygen species (ROS) occurs predominantly as by-products of cellular metabolism in the mitochondria of the cell (Terman*et al*., 2006). The process is associated with normal mitochondrial respiration and inevitable leaking of electrons to oxygen that are partially reduced to superoxide anion (Turrens, 2003; Terman*et al*., 2006). Nonetheless, mechanical damage and wounding on plant crops is widely reported to cause oxidative stress (Minibayeva *et al*., 2009). Oxidative stress is mainly caused by increased production of superoxide anions which in turn leads to the generation of other detrimental ROS, including hydroxyl radical, superoxide anion, hydrogen peroxide and various conjugated trienes (Rowan *et al*., 2001; Sabban-Amin *at al*., 2011). Lv *et al*. (2016) reported that bruise injury could induce the defence system of the plant material. This could be due to the fact that many of the genes that code the enzymes involved in the production of ROS and their metabolism are activated by wounding (Minibayeva *et al*., 2009). Earlier investigations have confirmed that oxygen species such as hydrogen peroxide and superoxide were generated in response to wounding in wheat roots and zucchini leaves (Minibayeva *et al*., 2009; Stoilkova *et al*., 2009). Furthermore, Lu and Finkel (2008) reported that rise in the intracellular ROS could contribute to the cellular senescence; hence leading to a conclusion by Li *et al*. (2010) that senescence caused by mechanical injury of fruit may be related to the accumulation of ROS. In their study, Li *et al*. (2010) revealed that quality of bruised pears decreased rapidly, and attributed this to the burst of ROS.

There are several putative physico-chemical and biochemical indicators that could be linked to the process involved in fruit bruising, as previously reported in mangosteen (Ketsa & Koolpluksee, 1993) and banana (Maia *et al*., 2011; Bugaud *et al*., 2014). However, no comprehensive study has been published on such indicators for pomegranate fruit. This study hypothesized that excessive impact loading on pomegranate fruit leading to mechanical stress, rupture of cell wall and membrane and subsequent bruising could provoke changes in membrane and cell wall integrity, causing oxidative stress and activate the phenolic oxidizing enzymes. This study assessed the physical, biochemical and microstructural changes associated with the pomegranate fruit bruising to provide information on the processes involved. Firstly, the study investigated the PPO activity of bruise damaged pomegranate fruit and determined its association with both the bruising and browning potential of the fruit. Secondly, changes in electrolyte leakage as an important physico-chemical indicator of membrane integrity after an induced impact were assessed. Furthermore, microstructural changes on the fruit peel and accumulation of ROS as a consequence of impact bruising was examined and quantified.

2. Materials and methods

2.1. Plant material

Pomegranate fruit (cv. Wonderful) were hand picking at commercial harvest maturity from an orchard located in Porterville, Wellington area (33° 38' S, 19° 00' E) in the Western Cape Province, South Africa. Fruit were in packed in a well-cushioned plastic crate and transported in a well-ventilated car on the same day to the Postharvest Technology and Research Laboratory located at the Stellenbosch University, in Stellenbosch. Upon arrival to the laboratory, fruit were pre-conditioned at ambient condition (22 ± 5 ºC, 60 ± 5 % humidity) for 24 h to avoid the effect of temperature variability.

2.2. Drop impact bruising of fruit

The total of 320 uniformly sized pomegranate fruit with a mass range of 270 - 300 g were randomly selected and sorted to ensure the use of fruit free from blemishes, cracking, sunburn or bruises. Fruit bruising was performed in the laboratory using a drop impact test method. The test was conducted by dropping individual fruit from pre-determined drop heights; 20, 40 or 60 cm for low, medium and high impact level, respectively onto a rigid impact surface. Each fruit was dropped twice from the same height onto two opposite sides of the fruit to allocate impact bruising at each of the two equidistant points on the fruit cheek position. A thin layer of white powdered chalk was spread onto the impact surface to ascertain the impact point on the fruit. Impact-bruised region on fruit was marked by permanent marker to outline the bruise boundary. Bruised and non-bruised (control) fruit were incubated at ambient (20 ± 3 ºC, 60 ± 5 % humidity) or cold condition (5 ± 1 ºC; 90 ± 5

% humidity) and further measurements of ROS production, peel electrolyte leakage, PPO enzyme activity, fruit peel browning and peel microstructural changes were performed at 4 or 48 h interval as described below (sections 2.3 – 2.7). Pomegranate fruit (bruised and non-bruised) were equally divided into 4 groups and sampling was performed at the following incubation temperature + time combinations: 4 h + 5 ºC, 4 h + 20 ºC, 48 h + 5 ºC and 48 h + 20 ºC. With the exception of drop impact bruising that was general for all experiments reported in this study, further fruit sample preparation was not consistent. Hence specific sample preparation details are described in relevant experiments below. In each experiment unless where stated otherwise, 5 - 6 fruit per drop height was used.

2.3. Measurement of ROS production by confocal microscopy

Reaction oxygen species of bruised and non-bruised (control) pomegranate fruit peels were detected using the fluorescent probe 2, 7-dichlorodihydrofluorescein diacetate (H2DCF-DA) as described by Macarisin *et al*. (2007) and Sabban-Amin *et al*. (2011). In principle, measurement of dichlorodihydrofluorescein diacetate (DCF) fluorescence is used to quantify general oxidative stress by means of florescent probe entering cells in the diacetate form, whereby reduced form (H2DCF) is hydrolysed by intracellular esterases, reacting with oxidants, and then resulting in the highly fluorescent DCF (Sabban-Amin et al., 2011). Peel slices (30 – 50 µm thickness) of pomegranate fruit (6 slices from the bruised spot of each fruit) was obtained for measurement of ROS production from 5 bruised fruit per drop height for each incubation temperature + time combination. Peel slices were affixed to a glass slide and then coated with poly-L-lysine to ensure better sticking. Peels from non-bruised fruit were included as a control for comparison. Slides containing slices were immediately immersed in a small Petri dish containing 10 mL of 10.0 µM H2DCF-DA (freshly prepared from a 20 mM stock solution in dimethyl sulfoxide, DMSO), in loading buffer (50 mM MES buffer, pH 6.5). To prevent light-inducible oxidation, the slices were incubated in the dark for 10 min. Slices were transferred to a new Petri dish containing loading buffer to thoroughly wash off excess DCF dye for about 2 min and were thereafter mounted with distilled water or fluorescent mounting medium (FFM) for better image quality. Examination of samples and image acquisition were performed using the inverted confocal laser-scanning microscope (Model IX 81, FLUOVIEW 500; Olympus, Japan) equipped with a 488 nm argon-ion laser. The fluorescent probe was excited with a 488 nm laser beam and the emission was collected through a BA 515–525 filter. An emission filter, BA 660 IF was used to filter an autofluorescence from samples. In order to increase magnification, the scanning laser beam was focussed onto the smaller area of the tissue. The transmitted-light images were obtained with Nomarski differential interference contrast (DIC) optics. The relative intensity of the fluorescence signal was estimated by calculating average pixel intensity from each successive focal plane of the pomegranate peel slice under examination, in 5 m steps, with MICA software (Multi-Image Analysis, CytoView, Israel). Fluorescence intensity was presented as mean (± standard error (SE)) of six fruit peel slices per treatment.

2.4. Measurement of peel electrolyte leakage

Peel electrolyte leakage (PEL) was determined according to Sayyari *et al*. (2009) and Safizadeh (2013). Using cork borer, 4 peel discs (10 mm thick) of pomegranate fruit (2 duplicate discs from each bruise spot) were obtained and pooled together from each of 6 bruised fruit per drop height and incubation temperature + time combination, including peel discs from non-bruised fruit. Peel discs were weighed, placed in 100 mL glass bottle, rinsed twice with deionized water and then incubated in 50 mL glass bottle containing 25 mL of 0.4 M mannitol at 25 ºC. The conductivity of the incubation medium (initial conductivity) was measured using conductivity meter (SensoDirect Con200, Dortmund, Deutschland) immediately after 4 h incubation under constant shaking. Bottles were then autoclaved at 121 ºC for 20 min to allow complete leakage of ions from the membranes (total conductivity) and cooled to 20 ºC before measuring total conductivity. The rate of PEL was calculated as the percentage of total conductivity and expressed as a percentage (equation 1).

$$\%\ \mathrm{PEL} = \frac{\mathrm{k_i}}{\mathrm{k_T} - \mathrm{k_i}} \times 100 \qquad (1)$$

where $\mathrm{k_i}$ and $\mathrm{k_T}$ are initial and final conductivity, respectively.

2.5. Polyphenol oxidase enzyme activity

2.5.1. Enzyme extraction

Sample preparation for polyphenol oxidase (PPO) enzyme extraction was performed on 5 randomly selected bruised pomegranate fruit per drop height, including non-bruised (control) fruit for each incubation temperature + time combination. 4 fruit peel discs of 10 mm thickness were obtained from each of the 6 bruised fruit per drop height. Discs from the two bruise spots of each fruit (2 duplicate discs per bruise spot) were finally pooled to one sample per fruit. Peel discs were immediately flash frozen in liquid nitrogen and stored at -80 °C before being freeze-dried (VirTis freeze dryer, SP Scientific sentry 2.0,Warminster, Pennsylvania, USA) and pulverization into a fine powder. Extraction was done by weighing homogenized peel powder (1.0 g) into centrifuge tube containing 10 mL (pH 7.0) of extraction buffer(made by adding 0.1 M L^{-1} potassium phosphate buffer (pH 7.0), 0.05 M L^{-1}ethylenediaminetetraacetic acid and 60 g L^{-1} of insoluble polyvinylpyrrolidone in 1:1:1 ratio). The mixture was vortexed by using the mixer (Model. G560E, Scientific Industries, USA) and sonicated using an ultrasonic bath (Ultrasonic Cleaner DC400H, MRC Ltd. Israel) at 5 °C for 10 min. This was followed by incubation for 2 h at 4 °C in the dark.The homogenates were centrifuged for 20 min at 10000 rpm using refrigerated centrifuge (Eppendorf Model 5810 R, Merck, Hamburg, Germany) set at 4 °C to prevent interference. The crude extract (supernatant) containing the enzyme was used to assay PPO activity.

2.5.2. Analysis of PPO activity

Enzyme extract was assayed for PPO activity according to Gonzalez *et al.* (1999), using pyrocatechol as a substrate. The PPO enzyme activity was measured spectrophotometrically with UV–vis spectrophotometer (Thermo Fisher Scientific, Madson, USA) at 25 °C by measuring the initial rate of increase in absorbance at 420 nm. The reaction mixture of 3 mL potassium phosphate buffer (0.2 M, pH 6.0) and 200 µL of prepared enzyme extract was prepared in a test tube, followed by addition of 300 µL of catechol (0.1M) to start the PPO activity. The mixture was immediately taken into a cuvette for absorbance reading at 420 nm. Enzyme activity (U g^{-1}) was expressed as the change in absorbance at zero time (initial rate) and after 3 min of reaction per minute per gram of the pomegranate peel powder in the 30 s intervals reaction (change in absorbance per gram of tissue of fresh weight, FW) using equation 2;

$$\mathrm{U} = (\mathrm{Abs\ at\ 3\ min} - \mathrm{Abs\ at\ 0\ min}) \times \mathrm{Total\ reaction\ vol/Time\ interval} \times 2 \quad (2)$$

2.6. Fruit peel browning

Characterization of the overall changes in the browning of pomegranate fruit peel after bruising was performed by calculating the browning score (BS), which represents the purity of browncolour (Pathare *et al.*, 2013) using the subjective/ visual colour assessment method of the total brown area. In duplicate, the BS was assessed on the pomegranate fruit peel surface of each of the 2 drop impact-bruise spots of 5 bruised fruit per drop height, including a set of non-bruised or control fruit for each incubation temperature + time combination. The scale used in BS assessment is as follows: 0 = no browning; 1 = trace browning; 2 = moderate browning; 3 = severe browning and 4 = extreme browning on the marked impact bruised surface of the fruit peel. The subjective browning score (BS) was further calculated from the subjective score values using equation (3) as shown below.

$$BS = \sum_{n=0....4} \frac{A_n \times n}{F_t} \quad (3)$$

where n is the level score of browning, A_n is the number of fruit evaluated in the n level score, and Ft is the total number of fruit evaluated per treatment.

The second method relied on the determination of the difference in colouron the fruit peel using the mathematical expression from the CIE L*a*b* coordinates values. The total colour difference (TCD) was measured to determine the colour disparity of impact bruise damaged region of the fruit between initial (immediately after impact) and 4 or 48 h after impact bruising (incubation time). The L*, a*, b* coordinates were measured with calibrated Minolta Chroma Meter (Model CR-400/410; Minolta Corp, Osaka, Japan) in 10 randomly selected and impact bruised fruit. Peel colour measurements were taken on two positions of the marked bruised spot of each individual fruit. The total colour difference was calculated using equation 4.

$$TCD = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \quad (4)$$

where, L_0^*, a_0^* and b_0^* are the 'reference' (initial) colour values of the fruit peel, while L*, a* and b* are the colour values of the bruised spot after 4 or 48 h (Pathare *et al*., 2013; Fawole & Opara, 2013c). Means ± S.E of 10 determinations (n = 10) were obtained from the 10 measured fruit.

2.7. Microscopic analysis of peel microstructure

Cellular microstructure of bruised and non-bruised control pomegranate fruit peel was investigated based on the method of Jiménez *et al*. (2016) with some modifications. Analysis of fruit peel microstructure using scanning electron microscopy was performed after 4 h and 48 h of incubation time at ambient incubation (4 h + 20 ºC; 48 h + 20 ºC) of drop impact bruising. In duplicate, 2 cross-section slices of the fruit peel (4–5 mm thickness) containing subcutaneous tissue directly below the peel surface were obtained from each of the 2 sides of bruise spot of 5 bruised fruit per drop height. Peel slices of non-bruised fruit were included as a control. Slices for histological observation were further sectioned transversely at 10-12 µm with a rotary microtome, mounted on glass slides and immediately fixed in 2.5 % glutaraldehyde solution in phosphate buffer (at a pH of 6.8) and stored in cold temperature (4 ºC) over night to ensure complete infiltration. Samples were then rinsed three times with fresh phosphate buffer to wash off all fixative, followed by dehydration series by replacing buffer solution with varying concentrations of ethanol (30, 50, 70, 90 and 100 %) for 15 min each. Dehydration in 100 % ethanol was repeated twice. This step was followed by a drying process using Hexamethyldisilizane (HMDS).Samples were transferred from 100 % ethanol into a 1:2 solution of HMDS: 100 % ethanol and left for 20 min, and then into a fresh solution of 2:1 HMDS: ethanol for 20 min. Finally, samples of fruit peels were transferred into 100 % HMDS for 20 min, this step was repeated twice. Samples were submerged in the final 100 % HMDS solution while capped loosely in a fume hood overnight to allow for complete dryness of samples. Dried histological preparations were sputter coated with carbon and examined using field emission scanning electron microscope (Merlin Zeiss GeminiSEM 500, Germany).Fruit peels of the same peel slices containing non-bruised tissue were also included as a control for comparison and to ensure that observed changes in the impacted region are due to bruising rather than histological processing.

2.8. Statistical analysis

Experiments were conducted using a completely randomized design, and statistical analysis of data performed using Statistica software version 13.2 (Statistica for Windows, Tulsa, OK, USA).Data were further subjected to factorial analysis of variance (ANOVA), main treatment means were compared using Duncan's multiple range test ($p < 0.05$). All results are presented as the mean ± standard error (S.E) of three independent determinations. Correlations between measured attributes were determined by Pearson correlation matrix method using XLSTAT version 2017.01 (Addinsoft, France). Bar and line charts were constructed using GraphPad Prism software version 5.02 (GraphPad software, Inc. San Diego, USA).

3. Results and discussion

3.1. Reactive oxygen species

Confocal laser scanning fluorescence imaging results showed that impact bruising influenced the production of reaction oxygen species (ROS) as shown in Fig. 1A. High ROS production and accumulation as green fluorescence were observed in medium (40 cm) and high (60 cm) impact-bruised fruit. In contrast, fruit bruised at lower impact (20 cm) or control (non-bruised) fruit had very little or no accumulation of ROS as indicated by fluorescence images (Fig. 1A, a - d). Further results of relative intensity of DCF fluorescence of quantified ROS in pomegranate fruit peel revealed that ROS intensity was the highest in high impact-bruised fruit and the lowest in non-bruised fruit. Results in Figure 1B showed that ROS production in low impact bruised pomegranate fruit was 70.5 % higher than non-bruised fruit. Furthermore, increase in drop impact from low to medium and medium to high impact level increased the ROS production by 59.1 and 38.9 %, respectively.

Pomegranate fruit bruised at medium and high drop impact showed damages that potentially induced the fruit defence system, hence triggering production of ROS (Lv *et al*., 2016). This corroborates with Li *et al*. (2010) who reported that pears bruised by dropping from a height of 15 cm induced 96 % higher ROS (H_2O_2) than non-bruised (control) fruit during the first 15 d of storage. During the same period, the authors observed the similar trend of superoxide radical production in bruised fruit, which was 22 % higher than that of non-bruised fruit.Similarly, Castro-Mercado *et al*. (2009) repeated that ROS were rapidly produced in avocado fruit 15 min after mechanical stress resulted by cutting. The levels of superoxide and H_2O_2 wereproduced in the mesocarp tissue and accumulated progressively during the time of the experiment. It is presumed that high levels of ROS are produced more in bruised than non-bruised fruit due to slight inhibition effect caused by detoxification enzymessuch as catalase(CAT), superoxide dismutase (SOD) and peroxidases (POD) in damaged tissues (Castro-Mercado *et al*., 2009; Li *et al*., 2010). Li *et al*. (2010) revealed that rapid declining quality of bruised pears was attributed to the detected burst of ROS, suggesting that mechanical damage of fruit is greatly associated to the accumulation of ROS as observed in ‘Wonderful’ pomegranate fruit.

3.2. Peel electrolyte leakage

The percentage peel electrolyte leakage (% PEL) was significantly affected by both the drop impact and the incubation temperature + time combination ($p < 0.05$). Lower percentages of PEL in fruit bruised at low drop impact (20 cm) or non-bruised (control) were observed in both incubation temperature + time combination treatments (Fig. 2 A). Results showed that non-bruised fruit had the lowest PEL of 27.4 and 21.7 % after 4 h incubation time, and 15.4 and 25.6 % after 48 h incubation at ambient and cold condition, respectively. The highest PEL was observed in peels of fruit bruised at the highest drop impact (60 cm), reaching the peak values of 43.2 after 4 h incubation in cold storage. A similar trend of increase in PEL with increasing level of drop impacts was observed across all incubation temperature + time combinations. At ambient condition, the PEL of 32.3 and 33.4 % were observed in high drop impact bruised fruit after 4 h and 48 h of incubation, respectively.

Results of the present study have shown that, in addition to the level of drop impact bruise damage, incubation temperature affects the discharge of electrolytes from the bruise damaged pomegranate fruit. Incubation in cold condition resulted in 13.9 and 25.4 % higher PEL in medium (40 cm) and high drop impact (60 cm) bruised fruit peel after 4 h incubation. An increase in electrolyte leakage was also observed during cold storage of no-treated 'Mollar de Elche' pomegranate fruit (Mirdehghan *et al*., 2007). However, this observation is contrary to Bugaud et al. (2014), who observed that both membrane permeability and PEL in bananas subjected to drop impact (impact energy: 20 - 200 mJ) were not affected by 18 ºC storage temperature. Likewise, Ratule *et al*. (2006) reported no significant difference in PEL between 10 and 15 ºC stored green bananas at the end of 16 d evaluation. The influence temperature on PEL is attributed to its effect on phase change of cell membrane lipids. In view of that, low temperature induces changes in the state of cell membrane lipids from liquid-crystalline to a solid-gel state, resulting in an increase in membrane permeability and leakage of ions (Gomez-Galindo *et al*., 2004; Mirdehghan *et al*., 2007).

In the current study, it could be suggested that the stress induced by drop impact bruising onto pomegranate fruit affected the membrane integrity of skin cells leading to increased electrolyte leakage. This is in support of several other studies reported in banana (Maia *et al*., 2011; Bugaud *et al*., 2014), pears (Zhou *et al*., 2007) and tomatoes (Lee *at al*., 2007). Bugaud *et al*. (2014) reported a positive correlation between bruise susceptibility and PEL and hence concluded that the latter was the physico-chemical indicator that best distinguished cultivars of banana by their susceptibility to bruising. Lee *et al*. (2007) showed that irrespective of ripeness stage, the PEL of 'Roma' tomato fruit impacted by dropping (> 40 cm) was 27 % higher than that of non-impacted fruit after 6 d storage. Similarly, Maia *et al*. (2011) reported that PEL in all mechanically damaged bananas (by cutting, impact or compression) was higher than that of non-bruised (control) fruit throughout the 9 d of the evaluation period. Overall, these findings confirm that the integrity of cell membranes as indicated by the degree of discharge of electrolyte ions is affected mechanical damage such as bruising.

Peel electrolyte leakage is the useful index to quantify the damage conceived by plant cell membrane (Zhou *et al*., 2007; Tareen *et al*., 2012), due to postharvest physiological changes such as senescence, ripening or physical damage (Lee *et al*., 2007; Zhou *et al*., 2007; Maia *et al*., 2011). In the present study, higher PEL observed could be due to mechanical damage mediated by increased free radical and loss of membrane integrity and/ or alteration of selective permeability, which subsequently resulted in more ion leakage (Maia *et al*., 2011).

3.3. Polyphenol oxidase enzyme activity

The polyphenol oxidase (PPO) enzyme activity of pomegranate fruit peel was significantly affected by the increasing level of drop impact on fruit and the combination of incubation temperature and time ($p < 0.05$). After 48 h, fruit incubated at ambient exhibited an increase PPO activity from the initial of 0.95 U g^{-1}FW for non-bruised to 1.15, 1.55 and 1.6 U g^{-1} FW for low (20 cm) medium (40 cm) and high (60 cm) drop impact bruised fruit, respectively. The increase in PPO enzyme activity of fruit incubated for 4 h followed the similar trend at the same incubation temperature (ambient, 20 ºC), but the activity was slightly lower than that measured after 48 h. During this period, pomegranate fruit subjected at 20, 40 and 60 cm drop impacts indicated increases in PPO activity by 34.6, 35.6 and 41.4 %, respectively (Fig. 2 B). During cold incubation, the PPO activity of fruit bruised at 20, 40 and 60 cm drop impacts increased by 5.4, 9.4 and 28.1 % respectively, after 4 h incubation time, and 10.5, 5.6, and 37 % after 48 h from the initial value of 0.87 and 0.85 U g^{-1}FW of non-bruised fruit, respectively. At the same incubation temperature (5 ºC), higher increases in PPO activity were observed for low drop impact (17.4 %) and medium drop impact bruising (25.8 %). Overall, these findings agree with the hypothesis that activation of physiological and biochemical mechanisms through the induction and/ or changes of enzyme activities by physical wounding or bruises can potentially occur within a few minutes, several hours, or a few days (Leon *et al*., 2001; Lee *et al*., 2007).

Findings of the present study have indicated that the increase in PPO activity of bruise damaged pomegranate fruit peel is the subject of the combined effect of incubation temperature and time ($p < 0.05$). For instance, the peak activity of PPO at ambient reached 1.6 U g^{-1}FW after 48 h for fruit bruised at high impact, 15.6 % higher than the activity measured at 5 ºC at the same bruise impact level and incubation time (Fig. 2 B). Martinez and Whitaker (1995) stated that the rate and intensity of enzymatic browning are determined by temperature among other factors. Accordingly, temperature affects the catalytic activity of PPO partly due to its influence on the solubility of oxygen (Valero and Garcia-Carmona, 1998). Similar to our findings, Segovia-Bravo *et al*. (2007) revealed that the browning of olive bruises caused by PPO oxidations was reduced in fruit kept at 8 º C, in comparison with those at 25 ºC. In their observations, the PPO enzyme activity of 'Manzanilla' olive fruit increased from 0.38 U min^{-1} at 8 ºC to 0.49 U min^{-1} at 25 ºC, although the activity was completely inhibited at pH values below 3.0 regardless of temperature. Zhang and Zhang (2008) reported that the PPO activity of 'Ganesh' pomegranate fruit peel decreased with the decreasing temperature during storage.

Overall, the findings from this study revealed that mechanical impacts resulting from the drop impacts could result in latent physical damages below the fruit surface at the cellular level in the pomegranate fruit. It is thus logical to suggest that, the activity of PPO is directly related to the resulting cellular damage as confirmed for other fruit including olive fruit (Segovia-Bravo *et al*., 2007), banana (Maia *et al*., 2011), persimmon (Lee *et al*., 2005), pawpaw (Galli *et al*., 2009) and longkong fruit (Lichanporn *et al*., 2009). This buttresses our findings that PPO activity increased with increasing impact level, which presumably increased the severity of cellular damage at impact. Upon impact, the contact between cytoplasmic enzymes (mainly PPO) and phenolic contents stored in the vacuole is initiated (Shewfelt, 1993; Lee *et al*., 2005; Jiménez *et al*., 2011), hence triggering the activities of PPO enzyme and onset of the browning.

3.4. Fruit peel browning

Changes in peel browning as the result of bruised damaged pomegranate fruit was significantly affected by the level of drop impact and incubation temperature + time combination ($p < 0.05$). Evaluation of peel browning made on the drop impact bruised fruit indicated that the worst browning score (BS) according to subjective visual assessment corresponded to that pomegranate fruit that were bruised at medium (40 cm) and high (60 cm) drop impact levels (Fig. 3A). Accordingly, extreme browning was observed on fruit bruised at medium and high impact levels after 48 h incubation both in cold and ambient temperatures, as indicated by the respective highest scores of 3.6 and 3.9. In comparison, severe (BS = 3.1) and trace (BS = 1) browning was observed in high impact bruised fruit after 4 h at ambient and cold condition respectively, indicating that the increase in browning intensity is associated with both temperature and time after impact. No significant difference ($p < 0.05$) in browning score between non-bruised (control) fruit and those bruised at low (20 cm) drop impact at both incubation temperature and time, with assessment ranged between a trace to no browning.

The total colour difference (TCD) showed the disparity in peel colour between the initial colour (measured immediately after drop impact) and ones after 4 h and 48 h incubation time. The observed changes in colour measured by TCD were influenced by both drop impact and fruit incubation temperature + time combined. The TCD increased significantly with drop impact level ($p < 0.05$), reaching the highest of 15.2 and 19.4 after 4 h and 48 h incubation time for fruit bruised at medium (40 cm) and high (60 cm) drop impacts incubated at ambient, respectively. In comparison, fruit incubated in cold condition showed lower values of TCD, irrespective of incubation time which ranged from 4.7 for control fruit and increased with drop impact levels to 11.5 for high drop impact bruised fruit (Fig. 3 A). These results suggest that temperature is closely associated with browning of bruise-damaged fruit, in part due to its effect on the activity of polyphenol enzyme activity induced by fruit tissue damages (Lichanporn *et al*., 2009). Overall, the present results showed a good correlation between TCD and fruit browning, thus suggesting its suitability as an important index for assessment of browning in pomegranate fruit, and to distinguish between bruised and non-bruised fruit.

Browning of pomegranate fruit peel has been mainly attributed to the development of some physiological disorders such as a chilling injury during storage (Defilippi *et al*., 2006) and loss of water through pores on the fruit peel (Tian *et al*., 2005; Lichanporn *et al*., 2009). Nonetheless, the results of this experiment have further established that browning reactions are also dependent on the mechanical integrity of cell membranes. In this accord, mechanical wounding triggers the contact between the phenolic compounds and enzymes stored within the cell vacuoles following the loss of cellular compartmentalization (Lichanporn *et al*., 2009: Taranto *et al*., 2017). Oxidations of phenolic compounds into quinones under aerobic conditions by PPO, and the quinones compounds polymerizing to form brown polymeric pigments, primarily lead to browning (Lichanporn *et al*., 2009; Zhang & Zhang, 2008).

For browning process to occur, there are three necessary conditions required: substrates (phenolic compounds), enzymes and oxygen (Lin *et al*., 2002; Taranto *et al*., 2017). Different fruits have specific phenolic compounds that are responsible as a substrate for enzymatic browning. Zhang and Zhang (2008) discerned that the main browning substrates in 'Ganesh' sweet pomegranate fruit were tannins and that the activities of PPO and POD in pomegranate peel correlated positively with the peel browning. In the present study, impact bruising of pomegranate fruit brought together the substrates concomitant to browning. These results are in corroboration with previous bruising mechanisms proposed for longkong (Lichanporn *et al*., 2009), olive (Sánchez *et al*., 2013), banana (Nguyen *et al*., 2003) and litchi (Jiang, 2000).The PPO enzyme was attributed with the browning of longkong pericarp due to noticeable increase browning effect with increasing PPO activity (Lichanporn et al., 2009). Similarly, the impact on the olives caused the rupture of tissues and put PPO into contact with polyphenols thereby giving rise to browning quinones (Sánchez *et al*., 2013).

Standardised Pareto charts were constructed to evaluate the effect of drop impact and incubation temperature + time combination on the total colour difference (TCD), peel electrolyte leakage (PEL), browning index (BI) and PPO enzyme activity (Fig. 4). Drop impact showed a positive significant effect on both measured parameters ($p < 0.05$) (Fig. 4 a-d), whereas incubation temperature-time combination had a positive effect on PEL (Fig. 4 b) and negative effect on BI (Fig. 4 c). Overall, fruit bruising showed the largest effect between the two variables, followed by the incubation temperature + time combination.

3.5. Peel microstructure

Scanning electron microscopy micrographs (Fig. 5, a-d) revealed the difference in microstructures between non-bruised and bruised peel tissues of pomegranate fruit. There were clear differences in peel microstructures between non-bruised (control) or low (20 cm) drop impact bruised fruit and medium (40 cm) and high (60 cm) drop impact bruised fruit. Peel tissue comprising cells in non-bruised fruit was characterised by the presence of intact intercellular spaces as shown in Fig. 5a. Microstructure image in Fig. 5b indicated minimal bruise damage to peel tissue of fruit bruised at low (20 cm) drop impact. Microstructure image of pomegranate fruit subjected to low drop impact showed clear evidence of bruise damages while the rest portion of the fruit had no such evidence, and tissues remained intact. This suggests that pomegranate fruit showed different responses to drop impact, probably due to its complex structure (Ekrami-Rad *et al*., 2011). Pomegranate fruit peel is the outermost tissue of the fruit that plays a crucial role in protecting the inner parts, the spongy mesocarp and edible portion (aril sacs) from physical defects such as mechanical impacts. The peel is composed of cuticle, epidermis and several layers of hypodermis, all of which act as a cushion against impact damage of inner parts of the fruit. This could also suggest low drop impact did not cause significantly greater damage of the peel tissues. Another source of this variation could be due to common natural variability in biological materials that potentially influences differences in mechanical properties of fruit even within the same batch of fruit cultivar (Van Linden *et al*., 2006; Ghaffari *et al*., 2015).

Microstructures of bruised tissue (Fig. 5 c – d) appeared to lose compactness apparently due to the drop impact suffered by the fruit. Bruised tissues exhibited some empty regions or 'damage holes' defined as regions that are not occupied by cells with defined shape (demarcated by dished arrows), and increased intercellular spaces especially in near-by bruised regions. Additionally, results in Fig. 5 showed greater size of damage holes in peels of high drop impact bruised fruit compared to medium impact bruise damaged ones. Similar results were found by Mitsuhashi-Gonzalez *et al*. (2010), who discovered that parenchyma cells of apple fruit suffered varying mechanical failures depending on the force applied. However, this study did not report which individual cells were more susceptible to impact bruising, mainly due to the nature of damage caused by impact.

Furthermore, scanning microscopy observations of bruise-damaged tissues revealed that observed changes in the bruised fruit peel were not dependent on time after bruise impact.

Evidence of damaged cells of the peel tissue was observable on scanning electron micrographs both at 4 and 48 h after the impact, with no noticeable difference in damage size between the two (data for 48 h incubation time not shown). De Martino *et al*. (2006) observed similar results on scanning microscopy micrographs for apricots. The authors found that squeezing of the cell layers under the peel was visible immediately after impact bruising. Our current results have shown that medium and high dropping impacts resulted in damage holes extending a few millimetres from the point of impact.

Overall, results of scanning microscopy of bruised pomegranate fruit observed shortly (4 h) after impact bruising provided clear evidence that development of browning on fruit due to bruising lags behind immediate changes occurring within the damaged internal tissues. Mitsuhashi-Gonzalez *et al*. (2010) defined a bruise as an area of discoloured tissue consisting of an array of undamaged, burst and crushed cells, along with cells that have not been physically damaged. However, previous findings by Kim and Hung (1990) revealed that colour changes and/ or browning reactions of bruise-damaged tissues are indirect indicators of fruit bruising. The authors further indicated that the use of discoloration of damaged tissues to indicate the location of the bruise-damaged region is quite subjective due to the fact that biochemical reactions leading to browning is usually not immediate. Therefore, microscopic observation on bruised tissue provided additional information to support the bruising mechanism of pomegranate fruit at the cellular level; i.e., drop impacts cause rupture of damages to whole fruit, starting from at the cellular level, thus giving rise to softening and release PPO enzymes responsible for browning reaction.

3.6. Pearson's correlation

Pearson's correlation analysis was performed to establish the relationship between measured variables (Table 1). Total colour difference (TCD) showed positive moderate correlations for PPO activity ($r = 0.59$) and ROS ($r = 0.67$). Furthermore, PPO activity was strongly related to PEL and moderately related to ROS with coefficient of 0.70 and 0.61, respectively. Thus, the apparent relationship between PPO activity and PEL suggests that membrane permeability could be one of the main factors contributing to the browning process as proposed by Bugaud *et al*. (2014). Similarly, moderate positive correlation between PPO activity and TCD indicates that PPO oxidizing enzymes localized on the thylakoid membranes of chloroplasts and the phenolic compounds are responsible for post-impact bruising browning (Rinaldo *et al*., 2010). However, Lee *at al*. (2005) explained that the PPO activity may not strictly correlate with tissue browning potential, because the latter is dependent on both qualitatively and quantitatively substrate. Increase in fluorescent units of ROS strongly correlated with BS ($r = 0.70$) and moderately related to TCD ($r = 0.67$), and PPO activity ($r = 0.61$). A strong relationship between ROS fluorescence units and BS, and a moderate between PPO activity and the former could be explained by the fact that many of the genes coding the enzymes involved in production of ROS and their metabolism are activated by physical wounding such as bruising (Minibayeva *et al*., 2009). In turn, sustained bruises provoke PPO enzyme activities and subsequent browning of injured tissues.

4. Conclusion

This study has investigated the physical and biochemical changes associated with impact bruising in pomegranate fruit. Our results revealed that impacts applied to pomegranate fruit initiated bruising which is exhibited by a combination of biochemical and physical changes, including of electrolyte leakage, ROS production, the PPO enzyme activity and browning of fruit peel. Bruising caused alterations in cell wall and membrane integrity as indicated by increasing electrolyte leakage of ions measured in fruit peels. This study has further demonstrated that higher levels of ROS productions are associated with membrane damage to fruit at medium and higher impact bruising. Similarly, fruit bruising resulted in significant increase in PPO activity, in comparison to non-bruised fruit. In comparison to incubation temperature, the effect of time lapse (incubation time) after bruise damage on fruit was not more pronounced particularly for the activity of PPO. Our findings showed that the PPO enzyme activity increased at ambient (20 ºC) compared to cold (5 ºC) stored fruit, irrespective of incubation time. Both the browning score and total colour difference relied more the on the level of drop impact damage, and the effect increase at higher storage temperature and prolonged fruit incubation time. Given the impact of browning on quality for fresh fruit market, these findings demonstrate the importance of additional care and handling of fruit during harvesting, transport and packhouse operations, most of which are performed at ambient temperature.

Microstructures of bruised and non-bruised (control) pomegranate fruit peels were qualitatively differentiated using scanning electron microscopy micrographs. Imaging of fruit peel from bruise-damaged fruit facilitated immediate visibility of damaged tissues ahead of chemical and physical changes that occur at a later stage. Scanning electron micrographs have further shown that microscopic observation on bruised pomegranate tissue could provide information to support the traditional bruising estimation methods that rely on the measurement of diameter, depth, weight or volume of browned tissue. Nevertheless, further research on image analysis to support the visual observation and provide numerical results for statistical analysis is warranted.

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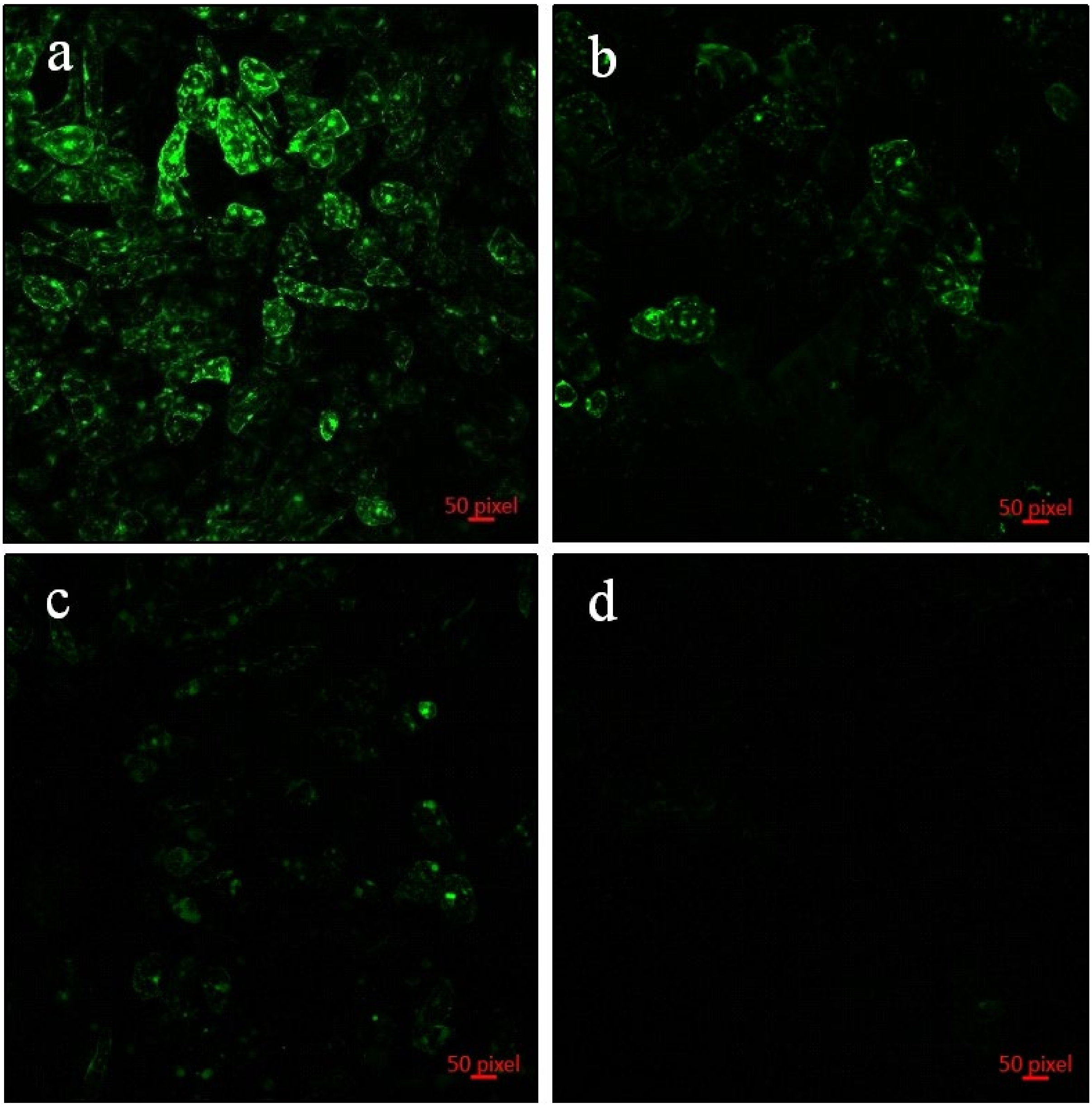

Fig. 1 A Confocal laser-scanning fluorescence images of 'Wonderful' pomegranate fruit peel. Images of reactive oxygen species (ROS) fluorescence are for bruised fruit at 60 cm (a), 40 cm (b) and 20 cm (c) drop impacts. Fig. 1d is the fluorescence image for control or non-bruised fruit peel. Images are projections of several optimal sections collected by confocal microscopy, showing DCF fluorescence. Pomegranate fruit peel slices were stained with H_2DCF-DA and viewed under constant-excitation light intensity.

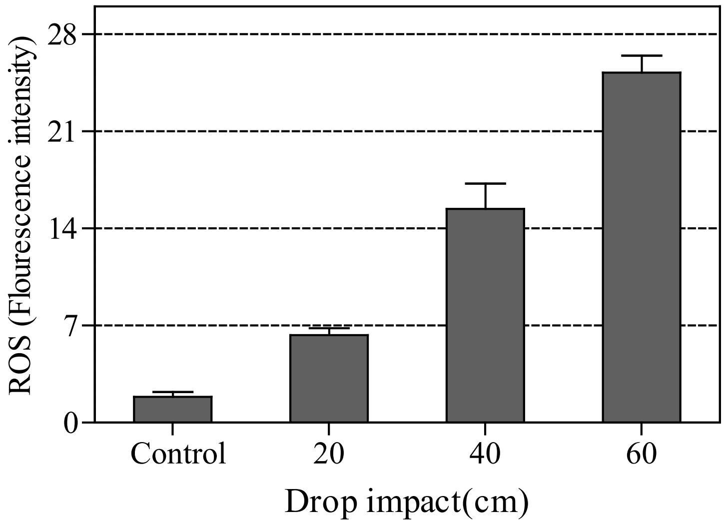

Fig. 1 B Relative intensity of dichlorodihydrofluorescein diacetate (DCF) fluorescence quantifying reactive oxygen species (ROS) in 'Wonderful' pomegranate fruit peel from non-bruised (control) and bruised fruit at varying drop impacts (20, 40 and 60 cm drop heights). Each bar represents mean value and standard error (± SE) of independent measurements of six different slices of the pomegranate fruit peel. Data were subjected to one-way analysis of variance (ANOVA). Mean values are statistically significant according to Duncan's multiple range test ($p < 0.05$).

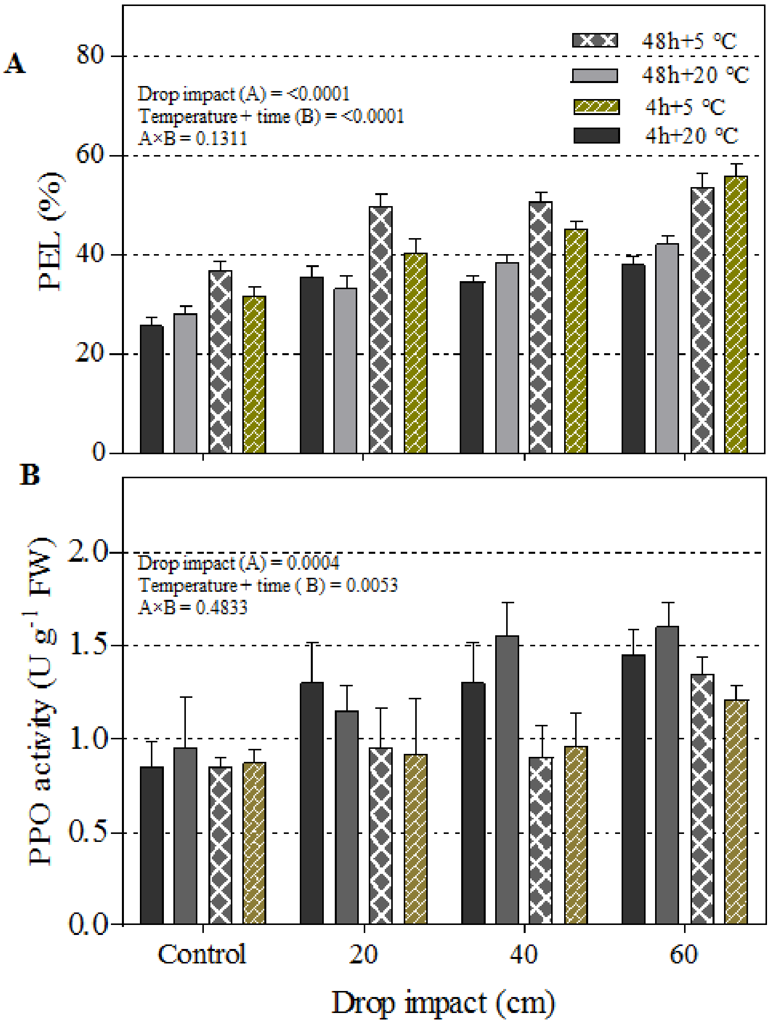

Fig. 2 Changes in electrolyte leakage (% PEL) (A), and polyphenol oxidase (PPO) enzyme activity (B) of flesh peels from bruised and control (non-bruised) 'Wonderful' pomegranate fruit. Values of PPO activity and PEL represent the means of independent measurements (± SE) for respective 6 different pomegranate fruit; and FW = fresh weight. Data were subjected to factorial analysis of variance (ANOVA) (factor A; drop height, factor B; temperature-time). Mean separation was carried out using Duncan's multiple range test ($p < 0.05$).

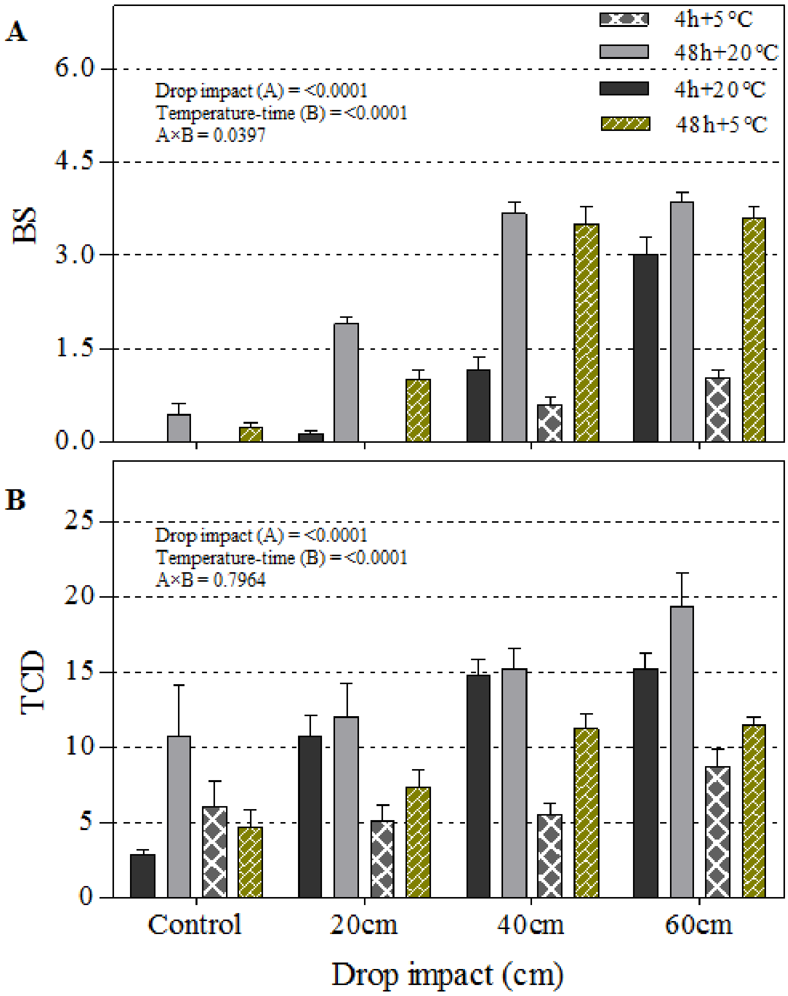

Fig. 3 (A): Browning score (BS), and (B): total colour difference (TCD), of control (non-bruised) and drop impact bruised 'Wonderful' pomegranate fruit incubated at different temperature and time combination. Values of BS and TCD represent the means of independent measurements (± SE) for respective ten different bruise impact bruise-spots of the pomegranate fruit. Data were subjected to factorial analysis of variance (ANOVA) (factor A; drop height, factor B; temperature-time). Mean separation was carried out using Duncan's multiple range test ($p < 0.05$).

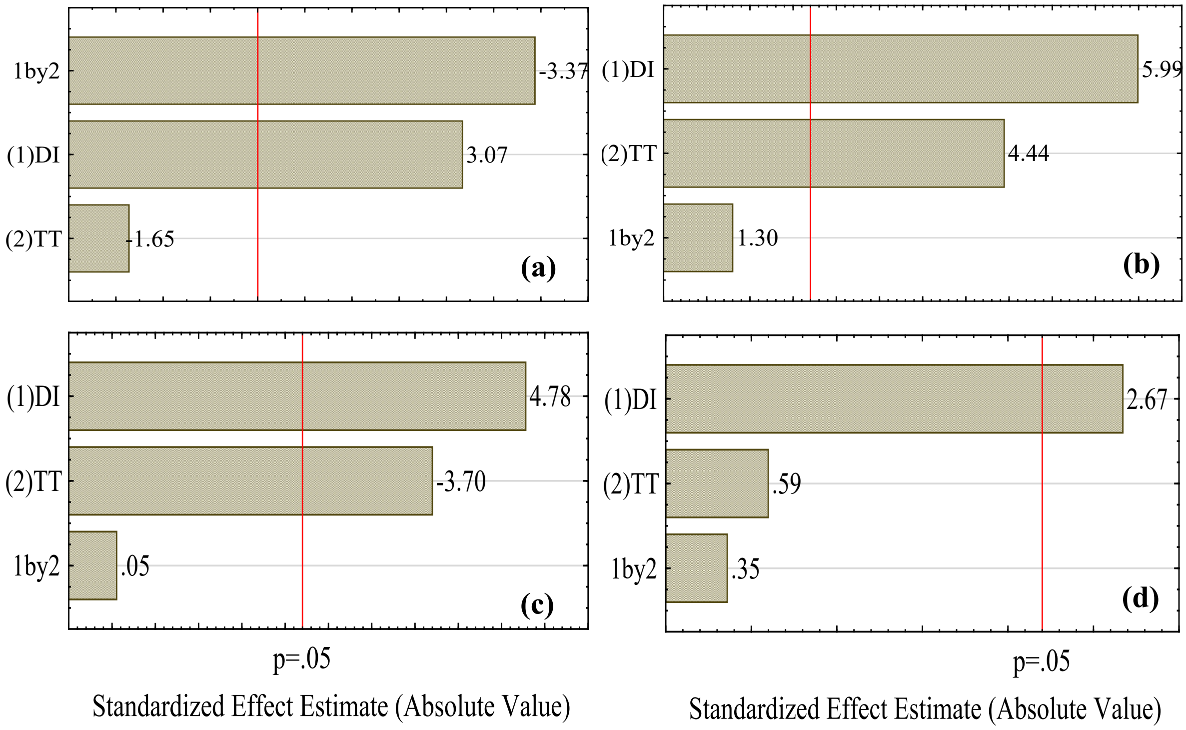

Fig. 4 Pareto chart showing the effect of drop impact (DI) and incubation temperature + time combination (TT) on the total colour difference (a), peel electrolyte leakage (b), browning index (c) and polyphenol oxidase enzyme activity (d). The dashed vertical line corresponds to the 95 % confidence level. The standardised effects with bars that go over this line are statistically significant ($p < 0.05$).

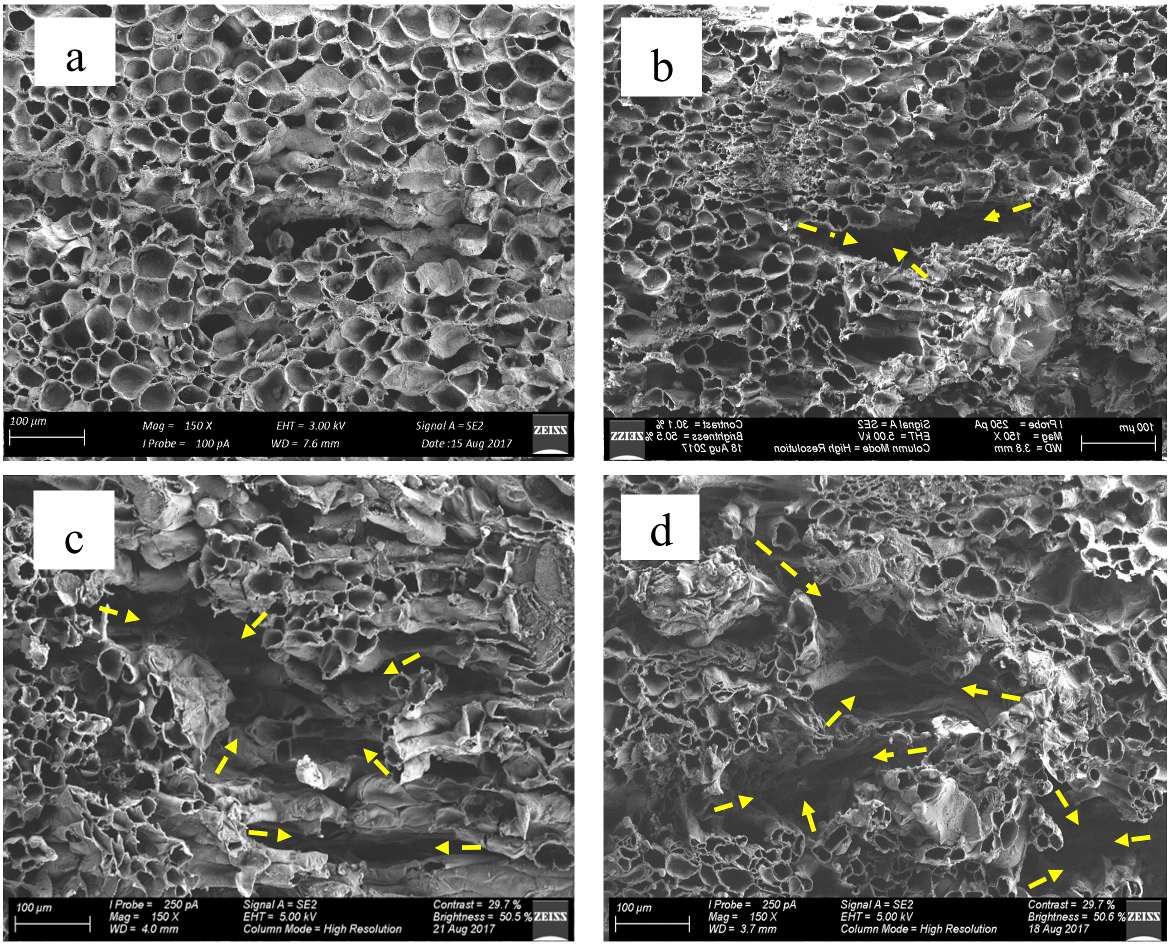

Fig. 5 Scanning electron micrographs of pomegranate fruit peels of unbruised (a) and bruised fruit peel cross-sections (b, c and d) impact-bruised at low (20 cm), medium (40) and high (60) drop heights, respectively. Arrows demarcate the region of bruised damage on the peel microstructure. (Scale bar = 100 µm).

Table 1 Pearson correlation coefficient matrix between physico-chemical and biochemical changes resulting to impact bruising of 'Wonderful' pomegranate fruit

Variables	TCD	BS	% PEL	PPO activity	ROS
BS	1				
BI	0.55	1			
% PEL	0.31	0.42	1		
PPO activity	**0.59**	0.44	**0.70**	1	
ROS	**0.67**	**0.70**	0.52	**0.61**	1

Correlation highlighted in bold are significant at $p < 0.05$. Total colour difference (TCD), browning score (BS), peel electrolyte leakage (% PEL), polyphenol oxidase enzyme activity (PPO) and ROS fluorescence intensity.

CHAPTER 7

BRUISE DAMAGE OF POMEGRANATE DURING LONG-TERM COLD STORAGE: SUSCEPTIBILITY TO BRUISING AND CHANGES IN TEXTURAL PROPERTIES OF FRUIT

Bruise damage of pomegranate during long-term cold storage: susceptibility to bruising and changes in textural properties of fruit

Abstract

This study evaluated the bruise susceptibility of pomegranate (cv. Wonderful) fruit and textural properties of impact bruised fruit during long-term storage. Pomegranate fruit were stored at 5 ºC and 85 % relative humidity for 90 d.Impact tests were performed by dropping fruit from three drop heights (20, 40, and 60 cm) onto a flat hard surface to obtain different impact energy levels. The energy absorbed at impact, bruise volume (BV, mm^3), bruise area (BA, mm^2) and bruise susceptibility (BS, mm^3/mJ) of pomegranate fruit were determined at 30 d interval for each impact dropped fruit. Textural properties for whole bruised and control (non-bruised) cold stored fruit were carried out at 14 d intervals to determine the mechanical strength based on compression, cutting and puncture resistance tests. Based to impact tests, the increase in BV and BA of pomegranate fruit with increased drop heights and duration of cold storage were found significant ($p < 0.05$) during the first 2 months of storage and then decreased in the last month of storage time. Pearson correlations indicated a strong linear relationship between increase in both BV and BA with an increase in impact energy throughout the 90 d storage period. Fruit puncture resistance and whole fruit cutting force, cutting energy and power as well as fruit compression strength properties; firmness, fruit compression energy and power, bioyield energy and modulus of elasticity increased with higher impact bruising and prolonged storage. The results obtained in this study demonstrate that long-term storage, indeed, influenced the susceptibility of pomegranate fruit to bruise, and this in turn, significantly ($p < 0.05$) altered fruit textural properties.

1. Introduction

Production of pomegranate (*Punica granatum* L.) fruit has become a widely grown commercial fruit to many parts of the globe. Large commercial orchards of pomegranate trees are presently grown in many geographical regions, from the Mediterranean basin in the northern hemisphere to the southern hemisphere (Holland & Bar-Ya'akov, 2008; Opara *et al*., 2009; Fawole *et al*., 2013). Consumption of pomegranate fruit has remarkably increased due in part to its unique sensory and nutritional properties coupled with medicinal benefits that are attributed to the fruit's high content of health-promoting phytonutrients and high antioxidant capacity (Opara *et al.,* 2008; Ayhan & Esturk, 2009; Hassan *et al*., 2012).

Mechanical damage occurring between harvesting and consumption has become one of the major contributing factors for the decreasing market value and quality loss of many fruits, including pomegranates (Opara & Pathare, 2014; Ghaffari*et al*., 2015; Shafie *et al*., 2015).Damage on fruit (i.e. bruises, cuts or abrasions) is caused mainly by mechanicalimpacts predominantly occurring during harvesting, handling andtransportation (Opara & Pathare, 2014; Tabatabaekoloor, 2013). Bruising is the most prevalent form of mechanical damage in many fruits which is caused largely by excessive impact and/ or compression forces onto the fruit surface against a rigid body or fruit against fruit mainly involved during mechanical handling (Kitthawee *et al*., 2011; Li & Thomas, 2014; Stropek & Gołacki, 2015). Bruising is defined as the damage to the fruit caused by subcutaneous tissue failure without which may not necessarily involve rupture of the skin of fresh produce, usually indicated by the discoloration of the injured tissue (Blahovec & Paprštein, 2005; Opara & Pathare, 2014; Shafie *et al*., 2015).

Studies have been carried out to assess the factors influencing bruise damage susceptibility of fruit during postharvest storage. Findings from previous research have shown that storage duration influences the impact or compression response of fruit (Yurtlu & Erdoúan, 2005; Kitthawee *et al*., 2011). Increase in bruise sensitivity with increasing storage time has been ascribed to the changes in fruit's physical characteristics during postharvest storage (Vursavus & Ozguven, 2003; Yurtlu & Erdoúan, 2005). In addition, factors such as temperature and humidity have a considerable effect on changes in overall quality, mechanical properties and bruise susceptibility of fruit (Yurtlu & Erdoúan, 2005; Ekrami-Rad *et al*., 2011; Albaloushi *et al*., 2012). Yurtlu and Erdogan (2005) showed that increasing duration of cold storage of apples and pears decreased the bioyield point force, elastic moduli, and fruit firmness and led to a subsequent increase in impact bruising (bruise area and bruise susceptibility) for both fruits. Vursavus and Ozguven (2003) evaluated the effect of duration on bruised susceptibility of peaches using pendulum impactor at the preselected amount of impact energy. Reported results revealed that while the mechanical properties such as modulus of elasticity, bio-rupture force and rupture stress decreased over storage, there was a non-linear relationship between the increase in bruise susceptibility and storage duration at each impact.

There is a dearth of published data on bruise damage susceptibility of pomegranate fruit, which might be partly explained by the complex structure of the fruit (Ekrami-Rad *et al*., 2011). Developing an understanding of bruise damage susceptibility of fruit during storage and handling is a complementary approach in developing improved harvesting and handling practices and could also influence the choice of storage methods as well as transportation systems (Ekrami-Rad *et al.,* 2011; Albaloushi*et al*., 2012; Shafie *et al*., 2015). Furthermore, analysis of the changes in mechanical properties of fruit following the impact bruising could also help in provision of useful recommendations to reduce further losses (Albaloushi*et al*., 2012). This highlights the need for in-depth understanding of bruise damage susceptibility of pomegranate fruit during postharvest handling. Additionally, it is still unknown to what extent bruising could affect the textural or mechanical properties of pomegranate fruit during long- term storage. This study investigated the effect of storage duration on bruise susceptibility of pomegranate and analysed the textural profile of bruise damaged fruit during a 3-month storage period.

2. Materials and methods

2.1. Fruit preparation and storage

Pomegranate cv. Wonderful fruit were handpicked at commercial maturity from Blydeverwacht commercial orchard located in Wellington (latitude 33° 38' S, 19° 00' E, altitude 252 m) in the Western Cape Province, South Africa. Fruit were packed in well-cushioned boxes to avoid on transit bruising and transported in an air-ventilated vehicle to the Postharvest Technology and Research Laboratory, Stellenbosch University. Upon arrival to the laboratory, fruit for the experiment were carefully sorted to ensure use of sound fruit free of any physical defects such as cracking, sunburn and husk scald.

2.2. Impact test and bruise measurement

In the first experiment, we studied the bruise susceptibility of pomegranate 'Wonderful' fruit as affected by storage condition and duration. Sorted fruit were kept in cold storage (5 º C, 85 ± 5 % relative humidity) for 90 d to simulate long-term storage. Sampling for impact test and bruise measurement was done at 14 d intervals for the duration of 12 weeks. Prior to testing, fruit were weighed individually using a Mettler weighing balance (± 0.01g), and the mass of individual pomegranate fruit varied from 250 to 300 g. Impact bruising of whole fruit was performed by a drop test technique using the method reported by Montero *et al*. (2009). 45 individual pomegranate fruit were bruised by subjecting to different levels of drop impacts by letting the fruit fall from 20, 40 or 60 cm drop heights (15 fruit per drop height) onto a rigid flat surface. In this particular experiment, each pomegranate fruit was dropped twice from the same drop height onto two opposite sides of the fruit while ensuring that the two impacts are allocated at equidistant points on the cheek position of the pomegranate fruit. 6 fruit were dropped per drop height to make 12 replications per drop height (i.e. 6 biological replicates; 6 fruit x 2 drop impacts per fruit for each drop height). In each test, the fruit wascaught by hand after the first rebound to avoid multiple impacts onto fruit. After each impact, the bruised region of each fruit was marked using a marker in order to facilitate the detection of bruise damage during measurement. To allow bruise manifestation on damaged tissue after impact tests, fruit were incubated at ambient condition (19 – 22 ºC, 60 ± 5 % relative humidity) for 48 h. Impact energy (E, mJ) resulting from each drop impact on fruit was calculated from equation (1);

$$\mathrm{Ei} = \mathrm{mgh} \qquad (1)$$

where m is the mass of each individual pomegranate fruit, g is the gravitational constant (9.81 m/s^2) and h is the drop height.

Bruise measurement was performed by slicing the fruit through the centre of the marked impact region. Bruise damage of the fruit was identified by the presence of visiblybruise damaged tissue on the marked region which was clearly distinguishable from other non-bruised parts of the same fruit. Measurement of bruise dimensions comprised; w_1 and w_2 for major and minor width, respectively, and d for bruise depth, all performed using a digital calliper (Mitutoyo, ± 0.02 mm). Results of bruise damage size were presented as bruise area (BA, mm^2), bruise volume (BV, mm^3) and bruise susceptibility (BS, mm^3/mJ) expressed as the ratio of bruise volume to the energy absorbed during impact using equations 2 – 4 (Opara and Pathare, 2014).

$$\mathrm{BA} = \left(\frac{\pi}{4}\right) * \mathrm{w}_1\mathrm{w}_2 \qquad (2)$$

$$\mathrm{BV} = \left(\frac{\pi}{8}\right) * \mathrm{w}^2\mathrm{d} \qquad (3)$$

$$\mathrm{BS} = \frac{\mathrm{BV}}{\mathrm{Ei}} \qquad (4)$$

2.3. Textural profile

In the second experiment, changes in some mechanical properties of bruised pomegranate fruit during cold storage were investigated. Prior to storage, a batch of sound pomegranate fruit were selected and pre-conditioned at 22 ± 5 ºC and 60 ± 5 % relative humidity for 24 h. After stabilization at this temperature, 90 fruit were subject to different drop impact heights by letting the fruit fall from 20, 40 or 60 cm drop heights (30 fruit per drop height) using similar procedures as described in section 2.2. For the batch of fruits used for textural profile experiments, each fruit was dropped only once from the same drop height onto a cheek position of the fruit. Dropping of fruit was controlled to ensure that the impact is allocated on the midst point of the cheek position of the fruit. After drop impact treatments, fruit were stored at cold temperature (5 ºC, 85 ± 5% relative humidity) for 90 d. Sampling for puncture resistance, cutting and compression tests of fruit was conducted at 14 d interval throughout the storage duration.

2.3.1. Puncture resistance test

Measurement of fruit puncture resistance was conducted using the fruit texture analyzer (GÜSS-FTA, model GS, South Africa), with a 5 mm cylindrical probe programmed to puncture 15 mm into the fruit at the speed of 10 mm/s. Ten randomly selected pomegranate fruit from each drop impact heights (20, 40, 60 cm) and 10 non-bruised (control) fruit were punctured on three different positions of the fruit placed on a steel test platform with the stem calyx axis parallel to the platform. An average puncture resistance for each fruit was determined. Peak force required to puncture the fruit surface was taken as puncture resistance and the values were presented as mean ± S.E of 8 replications.

2.3.2. Cutting test

Fruit cutting test was performed using texture profile analyzer XT Plus (Stable MicroSystem, Godalming, UK) with a blade set knife (a stainless steel cutting probe with sharpened edges). Ten randomly selected pomegranate fruit from each drop impact level (20, 40, 60 cm heights), each test was carried by cutting the pomegranate whole fruit into halves while positioned with its stem calyx axis parallel to the platform. The texture profile analyzer was set at 1 mm/s pre-test speed, 1 mm/s test speed, 10 mm/s post-test speed, 1000 N cutting force and 20 mm cutting distance. The later was set to prevent the cutter from touching the plate during downward movement of the probe. Data obtained from the textural profile analyzer was processed using Exponent v.4 software (Exponent v.4, Stable MicroSystem Ltd). Fruit cutting force, energy and power were all evaluated from the force–deformation curve generated. Cutting energy was calculated by measuring the area under the force-deformation curve, whereas power required for cutting the whole pomegranate fruit into halves was calculated using equation 5.

$$P_c = \frac{E_c R_c}{60000x} \qquad (5)$$

where P_c is the cutting power (Watt), E_c is the cutting energy (N.mm), and R_c and *x* are the rate of fruit cutting (probe test speed, mm /min) and cutting displacement (mm), respectively. Results (average values of 8 replication fruit) for cutting force, energy and power were expressed as mean ± S.E.

2.3.3 Compression test

Texture profile analyzer (TA-XT Plus, Stable Micro Systems, England, UK) was used to perform the fruit compression test. Prior to testing, the texture profile analyzer was calibrated with a 10 Kg load cell. Machine operating conditions were set at; pre-test speed 1.5 mm/s, probe test speed 1 mm/s, post-test speed 10.0 mm/s, compression force 1000 N and deformation distance 20 mm. Using a 75 mm diameter compression plate, fruit were compressed on the cheek position with the stem/ calyx axis parallel to the flat steel platform where each compression test was done per fruit. Ten fruit from each bruise drop impact height (20, 40, 60 cm) and eight non-bruised (control) fruit were randomly selected and compressed. For each compression test, a force-deformation curve was generated automatically and used to determine the compression properties of fruit using texture profile analyser software (Exponent v.4, Stable MicroSystem Ltd.). From the force-deformation curve, the initial slope which gives an indication of the fruit tendency to deform elastically when a force is applied (elastic modulus, N/mm^2), the maximum force (N) required to compress the fruit to a distance of 20 mm (firmness, N) and the energy required to compress the fruit (toughness, N mm) were obtained. Energy at bioyield, the point at the compression curve where the biological material is said to have failed in internal cellular structure (or permanent deformation) during compression at a given force was obtained by calculating area under the force-deformation curve (Arendse *et al*., 2014). Furthermore, the power (W) to compress the whole pomegranate fruit was determined by using the equation below;

$$C_p = \frac{E_{cp} R_{cp}}{60000x} \qquad (6)$$

where C_p is the compression power, E_{cp} is the compression energy (N mm), and R_{cp} and x are rate of fruit compression (probe test speed, mm /min) and compression displacement (mm), respectively. Results of eight replicates for compression force, compression energy and power were expressed as mean ± S.E.

2.4. Statistical analysis

Statistical analysis was carried out using Statistica software (Statistical version 13, StatSoft Inc., Tulsa, OK, USA). Factorial analysis of variance (ANOVA) was used to evaluate the effects of storage duration and bruising on bruise damage and fruit mechanical properties, respectively. Duncan's multiple range test (DMRT) was used to separate mean values of measured parameters based on their statistical differences.

3. Results and discussion

3.1. Effect of storage duration on pomegranate fruit bruising

The bruise size determined in each sampling day and impact test are shown in Fig. 1. Bruise volume (BV, mm^3) and bruise area (BA, mm^2) of pomegranate fruit increased in the first 2 months in storage. The BV of medium (40 cm) and high (60 cm) impact dropped fruit increased significantly from 5072 and 5803 mm^3 on day 0 to reach the respective peak values of 6867 and 8637 mm^3 after 2 months of cold storage (Fig. 1 A). During the same period, BV for low (20 cm) impact dropped pomegranate fruit also increased albeit at a much lower magnitude (from 3712 mm^3 day 0 to 4619 mm^3 after 2 months).At the end of 3-month storage, fruit impacted at low, medium and high drop heights displayed 22.9, 29.6 and 16.2 % respectively lower BV, suggesting increased resistance to bruising during storage. The bruise area measured in pomegranate fruit subjected at low and medium drop impacts followed a similar trend to that of BV (Fig. 1B). Consistent increase in BA for fruit dropped at medium and high drop impact was observed throughout the storage duration reaching 901 and 1010 mm^2 after 2 months from the respective initial values of 684 and 740 mm^2 on day 0.

Overall, increase in bruise size (BV and BA) of pomegranate fruit with increasing storage duration could be attributed to the decrease in turgor pressure in the course of cold storage (Shafie *et al*., 2015). Fruit containa large percentage of water that is responsible for turgidity in soft tissues (Singh *et al*., 2014), which tends to decline due to continuous moisture loss in the course of storage.Reduction in fruit turgor presumably leads to a consequential reduction in tissue sensitivity to bruise damage (Singh *et al*., 2014; Hussein *et al*., 2018). In the present study, the tendency of excessive loss in moisture is likely caused by impact bruising on pomegranate fruit at medium and high drop impacts. Lower sensitivity of pomegranate fruit to impact bruising observed in the end of three months storage could be the result of drying effect of the pomegranate fruit peel due to excessive moisture loss in fruit stored for more than two months leading to hardening of the fruit peel (Aktas *et al*., 2008; Polat *et al*., 2012).

The bruise susceptibility (i.e. ratio of the bruise volume to the energy absorbed during impact, mm^3/mJ) of pomegranate fruit followed the trend of BV for all drop impact levels as shown by sharp rise in the first month of storage followed by a consistent decline until the end of storage (Fig. 1C). The values of BS were expectedly higher in the order of 20 cm > 40 cm > 60 drop impacts for the first 2 months of storage. Accordingly, the relationship between bruise volume and the absorbed energy has been described as a simple linear function (Van Zeebroeck *et al*., 2007; Opara & Pathare, 2014). In the present study, the difference in impact energy due to differences in investigated drop impact heights (20, 40 and 60 cm) reduced the effect of BV for each calculatesthe ratio of BV to the impact energy measured ratio for two parameters. Thus, in the present study, the BS may not be suitable as the parameter to compare the sensitivity of pomegranate fruit to impact bruising.

3.2. Bruise size versus impact energy relationship during long-term storage

Fig. 2 represents the relationship between bruising and the impact energy absorbed at monthly interval. BV increased with impact energy throughout the storage duration, with high and positive correlation coefficients of 0.928, 0.971 and 0.934 after 1, 2 and 3 months of cold storage, respectively. The results also suggest that the linear change of BV of pomegranate fruit with absorbed impact energy increased after a month in cold storage and reached the peak after 2 months of storage. This tendency was also observed in BA - Ei relationship as shown in Figure 2B. Furthermore, Pearson correlations in Table 1 show that the plot of BS – Ei followed the trend like that of BV but in the opposing direction. The results revealed that higher values of BS were linearly associated with lower impact energy and vice versa. There was a general decline in linearity between the BS and impact energy with increasing duration of storage from strong lineal relation ($r = -0.964$) on day 0 to moderate ($r = -0.744$) at the end of 3 months storage.

Overall, these results highlight that freshly harvested pomegranate fruit tend to bruise less at higher impact energy absorbed compared to stored fruit as evidenced by lower values of bruise size (BV and BA) before storage corresponding to higher impact energies across all studied drop impact levels (Fig. 3).This could be explained by high strength of the cell wall of freshly harvested fruit tissue that presumably supersedes the effect of high turgor pressure on the cell walls of fruit prior to cold storage (Mirdehghan *et al*., 2006; Singh *et al*., 2014). Furthermore, this finding suggests that in order to reduce fruit bruising incidences, most handing of pomegranate fruit, such as packing, sorting and transportation, should be performed within a short time (< 1 month) after harvest. Energy absorbed at impact decreased during storage presumably due to decreasing fruit mass resulting from moisture loss. However, the decline in impact energy in the course of storage had no effect on the increase in bruise size in the first 2 months of storage as described in the previous section.

3.3. Puncture resistance

The effect of impact bruising ($p < 0.0001$) and storage duration ($p < 0.0001$) on fruit puncture resistance was significant. There was generally higher puncture resistance in medium and high drop impact bruised fruit throughout the 12-week storage period (Fig. 4). A significant difference in puncture resistance was observed between bruised and non-bruised (control) fruit from week 6 until the end of storage. During this period, increase in puncture resistance was significantly higher in medium drop impact bruised fruit (152.9 N), and high drop impact bruised fruit (148.3 N) in comparison to that of control (136.3 N) or low impact bruised fruit (131.2 N). During the same period, the effect of storage on fruit resistance to puncture was also significant ($p < 0.05$), reaching the peak value of 160.9 and 158.7 N for medium and high impact bruised fruit, compared to 143.8 and 143.2 N for control or low drop impact bruised fruit, respectively (Fig. 4). Changes in fruit resistance to puncture could be attributed to hardening of pomegranate fruit peel resulting from moisture loss during storage. In agreement to the current study, it has been reported that moisture loss from the fruit led to a hardening of the fruit peel during long term storage and increased the resistance of fruit to puncture (Mansouri*et al*., 2011; Arendse *et al*., 2014). This further suggests that the effect of bruising aided fruit moisture loss during storage, and hence, more hardening of fruit peel resulting in higher resistance to puncture compared to non-bruised control fruit.

3.4. Cutting force, energy and power

Fruit cutting characteristics, including force, energy and power for bruised and control (non-bruised) pomegranate fruit during storage in cold (5 ºC) condition is shown in Fig. 5. A significant difference in the maximum cutting force between drop impact bruised and non-bruised pomegranate fruit was evident from week 6 until the end of cold storage ($p < 0.05$). During this period, fruit bruised at medium and high drop impact had 6 – 11 % higher resistance to cutting than non-bruised or low drop impact bruised fruit that was maintained for the rest of storage duration. Similarly, storage time had a significant effect on the fruit cutting force ($p < 0.0001$). During the first 4 weeks of storage, average values of cutting force for medium and high impact bruised fruit were significantly lower than those observed in the subsequent weeks of storage.

The maximum cutting energy for medium and high drop impact bruised pomegranate fruit was generally higher than those of non-bruised or low impact bruised fruit. Pomegranate fruit bruised at medium (40 cm) or high (60 cm) drop impact heights had up to 36 – 39 % higher cutting energy than non-bruised fruit whereas no significant difference ($p < 0.05$) was observed between non-bruised and low impact bruised fruit until the end of 12-week storage time. The trend for cutting energy was also observed for cutting power (Fig. 5C). At the end of 12-week storage, the cutting power was the highest in medium (0.173 W) and high drop impact bruised fruit (0.176 W), and the lowest was recorded for non-bruised (0.127 W) and low impact bruised fruit (0.141 W). Similarly, there was a general increase in the cutting energy and power for pomegranate fruit with an increase in storage duration ($p < 0.0001$). The average cutting energy increased from 1472.9 N mm for fresh fruit before storage (BS) to average values of 2674.9 N mm (for non-bruised or low impact) and 3508.6 N mm (for medium and high impact bruised fruit). Overall, these results highlight that both storage time and bruise damage had a significant effect on the mechanical strength of pomegranate fruit and peel. It has been revealed that storage conditions such as temperature, humidity, and storage time have a considerable effect on changes of both the quality and mechanical properties of fruit (Bentini*et al*., 2009; Ekrami-Rad *et al*., 2011).

The present study has confirmed that impact bruising increase the resistance to fruit cutting that subsequently affected the force, energy and power required to cut the pomegranate fruit into two halves, a typical procedure of most pomegranate processing machines prior to removing the arils. Ekrami-Rad *et al*. (2011) found an increasing trend both in flavedo peak cutting force, force of cutting, whole fruit cutting energy and whole fruit cutting power with an increase in storage time. Nonetheless, the difference in cutting properties for pomegranate fruit between the previously reported data (Ekrami-Rad *et al*., 2011; Arendse *et al*., 2014) and the results of the present study highlights theinfluence of bruising on changes in mechanical properties of pomegranate fruit. For instance, Ekrami-Rad *et al*. (2011) reported 46 and 80 % less force of cutting and cutting power, respectively after 6 months of cold storage, in comparison to the results of the present study for medium and high drop impact bruised pomegranate fruit. Similarly, Arendse *et al*. (2014) reported 34 and 59 % lower cutting force and cutting energy, respectively for non-bruised 'Wonderful' pomegranate fruit stored at 5 ºC for 3 months. Therefore, the results of the present study demonstrated that impact bruising of pomegranate fruit could lead to increased cost of labour required for manual removal of arils for processing due to increased energy required to separate the arils from the fruit. Bruise damage could also lead to downgrading of fruit not destined for processing due to deformation resulting from peel hardening and concomitant visual appearance of fruit. In worst case scenario, fruit could be disposed due to excessive hardening of peel.

3.5. Fruit compression profile

3.5.1. Compression force, energy and power

Results of the compression test for whole pomegranate fruit is presented in Table 2 and 3. There was a significant ($p < 0.05$) difference in the force required to compress the fruit (firmness), the compression energy and power at different drop impacts (drop heights). After 6 weeks of cold (5 ºC) storage, firmness of bruised fruit at medium (40 cm) and high (60 cm) drop heights was significantly higher than low (20 cm) or non-bruised fruit ($p < 0.05$). During this period, the differences in firmness values between fruit bruised (at medium and high drop impacts) and non-bruised fruit ranged between 30.29 N to 39.85 N. Furthermore, there were differences in values of firmness albeit not significant between medium and high drop impact bruised fruit, and between low drop impact bruised and non-bruised fruit. The trend of change in compression energy and power of pomegranate fruit with both drop impact levels and storage duration was similar to the results obtained in fruit firmness. After the 4-week storage period, there were higher values of compression energy and power in medium and high drop impact bruised fruit in comparison to low drop impact bruised (20 cm) and non-bruised fruit that progressed until the end of the storage trials. For the compression energy and power only high drop impact bruised fruit exhibited a significant increase after 4 weeks of storage. High values of firmness, energy and power required to compress the fruit that were observed in bruised pomegranate fruit from the first 4-weeks storage period could be due to hardening of fruit peel caused by excessive moisture loss leading to increased fruit stiffness, which is characterised by toughening and increased mechanical strength (Ekrami-Rad *et al*., 2011).

Changes of fruit firmness, energy and power required to compress the fruit were significant ($p < 0.05$) with respect to the duration of storage. At the end of 12-week storage, medium and high drop impact bruised fruit had respective 1.41 and 1.29 - fold increase in firmness. The maximum firmness was reached after 10 weeks of storage for fruit bruised at low (205.21 N), medium (245.65 N) and high drop impact (240.31 N), which is similar to the results observed for fruit compression energy and power (Table 3). Furthermore, there was a general trend of decline in firmness, compression energy and power across all bruised and non-bruised fruit during the last week of storage. The present study has revealed that bruise damage influenced loss of moisture from the pomegranate fruit peel during storage in cold (5 ºC) condition that was associated with shriveling and an increase in the fruit stiffness. The results of an increase in pomegranate fruit firmness with storage time was similar to those obtained in pomegranate cv. Malas Saveh fruit (Ekrami-Rad *et al*., 2011) during the first 2-month storage.

Generally, the force required to compress the fruit is said to decrease with extended duration of postharvest storage, as reported in pomegranate fruit cv. Wonderful, during 5-month storage (Arendse *et al*., 2014) and cv. Mollar de Elche (Mirdehghan *et al*., 2006). Similarly, Ekrami-Rad *et al*. (2011) reported the decrease in fruit firmness during the last 4-month storage period (from 2 to 6 months) for ‘Malas Saveh’ pomegranates. The decrease in overall fruit stiffness during postharvest storage is attributed to the decrease in turgor pressure and loss of cell-wall integrity of pomegranate arils, resulting from the breakdown of pectin substances (Mirdehghan *et al*., 2006; Ekrami-Rad *et al*., 2011). Furthermore, decrease in firmness of pomegranate fruit during long-term cold storage is also attributed to chilling injuries leading to increased loss of cell-wall integrity (Arendse *et al*., 2014; Shafie *et al*., 2015). The present data have been compared with previously reported physico-mechanical data (Ekrami-Rad *et al*., 2011; Arendse *et al*., 2014) on the tendency of decreasing firmness of fruit during postharvest storage and found the contrasting results. Possibly, the reason for this disparity could be due to the effect of impact bruising reported in the present finding, in comparison to previously reported data. Changes in compression textural properties observed in the present study were caused by fruit hardening due to increased moisture loss in bruise damaged pomegranate fruit during storage.The implication of this could be more energy requirements for processing of bruise damaged pomegranate fruit that could subsequently lead to increased cost of processing as opposed to non-bruised fruit.

3.5.2. Modulus of elasticity and bioyield energy

Table 3 shows the modulus of elasticity and bioyield energy for bruised and non-bruised pomegranate fruit during the 12-week storage. Modulus of elasticity of fruit increased gradually and significantly ($p < 0.0001$) with prolonged storage, in particular, from 0 to 8 weeks. During this period, there were no significant differences ($p = 0.0684$) in modulus of elasticity between bruised and non-bruised fruit. Afterward, modulus of elasticity declined until the end of storage albeit not significant. The effect of pomegranate fruit bruising on the modulus of elasticity was evident during the last 4 weeks of storage duration. The highest and lowest values of elastic moduli were 32.86 N/mm and 26.13 N/mm observed on the eighth week of storage for medium drop impact bruised and non-bruised fruit, respectively. Bentini *et al*. (2009) stated that when the fruit is exposed under mechanical loading, it exhibits the visco-elastic behaviour which depends on both the amount of force applied, the rate of loading and the duration of storage. Fresh tissue is characterised by elastic, hard and brittle behaviour which could be affected by moisture loss and drying leading to loss of tissue elasticity and increased tissue deformability (Mayor *et al*., 2007). This could mean that the elastic behaviour of fruit tends to decrease with the storage duration. In the light of the present study, the postharvest storage of bruised pomegranate fruit did not influence fruit elastic behaviour. This could be explained by the fact that hardening effect of pomegranate fruit peel due to excessive moisture loss observed in bruised fruit resulted in toughening and an increase in mechanical strength of flavedo (the outer and coloured portion of the peel) while maintaining the freshness of the inner content (arils) of the fruit that maintained the elasticity of the fruit. An increase in the elastic modulus of fruit during the first 8 weeks of storage corroborates with the previous work for 'Malas Saveh' pomegranate fruit by Ekrami-Rad *et al*. (2011) who reported 13 % increase in modulus of elasticity with an increase in storage time from 0 to 2 months.

There was a significant increase in bioyield energy for bruised fruit, which was about 2-fold higher than the initial values (day 0) for medium and high drop impact bruised fruit. Increase in bioyield energy with increasing storage duration was also observed in low drop impact bruised and non-bruised fruit, although this change was not significant. Furthermore, pomegranate fruit bruised at medium and high drop impact generally had higher bioyield energy than non-bruised fruit. Furthermore, a significant difference between fruit bruised (at medium and high drop impact level) and non-bruised fruit was evident after 8 week of storage until the end of storage duration. From the force-deformation curve for the fruit compression test where bioyield points are determined, the bioyield point occurs where there is an increase in deformation with a decrease or no change in force. Polat *et al*. (2012) stated that the presence of bioyield point is an indication of initial cell rupture in the cellular structure of the material. Hence, this could mean that increase in bioyield energy demonstrates decreasing deformability of fruit to compression test as storage period progressed. The results in the present study conflicts with other previous findings, where increase in duration of storage was found to increase the fruit deformation in nectarine (Polat *et al*., 2012) and 'Wonderful' pomegranate (Arendse *et al*., 2014). Decrease in fruit deformation during storage could be the result of excessive moisture loss in bruise damaged pomegranate fruit, which was characterised by hardening of fruit peel and subsequent increase in resistance to fruit compression.

4. Conclusion

Changes in bruise damage susceptibility and mechanical properties of impact bruised 'Wonderful' pomegranate fruit were investigated during long-term cold storage. There was a general increase in susceptibility to bruising for pomegranate fruit within the first 2 months of storage and then decreased in the last month. These findings highlight the need to carry out most of the fruit handling operations such as sorting, packing and transportation as soon after harvest as possible to reduce bruising. The results of mechanical properties of pomegranate fruit such as puncture resistance and whole fruit cutting force, cutting energy and power as well as fruit compression properties showed that these parameters were influenced by both the level of drop impact bruising and duration of cold storage.Overall, the results of the present study suggest that there was a rapid increase in stiffness for bruised fruit peel with an increase in the duration of storage. This subsequently modified the mechanical strength of the fruit peel. The present work has also revealed that the studied mechanical properties and bruising susceptibility of pomegranate fruit are strongly related to the duration of storage. Hence, these results further demonstrate that the design of pomegranate fruit processing machines for separation of the arils from its rind and/ or compression of whole fruit to obtain juice should take into consideration the effect of bruising and storage duration altogether. Overall, the results of textural attributes of pomegranate fruit induced by impact bruising reported in this study highlight some important aspects of fruit processing such as energy requirements for fruit cutting and compression. Processing of bruise damaged pomegranates may require more energy that could lead to increased cost of processing, especially during manual removal of arils or processing. On the other hand, excessive moisture loss, hardening of fruit peel and shrinkage, which are all associated with pomegranate fruit bruising potentially alter the morphological appearance of fruit hence impairing the fruit visual quality and consumer acceptability. Hence the present results provided a contribution to the knowledge to better understand the extent of impact bruising on textural attributes of pomegranate fruit during long-term storage

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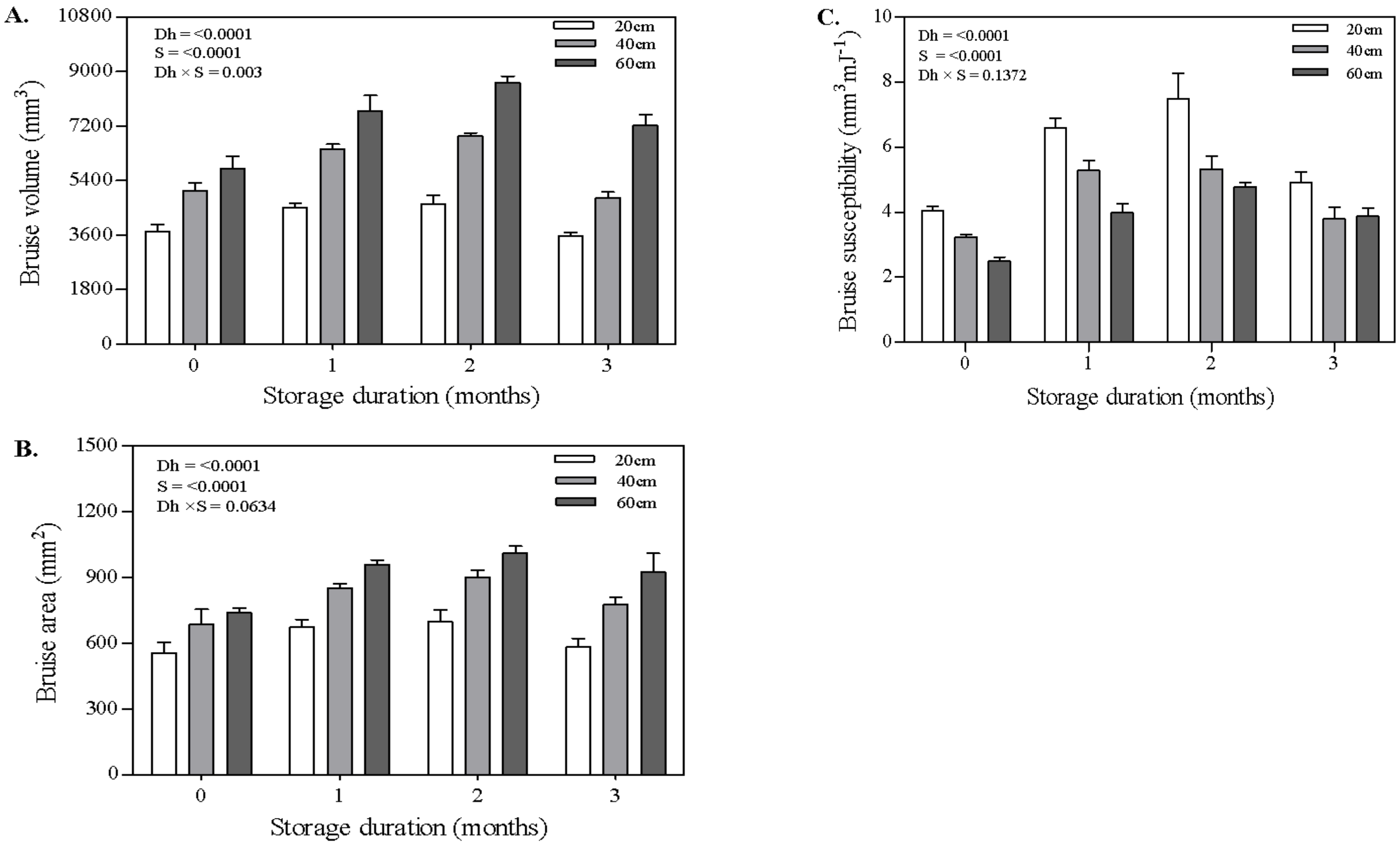

Fig. 1 Bruise size of 'Wonderful' pomegranate fruit; (A)bruise volume (B) bruise area, and (C) bruise susceptibility at different drop impact energy levels; low (20 cm), medium (40 cm) and high (60 cm) during three months cold (5 ºC) storage.0 = before storage (day 0), Dh = drop height (cm) and S = storage duration (months).

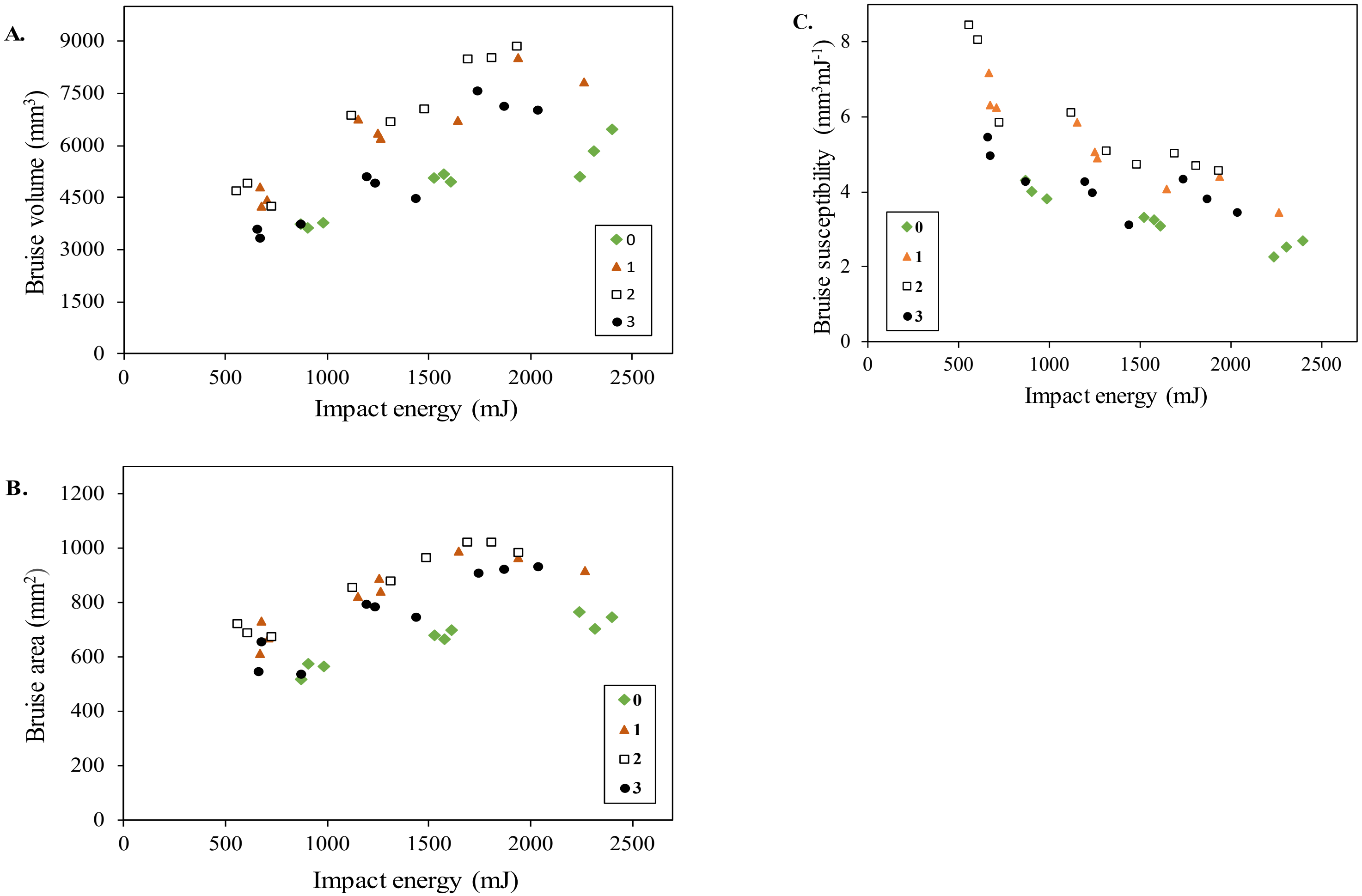

Fig. 2 Scatter pots for bruising of pomegranate fruit cv. Wonderful versus impact energy relationship for different storage durations; 0 = before storage (day 0); 1= month one; 2 = month 2 and 3 = month 3 of cold storage; (A) bruise volume, (B) bruise area and (C) bruise susceptibility.

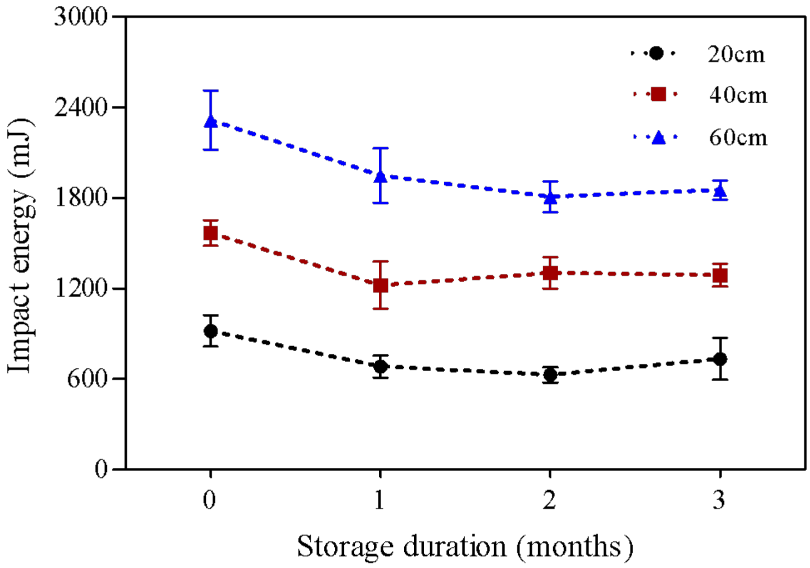

Fig. 3 Impact energy plotted against storage time at 5 ºC for pomegranate fruit cv. Wonderful dropped at low (20 cm), medium (40 cm) and high (60 cm) drop heights ; 0 = before storage (day 0).

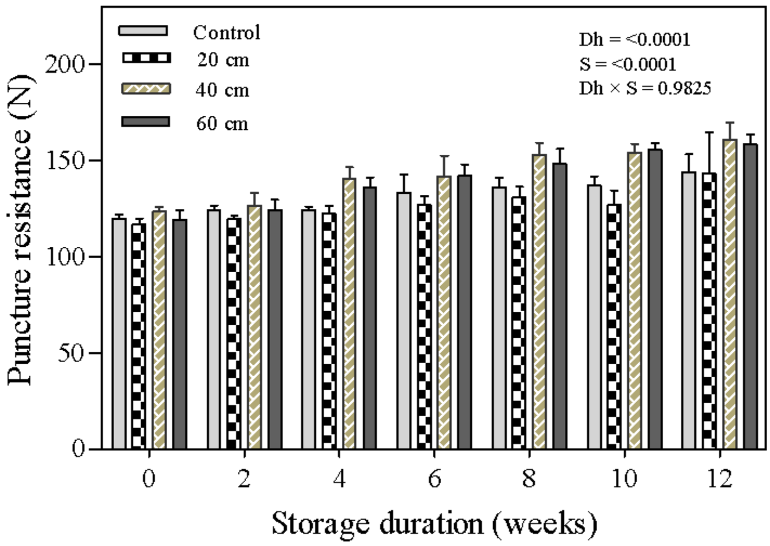

Fig. 4 Puncture resistance for pomegranate fruit cv. Wonderful subjected to low (20 cm), medium (40 cm) and high (60 cm) drop impact bruising and evaluated for 12 weeks in cold (5 C) storage. Non-bruised fruit were included as control; 0 = before storage (day 0)

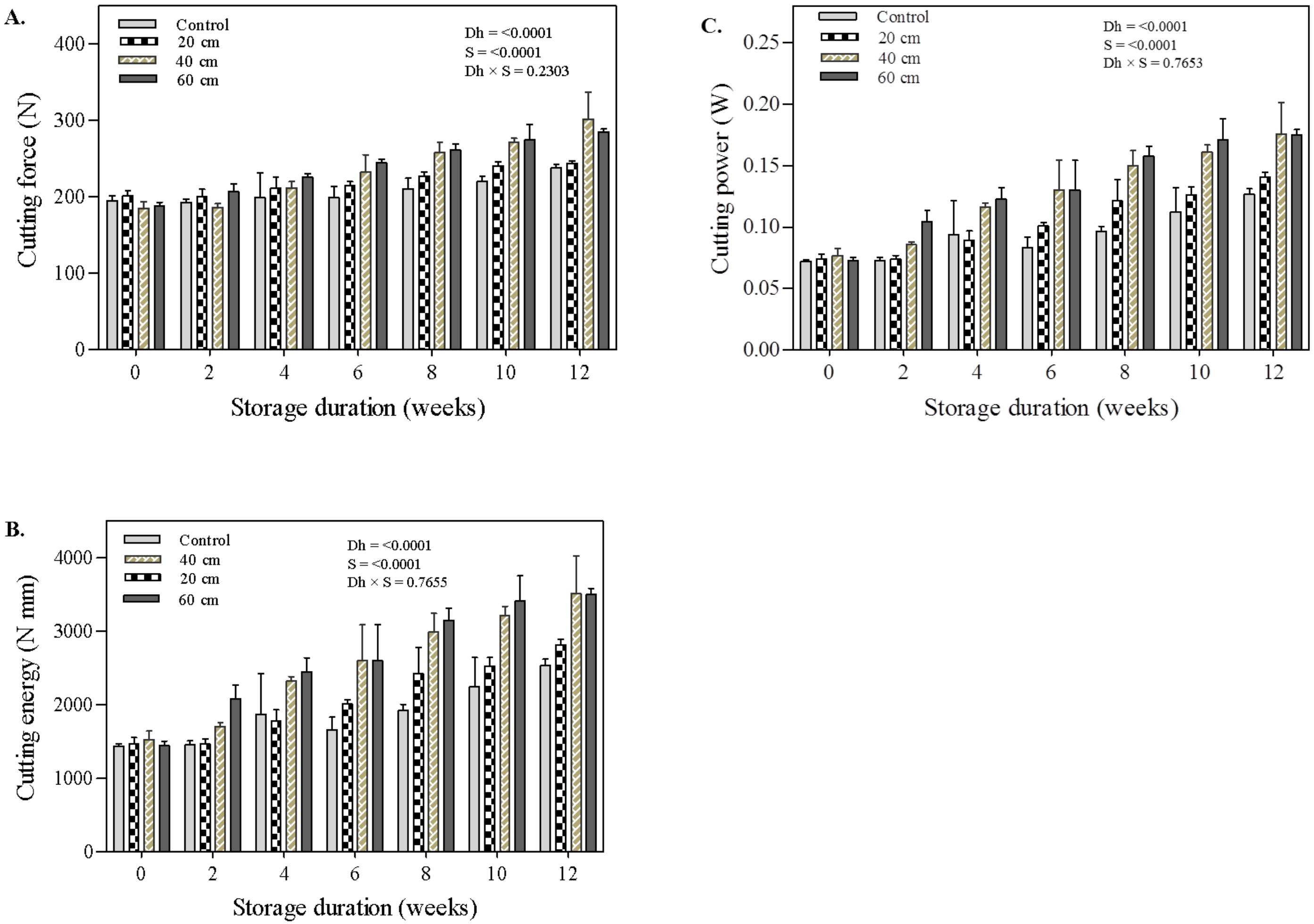

Fig. 5 Changes in maximum cutting force, cutting energy and power for whole pomegranate fruit cv. Wonderful subjected to low (20 cm), medium (40 cm) and high (60 cm) drop impact bruising and evaluated for 12 weeks in cold (5 ºC) storage. Non-bruised fruit were included as control; 0 = before storage (day 0).

Table 1. Pearson correlation coefficients and p-values of bruise size – impact energy relationship fitted by simple linear regression

Storage duration (months)	BV – Ei		BA – Ei		BS – Ei	
	r	p-value	r	p-value	r	p-value
0	0.934	0.0001	0.926	0.0002	-0.964	<0.0001
1	0.928	0.0002	0.862	0.0014	-0.944	<0.0001
2	0.971	<0.0001	0.963	<0.0001	-0.882	0.0008
3	0.934	0.0001	0.923	0.0002	-0.744	0.0108

P-values of all variables are statistically significant at $p < 0.05$. r = Pearson correlation coefficient; BV = bruise volume; BA = bruise area; BS = bruise susceptibility; Ei = impact energy.

Table 2. The compression strength properties for impact bruised pomegranate (cv. Wonderful) fruit during cold (5 ºC) storage for 12 weeks.

Storage duration (weeks)	Drop height (cm)	Firmness (N)	Compression energy (N mm)	Compression power (W)
0	Control	180.43 ± 6.10^{ih}	411.55 ± 11.67^{ih}	0.02 ± 0.00^{ih}
	20	176.30 ± 9.12^{i}	400.33 ± 5.76^{i}	0.02 ± 0.01^{i}
	40	170.88 ± 5.29^{gi}	395.16 ± 47.77^{gi}	0.02 ± 0.01^{gi}
	60	178.25 ± 0.50^{ih}	406.69 ± 5.01^{ih}	0.02 ± 0.00^{ih}
2	Control	184.34 ± 4.71^{ih}	470.56 ± 3.44^{ih}	0.02 ± 0.00^{ih}
	20	188.95 ± 6.21^{i}	458.63 ± 10.80^{i}	0.02 ± 0.00^{i}
	40	181.31 ± 7.61^{gie}	457.40 ± 8.62^{gie}	0.02 ± 0.00^{gie}
	60	201.09 ±7.61^{gif}	460.04 ± 15.27^{gif}	0.02 ± 0.01^{gif}
4	Control	188.10 ± 6.09^{gi}	518.30 ± 46.63^{gi}	0.03 ± 0.01^{gi}
	20	189.06 ± 7.09^{ih}	488.64 ± 31.21^{ih}	0.02 ± 0.01^{ih}
	40	208.40 ± 6.18^{gch}	567.81 ± 30.70^{gch}	0.03 ± 0.00^{gch}
	60	218.91 ± 3.61^{gic}	558.84 ± 6.07^{gic}	0.03 ± 0.00^{gic}
6	Control	199.36 ± 13.67^{gid}	539.66 ± 5.70^{gid}	0.03 ± 0.01^{gid}
	20	210.56 ± 12.06^{gic}	535.14 ± 3.87^{gic}	0.03 ± 0.00^{gic}
	40	220.89 ± 10.13^{a-f}	657.32 ± 54.50^{a-f}	0.03 ± 0.00^{a-f}
	60	225.95 ± 8.98^{a-e}	606.70 ± 22.87^{a-e}	0.03 ± 0.01^{a-e}
8	Control	218.67 ± 3.50^{gic}	637.35 ± 5.59^{gic}	0.03 ± 0.00^{gic}
	20	214.74 ± 5.29^{gid}	615.53 ± 12.46^{gid}	0.03 ± 0.00^{gid}
	40	228.34 ± 27.64^{a-d}	673.59 ± 18.45^{a-d}	0.03 ± 0.01^{a-d}
	60	237.34 ± 13.02^{ab}	705.82 ± 27.71^{ab}	0.04 ± 0.00^{ab}
10	Control	215.36 ± 3.46^{gch}	611.13 ± 5.45^{gch}	0.03 ± 0.00^{gch}
	20	200.54 ±5.64^{gch}	641.82 ± 56.46^{gch}	0.03 ± 0.00^{gch}
	40	245.65 ± 6.48^{a-c}	703.89 ±9.92^{a-c}	0.04 ± 0.00^{a-c}
	60	240.31 ± 6.67^{ab}	727.78 ± 6.30 ^{ab}	0.04 ± 0.00^{ab}
12	Control	209.04 ± 3.07^{gb}	588.80 ± 49.72^{gb}	0.03 ± 0.01^{gb}
	20	205.20 ± 5.52^{gb}	568.90 ± 48.63^{gb}	0.03 ± 0.00^{gb}
	40	240.36 ± 5.47^{ab}	680.24 ± 21.00^{ab}	0.04 ± 0.00^{ab}
	60	231.13 ± 12.07^{a}	661.50 ± 6.04^{a}	0.04 ± 0.00^{a}
Significance level	Drop height (A)	<0.0001	0.003	0.003
	Storage duration (B)	<0.0001	<0.0001	<0.0001
	A × B	0.5632	0.8919	0.8919

Values are presented as mean ± standard error (SE). Means of each parameters followed by different letters across drop impact level and storage duration differ significantly (p <0.05) according to Duncan's multiple range test.

Table 3 Elastic modulus and energy at bioyield point of the force-deformation curves for compressed bruised pomegranate 'Wonderful' fruit during cold (5 ºC) storage for 12 weeks.

Storage duration (weeks)	Drop height (cm)	Elastic modulus (N/mm^2)	Bioyield energy (N mm)
0	Control	19.42 ± 1.01^{ih}	261.93 ± 9.65^{ihe}
	20	20.33 ± 1.11^{i}	226.22 ± 21.17^{ih}
	40	19.26 ± 1.64^{gi}	281.39 ± 9.36^{gi}
	60	18.12 ± 0.97^{ih}	272.51 ± 13.90^{ih}
2	Control	20.73 ± 1.90^{ih}	252.35 ± 61.14^{ih}
	20	22.24 ± 1.53^{i}	229.64 ± 63.95^{i}
	40	22.90 ± 2.14^{gie}	317.11 ± 17.31^{gie}
	60	23.79 ± 1.55^{gif}	300.03 ± 14.59^{gif}
4	Control	23.34 ± 1.50^{gi}	282.16 ± 3.89^{gi}
	20	24.12 ± 1.01^{ih}	270.40 ± 60.43^{ih}
	40	23.12 ± 2.06^{gch}	362.95 ± 83.28^{gch}
	60	25.68 ± 0.95^{gic}	350.95 ± 22.38^{gic}
6	Control	25.14 ± 1.90^{gid}	345.02 ± 10.38^{gid}
	20	28.85 ± 2.54^{gic}	347.32 ± 102.62^{gic}
	40	27.56 ± 1.64^{a-f}	422.17 ± 25.92^{a-f}
	60	27.92 ± 3.29^{a-e}	435.66 ± 9.53^{a-e}
8	Control	27.42 ± 1.80^{gic}	355.04 ± 45.45^{gic}
	20	29.14 ± 0.59^{gid}	345.27 ± 13.61^{gid}
	40	32.87 ± 2.31^{a-d}	448.52 ± 16.05^{a-d}
	60	30.43 ± 1.83^{ab}	496.90 ± 8.00^{ab}
10	Control	26.14 ± 1.71^{gch}	370.83 ± 9.10^{gch}
	20	27.83 ± 3.19^{gch}	368.26 ± 18.13^{gch}
	40	31.57 ± 1.53^{a-c}	475.19 ± 16.64^{a-c}
	60	31.14 ± 2.26^{ab}	505.29 ± 14.43^{ab}
12	Control	26.84 ± 1.65^{gb}	401.67 ± 53.63^{gb}
	20	25.92 ± 4.32^{gb}	405.87 ± 34.49^{gb}
	40	29.67 ± 2.83^{ab}	525.17 ± 13.60^{ab}
	60	30.14 ± 2.29^{a}	536.11 ± 28.39^{a}
Significance level	Drop height (A)	0.0684	<0.0001
	Storage duration (B)	<0.0001	<0.0001
	A × B	0.9559	0.9884

Values are presented as mean ± standard error (SE). Means of each parameters followed by different letters across drop impact level and storage duration differ significantly ($p < 0.05$) according to Duncan's multiple range test.

CHAPTER 8

INVESTIGATING THE EFFECTS OF BRUISING AND STORAGE DURATION ON PHYSIOLOGICAL RESPONSE, PHYSICO-CHEMICAL CHANGES AND ANTIOXIDANT CONTENTS OF POMEGRANATE FRUIT

Investigating the effects of bruising and storage duration on physiological response, physico-chemical changes and antioxidant contents of pomegranate fruit

Abstract

Mechanical damage, mainly bruising of fresh fruit has become the most important problem in the horticultural industry, predominantly, due to the magnitude of occurrence at all stages of postharvest handling chain, from harvest, transport and postharvest handling. The objective of this study was to examine the effects of impact bruise damage on the postharvest physiological, response, physicochemical quality and antioxidant properties of pomegranate (*Punica granatum* L) fruit cv. Wonderful. Fruit were subjected to low (20 cm, medium (40 cm) and high (60 cm) drop impacts by falling freely only once onto the cheek position to a hard surface. Bruised and control (non-dropped) fruit were further stored at 5 ± 0.5 ºC for 90 dplus an additional 4 days at 20 ± 2 °C to simulate shelf life storage. On each sampling day at 14 d intervals, fresh arils (50 g) were obtained from the bruise-damaged site of the fruit of both impact levels, as well as control fruit followed by analysis for physico-chemical and phytochemical quality attributes. Impact bruising induced substantial losses in fresh weight of whole fruit, and significantly doubled the respiratory activity of the arils at least during the first 30 d of storage. Changes in total soluble solids (TSS), titratable acidity (TA), Brix-to-acid ratio (TSS:TA) and BrimA were induced by bruising at medium and high drop impact. High impact bruising resulted in 30 % of both decay incidence and internal fruit decay of fruit after 12-week storage. Qualitative colour modification of fruit peel and arils; colour intensity (chroma, C*) and redness (a*) were significantly ($p < 0.05$) modified by medium and high impact bruising. However, the colour disparity among various drop impacts bruising was higher in fruit peel than arils. Changes in firmness and aril compression energy (toughness) of arils were only significant for storage duration. Mechanical stress induced on fruit at medium and high drop impact bruising increased the radical scavenging activity in DPPH and the total phenolics content towards the end of 12-week storage. Principal component analysis (PCA) revealed that significant and quantified changes observed for both measured attributes were associated with medium and high drop impact bruising.

1. Introduction

Mechanical damage on fruit is the common cause for quality deterioration and postharvest losses during handling and storage (Sablani *et al*., 2006; Jahanfar *et al*., 2011; Hussein *et al*., 2017). Bruise damage on fruits is largely by one or the combination of such loadings; as compression, impact and vibration owing to mechanical forces resulted during harvest, transport as well as handling operations (DeMartino *et al*., 2002; Ergun, 2017; Costa*et al*., 2018; Hussein *et al*., 2018). Bruising begins when shear stress is induced in the fruit by either dynamic or static loading exceeds the failure stress of the fruittissue (Jahanfar *et al*., 2011; Ahmadi*et al*., 2014). Impact and compression loading are prevalent during collisions of fruit againstother fruits, parts of the trees, containers, parts of packhouse equipments or uncushioned surfaces (Ruiz Altisent, 1991; Li & Thomas, 2014; Opara & Pathare, 2014). Bruise damage contributes to the downgrading and rejection of produce, thereby contributing to postharvest losses (Hussein *et al*., 2017). Studies have shown that bruising does not only affect external fruit characteristics such as colour and absence of blemishes, but is related to internal quality losses, alterations of physiological processes prevalence of postharvest rot and decay (Zhao, 2005; Elshiekh & Abu-Goukh, 2008; Montero *et al*., 2009; Scherrer-Montero *et al*., 2011). Bruise damage causes produce quality deterioration and subsequent economic losses due to decay and microbial spoilage, loss in fresh weight, change in pericarp colour and visual quality (Stropek & Gołacki, 2015; Hussein *et al*., 2018). Subsequently, losses due to bruising include the quantitative decrease of fruit and vegetable weight as well as qualitativedecrease of their market value (Gołacki&Kolodziej, 2011). Earlier research by Crisosto *et al*. (1993) reported that economic loss to the fruit growers could result even when as little as 8 % of the fruit fresh weight is lost in addition to unsightly shrivelling.

Bruise damage modifies physiological and metabolic processes that lead to faster ripening, internal browning and eventually quality losses (Opara & Pathare, 2014; Costa *et al*., 2018). Studman (1997) explained that the postharvest losses for apples that could result from bruising could be as high as 50 %, even though most commonly, the losses are in the range of 10–25%. Minor or hidden bruises on fruit are hardly noticed during visual inspection, hence usually neglected. This increases the danger of consequent fungal infections to the bruised fruit (Zeebroeck *et al*., 2007; Ergun, 2017). Minor bruise damage on ‘Galaxy’ apple resulted in a decrease in firmness and fruit browning (Ergun, 2017). Similarly, bruising damage accelerated the decrease of firmness and the ratio of sugar to acid for Yali pears (Li *et al*., 2012). Impact bruising resulted in both the qualitative internal and minor external changes on tangerines, including losses of citric acid, soluble solid and losses of ascorbic acid (Montero *et al*., 2009).

Like many other fruit species, pomegranate (*Punica granatum* L) is prone to bruising as the result of excessive loading due to improper handling, poorly designed equipment or improper packaging (Shafie *at al*., 2017). Despite the characteristic hard rind of pomegranate fruit, the increasing use of mechanical equipments for the harvesting and postharvest handling operations potentially predispose the fruit into excessive mechanical forces that cause bruising (Shafie *et al*., 2015; Hussein *et al*., 2017). Nevertheless, there is a dearth of detailed information on quality losses of pomegranate fruit caused by impact bruising. Limited availability of pomegranate research data on the subject could, in part, due to the complex structure of the fruit, in comparison to stone fruits such as apples and pears. This study investigated the effect of impact bruising on the physiological response, physico-chemical and textural changes and antioxidant properties of pomegranate fruit cv.Wonderful.

2. Material and methods

2.1. Plant material and sample preparation

Pomegranate fruit (cv. Wonderful) were harvested by hand picking at commercial maturity from an orchard located in Wellington area (33°38' S, 19°00' E) in the Western Cape Province, South Africa. Fruit were transported by car on the same day to the Postharvest Technology and Research Laboratory located at Stellenbosch University. Fruit were sorted to get rid of damaged ones, and then pre-conditioned at 22 ± 5 ºC and 60 ± 5 % relative humidity for 24 h in order to avoid the effect of temperature variability. Healthy fruit were subjected to different degrees of impact to cause bruising by dropping individual fruit from pre-selected drop heights (20, 40 and 60 cm for low, medium and high impacts, respectively). Each fruit was dropped once onto a rigid surface on the cheek region along the equatorial axis. Following impact treatments, bruised fruit was stored in cold storage (5 ± 0.5 ºC) for 90 d. Non-bruised pomegranate fruit was also included as control. Sampling for analyses of physico-chemical attributes, antioxidant properties, fruit decay and weight loss was randomly done at 14-day intervals by taking a sample of 5 to 15 fruit from each treatment. Measurement of respiratory activity of whole pomegranate fruit was performed at 30 d interval for 90 d. A baseline analysis to investigate the physiological response, physico-chemical quality attributes and antioxidant properties of pomegranate fruit samples was conducted on control fruit prior to storage. Pomegranate fruit for chemical analysis were hand processed by cutting fruit into two equal halves to remove arils (without crushing). Approximately 50 g of fresh arils from a half of bruise-damaged fruit (on the impact region) were obtained and juiced using a cheese cloth.

2.2. Decay incidence and fruit internal decay

Decay incidence was recorded by visual assessment of 20 individual fruit. Fruit showing external surface mycelia development or bacterial lesions were considered decayed and discarded. Fruit were further examined for internal decay but cutting fruit through the bruised area. Care was taken to distinguish between common pomegranate internal fruit decay caused by *Aspergillus niger* such as heart rot, also known as black heart, and that caused by mould or bacteria as a result of impact bruise-induced wounding. Generally, fruit observed with internal visible lesions were considered decayed.Results were presented as a percentage of decay incidence or fruit internal decay and calculated using equation 1;

$$\text{Decay} = \frac{N_d}{N_T} \times 100 \qquad (1)$$

where, N_d is the number of fruit externally or internally found decayed on each sampling, and N_T is the total number of fruit on each sampling day.

2.3. Physiological response

2.3.1. Weight loss

Cumulative change in fresh weight for pomegranate fruit was determined for each bruising and storage condition using method described by Arendse et al. (2014). Initial fresh weight of 20 randomly selected pomegranate fruit samples was taken after inflicting bruise impact. Fruit were then numbered and weighed at 14 d intervals until end of storage duration. Non-bruised fruit were also included for control. Changes in fresh weight of fruit was measured with respect to drop heights and storage duration using an electronic scale (Mettler Toledo, Model ML3002E, Switzerland, 0.0001 g accuracy), and percentage weight loss calculated using equation 2.

$$W_L = \frac{W_i - W_f}{W_i} \times 100 \qquad (2)$$

where, W_L is the cumulative weight loss (%) of fruit; W_i initial weight (g) of the fruit at the beginning of storage; and W_f weight (g) of the fruit at the time of sampling during storage.

2.3.2. Fruit respiration rate

Respiration rate of pomegranate fruit 'bruised' or 'non-bruised' (control) was measured at intervals using the closed system method as previously described by Caleb *et al*. (2012). Pomegranate fruit cv. Wonderful were bruised by drop impact at 20, 40 and 60 cm drop heights using previously described procedures. In triplicate, 3 bruised fruit (for each drop height) or non-dropped (control) fruit were placed inside 2 L air-tight glass jars (previously equilibrated to the temperature and relative humidity of the experimental storage conditions) with lid containing a rubber septum. Glass jars were kept at 20 ºC on the laboratory bench or 5 ºC in a cold chamber for 5 days. Gas (O_2 and CO_2) composition was monitored every hour for 4 hours consecutively by drawing gas sample from glass jar headspace using O_2/CO_2 gas analyser (Checkmate 3, PBI Dansensor, Ringstead, Denmark) with an accuracy of 0.5%. The rate of respiration was worked out from the jar volume, fruit mass and the time that the jars were closed (60 minutes). Five (5) respiration measurements were performed per day over 5 consecutive days and results presented as mean ± S.E (mL CO_2 $kg^{-1}h^{-1}$) of fivedeterminations.

2.4. Physico-chemical and textural attributes

2.4.1. Fruit peel and aril colour

Fruit and aril colour were measured on basis of CIE *L**, *a** and *b** colour system by Commission International del' Eclairage (CIE), using a pre calibrated digital colour meter (Minolta Chroma Meter, CR-400, Japan). Peel colour of 6 bruised fruit (for each drop height) was measured on the marked region of the drop impact spot, whereas that of non-bruised control fruit was measured on the cheek position alongside the fruit equatorial axis. Fruit arils were obtained from marked region of 6 bruise-damaged fruit (for each drop height) and non-bruised control fruit and spread to cover a petri dish and colour measurements were taken from five different points of arils. Colour coordinates, *L**, *a** and *b** for lightness, redness/greenness and yellowness/blueness, respectively were recorded for each measured point and means of all measurements were determined. The results were presented as the colour intensity (chroma, *C**) and color appearance parameter (hue, h^o) using equations 3-4 (Pathare *et al*., 2013).

$$C^* = \sqrt{a^{*2} + b^{*2}} \qquad (3)$$

$$h^\circ = \tan^{-1}\left(\frac{b^*}{a^*}\right) \qquad (4)$$

The difference in colour between fruit peel and arils was expressed as total colour difference (TDC), calculated using equation 5.

$$\text{TCD} = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \qquad (5)$$

where L_0^*, a_0^* and b_0^* are the 'reference' colour values of the fruit peel, while L*, a* and b* are the colour values of the arils (Pathare *et al*., 2013; Fawole & Opara, 2013c). Means ± S.E of 10 determinations (*n* = 10) were obtained from the 10 measured fruits.

2.4.2. Aril compression test

Aril compression test was performed using a texture profile analyzer XT Plus (Stable MicroSystem Ltd., Godalming, UK) as reported by Arendse *et al*. (2015). Compression test was performed on arils from 6 bruised fruit (for each drop height) and non-bruised fruit using a 35 mm diameter cylindrical compression probe set at 1.5 mm/s pre-test speed, 1 mm/s probe test speed, 10.0 mm/s post-test speed, 10 N compression force and 10 mm compression distance (Fawole & Opara, 2013b). Data from compression test were interpreted using software Exponent v.4 (Stable MicroSystem Ltd., Godalming, UK), by running the macro to obtain the hardness (N) and toughness (N mm) of the tested aril. Hardness (firmness) was presented as the the maximum force (N) required to compress the pomegranate aril to a distance of 10 mm, and is usually defined as the resistance to indentation, which is determined by measuring the permanent depth of the indentation such that the smaller the indentation, the harder the food material (Yamashita, 2008). Toughness is the energy required to compress the aril, presented as the area underneath the curve in a force–displacement graph (Yamashita, 2008; Arendse *et al*., 2015). In each test, about 20 randomly selected arils from each treatment (bruised and unburied fruit) were compressed individually and the results presented as the mean (± S.E) of 20 determinations.

2.4.3. Titratable acidity, total soluble solids, TSS/TA ratio and BrimA

Titratable acidity (TA) of aqueous diluted pomegranate juice (PJ) from 6 bruised fruit (for each drop height) and non-bruised fruit was determined potentiometrically by titration with 0.1N NaOH (Merck) to an end point of pH 8.2 using a Metrohm 862 compact auto titrosampler (Herisau, Switzerland). Titratable acidity was expressed as milligrams of citric acid (CA) per a hundred millilitres of crude PJ (mg CA/100mL). Total soluble solid (TSS) of PJ was measured using digital refractometer expressed as ºBrix (Atago, Tokyo, Japan). Using values of TSS and TA obtained above, TSS/TA ratio and BrimA values were calculated. The latter is an important scale proposed as a criterion for consumer acceptability in fruit (i.e. measures the balance between sweetness (ºBrix) and sourness (acidity) (Jordan *et al*., 2001) was calculated using equation 6 (Jordan *et al*., 2001; Harker *et al*., 2002).

$$BrimA = {}^{\circ}Brix - k \times TA \qquad (6)$$

where k is a constant that ranges from 2 - 10, mainly due to differing mixes of acids and sugars in different fruit species/cultivars. The k value of 2 was used to avoid a negative BrimA index (Fawole & Opara, 2013a).

2.5. Antioxidant content

Analysis of antioxidant content of 6 bruised fruit (for each drop height) and non-bruised fruit was performed in triplicates using pomegranate juice (PJ). Prior to analyses, 0.5 mL of PJ was mixed with 14.5 mL of 50 % methanol in centrifuge tubes. The mixture was sonicated in cold (10 ºC) water and centrifuged for 25 min. The supernatant of PJ methanoic extract was carefully poured in clean tubes and used for analysis of total phenolic content and antioxidant capacity.

2.5.1. Total phenolics content

Total phenolics content (TPC) was determined using the Folin–Ciocalteu (Folin–C) method as described by Makkar (2000) with slight modification (Fawole *et al*., 2012). Diluted PJ extract (50 µL) of 6 bruised fruit (for each drop height) and non-bruised fruit was mixed with 450 µL of 50 % methanol in the test tube followed by the addition of 500 µL Folin–C reagent. After 2 min, 2.5 mL of sodium carbonate solution was added to the mixture of methanolic PJ extract and Folin – C. The mixture was vortexed and incubated in dark chamber for 40 min at room temperature (25 ºC). The absorbance of the solution mixture was measured at 725 nm using a UV–visible spectrophotometer (Thermo Scientific Technologies, Madison, USA) and compared with values from the Gallic acid standard curve (0.02 – 0.10

mg/mL). Total phenolics content was expressed as milligram gallic acid equivalent per 100 mL (mg GAE/100 mL) of crude PJ.

2.5.2. DPPH Radical-scavenging activity

The ability of PJ to scavenge 2, 2-diphenyl-1-picryl hydrazyl (DPPH) radical was analysed colorimetrically. The DPPH assay (Sigma Chemical Co.) was carried out according to the method reported by Fawole et al. (2012). Methanolic extract of PJ (15 µL) of 6 bruised frui (for each drop height) and non-bruised fruit was diluted with 735 µL 100 % methanol in Eppendorf tubes. Exactly 750 µL of 0.1 mM solution of methanolic DPPH was added. The mixture was incubated for 30 min in the dark at room temperature followed by measuring the absorbance at 517 nm using a UV–visible spectrophotometer. Free-radical scavenging capacity of PJ based on DPPH reaction was determined by extrapolation using trolox standard curve (0–20 µM). The results were expressed as micro molar ascorbic acid (AA) equivalent per millilitre of crude pomegranate juice (µM AAE/mL PJ).

2.5. Statistical analysis

Data for fruit physiological responses, physico-chemical quality attributes and antioxidant properties were analysed using Statistica software (Statistica 14.0, Statsoft, USA). Data were subjected to factorial Analysis of Variance (ANOVA) and analysed for the main effects (impact bruising and storage duration).Post-hoc test was used to test for statistical significance such that observed differences at $p < 0.05$ were considered significant according to Duncan's Multiple Range Test. Relationship and variability among the measured parameters were determined by subjecting data to principal component analysis (PCA) using XLSTAT software version 2015.04.1(Addinsoft, France).

3. Results and discussion

3.1. Decay incidence and fruit internal decay

Decay incidence of pomegranate fruit was influenced by impact bruising and prolonged storage duration.Decay started occurring after 4 weeks of storage in medium and high drop impact bruised fruit with 5 and 10 % decay incidences, respectively (Fig.1 A). Fruit decay continued to increase in medium and high drop impact bruised fruit, and after 8 weeks of storage, decay (5 %) was observed in low drop impact bruised fruit. No further decay was in week 10 of storage, regardless of drop impact, however, at the end of storage (12 weeks), non-bruised fruit had 10 %; low impact bruised fruit had 10 %; medium impact bruised fruit had 15 % while high impact bruised fruit had 30 % decay incidences. Similar to our finding, Li *et al*. (2011) reported 42.8 % higher decay incidence of bruised pears than that of non-bruised (control) pears after 60 d storage.

Similarly, both impact bruising and storage duration had an influence on fruit internal decay with a similar to that of external day. However, internal decay incidence was lower than that of external decay (Fig. 1B). At the end of 12 week storage, internal decay recorded in fruit was 4 % for control and low impact, 20 % for medium and 30 % for high impact bruising. The observed internal decay of pomegranate fruit was predominantly gray mould (Botrytis cinerea) or blue mould (*Penicillium spp*.). Palou *et al*. (2013) has revealed that postharvest gray mold of pomegranate originate both from wound and latent infections, whereas the blue mould is caused by latent infections. Overall, factors such as temperature (5 – 8 °C), high relative humidity (90 – 95 %), and nutrients from the fruit surface critically favour the activity of postharvest pathogens. Additionally, softening of fruit tissue due to mechanical injury presumably stimulated further susceptibility to infection (Van Zeebroeck *et al*., 2007; Sivakumar *et al*., 2011). Pathogens typically enter through susceptible (dead or wounded) tissue in the epidermis and initiate infection before contaminating the rest of the fruit (Spotts *et al*., 1998; Fischer *et al*., 2009). In addition, it has been revealed that, for decay pathogens to assume control in mechanically damaged fruit, the resulting wounds must exceed a certain size (Van Zeebroeck *et al*., 2007). This could be attributed to the least decay incidence and internal decay that was observed in control and low impact bruised fruit. Accordingly, the decay incidence in low impact bruised fruit was characterised by a mass of black arils, which could be described as heart rot, commonly known as black heart disorder, which is caused by*Aspergillus niger*and *Alternaria* spp. (Palou *et al*., 2013;Yehia, 2013). These specific internally decayed pomegranates had no characteristic external symptoms except for slight rind discolouration.

3.2. Fruit physiological response

3.2.1. Weight loss

Impact bruising, and the interaction between storage duration and bruising had a significant influence on weight loss of whole pomegranate fruit ($p < 0.001$).The average fresh weight loss varied between non-bruised (control) fruit, low (20 cm), medium (40 cm) and high (60 cm) drop impact bruised fruit (Fig. 2A). Medium and high impact bruised fruit had higher WL than low drop impact and control fruit and increased with prolonged storage. At the end of cold storage, the highest cumulative weight loss reached 39.27 and 38.61 % for the medium and high drop impact bruised, respectively. During that period, the difference in weight loss between control and low impact bruising was not significant ($p > 0.05$). Overall, the difference in weight loss between control and bruised (medium and high drop impact) fruit ranged between 33.3 – 36.3 % at the end of the investigated storage period (12 weeks). The difference in weight loss among treatments could be explained by the impact intensity (Montero *et al*., 2009). Pomegranate fruit peel has high porosity that permits free movement of water vapour, the fact that makes the fruit highly susceptible to weight loss (Fawole & Opara, 2013a). Moreover, tissue damage due to bruising modifies the permeability of cell wall tissues by creating small cracks that connect the internal and external atmosphere of the fruit, permitting even more free exchange of water vapour (Martinez-Romero, 2003). However, the susceptibility to weight loss could also depend on other factors such as storage conditions, plant species and cultivar (Kasat *et al*., 2007; Fawole & Opara, 2013a). Montero *et al*. (2009) found that the weight loss in 'Rainha' tangerines dropped at 40 and 60 cm were 8.4 and 8.8 %, respectively only after 7 d at 20 ºC. Elsewhere, Sanches *et al*. (2008) did not find any change in fresh weight loss of avocados in impacted by bruising. It is also worth mentioning that when a fruit is bruised, the stress leads to an increase in respiration rate, which in turn, brings about loss of water, and hence weight loss.

3.2.2. Respiration rate

Changes in the respiration rate (RR) of whole pomegranate fruit were significantly affected by impact bruising and storage duration ($p < 0.05$). After 4 weeks of storage, the RR of medium and high drop impact bruised fruit increased to reach their corresponding peak values of 17.57 and 18.50 mL CO_2 $kg^{-1}h^{-1}$ from initial (before storage) values of 10.49 and 11.49 mL CO_2 $kg^{-1}h^{-1}$ for control and low impact bruised fruit (Fig. 2B). During this period, there was a 1.4-fold average increase in RR in medium and high impact bruised fruit compared to the control and low impact bruised fruit. Afterward, the RR declined gradually in both treatments (drop impacts) until the end of storage. At the end of 12 weekstorage, the RR of fruit declined to reach 11.81 and 3.11 mL CO_2 $kg^{-1}h^{-1}$ for medium and high impact bruised fruit, respectively. This was consistent with the RRfor control and low impact bruised fruit which declined to 9.46 and 8.023 mL CO_2 $kg^{-1}h^{-1}$, respectively.

Overall, the present work has revealed that, when the highest impact treatments were applied, the rates of CO_2 production were highest, indicating higher RR for medium and high impact bruised fruit throughout the storage duration. Increase in RR during the first 4 weeks of storage could be attributed to increased fruit stress resulting from impact damage coupled with increased metabolic activities (Scherrer-Montero*et al.*, 2011). These results reaffirm those reported in plums by Pérez-Vicente *et al.* (2002) and 'Tahiti' limes by Durigan *et al.* (2005), who both found that the impact bruised fruit had up to three times higher respiration rate than control fruit. Furthermore, it has been found that respiration of pomegranate fruit increases with prolonged storage irrespective of treatment or fruit condition (Fawole & Opara, 2013a; Mphahlele *et al.*, 2016). Increase in RR is spurred by an increase in the metabolic activity, associated with tissue senescence in the course of storage (Sánchez-González *et al.*, 2011; Fawole & Opara, 2013a). Hence, careful handling of pomegranate fruit is critical to avoid mechanical damages during long-term storage and slow down the physiological changes and improve fruit storability.

3.3. Physico-chemical and textural quality

3.3.1. Fruit peel and arilcolour

Peel colour of pomegranate fruit was significantly ($p < 0.05$) affected by drop impact level and storage duration (Fig. 3 and 4). During the first 4 weeks of storage,the L^* value (lightness) of the bruised region of fruit peel decreased gradually for fruit subjected to bruising at low (20 cm), medium (40 cm) and high (60 cm) drop impact levels, and stabilized for the rest of the storage duration only for low impact bruised and control (non-bruised) fruit (Fig. 3A). During the first 4 weeks of storage, lightness (L^*) values of the fruit bruised region decreased gradually for fruit subjected to bruising at low (20 cm), medium (40 cm) and high (60 cm) drop impact levels.Afterward, L^* of non-bruised and low impact bruised fruit stabilized for the rest of the storage duration.After 12 weeks, L^* values for medium and high impact bruised fruit declined from 37.90 to 14.12 and 33.09 to 14.66, respectively. In contrast, a continuous increase in redness (a^*) was observed after 4 weeks of storage from 25.96 to 45.49 and 19.35 to 49.83 for medium and high impact bruised, respectively (Fig. 3C). Overall, the observed trend of decrease in $L^*$and increase in a^* values for medium and high impact bruised fruit indicates the perceptible browning colour of pomegranate fruit with the course of storage (Tijskens *et al.*, 2010). Similar to our findings, the smaller L^* values and relatively larger a^* values were also reported for bruised Yali pears fruit (Li *et al.*, 2012).

Ergun (2017) reported a similar trend of higher *a** value than non-bruised Galaxy' apple tissue compared to bruised tissue of the same fruit.

With respect to the arilcolour, the aril lightness (*L**) declined progressively until the last day of storage for all impact bruising treatments (Fig. 3 B). The rate of reduction in colour lightness of arils for medium and impact bruised fruit was significantly higher ($p < 0.05$) than that of non-bruised and low impact bruised fruit. At the end of 12 week storage, the lightness of arils changed from an average of 44.65 before storage to 38.31for non-bruised and low impact bruised fruit, and 41.80 to 27.53 for medium and high impact bruised fruit (Fig. 3B).There was a significant decline in aril redness (*a**) with increased storage duration ($p < 0.0001$) across all impact bruising treatments until the end of 12 week storage. The redness colour of arils decreased from the initial (before storage) values of 52.13 to 47.43 for non-bruised fruit and 51.10 to 44.31 for low impact bruised fruit. Similarly, during the same period, the aril redness declined by 1.27 and 1.17- folds from the initial values of 42.24 and 46.82 for medium and high impact bruised fruit, respectively.However, the redness colour remained significantly higher ($p < 0.05$) in non-bruised and low impact fruit arils throughout the duration of storage until the storage was terminated (12 weeks).

There was no significant difference ($p > 0.05$) in chroma (C*) between bruised and non-bruised in the first 4 weeks of storage. The colour intensity (C*) picked up to reach the maximum at the end of 12 week storage reaching 49.43 and 51.32 for medium and high impact bruised fruit (Fig. 4 A). The change in colour intensity (C*) observed during this period was significantly higher for medium and high impact bruised fruit that non-bruised or low impact bruised fruit, which indicated the increase in browning intensity of medium and high impact bruised fruit. This could be explained by the intensification of enzymatic browning in bruise damaged tissue of the fruit peel in the course of storage. Discoloration of peel tissue of bruised pomegranate (cv.Wonderful) fruit was activated by release of intra-membrane cell content (mainly phenolic compounds) into intercellular space presumably as the result of cell rupture (Mitsuhashi-Gonzalez *et al*., 2010; Hussein *et al*., 2018). Following that, polyphenol oxidase enzyme acts on sequestered substrates, leading to tissue browning (Li *et al*., 2012; Jiménez *et al*., 2016). However, other processes such as chilling injuries and moisture loss through pores on the fruit peel during cold storage of pomegranate fruit cannot be ignored as the cause for the increase in fruit browning (Defilippi *et al*., 2006; Lichanporn *et al*., 2009).

Colour purity (hue angle, h°) of the pomegranate fruit peel declined slightly across all treatments in the first 4 weeks, and then decreased sharply until the eighth week followed by gradual increase in control and low impact bruised fruits during the remainder of the storage duration (Fig. 4 C). During the same period, changes in hue anglewas significant for medium and high impact bruised fruit. Thereafter, there was a steep decline in h°colour index for the next 4 weeks in both bruised and non-bruised fruit followed by stabilization until the end of 12 week storage. Decline in colour purity (h°) indicated the decline in the characteristic red colour of the fruit peel that could be due to enzymatic browning of bruise damaged tissue. During the last 4 weeks of storage, non-bruised and low impact bruised fruit had significantly ($p <0.05$) higher values of h° compared to the medium and high impact bruised fruit. This result highlighted that the rate of reduction incharacteristic red colour for non-bruised or low impact bruised fruit was lower than that medium or high impact bruised fruit. Similarly,the colour purity (h°) and colour intensity (C*) followed similar fashion to aril redness, and was significantly higher in control and low impact bruised fruit (Fig. 4 B & D). Overall, the differences in aril redness colour, purity and the colour intensity observed between bruised and non-bruised or low impact bruised fruit demonstrates the effect of impact bruising in in changes of aril colour attribute.

3.3.2. Total colour difference

The colour disparity of pomegranate fruit peel between the initial (before storage) and successive storage weeks presented as total colour difference (TCD) is shown in Figure 4a. Totalcolour difference increased progressively for both bruised and non-bruised fruit, reaching the highest values of 32.64 for medium impact bruised fruit and 33.35 for high impact bruised fruit at the end of 12 week storage. In contrast, lower TCD was observed in non-bruised (12.13) low impact bruised (19.30) fruit. With respect to the fruit arils, the TCD between measured initial and the end of storage values was 8.88 for non-bruised fruit and 2.29 for low impact, and an average of 5.16 for medium and high impact bruised fruit. Overall, these results suggest that changes in colour of the fruit peel in the course of storage as the result of impact bruising were higher than those observed in fruit arils. These findings were buttressed by images of whole fruit and arils presented in Figure 5. The differences in discoloration of bruise damaged pomegranate fruit peel was vividly perceptible among the three levels of drop impact treatments and were clearly distinguishable from each other as well as the control fruit (Fig.5A-D). Comparatively, the observed colour disparity in fruit arils between the three impact levels was not as noticeable as the peel colour, as displayed in Fig. 5A-D. However, a large difference in aril colour was observed between control and high drop impact bruised fruit. Hence, this could rationalize the lack of major changes in most of the measured chemical quality attributes at lower impact level, which are directly linked to bruise damages of arils.

3.3.3. Titratable acidity, total soluble solids, TSS/TA ratioand BrimA

Results in Table 2 show that impact bruising ($p < 0.0063$) and storage duration ($p < 0.0070$) had a significant influence on the content of titratable acidity (TA) of pomegranate fruit arils. In the first 4 weeks of storage, medium (40 cm) and high (60 cm) impact bruised fruit arils had the highest content of TA, about 25.7 and 39.4 % higher compared to that of low impact and non-bruised (control) fruit, respectively. Increase in TA could be due to the concentration effect of acid content in fruit due to excessive moisture loss in bruise damaged fruit. After 12-week storage, the TA of fruit arils declined from peak values of 0.99, 1.10 and 1.30 % that were reached on the fourth week, to 0.52, 0.29 and 0.86 % for low, medium and high impact bruised fruit, respectively (Table 2). The lowest decline in TA (0.82 to 0.461 %) was noticed in non-bruised pomegranate fruit arils. The decline in acid content could be related to higher respiratory activity in bruised fruit during storage where organic acid could be used as a substrate in the process (Montero *et al*. (2009). Changes in the content of total soluble solids (TSS) for bruised and non-bruised pomegranate fruit arils are shown in Table 2. No significant difference ($p = 0.8190$) in TSS was noticed between impact bruising treatments. In contrast, the duration of storage had a significant effect ($p < 0.0001$) on TSS of fruit arils. The highest content of TSS observed after 2 weeks of storage was 19.40, 19.43 and 19.10 ºBrix for arils of low, medium and high impact bruised, respectively. Afterward, the TSS of both bruised and non-bruised fruit declined gradually until the end of 12-week storage (Table 2). Overall, bruise damage could lead to the concentration effect of sugar contents in fruit due to excessive moisture loss, whereas decline in the later stage of storage could be due to use these contents as substrates during high respiratory activity of stressed bruise damaged fruit (Sanches *et al*., 2008; Montero *et al*., 2009).

The ratio Brix-to-Acid (TSS/TA) ratio was highest in control and low drop impact bruised fruit, in similar fashion to TSS, for the investigated storage duration. The later increased from corresponding initial values of 23.48 and 19.59 to reach peak values of 39.78 and 35.22 after 10 and 12 weeks of storage. Medium and high drop impact treatments significantly ($p < 0.05$) reduced the Brix-to-Acid ratio, being about 40.1 and 49.1 %, respectively lower than that of control fruit. However, this ratio increased gradually with the increase in duration of storage, reaching 21.44 and 18.22 ºBrix from initial values of 13.79 and 14.14 ºBrix, for the former and the later treatments, respectively. These results are attributed to the relatively higher acid content (TA) and corresponding lower soluble solids (TSS) observed during storage as shown in Table 2.

Changes in BrimA as the result of impact bruising and storage duration are presented in Table 2. The effect of impact bruising had no significant ($p = 0.5295$) influence on changes in BrimA. However, the effect of storage duration had significant ($p < 0.0107$) influence on the observed changes in BrimA. After 2 weeks of storage, the highest BrimA (high sugar and low acid content) was observed for non-bruised and low impact bruised fruit. There was a gradual decline in BrimA during the later period of storage, reaching 15.18 and 14.83 from peak values of 17.68 and 17.40, for non-bruised and low impact bruised fruit, respectively. The decline in BrimA in bruised fruit for the majority of storage duration could be attributed to the decrease in sugar contents occurred during storage.

3.3.4. Aril firmness and compression energy

Firmness is one of the important attribute most frequently measured to evaluate the quality of fresh fruit (Moggia *et al*., 2017). A gradual decline in aril firmness was observed in the course of 12- week storage across all impact bruising treatments (Table 1). However, there was no significant difference ($p < 0.05$) in aril firmness between the initial value (before storage) and that of bruised fruit for the first 6 weeks of storage. Subsequently, the firmness of arils from bruised fruit declined progressively until the end of storage, from 194.69 to 155.57N, 191.1 to 160.52 N and 193.85 to 151.93 N for respectively low, medium and high impact bruised fruit. Similarly, the firmness of arils from non-bruised fruit had1.24-fold decline at end of 12 week storage.Similar to the present findings, Li *et al*. (2011) found that after 60 d of storage, the firmness of bruised 'Yali' pears declined by only 4.2 % in comparison to non-bruised fruit On the contrary, there were no significant differences in aril firmness between control and bruised fruit at all drop impact levels ($p > 0.05$). Similarly, the energy required to compress the pomegranate fruit arils changed significantly ($p < 0.05$) during the 12-week storage (Table 1). The compression energy of arils from both non-bruised and bruised pomegranate fruit remained unchanged for the first 6 weeks. Afterward, the aril toughness declined significantly until the end of storage duration. During this period, the compression energy of pomegranate fruit arils declined 207.38 to 164.42 N/mm and 204.46 to 164.31 N/mm for medium and high impact bruised fruit. Similarly, the compression energy of fruit arils from non-bruised and low impact bruised fruit declined from 220.78 to 170.68 N/mm and 219.44, to 169.54 N/mm, respectively.

Overall, the decrease in textural profile (firmness and compression energy) of pomegranate fruit could be ascribed to the loss in cell-wall integrity of pomegranate fruit arils that could occur in the course of storage (Ekrami-Rad *et al*., 2011; Arendse *et al*., 2014). In agreement with these current findings, Arendse *et al*. (2014) reported the decline in energy required to compress arils of pomegranate (cv.Wonderful) fruit with a reduction in aril firmness. In the present study, the impact bruising did not significantly alter the firmness of pomegranate fruit arils, probably due to, partly the degree of bruising (drop impact heights) which localized over a very restricted depth. Additionally, the complex structure of pomegranate fruit could also be the reason for less effect of impact bruising on the investigated mechanical profile of fruit arils. Other studies have shown that bruise damage accelerates fruit softening during storage. Moggia *et al*. (2017) found significant differences between bruised and non-bruised blueberries during 35 d of cold (0 ºC) storage. The authors compared firmness of bruised and non-bruised 'Brigitta' blueberries and revealed that the former appeared to be as high as 14 % softer than the later. Pectin has been associated with the mechanical strength of plant cell walls. Previous research in other fruits have revealed that rapid softening caused by bruise damage has been associated with pectin solubilisation, loss of membrane integrity and increased polygalacturonase activity (Li *et al*., 2011;Buccheri & Cantwell, 2014; Moggia *et al*., 2017).

3.4. Antioxidant properties

3.4.1. Total phenolic content

The influence of impact bruising on total phenolic content (TPC) of pomegranate fruit was not significant ($p > 0.05$) as presented in Fig.6A. However, the content of total phenolics observed in medium and high impact bruised fruit towards the end of storage was high compared to non-bruised or low impact bruised fruit content. Changes in TPC in the course of storage were significantly pronounced in the last 2 weeks of 12-week storage. At the end of 12-week storage, the content of TPC increasedfrom the initial (before storage) content of 70.77 μg GAE/mL to 92.14, 96.08, 109.04, 116.17 μg GAE/mL for respectively non-bruised, low, medium and high impact bruised fruit. Overall, higher TPC observed in pomegranate fruit could be attributed to the concentration effect due to higher moisture loss in fruit (Mphahlele *et al*., 2016). Higher TPC observed at the end of 12 week storage in medium and high impact bruised fruit could be linked to higher concentration effect compared to non-bruised or low impact bruised fruit. Bruise damage modifies the tissue permeability of fruit by creating macro cracks that lead to increased loss of moisture during storage (Martinez-Romero, 2003; Hussein *et al*., 2019). Other findings have established that cell disruption of plant tissue due to mechanical damage provokes de novo synthesis of phenylalanine ammonia-lyase (PAL) that is responsible for biosynthesis of phenolics (Tomas-Barberan & Espin, 2001; Lichanporn *et al*., 2009). However, the present findings indicated that impact bruising did not influence the significant increase in the content of total phenolics in pomegranate fruit. It is, therefore, possible that the level of impact bruising in the present study was not sufficient enough to cause the biosynthesis of phenolics.

3.4.2. Radical scavenging activity

Radical scavenging activity of pomegranate fruit juice in DPPH assay increased in pomegranate fruit with advancement in storage duration. Similarly, increase in the DPPH activity was higher in medium and high impact bruised fruit than in non-bruised and low impact bruised fruit. There were clear differencesbetween radical scavenging activity of bruised (medium and high impact bruised) and control or low impact bruised fruit (Fig. 6A). The increase in the level of radical scavenging activity in medium and high impact bruised fruit was approximately 2-fold the initial (before storage) value at the end of 12 week storage. In the period between 4 to 8 weeks of storage, the difference in RSA between both the medium and high impact bruised fruit and that of control or low impact bruised fruit reached 18.5 – 34.4 %, with the former being significantly higher than the later. Furthermore, the increase in radical scavenging activity with storage duration from week 6 until the end of storage was evident in both impact bruising treatments. This observation in similar to report a previous reported by Mphahlele *et al*. (2016), who observed a 2-fold increase in the radical scavenging activity of pomegranate (cv. Wonderful) fruit after 4 months of storage. Increase in the level of radical scavenging activity of pomegranate fruit juice is often linked to higher polyphenol concentration of the fruit (Viuda-Martos *et al*., 2010). It has been established that some phenolic compounds potentially contribute towards the radical scavenging ability (Minibayeva *et al*., 200; Mphahlele *et al*., 2016). Minibayeva *et al.* (2009) stated that mechanical wounding on plant tissues causes oxidative stress. Thus, the modifications in the levels of antioxidants in pomegranate fruit could also coincide to impact-mediated stress that in part, triggers the changes in metabolite and production antioxidant enzymes subsequently affecting the antioxidant ability of the plant tissue (Minibayeva *et al*., 2009; Li *et al*., 2010).

3.5. Principal component analysis

Quality attributes corresponding to varying levels of impact bruising were subjected to principal component analysis (PCA). The PCA was carried out to establish a clear relationship and variability between measured pomegranate fruit attributes as affected by varying levels of impact bruising and storage duration. Fifteen factors (F1–F15) described the total variability of the original variance in the data set of physico-chemical and phytochemical attributes. However, with respect to eigenvalues ≥1, the first two principal factors (F1 = 59.18 %, and F2 = 20.02 %) explained 79.20 % of this variability (Fig. 7A), hence indicating that acceptable conclusion on existing variability among measured attributes at different impact bruising and storage duration is explained by first factor (F1) (Fig. 7A and B). Evident observations on the PCA (Fig. 6A) showed that total phenolic content (TPC), radical scavenging activity, colour redness (a*), colour intensity (chroma, C*), fruit decay and internal decayhad positive correlations that corresponded to high positive scores (r ≥ 0.76) along F1. Based on the factor loadings (Fig. 6 B and Table 3A), these scores corresponded to high impact bruising and maximum 12-week storage (12W_HI). Negative scores observed along F1 (Fig. 6A and B) corresponded to TSS, BrimA, toughness, hardness, colour lightness and hue angle (hº) with the negative association to the medium or high impact bruising. Further significant relationships between the indices on PCA were evidenced by close proximities between them (Fig. 6A and B). Observed distance on the current PCA between TPC and radical scavenging activity, a* and C*, and between TSS and BrimA suggested a significant contribution of each individual index on the other (Fawoleand Opara, 2013c). Overall, it is evident based on the PCA that pomegranate fruit subjected at medium to high impact bruising and cold (5 ºC) stored for 12 weeks are characterised by a significant increase in weight loss as well as changes both in physicochemical and antioxidant properties.

4. Conclusion

This study provided scientific evidence that over the course of storage duration, impact bruise damage caused by dropping of pomegranate fruit had important direct effects on several quality attributes mainly fruit colour, weight loss, and respiratory activity. Excessive weight loss in bruised pomegranate fruit reached about 40 % during cold storage. These findings inform the pomegranate fruit industry of how much economic losses that could be incurred due to bruise damage problem. Additionally, medium and high impact bruising induced perceptible colourchanges more on the fruit peel than arils. The former is the key observation due to its effect on perceived visual quality of pomegranate fruit fresh market. Similarly, there was a significant reduction in chemical quality attributes; TSS, acidity, Brix-to-acid ratio and BrimA of pomegranate juice of medium or higher drop impact bruised fruit. The effect of impact bruising on the aforementioned chemical quality attributes is worthy taking into account, due to its consequence on the potential modification of the pomegranate fruit taste. Nonetheless, elucidation of the effect of drop impact bruising on sensory quality and changes of volatile organic compounds of pomegranate fruit requires further research. In addition, significantly higher incidences of decay and internal fruit decay were observed at medium to high drop impact levels. Changes in total phenolic content and the radical scavenging activity were induced mainly by the concentration effect due to increased moisture loss over the course of storage. The Principal component analysis (PCA) has successfully established clear relationships between impact bruising and subsequent changes in physiological responses, physico-chemicalattributes as well in the antioxidant properties of pomegranate fruit stored at 5 ± 0.5 ºC for 12 weeks. It is generally evident that subjecting pomegranate fruit to as high as 40 cm drop impact bruising could influence changes in overall fruit quality. Hence, given the high incidences of postharvest handling damages associated with mechanised harvesting, transporting and packhouse operations, it is important that harvesters and fruit packers limit exposure of pomegranates to impact levels below 40 cm drop impacts, during harvest and postharvest activities and operations.

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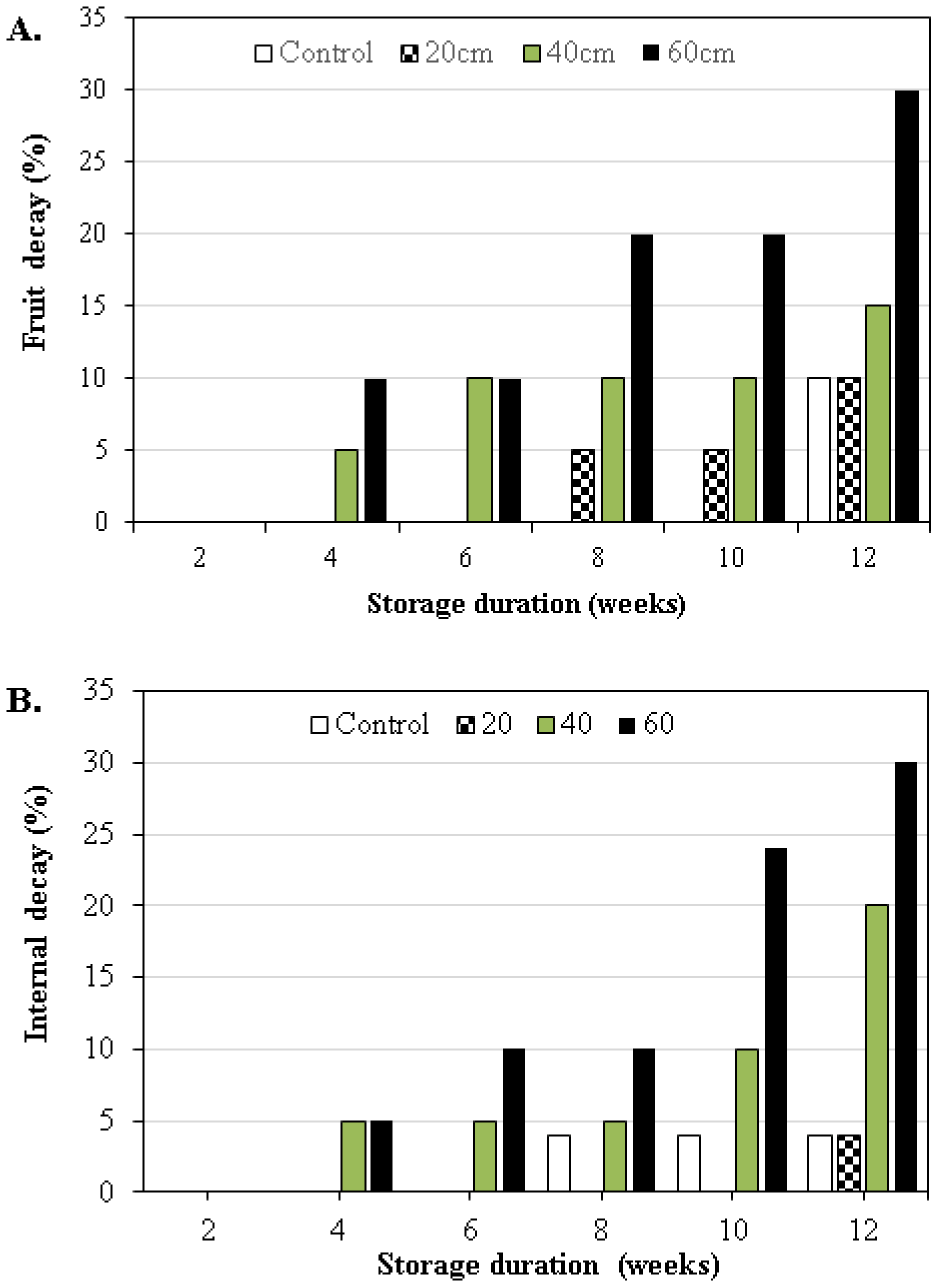


Fig. 1 Influence of impact bruising on fruit decay (A), and internal decay (B) of pomegranate (cv. Wonderful) fruit during storage at 5 ± 0.5 ºC. Control, 20, 40 and 60 cm represent non-bruised, low, medium and high drop impacts, respectively.

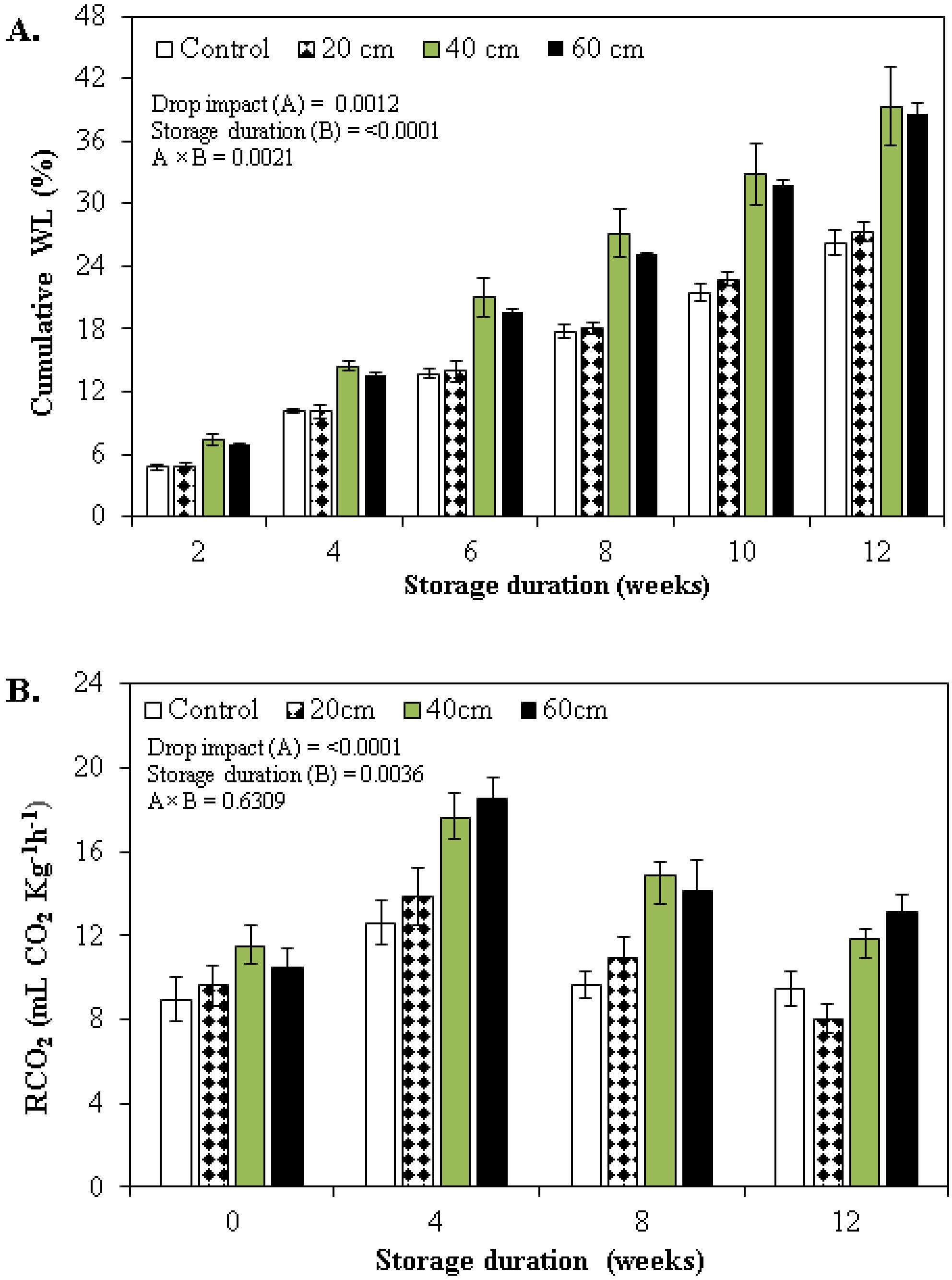


Fig. 2 Cumulative weight loss (WL) (A), and respiration rate (RCO_2) of control (non-bruised), low (20 cm), medium (40 cm) and high (60 cm) drop impact bruised pomegranate (cv. Wonderful) fruit during 12 weeks of cold (5 ± 0.5 ºC) storage. Error bars represent standard error (SE) of the mean values ± S.E. of five measurements.

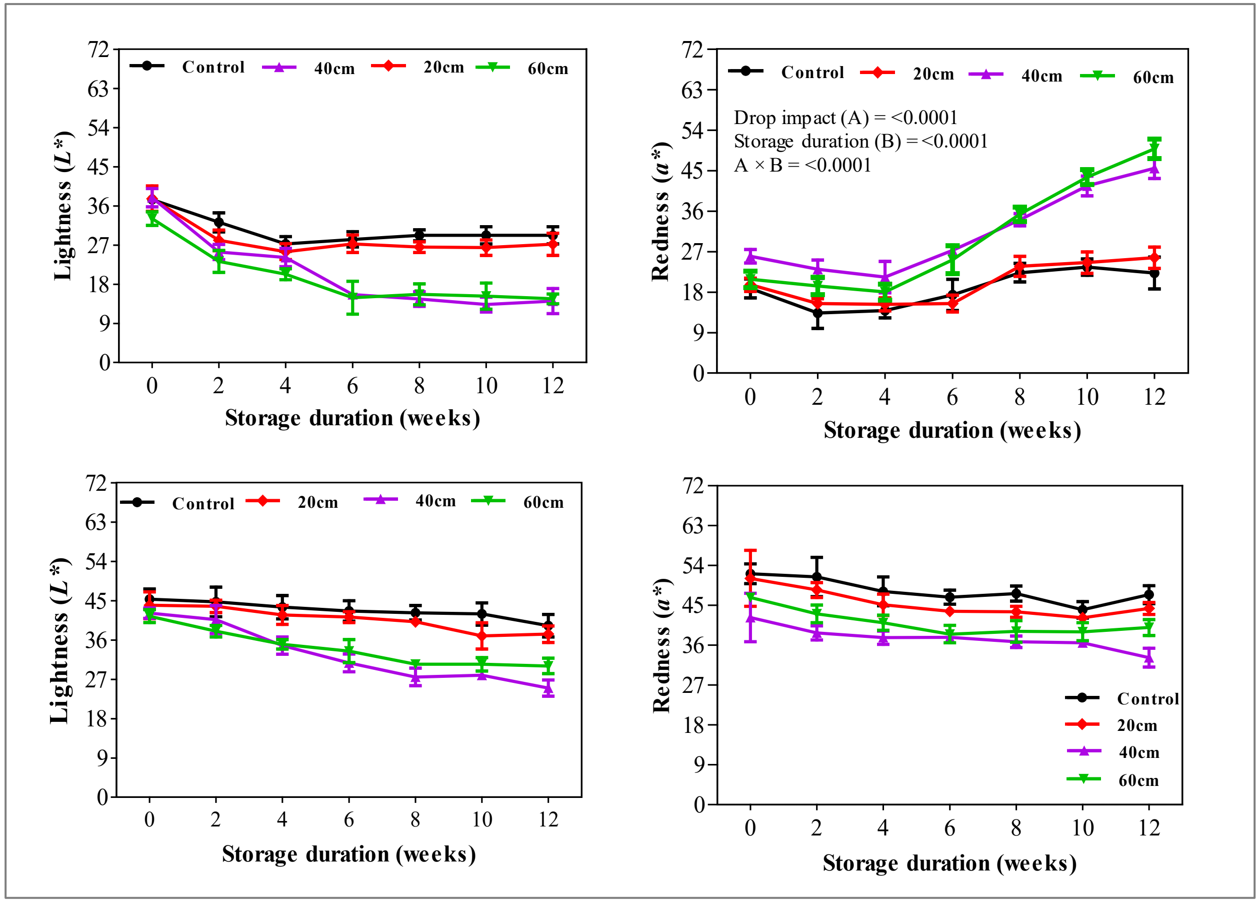


Fig. 3 Changes colour attributes of impact bruised pomegranate (cv. Wonderful) fruit and arils. Fruit were subjected to low (20 cm), medium (40 cm) and high (60 cm) drop impact bruising and stored at 5 ± 0.5 ºC for 12 weeks. Non-bruised pomegranate fruit included as control. Peel lightness (A), aril lightness (B), peel redness (C), and aril redness (D). Error bars represent standard error (SE) of the mean values ± S.E. of five replicates.

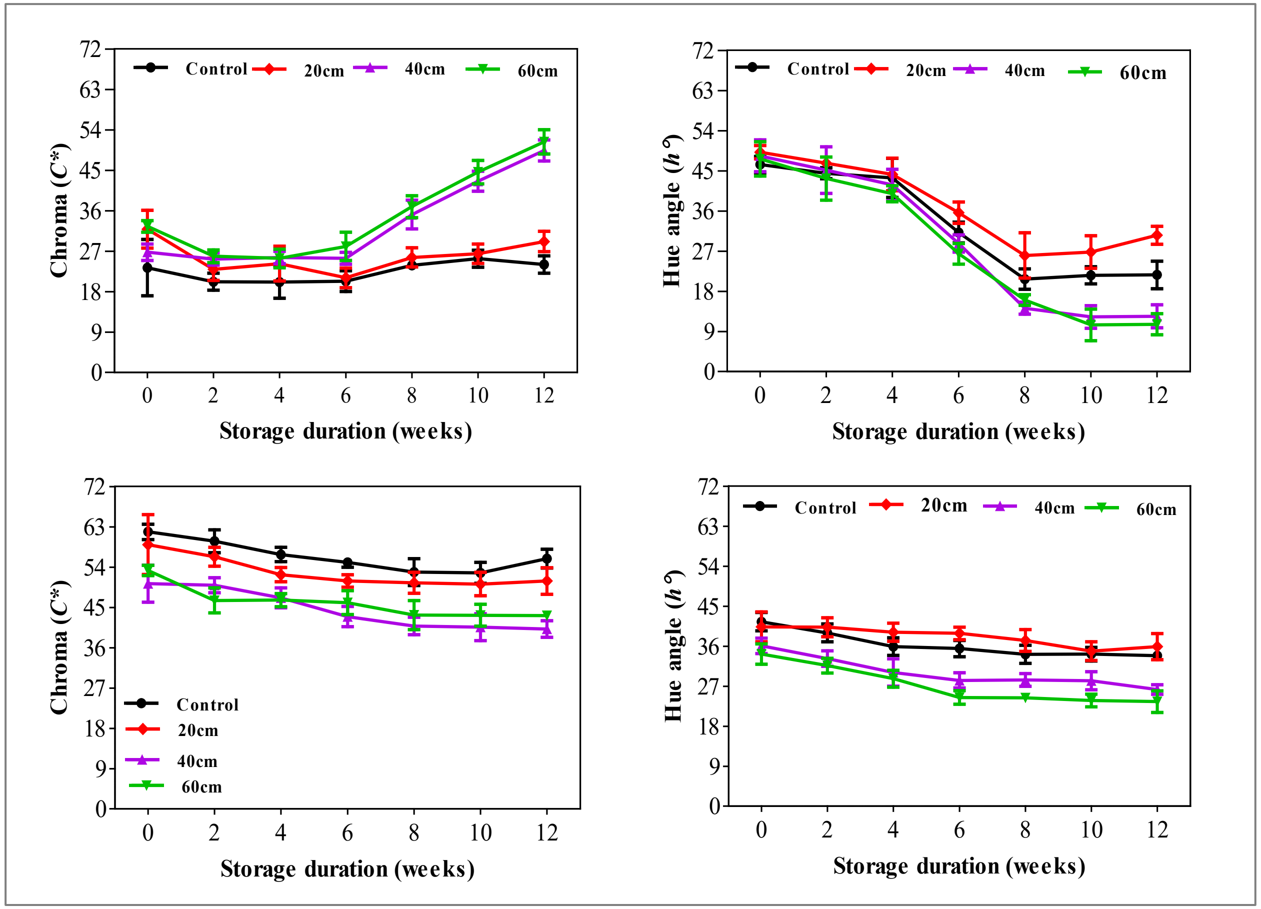


Fig. 4 Changes in the colour intensity and purity of whole fruit and arils for bruised pomegranate (cv. Wonderful). Fruit were subjected to low (20 cm), medium (40 cm) and high (60 cm) drop impact bruising and stored at 5 ± 0.5 ºC for 12 weeks: Non-bruised pomegranate fruit included as a control. Peel chroma (A), aril chroma (B), peel hue angle (C), and aril hue angle (D). Error bars represent standard error (SE) of the mean.

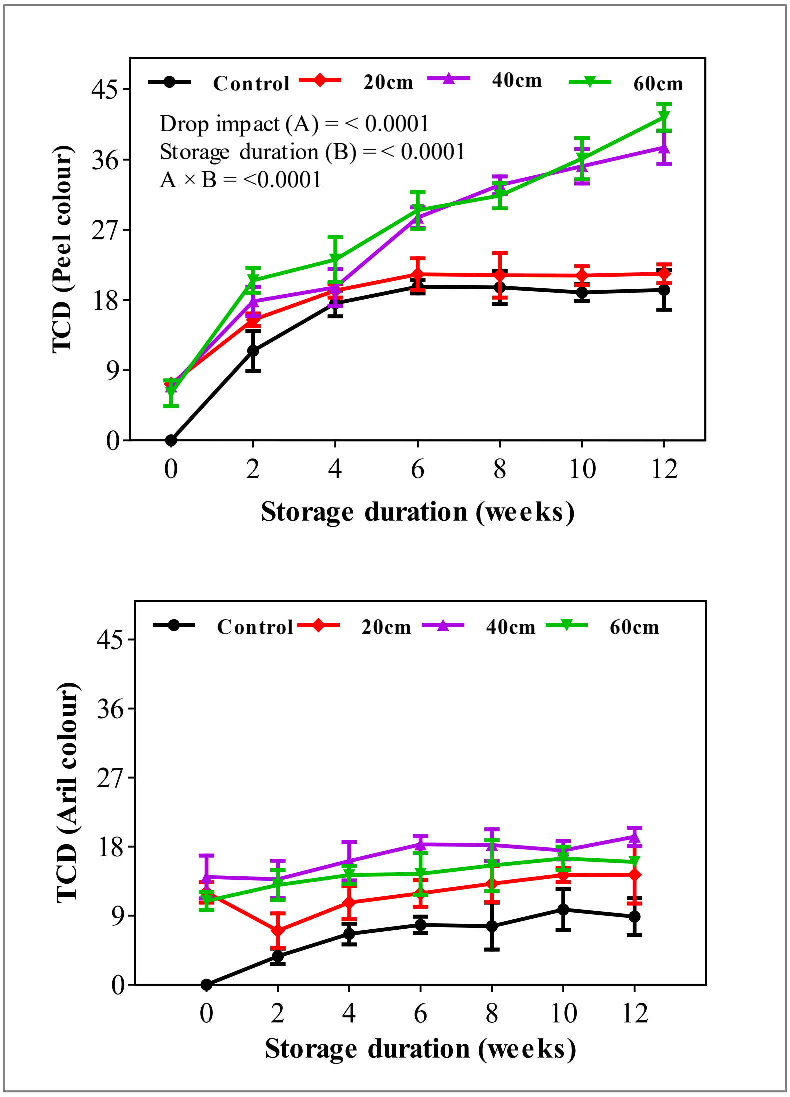
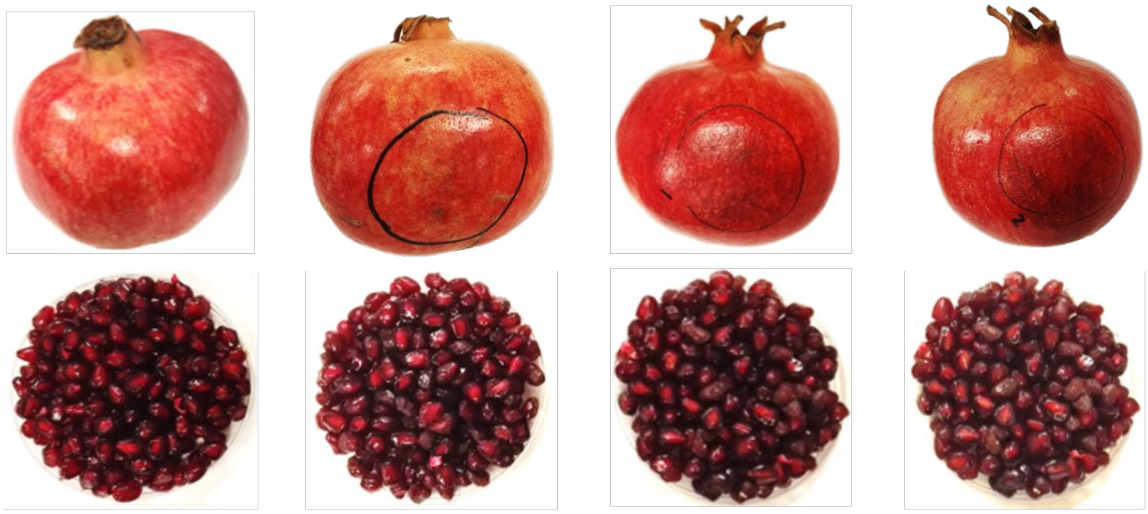


Fig. 5 Total colour difference (TCD) for the fruit peel (A) and arils (B) of pomegranate (cv.Wonderful) fruit bruised at low (20 cm), medium (40 cm) and high (60 cm) drop impacts and stored at 5 ± 0.5 ºC for 3 months. Non-bruised fruit is included as control. Error bars represent standard error (SE) of the mean values ± S.E. of five replicates.



(A) **(B)** **(C)** **(D)**

Fig. 6 Images of pomegranate cv. Wonderful fruit and arils displaying changes in colour after being submitted to various level of impact bruising and stored for three months at 5 ºC. A, B, C and D are pair of whole fruit and arils from non-bruised (control), low (20 cm), medium (40 cm) and high (60 cm) drop impact bruising, respectively. Images of whole fruit were taken on the bruise-damaged side of the fruit, whereas those of fruit arils were taken from arils obtained on the bruise-damaged site of the frui

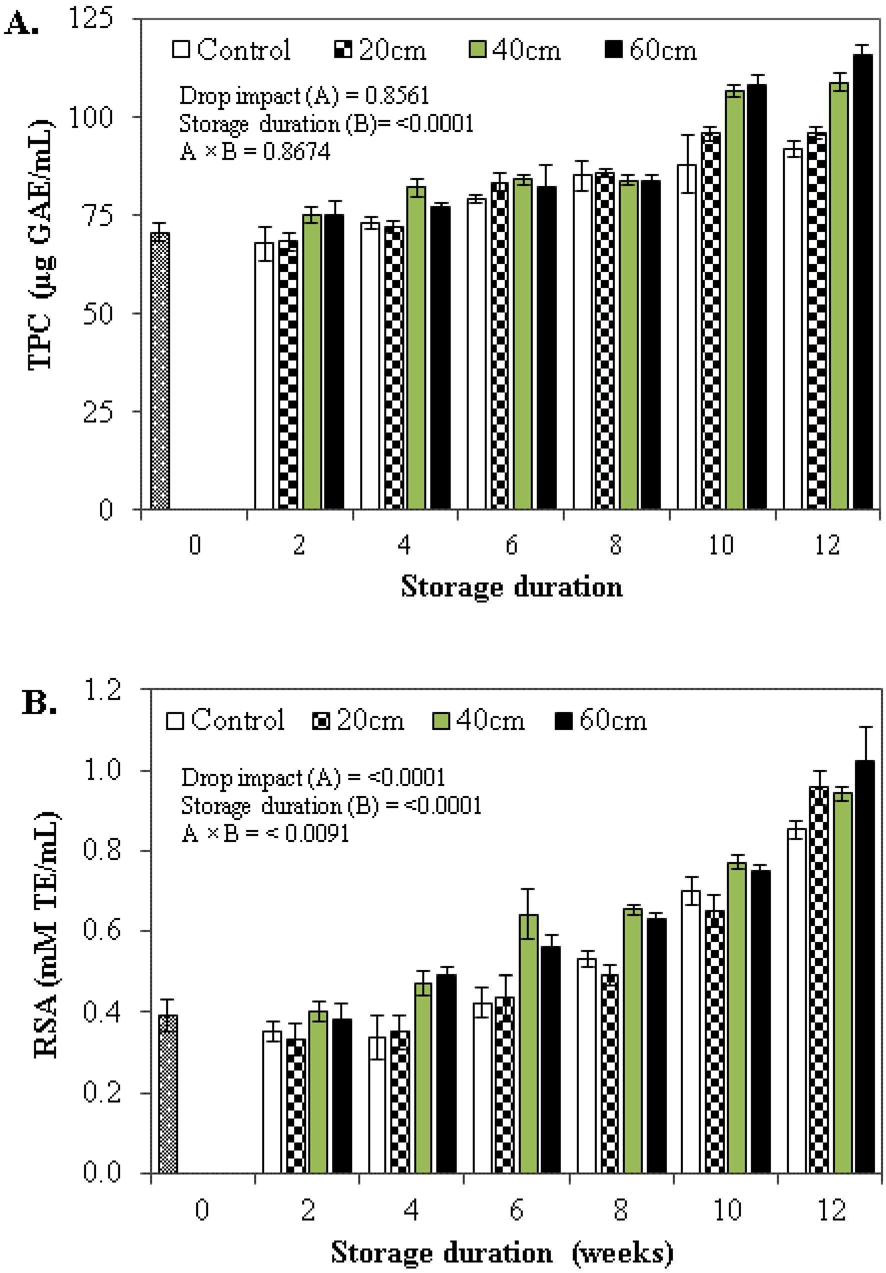


Fig. 7 Changes in total phenolics content (A),and radical scavenging activity of DPPH (B) or pomegranate (cv. Wonderful) fruit submitted at low (20 cm), medium (40 cm) and high (60 cm) drop impacts and stored at 5 ± 0.5 ºC for 3 months. Non-bruised fruit is included as control. Error bars represent standard error (SE) of the mean values ± S.E. of five replicates.

Table 1 Textural profile of pomegranate (cv.Wonderful) fruit arils after whole fruit drop impact bruising and cold (5 °C) stored for 12 weeks

Textural properties	**Impact (cm)**	**Storage duration (weeks)**						
		0	2	4	6	8	10	12
Firmness (N)	Control	211.11 ± 7.21	207.01 ± 3.59^{a-c}	208.15 ± 1.65^{a}	204.82 ± 9.64^{ab}	197.28 ± 2.63^{a-c}	180.47 ± 4.17^{d-f}	162.21 ± 3.28^{he}
	20		181.63 ± 1.96^{d-e}	188.85 ± 4.21^{ad}	194.69 ± 3.13^{a-c}	194.22 ± 13.74^{ad}	179.01 ± 9.17^{d-f}	155.57 ± 4.01^{hg}
	40		185.54 ± 3.74^{db}	192.24 ± 1.08^{ad}	191.1 ± 3.06^{ad}	185.62 ± 11.60^{db}	172.47 ± 6.50^{d-g}	160.52 ± 3.20^{hf}
	60		187.69 ± 3.40^{ad}	189.26 ± 3.46^{ad}	193.85 ± 3.34^{ad}	184.3 ± 11.42^{db}	179.37 ± 4.99^{d-f}	151.93 ± 4.74^{h}
Compression energy (N/mm)	Control	215.23 ± 2.44	218.71 ± 2.80^{a-c}	220.38 ± 1.18^{a}	220.78 ± 1.83^{ab}	211.64 ± 4.17^{ad}	200.41 ± 4.38^{d-f}	170.68 ± 4.72^{he}
	20		222.42 ± 7.32^{c-e}	221.04 ± 4.26^{a}	219.44 ± 4.21^{ab}	210.4 ± 9.94^{ad}	197.34 ± 9.47^{d-f}	169.54 ± 3.89^{hg}
	40		206.27 ± 4.66^{db}	206.78 ± 4.46^{ad}	207.38 ± 6.32^{ad}	200.14 ± 3.42^{ad}	184.15 ± 4.56^{d-g}	164.42 ± 3.10^{h}
	60		201.78 ± 3.91^{ad}	202.37 ± 3.77^{ad}	204.46 ± 9.91^{ad}	197.18 ± 4.93^{db}	185.33 ± 3.91^{d-g}	164.31 ± 3.92^{h}

Level of significance

Parameter	**Drop impacts (A)**	**Storage duration (B)**	**A × B**
Firmness (N)	0.5506	<0.0001	<0.0359
Toughness (N/mm)	0.2134	<0.0001	<0.0278

Mean values are presented as mean ± SE. Means presented in the same column with different letters indicate significant differences between impacts ($p < 0.05$). Means presented in the same row with different letters indicate significant differences between storage duration ($p < 0.05$), according to Duncan's multiple range test.

Table 2 Influence of impact bruising on chemical quality attributes of pomegranate (cv. Wonderful) fruit submitted to impact drops and kept in cold (5 °C) storage for 12 weeks.

Chemical properties	Drop impact (cm)	Storage duration (weeks)						
		0	2	4	6	8	10	12
TA	0	0.64 ± 0.02	0.82 ± 0.02^{ac}	0.80 ± 0.01^{ab}	0.62 ± 0.25^{ac}	0.64 ± 0.28^{ac}	0.54 ± 0.22^{cb}	0.46 ± 0.05^{c}
(% citric acid)	20		0.80 ± 0.32^{a}	0.99 ± 0.33^{ab}	0.80 ± 0.04^{ab}	0.85 ± 0.05^{ab}	0.93 ± 0.33^{cb}	0.52 ± 0.06^{cb}
	40		1.31 ± 0.04^{a}	1.10 ± 0.08^{ab}	0.80 ± 0.45^{cb}	0.71 ± 0.36^{cb}	0.65 ± 0.03^{cb}	0.29 ± 0.01^{cb}
	60		1.28 ± 0.04^{a}	1.30 ± 0.11^{ab}	1.22 ± 0.16^{ac}	0.92 ± 0.32^{ac}	0.92 ± 0.12^{cb}	0.86 ± 0.05^{d}
TSS (°Brix)	0	17.47 ± 1.59	17.13 ± 1.62^{b}	17.63 ± 0.97^{b}	17.20 ± 0.23^{ab}	17.20 ± 0.35^{ab}	15.63 ± 0.96^{ab}	16.10 ± 0.29^{ab}
	20		19.40 ± 1.25^{ab}	17.30 ± 0.06^{b}	17.43 ± 0.68^{b}	17.27± 0.68^{b}	16.23 ± 0.12^{b}	15.73 ± 0.24^{ab}
	40		19.43 ± 1.12^{b}	17.77 ± 0.52^{b}	16.30 ± 0.64^{ab}	16.03 ± 0.87^{ab}	16.13 ± 1.21^{b}	15.83 ± 0.76^{a}
	60		19.10 ± 1.65^{b}	18.40 ± 0.31^{b}	17.07 ± 0.78^{b}	17.17 ± 0.26^{b}	16.27 ± 0.46^{bAC}	14.47 ± 0.09^{b}
TSS:TA	0	27.14 ± 2.11	20.76 ± 1.55^{ac}	21.98 ± 1.44^{ab}	26.20 ± 6.43^{ab}	36.40 ± 5.15^{ab}	36.96 ± 9.8^{cba}	35.79 ± 3.85^{ac}
	20		37.41 ± 8.20^{a}	24.58 ± 7.01^{ac}	21.86 ± 1.11^{ab}	20.52 ± 1.84^{ac}	28.17 ± 9.21^{cb}	31.04 ± 3.49^{cb}
	40		14.80 ± 0.73^{ab}	16.42 ± 1.81^{ac}	18.04 ± 7.15^{cb}	33.65 ± 9.93^{cb}	24.48 ± 0.90^{cb}	45.43 ± 3.94^{ac}
	60		14.92 ± 1.40^{ab}	14.37 ± 1.48^{ab}	14.47 ± 2.08^{cb}	27.93 ± 9.72^{ac}	18.26 ± 2.55^{cb}	16.94 ± 0.97^{c}
BrimA	0	16.18 ± 1.56	15.49 ± 1.58^{ac}	16.03 ± 0.98^{ab}	15.97 ± 0.55^{ab}	15.91 ± 0.77^{ab}	14.55 ± 0.53^{cb}	15.18 ± 0.34^{ac}
	20		17.81 ± 1.79^{a}	15.33 ± 0.61^{ac}	15.83 ± 0.66^{ab}	15.57 ± 0.77^{ac}	14.37 ± 0.73^{cb}	14.69 ± 0.22^{cb}
	40		16.81 ± 1.07^{ab}	15.57 ± 0.68^{ac}	14.71 ± 0.58^{cb}	14.61 ± 0.40^{cb}	14.73 ± 1.16^{cb}	15.26 ± 0.77^{ac}
	60		16.53 ± 1.67^{ab}	15.79 ± 0.46^{ab}	14.62 ± 0.96^{cb}	15.33 ± 0.63^{ac}	14.42 ± 0.58^{cb}	12.75 ± 0.02^{c}

Level of significance

Parameter	Drop impact (A)	Storage duration (B)	A × B
TA (% citric acid)	<0.0063	<0.0070	0.9338
TSS (°Brix)	0.8190	<0.0001	0.7360
TSS:TA	<0.0241	<0.1165	0.3069
BrimA	0.5295	<0.0107	0.7915

All values are presented as mean ± SE. Means presented in the same column with different letters indicate significant differences between drop impacts ($p < 0.05$). Means presented in the same row with different letters indicate significant differences between storage duration ($p<0.05$), according to Duncan's multiple range test.

Table 3 Factor scores, loadings, eigenvalues and cumulative variance (%) for the first two Principal component factors (F1 – F2) based on physico and phytochemical quality attributes of pomegranate fruit cv. Wonderful subjected to different drop impacts and stored for 12 weeks

(A)			**(B)**	**Factor scores**	
Loadings	**F1**	**F2**	**Observation**	**F1**	**F2**
2W_C	-3.456	-0.454	RSA	0.927	-0.251
2W_LI	-3.796	-0.745	TPC	0.939	-0.103
2W_MI	-2.822	2.554	TA	-0.376	0.853
2W_HI	-2.656	2.311	TSS	-0.790	0.396
4W_C	-3.336	0.071	TSS:TA	0.194	-0.820
4W_LI	-2.611	0.163	BrimA	-0.778	0.026
4W_MI	-1.458	1.888	C*	0.922	0.203
4W_HI	-1.519	2.539	h^0	-0.877	0.196
6W_C	-2.578	-1.610	L*	-0.701	-0.508
6W_LI	-2.455	-0.864	a*	0.950	0.154
6W_MI	0.353	0.951	Firmness	-0.775	0.177
6W_HI	0.214	2.271	Toughness	-0.880	0.099
8W_C	-1.227	-1996	WL	0.286	0.817
8W_LI	-1.214	-0.472	Fruit decay	0.821	0.375
8W_MI	1.545	-0.468	Internal decay	0.765	0.400
8W_HI	1.798	0.898			
10W_C	0.242	-3.067			
10W_LI	0.222	-1.199			
10W_MI	3.737	0.101			
10W_HI	4.647	2.247			
12W_C	1.478	-2.731			
12W_LI	1.938	-2.539			
12W_MI	5.396	-1.496			
12W_HI	7.559	1.646			
Eigenvalue	8.878	20.015			
Variability (%)	59.184	3.002			
Cumulative %	59.184	79.199			

(A) Loadings: 2W, 4W, 6W, 8W, 10W and 12W = respective number of storage weeks, C = control (non-dropped), LI = low impact (20cm), MI = medium impact (40cm), HI = high impact (60cm).

(B) Observation: RSA = radical scavenging activity, TPC = total phenolics content, TA = titratable acidity, TSS = total soluble solids, TSS:TA = Brix-to-acid ratio, C* = chroma, h^0 = hue angle, L* = lightness, a* = redness, WL = weight loss.

CHAPTER 9

GENERAL DISCUSSION AND CONCLUSION

General discussion and conclusion

1. Introduction

Pomegranate (*Punica granatum* L.) fruit is subjected to bruising, a major form of mechanical damage due to the action of various forces during postharvest handling (Shafie *et al.,* 2015; 2017). Bruise damage of fruit could potentially occur from harvest and all along the postharvest handling chain (Ahmadi *et al.,* 2010; Ghaffari *et al.,* 2015: Shafie *et al*., 2017).Bruise damage is not only one of the most decisive factors determining the external quality and marketability of fresh fruit, but also limits the successful mechanization and automation of harvesting and postharvest handling operations.

An in-depth understanding of bruising susceptibility of pomegranate fruit and the effects of bruising on the fruit quality could be useful for the prospects of the South African pomegranate industry. The aim of the present study was two-fold. Firstly, it attempted to investigate the bruise threshold and bruise damage susceptibility of selected pomegranate fruit cultivars grown in South Africa, and then to detect and characterize the bruise damage using both destructive and non-destructive methods. Secondly, the study explored the impact of bruise damage on the overall fruit quality and antioxidant content of pomegranate fruit during long term storage.

This dissertation was structured into eight chapters as discussed below.

2. General discussion

Literature review in **chapter 2** (published in Scientia Horticulturae) provides a detailed overview of preharvest factors that potentially influence bruise damage susceptibility with reference on various fresh fruits. Focusing on preharvest factors, the review has clearly enlightened that higher degree of fruit bruising occurs at harvest and during postharvest handling (i.e during grading, packing, and transportation/distribution). However, during fruit development, a wide range of factors modulate fruit response to various mechanical loading at harvest and/ or during postharvest handling. Such factors as cultivar and/or rootstock (Stropek & Golacki, 2013; Jiménez *et al*., 2016) and climatic conditions during the growing season (Tahir *et al*., 2009; Lv *et al*., 2016) potentially influence the severity and incidence of fruit bruising. Additionally, the review has revealed the potential role of proper orchard management practices both in improving fruit yield, controlling fruit maturity and quality, as well as modulation of fruit resistance to mechanical bruising (Opara, 2007; Eckhoff *et al*., 2009).Therefore, in an attempt to reduce the problem of fruit bruising and the associated economic losses after harvest, the review highlighted the need for orchardists to be informed about and control factors influencing fruit susceptibility to bruising. Control of these factors would influence the change in composition and structure and modify the mechanical strength of fruit.

The review in **chapter 3** has revealed that bruising is also dependenton a number of harvest factors such as harvest methods, produce maturity, ripening, harvest time (during the day or season) and time-lapse after harvest (Abbott *et al*., 2009; Hu *et al*., 2017). In addition, postharvest factors comprising of environmental conditions such as temperature, humidity, and several other postharvest treatments have the potential to alter the produce physiological and biochemical properties that influence the fruit sensitivity to bruise damage (Gunes *et al*., 2002; Van Zeebroeck, 2006; Ferreira *et al*., 2009). In combination, both literature reviews have established a clear understanding of the relationships amongst preharvest, harvest and postharvest, and how they influence fruit susceptibility to bruise. The reviews further offer a suitable approach to reduce the high incidence of postharvest and economic losses of fresh fruits resulting from bruise damage. At preharvest stage, the correct application of orchard management practices such as choice of fertilization formulas, irrigation schedules and pruning routines based on specific plant requirement has the potential to reduce the fruit susceptibility to bruising. Furthermore, appropriate and consistent temperature and humidity control after harvest, and use of postharvest treatments alone or in combination with other storage methods could be suitable operating strategies to reduce incidences and severity of bruise damage.

Literature evidence demonstrates that bruise damage susceptibility of fresh fruit is linked to the fruit intrinsic elements such as physiological and biological makeup of the fruit, that characterize the existing differences between cultivar and/ genetic (species/genotype) (Mohsenin, 1986; Strehmel *et al.*, 2010). Therefore, the objective of the study reported in **chapter 4** was to explore the extent to which bruise susceptibility of pomegranate fruit is dependent on drop impacts, cultivars, storage condition and duration, and to determine the minimum impact energy level which could result to bruise damage during handling of pomegranate fruit. Impact bruising at minimal drop heights (0.1, 0.15, 0.2 m) revealed 'Wonderful' had the lowest impact threshold (equivalent drop height, EDH = 0.1 m; 371.87 mJ) at which bruising occurred which indicates the highest susceptibility to bruising compared to 'Acco' (EDH = 0.15 m; 406.26 mJ) and 'Herskawitz' (EDH = 0.15 m; 511.57 mJ). Similarly, bruise damage susceptibility i.e. bruise volume (BV, mm^3) and bruise area (BA, mm^2) at impact levels above threshold i.e. low (20 cm), medium (40 cm) and high (60 cm) was also cultivar dependent in the order of 'Wonderful' > 'Herskawitz' > 'Acco'. These findings highlight that the differences in susceptibility to impact bruising were attributed to differences in the cultivars' mechanical and physico-morphological attributes such as firmness, peel thickness and fruit's radius of curvature (Shafie *at al*., 2015; Hussein *et al*., 2018). The present results identified 'Wonderful' as the fruit cultivar that requires additional care during harvest and postharvest handling due to its critically lower bruise threshold. Nonetheless, careful handling of other investigated pomegranate fruit cultivars is equally highly recommended to reduce bruise damage.

Further findings indicated that bruise size of cold (5 ºC) conditioned pomegranate fruit was significantly higher than that of fruit at ambient (20 ºC) condition. This demonstrates the importance of improving handling practices of pomegranate fruit in cold chain to reduce incidence and severity of bruising. Additionally, the study has shown that drop impact bruising had a larger effect on the fruit physiological response (respiration rate and weight loss) for bruised fruitin comparison to non-bruised fruit, even during short term storage (10 d). Fruit impacted at higher drop impact levels (0.4 or 0.6 m) exhibited2 to 3- fold higher respiration rate than fruit bruised at lower impact level (0.2 m) or non-bruised fruit. Respiration rate and weight loss increased with prolonged storage period and temperature, both in bruised and non-bruised fruit.

The presence of bruise damage on pomegranate fruit is hardly detectable by flattening, softening or even by visual appearance. The signs of bruising may be apparent on damaged fruit until at a later stage in the handling chain. Hence, the availability of accurate and cost-effective non-destructive assessment method both for the field and laboratory measurement could be a solution to maintain the quality of fruit for fresh market by preventing additional economic losses that may arise from down-grading of exported fruit (with latent or hiden bruises) in the international markets. In addition, availability of non-destructive assessment methodfor in-line sorting could assist in reducing further losses by sorting out bruised fruit which are predisposed to other disorders such as decay. Packing of decayed fruit could affect healthy fruit, and/ or contaminate the whole batch of fruit and hence compromise quality of exported fruit.

The study in **chapter 5** explored the potential of application X-ray micro computed tomography (X-ray μ CT) in detection and characterization of pomegranate fruit bruising. The results of visual assessment of two-dimensional (2D) X-ray μCT images revealed that fresh bruises (i.e. immediately bruised fruit) and fruit scanned 48 h, 3 d and 5 d after bruising showed no evidence of bruise damage. However, 2D images of pomegranate fruit scanned 7 d days after impact bruising showed a promising evidence of bruise damage, characterized by relatively darker region of the fruit image. The conclusion was made that X-ray μCT is not a suitable non-destructive method for early detection of fresh or imediately bruised fruit damage on pomegranate fruit. Quantitate results of μCT analysis that were based on changes of fruit density and corresponding gray values supported visual assessment outcomes. The quantitative data obtained in this study could be a starting point for future research to develop algorithms suitable for detection of fresh bruises on pomegranate fruit.

Chapter 6 presents findings on the polyphenol oxidase (PPO) enzyme activity of bruise damaged pomegranate fruit and its association with both the impact bruising and browning potential of the fruit. Peel electrolyte leakage, an important chemical indicator of membrane integrity of an impact bruised plant tissue was also assessed. Furthermore, microstructural changes in the fruit peel and accumulation of reaction oxygen species (ROS) as a result of impact bruising was examined and quantified. The effects of impact bruising (at 20, 40 and 60 cm drop heights) on the physical, biochemical and cellular microstructural changes of 'Wonderful' pomegranates were investigated as reported in **chapter 7**.The results of this study showed that physical and biochemical changes such as colour browning, peel electrolyte leakage (PEL), polyphenol oxidase (PPO) enzyme activity and accumulation of reaction oxygen species (ROS) measured in pomegranate fruit peel were dependent on drop impact bruising. This study demonstrated that bruising affected the membrane integrity of fruit skin cells and defence system, hence triggering production of high ROS and increased PEL (Zhou *et al*., 2007; Bugaud *et al*., 2014). Additionally, the influence of temperature had a significant effect on the measured physical and biochemical changes such as browning and PPO activity which was higher in ambient (20 ºC) than cold (5 ºC) conditioned fruit. Fruit browning due to impact bruising was influenced by the level of drop impact and increased at higher storage temperature and prolonged fruit incubation time. However, micrographs of scanning electron microscope showed that differences in cellular microstructural changes between bruised and non-bruised fruit tissues were visible both after 4 and 48 h of drop impact. These results proved the hypothesis that surface browning on bruise-damaged region of the fruit lag changes occurring inside the damaged inner tissues (Samim & Banks, 1993).Furthermore, this study contributes to the understanding of physical and biochemical changes characterizing putative indicators of pomegranate fruit bruising.

Evidence from literature has shown that bruising on fruit modifies physiological and metabolic processes that lead to internal browning and eventually quality losses (Costa *et al*., 2018). Bruise damage causes produce quality deterioration and subsequent economic losses due to decay and microbial spoilage, loss in fresh weight, change in pericarp colour and visual quality (Stropek & Gołacki, 2015; Hussein *et al*., 2018). Limited research has been conducted to evaluate the effect of impact bruising on quality losses and physiological responses of pomegranate fruit.

Developing an in depth understanding of bruise damage susceptibility of pomegranate fruit during storage and handlingis a complimentary approach in developing improved harvesting and handling practices and could also influence the choice of storagemethods as well as transportation systems. The study in **chapter 7** evaluated the bruise susceptibility of pomegranate fruit and textural properties of impact bruised fruit during long-term storage. The findings showed that storage duration significantly ($p < 0.05$) influenced bruise susceptibility of pomegranate fruit.Bruise volume (BV) and bruise area (BA) measured in bruised damaged pomegranate fruit increased with increasing drop heights and duration of cold storage during the first 2 months of storage and then declined in the last month of storage time. Increase in bruise sensitivity of pomegranate fruit was attributed to a reduction in fruit turgor in the course of cold storage (Singh *et al*., 2014; 2015).Lower bruise size observed in fruit bruised before storage indicated that freshly harvested pomegranate fruit would bruise less at higher impact energy absorbed compared to stored fruit. This highlights the importance of timely and careful handling operations of fruit such as sorting, packing and transportation soon after harvest to reduce incidences of bruise damage. Furthermore, excessive moisture loss in fruit stored for more than two months led to hardening of pomegranate fruit peel.This led to increase resistance of fruit to impact bruising (i.e. lower BV and BA) at the end of 3 months cold storage. Similar effects of excessive moisture in bruised fruit were attributed to the observed increase in mechanical properties of bruised pomegranate fruit during storage. Results of textural profile of bruise damaged pomegranate fruit showed that increases in puncture resistance, cutting and compression strength properties were dependent on drop impact bruising and storage duration, especially for the first 8 weeks of storage. From a practical viewpoint, this falls within the fruit export (3-6 weeks) and retail windows (2 weeks), a total timeline of ± 8 weeks. This suggests that bruise damage would result in significant changes in fruit textural attributes and hence arrival of hardened fruit in the export markets. Consequently, such fruit will have low consumer appeal or require higher mechanical energy during processing i.e. fruit cutting and compression for juicing.

The study reported in **chapter 8** investigated the effect of bruising and long-term storage on the physiological response, physico-chemical quality attributes, textural changes and antioxidant content of pomegranate fruit. Weight loss and the respiration rate were highest in medium and high impact bruised fruit. Increase in respiratory activity of bruise-damaged pomegranate fruit was attributed to increased fruit stress due to impact bruising (Scherrer-Montero*et al*., 2011), while weight loss was linked to bruise damage of fruit tissue that modified the permeability of cell wall tissues (Martinez-Romero, 2003). Hence, careful handling of pomegranate fruit to avoid mechanical damages during long-term storage could be a suitable strategy to slow down the physiological responses and improve fruit storability.

The results showed significant increase in the values of chemical quality attributes with storage duration. Total soluble solids (TSS), titratable acidity (TA), Brix-to-acid ratio (TSS:TA) and BrimA for medium and high drop impact bruised were all significantly ($p < 0.05$) higher compared to non-bruised pomegranate fruit. This study revealed that there were increases in total phenolic content and the radical scavenging activity of fruit bruised at medium (40 cm) and high (60 cm) drop heights. The results further revealed that changes in antioxidant content and chemical quality attributes of bruised pomegranate fruit were, in part, due to the concentration effect due to increased moisture loss during the investigated 12-week storage period. Additionally, high drop impact bruising resulted in 30 % of both decay incidence and internal fruit decay after 12-week storage, compared to 10 % decay incidences for non-bruised fruit.Internal fruit decay was 20 and 30 % for medium and high drop impact bruised fruit, respectively, after 12 week storage.The literature evidence of the relationship between bruise damage and fruit decay demonstrates that softening of fruit tissue due to bruise injury stimulate further susceptibility to infection via the open wound and cracks (Fischer *et al*., 2009; Sivakumar *et al*., 2011).

Perceptible changes in pomegranate fruit colour from reddish to browning was observed with prolonged period of storage. Impact bruising resulted in significant changes in aril colour. Additionally, the total colour difference results suggest that changes in colour of the fruit peel during storage, due to impact bruising, were higher than those observed in fruit arils. From a practical point of view, the finding revealed that bruising could indeed affect the visual quality of pomegranate fruit during storage, which potentially could result to downgrading of fruit market value or complete fruit loss

3. General conclusion and future prospects

This study represents the piloted research findings aimed at providing scientific knowledge to broaden the understanding of pomegranate fruit susceptibility to bruising and associated impacts on fruit quality. The coverage of this dissertation extends from the assessment of bruise damage susceptibility and bruise threshold of selected cultivars, to characterization and measurements of bruising using destructive and non-destructive methods. The findings in this dissertation have established the impact threshold for bruising and the fruit cultivar with minimum impact threshold among the studied cultivars Acco, Herskawitz and Wonderful.

The study to explore the application of non-destructive X-ray micro CT in detection of bruise damage in pomegranate fruit was limited by its inability to detect bruises at early stages of development (young bruises). Hence, improvement of X-ray CT to develop algorithms that could exploit the properties of fruit with hard rind to detect and segment bruises is a crucial requirement. In addition, the need for future studies to explore alternative non-invasive techniques such as hyperspectral imaging system for detection of fresh bruises of pomegranate is of utmost importance.

The findings in this dissertation have established that bruise susceptibility of pomegranate fruit is dependent on the level of drop impact, cultivar, storage condition and duration. Furthermore, this study showed that bruising, storage conditions and duration play a crucial role on physiological responses (i.e. respiration rate and weight loss), mechanical properties and chemical quality attributes of whole pomegranate fruit and arils. The impact of bruising on chemical quality attributes observed in this study is noteworthy, due to its potential to alter the characteristic pomegranate fruit taste. However, this study did not establish the impact of bruising on sensory quality and changes of volatile organic compounds of pomegranate fruit and may require further research.

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