# Effect of variety, harvest and storage time, defoliation and nitrogen application on the physical and biochemical properties of potato tubers in relation to bruise susceptibility

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Submitted in accordance with the requirements for the degree of

Doctor of Philosophy

The University of Leeds

School of Food Science and Nutrition

October, 2014

The candidate confirms that the work submitted is her own and that appropriate credit has been given where reference has been made to the work of others.

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# Publications

## List of accepted abstracts

Scharf, R., Watson, N., McManus, C., Orfila, C., Povey, M. J. W. and Ho, P. 2012. *Assessment of potato bruising*. Sutton Bridge Storage Day, 29 November 2012, Sutton Bridge, UK.

Scharf, R., Orfila, C. and Ho, P. 2013. *Extraction of 'free' and 'trapped' phenolics from potato fibre.* Industry day, 14 January 2013, Leeds, UK.

Scharf, R., Orfila, C. and Ho, P. 2013. *Effect of harvest period on the mechanical properties and microstructure of potatoes*. ESPCA/São Paulo School of Advanced Science, Advances in Molecular Structuring of Food Materials, 1-5 April 2013, Pirassununga, Brazil.

Scharf, R. and Orfila, C. 2013. *Cell wall microstructure of potato cortex tissue and correlations with bruise susceptibility upon harvest and storage*. The XIIIth Cell Wall Meeting, 7-12 July 2013, Nantes, France.

Scharf, R. 2013. *The relationship between bruising index, tuber deformability and composition of phenolic acids*. AHDB Crop Research Conference, 25 September 2013, London, UK.

Scharf, R., Ho, P., Maycock, J. and Orfila, C. 2014. *The effect of variety, defoliation, application of nitrogen, harvest and storage on bruising incidence of potato tubers*. 50 years of Storage Research, 3 July 2014, Sutton Bridge, UK.

## Acknowledgements

Watson, N., Povey, M., Corona, E, Benedito, J. and Parker, N. 2012. Acoustic microscopy in the food industry. International Symposium on Ultrasound in the Control of Industrial Processes (UCIP 2012). IOP Publishing IOP Conf. Series: Materials Science and Engineering, 42.

## List of papers produced (manuscripts in preparation)

Scharf, R., Ho, P., Maycock, J. and Orfila, C. 2014. Characterization of potato cell wall sugars by HPAEC-PAD: enhancing extraction and analysis. In preparation.

Scharf, R., Ho, P., Maycock, J. and Orfila, C. 2014. Extraction of 'free' and 'trapped' phenolics from potato fibre. In preparation.

Scharf, R., Ho, P., Maycock, J. and Orfila, C. 2014. The effect of variety, harvest and storage on bruising incidence of potato tubers. In preparation.

Scharf, R., Ho, P., Maycock, J. and Orfila, C. 2014. The effect of variety, harvest and defoliation on bruising incidence of potato tubers. In preparation.

Scharf, R., Ho, P., Maycock, J. and Orfila, C. 2014. A comparison of methods to assess bruising of potato tubers. In preparation.

*“The potato is on the frontline in the fight against world hunger and poverty.”*

Jacques Diouf

Director-General FAO from 1994 to 2011

## Acknowledgements

First and foremost, praises and thanks to the Lord, the Almighty, for His showers of blessings throughout my research work to complete the research successfully.

I would like to thank for the help and support of the kind people around me, to only some of whom it is possible to give particular mention here. I deeply acknowledge my supervisors, Dr Caroline Orfila, Dr Peter Ho and Dr Joanne Maycock, for having trusted in my ability to complete this project successfully. I am grateful to Dr Orfila, my main supervisor, for having invited me to start a PhD and for having supported this decision. I thank her for providing the invaluable guidance throughout this research, especially for having taught me how to report my work in an interesting and successful way. I also wish to express my deepest gratitude to Dr Ho, my collaborative supervisor, for having been so supportive, dedicating me his time whenever I needed his advice. Dr Maycock, my third supervisor, for her encouragement and good advice on writing. To my external supervisor, Dr Sue Cowgill, for her support and excellent advice. Undoubtedly, it was a great privilege and honour to work and study under my supervisors guidance.

I would like to thank the British Potato Council and the Yorkshire Agricultural Society for funding, Cambridge University farm, namely Dr Mark Stalham and Sutton Bridge Crop Storage Research, specially Dr Glyn Harper, for performing field and storage trials respectively, and Professor Paul Knox (Centre for Plant Sciences, University of Leeds) for kindly providing monoclonal antibodies. Dr Nik Watson for assistance with the high speed

camera. I thank very much my colleagues and staff of the Food Science and Nutrition School who were present beside me during this experience.

I would like to thank my husband Junior Roberti for his love and unequivocal support throughout, as always, for which my mere expression of thanks likewise does not suffice. My son Pietro and my daughter Julia for being the inspiration of my days. To my family, especially my parents Rogério and Lucia and my brother Luciano, for their personal support and great patience at all times. To the Bridge Street Church family, for their love, prayers and caring. Finally, my thanks go to all the people who have supported me to complete the research work directly or indirectly.

## Abstract

Bruising of potato tubers leads to losses of 20% of the UK annual crop. The relationship between bruising, tuber physical and mechanical properties, and composition of phenolic acids, tyrosine and cell wall monosaccharides was explored in this thesis. Three field trials were undertaken and the varieties Lady Rosetta (LR), Maris Piper (MP) and Russet Burbank (RB) were grown in replicate field plots. Field trial 1 was designed to investigate the effect of harvest time and defoliation; field trial 2 was designed to investigate the effect of harvest and storage time and a third field trial was undertaken to investigate the effect of nitrogen application to soil (in variety LR only).

Bruising was induced using a falling bolt for severe bruising and bruising index assessment. Weight, specific gravity and oxidative potential were also measured. Cortex and skin mechanical properties were measured using a TA.XT2i Texture Analyser. Phenolic acids, tyrosine and cell wall monosaccharides were analysed chemically using HPLC.

The results from the field trials showed that tubers harvested ~ 150 days after planting varied in susceptibility to bruising for MP (11-60%), LR (14-52%) and RB (50-92%). Earlier harvest (98-139 days) showed lower incidence of bruising for MP (0-16.7%) and LR (17-23%) but not always for RB (0-66.7%). Late harvest (180 days) presented high incidence of bruising for all varieties varying from 81-88%.

Short storage periods (until January) did not increase bruising significantly. Long storage periods (March) increased incidence of bruising for all

varieties, and is associated with higher specific gravity, higher tissue deformability and higher phenolic acid and tyrosine levels.

Potato plants defoliated 49 days before harvest showed lower bruising incidence than undefoliated samples, but had significantly ($p<0.05$) lower weight. Application of nitrogen increased weight of tubers and was associated with higher bruising incidence of LR when tubers were harvested later than 92 days after planting.

Tyrosine levels or specific gravity were not always associated with highest bruising incidence. Hot dry conditions during tuber development (observed in field trial 2) was associated with early plant senescence and high tuber bruising incidence.

In conclusion, bruising is affected by agricultural and post-harvest practices, and is determined by a number of physical and biochemical factors that vary between variety. The factors determining bruising seem to be dependent upon variety and the maturity of the tubers at harvest. Understanding these factors will help growers manage their crop to optimize quality and minimize waste.

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# Abbreviations and units

Ara……. *L*-arabinose

CA……..…..caffeic acid

DAD.....diode array detector

Fuc…..…….……...*L*-fucose

Gal……..……..…*D*-galactose

Glc……..…… *D*-glucose

GlcA……..……*D*-glucuronic acid

h.....hour(s)

ha.....hectare

HC.....hydroxycinnamic acids

HPLC……....high pressure liquid chromatography

Man……..…..…*D*-Manose

min.....minutes

mg….....milligram

ml.....millilitre

Rha……...*L*-Rhamnose

R.E.....relative error

R.S.D....relative standard deviation

Rt .....retention time

s .....second

SD .....standard deviation

SE……..…standard error

t.....tonnes

Xyl……..…..……*D*-xylose

U.....enzyme units

µl .....microlitre

µg.....microgram

>.....greater than

<.....less than

# 1 Introduction

## 1.1 Origin and physiology

The potato (*Solanum tuberosum*) was originally cultivated in Peru and was brought to Spain and Portugal in the late 1500s, from where it dispersed to other parts of Europe (Pringle *et al*., 2009). The potato is the fourth most important food crop in the world behind maize, wheat and rice, with over 300 million tonnes produced annually (CIP, 2007).

Potatoes are swollen stolons attached to the stems of potato plants, which act as storage organs. The stolon tips begin to swell as tuber initiation begins. New cells are created through cell division and starch is deposited after conversion from translocate sugars. The tuber periderm cells of skin are formed combining the process of laying down of stacked periderm cells with the deposition of suberin within and under these cells to form a protective barrier against disease and water loss (Pringle *et al*., 2009).

## 1.2 Importance of potato in world economy

The world potato sector is undergoing major changes. Europe, North America and countries of the former Soviet Union were the bigger producers and consumers until the early 1990s. Since then, the expansion of potato production and consumption in Asia, Africa and Latin America, where output rose from less than 30 million tonnes in the early 1960s to more than 165 million tonnes in 2007 (FAO, 2010). There has been a rise of 3.8% per annum in developing countries and 1.8% decrease in production within

industrialized countries, making the world potato production static. In the industrialised countries, 12% of production is exported while in developing countries the quantity is less than 2% of the world production (Pringle *et al*., 2009). Presently, China is the biggest potato producer, followed by India and Russian Federation (FAOSTAT, 2013). However, yields in North America and some European countries are over 40 tons/hectare (ha); even 70 to 80 tons/ha can be produced in experimental plots. The yield in developing countries is less than 20 tons/ha, even less than 10 tons/ha in some countries (Wang, 2008).

Almost half of the world potato supply is consumed in Asia, but its big population means that consumption per person was a modest 24 kg in 2005. The potato eaters are Europeans, 88 kg per capita. Africa and Latin America have the lowest per capita consumption, 14 and 21 kg per capita respectively, but the consumption in these continents is increasing (FAO, 2010). Several factors can be attributed for the expansion of production and consumption of potatoes, as the inherent plasticity of the crop, international training, technical programs, technology transfer, the ecological facility and overarching political-economic transformations in income and trade, especially via the fast-food industry (Kiple and Ornelas, 2000).

The continuous supply to market all year around is provided by strategies that include short storage non-environmentally controlled cellars (1-2 months) to protect crops from insects and animals; storage for 6 to 9 months under ambient-air cooling to prevent sprout growth and to minimize disease development (Pringle *et al*., 2009) and the use of sprout suppressants as hot

fogging with chloropropham (CIPC), maleic hydrazide or ethylene (Briddon, 2006).

In the UK, planting date depends on final marketing, seasonal weather, latitude and longitude. The earliest planting occurs in the south-west region, usually in January/February and the latest in Ireland and Scotland, usually in May/June. Harvest of early potatoes will start in April/May, while main crop and seed crop will span September to November. Late harvest crops tend to have more disease than early harvested crops. However, early harvested crops produce more heat during storage than late crops and require ventilation to prevent subsurface condensation (Pringle *et al*., 2009).

Storage may be a marketing decision based on the rise in potato price over the storage period and to satisfy processors and pack house requirements for a continuous supply of material all year. A typical pattern is found during the season; however it is dependent on the level of supply. From July to end-October, Weekly Average Price (WAPS) show that prices usually decline as supply increases, shown in figure 1.1. If harvesting progresses slowly, sometimes summer prices are steady to early September. A gradual increase on crop price is expected once lifting is complete. Post-Christmas is a period of weaker demand but with increases in prices from March onwards. As the new crop becomes available from May, old crop prices may drop sharply, but if the new crop is late, they may remain strong into the new season (BPC, 2013).

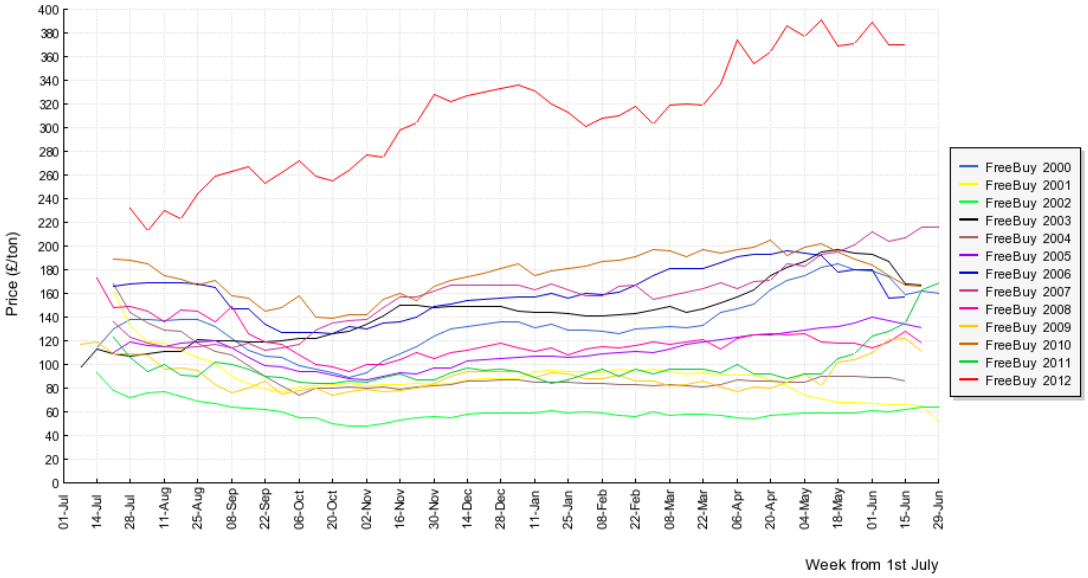



Figure 1.1 Free market weekly average price index 2000-2012 (BPC, 2013).

## 1.3 Quality of potato

The definition of ”quality” can depend on the processing condition and type of end products to be produced, from harvesting to handling, purchase from grower to purchase by final consumer (Pardede, 2005).

In industry, initial sampling of potatoes establishes whether a particular batch of potatoes is suitable for the proposed end market. Tests may assess size distribution, damage index, blackspot bruise, growth deformities, presence of sprouts, pest damage, disease, bloom and potential to rot. Additional tests for the processing market are dry matter and sugar content. For the purposes of this thesis I shall refer to 'blackspot bruise' as simply 'bruising' which is the more widely used term at this time.

Bruising of potato tubers represents a major problem for the potato supply chain, being the biggest single cause of consumer complaints (BPC, 2011).

A 20% loss in production has been reported, costing the industry £26 million in 2004 of the UK annual crop (BPC, 2011). Its avoidance increases production costs (McGarry *et al*., 1996).

## 1.4 Bruising

Bruising can be caused by one or more types of contact, such as: 1) impact: which happens when tubers are hit by a stone, other tubers or dropped on to a hard surface; 2) compression: when exposed to pressure damage from weight of crop above and 3) vibration: which usually occurs during transportation and is difficult to avoid (Ophara and Pathare, 2014).

The impact may be dissipated in different ways due to structural or mechanical properties of the tuber tissue (Peterson and Hall, 1975), and is dependent upon velocity and energy of impact (Skrobacki *et al*., 1989).

The mechanical impact damages the cortex and the medullar cells just beneath the skin, without actually breaking them (Burton, 1989). It is invisible to the inspection staff unless the tuber is sliced open. Bruised tubers are therefore very difficult to remove on a pack house inspection line; so whole crops are often rejected even if only a few bruised tubers are presented.

Previously, Reeve (1968) observed using light microscopy that the cytoplasm became dark and granular in bruised tissue. No obvious structural damage was noted and melanin was identified as forming on the intracellular surfaces of protoplasts and inner cell walls.

Edgell *et al*. (1998) demonstrated that impact results in a loss of intracellular compartmentation followed by an increase in the ribosomal and mitochondrial abundance within the cytoplasm, an increase in density of cytoplasm adjacent to the cell wall and surrounding amyloplasts, and the development of melanin in bruised cells. Indeed, a subcellular redistribution of polyphenol oxidase (tyrosinase, ECl.14.18.1) 12 hours after impact has been demonstrated by Partington *et al*. (1999), which coincides with a loss of membrane integrity and is associated with melanin deposits as the bruise developed.

It is therefore generally accepted that a physical impact disrupts cellular membranes sufficiently so that the enzyme polyphenol oxidase (PPO) localised within plastids (chloroplasts and amyloplasts) comes into contact with phenolic compounds present in the vacuole and start the reactions (Corsini *et al*., 1992, Blessington *et al*., 2010 and Strehmel *et al*., 2010a).

PPO is a copper-containing enzyme that catalyzes two different reactions involving molecular oxygen with various phenolic substrates: the *o*-hydroxylation of monophenols (e.g. tyrosine) to *o*-diphenols by monooxigenase or cresolase activity and the subsequent oxidation of *o*-diphenols to *o*-quinones by diphenolase or catecholase activity. The formation of a heterogeneous group of melanins, the black pigment compounds, is the result of the polymerization of the quinones (Falguera *et al*., 2010).

Polymeric polyphenolic compounds seem to be more toxic to potential phytopathogens than phenolic monomers from which they are derived. The

polyphenol oxidase (PPO) catalysed polymerization helps to seal the injured plant surface and begin the healing process, analogous to the formation of fibrin blood clots in injured humans (Friedman, 1997).

### 1.4.1 Bruising assessment

In order to compare the occurrence of internal damage, bruise quantification can be carried out using destructive manual measurements and subsequent analysis, or using a range of non-destructive techniques.

An impact test method is used to simulate bruising that occurs normally in harvesting and handling operations. Drop tests of bulk samples or individual tubers, where samples are dropped from a known height onto a hard surface, are dependent on the mass, size and shape of the tubers (Baritelle *et al*., 2000). Impact tests consist of holding tubers steady and damaging them with a moving mass such as a guided falling bolt (Corsini *et al*., 1999, Stalham and Allen, 2006) or a pendulum (Noble, 1985). Another impact-based method is shaking tubers in a rotating barrel (Mohsenin, 1986). This is commonly used in the potato industry.

One of the challenges of drop and impact tests is the difficulty of estimating the rebound height, which is needed to calculate the actual impact energy absorbed to cause damage. To address this problem in bruise testing, Opara *et al.* (2007) designed and developed a device using a standard video camera for automatic recording of the rebound height determination during a pendulum test.

Recently, Jiménez-Jiménez *et al*. (2013) designed an impact device to cause controlled and reproducible impact by dropping the olive fruit onto a metal plate and the impact parameters were recorded with a piezoelectric load cell attached to the metal surface. The authors observed that mechanical damage from impact (bruising) in three table olive cultivars was directly related to the impact energy level and the time after impact. Geyer *et al*. (2009) described a similar apparatus equipped with an impact force sensor to record impact force versus time (samples rate of 10,000 Hz) to determine bruising mechanical impact in tubers.

Bruising develops over a period of 1–3 days following mechanical impact (Strehmel *et al*., 2010a), depending on temperature. To accelerate the identification of potential bruising from mechanical damage, samples can be put in to a hot box and kept at high relative humidity (RH) by circulating air over a water bath. The hot box is maintained at a temperature of 34-36°C and RH 95-98% and the bruise will develop within 12-14 hours (h). This procedure is applied at Sutton Bridge Crop Storage Research.

Another way to accelerate the speed of bruise formation is to use a more complex method that consists of storing potatoes under oxygen at high pressure (1.4 kg/cm$^2$). The time required for maximum pigmentation is regularly in the order of 2 h (Duncan, 1973). However, due to the relative simplicity of the hot box, growers and pack house staff routinely use the hot box for rapid bruise detection.

The most sensitive part of the tuber is the vascular region of the potato tuber and the stolon end of the tuber (Adams and Brown, 2007). A classification

system for impact-related defects in potato tubes puts the tuber into one of seven categories: no bruise, bruising, crush, white spot/white knot, internal shatter, external shatter, external cracking. The damage index is the amount of tuber that will be lost to remove the on the damaged area (Baritelle *et al*., 2000). This research focused in bruising and external cracking (skin damage).

The manual primary measurement of bruised tissue depth and volume. To assess the depth of bruise damage, the tubers should be peeled to reveal bruising. The symptoms are usually limited to a zone 5-10 mm of diameter located about 2 mm beneath the surface of the tuber (Burton, 1989). The categories are nil bruising, slight bruising (less than two peelers) and severe bruising (needs more than two peelers to remove) (procedure developed at the Sutton Bridge Crop Storage Research, 2008).

Several mathematical formula can be used estimating bruise volume, such as bruise thickness method, full-depth method, ellipsoid method, enclosed volume, unbruised volume removed method, sphere bruise shape, and semi-oblate spheroid bruise shape (Opaha and Pathare, 2014). The area of bruised tissue can also be estimated by the use of a software, such Optimas 6.0 (BioScan Inc, Bothell Washington, USA) (Sthehmel *et al*., 2010).

The manual method assuming cylindrical shaped bruise volume of bruised tissue is calculated measuring bruise depth, width and visual assessment of the bruise pigment intensity (BPC_LINK 240, 2007). The visual rating of bruise pigment for bruising assessment is commonly based on a visual rating scale which is not consistent among researchers.

Methods such as abrasive peeling (Dean *et al*., 1993, Corsini *et al*., 1999) and optical density (oxidative potential) (Sabba and Dean, 1996, Dean *et al*., 1993) do not measure susceptibility to internal damage caused by an impact, but are useful for comparing the speed and extent of browning responses and are useful indications of susceptibility to impact, when the limiting mechanisms are primarily biochemical oxidation of tuber homogenates (Dean *et al*., 1993). Dean *et al*. (1993) suggested that significant but not large correlations have been found between measurements of bruise resistance by the homogenization technique and impact type bruise techniques (R = 0.35 to 0.37).

Novel and emerging non-invasive technologies for bruise measurement of fresh horticultural produce include visible and near infrared (Vis–NIR) spectroscopy (Jiménez-Jiménez *et al*., 2012), nuclear magnetic resonance imaging (Thybo *et al.*, 2004), hyperspectral imaging and thermal imaging (Opara and Pathare, 2014).

Simulation of the vibration force created during transportation is also been studied. A comparison of the package-cushioning materials to protect bruising damage to apples was investigated using an exciter vibration table with a force transducer (Eissa and Hafiz, 2012).

The instrumented spheres such as electronic potatoes permit real time monitoring and evaluation of packing lines to characterize the bruise potential postharvest handling systems (Van Canneyt *et al*., 2004). This kind of device helps to monitor potato trajectories through the chain and identify the critical control points to reduce the incidence and magnitude of

mechanical damage of fresh horticultural produce. Electronic potatoes are useful to estimate the pressure of bruising on tubers located at lower depths in bins of stored potatoes.

Different methods can give a variation in bruising susceptibility due to many terminologies and ranges of subjective bruise scores used to characterise visual bruising (McGarry *et al*., 1996, Baritelle *et al*., 2000). In the present study, different methods to assess the extent of bruising have been explored.

## 1.5 Factors influencing bruising in potatoes

### 1.5.1 Genotypic characteristics

Whilst no single factor determines bruising susceptibility, genotypic differences among cultivar varieties have a strong influence on the frequency and extent of bruising (Corsini *et al*., 1992, McGarry *et al*., 1996). Varietal characteristics influence the shape and size of the tubers (Partington *et al*., 1999) which are important factors to consider in relation to bruising susceptibility.

In this present study, the varieties Maris Piper (MP), Lady Rosetta (LR) and Russet Burbank (RB) were investigated. These UK varieties of potatoes are known to differ in their tendency toward bruising. MP and LR present a bruising susceptibility score of 6, and RB a bruising score of 4 in ratings ranging from 0 (most susceptible) to 9 (least susceptible) (Carnegie *et al*., 2005, BPVD, 2012). These ratings are derived by linear transformation using

varieties with known consistent susceptibilities and resistant reactions to bruising as fixed reference points after assessment made with a standard force applied to the heel end and the depth of damage at the point of impact measured (Carnegie *et al*., 2005).

MP is a white to yellow skin colour, it is one of the best known and popular potato varieties on sale and it is grown in high numbers across the UK (BPVD, 2012). LR is a red skinned, round shaped potato and it is specially used as a crisping variety (BPVD, 2012). RB is a large brown-skinned, white-fleshed potato cultivar majorly used for making chips or baking (Carnegie *et al*., 2005, BPVD, 2012).

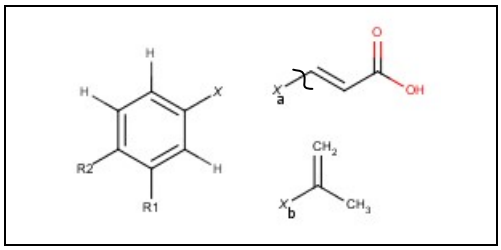
### 1.5.2 Phenolic acids

A phenolic is a compound with an –OH group attached directly to a benzene ring (Fry, 2000), shown in table 1.1. Phenolic acids contain two distinguishing constitutive carbon framework: the hydroxycinnamic acid (Xa) and hydroxybenzoic acid (Xb) structures. Hydroxycinnamic acids are the most widely distributed in potatoes, constituted of series of trans-phenyl-3-propenoic acids, differing in their ring substitution (Robbins, 2003).

Both benzoic and cinnamic acid derivatives have their biosynthetic origin from the aromatic amino acid *L*-phenylalanine, itself synthesized from chorismate, the final product in the shikimate pathway shown in figure 1.2 (Jensen, 1986; Robbins, 2003). The subsequent conversion of *L*-phenylalanine to the various hydroxycinnamic acids involves a three-step

sequence referred to as the “general phenylpropanoid metabolism” (Robbins, 2003).

Table 1.1 Structure of the naturally occurring phenolic acids in potatoes where Xa is a hydroxycinnamic acid and Xb is a hydroxybenzoic acid.




| R1 | R2 | X | Common name |
|---|---|---|---|
| H | -OH | a | *p*-coumaric acid |
| $-OCH_3$ | -OH | a | ferulic acid |
| -OH | -OH | a | caffeic acid |
| -OCH3 | -OH | b | vanillic acid |

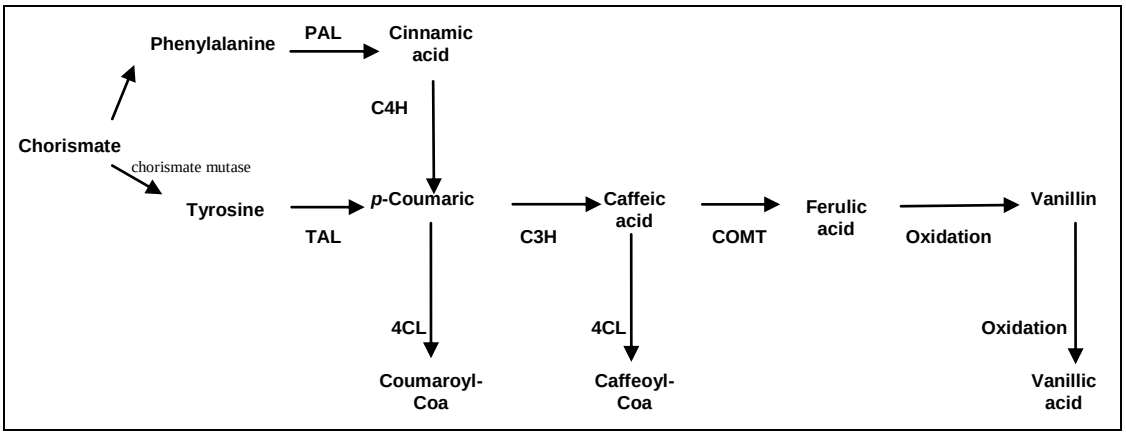



Figure 1.2 The phenolics biosynthetic pathway. The enzymes are: PAL phenylalanine ammonia-lyase; TAL tyrosine ammonia-lyase; C4H cinnamate 4-hydroxylase; C3H 4-hydroxycinnamate 3-hydroxylase; COMT caffeic acid 3-O-methyltransferase; 4CL 4-coumarate: (Adapted from Jensen, 1986, Spangenberg *et al*., 2001 and Converti *et al*., 2010).

The major phenolic acid compound in potato tubers is chlorogenic acid (5-*O*-caffeoylquinic acid or 5-CQA). Chlorogenic acid contributes up to 90% of the total phenol content of potatoes tubers and most of the discussion has centred on this compound (Friedman, 1997). Other common compounds found in potatoes are 3-CQA and 4-CQA which are 5-CQA isomers differing in their position of caffeic acid attachment on quinic acid as shown in figure 1.3. The structure of ferulic acid (3-methoxy-4-hydroxy), caffeic acid (3, 5 dihydrocinnamic acid), *p*-coumaric acid (4-hydroxy) and vanillic acid are shown in table 1.1 (Ramamurthy *et al*., 1992, Dao and Friedman, 1992, Desotillo *et al*., 1994, Hale, 2003, Shakya and Navarre, 2006 and Blessington *et al*., 2010) .

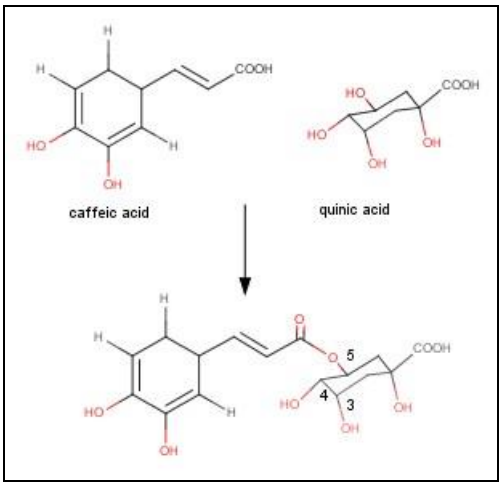



Figure 1.3 Structures of caffeoylquinic acid (5-CQA). CQA isomers differing in their position of caffeic acid attachment on quinic acid as indicated.

Phenolic compounds are distributed mostly between the cortex and skin (peel) tissues of the potato (Reeve *et al*., 1969). Chlorogenic acids are much

more concentrated in outer than in inner tissue zones and is also highly concentrated in the phloem and phloem parenchyma tissues of both cortex and perimedullar zone (Reeve *et al*., 1969).

The phenolic compounds are widely distributed in most tissues as conjugates in plant material but seldom as their free acids (Karakaya, 2004). The conjugates are bound to polysaccharides or other high molecular weight molecules. Ferulic acid and related compounds also occur in vacuoles in the form of *β*-glucosyl esters, which are much more water-soluble than the free acids (Fry, 2000). Some sugars in growing cell wall polysaccharides are also found with traces of ester bonds with phenolic acids, such as ferulic acid, coumaric acids and *p*-hydrobenzoic acid (Albersheim *et al*., 2011). These link the –COOH group of phenolic acid to specific –OH groups on particular sugars of certain polysaccharides. The traces of phenolics that are present may nevertheless act as important cross linking sites between polysaccharides (Fry, 2000).

Ferulic or *p*-coumaric acid can also have ether bond with wall polymers especially lignin. There are also reports of ferulic acid being amide-linked via its –COOH group to $NH_2$- of the protein. Cells with suberin also contain phenolic material, but their cross-links are very poorly understood (Fry, 2000).

Phenolic compounds, at low concentration, may act as antioxidants and protect foods from oxidative deterioration, providing resistance of plants (Friedman, 1997; Shakya and Navarre, 2006). The metabolism of phenolics in plants has been associated with injuries, thermal stress, exposure to UV

rays, ozone and biotic stress (Ngadze *et al*., 2014). At high concentrations, phenolic acids or their oxidation products may interact with proteins, carbohydrates and minerals (Karakaya, 2004; Karakaya and El, 2006).

Phenolic content is likely to be an important factor in determining bruise development. Ramamurthy *et al*. (1992) observed that during wound healing tubers greatly increased the content of chlorogenic acid, caffeic acid, *p*-coumaric and ferulic acid. In addition, the 3-CQA and 4-CQA isomers of 5-CQA accumulated in the tissue.

Mondy *et al*. (1987) found that bruised tissue contained significantly more phenolics than unbruised tissue following damage and storage at either 5°C or 20°C for 1, 3, 6, and 12 weeks. However, Dale *et al*. (1998) observed that the rate of accumulation of chlorogenic acid in response to damage-induced stress is genotype dependent. The cultivars Brodick and Torridon presented a small significant increase in chlorogenic acid levels after damage while the other cultivars, Ailsa, Eden and P Dell exhibited no appreciable differences.

Among phenolic acids found in potatoes, the compounds chlorogenic acid and caffeic acid are known to be relevant in the bruising of potatoes (Lærke *et al*., 2002). However, contrasting findings suggest that the contribution of other phenolic compounds to bruising formation remains unclear (Corsini *et al*., 1992, Mondy and Munshi, 1993, Friedman, 1997).

Bruising pigments isolated from two commercial cultivars have shown that the pigment is made of a protein matrix with covalently bound constituents which give the polymers an absorbance through the visible spectrum. The

results indicated that the pigments of one of the cultivars incorporated the endogenous *o*-diphenol chlorogenic acid (Stevens *et al*., 1998). Besides, the formation of iron-chlorogenic acid-protein chelates have been suggested to contribute to the internal discolouration of potato tuber tissue (Strehmel *et al*., 2010a).

Gosselin and Mondy (1986) observed that the varieties which produce more colour on the bruised tissue were higher in phenol content. However, the literature has contradictory findings about PPO and substrates. Early in the literature, Mondy *et al.* (1959) observed that the variety most susceptible to discolouration showed a greater increase in phenolic content during storage and suggested that the accumulation of phenolics was resultied from decreased activity of PPO.

Lærke *et al.* (2000) observed that MP tubers were 10 times more bruising-susceptible than the variety Colmo (CM) but the activity of PPO found in CM was more than three times higher compared to MP, which is unexpected.

McNabnay *et al*. (1999) suggested that PPO is absolutely necessary for the formation of the dark coloured compounds but the level of the enzyme is usually not limiting in commercial cultivars. In contrast, McGarry *et al*. (1996) observed that the discolouration reaction strongly depends on PPO activity on its substrates. According to Croy *et al*. (2000), PPO is a major limiting factor for bruising pigment formation. In tuber extracts, the addition of free tyrosine had little or no effect on pigment development, whereas addition of minute quantities of the enzyme caused a large increase in pigment formation within 60 minutes.

It has been shown that the role of PPO activity is predominant however other enzymes like cytochrome oxidase activity (Mondy *et al*., 1959) and peroxidases are possibly involved in the browning phenomenon (Partington *et al*., 1999, Urbany *et al*., 2011). In addition to PPO and its substrates, reducing agents such as ascorbate influence tuber bruising (El-Shimi, 1993).

### 1.5.3 Tyrosine

Another phenolic compound related to bruising is free tyrosine, which has been recognized as the main substrate for polyphenol oxidase-catalysed conversion (Dean *et al*., 1993; Mondy and Munshi, 1993 and Stevens *et al*., 1998). Tyrosine is synthetized via the shikimate pathway, of which chorismate mutase is a key regulatory enzyme as shown in figure 1.2 (Jensen, 1986).

Previously, Belknap *et al*. (1990) reported that wound-induced synthesis of proteins, such as ubiquitin and phenylalanine ammonia lyase was associated with bruising and cell damage. Rhamamurthy *et al.* (1992) also reported an increase in phenylalanine ammonia-lyase activity accompanied by a parallel rise in formation of phenolics after damage.

However, Dean *et al.* (1992) suggested that phenylalanine plays little or no role in determining the extent of bruising. Sabba and Dean (1994) observed that post mechanical impact, higher levels of free tyrosine were present due primarily to the increased activity of endopeptidases and it was not due to increased traffic through the biosynthetic pathway for tyrosine.

Strehmel *et al.* (2010a) explored the metabolic changes induced after mechanical stress and observed that the tyrosine and phenylalanine pools does not increase significantly after mechanical impact prior to bruising formation nor decrease concomitantly, what exclude bruising susceptibility being mediated by precursor accumulation or limitation. Also Strehmel *et al*. (2010a) observed that the absolute level of tyrosine before impact was higher in the non-sensitive cultivar studied.

The presence of tyrosine is higher in the stolon end of the potato (Mondy and Munshi, 1993), a fact that leads to increase in sensitivity to bruising in this area (Adams and Brown, 2007). Immature tubers present low levels of free tyrosine, being 0.081 and 0.038 mg/100g fresh weight for the varieties Pontiac and Ontario harvested 9 weeks after planting and then increasing progressively, incrementing by 0.5 mg/100g fresh weight when harvested after 11 weeks of planting. Evidence shows that the concentration of tyrosine in stored tubers is highly dependent on tuber maturity at harvest (Mondy and Munshi, 1993). A threshold concentration of tyrosine of 4 µmole per gram fresh weight has been proposed below which no bruise pigments are formed in response to mechanical impact (Corsini *et al.*, 1992).

Several studies have explored the correlations between tyrosine levels and bruising but they are contradictory (Corsini *et al*., 1992; Dean *et al*., 1992 ; Dean *et al*., 1993 and Stevens and Davelaar, 1997).

A higher content of free tyrosine was associated to the significant increase of the total potential to form dark colour measured at the optical density 475 nm of homogenized tissue in potassium deficient treatments (McNabnay *et al.,*

1999). *In vitro* assay with desalted polyphenol oxidase (PPO) showed that tyrosine contributed more to oxidation of tissues than chlorogenic acid (Kim and Dean, 1998). Sabba and Dean (1994) reported that bruising susceptibility had strong positive correlation (R=0.88) to tyrosine levels in certain varieties. Experiments from Stevens and Davelaar (1997) showed a good linear correlation with tyrosine and bruising pigments (*in vitro*) but not with bruising susceptibility following impact with a pendulum. The results show that tyrosine content was largely dependent and could be decreased by the combined supplementation of nitrogen.

The researchers Mondy and Munshi (1993) also found a positive correlation between bruising and free tyrosine within a cultivar, but suggested that tyrosine levels were not the predominant factor in determining bruising susceptibility in potatoes as a whole due to the fact that their cultivar with high bruise susceptibility had overall considerably lower levels of tyrosine than their cultivar with high bruise resistance.

Similarly, in diploid hybrids resistant to bruise, the content of *L*-tyrosine was not significantly correlated with bruising (Hara-Skrzypiec and Jakuczun, 2013). Corsini *et al.* (1992) noted there was an inverse relationship between the amount of protein-bound tyrosine with free tyrosine, suggesting that protein biosynthesis affected bruising susceptibility by reducing the pool of free tyrosine available for use by PPO. Partitioning of tyrosine between tuber protein and the free amino acid pool varies with genotype and appears to be a major determinate of bruising resistance (Corsini *et al*., 1992; Mondy and Munshi, 1993; Friedman, 1997). Tyrosine becomes elevated either through

de novo synthesis or by liberation from protein via proteases (McNabnay *et al*., 1999).

Contrasting findings suggest that the impact on discolouration with tyrosine and the partitioning of tyrosine between tuber protein and the free amino acid pool remains unclear.

### 1.5.4 Cell walls

The gross cell wall composition of potato tubers is typical of parenchymous tissue. Parenchyma in plants tissue is composed of living cells that are thin-walled, unspecialized in structure, and therefore adaptable, with possible differentiation to various functions (Ross *et al*., 2011a, b).

Cell walls of potatoes have an important role in maintaining freshness of potato tubers during storage and also influence the textural quality of many processed products (Jarvis *et al*., 2003). The cell wall is a rigid structure encasing plant cells which resists turgor pressure and mediates cell-cell adhesion (Fry, 2000).

The primary walls consist of cellulose microfiblis embedded in a matrix of polysaccharides and often, not always, glycoproteins, hemicellulose, phenolics and aqueous phase (70%) (Albersheim, 2011). Pectin and hemicelluloses are the noncellulosic polysaccharides of the primary cell wall (Fry, 2000). Figure 1.4 shows the most important monosaccharides of potato cell wall which account for monomer subunits of the matrix polyssacharides.

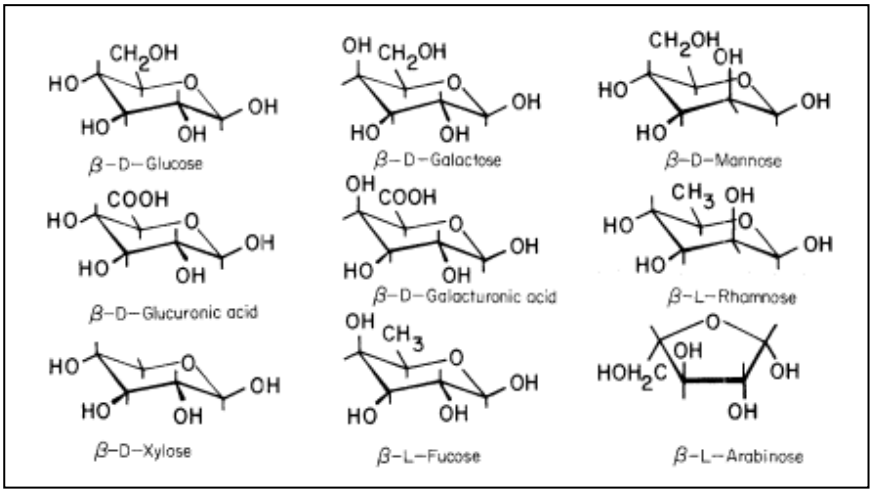



Figure 1.4 Sugars of plant cell walls (Smith, 1977). The sugar monomers of plant cell walls include *β*-D-Glucose, *β*–D-Galactose, *β*–D-Mannose, *β*-D-Glucuronic acid, *β*-D-Galacturonic acids, *β*-L-Rhamnose, *β*-D-Xylose, *β*-L-Fucose and *β*-L-Arabinose.

### 1.5.4.1 Pectin

Pectins are the main polysaccharides in potato cell wall and contributes between 52-55% of total polysaccharides (Ross *et al*., 2011a, b). The synthesis of pectic polysaccharides is estimated to involve at least 67 different enzyme activities, including glycosyl-, methyl-, and acetyltransferases (Mohnen, 2008; Harholt *et al*., 2010). Degradation of pectin is catalysed by pectinases through depolymerisation (hydrolases and lyses) and deesterification (esterases) reactions (Pedrolli *et al*., 2009).

Synthesis and modification of cell wall pectins occurs in the *cis*-Golgi apparatus, where activated nucleotide sugars, such as uridine diphosphate glucose (UDP-Glucose), are required for pectin biosynthesis (Caffall and Mohnen, 2009). There may be branching and esterification of pectin in the

*trans*-Golgi. However, relatively unesterified pectin can also be inserted into the cell wall (Ridley *et al*., 2001).

Pectin is present in much larger quantities and surround growing and dividing cells in primary walls compared to secondary walls (Caffall and Mohnen, 2009), suggesting that pectin has a function in plant growth (Mohnen, 2008). They are highly hydrophilic polysaccharides, and the water that they introduce into the matrix may loosen the wall, enabling the skeletal cellulose microfibrils to separate – necessary for cell wall expansion (Fry, 2000). On the other hand pectin can form cross-links via calcium bridges that may serve the opposite function of resisting the expansion of the cell wall (Fry, 2000). Pectin is also present in the soft parts of the plant, cell corners and largely in the middle lamella, where they presumably serve the function of cell–cell adhesion (Fry, 1986; Jarvis, 1998). Other evidence indicate a role for pectin in defence (White and Broadley, 2003), binding of ions (Vincken *et al*, 2003), wall porosity, morphogenesis, signalling enzymes, seed hydration, leaf abscission, fruit development (Mohnen, 2008), and structural providing a plastic behaviour (Zdunek *et al*., 2004).

Pectins are a family of covalently linked galacturonic acid-rich plant cell wall polysaccharides. Galacturonic acid comprises approximately 70% of pectin, and all the pectic polysaccharides contain galacturonic acid linked at the *O*-1 and the *O*-4 position. Pectins are highly complex polysaccharides and are composed of subclasses: homogalacturonan (HG), rhamnogalacturonan I (RG-I) and RG-II (Mohnen, 2008), shown in figure 1.5. The content of subclasses is variable. Typically HG is the most abundant polysaccharide

constituting up to 65% of the pectin, and RGI constitutes 20% to 35% (Mohnen, 2008).

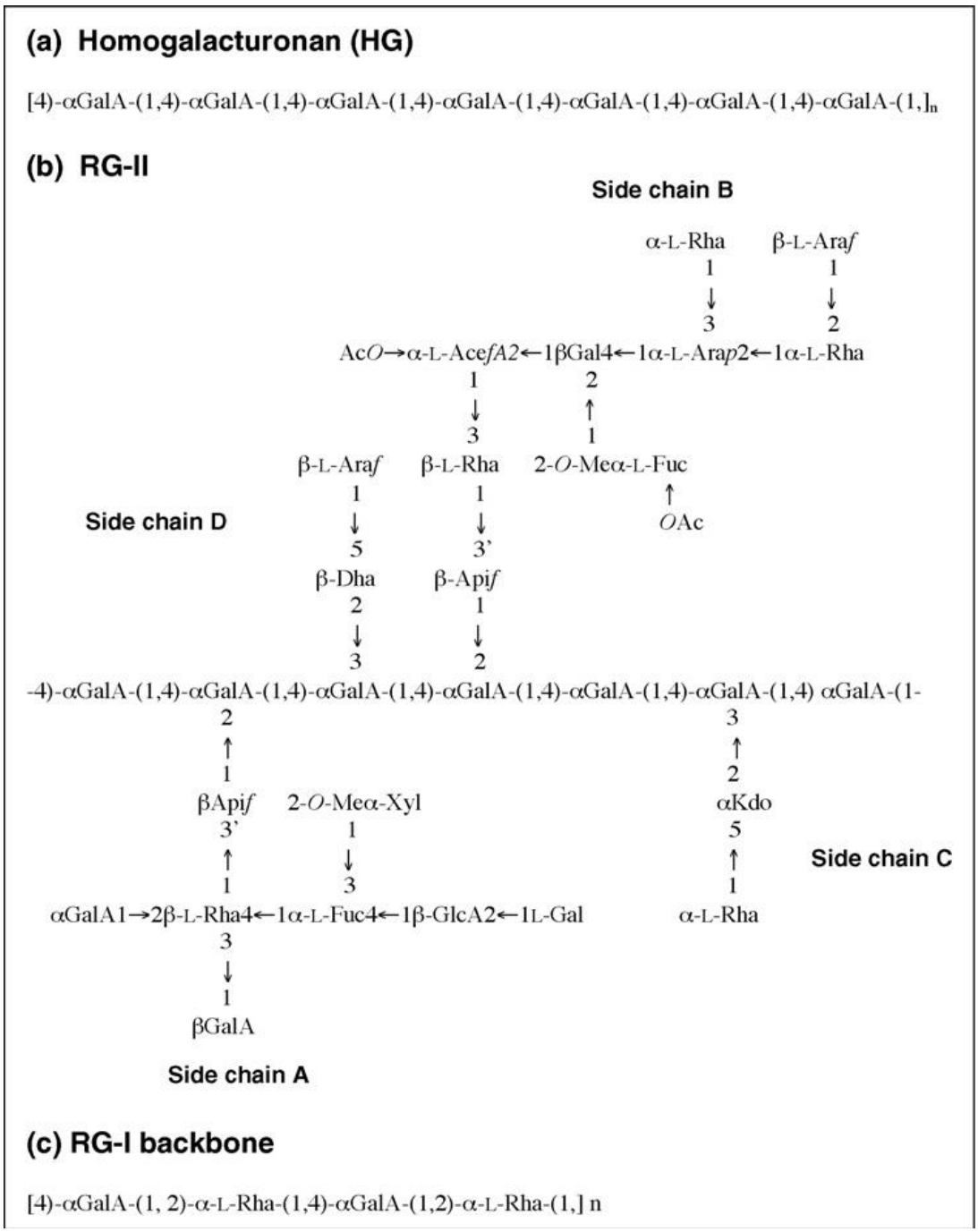



Figure 1.5 Selected schematic structure of pectin showing the regions of representative pectic polysaccharide: (a) Homogalacturonan, HG, (b) Rhamnogalacturonan II, RG-II, (c) Rhamnogalacturonan I, RG-I, (d) Selected RG-I backbone side chains. The bottom structure is a so-called type II arabinogalactan since similar structures are also found in abinogalactan proteins (Mohnen, 2008).

**Homogalacturonan**

The HG is abundant in potato primary cell walls and, is particularly dense in the middle lamellae (Caffall and Mohnen, 2009). HG consists of *α*-1,4-linked galacturonic acid residues that can be methyl esterified at C-6 carboxyl group (COOH) , and/or acetylated at *O*-2 or *O*-3 (Vincken *et al*., 2003).

Due to changes in the physical and chemical properties of pectin to a large extent, the methyl-esterification has gained a lot of attention over the years. Pagel and Heitefuss (1989) observed a high correlation was found between the content of non-esterified homogalacturonan and resistance to bruising in potatoes. Additionally, the reduction of bruising severity is linked with increase in calcium concentrations in potatoes tissue (Ozgen *et al*., 2006).

The reasons for low calcium levels correlated with internal browning in raw fruits and vegetables are not fully understood at present (Adams and Brown, 2007). It is known that cytosolic (cyt) calcium concentrations has crucial importance as it stabilizes cell membranes, and is a key regulator of plant defences to mechanical perturbation, cooling, heat shock, acute salt stress, hyper-osmotic stress, anoxia, and exposure to oxidative stress elicitors. An immediate transient increase in cyt calcium in plant cells is followed by a more prolonged elevation of cyt calcium lasting many minutes or hours (White and Broadley, 2003). Thus, under low calcium conditions, cellular defence regulation may be disrupted and this leads to accumulation of

reactive oxygen species that cause oxidation of phenolic compounds and the resulting discolouration (Adams and Brown, 2007).

The formation of calcium crosslinks between HG chains is impaired by acetylation of galacturonic acid (Renard and Jarvis, 1999). The degree of acetylation (DA) on potato cell wall material has been estimated to be around 40-45% (van Marle *et al*., 1997) with heterogeneous distribution of acetyl along the pectic backbone (Orfila *et al*., 2012). Acetyl substitution also affect the enzymatic activity on HG by some endopolygalacturonases (endoPGs) (Bonnin *et al*., 2003).

**Rhamnogalacturonan I**

RG I is a branched polymer with a backbone of disaccharide (*α*-1,4-*D*-GalA-*α*-1,2-*L*-Rha) (Harholt *et al*., 2010). Up to 80% of the rhamnosyl residues are substituted at *O*-4 with arabinan, galactan, and/or arabinogalactan I (AG I) side chains, depending on the plant source and method of isolation (Caffall and Mohnen, 2009). Other substituents in small amounts are *L*-fucosyl and *D*-glucosyluronic acid residues (Albersheim, 2011).

The arabinans consist of a *α*-1,5-*L*-Arabinose backbone, which can be substituted with *α*-1,2-Ara-*α*-1,3-; and/or *α*-1,3-Ara-*α*-1,3-Ara side chains (Vincken *et al*., 2003). Galactans account to 67% of RGI. AG-I is composed of a *β*-1,4-galactosil backbone where arabinose residues can be attached to the *O*-3 of the galactosyl. Other substituents at *O*-3 in AG-I can be ferulic and coumaric acid (Orfila *et al*., 2012). Side chains can also crosslink to other wall components such as xylans, xyloglucans, proteins, and lignins

(Caffall and Mohnen, 2009). The degree of substitution, branching and integration in the cell wall may differ and have great impact in tissue properties (Harholt *et al.*, 2010).

Side chains of RG-I of pectin may also play some role in cell wall firmness. Ulvskov and co-workers (Oomen *et al*., 2002, Skjøt *et al*., 2002, Ulvskov *et al*., 2005, Orfila *et al*., 2012) proposed that the components of RGI (galactan and arabinan) transmit stresses in the wall and hence play a direct role in wall rheological properties. The force to fracture cylinders of tuber tissue decrease when levels of galactan and arabinan are reduced due to expression of fungal pectin-digesting enzymes. The elastic properties of the tubers were also altered, with a stiffening of the cell wall (Orfila *et al*., 2012). Loss of arabinan and galactan was also associated the loss of firm texture in apples (Pena and Carpita, 2004). Alterations in arabinan content have been associated with cell adhesion defects in tomatoes (Orfila *et al*., 2001). Mitsuhashi-Gonzalez *et al*. (2010) observed in apples that the greater the amount of intercellular space present in the tissue, the more tissue damage from bruising occurred.

Salato *et al.* (2013) observed that soft cherries had lower wall contents together with higher neutral sugar rich-pectin side chains compared to firm cherry, factors that the authors suggested may be involved in the differences in firmness.

A study of the motilities of polysaccharides in a cell-wall suggested that arabinans and galactans are among the most freely mobile polymers in hydrated, RGI-rich primary walls (Tang *et al.,* 1999). Side chains of RG-I

galactan and arabinan can interact with cellulose microfibrils in primary cell walls (Zykwinska *et al*., 2007), and can be covalently linked to xyloglucan as noted in Arabidopsis cell cultures (Popper and Fry, 2008). The galactosyl [1]containing side chains of xyloglucan contribute to the tensile strength of cell walls (Caffall and Mohnen, 2009).

**Substituted galacturonan: rhamnogalacturonan II (RG-II)**

HGs can contain clusters of four different (heterooligomeric) side chains attached onto the *O*-2 or *O*-3 position in the galacturonan backbone with very peculiar sugar residues (such as *D*-Apiose, aceric acid, 2-keto-3-deoxy-*D*-lyxo heptulosaric acid (Dha) and 2-keto-3-deoxy-*D*-manno octulosonic acid (Kdo)) to form RGII (Harholt *et al*., 2010). These side chains are composed of 12 types of glycosyl[2] residues linked together (Mohnen, 2008, Harholt *et al*., 2010) by at least 22 different glycosidic bonds (Harholt *et al*., 2010). RG-II domains can form crosslinks to other RG-II molecules via borate diester linkages, to form RG-II dimers that contribute to wall strength (Peña and Carpita, 2004) and affect pore size and flexibility of the pectic network (Caffall and Mohnen, 2009). Greater than 95% of RG-II molecules participate in dimer complexes of RG-II (Caffall and Mohnen, 2009). RG-II ,

[1] When monosaccharides become incorporated into a polysaccharide, one water molecule is lost for each glycosyl link that is formed, the corresponding sugar residue that is incorporated is identified by the suffix –osyl (Fry, 2000).

as HG, is a key feature controlling cell-cell adhesion (Jarvis *et al*., 2003). Another linkage not yet established is whether XGA can harbor RG-II elements (Vincken, 2003).

Although pectin plays a role in cell wall firmness, the role of the pectin in relation to bruising has not yet been established. Wulkow (2009) has previously investigated if the pectin concentration of potato tubers influences bruising. Wulkow (2009) reported that there was no correlation between dry cell wall material, the total pectin nor the nonpectin (celluloses, hemicelluloses) concentration and blackspot susceptibility ($p>0.05$) in tubers of various specific gravities. Also, there was no correlation found between the degree of esterification of the pectin to the blackspot susceptibility index of tubers.

### 1.5.5 Mechanical properties

The mechanical parameters are known to be associated with potato bruising damage such as the amount of physical deformation, the transmission of impact energy and the predisposition to fracture (McGarry *et al*., 1996).

The mechanical properties of plant organs depend upon anatomical features such as cell size, cell wall thickness, skin thickness, cell-cell adhesion, turgor pressure and the strength of the plant cell wall (McGarry *et al*., 1996; Zdunek *et al*., 2004; Singh *et al*., 2005; Jarvis, 2011).

The turgor seems to play an important role on the texture. Changes in water status was responsible for appreciable changes in the fracture properties of the varieties King Edward and Record when the turgor cell wall was

manipulated to three different states (turgid, fresh and flaccid) (Hiller *et al.,* 1996).

Tuber turgidity and cell wall strength have also been reported to influence impact bruise susceptibility in potatoes. Higher turgor pressure ruptures the cell walls, which is responsible for damage to cells, leading to bruising formation (Singh, 2014). Also, Praeger *et al*. (2010) reported that decline in turgor pressure of stored potato tubers was accompanied by a decrease in bruising susceptibility.

Softening, which has been referred to as a long term storage effect of fruits and vegetables, is usually represent by a decrease in the firmness of the tissue (Pardede, 2005). These changes in texture are related to a decrease in turgor and concomitant changes in the composition and structure of matrix components due to cell wall-degrading enzymes acting on cell carbohydrates leading to disassembly of cell wall adhesion. Softening in fruits and vegetables is accompanied by pectin solubilisation, by action of cell wall hydrolases as polygalacturonases, pectinmethylesterase, *β*-galacturonase and glycase. Activity of hemicellulose and cellulose related enzymes are also involved, as the increase of xyloglucan endotransglycosylase (XET) (Pardede, 2005). Puncture and penetration methods are widely used for the measurement of textural parameters of fruits and vegetables.

Anzaldúa-Morales *et al.* (1992) observed that the cortex tissue in raw potato was about 50% firmer than the pith measured by means of puncture force between potato tissues using puncture test with a cylindrical probe 2.5 mm

diameter at a speed of 50 cm/ min. The method also detected differences in firmness in potatoes of three cultivars.

Grotte *et al.* (2001) observed decreases in firmness and in deformation by 10 and 50% respectively in apple flesh and during the cool storage at 2°C, and 96% relative humidity using the puncture test.

A reduction in firmness (78.2–68.9 N) was also observed in the potato cultivars Spunta and Agria after 90 days of storage at 5°C and 90% relative humidity using a hand-operated penetrometer with 0.5 cm probe (Arvanitoyannis *et al*., 2008).

Garcia and Altisent (1993) found the deformation by skin puncture was the physical parameter most related to fruit turgidity and this parameter was related to bruise susceptibility in apples and pears. The maximum force at skin puncture decreased after storage in pears, but remained fairly constant in apples. However, maximum deformation by skin puncture increased after storage in both apples and pears using puncture test with a cylinder probe 0.5 mm diameter at 20 mm/min.

Recent research from Mahto and Das (2014) found that increasing gamma irradiation up to 0.12 kGy progressively reduced the textural deterioration in the tubers during storage. The samples treated retained their puncture force required to puncture the tubers using a cylindrical probe of 6 mm diameter at a speed of 0.25 mm/s throughout the storage period.

However, few reported studies provide convincing evidence of the mechanical properties relating to bruising sensitivity. Bajema *et al.* (1998)

demonstrated that failure properties after dynamic compression of potato tuber tissue can be used to characterize differences with regard to bruising sensitivity between potato cultivars.

Hironaka *et al.* (2007) observed that bruise-resistant potatoes required more force, deformation and energy to break the skin using a penetration test with a 2 mm cylindrical probe at 50 cm/min. The deformation and energy results of the five japonese varieties investigated correlated significantly with blackspot index following 100 g round plug damage.

Mechanical properties were also explored in a research undertaken through a BPC_LINK 240 (2007) project. It was observed that the bruise susceptible cultivar (Russet Burbank) is more brittle than resistant tissue (Cara) which is able to diffuse the stress across a larger number of cells.

Considering the necessity for understanding the mechanical properties of the varieties and bruising, the mechanical properties of the skin and cortex were explored.

### 1.5.6 Specific gravity

The specific gravity of tubers is a method used to estimate the dry matter content using by a first regression equation (Haase, 2003/4). The method consists in measuring a known weight of potato attached to a float displaced a volume of water relative to dry matter (Fong, 1973). Other forms to measure the dry matter content is by comparing the weight of an oven-dried sample with its original weight, flotation in salt (Wright *et al*., 2005) and near infrared (NIR) measurements (Haase, 2003/4).

The term dry matter refers to all substances of the potato tuber, except water. Dry matter can vary from variety, plant to plant within a crop and also from tuber to tuber on a single plant (Lisinka and Leszczynski, 1989). Even within an individual tuber, dry matter is higher near scar stolon. The variation can be also due the amount of intercepted radiation during crop growth (sunshine hours), water availability and fertilizer rates (Stalham, 2008).

Dry matter increases over time and reaches a peak 4-6 weeks before defoliation. Thereafter, dry matter may remain constant or change in either direction. Starch contributes approximately 80% of dry matter content of potato which represents 10-25% of the storage compound of potato tubers (Ross *et al*., 2011a).

The presence, number, size and angularity of starch grains may be important simply on the basis of their potential for physical damage to membranes when cells are deformed by impact and thereby affect cellular stability and tuber bruising (Wulkow, 2009; Urbany *et al*., 2011).

It may be assumed that a fully turgid vacuole presses protoplasm and starch granules against the cell walls more than in flaccid cells. Compared to flaccid cells this may increase the shock wave speed after impact. The cell walls could compensate the acceleration of shock wave due to the chemical interactions between the various cell wall components or transferred the impact energy into the parenchyma (Bajema *et al*.1998).

The susceptibility to bruising might increase in tubers with high dry matter because of a reduced cell wall tension and/or a higher concentration of

starch granules (Bajema *et al*., 1998). Therefore in flaccid cells the specific gravity influences impact susceptibility (Wulkow, 2009).

The way in which impact energy is dissipated through a tuber is also affected by the tissue porosity, cell size, orientation and packing (Stalham, 2008).

SG has been shown to influence the mechanical properties. Puncture force increased with increases in SG of potato cultivars Atlantic and Chieftain (Anzaldúa-Morales *et al.,* 1992). SG showed a close relationship with dry matter content in this experiment.

Kaaber *et al*. (2001) observed that the dry matter content decreased significantly during storage at 4 °C, but increased at 8 °C due to evaporation. Moreover, Baritelle and Hyde (2003) found in the variety Russet Burbank that a higher volume of bruised tissue was associated with higher SG, with increments of about 1 $cm^3$ comparing potatoes with SG <1.080 with potatoes with SG on the range 1.090-1.100. Wulkow (2009) also observed increase in blackspot index (BSI) as specific gravity increased within eight cultivars of potatoes.

However, the authors Wright *et al*. (2005) reported no simple consistent relationship between specific gravity and bruise score across cultivars using falling bolt test. Similarly, research conducted by Stalham (2008) have not shown significant correlation between bruise score and tuber dry matter within varieties studied, either at individual harvests or over the course of the season.

Results from Fellows (2004) suggested that varieties with a higher total oxidative potential are likely to have a lower dry matter percentage and a higher susceptibility to bruising. However, within any one variety there were no significant correlations between the percentage of tubers bruised and total oxidative potential suggesting that total oxidative potential is unlikely to be a useful indicator of bruising susceptibility.

Praeger (2010) reported higher bruising susceptibility for the variety Milva with low content of starch compared to Afra.

### 1.5.7 Enviromental influences

Besides the physical aspects and potato genotype, observations suggest that environmental conditions also affect bruising. (McGarry *et al*., 1996).

Bruising susceptibility tends to be higher in long, hot, and dry growing seasons apparently due to physiologically older tubers have higher levels of tyrosine (Corsini *et al*., 1999), the main substrate for the enzyme polyphenol oxidase (PPO).

Water stress can affect the potato crop in a number of ways. It is particularly important from a quality perspective at tuber initiation and in the days and weeks that follow initiation. During this stage of growth the tuber periderm is not fully developed and common scab can affect tubers (Pringle *et al*., 2009). The temperature is also an important factor correlated to bruising. Bruising has strongly increased when tubers were handled under chilled conditions (Corsini *et al*., 1999).

### 1.5.8 Agricultural practice

#### 1.5.8.1 Maturity at the harvest time

The maturity status before harvest is a relevant factor in bruising. Maturity is related to the time when the plant will desiccate and tuber skin is set (Pringle *et al.*, 2009). It is known that the content of phenolic substrates (e.g free tyrosine) for PPO tend to be less abundant in early (immature tubers) than in late-season (Lisinska and Leszczynski, 1989; Mondy and Munshi, 1993).

Maturity at harvest and evaporation during the storage appears to influence periderm cells collapsing, prone to microscopic cracking and cause poor bloom (Wiltshire *et al*., 2005).

In addition, the maturity at harvest time is the predominant factor influencing processing quality of potatoes throughout storage (Groves *et al*., 2005).

#### 1.5.8.2 Storage

Storage length and temperature influences tuber physiological age (Burton, 1989). Temperature and humidity control are important during storage to control the rate of respiration and evaporation (Mohsenin, 1986).

The biochemical process of respiration requires oxygen and is proceeding by a conversion of starches to sugar. In the mitochondria, the tuber cells combustion chambers, glucose is oxidized into nutrient that is required by the tuber to stay alive and produce water carbon dioxide and heat energy as by-product. The heat produced reduces the relative humidity (RH) of the air within the voids, increasing its water-holding capacity, and contributes to

moisture loss through evaporation of water from the tuber skin (Pringle *et al*., 2009).

Respiration varies between varieties, increases rapidly immediately after harvest, particularly in immature tubers, followed by a fall. In 3–6 weeks rate of respiration declined until a minimum was reached followed by an increase which was particularly marked through to sprouting/dormancy break (Schippers, 1977). Respiration also increases after tubers have been handled, washed, transported over rough tracks or with damage as bruise and cuts (Schippers, 1977).

Respiration therefore influences tissue properties. The cell walls within the tuber become weak and membranes leak as tubers age (Mohsenin, 1986), releasing substrates to PPO. Tubers also suffer moisture loss resulting in low tuber turgidity and this increases bruising susceptibility, as demonstrated in Russet Burbank after 4 months storage at 7 °C (Corsini *et al*., 1999). Mondi and Munshi (1993) observed greatest increases in tyrosine levels in tubers harvested at 7 (Ontario) and 9 weeks (Pontiac) following 24 weeks of storage.

Ninety-eight per cent of the moisture that leaves a tuber during storage is lost through its skin by evaporation, only 2.4% leaves the tuber via the lenticels along with the carbon dioxide produced by respiration (Burton, 1989).

A experiment using film wrap Cryovac D-955 shrink reduced potato weight loss (water loss) and also bruising when stored at 24°C but it did not alter

the rate of respiration or the endogenous oxygen or carbon dioxide levels and it is not involved with inhibition of polyphenol oxidase. It was suggested that decrease moisture loss thereby maintain membrane integrity and may dissipate some of the forces of impact, spread the impacting force over a wider area and so diminish the intensity of bruising (Shetty *et al*., 1991).

The relationship between the periderm lipid coverage and the water transpiration properties is not fully understood. The molecular arrangement and precisely localised deposition of suberin within the cell wall must contribute to the efficiency of suberin as a barrier to water transport (Schreiber *et al*., 2005). Shrinkage and flaccidity occur in tubers if the protection afforded by the periderm against water loss is compromised (Lulai *et al*., 2006). The periderm is one aspect of potato tubers that has been widely studied tubers because of the latter's great agronomic significance (Sawyer and Collin, 1960; Strehmel *et al*., 2010b; Lulai *et al*., 2006; Schreiber *et al*., 2005).

#### 1.5.8.3 Defoliation

One of the factors analysed in this study was defoliation. Defoliation is a commonly used agricultural practice to prepare the field for the harvest of potatoes. Methods to defoliate the plants include mechanical or use of chemical defoliant, such as acid, reglone, glyphosate and triazolinone. The artificial defoliation is a common technique used to investigate the correlation between damage caused by either hail or insecccts and yield (Croy *et al*., 2000).

Defoliation at flowering and tuber formation considerably affect yield of tubers, however, barely affect the yield of tubers completed grown (Irigoyen, 2011). Sight *et al*. (2001) observed reduction of 34.3 to 51.8% in tuber yield and 40.3-50.1% in dry matter.

A previous Potato Council project (Fellows, 2004) reported that the varieties Marfona and in Maris Piper presented association between the time of defoliation and lifting related to susceptibility to bruising but there was no trend evident with the other variety studied (Cara).

Stalham (2008) found that more bruising occurred in crops harvested three to five weeks after defoliation (21 and 35 days respectively).

#### 1.5.8.4 Nitrogen

The use of the fertilizer nitrogen (N) plays a key role in vegetative growth and in tuber production, having a significant effect on a number of physiological processes in potatoes, as influence on crop senescence, skin set and dry matter (Mondy and Koch, 1978; Kunkel and Dow, 1961; McGarry, 1996; Sun *et al*., 2012).

Symptoms of N deficiency include early senescence and lower yields. Excess nitrogen can delay maturity, reduce potato yield and delay the crop in achieving the dry matter content (Sun *et al*., 2012), adversely affecting processing quality.

Hole (1997) suggested that because the maturity of harvest can affect the incidence of bruising, tubers treated with nitrogen become less susceptible

to bruising. However, there is no agreement in the literature about the effect of N and bruising incidence. Upon application of N, references found an increase in bruising (De Bruyn, (1929) summarized by Mondy and Koch (1978), Koblet, *et al*. (1948) in McGarry, (1996)). Kunkel and Dow, 1961 found a decrease in bruising while Rogers-Lewis (1980) and Silva *et al*., (1991) observed no effect on bruising.

## 1.6 Aim

Considering that bruising is a significant problem in potatoes, the main aim of this project was to investigate the relationship between bruising and the physicochemical properties of potato tubers in three varieties of potatoes known to differ in their tendency toward bruising: Lady Rosetta (LR), Maris Piper (MP) and Russet Burbank (RB). Three field trials were undertaken to investigate the effect of agricultural and storage practices on bruising incidence and tuber properties. Field trial 1 was designed to investigate the effect of harvest time and defoliation; field trial 2 was designed to investigate the effect of harvest and storage time and the field trial 3 was undertaken to investigate the effect of nitrogen application to soil (in variety LR only). The knowledge from this project would enable growers to better manage crops to achieve the necessary standards for optimum quality of fresh and stored potatoes.

Additionally the research seeks to establish whether physiological and biochemical characteristics, such as weight, specific gravity, mechanical properties, phenolic acids, tyrosine and cell wall composition of skin and

cortex tissue are factors that may be used as predictive indicators of bruising at harvest time and for stored potatoes.

## 1.7 Objectives

The research objectives are:

1 To test different methods for bruising assessment.

2 To investigate the bruising potential in three UK varieties (LR, MP and RB).

3 To develop methodology for analysing the mechanical properties of cortex and skin tissue.

4 To investigate the potential for using physical and biochemical measurements as indicators of bruising at harvest and storage.

5 To improve the understanding of the influence of defoliation, harvest and storage time on the incidence of bruising.

6 To determine if application of nitrogen fertilizer increases bruise susceptibility.

## 1.8 Hypotheses

The hypotheses tested were:

1 The variety RB will bruise more than MP and LR.

2 Potatoes harvested later in the season will show more bruising.

3 Potatoes supplied with nitrogen fertilizer will show more bruising along harvest.

4 Potatoes from defoliated plants will show more bruising in crops harvested three to five weeks after defoliation (21 and 35 days respectively).

5 Stored potatoes harvested in September will show less bruising than stored tubers harvested in October.

6 The content of phenolics will increase along harvest and storage time, and will be associated with increased bruising incidence.

7 Phenolic substrates will be higher in defoliated and in nitrogen treated tubers.

8 Specific gravity will increase along harvest and storage, and will be associated with increased bruising incidence.

9 Specific gravity is higher in tubers supplied with N and is associated with increased bruising incidence.

10 The mechanical properties of the tuber will influence bruising and these properties are influenced by the cell wall composition of cortex cells at harvest and storage.

11 Cell wall of tubers presenting more arabinose and galactose will require more force to break the tissue.

12 Cell wall of tubers presenting less methylation on the homogalacturonan pectin will require more force to break the tissue.

# 2 Materials and Methods

## 2.1 Sampling

### 2.1.1 Field trial 1

Potato plants from three different cultivars Maris Piper (MP), Russet Burbank (RB), and Lady Rosetta (LR) were grown at Cambridge University Farm (CUF), planted on 23 April 2010 and tubers harvested at four time points. Before the harvest, defoliation was carried out at two time points (early defoliation and late defoliation), as indicated in table 2.1. Trials were randomised with two factors (variety, defoliation) with three replicate plots. Ten tubers per plot were collected and shipped to Leeds on harvest day.

Table 2.1. Field trial 1 - Year 2010

| **Trial 1** | **Harvest and Defoliation period** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Harvest** | **1st Harvest (H1)** | | | **2nd Harvest (H2)** | | | **3rd Harvest (H3)** | | | **4th Harvest (H4)** | | |
| **Date** | 2nd August | | | 16th August | | | 9th September | | | 20th September | | |
| **Days after planted** | 101 | | | 115 | | | 139 | | | 150 | | |
| **Defoliation** | D1 | D2 | UND | D1 | D2 | UND | D1 | D2 | UND | D1 | D2 | UND |
| **Days after defoliation** | 0 | / | / | 14 | 0 | / | 38 | 24 | / | 49 | 35 | / |

### 2.1.2 Field trial 2

Potato plants from three different cultivars Maris Piper (MP), Russet Burbank (RB), and Lady Rosetta (LR) were grown at Cambridge University Farm (CUF), planted on 15 April 2011, and harvested at two time points and stored for three time points. Harvest and storage dates and periods are indicated in table 2.2. Trials were randomised with three factors (variety, harvest and storage) with three replicate plots. Twenty tubers per plot were collected and either shipped to Leeds or Sutton Bridge Crop Storage Research (Sutton Bridge, Suffolk) on harvest day. The tubers were stored in trays within temperature and moisture controlled storage chambers, at temperature below of 10º C and 95+% Relative Humidity (RH). At the end of the storage period, tubers were shipped to Leeds.

Table 2.2 Field trial 2 - Year 2011/2012

| **Trial 2** | **Harvest and Storage period** | | | | | |
|---|---|---|---|---|---|---|
| **Harvest** | **1st harvest (H1)** | | | **2nd harvest (H2)** | | |
| **Date of harvest** | 19th September | | | 12th October | | |
| **Days after planted** | 157 | | | 180 | | |
| **Storage** | Storage 1 (S1) | Storage 2 (S2) | Storage 3 (S3) | Storage 1 (S1) | Storage 2 (S2) | Storage 3 (S3) |
| **Date of sampling** | 14th January | 26th March | 11th May | 14th January | 26th March | 11th May |
| **Days after planted** | H1-117 | H1-189 | H1-235 | H2-94 | H2-166 | H2-212 |

### 2.1.3 Field trial 3

Potato plants from cultivar Lady Rosetta (LR) were grown at Cambridge University Farm (CUF), planted on 15 April 2013, and harvested at four time points. Harvest dates are indicated in table 2.3. Controls and application of 200kg/hectare of nitrogen on the soil were studied. Trials were randomised with two factors (harvest and nitrogen application) with six replicate plots. Ten tubers per plot were collected and sent to Leeds on harvest day.

Table 2.3 Field trial 3 – Year 2013

| **Trial 3** | **Harvest period** | | | |
|---|---|---|---|---|
| **Event** | 1st harvest (H1) | 2nd harvest (H2) | 3rd harvest (H3) | 4th harvest 4 (H4) |
| **Days of harvest** | 22th July | 5th August | 22th August | 5th September |
| **Days after planted** | 98 | 112 | 129 | 143 |

### 2.1.4 Tuber preparation for analysis

Tubers were cleaned upon arrival in Leeds to remove soil. Measurements of physical and mechanical properties and bruising assessment (except oxidative potential) were conducted with fresh tubers. Fresh tissue to be used for microscopy was processed by exposure to fixative as described in section 2.6.1.

Potatoes used to analyse biochemical properties (including oxidative potential) were prepared as follows: potatoes were cut transversely into slices of 1 cm thickness and the stolon and bud ends were discarded. The skin was peeled and the cortex was separated from the internal section

(medullar layer) using a blade, Typical anatomy of the tubers is shown in figure 2.1. The medulla was cut in cubes (0.5 $cm^3$). The three different tissues were fast frozen in liquid nitrogen, kept frozen at -20ºC and freeze dried for 48 h (SB4 Freeze drier, temperature -30°C, pressure 1,5 Torr). Each sample used in further analysis (oxidative potential, tyrosine, phenolic acids and cell wall composition) was obtained by mixing equal proportions from 3 potato tubers from each plot. The freeze dried tissue was ground to a fine powder using a food processor for 5 min and stored at -20°C until use.

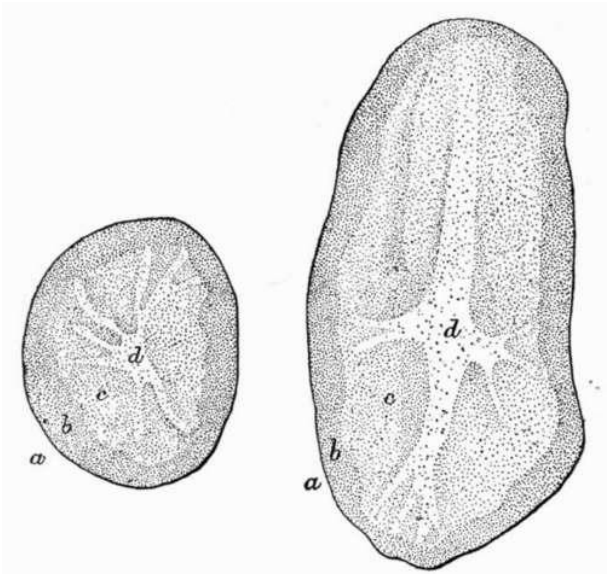



Figure 2.1 Transverse and longitudinal sections of the potato tuber: (a) skin, (b) cortical layer, (c) outer medullary layer and (d) inner medullary area (Grubb and Guilford, 1912).

## 2.2 Bruising assessment

### 2.2.1 Assessment of severe bruising using the falling bolt method – field trial 1

Incidence of bruising was assessed following the falling bolt damage test (Stalham, 2008). Tubers were cooled down to 6 °C in the fridge. The impact was made using a steel coach bolt of 182.6 g in weight with a regular hexagon end with diameter of 13 mm, and overall bolt height of 11.5 mm. The bolt was dropped from a height of 335 mm inside an aluminium guide tube of 40 mm internal diameter onto the flat surface of the tuber leading to a force applied of 0.6 J. The guide tube was held by a pair of retort stand clamps, one acting as a guide, the other clamping the tube at the correct height above the tuber. The impacting surface was a MDF work surface as shown in figure 2.2.

Figure 2.2 Aluminium guide tube for assessment of severe bruising using the falling bolt method

‘Hot boxing’ was performed by placing the potatoes following impact for 48 hours in an incubator at 33 °C, >95% RH. The potatoes were brought to room temperature two hours before being examined by peeling. A single peel (depth 1.2-1.5 mm) was removed at the site of impact using an Oxo Good Grips Swivel Peeler and then a further three peeler strokes were made to detect deeper damage. Calibrations were performed on the peel thickness by measuring 50 random peel slices with a Mercer England Thickness Gage (reading 0.1mm). Bruising was classified following peeling based on a procedure developed at the Sutton Bridge Crop Storage Research (2008).

- No bruise: no visible bruise following initial exploratory peel with a domestic peeler.

- Slight bruise: no visible bruise after two additional strokes of a domestic peeler following initial exploratory peel.

- Severe bruise: bruise visible after two additional strokes of a domestic peeler following initial exploratory peel.

### 2.2.2 Assessment of severe bruising using the falling bolt method – field trial 2 and 3

Adaptations of the method from year 1 were made due to a high incidence of bruising on field trial 2. Impact tests were carried out at 20-22 °C with the steel coach bolt used in field trial 1, impacting on the stolon end of potatoes. Different energy level (0.3 J) and incubation time (25 °C for 20 h, humidity > 95% RH) was used. Following bruising development, potatoes were analysed as previously described on 2.2.1. Determination of Bruising Index

(BI) was also carried out (BPC_LINK 240, 2007). The impact zone of each tuber was analysed and a measure of the width and the depth of pigmented tissue was taken with a visual assessment of the bruise pigment intensity compared with the surrounding tissue, using the following scale:

0 – No visible pigmented tissue at site of mechanical impact

1 – Low level of pigmented tissue (typically pink, red, red-brown, grey)

2 - Intermediate level of pigmented tissue (typically brown or brown-black)

3 - High level of pigmented tissue (typically blue-black or black)

Colour intensity was classified using Munsell Atlas Hue 9R to standardize the assessment of colours as shown in figure 2.3.

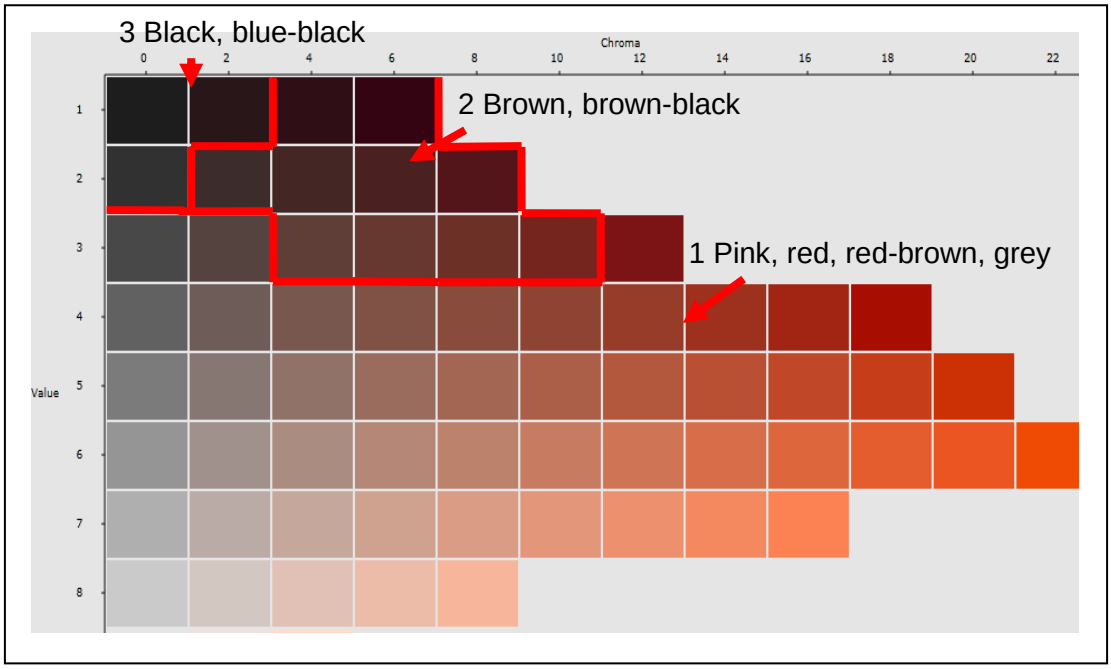



Figure 2.3 Scale used to classify colour intensity of pigmented tissue after falling bolt impact and incubation.

The mean for bruise depth, width and pigment assessment were calculated and used for comparing bruise susceptibility between different varieties, harvests, storage (trial 2), nitrogen application (trial 3) and also on tubers from the same variety. Bruising index was calculated using equation 1:

**Equation 1** $$\frac{\pi \times \frac{1}{2}(\text{bruise width})^2 \times \text{bruise depth} \times \text{bruise pigment intensity}}{235.6}$$

This assumes a cylindrical shaped bruise zone and by dividing by 235.6 this compares the bruise indices on a scale of 0 – 10 to a bruise with diameter 10 mm, depth 10 mm and bruise intensity 3 – the highest value observed in practice.

### 2.2.3 Assessment of external damaged skin using the falling bolt method

After falling bolt impact and hot box incubation of samples for assessment of bruising, samples were examined individually and classified as damaged when skin was broken with or without flesh damage. The percentage of samples damaged was calculated from the total of samples assessed.

### 2.2.4 Falling bolt impact captured by high speed camera

Images of falling bolt impact were recorded with a Phantom v.90 high speed camera V9 (Dantec), at a setting of 1000 frames-per-second (FPS) at the School of Mechanical Engineering, University of Leeds. Samples from harvest 2 (H2) stored for 212 days (S3) were used, using 3 replicates for each variety. The assessment of bruising of these tubers followed the protocol described in 2.2.2.

### 2.2.5 Spectrophometric assessment of oxidative potential

The method was adapted from McNabnay *et al*. (1999). The oxidative potential of tuber tissue to bruising development was determined by measuring the extent of colour development of homogenised tissue under controlled conditions. Assessments were performed using lyophilized cortex tissue samples with three replicates performed per sample. 0.2 g of lyophilized cortex was suspended in 3 mL of 0.05 M phosphate buffer (pH 6.5) and mixed vigorously using a vortex mixer for 1 minute. The homogenate was allowed to oxidise at room temperature for 20 hours. Samples were filtered through a Whatman 4 filter paper and oxidative potential was measured at 475 nm with a spectrophotometer (Cecil CE 7200, Cecil Instruments Limited, Cambridge, UK).

## 2.3 Physical properties

### 2.3.1 Weight and specific gravity

The weight of individual potatoes was measured using a semi-analytical scale (field trial 1 n=9 and field trial 2 and 3 n=30, 10 each per plot).

Specific gravity was determined on individual potatoes from field trial 2 and 3 (n=30 per variety, 10 each per plot) using weight in air and weight in water method (Fong, 1973). Specific gravity was calculated by using equation 2.

**Equation 2** **Specific gravity =** $\frac{\text{Weight in air (g)}}{\text{Weight of the water displaced by the tuber (g)}}$

## 2.4 Mechanical properties

### 2.4.1 Energy required to break the potato skin and cortex tissue

Mechanical properties were investigated using a penetration test with a TA.XT plus Texture Analyser (Stable Micro Systems Ltd, Surrey, UK). For sample preparation, a tuber was taken from ambient temperature, cut transversely in slices of 1 cm in thickness and the slice from the middle of the tuber was separated for the penetration test. The probe used to perform the test was a cylinder with 2 mm diameter, test speed was 20 mm/sec with tagged mode distance 5 mm, with trigger type auto (force) and trigger force 0.5 N. For each potato one test was carried out on the cortex and one test on the skin as shown in figure 2.4. To perform the test on the skin, 1 cm of the side of the potato was cut and placed vertically on the texture analyser plate. Three samples were measured for each plot on potatoes from field trial 1, ten replicates per plot on potatoes from field trial 2 and five replicates from each plot from field trial 3. Force and distance were obtained from the curve plotted from the software of the TA.XT plus Texture Analyser at the yield point (point where rupture of tissue occurs). Energy (mJ) was calculated by multiplying force (N) and distance (mm).

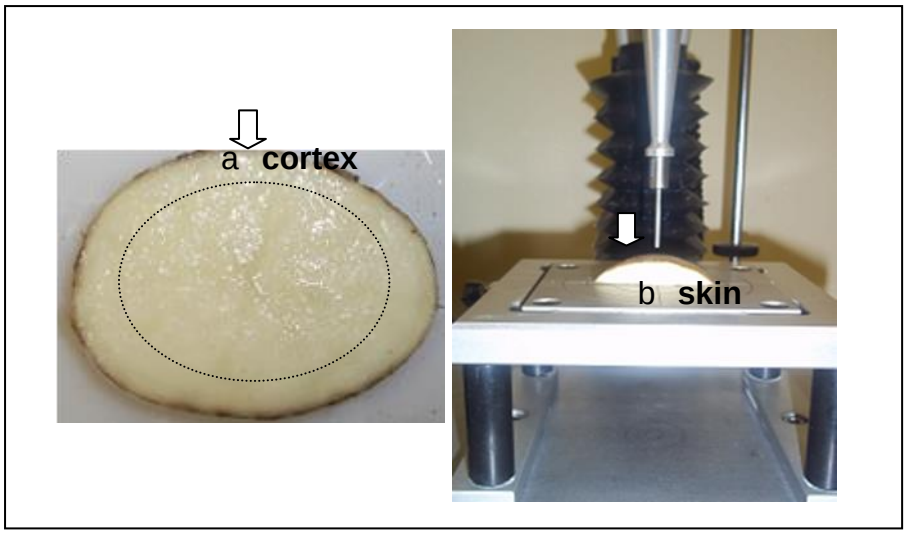



Figure 2.4 Transverse section with the points analysed with penetration test, (a) cortex and (b) skin cortical layer.

## 2.5 Phenolic composition

Chemicals ethylenediaminetetraacetic acid (EDTA) and formic acid were purchased from Fisher Bioreagents and methaphosporic acid from Alpha Aesar. Acetonitrile HPLC grade was purchased from VWR. Milli-Q purified water was used for dilutions and solvent preparation. Phenolic acid standards were purchased from Sigma. A representative range of phenolic acids known to be present in potato was selected for this study namely chlorogenic acids (3-, 4-, and 5-caffeoylquinic acid (CQA)), ferulic acid (FA), vanillic acid (VA), caffeic acid (CA) and *p*-coumaric acid (*p*Cou). Sinapic acid was used as internal standard. Stock solutions of phenolic acids were prepared in duplicate at a concentration of 10 mg/mL in 50% ethanol and the dilutions made with Milli-Q purified water. The stock solution of *dl*-tyrosine was prepared in duplicate at concentration of 10 mg/ml in 0.1 N HCl and dilutions made with Milli-Q purified water. The stock solutions were stored in darkness at 4 °C. The external standard method of calibration was used,

with each curve prepared from 7 different concentrations of standard solutions. The range of standards concentration used was as follows: 5-CQA (3.13 -200 µg/mL), 3 and 4-CQA (0.78 – 50 µg/mL) and CA, VA, *p*Cou and FA (0.16-10 µg/mL). The external standard (sinapic acid) concentration used was 200 µg/ml .

### 2.5.1 Extraction of phenolic compounds

The method of extraction was adapted from Shakya and Navarre (2006). Phenolic compounds were extracted in triplicate from the freeze dried cortex of three tubers with 1.5 mL of extraction buffer (50% MeOH, 2.5% metaphosphoric acid, 1 mM EDTA) and 500 mg of glass beads 1.0 mm in diameter. Tubes were shaken using a vortex for 10 min and sonicated at 10 °C for 10 min. After sonication, tubes were shaken again with the vortex for 10 min. Tubes were centrifuged at 4000 rpm at 4 °C for 10 minutes and the supernatant was collected. Extractions were repeated three times and supernatants combined. The supernatants were dried under vacuum using a centrifugal evaporator at room temperature and low boiling point (BP) condition (Genevac SP Scientific, Ipswich, Suffolk, UK), resuspended in 0.5 mL of Milli-Q purified water and filtered using a 0.45 µm PTFE filter prior to HPLC analysis. Samples were kept chilled at all times and not exposed to bright light.

### 2.5.2 Analysis of phenolic acids using high performance liquid chromatography

Analysis of phenolic compounds was performed according to the method of Farrel *et al.* (2011) using a reversed phase HPLC Agilent 1200 Series HPLC consisting of a solvent degassing unit, binary pump, autosampler, thermostatic column oven and diode array detector. The column used was an Agilent Zorbax Eclipse plus C18, 4.6 mm x 100 mm, 1.80 micron internal diameter and 600 bar maximum pressure. Column temperature was 35 °C, flow rate of 0.26 mL/min and injection volume of 5 µL. The 61-min elution program consisted of a isocratic elution from 0-17.5 min with 100% solvent A (0.1% formic acid, 5% acetonitrile and 94.9% water), followed by linear gradient from 17.5-51 min to 25% solvent B (0.1% formic acid, 5% water and 94.9% acetonitrile), linear gradient from 51-51.1 min up to 100% solvent B, isocratic elution from 51.1-56 min with 100% solvent B, linear gradient from 56-56.1 to 0% solvent B and isocratic elution from 56.1-61 min with 0% solvent B. The photo-diode array detection spectra was recorded at wavelengths of 220, 260, 280, 300, 310 and 325 nm.

### 2.5.3 Analysis of tyrosine using high performance liquid chromatography

Reversed phase HPLC Shimadzu (Prominence) consisting of a solvent delivery unit, column oven, autosampler, UV-Vis detector, photo-diode array detector, and on-line degassing unit was used to analyse tyrosine. The column used was Phenomenex Onyx, 4.6mm x 150 mm, 5 micron internal diameter. Column temperature was 30 °C, flow rate of 1.5 mL/ min and

injection volume of 10 µL. The 22-min elution program consisted of isocratic elution from 0-9 min with 100% solvent A (10 mM formic acid, pH 3.5, with ammonium hydroxide), followed by a linear gradient from 9-10.5 min 35% buffer B (100% methanol with 5 mM ammonium formate), linear gradient from 10.5 -14 min with 65% solvent B; linear gradient from 14-16.5 min up to 100% solvent B, linear gradient from 16.5 -18 min to 0% B and isocratic gradient from 18-22 min with 0% solvent B. UV-VIS detection spectra was recorded at a wavelength of 280 nm. The external standard method of calibration was used, with each curve prepared from 7 different concentrations of standard solutions. The range of standard concentration used was (0.58-300 µg/mL). The external standard (sinapic acid) concentration used was 200 ug/ml .

## 2.6 Cell wall ultrastructure and composition

### 2.6.1 Immunofluorescence localization of cell wall polymers

Fresh tuber specimens (0.5 $mm^3$) were fixed in 4% formaldehyde in PEM buffer (50 mM Pipes, 5 mM $MgSO_4$ and 10 mM EGTA, pH 6.9). Fixative was removed with PEM and the samples was washed with phosphate buffered saline (PBS), dehydrated in ethanol 30-70% series and embedded in Steadman wax (9:1 polyethylene glycol 400 distearate and 1-hexadecanol). Wax embedded periderm samples were sectioned using a microtome with blade at 11 degrees and 50 µm thickness. Sections were placed onto polysine-coated glass slides, followed by dewaxing with ethanol 97-50% series. Prior to the labelling procedure, sections were incubated with 150 µL of 3% (w/v) milk protein in PBS for 1 hour to reduce nonspecific binding.

Monoclonal antibodies JIM5 and JIM7 were kindly provided from Professor Paul Knox (Centre for Plant Sciences, University of Leeds, UK). The sections were incubated overnight at 4°C in the primary antibodies, diluted 1:5 in PBS with milk. Control sections were incubated in PBS alone. Samples were washed twice with 0.1% v/v Tween 20 in PBS for 10 min. All sections were incubated at room temperature for 1 hour in secondary antibody anti-rat FITC (Sigma), diluted 1:100 in PBS. Samples were washed 10 min with 0.1% v/v Tween 20 in PBS plus 10 min in PBS. Samples were then stained with 0.1% Toluidine Blue for 10 min, washed for 10 min in PBS, mounted with anti-fading glycerol phosphate buffered solution (Citifluor AF1, Agar Scientific, UK) and covered with a glass cover slip. Observations were made with a BH2 Olympus microscope equipped with blue epifluorescence and Confocal Zeiss Axioplan Imaging LSM 510 Meta.

### 2.6.2 Optical localization of biological wall membranes

Fresh samples were hand cut and embedded samples (as described in 2.6.1) were used. Samples were cut using a microtome to thickness varying between 12-35 μm. Fresh and dewaxed samples were stained with 0.1% Toluidine Blue for 10 min, washed for 10 min in PBS, mounted on glass slides and covered with a glass cover slip. Observations were made using light illumination with optical BH2 Olympus microscope.

## 2.7 Analysis of Cell Wall Material (CWM)

### 2.7.1 Extraction

Isolation of the cell wall material was achieved using adapted methods by Jardine *et al*. (2002); Øbro *et al.* (2004); Ross *et al.* (2011a) and commercial enzymatic protocols (Megazyme methods) to analyse total starch (amyloglucosidase/ *α*-amylase method) and total dietary fibre. The enzymes used to hydrolyse starch and protein are summarised in table 2.4. Lyophilised cortex (1 g) was homogenised using a homogeniser (Ultra Turrax , IKA, Staufen, Germany) at 13.500 rpm with 5 mL of mixed-cation buffer (MCB) (10 mM NaOAC, 3 mM KCl, 2 mM $MgCl_2$ and 1 mM $CaCl_2$, pH 6.5) containing Triton X-100 (2 mg/ml). The adequate disruption was achieved with up to 5 minutes of homogenisation and checked under the light microscopy using Toluidine Blue for staining the cells' membranes. All procedures were carried out at 4 ˚C. The detergent suspension was removed by washing through a 45 µm metal sieve with 10 mL of chilled MCB without Triton X-100. The residue was washed with 10 mL of 50% chilled acetone. To deproteinate samples, two procedures were tested. On the first, the washed residue was stirred with 80% (v/w) saturated phenol for 30 minutes following by filtration and washes with MCB. After this step, gelatinisation was carried out in 10 mL MCB at 80 °C for 45 minutes following by incubation with *α*-amylase as described below. On the second method tested the washed residue was gelatinised with 10 mL MCB at 80°C for 45 minutes and incubate at 40 °C for 45 min with 400 µl of pancreatin solution (10 mg/mL) (P7545 Sigma) before starch digestion with 11.700 U

heat stable *α*-amylase (A3306 Sigma)**.** The temperature was cooled down to 20 °C following incubation for 2 h at 20 °C.

After the *α*-amylase incubation step, the suspension was adjusted to pH 5.0 with 1M acetic acid and a combination of pullulanase (12 U) (P5420- Sigma) and amyloglucosidase (12 U) (A9913 Fluka) were added to enzymatically degrade branched starch. After incubation for 14 hours at 25 ˚C, the presence of starch was monitored by removing small aliquots of the insoluble material and staining with 0.2% iodine to visualise the starch using light microscope. The cell suspension was washed using a metal sieve 45 µm with 2 L of water and 10 mL of 50% acetone.

Table 2.4 The enzymes involved in the hydrolysis of starch and protein

| **Source** | **Substrate** | **Specific Activity Unit/ Portion** | **Optimum pH** | **Stable pH** | **Optimum temperature** | **Stability temperature** |
|---|---|---|---|---|---|---|
| ***α* – Amylase – One unit will liberate 1 mg of maltose per min** | | | | | | |
| *Bacillus licheniformis* | p-nitrophenyle maltoheptaoside | 39,000 U/mL | 6.9 | 5.1-8.2 | 20 °C | < 75 °C |
| **Amyloglucosidase – one unit liberate 1.0 mg of glucose per min** | | | | | | |
| *Aspergillus niger* | starch | 2,725 U/mL | 5.0 | | 25 °C | |
| **Pullulanase – one unit liberate 1 umole of maltotriose per min** | | | | | | |
| *Klebiela pneumonia* | pullulan | 32,877 U/mL | 5.0 | | 25 °C | |
| **Pancreatin 8 × USP specifications - amylase, trypsin, lipase, ribonuclease and protease**. | | | | | | |
| Porcine pancreas | proteins, starch and fats | >250/mg solid | 7.5 | | 40 °C | |

After washing, the purified cell wall material (CWM) was dried overnight in an oven at 35 °C. CWM hydrolysis was performed in duplicate in two steps. 2 mg of CWM were first hydrolysed with 1 mL of 0.1 M trifluoroacetic acid (TFA) for 1 h at 100 °C. Samples were centrifuged at 4000 rpm at 4 °C for 10 minutes and the supernatant was collected. The CWM solid residue from step one was then hydrolysed with 2 M TFA for 1h at 100°C. Tubes were centrifuged at 4000 rpm at 4°C for 10 minutes and the supernatant was collected. 500 µl from each supernatant (0.1 and 2 M TFA) were combined and the TFA was removed using a centrifugal evaporator (Genevak, Surrey, UK). Dried samples were resuspended with 1 mL of milli-Q purified water and filtered using a 0.45 µm nylon filter prior to Dionex analysis. Samples were kept chilled at all times and not exposed to bright light.

### 2.7.2 Analysis of monosaccharide composition using high performance anion exchange chromatography amperometric detection (HPAEC - PAD) - Dionex

The method used was adapted from Øbro *et al*. (2000). The monosaccharide composition was determined with high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). The column used was PA20 (Dionex, Thermo Scientific). Column temperature was 30 °C, flow rate of 0.30 mL/min and injection volume of 10 µL. The 65-min elution program consisted of linear gradient from 10 uM to 5µM NaOH from 0 – 1.5 min, followed by isocratic elution with 5 µM NaOH from 1.5 – 30 min, linear gradient up to 1 M NaOH from 30-40 min, column

washing with 1 M from 40-45 min, linear gradient to 10mM from 45-55 min following equilibration of the column with 10 mM NaOH from 55 to 65 min. Monosaccharides were detected using a pulsed amperometric detector with gold working electrode and silver reference electrode. Monosaccharide standards were *L*-Fucose, *L*-Rhamnose, *L*-Arabinose, *D*-Galactose, *D*-Glucose, *D*-Xylose, *D*-Manose, *D*-Galacturonic acid and *D*-Glucuronic acid. Fructose was used as an internal standard. A standard mixture run was performed before sample analysis to determine response factor. The external standard method of calibration was used, with each curve prepared from 7 different concentrations of standard solutions. The range of standards concentration used was from 0.39-100 µg/mL. The internal standard (fructose) concentration used was 200 µg/ml .

### 2.7.3 Linearity, precision and accuracy

Method validation for analysis of sugars was performed according to ICH recommendations and the European Commission Directive for the performance of analytical methods including linearity, precision and accuracy, which are principal components of quantification. Linearity was investigated by analysis of peak area response versus concentration over a range of 11 ng/mL to 150 µg/mL. For calibration curves, the peak areas of the Dionex chromatogram were plotted against on-column amount and analysis was performed on 2 separate occasions with triplicate injections of each concentration. Precision and accuracy were evaluated for galactose at 3 quality control (QC) concentration (25, 50 and 100 µg/mL). The lower QC is representative of the lower concentration of hydrolysed sugars from CW samples, and the high QC level is near the upper boundary of the standard

curve. Intra-day precision and accuracy was calculated from triplicate injection of the 3 concentrations on the same day. Inter-day precision and accuracy was determined by analysis of triplicate injections of the 3 concentrations on the 3 separate days. Values for precision are expressed as relative standard deviation (R.S.D) and relative error (R.E.) for accuracy.

### 2.7.4 Stability of monosacharides at 10° C storage

To assess stability at 10 °C, which is the temperature of the Dionex autosampler, monosaccharides arabinose, galactose and glucose were diluted from concentrated stock to final concentration of 25 µg/ml. Standards were combined and refrigerated (10 °C) for 3 days (n=3). The initial concentration was determinate to be > 95% of the expected value and the percentage of initial concentration remaining was determined at 3 d storage (10 °C).

### 2.7.5 Recovery efficiency for hydrolysis studies

Extraction efficiency experiments were performed with combined monosaccharides and diluted from concentrated stock to 25 µg/mL. Aliquots of 1 mL were dried (n=3) and hydrolysed in 0.1 M and 2 M TFA at 100°C for 1h. After hydrolysis, samples were dried using a centrifugal evaporator (Genevac SP Scientific, Ipswich, Suffolk, UK) ressuspended in Milli-Q purified water and the recovery was analysed by comparison with initial concentration (n=3 injections).

### 2.7.6 Percentage of sugar released from CW in each step of hydrolysis

The percentage of monosaccharides released from CW was investigated after partial hydrolysis with 0.1 and 2 M TFA and total hydrolysis with 1M $H_2SO_4$. The total amount and percentage of sugars released from potato cortex cell wall under the sequential hydrolysis was calculated and the percentage of each step of hydrolysis was estimated.

## 2.8 Statistical analysis

Mixed effects analysis of variance ANOVA was explored for harvest time (field trial 1, 2 and 3) and storage time (only field trial 2) being random factors and the variety (field trial 1 and 2), defoliation (field trial 1) and supplement of nitrogen (field trial 3) considered as fixed factor. Multiple comparisons have been performed with Student-Newman-Keuls (SNK) and confirmed with REGWQ - Ryan/Einot and Gabriel/Welsch test procedure. Effects on individual variety were explored using a factorial 2-way ANOVA and Tukey multiple comparison test to analyse the effect of factors harvest and defoliation (field trial 1), storage (field trial 2) or supplement of nitrogen (field trial 3). The relationships between results were summarised using Principal Component Analysis (PCA). Statistical analysis is performed using R for Windows (R Core Team, 2014).

The Student's t test (Excel, Microsoft 2010) was performed to compare two samples on method development. Error bars shown on the graphs are standard errors of the mean.

# 3 Method development

## 3.1 Introduction

Many different approaches need to be adopted to analyse the structure and composition of potatoes. It was therefore necessary to test methods according to the material and equipment available in the laboratory, requiring optimisation and adaptation of published protocols.

This chapter describes the development of methodologies for the analysis of potato samples, showing adaptations and improvements achieved.

### 3.1.1 Aim

Adaptation and development of reliable and reproducible methods to analyse structure and composition of potatoes that could be linked to bruising incidence.

### 3.1.2 Objectives

The chapter objectives are:

- To test different methods used for bruising assessment.
- To establish a standard classification of colours developed in bruised tissues.
- To understand differences in mechanical properties when penetration test was applied through cortex and skin tissue.
- To determine the complete extraction of phenolic acids .

- To adapt a method for tyrosine analysis HPLC-UV.
- To optimize a method for extraction of cell wall material.
- To adapt a method for cell wall sugar analysis using HPAEC - PAD .

## 3.2 Methods optimized

### 3.2.1 Bruising assessment

A high speed camera was used to study how the bolt impacts the potatoes when using falling bolt method to damage tubers. It was found that the bolt impacts the tubers twice when it is dropped on the stolon of potato as indicated in figure 3.1, transmitting to the tuber more energy than predicted (0.3-0.6J) by potential energy (E=m.g.h). This questions the suitability of the current bruising methods.

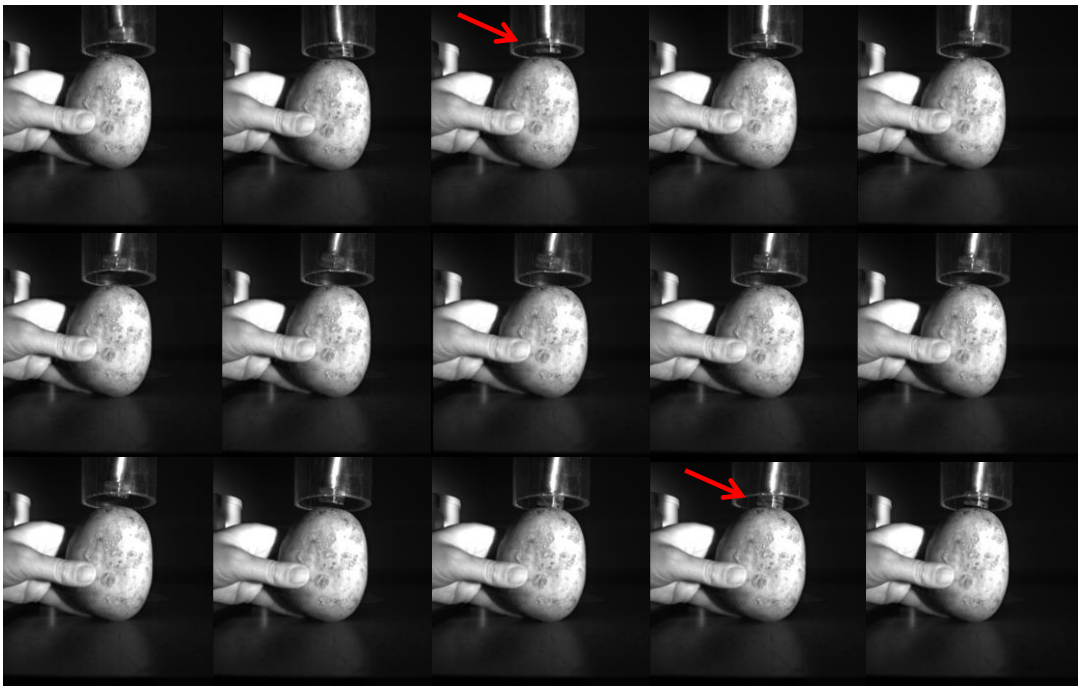


Figure 3.1 Impact on the stolon end of the potato using falling bolt. Images from high-speed camera every 0.02 sec. Arrows indicate contact between bolt and tuber.

To understand if the second impact was dependent on the mechanical properties, the coefficient of restitution was calculated from the square root of the ratio of the bounce height after first impact to the drop height (Robertson *et al*., 2013) and correlated with bruising index as shown in figure 3.2.

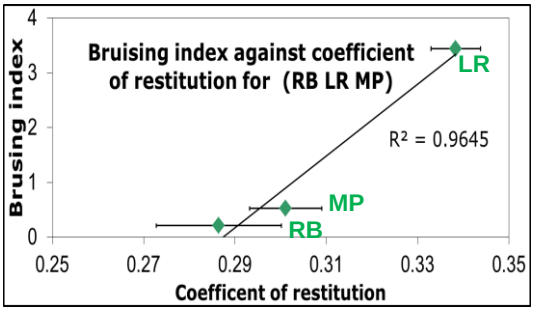



Figure 3.2 Coefficient of restitution calculated from images recorded using high speed camera and bruising index of potatoes damaged with falling bolt 0.3 J energy and incubated at 25 °C for 20 h (n=3).

Based in figure 3.2, mechanical properties played a role on the impact of the bolt on the stolon as the height of the bolt reached after first impact was different for the varieties studied. Due to limited number of samples tested, it was not possible to establish a factor for correction on bruising data based on mechanical properties but it will be further explored with results from the field trials.

From pictures of the high speed camera it was also possible to understand how the bolt was impacting the lateral and the stolon end of potatoes. It was found that the bolt impacts the tubers at a variety of angles resulting in variable distribution of energy of impact. Samples impacted on the side of potatoes (figure 3.3) were more evenly impacted (7 out of 9) than samples

impacted at the stolon (3 out of 9) as seem in figure 3.4. The angle of the bolt was also dependent of the curvature of the potato as shown in figures 3.3 and 3.4.

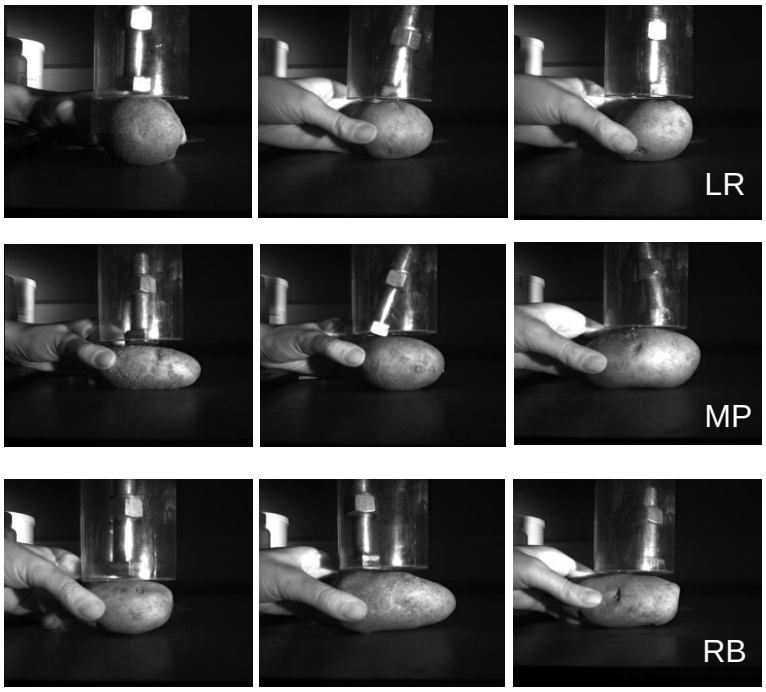



Figure 3.3 Images of the impact on the lateral of tubers

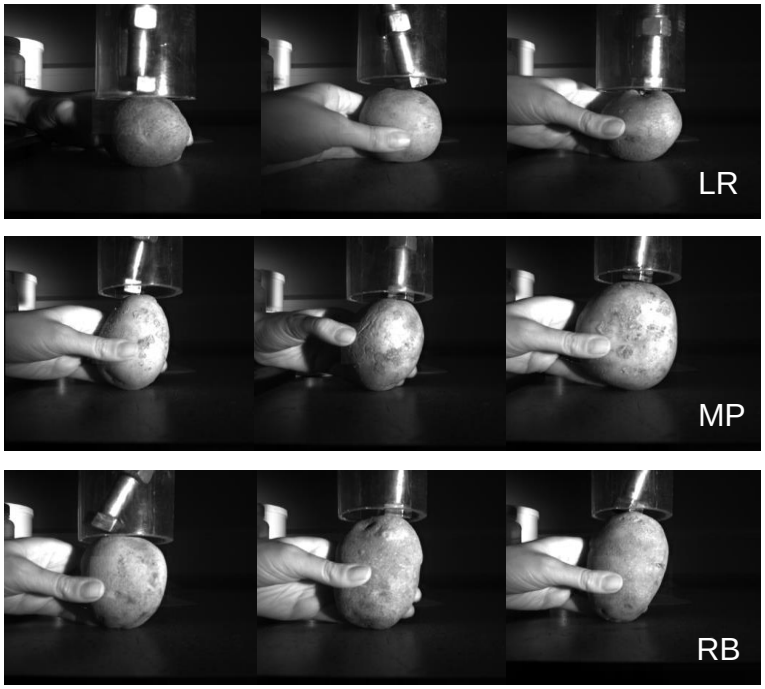



Figure 3.4 Images of the impact on the stolon of tubers.

Although samples impacted on the side of potatoes were more evenly impacted, no bruising was found in potatoes damaged at room temperature with 0.3 J impact and incubated at 25 °C for 20 h. These results could give a better understanding about of the results from the first year trial (chapter 4), where no bruising was found up to the third harvest. Bruising results from the first year trial were achieved by applying the falling bolt to the lateral side using 0.6 J impact energy on potatoes previously refrigerated at temperature below 10 °C following by incubation at 33 ° C for 48 h. From the results presented here it is possible to conclude that the cortex at the lateral side has the biochemical apparatus for bruising but is less biologically active than the stolon.

Moreover, when samples impacted on the stolon had a flat contact with the surface of the potato, the width of bruised tissue was higher than in samples

with angle contact as indicated in table 3.1 (results in bold). These results do not always correspond to deeper of bruised tissue or higher formation of colour. For this reason, it was important to keep two types of assessment of bruising: (1) assessment of severity of bruising as Potato Council protocol, where only depth of bruised tissue was analysed and (2) assessment of bruising index, where volume of area affected were integrated with colour formation.

Table 3.1 Analyze of depth, width and colour intensity of samples impacted on the stolon (n=3) as indicated on figure 3.4. Samples shown in bold had flatter contact with the bolt.

| Samples | Depth (mm) | Width (mm) | Colour intensity |
|---|---|---|---|
| LR 1 | 3.5 | 9.6 | 2 |
| LR 2 | 4.4 | 7.5 | 3 |
| LR 3 | 6.9 | 13.1 | 3 |
| Average | 4.9 | 10.1 | 2.7 |
| | | | |
| MP1 | 2.6 | 5.7 | 3 |
| MP2 | 0.7 | 4.2 | 1 |
| MP3 | 3.2 | 9.0 | 3 |
| Average | 2.2 | 6.3 | 2.3 |
| | | | |
| RB1 | 1.5 | 4.6 | 1 |
| RB2 | 2.5 | 5.2 | 2 |
| RB3 | 3.7 | 4.9 | 1 |
| Average | 2.5 | 4.9 | 1.3 |

On bruising index assessment, a relevant point is to develop a reliable method to classify the colour developed on bruised tissue after damage and incubation. This classification could vary with local illumination and also due personal interpretation. For this reason, a standard method was developed and bruised samples were classified using the Munsell Atlas Hue 9R (figure 2.3). As colour on the computer screen and printed paper can change with

screen/print inject used, the same printed version was kept to classify bruising intensity on field trial 2 and 3. Figure 3.5 shows examples of bruised samples and the category classified.

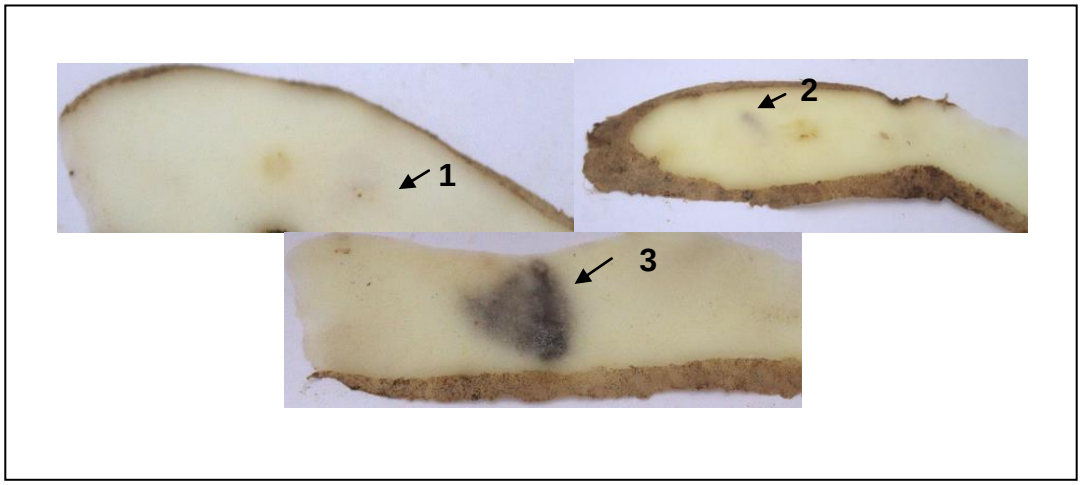



Figure 3.5 Bruising colour classification according to Munsell Atlas Hue 9R shown in figure 2.3, variety RB. Numbers indicate the classified intensity of colour.

### 3.2.2 Mechanical properties

#### 3.2.2.1 Energy required to break the skin and cortex tissue

To assess the texture of tubers along harvest and storage times and with application of nitrogen, a penetration test was carried on the cortex and skin tissue of tubers. Force and distance to break the potato tissue (called yield point) were obtained from the curve plotted from the software of the TA.XT plus Texture Analyser as shown in figure 3.6. Energy (mJ) was calculated by multiplying force (N) and distance (mm) at yield or break point.

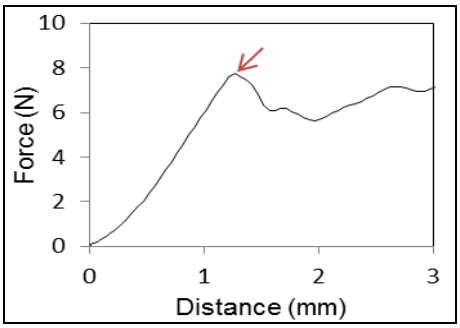



Figure 3.6 Typical graph from Texture Analyzer. Arrow indicates the yield point and force (N) and distance (mm) were collected from the TA software.

It was possible to estimate the number of cells in contact with the probe at yield point by dividing the circle area of probe (π.r= 3.14 mm) by the ellipse area of cells from cortex (π.a (65 µm) .b (35 µm). Diameters of potato cell were estimated from micrographs shown in figure 3.7. It was found that about 1758 cells from cortex were broken at the yield point.

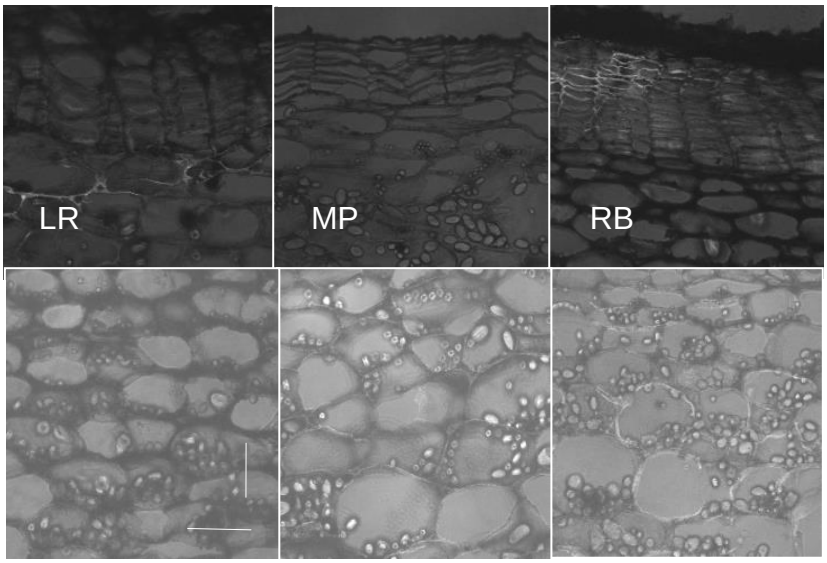



Figure 3.7 Optical microscopy of potato skin (top micrographs) and cortex (bottom micrographs) tissue stained with toluidine blue. Magnification 20x. Error bar 50 µm.

Varietal differences were found at the microscopic level of the skin of potatoes. Maris Piper presented cells which were not regularly stacked on top of each other in the skin and higher energy (~1.5X) was required to break the tissue, while Russet Burbank, had the most regular structural organisation (cells stacked in regular rows) required lower energy to break the skin (data not shown). This observation indicates that greater force was required to penetrate tissue across cells (therefore breaking through cell walls) than between cells. It can be concluded that the method chosen was sensible to analyse varietal differences on the skin.

## 3.2.3 Phenolic compounds

### 3.2.3.1 Extraction of phenolic compounds

A variety of diverse solvents are used to extract plant phenolics. Some examples of extraction solvents used are methanol (Hale *et al*., 2003,

Blessington *et al*., 2010, Sotilho *et al*, 1994 and Rhamamurthy *et al*., 1992), methanol with acid (Lewis *et al*., 1988, Shakia and Navarre, 2006), ethanol (Malmberg and Theander, 1985) and hexane (Dao and Friedman, 1992). Shakya and Navarre (2006) have found better recoveries of chlorogenic acid and tyrosine in samples extracted with acidified 50% methanol, when comparing with extractions made with 90% ethanol, 80% acetone and 80% methanol. They also obtained equivalent of superior extraction with freeze-dried tissue using two sequential 15 min extractions with mini-beadbeater96 when comparing with shaking samples 1-24 h in the dark either at room temperature or 4 °C or extractions in which tissue was boiled.

Due these facts and no availability of mini-beadbeater96 in the school, adaptations of the method were tested. First, extractions were carried out using either one of two different instruments: the Ultraturrax homogenizer and vortex mixer using 50% methanol (MeOH) acidified with 2.5% metaphosphoric acid as extractant of phenolic compounds. Secondly, extractions were carried out with either 50 or 70% of MeOH acidified with 2.5% metaphosphoric acid using vortex alone. For both methods tested the sequence of procedures was as follows: 10 minutes of either disruption in ultraturrax or shaking in vortex, followed by sonication for 10 minutes and return to the instrument for more 10 minutes extraction. This sequence was repeated twice.

The method using acidified 50% methanol as a solvent and using vortex presented superior extraction, as shown in Table 3.2.

Table 3.2 Method for extraction of phenolic acid tested. Extraction with 50% MeOH using ultraturrax and 50 and 70% MeOH using vortex. Values represent average ± SD (n=4).

| **Method** | **Ultraturrax with acidified 50% MeOH** | **Vortex with acidified 50% MeOH** | **Vortex with acidified 70% MeOH** |
|---|---|---|---|
| Compound | mg/100g dry weight | mg/100g dry weight | mg/100g dry weight |
| **3-CQA** | 0.99 ± 0.01 | 1.08 ± 0.18 | 0.80 ± 0.04 |
| **5-CQA** | 29.84 ± 0.56 | 39.12 ± 3.79 | 21.87 ± 0.34 |
| **4-CQA** | 2.48 ± 0.06 | 2.77 ± 0.43 | 1.55 ± 0.06 |

Sinapic acid was chosen as internal standard because it is a common compound in broccoli, kale, other leafy brassicas and citrus juice, but not in potatoes. It was found that after repetition of randomised samples, qualitative and quantitative analyses of extracts were reproducible, allowing the phenolic acids to be meaningfully compared among extracts from different varieties and treatments.

To certify that extraction of phenolic acids were complete, four extractions of the same samples were made. The percentage obtained in each extraction is shown of table 3.3. According to the results from four extractions, there were some chlorogenic acid after three extractions remaining in the sample, however the forth extract contained maximum 3.85% of total extracted yield. For this reason it was decided to carry on with only three extractions to analyse phenolic compounds of samples.

Table 3.3 Percentage of phenolic acid released in each step of extraction with acidified 50% MeOH and using vortex method.

| Extraction/ Compound | 1st Extraction | 2nd extraction | 3rd extraction | 4th extraction |
|---|---|---|---|---|
| 3-CQA | 69.59 ± 4.28 | 18.47 ± 0.11 | 8.10 ± 1.06 | 3.85 ± 0.31 |
| 5-CQA | 71.05 ± 0.10 | 19.15 ± 0.08 | 7.97 ± 0.08 | 1.83 ± 0.02 |
| 4-CQA | 71.14 ± 1.82 | 19.81 ± 2.64 | 7.01 ± 0.02 | 2.05 ± 0.02 |

#### 3.2.3.2 Analysis of phenolic acids using high performance liquid chromatography

Good separation of phenolic acids investigated was found when a method previously optimized for characterization of hydroxicinnamic acids conjugates from Farrell *et al*. (2011) was applied. Typical HPLC-DAD chromatograms are presented in figure 3.8.

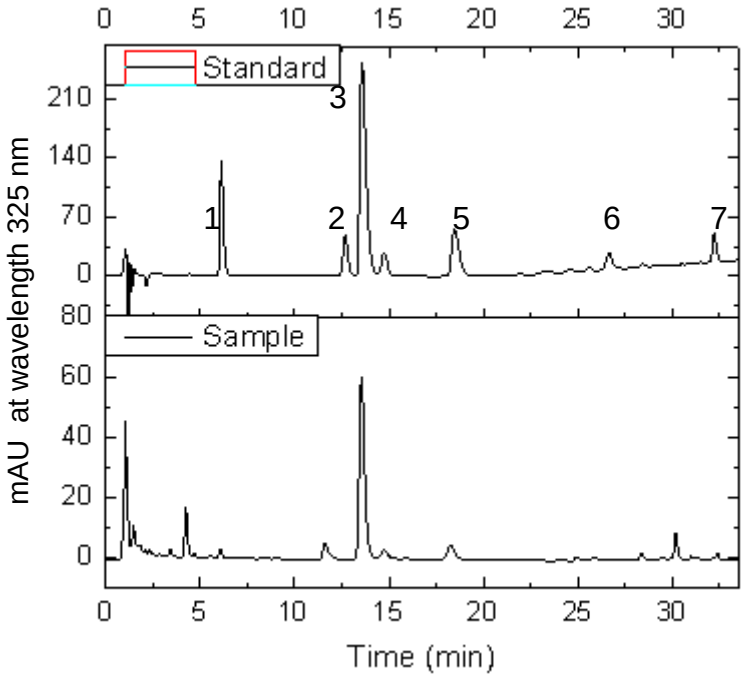



Figure 3.8 HPLC separation of standards and sample profile detected at wavelength 325 nm. Peaks: (1) 3-CQA, (2) VA, (3) 5-CQA, (4) CA, (5) 4-CQA, (6) p-Cou and (7) FA.

The response factors of phenolic acids investigated by HPLC–DAD are summarised in Table 3.4. The limit of quantification (LOQ) of chlorogenic acids, the predominant phenolic acids found in potatoes, was in a range between 0.71-2.82 µg/mL. Minor compounds presented LOQ of 0.14 ug/ml. Maximum wavelengths of each compound was determined. The majority of compounds presented higher absorption spectra at 325 nm and only vanillic acid absorbed at 220 nm.

Table 3.4 HPLC-DAD characterization of phenolic acids, limit of quantification (LOQ), calibration (R) and spectra of maxima absorbance. (a) Mean of retention times (Rt) ± standard deviations of 10 replicates and (b) Relative standard deviations (RSD) of retentions times (%).

| Peak no. | Compound | Rt (min) Mean ± SD [a] | RSD (%)[b] | LOQ (ug/ml) | $R^2$ | Spectral λ max |
|---|---|---|---|---|---|---|
| 1 | 3-CQA | 6.51 ± 0.01 | 0.12 | 0.74 | 0.9994 | 325 |
| 2 | Vanillic acid | 13.30 ± 0.01 | 0.04 | 0.14 | 0.9996 | 220 |
| 3 | 5-CQA | 14.98 ± 0.03 | 0.22 | 2.82 | 0.9991 | 325 |
| 4 | Caffeic acid | 15.56 ± 0.02 | 0.10 | 0.14 | 0.9975 | 325 |
| 5 | 4-CQA | 20.43 ± 0.01 | 0.06 | 0.71 | 0.9991 | 325 |
| 6 | p-Coumaric acid | 27.36 ± 0.03 | 0.12 | 0.14 | 0.9982 | 325 |
| 7 | Ferrulic acid | 32.69 ± 0.02 | 0.08 | 0.14 | 0.9986 | 325 |
| 8 | Sinapic acid | 34.53 ± 0.02 | 0.06 | - | - | 325 |

### 3.2.3.3 Analysis of tyrosine using high performance liquid chromatography

Two columns were tested during process of selecting method for high-throughput analysis of tyrosine following Shakya and Navarre (2006) method. Initially a rapid resolution column Zorbax XDB RR HT, size 4.6 x 50mm, pore size 1.8 at flow rate ranged from 0.4 – 1 ml/min was tested. With Zorbax column ascorbic acid and tyrosine were coeluting and even reducing flow rate, attempting to spread the elution of both compounds, it was not giving good resolutions of compounds as retention time of both compounds was varying from 0.5 and 1 min. The second column tested was Phenomenex Onyx, 4.6mm x 150 mm, 5 micron internal diameter, flow rate 0.4 to 2 ml/min. Phenomenex column separated tyrosine and ascorbic acid. Good sensitivity and peak sharpness of tyrosine was achieved with flow rate of 1.5 mL/ min as shown in figure 3.9

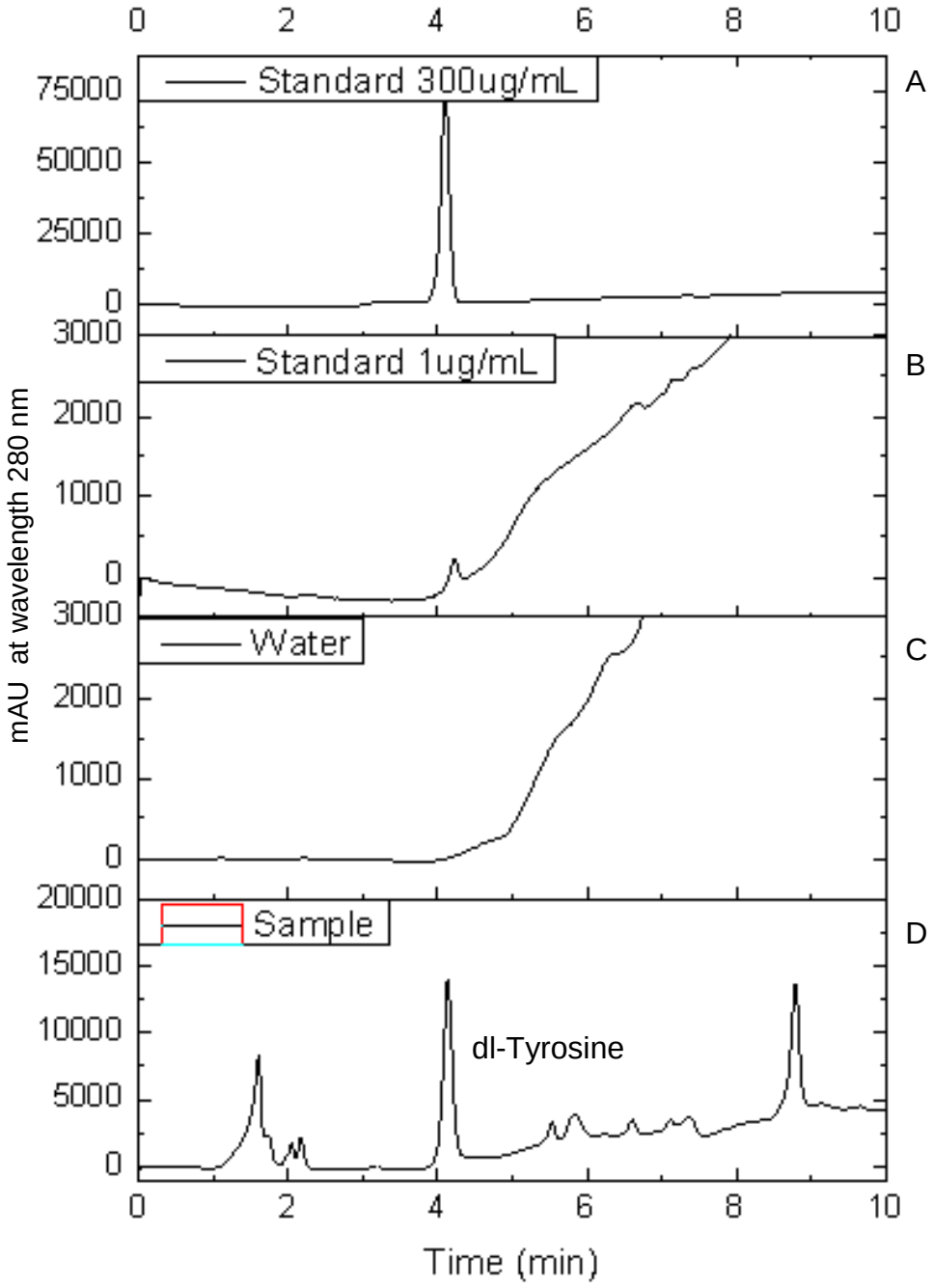



Figure 3.9 HPLC-UV analysis of standard detected at 280 nm. (A and B) Upper and lower boundaries of standard curve, (C) water profile and (D) sample profile.

Tyrosine presented mean of retention time 4.12 min ± 0.05 (SD), relative standard deviations of retentions times 0.01%, limit of quantification 1 µg/ml, linearity of curve 0.9994 and UV spectra maximum at 280 nm. It was found that standards with concentration below 1 µg/mL were causing reduction of

linearity of the standard curve. It may be due the increase of methanolic buffer was affecting the baseline (water profile in figure 3.9) and thereafter the area of the compound.

### 3.2.4 Cell Wall composition

#### 3.2.4.1 Microscopy and immunolocalisation

Structural studies can be done using specific localising reagents (for example, stains, lectins, or antibodies) together with bright-field, fluorescence, or electron microscopy to localise specific epitopes within the cell wall structure (Albersheim *et al*., 2011).

Microscopy techniques (e.g. optical or light, electron and atomic microscopy) vary in methods of image production, resolution, and type of signal detected, and give a particular type of structural information that is unique to the technique used. Bright field, polarizing, and fluorescence microscopy techniques are used most frequently. In conventional bright-field microscopy, illumination is transmitted sequentially through a condenser. If the specimen is not highly coloured, contrast must be introduced to make it visible. This is commonly achieved by the use of dyes or stains of known specificity for different components of the specimen (Kaláb *et al*., 1995).

Advances in instrumentation have been made in light microscopy, most notably in the development of confocal laser scanning microscopy (CLSM). This method not only provides an image with better resolution than conventional light microscopy or fluorescence microscopy, but also provides

an opportunity to observe a 3-dimensional image without creating the need to physically section and observe the same sample in the z-direction. In CLSM, a laser source is focused by the objective lens to illuminate a single, precisely defined point in the specimen (the focal point). A scanning device deflects the beam in the X/Y, X/Z, or Y/Z dimension, thereby scanning the focused spot on the specimen to create an image of the X/Y, X/Z, or Y/Z focal plane. Reflected and fluorescent light returns via the illumination path, and is then focused by the optics of the microscope at the confocal point at the center of a pinhole. Since the spot on the pinhole and the spot on the specimen are both located in the focal plane of the imaging lens, they are said to be confocal. The CLSM is most advantageous in its ability to provide extraordinarily thin, in focus, high-resolution optical sections through a thick specimen (Aguilera and Stanley, 1999). CLSM has been used to examine the pectin deposition in relation to pit fields at the plasma-membrane-face of tomato pericarp cell walls with the use of monoclonal antibody JIM5 (Casero and Knox, 1995).

#### 3.2.4.2 Monoclonal antibodies

Several monoclonal antibodies to plant cell wall polymers have been generated (Knox, 2014) to investigate the anatomical characteristics of the cortical tissue by immunofluorescence microscopy. Specific to pectin, the antibody JIM5 binds to completely de-esterified homogalacturonan domain of pectic polysaccharides (Willats *et al*., 2000; Knox *et al*., 1990). Another antibody used to investigate pectin is JIM7, which binds to the homogalacturonan domain of pectic polysaccharides, with a range of esterified pectin from about 15 to 80% (Willats *et al*., 2000) but does not bind

to un-esterified homogalacturonan (Knox *et al*., 1990), shown in figure 3.10. These antibodies can recognise pectic polysaccharides in several species. For both antibodies there is no known cross-reactivity with other polymers (Knox *et al*., 1990).

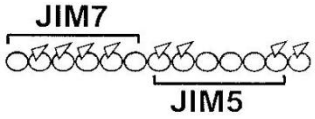



Figure 3.10 JIM5 and JIM7 recognise a range of partially methyl-esterified HG structures with representative's epitopes shown above (Knox, 2014)

Knox *et al*. (1990) used monoclonal antibodies for un-esterified pectin (JIM5), with the degree of esterification (DE) above 35% and methylesterified (JIM7) with the range of 35 to 90% DE, to detect pectin in the root apex of carrot. In a study by Parker *et al*. (2001) JIM5 and JIM7 were also used to investigate the distribution of pectic polysaccharides in the separated cells at the potato surface.

To investigate pectin localization on cortex tissue, samples were embedded in wax following protocol used with other agricultural products. Sections of embedded tissue were probed with monoclonal antibodies to analyse methylation of pectin.

First results showed that embedding time in wax (2 x 1h) was not enough for the wax to penetrate into tissue. Samples were crumbling when sectioned using microtome with blade at 11 degrees and 12 µm thickness.

A second test was made where thicker sections of 32 um were tested. Little improvement was achieved but until not enough to analyse samples.

A third method was tested were samples were embedded in wax for longer period (2 x 2h), sectioned 50 µm thick, labelled and then membranes were stained with 0.1% Toluidine Blue to reduce autofluorescence from phenolic acids attached to the cell wall. Thicker sections required the use of confocal microscopy due high-resolution through a thick specimen.

Results from the methods tested are summarised in figure 3.11.

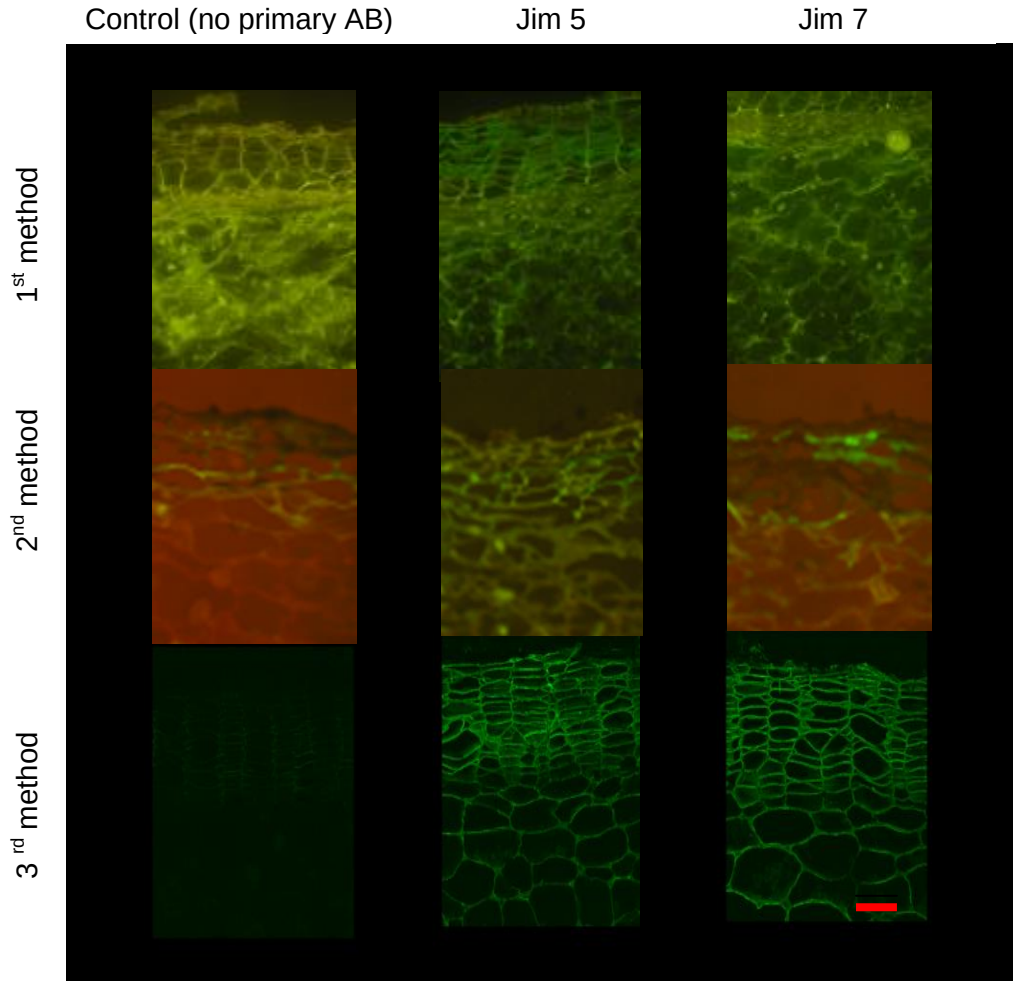



Figure 3.11 Fluorescent microscopy of potato tissue labelled with JIM5 or JIM7. Control indicates no primary antibody. Sections of method 1, 2 and 3 were 12, 32 and 50 µm thickness. Magnification 20X. Scale bar: 50 µm.

### 3.2.4.3 Optical localization of membranes

Handcut sections of fresh tissue were made. Samples were stained with 0.1% Toluidine Blue, 0.05% Iodine and Calcofluor and analysed with optical microscope. Pictures obtained were not clear and hand sectioning of fresh tissue method was no longer used. Results are presented in figure 3.12.

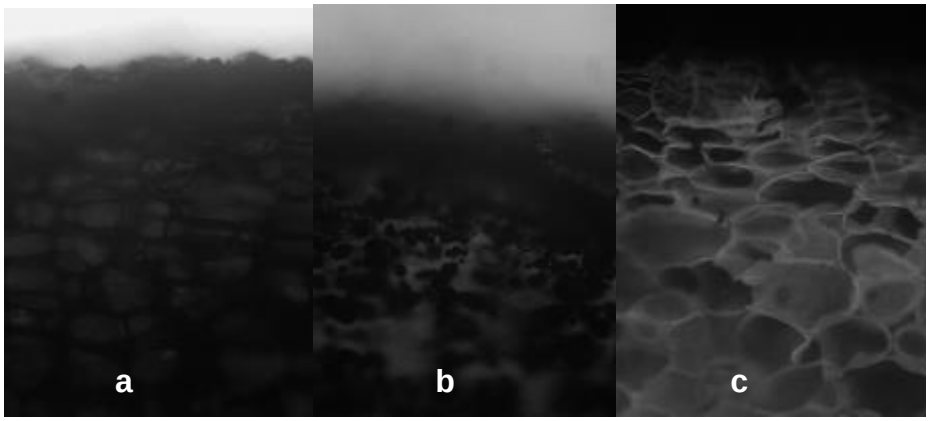



Figure 3.12 Handcut stained with (a) toluidine blue, (b) iodine and (c) calcofluor UV, magnitude 20X.

### 3.2.4.4 Analysis of Cell Wall Material (CWM)

### 3.2.4.5 Extraction

Analyses of cell wall monosaccharides have been restricted by difficulties encountered with methods for extraction that usually require several steps. Although potatoes have less than 2% protein, deproteination is an important step for the purification of potato cell wall. According to the literature, some proteins from glycoproteins and some enzymes can be removed in cold water, other enzyme and lectins and newly-deposited extensins are ionically-bond to the acidic polysaccharides and can be extracted with salt (e.g. NaCl). The non-covalently bound membrane proteins require the use of detergents and more powerful solvents to be removed such Triton X-100,

SDS or CHAPS, but usually hash solvents are used to purify extract proteins from the potato cell wall e.g. saturated phenol (Fry, 2000) or phenol/acetic acid/$H_2O$ (2:1:1) (PAW) (Jardine *et al*., 2002). In this study an enzymatic digestion of proteins using pancreatic protease was tested compare with 80% (v/w) saturated phenol. The results are shown in table 3.5.

Table 3.5 Monossacharide concentration of CWM extracted with protease or 80% (v/w) saturated phenol and hydrolysed with 1M $H_2SO_4$. Concentrations are expressed per %*mol* (n=2).

| Monossacaride | Protease % mol | Phenol %mol |
|---|---|---|
| **Fucose** | 0.4 | 0.2 |
| **Rhamnose** | 1.8 | 1.9 |
| **Arabinose** | 12.3 | 11.9 |
| **Galactose** | 72.3 | 71.0 |
| **Xyllose** | 6.8 | 9.5 |
| **Galacturonic acid** | 6.5 | 5.5 |

Results from enzymatic approach indicate minimal differences comparing extraction using protease and phenol 80% (v/w) after hydrolysis with 1M $H_2SO_4$, except for Xyl. The most abundant type of pectin in potatoes is rhamnogalacturan I rich in arabinose and galactose. Recovery of these sugars was similar for both methods. Other relevant factors were the extensive number of samples to be extracted and the possibility to avoid the use of hash toxic chemicals (phenol) in the laboratory. For these reasons, enzymatic removal of protein was chosen.

#### 3.2.4.6 Analysis of monosaccharide composition using high performance anion exchange chromatography amperometric detection (HPAEC - PAD) - Dionex

Initially, the method described by Øbro *et al.* (2000) was tested but not good separation was achieved due the use of different equipment. Due mainly to coeluition of compounds, decrease on flow rate (from 0.5 to 0.3 mL/min) was tested. Also, the isocratic gradient from 1.5 min with 5mM was extended to guarantee elution of 9 compounds (from 20 to 30 minutes). It was found that column were not totally cleaned with 800mM NaOH so the concentration of NaOH was increased to 1M and the duration was extended to 5 minutes at this higher concentration.

With these adaptations, the 9 sugars from cell wall plus one internal standard were separated. Typical ion exchange chromatograms are presented in figure 3.12 showing all 10 compounds eluted within 65 min using water and NaOH gradient.

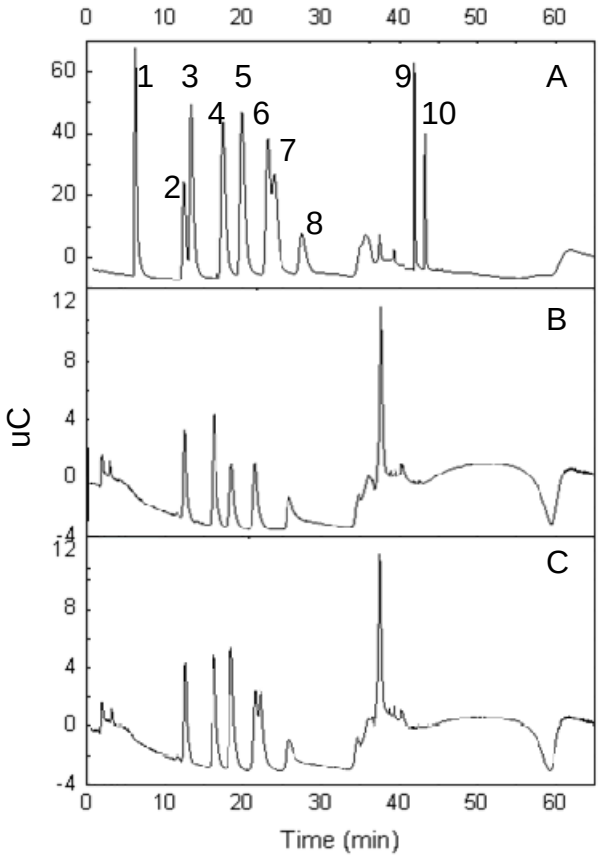



Figure 3.12 Ion exchange peaks of (A) standards 25ug/mL (1) Fucose, (2) Rhamnose, (3) Arabinose, (4) Galactose), (5) Glucose, (6) Xylose, (7) Mannose, (8) Galacturonic acid and (9) Glucuronic acid. (B) Sample and (C) sample spiked with mannose.

High-Performance Anion-Exchange Chromatography (HAPEC) operating conditions were optimized also to achieve excellent sensitivity. Limit of detections ranged from 47 ng/mL for 6 out of 9 standards and 93 ng/mL for the remaining. Intra-day and inter-day precision and accuracy was calculated at < 6.3% and <-9.1% respectively, as shown in table 3.6.

Table 3.6 Ion exchange optimization and characterization of sugars

| Peak | Compound | Rt (min) Mean ± SD [a] | RSD (%)[b] | LOQ (ng/mL) | $R^2$ |
|---|---|---|---|---|---|
| 1 | Fucose | 6.15 ± 0.18 | 2.88 | 47 | 0.9999 |
| 2 | Rhamnose | 12.09 ± 0.46 | 3.79 | 47 | 0.9996 |
| 3 | Arabinose | 13.10 ±0.45 | 3.46 | 47 | 0.9999 |
| 4 | Galactose | 16.81 ± 0.63 | 3.77 | 47 | 0.9997 |
| 5 | Glucose | 19.09 ± 0.72 | 3.79 | 93 | 0.9993 |
| 6 | Xylose | 22.26 ±0.81 | 3.65 | 93 | 0.9998 |
| 7 | Mannose | 23.08 ± 0.83 | 3.62 | 47 | 0.9999 |
| 8 | Fructose | 26.62 ± 1.06 | 4.00 | 47 | |
| 9 | Galacturonic acid | 41.80 ±0.05 | 0.13 | 93 | 0.9823 |
| 10 | Glucuronic acid | 43.10 ± 0.03 | 0.69 | 93 | 0.9999 |

n=2, 3 repetitions each

### 3.2.4.7 Linearity, precision and accuracy

For all compounds detected in Table 3.6, peak area varied linearly with on-column amount over the ranges ($R > 0.98$). Intra-day and inter-day precision was calculated for galactose as R.S.D. <4.6%, <1.7% and <6.4% for 25ug/ml, 50µg/ml and 100 µg/ml concentrations respectively. Good intra-day and inter-day accuracy was demonstrated across the concentration range with relative error <-7%. The precision and accuracy meet performance criteria for analytical methods, which indicate precision (R.S.D.) and accuracy (R.E.) must be within ±15%, or for the lower limit of quantification, values within ±20% are acceptable.

### 3.2.4.8 Stability of monosacharides at 10° C storage and recovery efficiency for hydrolysis studies

Extraction efficiency of investigated sugars in 0.1 and 2 M TFA was assessed after hydrolysis for 1h at 100 ºC. All compounds showed degradation in 0.1M TFA and further degradation upon higher molality of TFA (2 M), shown in table 3.7. The stability of standards samples prepared for ion exchange analysis was investigated and data indicate some degradation following storage of standards with concentration of 25 µg/mL in the chilled auto-sampler conditions for 3 days.

Table 3.7 Extraction efficiency of sugars with 0.1 M TFA and 2 M TFA at 100 °C (1h) and stability of rhamnose, galactose and glucose (25 µg/ml) in water after 3 days storage at 4 °C. Values represent average ± SD (n=3).

| Monos. | Extraction efficiency % of initial concentration | |
|---|---|---|
| | **0.1M TFA** | **2M TFA** |
| **Fuc** | 71.1 ± 0.2 | 66.3 ± 0.7 |
| **Rha** | 67.8 ± 0.6 | 62.1 ± 0.9 |
| **Ara** | 61.6 ± 0.4 | 57.9 ± 0.6 |
| **Gal** | 59.1 ± 0.4 | 55.5 ± 0.5 |
| **Glu** | 59.2 ± 0.7 | 56.1 ± 0.5 |
| **Xyl** | 75.2 ± 0.9 | 70.6 ± 0.4 |
| **Man** | 38.3 ± 0.4 | 36.3 ± 0.6 |
| **GalA** | 56.7 ± 0.8 | 55.8 ± 0.9 |
| **GluA** | 51.0 ± 1.0 | 50.0 ± 0.8 |
| | **Stability at 4 ºC % of initial concentration** | |
| **Ara** | 95.6 - 100.5 | |
| **Gal** | 95.4 - 100.5 | |
| **Glu** | 94.5 - 100.6 | |

### 3.2.4.9 Percentage of sugar released from CW in each step of hydrolysis

After partial hydrolysis with 0.1 and 2 M TFA, a further hydrolysis with 1 M $H_2SO_4$ hydrolysis was carried out to determine if there were any remaining pectic monosaccharides. The percentage of sugars released from CW in each step of hydrolysis is presented in table 3.8.

Table 3.8 Percentage of cell wall sugars released from potato cortex under the sequential hydrolysis. Bold results shows which stage the monosaccharide was released more (n=3).

| | % released | | |
|---|---|---|---|
| Monos. | 0.1 TFA | 2M TFA | 1M $H_2SO_4$ |
| Fuc | 26-32 | 45-74 | 0-23 |
| Rha | 55-77 | 23-45 | 0 |
| Ara | 91-97 | 3-7 | 0-2 |
| Gal | 59-73 | 26-40 | 1-2 |
| Glu | 8-38 | 40-46 | 22-46 |
| Xyl | 13-15 | 62-75 | 10-25 |
| Man | 0 | 0 | 100 |
| GalA | 38-64 | 11-27 | 24-35 |
| GlcA | 72-86 | 5 | 9-23 |

As higher percentages of most of the monosaccharides from pectin were extracted using 0.1 and 2 M TFA and further characterization of cell walls followed the 2 steps hydrolysis methods.

# 4 Effect of defoliation on bruising along harvests – field trial 1

## 4.1 Introduction

Previous studies suggest that both potato genotype and environmental conditions affect bruising (McGarry *et al*., 1996). Potato cultivars Maris Piper (MP), Lady Rosetta (LR) and Russet Burbank (RB) have been shown to vary in their susceptibility to bruising in potatoes grown under controlled conditions (Carnegie *et al.*, 2005; BPVD, 2012). In terms of environmental conditions, the maturity status before the harvest is a factor in bruising. Maturity is related to the time when haulm will desiccate and tuber skin is set (Pringle *et al*., 2009). It is know that the content of phenyl substrates such as tyrosine and other phenolic acid compounds such as chlorogenic acid, as well as levels of polyphenol oxidase (PPO), tend to be less abundant in early (immature tubers) compared to late-season tubers (Lisinska and Leszczynski, 1989).

A previous Potato Council project (Stalham, 2008) found that more bruising occurred in crops harvested three to five weeks after defoliation (21 and 35 days respectively) compared to undefoliated. However, the determinants of bruising were until not fully understood. According to previous studies presented in the introduction (chapter 1), these factors include the concentration of phenolic substrates, the mechanical properties of tuber and the composition of the plant cell wall.

### 4.1.1 Aim

The main aim of this chapter was to investigate the effect of harvest and defoliation on bruising in three varieties of potatoes. This knowledge would enable growers to better manage crops to achieve the necessary standards for the quality of potatoes. Additionally the research seeks to establish whether physiological and biochemical characteristics, such as mechanical properties, phenolic acids, tyrosine and cell wall composition are factors that influence bruising and may be used as predictive indicators of bruising.

In this present study, three varieties Maris Piper (MP), Lady Rosetta (LR) and Russet Burbank (RB) were investigated. These UK varieties of potatoes are known to differ in their tendency toward bruising. According to the Potato Council independent variety trials, MP and LR have been assigned with bruising susceptibility score of 6, whereas RB was assigned a bruising score of 4 in ratings ranging from 0 (most susceptible) to 9 (least susceptible) (Carnegie *et al*., 2005, BPVD, 2012), as mentioned before (Chapter 1, section 1.5.1). The cultivars studied were grown at Cambridge University Farm (CUF), planted on 23 April 2010 and harvested at four time points. Before the harvest, defoliation was performed at two time points (early defoliation and late defoliation), as indicated in table 2.1. Trials were randomised with two factors (variety and defoliation) with three replicate plots. Ten tubers per plot were analysed.

### 4.1.2 Hypotheses

The hypotheses tested were:

1) Potatoes from defoliated plants show more bruising in crops harvested three to five weeks after defoliation (21 and 35 days respectively).

2) Potatoes harvested later in the season show more bruising, and this may be due to accumulation of phenolic substrates.

3) The mechanical properties of the tuber influence bruising and these properties will be influenced by the cell wall composition of cortex cells.

### 4.1.3 Objectives

1) To improve the understanding of the influence on defoliation of crop and harvest date on the incidence of bruising.

2) To investigate the potential for using physical and biochemical measurements as indicators of bruising.

## 4.2 Results

### 4.2.1 Field phase

#### 4.2.1.1 Meteorological data

The 2010 season was characterized by short periods of bright days throughout June and July, interspersed with longer spells of more average radiation resulting in significantly higher evaporative demand for these two

months than average. Reference $ET_0$ (Evapotranspiration (ET) - is the sum of soil water evaporation (E) and plant transpiration (T)) was greater in June and July, with a mean daily $ET_0$ of 3.52 and 3.90 mm/day respectively and only 2.57 mm/day in August.

The temperature for normal tuber growth was beyond boundaries recommended by FAO (2013) with mean daily temperatures of 18 to 20°C and soil between 15 to 18°C. In general a night temperature of below 15°C is required for tuber initiation (FAO, 2013).

Around the time of tuber initiation there was rainfall but only 20 mm of rain from 10 June to 31 July. August was dull with a low evaporative demand and there was heavy rain in the month, thought to be important to reduce bruising (Stalham, 2008).

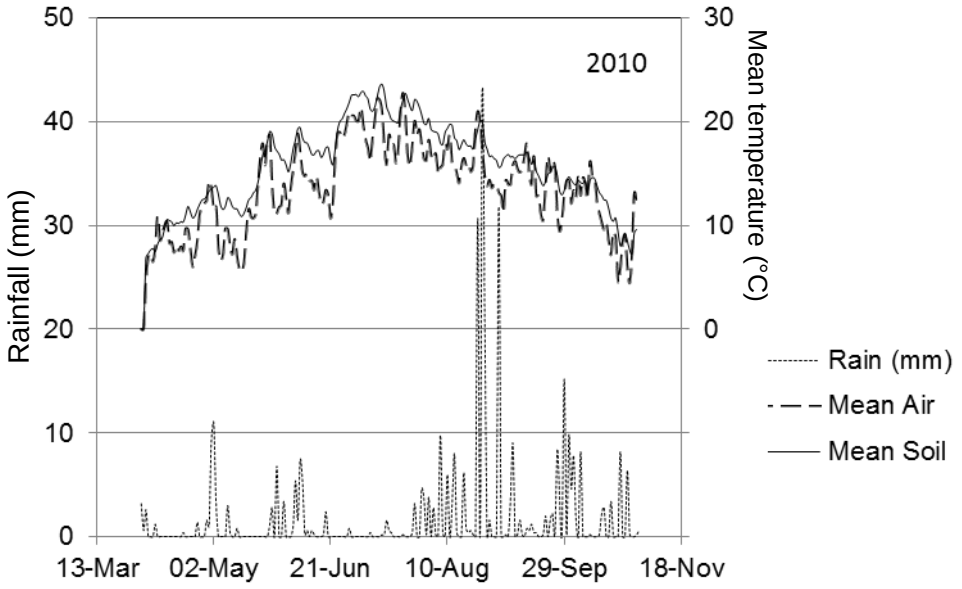



Figure 4.1 Rainfall (mm) and mean temperature of the air and soil (secondary axis) in field trial 1, 2010.

### 4.2.1.2 Green canopy cover (%)

Temperatures were colder than average in June and ground cover was slower to develop than normal. By the commencement of the measurement period (1st June), ground covers were 5- 8.4% in LR, 2-3% in MP and 2.7-4.3 in RB. All the varieties achieved 100% canopy by 13th July.

Measurements of decline of canopy were taken as key indicators of the effect of defoliation on the physiological characteristics of the crop. In particular, the stage of senescence reached by the date of defoliation was studied (table 4.1).

Table 4.1 Green canopy cover (%) at defoliation time of the varieties LR, MP and RB of tubers harvested in August (H1 and H2) and September (H3 and H4), 2010.

| Variety | LR D1 | LR D2 | LR UN |
|---|---|---|---|
| **H1** | 100.0 | 100.0 | 99.7 |
| **H2** | 0 | 99.3 | 99.0 |
| **H3** | 0 | 0 | 88.7 |
| **H4** | 0 | 0 | 55.0 |
| | **MP D1** | **MP D2** | **MP UN** |
| **H1** | 100.0 | 100.0 | 99.7 |
| **H2** | 0 | 99.7 | 99.7 |
| **H3** | 0 | 0 | 98.0 |
| **H4** | 0 | 0 | 92.0 |
| | **RB D1** | **RB D2** | **RB UN** |
| **H1** | 100 | 99.7 | 100.0 |
| **H2** | 0 | 99.7 | 100.0 |
| **H3** | 0 | 0 | 95.7 |
| **H4** | 0 | 0 | 59.0 |

The data show varietal difference on green canopy cover at defoliation. Delay in senescence was found in Maris Piper. All varieties had >99 %

ground cover at defoliation on H1 and H2. LR and RB had senesced almost half by mid-September (H4), with 55% and 59% ground cover respectively while MP until had 92% ground cover by this time.

### 4.2.2 Bruising assessment

#### 4.2.2.1 Assessment of severe bruising using the falling bolt method

For potatoes harvested early in the season (H1 and H2), no bruising was observed amongst any varieties or defoliation regime studied (figure 4.2). This may be because tubers may not be biochemically susceptible to bruising at this time, explored in section 4.2.5. In addition, these early potatoes were not subjected to 'hot boxing'. Hot boxing was applied to potatoes of the 3$^{rd}$ (H3) and 4$^{th}$ harvest (H4). The results from the 3$^{rd}$ and 4$^{th}$ harvest are presented in figure 4.2 and expressed in percentage of severe bruising according to the Potato Council protocol after falling bolt damage (equivalent to energy of 0.6 J) and for 48 hours incubation at 33°C and humidity >95%.

At the 3$^{rd}$ harvest, RB showed higher percentage of severe bruising (50-67%), followed by MP (17-33%) and LR (0-17%). Assessment of the effect of defoliation on bruising status of the tubers indicated that defoliated plants from all varieties presented similar or higher incidence of severe bruising than undefoliated, with exception of RB D2. At the 3$^{rd}$ harvest, samples were harvested 24 (D2) and 38 (D1) days after defoliation. These results are in accordance with the hypothesis, where more bruising would occur in the defoliated crops harvested 21 to 35 days after defoliation (3 to 5 weeks).

At the 4th harvest time, LR presented an increase in the percentage of bruised tubers (18-56%), while both RB (6-53%) and MP (6-17%) showed decreased levels compared to the 3rd harvest. These observations were also made in defoliated samples. Samples defoliated either early (D1) or later August (D2) were associated with less severe bruising for RB and LR and for MP defoliated early August (D1). By this time, tubers were harvested 35 (D2) and 49 (D1) days after defoliation.

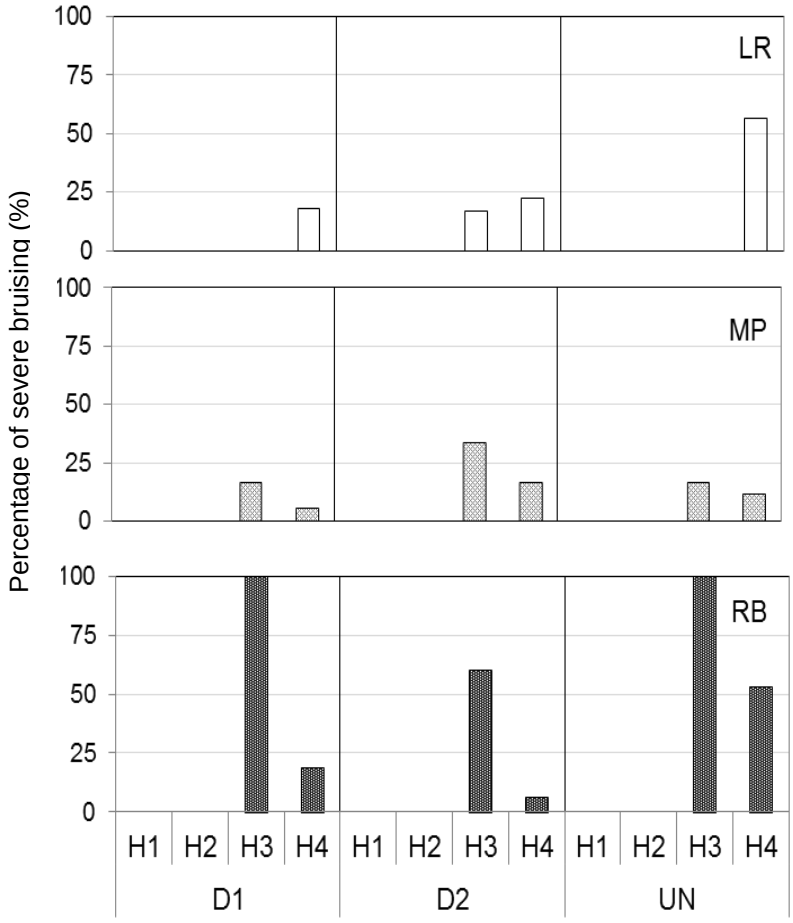



Figure 4.2 Effect of variety, harvest time and defoliation regime on percentage of severe bruising (%) following damage using a falling bolt method in potatoes from crops harvested in early August (H1), late August (H2), early September (H3) and late September (H4), defoliated early August (D1), late August (D2) and undefoliated (UN), field trial 1. No bruising was found at H1 and H2, measured without hot boxing. Values show percentage of severe bruising (%) (H1-H3 n=9 and H4 n=18).

In order to have more detail about the damage to potatoes, slight bruising was measured as well as severe bruising. Slight bruising is defined as requiring less than two peelers to remove the bruised tissue following initial exploratory peel based on a procedure developed at the Sutton Bridge Crop Storage Research (2008), shown in figure 4.3.

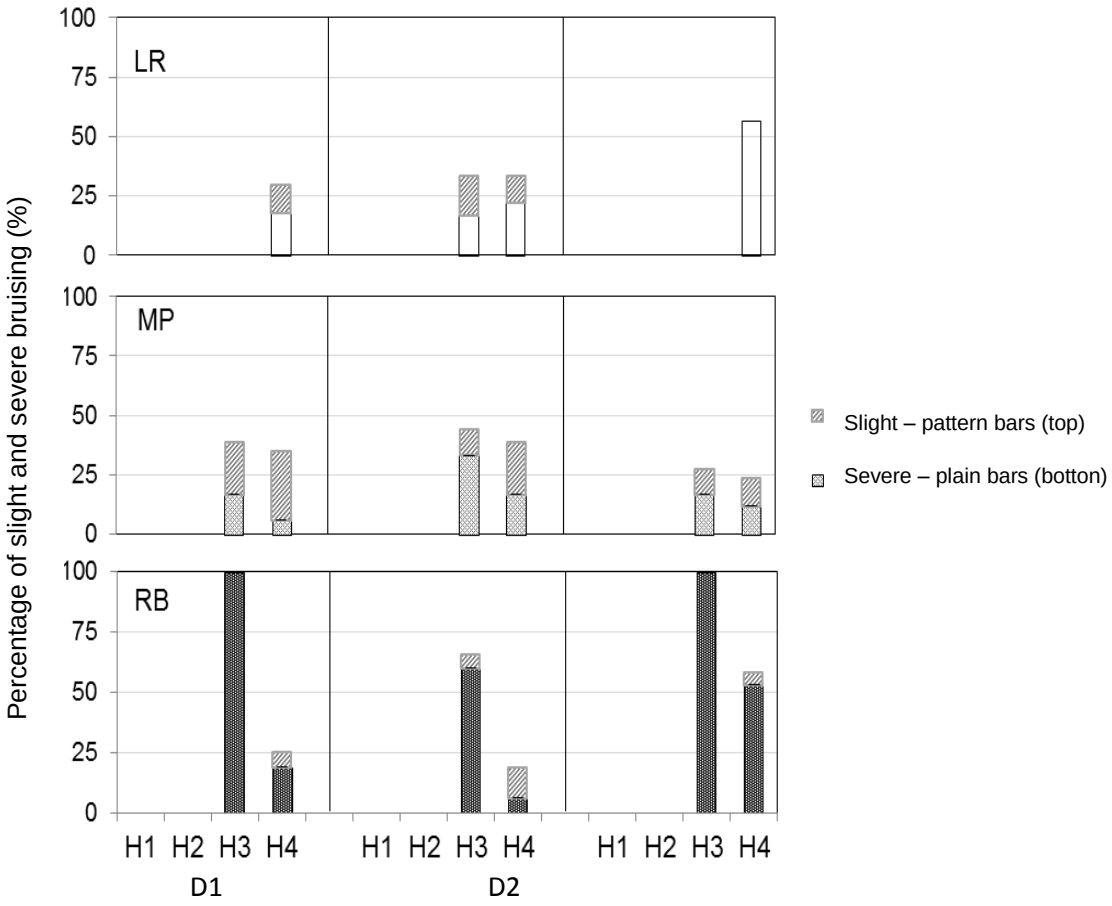



Figure 4.3 Effect of variety, harvest time and defoliation regime on percentage of slight (pattern bars) and severe bruising (plain colour bars) following damage with falling bolt in potatoes from crops harvested in early August (H1), late August (H2), early September (H3) and late September (H4), defoliated early August (D1), late August (D2) and undefoliated (UN), field trial 1. No bruising was found at H1 and H2, measured without hot boxing. Values show percentage of severe bruising (%) (H1-H3 n=9 and H4 n=18).

Trends were similar when accounting slight and severe bruising of the varieties at the 3rd and 4th harvest as shown in figure 4.3. However defoliation presented a higher incidence of bruising for MP in both defoliated treatments, early August (D1) and later August (D2) harvested early and later September compared with undefoliated. The other varieties, LR and RB, harvested after 35 days of defoliation (H4) bruised less than undefoliated samples.

#### 4.2.2.2 Damage

Samples that presented cracking on the external tissue following the falling bolt method and incubation were classified as having “breaking damage” which was excluded from the bruising assessment. Results showed varietal differences on the sensitivity of external tissue towards the bolt damage (figure 4.4). At 3rd harvest, the variety RB showed higher incidence of external damage, followed by LR and MP.

However, at the 4th harvest, RB had a lower incidence of potatoes with breaking damage when compared with 3rd harvest. The percentage of damaged samples at the 3rd harvest for RB D1, D2 and UN were 50, 16 and 33%, respectively, while at the 4th harvest a decrease to 11, 11 and 5%, occurred respectively. Incidence of skin damage of LR was in the range of 0-17% and for MP 0-6% for both harvests and defoliation regime. Variations in skin damaged of LR and MP were in the range of 0-6% comparing H3 and H4, independently of defoliation regime.

It was expected to find less damage in defoliated tubers as skin should set earlier. Very little skin damage was observed in LR and MP, with slightly less occurrence of damage in tubers from undefoliated plants. In RB, more damage was observed in tubers from plants defoliated early (D1) and less in tubers from plants defoliated later (D2) compared to control. This pattern is similar to bruising observations, indicating that RB is more susceptible to mechanical damage resulting in either bruising or skin damage.

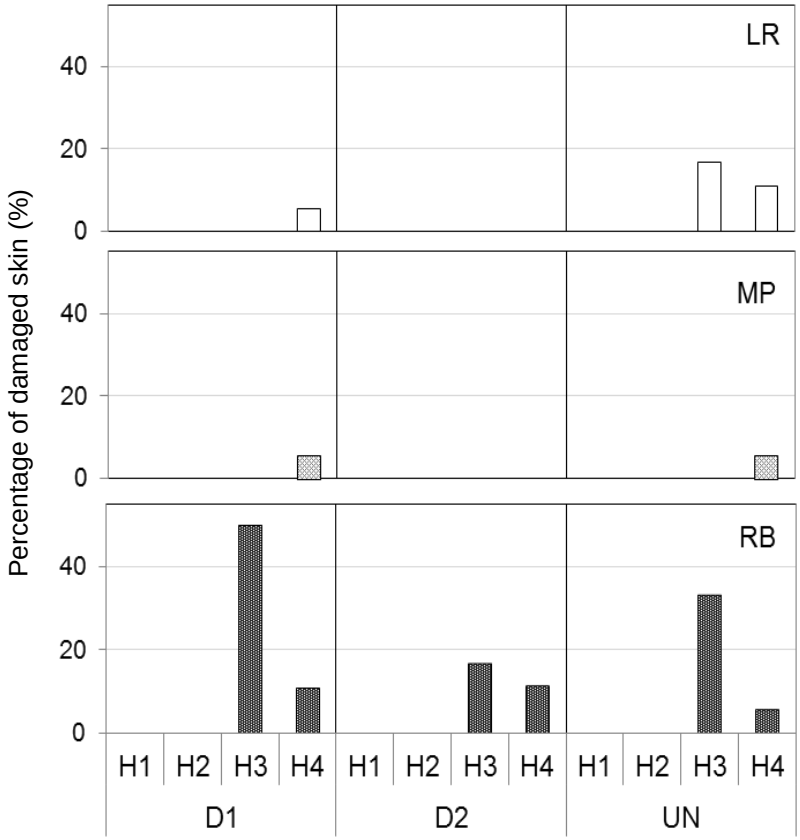



Figure 4.4 Effect of variety, harvest time and defoliation regime on percentage of damaged skin following damage using a falling bolt method in potatoes from crops harvested in early August (H1), late August (H2), early September (H3) and late September (H4), defoliated early August (D1), late August (D2) and undefoliated (UN), field trial 1. No damaged skin was found at H1 and H2, measured without hot boxing (H1- H3 n=9 and H4 n=18).

#### 4.2.2.3 Spectrophometric assessment of oxidative potential

Oxidative potential provides a measure of the amount of pigment developed which could potentially develop in bruised tissue. One of the objectives of this experiment was to correlate the pigment formation following oxidation with bruising. This is not a specific enzyme assay to identify a single enzyme activity but considered a more 'global' biochemical test which might take into account all metabolic processes within a given variety. Following this approach, the progress of pigment formation was followed spectrophotometrically directly in simple buffer extracts from tubers. The logic behind these experiments is that all the components needed for pigment formation including substrate, enzymes and oxygen will be present in these extracts - a tuber extract slowly turns brown on incubation due to the presence of polyphenol oxidase (PPO). Assuming that all the reactants are present in such an extract the concentration of brown polyphenol pigment synthesised should be representative of what takes place in the tuber following impact damage and thus may be correlated with the bruising potential of the tuber material.

The experiments were determined at different stages of tuber physiological maturity along the four harvest times and the results are shown in figure 4.5.

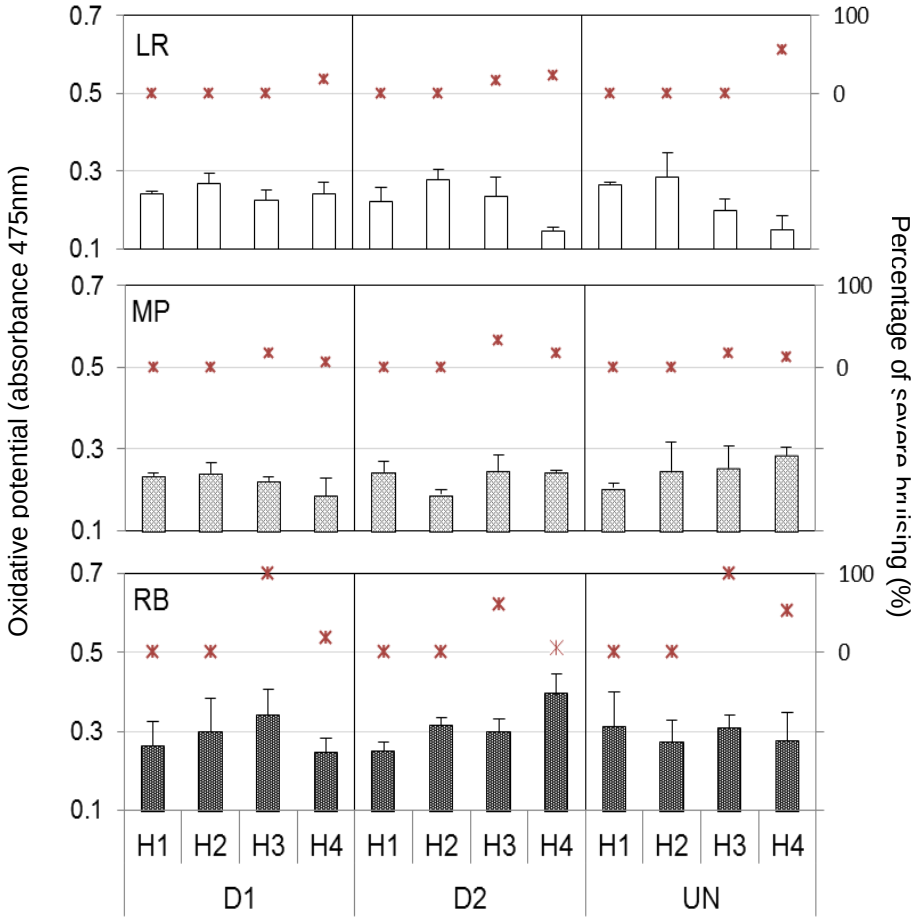



Figure 4.5 Effect of variety, harvest time and defoliation regime on oxidative potential following 20 hours oxidation (bars) and percentage of severe bruising (%) (scatter) from crops harvested in early August (H1), late August (H2), early September (H3) and late September (H4), defoliated early August (D1), late August (D2) and undefoliated (UN), field trial 1. Bars show means (n=3), errors bars are SE and scatter shows percentage of severe bruising (%) (H1-H3 n=9, H4 n=18).

Among the varieties, RB tubers presented significantly ($p<0.05$) higher oxidative potential along the harvest times, varying from 0.25-0.40 absorbance at 475 nm, compared to MP (0.18-0.28) and LR (0.15-0.28). There was not a significant change in oxidative potential along harvest times for any variety ($p>0.05$). Defoliation also did not affect bruising potential during the time investigated ($p>0.05$).

From these results, a clear pattern was not found in levels of oxidative potential when compared with the percentage of severe bruising along harvest times. Negative strong correlation was found for LR (R=- 0.49) and negligible relationship for the other varieties (R<0.11). Similar results were found for Stevens and Davelaar (1997) where the susceptibility of potato tubers to bruising was not correlated with the biochemical potential for pigment synthesis. The extent of the oxidative potential suggests that other factors are relevant for bruising susceptibility.

## 4.2.3 Physical properties

### 4.2.3.1 Weight

Results showed that the weight of all varieties studied were significantly affected by early defoliation comparing to undefoliated samples ($p<0.05$), figure 4.6. The differences in weight of samples defoliated early (D1) and undefoliated at the $4^{th}$ harvest were 33, 31 and 14 % respective to MP, LR and RB. Smaller differences were found when plants were defoliated later (D2), being 26, 14 and 2% for MP, LR and RB respectively. MP showed the largest differences among the varieties studied.

Significant differences in weight along the harvest times were found. LR and RB showed significant increase in weight during the interval of H2 and H3 ($p<0.05$). There was an increase in weight of tubers for MP over the harvest times but no significant differences were found when comparing time intervals along the harvest period. Correlation between size and bruising

was found was found for RB (R=0.50), but weak correlation for LR (R=0.38) and MP (R=0.21). So, larger tubers did not necessarily bruise more.

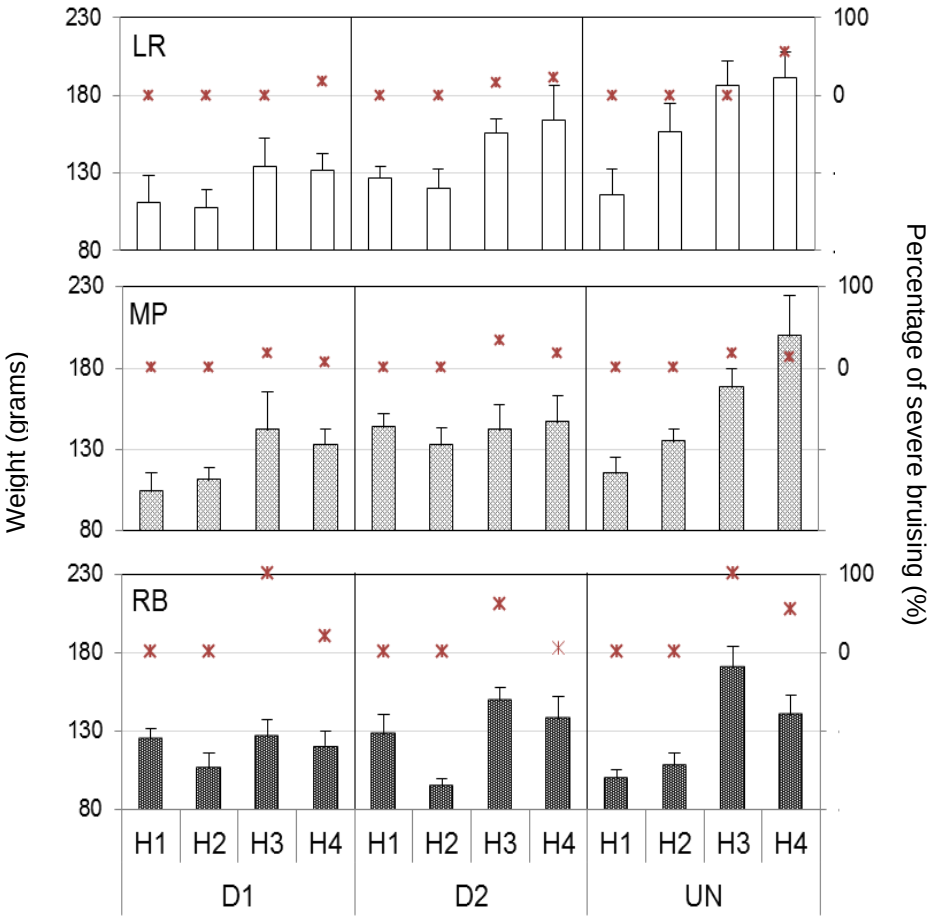



Figure 4.6 Effect of variety, harvest time and defoliation regime on weight of samples in grams (bars) and percentage of severe bruising (%) (scatter) from crops harvested in early August (H1), late August (H2), early September (H3) and late September (H4), defoliated early August (D1), late August (D2) and undefoliated (UN), field trial 1. Values bars show mean (n=9), errors bars are SE and scatter shows percentage of severe bruising (%) (H1-H3 n=9, H4 n=18).

## 4.2.4 Mechanical properties

### 4.2.4.1 Energy required to break the skin tissue

In field trial 1 measurements were taken of defoliated and undefoliated samples to assess differences in energy to break the tissue attempting to correlate with differences in bruising.

Amongst the varieties the skin of the RB, variety that bruised the most, required on average lower energy to break the skin tissue, followed by LR and MP along harvest times, as shown in figure 4.7. Significant differences in energy measures were found at different harvests when comparing varieties as shown in table 4.2. LR was significantly different to RB along all harvests, even when presenting similar incidence of severe bruising as at H4. LR was also significant different from MP at H1 and H4. MP and RB showed significantly different results at H2 and H3 but on the first and last harvest (H1 and H4) no difference was found.

Table 4.2 P-values of multiple comparisons of the results from the energy required to break the skin tissue performed with Student-Newman-Keuls (SNK). NS means no significant difference.

| Variety<br>Harvest | LR-MP | LR-RB | MP-RB |
|---|---|---|---|
| **H1** | <0.01 | <0.001 | NS |
| **H2** | NS | <0.001 | <0.05 |
| **H3** | NS | 0.01 | <0.01 |
| **H4** | <0.05 | <0.05 | NS |

In general, the energy required to break the skin decreased along harvests. LR presented a trend in all treatments investigated, decreasing the energy to break the skin until H3, followed by slight increase at H4. This is explained by weaker skin required for tuber growth until H3, followed by skin setting. In RB, the weaker skin at H3 was associated with more skin damage and more bruising. Similar observations have been described previously for other potato varieties. Strehmel *et al*. (2010a) observed that when tissue cracks upon collision, the impact energy is less strongly distributed throughout a larger area of the tissue.

Comparing all varieties, there was no significant effect of defoliation on the energy required to break the skin along the harvest times. Defoliation was expected to speed up skin setting, but this was not observed using this method of measurement.

The skin strength was measured by the force (N) at the point when the tissue breaks and the deformability was measured by the distance (mm) to rupture the tissue as shown in figures 4.8 and 4.9 respectively. It was observed that the trend in energy to break the skin tissue was not dependent only on one factor but on a combination of force and distance to break the tissue.

A strong negative correlation was found for MP (R=-0.52) when analysing correlations between the energy to break the skin tissue and the incidence of bruising but not found for LR (R=0.06) and RB (R=-0.28). Weak correlations were found between force to break the cortex with bruising incidence for all varieties (R <0.18). Strong negative correlation was found between distance

to break MP cortex tissue (R=-0.43) with percentage of severe bruising but not for LR (R=-0.26) and for RB (R=-0.01).

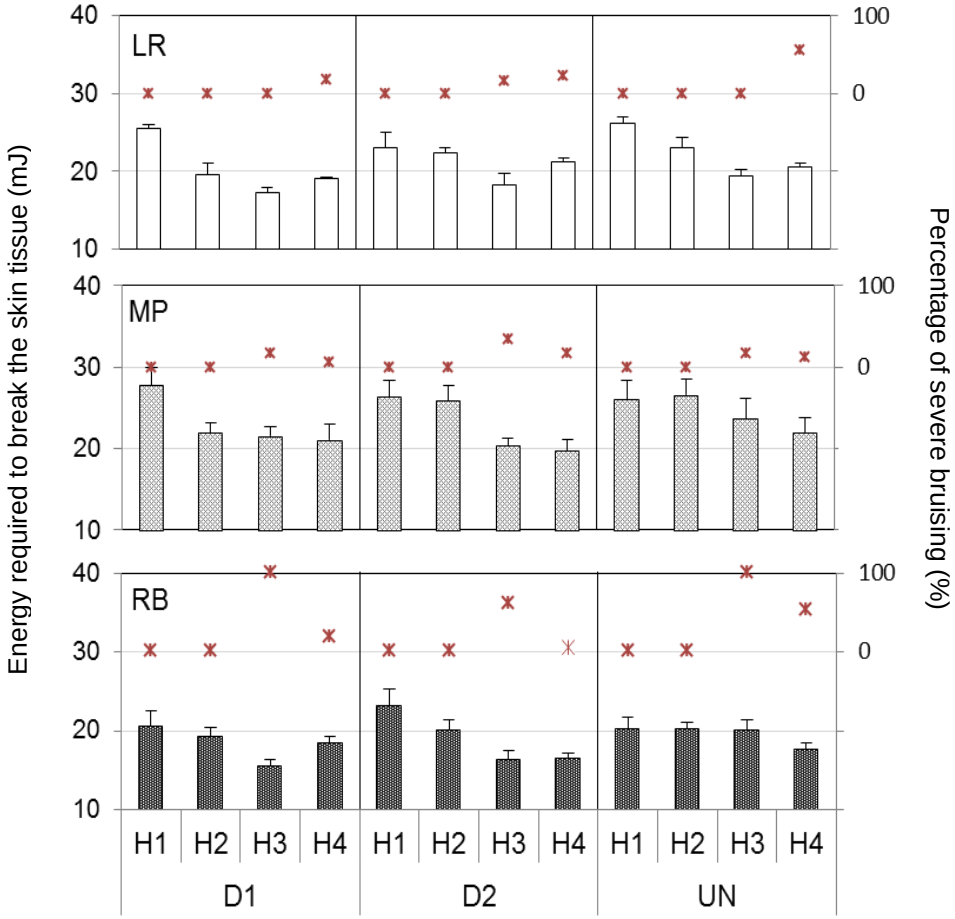



Figure 4.7 Effect of variety, harvest time and defoliation regime on the energy to break the skin tissue in mJ (bars) and percentage of severe bruising (%) (scatter) from crops harvested in early August (H1), late August (H2), early September (H3) and late September (H4), defoliated early August (D1), late August (D2) and undefoliated (UN), field trial 1. Values bars show mean (n=9), errors bars are SE and scatter shows percentage of severe bruising (%) (H1-H3 n=9, H4 n=18).

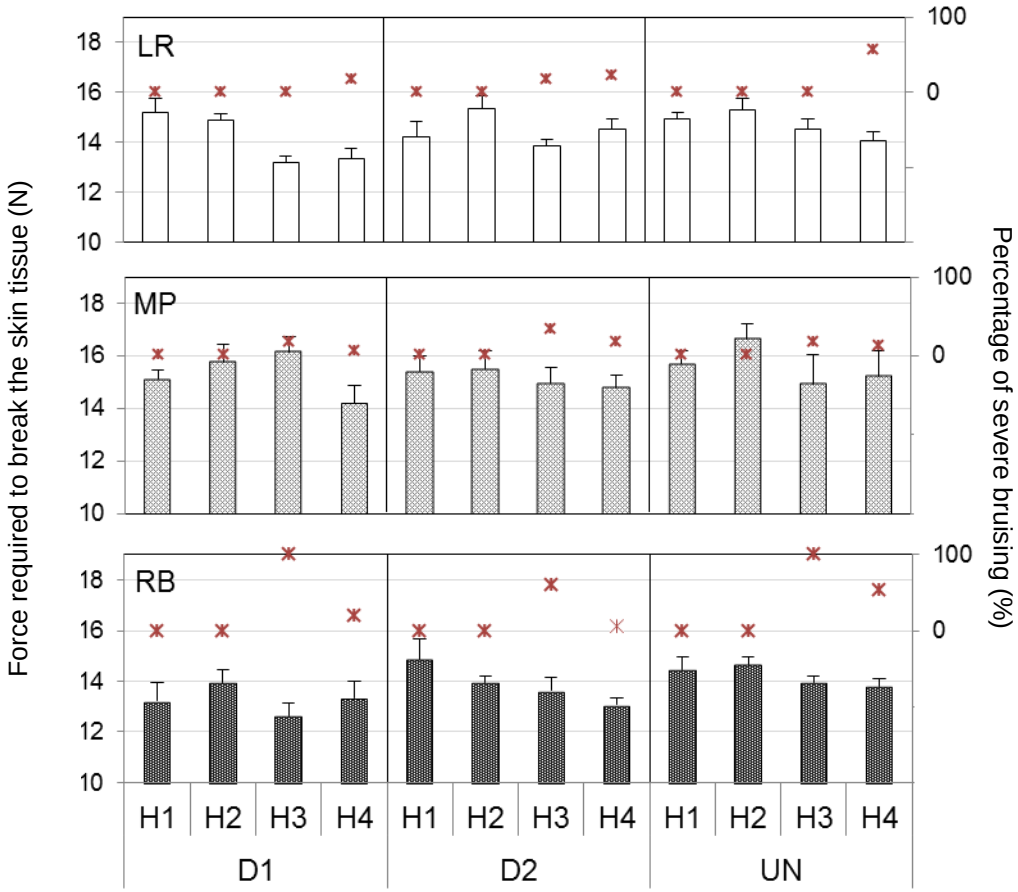



Figure 4.8 Effect of variety, harvest time and defoliation regime on the force to break the skin tissue in N (bars) and percentage of severe bruising (%) (scatter) from crops harvested in early August (H1), late August (H2), early September (H3) and late September (H4), defoliated early August (D1), late August (D2) and undefoliated (UN), field trial 1. Values bars show mean (n=9), errors bars are SE and scatter shows percentage of severe bruising (%) (H1-H3 n=9, H4 n=18).

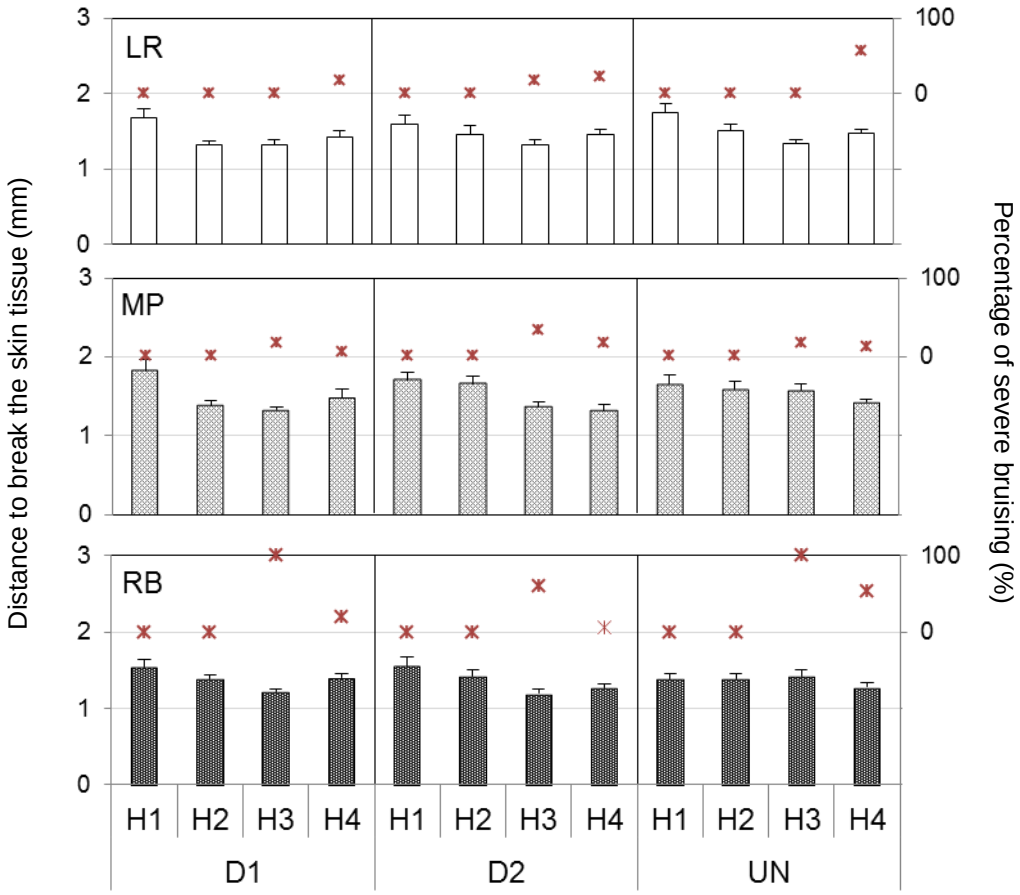



Figure 4.9 Effect of variety, harvest time and defoliation regime on the distance to break the skin tissue in mm (bars) and percentage of severe bruising (%) (scatter) from crops harvested in early August (H1), late August (H2), early September (H3) and late September (H4), defoliated early August (D1), late August (D2) and undefoliated (UN), field trial 1. Values bars show mean (n=9), errors bars are SE and scatter shows percentage of severe bruising (%) (H1-H3 n=9, H4 n=18).

#### 4.2.4.2 Energy required to break the cortex tissue

Measurement of mechanical properties of the cortex showed a decrease in the energy required to break the tissue along harvests, with lowest energy required for tubers from H4. Different levels of energy was required to break the cortex between the varieties studied ($p<0.05$), being lowest for RB, followed by MP and LR. Significant differences were found in the different time points of the harvest when comparing varieties as shown in table 4.3.

Table 4.3 P-values of multiple comparisons of results from energy required to break the cortex tissue performed with Student-Newman-Keuls (SNK). NS means no significant difference.

| Variety Harvest | LR-MP | LR-RB | MP-RB |
|---|---|---|---|
| **H1** | ns | <0.001 | <0.001 |
| **H2** | ns | <0.001 | <0.001 |
| **H3** | <0.05 | <0.001 | <0.01 |
| **H4** | <0.05 | <0.01 | NS |

According to table 4.3, LR was significantly different to RB along all harvests and different from MP at H3 and H4. MP and RB showed significantly different results from H1 to H3. However, comparing all varieties, there was not a significant difference ($p>0.05$) between undefoliated and defoliated samples along the harvest times.

The changes in energy to break cortex tissue along harvest were strongly dependent of the force (figure 4.11) and distance (figure 4.12) until break of the cortex, with pronounced decreases in distance along harvest for RB and LR, showing less deformable tissue.

A strong negative correlation between the energy required to break the cortex tissue and severe bruising incidence was found for the varieties MP ($R=-0.51$) and LR ($R=-0.48$) but not found for RB ($R=0.13$). Weak correlations were found between force to break the cortex with bruising incidence ($R<-0.29$). Strong negative correlation were found between distance to break MP cortex tissue ($R=-0.43$) and LR cortex tissue ($R=-0.50$) but not for RB ($R=-0.16$) with percentage of severe bruising.

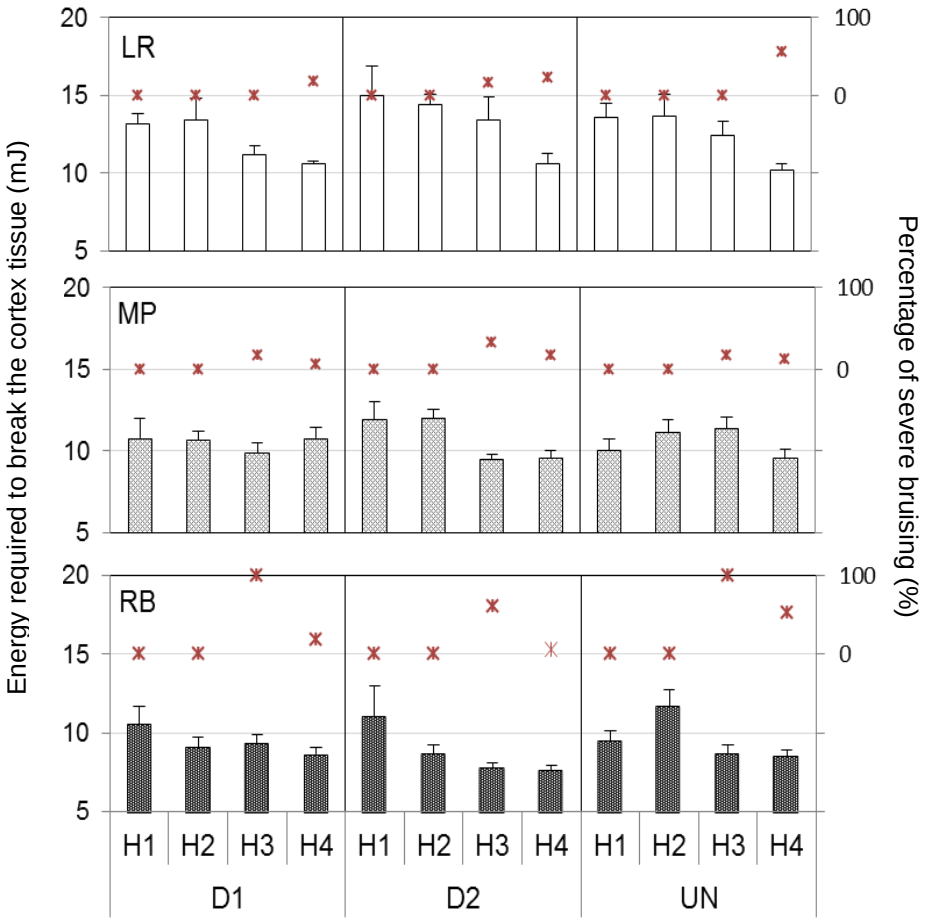



Figure 4.10 Effect of variety, harvest time and defoliation regime on energy to break the cortex tissue in mJ (bars) and percentage of severe bruising (%) (scatter) from crops harvested in early August (H1), late August (H2), early September (H3) and late September (H4), defoliated early August (D1), late August (D2) and undefoliated (UN) , field trial 1. Values bars show mean (n=9), errors bars are SE and scatter shows percentage of severe bruising (%) (H1-H3 n=9, H4 n=18).

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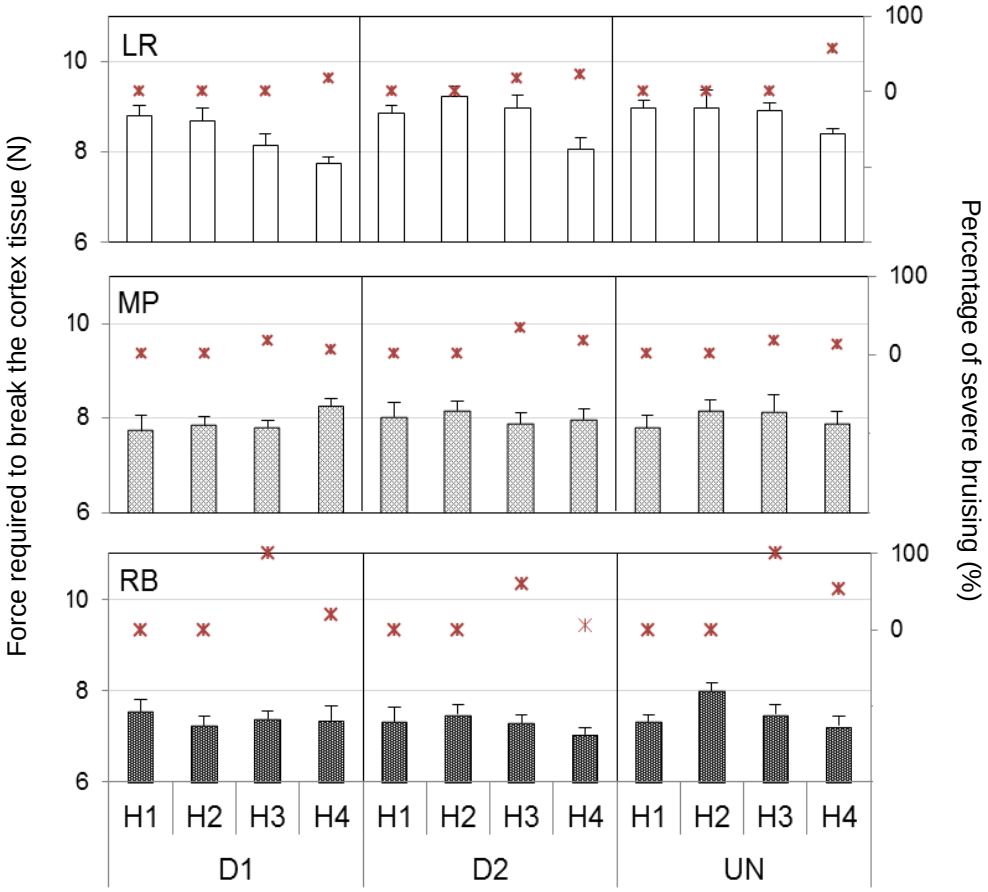


Figure 4.11 Effect of variety, harvest time and defoliation regime on the force to break the cortex tissue in N (bars) and percentage of severe bruising (%) (scatter) from crops harvested in early August (H1), late August (H2), early September (H3) and late September (H4), defoliated early August (D1), late August (D2) and undefoliated (UN), field trial 1. Values bars show mean (n=9), errors bars are SE and scatter shows percentage of severe bruising (%) (H1-H3 n=9, H4 n=18).

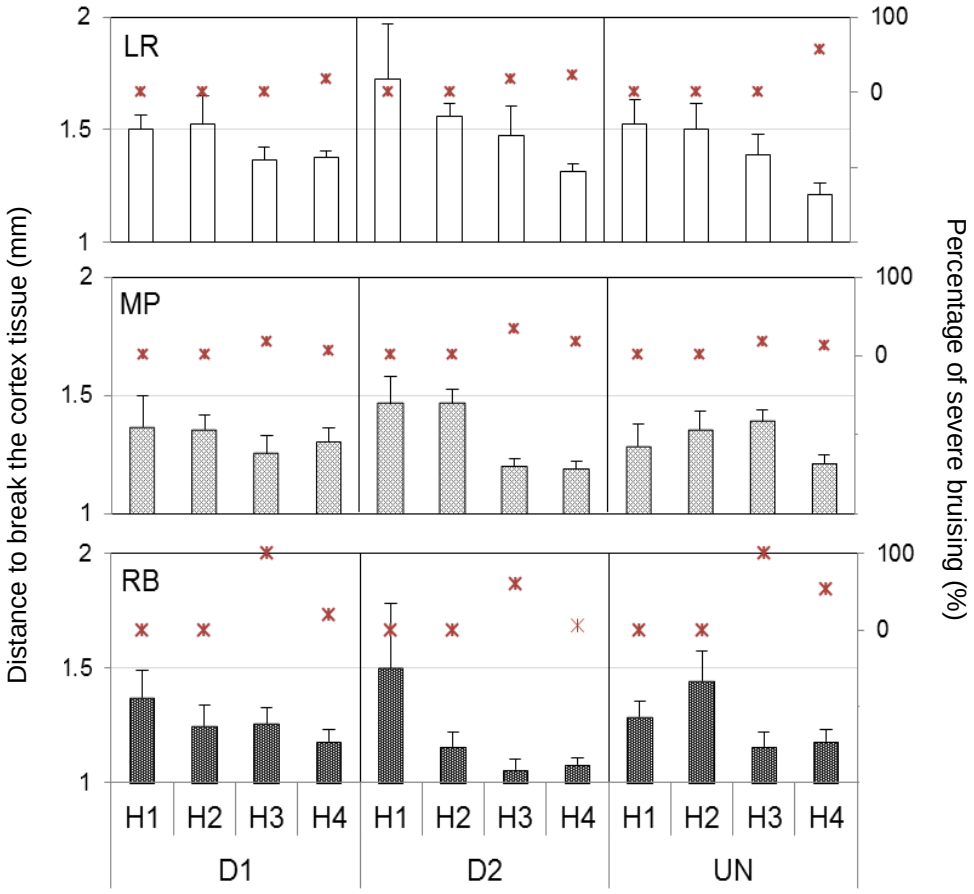


Figure 4.12 Effect of variety, harvest time and defoliation regime on the distance to break the cortex tissue in mm (bars) and percentage of severe bruising (%) (scatter) from crops harvested in early August (H1), late August (H2), early September (H3) and late September (H4), defoliated early August (D1), late August (D2) and undefoliated (UN) , field trial 1. Values bars show mean (n=9), errors bars are SE and scatter shows percentage of severe bruising (%) (H1-H3 n=9, H4 n=18).

## 4.2.5 Phenolic composition

### 4.2.5.1 Phenolic acids

The most abundant compound found for all varieties was 5-*O*-caffeoylquinic acid (5-CQA), referred to as chlorogenic acid (CQA) (Clifford, 2000), and has previously been reported (Shakya and Navarre, 2006). CQA constitutes between 62 to 84% of the total phenolic acids analysed and most of the discussion centres on this compound.

A high level of variation between the phenolic acids among varieties was observed and the total amount of phenolic acids extracted showed large differences, but most of them showed the same trend. Particularly, 5-CQA concentration increased up to the 3$^{rd}$ harvest time and diminished from the 3$^{rd}$ to the 4$^{th}$ harvest for all varieties and defoliations. It was expected that CQA levels be minimal in early-season compared with those in that late-season and the lower concentration of 5-CQA at H1 and H2 could be a factor contributing to the lack of bruising in this period. The decrease in chlorogenic acid content at the 4$^{th}$ harvest for all varieties and defoliation regimes studied was not expected, shown in figure 4.13. This could be due to the metabolic utilisation of CQA by the ageing tuber as an antioxidant.

Of the varieties examined, the highest amount of 5-CQA was found in LR, followed by RB and MP. Significant differences were found for each variety when comparing the harvest times, with the exception of LR D1 along all harvest times, LR D2 from H2 to H4 and RB D1 from H1 to H2, as shown in table 4.4.

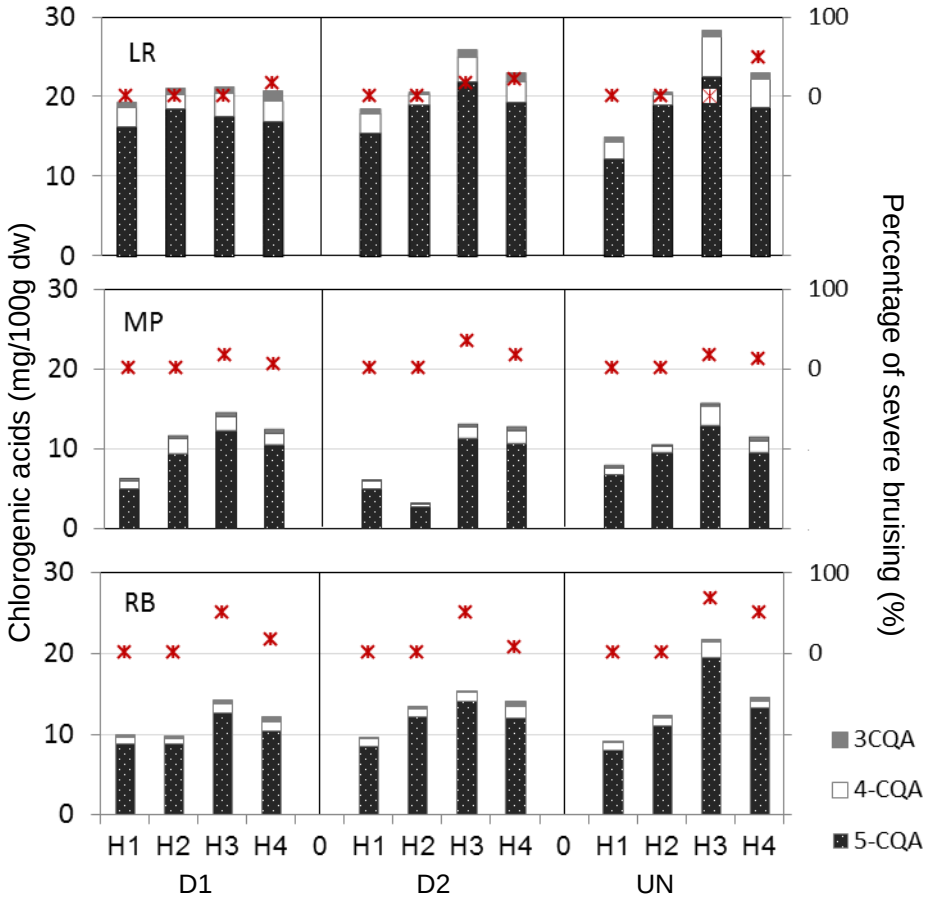


Figure 4.13 Effect of variety, harvest time and defoliation regime on chlorogenic acids (3-, 4- and 5-CQA) of lyophilized cortex (mg/100 g dw) and percentage of severe bruising (%) from crops harvested in early August (H1), late August (H2), early September (H3) and late September (H4), defoliated early August (D1), late August (D2), and undefoliated (UN), field trial 1. Values bars show mean (n=3), errors bars are SE and scatter shows percentage of severe bruising (%) (H1-H3 n=9, H4 n=18).

Table 4.4 Map of significant variances along harvests and comparison of defoliated samples to undefoliated at the 3rd and 4th harvest time. Cell shaded grey were significant different ($p<0.05$). Signals "+" and "-"means there was higher (+) or lower (-) levels in defoliated when compared with the respective undefoliated samples. Both signals "-/+" was applied when some results were lower and others higher than undefoliated samples.

| Varieties/ defoliation | Compounds | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 5-CQA | | | 4-CQA | | | 3-CQA | | |
| Harvests | H1-H2 | H2-H3 | H3-H4 | H1-H2 | H2-H3 | H3-H4 | H1-H2 | H2-H3 | H3-H4 |
| LR D1 | | | | | | | | | |
| LR D2 | | | | | | | | | |
| LR UN | | | | | | | | | |
| MP D1 | | | | | | | | | |
| MP D2 | | | | | | | | | |
| MP UN | | | | | | | | | |
| RB D1 | | | | | | | | | |
| RB D2 | | | | | | | | | |
| RB UN | | | | | | | | | |

| | Varieties – comparisons with undefoliated samples at H3 and H4 | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Defoliation | LR | | MP | | RB | | LR | | MP | | RB | | LR | | MP | | RB | |
| Harvests | H3 | H4 | H3 | H4 | H3 | H4 | H3 | H4 | H3 | H4 | H3 | H4 | H3 | H4 | H3 | H4 | H3 | H4 |
| D1 | + | | - | + | - | - | - | - | - | | - | + | | + | | - | + | + |
| D2 | | | - | -/+ | - | - | - | - | - | | | + | + | + | - | | - | + |

Defoliation significantly affected the content of 5-CQA for the variety MP compared to undefoliated samples ($p<0.01$) at all harvest times with lower amounts found in H3 and higher at D1 at the H4 compared to undefoliated. Defoliation significantly affected 5-CQA content for variety RB, particularly for later harvests (H3 and H4), with defoliated samples showing significantly lower levels of this compound in defoliated versus undefoliated samples. For variety LR, 5-CQA varied significantly along harvest time in defoliated samples, but was not significantly different than undefoliated samples (apart from LR D2 at H3).

Of the other CQA's quantified, the isomer cryptochlorogenic acid (4-CQA) was more abundant than neochlorogenic acid (3-CQA) in all varieties and ranged from 6-17% of the total CQA content, whereas 3-CQA comprised of 1.5 to 6% of the total. Different profiles were found for the isomers as 4-CQA presented a higher concentration at the 3$^{rd}$ harvest time whereas 3-CQA presented higher concentration at the 4$^{th}$ harvest for all varieties and defoliation regimes.

Defoliation showed significant differences in the composition of the isomers of CQA compared to undefoliated samples at the 3$^{rd}$ and the 4$^{th}$ harvest times as shown in table 4.4. In general defoliated samples presented an increased the content of 4-CQA and decreased the content of 3-CQA.

Some correlation between the incidence of severe bruising and concentration of CQAs in the cortex was found for the varieties RB (R=0.64) and MP (R=0.52), but not for LR (R=0.09).

Besides CQAs, caffeic acid (CA) is also known to be relevant in the bruising of potatoes (Lærke *et al*., 2002). CA was detected in all varieties with up to a 12 fold difference among varieties at H3. The concentration of CA ranged from 0.35-2.26 mg/100g dw in MP, 0.08-0.98 mg/100 g dw in LR and 0.12-0.48 mg/100g dw in RB along the harvest times (figure 4.14).

Additional compounds were measured in tubers including vanillic acid (VA), ferulic acid (FA) and *p*-Coumaric (pCou). All compounds were detected in all varieties (figure 4.14). LR and RB presented higher concentration of FA than MP. FA presented similar or higher amounts in more mature tubers (H4)

whereas VA and *p*Cou were higher in the more immature tubers (H2). As these compounds were considerably less abundant than 5-CQA and so would not offset the overall decrease seen during harvest times.

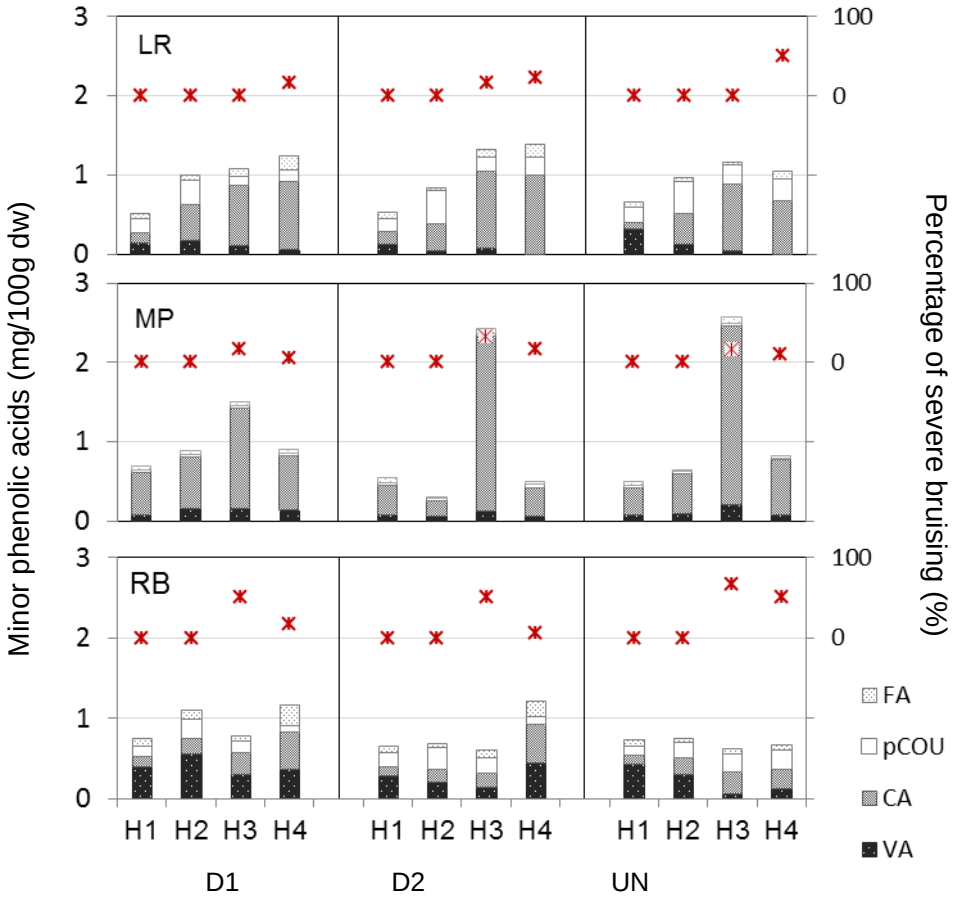


Figure 4.14 Effect of variety, harvest time and defoliation regime on minor phenolic acids (FA, *p*COU, CA, VA)) of lyophilized cortex (mg/100 g dw) and percentage of severe bruising (%) from crops harvested in early August (H1), late August (H2), early September (H3) and late September (H4), defoliated early August (D1), late August (D2), and undefoliated (UN), field trial 1. Values bars show mean (n=3), errors bars are SE and scatter shows percentage of severe bruising (%) (H1-H3 n=9, H4 n=18).

#### 4.2.5.2 Tyrosine

HPLC was used to determine the free tyrosine content in the methanolic extracts. Figure 4.15 shows the concentration of tyrosine in cortex tissue (mg/100g dw).

Significant variations in free tyrosine content was observed among all varieties ($p<0.001$). The higher amount of free tyrosine was found in RB, varying from 19.8-59.6 mg/100 g dw, followed by MP with 7.3-55.4 mg/100 g dw and LR from 4.1-30.1 mg/100g dw. These results were slightly lower than cited by other the literature, with variation from 9 to 319 mg/ 100g of dw (Lisinka and Leszczynski, 1989), however, tyrosine content in early-season cultivars tended to be lower than that in late-mature potatoes, as previously reported (Lisinka and Leszczynski, 1989). Although significant differences in the content among varieties were found, all of them showed the same trend with small variation when comparing H1 and H2 and increase in the content from H2 to H4.

Statistical analysis of variance showed significant differences, either in samples defoliated in early August (D1) and late August (D2) when compared to undefoliated samples ($p<0.01$) for all varieties studied. A higher content of tyrosine was found in potatoes defoliated early and later (D1 and D2 respectively) than undefoliated samples.

A weak correlation between incidence of severe bruising and tyrosine content was found for LR ($R=0.41$), but not for the varieties ($R=0.10$ for MP and RB).

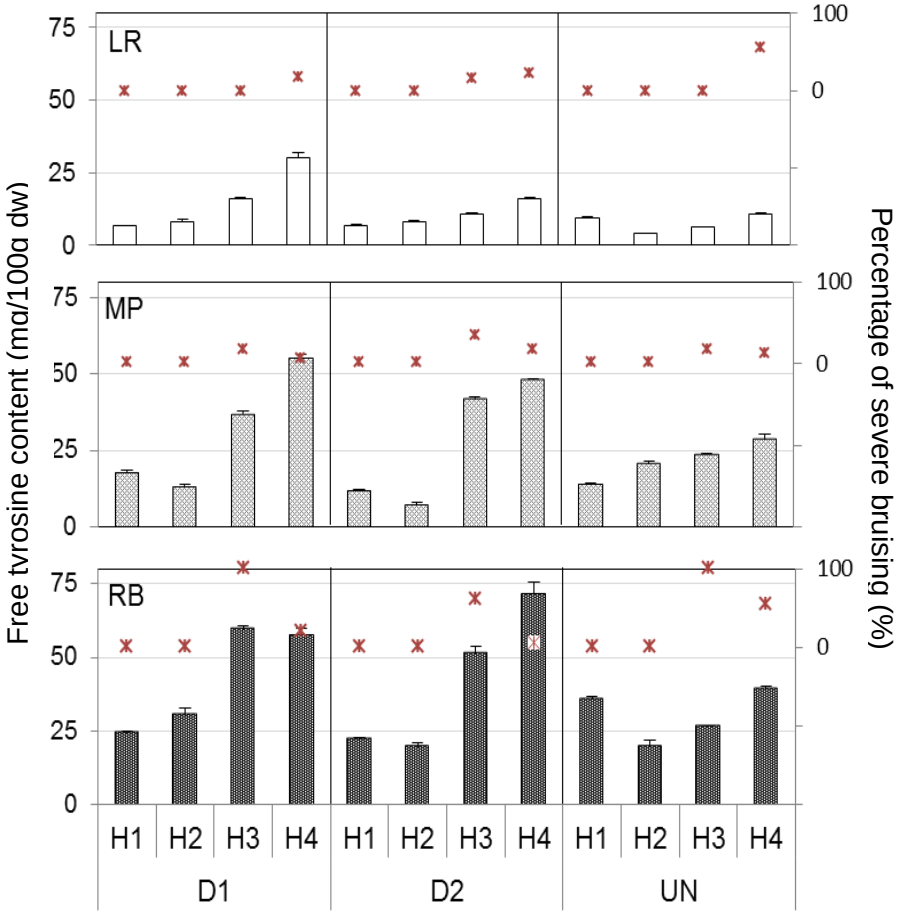


Figure 4.15 Effect of variety, harvest time and defoliation regime on free tyrosine levels of lyophilized cortex in mg/100 g dw (bars) and percentage of severe bruising (%) (scatter) from crops harvested in early August (H1), late August (H2), early September (H3) and late September (H4), defoliated early August (D1), late August (D2) and undefoliated (UN), field trial 1. Values bars show mean (n=3), errors bars are SE and scatter shows percentage of severe bruising (%) (H1-H3 n=9, H4 n=18).

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### 4.2.6 Cell wall ultrastructure and composition

#### 4.2.6.1 Optical localization of cell membranes

General anatomical features of potato tissue were investigated by light microscopy in order to identify the differences between varieties and characteristics of different tissues (e.g. skin and cortex). Wax embedded sections were stained with toluidine blue to show cell membranes as presented in figure 4.16.

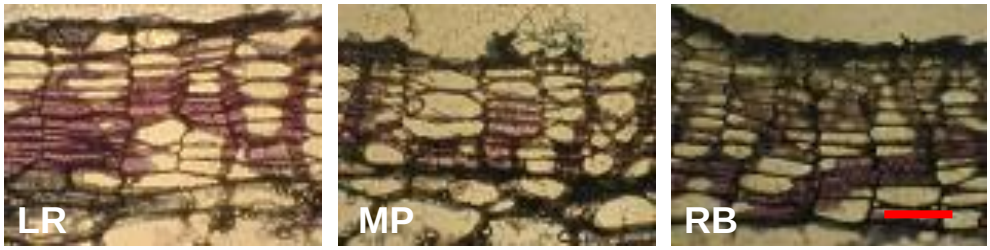


Figure 4.16 Toluidine blue staining of membranes in wax-embedded sections of skin. Magnitude 20X. Scale bar =50 µM

On the skin, RB presented suberized cells stacked with adjacent cells compressed together whereas LR and MP presented a 'ragged' appearance. The "ragged" organisation may act to prevent transmission and dissipation of forces throughout the potato, leading to more energy to be absorbed at the point of impact rather than transmitting and distributing it to the rest of the tuber. Comparing the structural characteristics with the results from the energy to break the skin tissue, LR and MP required respectively 25.5 and 33.3% more energy than RB to break the skin respectively (H4).

#### 4.2.6.2 Cell wall composition of potatoes from 4th harvest

The monosaccharide content from potatoes from the 4th harvest was characterised after sequential hydrolysis of cell wall material (CWM) using 0.1M and 2M Trifluoroacetic acid (TFA). The objective of this study was to analyse differences in composition and distribution of pectic polysaccharides among the varieties defoliated 49 days (D1), 35 days (D2) and undefoliated samples harvested in later September (H4). Results are summarized in table 4.5 and concentrations are expressed in %mol. The yield of extraction comprised between 0.27 and 3.71% of the dry matter. The huge range found was due to differences in starch content among varieties.

Also, despite several washings and sieving steps (mesh 45 µm), large granules of starch were left in the CWM together with starch granules in some small cells, which were not broken during gridding or ultraturrax homogenization. Due to starch remaining in the same samples, glucose amounts were not considered in the composition of the cell wall and the results were not presented to prevent misunderstanding about the source of glucose. Fucose and mannose were not detected in the chromatograms from the potato cell wall hydrolysates.

From the results, the percentage mol (%mol) of sugars of the CWM from the LR variety were significantly different to MP and RB ($p<0.001$), with exception of the xylose content ($p>0.05$). The %mol of the CWM from the varieties MP and RB were not significantly different ($p>0.05$). Although LR was significant different, the molar composition of the CWM was comparable among the varieties.

Galactose and arabinose have previously been shown to be the most abundant sugars in potato cell walls (Jarvis *et al*., 1981). The results of this study found higher content of arabinose than galactose. This may be due preliminary step of the hydrolysis using 0.1 M TFA which preserves arabinose. This was previously reported by Fry (2000) who showed that the hydrolysis of non-cellulosic polysaccharides with 0.1 M at 100 °C TFA for 1 h typically released arabinose, fucose and rhamnose and 1 M TFA release glucose, galactose, mannose, xylose, arabinose, glucuronic acid, galacturonic acid. In wall polymers, most arabinose residues are found in side chains to other polymers (in RG I side-chains, arabinoxylan and arabinogalactan) (Fry, 2000). Results have shown that among the varieties, arabinose concentration was lower in the LR potatoes, averaging from 33.9-37.1% mol while for MP and RB variations ranged from 42.2-43.0% mol and 38.2-41.8% mol respectively. The rhamnose content showed the same pattern as arabinose, with less concentration in LR samples (0.4-0.8% mol) and higher in MP and RB (0.9-1.1% mol). Rhamnose is a neutral sugar found in the backbone of RG I. For both sugars, arabinose and rhamnose, a slightly higher concentration was found in D1 samples, but significant differences were found only in LR D1 compared to undefoliated samples ($p<0.01$).

Another monosaccharide present as a side chain is galactose. Among the varieties, the concentration of galactose was lower in LR potatoes, averaging from 19.9 to 21.7% mol while for MP and RB galactose content ranged the levels from 21.3-24.4% mol. Early defoliation (D1) appeared to have significant effect only for RB when compared to undefoliated samples

($p<0.01$), showing lower content. Higher concentrations of galacturonic acid and glucuronic acid concentrations were observed in the LR potatoes. Defoliation had a significant effect for both monosaccharides only for the RB samples comparing D1 and UN ($p<0.01$), where galacturonic acid presented slight increase on the content and glucuronic acid slight decrease.

Table 4.5 Monosaccharide concentrations (rhamnose (Rha), arabinose (Ara), galactose (Gal), Xyose (Xyl), galacturonic acid (GalA) and glucuronic acid (GluA) from CWM of the cortex tissue of the cultivars LR, MP and RB from tubers harvested in later September 2010 (H4), defoliated early August (D1), late August (D2) and undefoliated (UN) after sequential hydrolysis with 0.1 M and 2 M TFA. Concentrations are expressed per %mol. Values represent average ± SEM (n=4).

| | **Variety and defoliation (% mol)** | | |
|---|---|---|---|
| **Monosaccharides** | **LR D1** | **LR D2** | **LR UN** |
| **Rha** | 0.8 ± 0.0 | 0.6 ± 0.0 | 0.4 ± 0.0 |
| **Ara** | 37.1 ± 0.6 | 34.1 ± 0.5 | 33.9 ± 0.3 |
| **Gal** | 21.7 ± 0.8 | 19.9 ± 0.8 | 20.3 ± 0.4 |
| **Xyl** | 11.5 ± 0.2 | 10.7 ± 0.5 | 9.8 ± 0.2 |
| **GalA** | 22.3 ± 0.8 | 26.8 ± 1.6 | 24.2 ± 0.3 |
| **GluA** | 3.9 ± 2.0 | 3.1 ± 0.0 | 3.4 ± 0.2 |
| **Monosaccharides** | **MP D1** | **MP D2** | **MP UN** |
| **Rha** | 1.1 ± 0.0 | 0.8 ± 0.1 | 0.9 ± 0.1 |
| **Ara** | 42.5 ± 0.5 | 43.0 ± 0.8 | 42.2 ± 0.9 |
| **Gal** | 21.8 ± 1.1 | 24.2 ± 0.2 | 24.4 ± 0.7 |
| **Xyl** | 11.5 ± 0.9 | 10.0 ± 1.5 | 11.0 ± 1.3 |
| **GalA** | 22.3 ± 0.7 | 21.1 ± 0.9 | 20.7 ± 0.9 |
| **GluA** | 0.8 ± 0.1 | 1.0 ± 0.1 | 0.7 ± 0.1 |
| **Monosaccharides** | **RB D1** | **RB D2** | **RB UN** |
| **Rha** | 1.1 ± 0.0 | 0.7 ± 0.1 | 1.0 ± 0.0 |
| **Ara** | 41.8 ± 0.8 | 38.2 ± 0.7 | 41.2 ± 1.1 |
| **Gal** | 21.3 ± 1.0 | 21.3 ± 0.6 | 24.4 ± 0.6 |
| **Xyl** | 10.5 ± 1.0 | 13.1 ± 0.6 | 11.0 ± 1.6 |
| **GalA** | 22.8 ± 0.6 | 22.8 ± 0.8 | 19.2 ± 1.1 |
| **GluA** | 1.1 ± 0.1 | 1.2 ± 0.0 | 1.4 ± 0.1 |

The molar ratio of the neutral sugars (arabinose + galactose) to uronic acids (glacturonic acid + glucuroic acid) can give information about the branching of the pectic polysaccharides, assuming that all neutral sugars are present as side chains (van Marle *et al*., 1997). The molar ratio of arabinose + galactose to uronic acids was calculated for the cultivars and presented in table 4.6.

Table 4.6 Neutral sugars (rhamose+arabinose+galactose (Rham+Ara+Gal)), branching (molar ratio of arabinose + galactose to uronic acids (Ara+Gal/UA)) and number of side chains (uronic acids (UA)/rhamnose) in CWM of the cultivars LR, MP and RB harvested in later September 2010 (H4), defoliated early August (D1), late August (D2) and undefoliated (UN) after sequential with 0.1 M and 2 M TFA.

| | **Varieties and defoliation** | | |
|---|---|---|---|
| | **LR D1** | **LR D2** | **LR UN** |
| **Neutral sugars (Rham+Ara+Gal)** | 59.5 | 54.6 | 54.7 |
| **Molar ratio (Ara+Gal/UA)** | 2.2:1.00 | 1.8:1.00 | 2.0:1.00 |
| **UA/Rhamnose** | 31.3:1.00 | 49.1:1.00 | 62.1:1.00 |
| | **MP D1** | **MP D2** | **MP UN** |
| **Neutral sugars (Rham+Ara+Gal)** | 65.4 | 68.0 | 67.5 |
| **Molar ratio (Ara+Gal)/UA** | 2.8:1.00 | 3.0:1.00 | 3.1:1.00 |
| **UA/Rhamnose** | 21.6:1.00 | 27.7:1.00 | 23.3:1.00 |
| | **RB D1** | **RB D2** | **RB UN** |
| **Neutral sugars (Rham+Ara+Gal)** | 64.2 | 60.3 | 66.6 |
| **Molar ratio (Ara+Gal)/UA** | 2.6:1.00 | 2.0:1.00 | 3.2:1.00 |
| **UA/Rhamnose** | 21.9:1.00 | 33.7:1.00 | 19.8:1.00 |

A higher molar ratio of arabinose + galactose to uronic acids indicates the presence of more and/or longer neutral sugar side chains. The extended side chain cannot discriminate between either a small number of relatively long polymerization degree (DP>) side chains or a large number of relatively small (DP<) side chains. Comparing varieties, MP and RB presented more

or longer side chains than LR samples. Among all varieties, the molar ratio of arabinose+galactose to uronic acids ranged from 1.8 to 3.2. The results found were comparable with the potato varieties Nicola and Irene with molar ratios of 2.3 and 2.6 respectively when CWM were hydrolysed with 2M TFA (van Marle *et al*., 1997).

The molar ratio of uronic acids to rhamnose (shown in table 4.6) can be used as an indication of the number of side chains and represent the measure of linearity of the cell wall pectin (van Dijk *et al*., 2002). It should be kept in mind, however, that this ratio may not be reliable, since only part of the rhamnose residues are substituted (Carpita and Gibeaut, 1993) and furthermore the rhamnose content of the cell wall is relatively low.

Among the varieties, LR presented higher linearity of pectin than MP and RB. Early defoliation affected significantly ($p<0.001$) the amount of arabinose in LR and the amounts of galactose and uronic acids in RB but no pattern was found when analysing defoliation. From these results we can conclude that the pectin structure of LR was more linear with the presence of less or smaller side chains attached to the structure $\alpha$-1,4-linked galacturonic acid chain of pectin.

When comparing severe bruising with arabinose content, galactose content, degree of branching and molar ratio of all varieties, no relationship were found ($R<0.16$).

### 4.2.7 Relationship between analyses

Pearson's correlation using the coefficient of determination R and significance value P are summarized in table 4.7. These correlations were conducted to study the relationship between physiological and biochemical characteristics of the crop during harvest times and under defoliation regime with severe bruising. The purpose of this exercise was to establish whether physical/composition aspects of the crop might act as predictive indicators of severe bruising.

Table 4.7 The relationships (R) and significance P value of severe bruising with physical, mechanical and compositional aspects of the varieties LR, MP and RB under defoliation regime along harvest times, field trial 1.

| **Variety and correlation sample size** | **LR (n=8)** | | **MP (n=8)** | | **RB (n=8)** | |
|---|---|---|---|---|---|---|
| **Assessment , sample size/R and P value** | **R** | **P value** | **R** | **P value** | **R** | **P value** |
| Oxidative potential (OP) | **-0.49** | 0.11 | **0.11** | 0.74 | 0.01 | 0.97 |
| Weight (n=9) | 0.39 | 0.21 | **0.21** | 0.51 | 0.50 | 0.09 |
| Energy to break skin tissue (n=9) | -0.06 | 0.85 | **-0.52** | 0.83 | -0.28 | 0.38 |
| Energy to break cortex tissue (n=9) | **-0.48** | 0.11 | **-0.51** | 0.09 | -0.13 | 0.69 |
| Chlorogenic acids content (n=3) | 0.09 | 0.78 | **0.52** | 0.08 | **0.64** | **0.02** |
| Tyrosine content (n=3) | **0.41** | 0.19 | **0.10** | 0.76 | 0.10 | 0.76 |

Although Person's coefficient (R) indicates some correlations, most of them were not statistically significant ($p>0.05$), what reflect a very small correlation sample size (n=8). So, Pearson coefficient was used as an indicator of the general trend. The summary of the correlations indicated that there was strong correlation between severe bruising of LR with tyrosine, with oxidative potential (negative) and cortex tissue. MP presented different correlations to physical aspects as energy to break the tissues and some correlation with chlorogenic acids content. RB presented some correlation between

incidence of severe bruising and concentration of chlorogenic acids in the cortex. From these results it is possible to observe that no a single factor could be used to predict bruising as each variety presented different factor of correlation. In addition, although there were some scatter correlations found among the varieties, no a strong relationship was found.

Further correlation analyses were conducted to investigate the relationships. The results for the three varieties studied under four harvests and two regimes of defoliation are summarized in the PCA bi-plot (figure 4.17). The labels are indicated in table 4.8. These analyses generated substantial number of correlations and the model of PCA explained about 59% of the data variance. The components allowed discrimination of the varieties. Investigation into the relative contribution (loadings) of individual variables in the PC1 dimension highlighted components with a significant impact on bruising. Severity of bruising (SB) and the mechanical properties of the skin tissue dimensions energy (ES), force (FS) and distance (DS) were strongly negative correlated  on this study, providing evidence of a useful link between bruising and mechanical properties. Oxidative potential was well correlated with tyrosine levels. In fact, the order of oxidative potential and concentration of tyrosine among varieties was RB>MP>LR.

Table 4.8 Labels in the PCA graphs from LR, MP and RB crops harvested in early August (H1), late August (H2), early September (H3) and late September (H4), defoliated early August (D1), late August (D2) and undefoliated (UN), field trial 1.

| Letter | Assessment | No. | Label | No. | Label | No. | Label |
|---|---|---|---|---|---|---|---|
| A | Severe Bruising | 1 | LR D1 H1 | 13 | MP D1 H1 | 25 | RB D1 H1 |
| B | Optical density | 2 | LR D1 H2 | 14 | MP D1 H2 | 26 | RB D1 H2 |
| C | Energy to break the skin tissue | 3 | LR D1 H3 | 15 | MP D1 H3 | 27 | RB D1 H3 |
| D | Force to break the skin tissue | 4 | LR D1 H4 | 16 | MP D1 H4 | 28 | RB D1 H4 |
| E | Distance to break the skin tissue | | | | | | |
| F | Energy to break the cortex tissue | 5 | LR D2 H1 | 17 | MP D2 H1 | 29 | RB D2 H1 |
| G | Force to break the cortex tissue | 6 | LR D2 H2 | 18 | MP D2 H2 | 30 | RB D2 H2 |
| H | Distance to break the cortex tissue | 7 | LR D2 H3 | 19 | MP D2 H3 | 31 | RB D2 H3 |
| I | Vanillic acid | 8 | LR D2 H4 | 20 | MP H2 H4 | 32 | RB D2 H4 |
| J | Caffeic acid | | | | | | |
| K | *p* Coumaric acid | 9 | LR UN H1 | 21 | MP UN H1 | 33 | RB UN H1 |
| L | Ferulic acid | 10 | LR UN H2 | 22 | MP UN H2 | 34 | RB UN H2 |
| M | 3-CQA | 11 | LR UN H3 | 23 | MP UN H3 | 35 | RB UN H3 |
| N | 4-CQA | 12 | LR UN H4 | 24 | MP UN H4 | 36 | RB UN H4 |
| O | 5-CQA | | | | | | |
| P | Tyrosine | | | | | | |

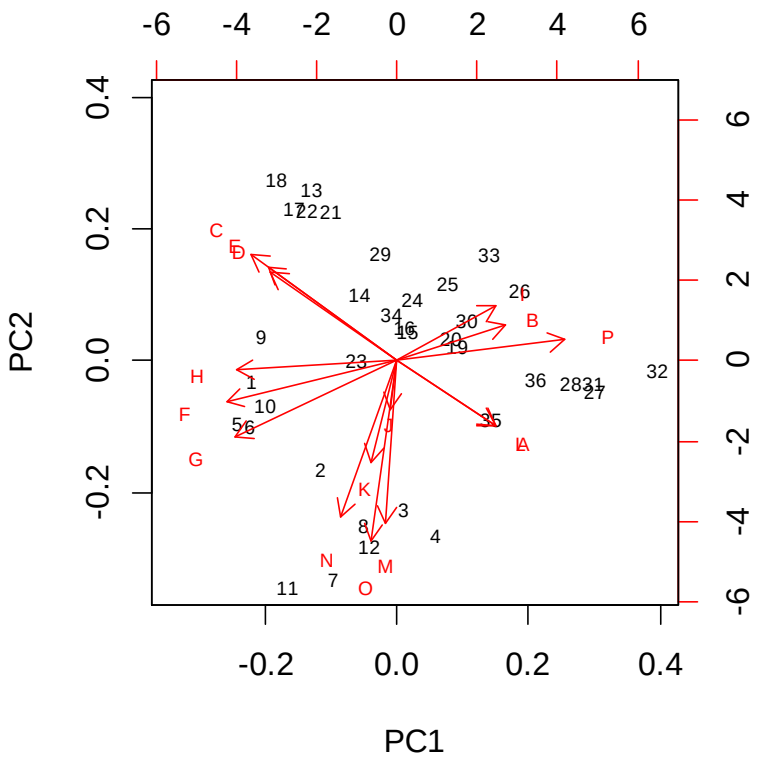


Figure 4.17 PCA bi-plot of data from potatoes harvested in early August (H1), late August (H2), early September (H3) and late September (H4), defoliated early August (D1), late August (D2) and undefoliated (UN), field trial 1. PC1 explains 35 % of variance and PC2 24%.

## 4.3 Discussion

The main aim of this study was to investigate the effect of harvest and defoliation on bruising incidence in three varieties of potatoes. Additionally the research correlated results to establish whether physiological and biochemical characteristics, such as mechanical properties, phenolic acids, tyrosine and cell wall composition are factors that influence bruising and may be used as predictive indicators of bruising. This knowledge would enable growers to better manage crops to achieve the necessary standards for the quality of potatoes.

Potatoes harvested in August (H1 and H2) have not presented bruising. The incidence of bruising in potatoes was lower in early-season than that in late-season and at these two occasions the potatoes were damaged and left over a period of 48h to develop bruising at room temperature. This observation agrees with the work of Strehmel and her co-workers (2010a) that showed that bruising development will depend on temperature and that room temperature was not hot enough to develop bruising in samples. Potatoes were therefore cooled down to 6°C as it is known that potatoes bruise more when damaged at temperatures below 10°C. To accelerate the identification of bruising from mechanical damage, samples can be put into a hot box, were the temperature was maintained of 33°C and RH 95-98%. Bruise normally occurs within 12-14 h but in this experiment 48 h was applied as 12-14h was not enough to develop bruising. The use of hot box would be recommended for all subsequent bruising measurements (field trial 2 and 3).

Among the varieties, the highest incidence of bruising was expected for RB. This was observed at the third harvest, with a decrease at the fourth harvest. However, the highest incidence of severe bruising was found for LR at the fourth harvest. In comparison to LR, MP showed lower levels of bruising during that growing season. This indicates that within the same field, different varieties have different bruising behaviours which may depend on their rate of maturity and their ability to respond differently to environmental conditions.

The green canopy cover can be used as an indication of crop maturity in the field (Stalham, 2008). The physiological age of LR and RB was different from

that of MP, where about 50% senescence was observed in the middle of September for LR and RB and only 8% senescence was observed for MP by this time. Although LR and RB were already senescent, RB showed more bruising at H3 followed by a decrease to H4 and LR showed more bruising from H3 to H4.

The huge decrease in bruising for RB comparing H3 to H4 was unexpected, but can be explained by a larger sample size used on the 4$^{th}$ harvest and subsequent decrease in the percentage of the potatoes with ‘breaking damage’ which were excluded from the bruising analysis. Physiological characteristics observed using microscopy can be a factor that explains more damage on skin of RB, where cell are arranged into uniform stacks, breaking easily between cells rather than through cells.

The general trend found about defoliation was higher incidence of bruising in defoliated samples at H3 (24 (D2) and 38 (D1) days after defoliation). This is in accordance with a previous study by Stalham (2008) which showed higher incidence of bruising when potatoes where harvested 3 to 5 weeks after defoliation. However, tubers from defoliated plants presented lower incidence of bruising when compared with undefoliated at H4 harvest (35 and 49 days after defoliation).

In terms of inspection in the industry, severe bruising is usually an indication to reject bruised crops, however, record of slight bruising was also taken. The analysis of slight bruising attempted to find out if a different trend in bruising would occur between varieties along the harvest periods. This was observed in the case of defolaliated MP samples, which showed higher

bruising in defoliated samples when compared with undefoliated for both harvests (H3 and H4). Comparing with the results of green coverage, it is observed that at H4 LR and RB were already senescent and tubers of undefoliated plants showed higher incidence of bruising. It can lead to think that there is an optimum “maturity” for best harvest time ahead defoliation. Tubers from undefoliated plants of MP could bruise more than defoliated if the harvest time was extended.

The lower incidence of bruising found in defoliated samples after 5 weeks of defoliation can be explained by the skin set when tubers were left into the ground without the plant (Wiltshire *et al*., 2005). The lower incidence of bruising was not associated with skin strength. However, this may not be of practical application for growers as other diseases can affect the potato when left into the ground for long period.

Results from the oxidative potential studies show that the assay does work sufficiently to show differences between the most and the least susceptible cultivars as higher amounts of pigment were formed for the variety RB, followed by MP and LR. However it was not possible to detect variations along harvest or defoliation time. This may be due to the fact that the available substrate is present in excess whereas the enzyme is only present in specific quantities and possibly other factors are limiting the extracts and may prevent a full estimate of the bruising activity.

Of the factors studied, varietal difference is one of the most important factors that has an effect on bruising. The variety RB was less deformable requiring less force and energy to break the skin and the cortex tissues. These results

are in accordance to a research undertaken through a BPC_LINK (240) project which investigated the amount of energy absorbed by tuber cortical tissue in response to uniaxial compression. The results from the LINK study indicated that tissue from a bruise susceptible cultivar (Russet Burbank) is more brittle than resistant tissue (Cara) which is able to diffuse the stress across a larger number of cells. Mechanical properties were also explored in the present study. Comparisons of increased harvest times did not lead to a clear relationship between severe bruising and energy required to break the tissues, apart for MP which presented strong negative correlation with energy to break the skin and moderate negative correlation with the cortex tissue. So the stronger the skin, the less bruising was observed in this variety. This suggests that for this variety, strong mechanical properties of the tissue maybe protecting against bruising.

For RB and LR, energy required to break the skin decreased along harvest time, and this was associated with more bruising. However, only energy required to break LR tissue was statistically significant associated. Defoliation was expected to lead to earlier skin setting, but these changes in skin properties were not detected using the adopted methodology. Nevertheless, overall, the PCA plot indicates that mechanical properties of the break the skin were negatively correlated with bruising, where stronger skin is associated with increased frequency of bruising. From these results, it is possible to conclude that small changes in the mechanical properties of tissue can have a large influence on bruising.

Cell wall composition can affect the mechanical properties of tissues, and also change in time due to changes in cell wall synthesis or degradation (Pena and Carpita, 2004; Orfila *et al.*, 2001)

Arabinose was the most abundant neutral sugar found in the potatoes cell walls used in this study. Previously it was reported that galactose is the most abundant neutral sugar (Jarvis *et al*., 2000). This shows that the extractability of potato pectic sugars is strongly dependent on the method of hydrolysis. In this study sequential hydrolysis was applied, extracting more arabinose on the first step and galactose on the second step of hydrolysis (showed previously in method development).

Differences were found in the sugars composition between varieties. These differences were mainly due the presence of more rhamnose, arabinose, galactose and less uronic acids (UA) in MP and RB compared to LR. The higher ratio of Arabinose + Galactose to UA in MP and RB and the higher ratio of UA to rhamnose for LR indicate the presence of more and or longer side chains in MP and RB than LR. The relative lower amounts of sugars on side chains (Rham+Ara+Gal) in LR gives additional indication that primary cell walls of LR has a less branched structure than the other varieties but it was not directly correlated to bruising (R=0.25). Decrease in branching should allow more opportunities for cross-linking of unmethylated acidic (unbranched) homogalacturonan (HG). The degree of methylation of HG was meant to be investigated during field trial 1 using immunofluorescence microscopy, however this was not possible due to technical difficulties with embedding potato tissue in wax. These difficulties were addressed and immunofluorescence was carried out on samples from field trials 2.

Side chains of pectin type rhamnogalacturonan I (RG-I) galactan and arabinan and can interact with cellulose microfibrils in primary cell walls (Zykwinska *et al*. 2007) and can be covalently link to xyloglucan as noted in Arabidopsis cell cultures (Popper and Fry, 2008). The galactosyl containing side chains of xyloglucan contribute to the tensile strength of cell walls (Ryden *et al*., 2003; Caffall and Mohnen, 2009).

This contribution to the tensile strength was supported by Ulvskov and co-workers (Oomen *et al*., 2002, Skjøt *et al*., 2002, Ulvskov *et al*., 2005, Orfila *et al*., 2012). They proposed that the components of RGI (galactan and arabinan) transmit stresses in the wall and hence play a direct role in wall rheology properties. The force to fracture cylinders of tuber tissue decreased when in galactan and arabinan deficient tubers were studied. These potatoes were obtained by expressing fungal pectin-digesting enzymes. The elastic properties of the tubers were also altered, with a stiffening of the cell wall. These suggest that less RGI-I side chains are associated with more brittle tissue (stiff but easier to fracture). These studies were done on young (grown for 16 weeks) tubers where the degree of methylation of HG is likely to be high, and therefore RGI may have a bigger impact on mechanical properties than HG. It is known that the degree of methylation decreases along tuber development, allowing for more calcium cross-links between HG. HG may have a larger contribution to cell wall properties later in development.

From these results, it was expected that LR, which presented lower content of side chains, would require the more force but less distance to break the tissue (more brittle). In fact, LR required on average more force and the

probe reached higher distance to rupture the cortex tissue than MP and RB at H4. However, at this stage, the tubers are more mature compared to previous studies, and the degree of methylation of HG is likely to be lower, allowing calcium cross-links to occur leading to a stronger tissue. Although the differences found between pectic structures were not enough to explain mechanical changes at one time point of harvest, more pronounced changes were found upon storage and are shown in chapter 5.

Different structural characteristics were observed across varieties that would influence the incidence of external damage of the skin, texture and transmission of energy across the cells but it was difficult to single out any factors that might mostly influence bruising.

Moving from physical to biochemical aspects involving bruising, it was observed that the content of phenyl substrates such as tyrosine and other phenolic acid compounds such as chlorogenic acid tend to be less abundant in early than in late-season, what is in accordance to Lisinka and Leszczynski (1989). Studies from Lærke *et al*. (2002) showed that phenolic acid compounds such as chlorogenic acid or caffeic acid are known to be relevant in the bruising of potatoes. Stevens and Davelaar (1996) indicated that chlorogenic acid may take part in bruising formation, but is not essential for the discolouration.

It was found that the varieties MP and RB showed correlations between severe bruising and chlorogenic acids (R=0.52 and 0.64 respectively), supporting the hypothesis that chlorogenic acid may have a role in bruising formation in these varieties. Further chlorogenic acids analysis using the

bruised tissue would be interesting to confirm and understand how the chlorogenic acid participate in bruising formation.

Traces of phenolic acids, such as ferulic, and *p*-coumaric acids were detected. These traces of phenolics that are present may nevertheless act as important cross linking sites between polysaccharides (Fry, 2000), where –COOH group of phenolic acid can be linked via ester bonds to some sugar –OH groups in cell wall polysaccharides (Albersheim *et al*., 2011). FA can also participate in the building network such as those of diferulic acid esters and galacturonyl esters or with proteins that lead to decrease in cell wall porosity by covalent linkages (Rondeau-Mouro *et al.*, 2008).

In this study no strong correlation was found when comparing tyrosine levels with the incidence of severe bruising ($R < 0.41$). In fact, a low concentration of tyrosine was found in field trial 1 (<56 mg /100g dw), which was below the average of 72 mg/100g dw suggested by Corsine *et al*. (1992).

A large proportion of the measurements showed significant changes in the defoliation with respect to the undefoliated plants. This observation suggests that the treatment is responsible, at least in part, for the bruising variation observed. Nevertheless, individual variability must be taken into account, because high compositional heterogeneity has been found.

In conclusion, for each crop, there is an optimum “maturity” for best harvest time ahead defoliation. In this field trial, RB matured quickly (by H3), followed by LR (mature at H4) and then MP (not reached by H4). Along the harvest time, substantial changes in the phenolic substrates for polyphenol

oxidase can influence bruising, especially the main chlorogenic acids. The composition of cell wall may explain some of the tissue mechanical properties. On the basis of this information it is possible to conclude that not a single factor is predominant in determining the bruising for all varieties. RB seems to be more dependent on biochemical properties (maybe due to faster maturation), while MP is more dependent on tuber mechanical properties. The study did support previous observations about harvest time after defoliation. The study has significantly increased the understanding of the processes involved in bruise development.

# 5 Effect of harvest time on bruising upon storage - Field Trial 2

## 5.1 Introduction

In the previous chapter, variations in susceptibility to bruising of potato cultivars (Maris Piper (MP), Lady Rosetta (LR) and Russet Burbank (RB)) under defoliation regime along harvests time were examined. In terms of environmental conditions, tuber maturity (as measured by canopy senescence) has been shown to have an effect on bruising at harvests. Maturity at harvest time is also the predominant factor influencing processing quality of potatoes throughout storage (Groves *et al.*, 2005). The theory is that the onset of bruising is largely related to senescence – the earlier and more rapidly this happens, the more severe bruising tends to be (Stalham, 2008).

The length of storage and temperature influences the tuber physiological age (Burton, 1989) and the level of respiration and evaporation of tubers (Mohsenin, 1986). Respiration rate of potatoes are less stable at storage temperatures higher than 20°C, but when storage temperatures are kept at 10°C or below in practice respiration over the storage periods is relatively constant (Pringle *et al*., 2009). Ninety-eight per cent of the moisture that leaves a tuber during storage is lost through its skin by evaporation, only 2.4% leaves the tuber via the lenticels along with the carbon dioxide produced by respiration (Burton, 1989). So long as the pressure within the cells of the tuber skins and the vapour pressure of the air in the voids

surrounding the tubers are the same, no evaporation will take place. For this balance to occur, the relative humidity of the air in the voids between the tubers has to be 97.8%. Ventilation of the crop with air cooler than the crop, no matter how humid, will always result in moisture loss through evaporation (Pringle *et al*., 2009).

Respiration and evaporation therefore influence tissue properties (Mohsenin, 1986). The cell walls within the tuber become weak and membranes leak as tubers age, releasing substrates to polyphenol oxidase. As presented in chapter 4, the content of phenolic substrates (tyrosine and phenolic acids) in potatoes tends to be less in early than in late-season. The content of phenolic compounds during storage is expected to increase, but not necessarily resulting in bruising, depending on storage temperature, as polyphenol oxidase activity is low at low temperatures (Friedman, 1997).

The starting point for this field trial was to investigate the changes which take place in physical and biochemical properties during growth and during post-harvest storage as it has not been clearly defined.

### 5.1.1 Aim

The aim was to test the relative importance of tuber maturity at harvest times in relation to bruising in stored potatoes. The same three varieties were investigated: MP, LR and RB. This knowledge would enable growers to better manage crops to achieve the necessary standards for the storage of potatoes. The research also aimed to understand whether physiological and biochemical characteristics, such as mechanical properties, weight, specific

gravity, phenolic acids, tyrosine and cell wall composition are factors that influence bruising and may be used as predictive indicators of bruising at harvest time for stored potatoes. As mentioned before (Chapter 1, section 1.5.1), these varieties of potatoes are known to differ in their tendency toward bruising where MP and LR present a bruising susceptibility score of 6, and RB a bruising score of 4 in ratings ranging from 0 (most susceptible) to 9 (least susceptible) (Carnegie *et al*., 2005; BPVD, 2012).

The cultivars studied were grown at Cambridge University Farm (CUF), planted on 15$^{th}$ April 2011 and harvested at two time points and stored for three time points. Harvest and storage dates and periods are indicated in table 2.2. Trials were randomised with three factors (variety, harvest and storage) with three replicate plots. Twenty tubers per plot (larger sample size than field trial 1) were collected and either shipped to Leeds or Sutton Bridge Crop Storage Research (Sutton Bridge, Suffolk) on harvest day. The tubers were stored in trays at temperature below 10º C and 95+% Relative Humidity (RH) at Sutton Bridge Crop Storage Research, and shipped to Leeds after specific storage times.

### 5.1.2 Hypotheses

The hypotheses tested are:

1) Potatoes harvested in September show less bruising than crops harvested in October, 24 days later.

2) Stored potatoes harvested in September show less bruising, and this may be due to lower content of phenolic substrates at harvest and storage.

3) Cell wall composition of cortex cells will influence the mechanical properties and therefore will influence bruising at harvest and storage.

### 5.1.3 Objectives

1) To investigate the potential for using physiological measurements at harvest as indicators of storage on the incidence of bruising.

2) To improve the potential for using physical and biochemical measurements as indicators of bruising.

## 5.2 Results

### 5.2.1 Field phase

#### 5.2.1.1 Meteorological data

The temperature of the air and soil between when the potatoes were planted and end of May were slightly higher than average years (Section 4.2.1.1 and 6.2.1.1) and are shown in figure 5.1. The temperature in this season was higher than other years studied but within average temperature for normal tuber growth. The optimum mean daily temperatures are 18 to 20°C and yields of crops are affected by temperature. Optimum soil temperature for normal tuber growth is between 15 to 18 °C. Tuber growth is sharply inhibited when below 10 °C and above 30°C. In general a night temperature of below 15 °C is required for tuber initiation (FAO, 2013).

Water stress can affect the crop in a number of ways. It is particularly important from a quality perspective at tuber initiation and in the days and weeks that follow initiation to prevent common scab that can infect tubers (Pringle *et al*., 2009). Rainfall was appreciably low (<0.8 mm) between when the potatoes were planted and the end of May as shown in figure 5.1. For high yields, the crop water requirements (ETm) for a 120 to 150 day crop are 500 to 700 mm, depending on climate (FAO, 2013). Water deficits in the middle to late part of the growing period thus tend to reduce yield more than in the initiation of tubers (FAO, 2013). Soil moisture deficits for growth could also lead to an increase in bruising (Stalham, 2008). In 2011 the late season watering sites have generally been wet enough not restrict crop growth.

Reference $ET_0$ (Evapotranspiration (ET) - the sum of soil water evaporation (E) and plant transpiration (T)) was greater in June and July than long-term averages, with a mean daily $ET_0$ of 5.30 mm/day and only 4.17 mm/day in August.

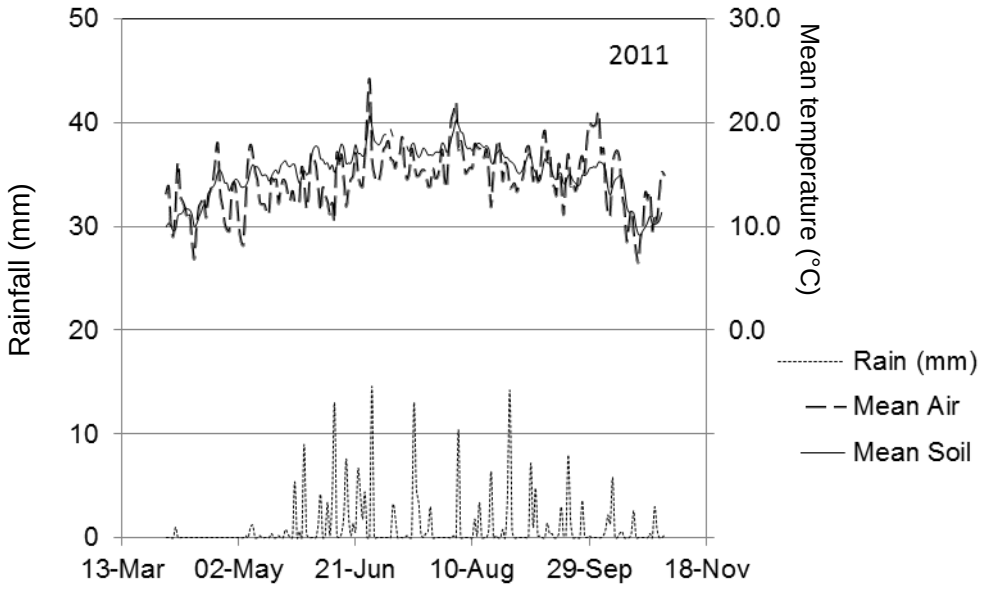


Figure 5.1 Rainfall (mm) and mean temperature of the air and soil (secondary axis) in field trial 2, 2011.

#### 5.2.1.2 Green canopy cover

A measurement of decline of canopy was taken as key indicators at harvest time of the physiological characteristics of the crop. In particular, the stage of senescence reached by the date of defoliation was studied previously and suggested a link between measure of maturity and crop quality (field trial 1). As the weather was unusual in 2011, early senescence was observed in this year compared to field trials 1 and 3 (Sections 4.2.1.2 and 6.2.1.2), shown in table 5.1. LR and RB harvested in September (H1) presented senescence in mid-August. The senescence in MP for both harvests and LR and RB in potatoes harvested in October (H2) was observed at the end of August.

Table 5.1 Date of the total senescence measured by the green canopy cover (%) of the varieties LR, MP and RB of tubers harvested in September (H1) and October (H2) 2011.

| Variety | LR | MP | RB |
|---|---|---|---|
| **H1** | 16th August | 30th August | 16th August |
| **H2** | 23rd August | 30th August | 30th August |

The data shows that all varieties were mature at harvest when measured by canopy senescence. This information is important, as it suggests no likely relative effect on tuber maturity at the point of harvest and store. However, the effect of defoliation may play a role towards bruising at the harvest time and in stored tubers. The varietal observed in defoliation were: LR was harvested 34 (H1) and 50 (H2) days after full senescence; MP was harvested 20 (H1) and 43 (H2) days after full senescence and RB was harvested 34 (H1) and 43 (H2) days after full senescence.

## 5.2.2 Bruising assessment

### 5.2.2.1 Assessment of severe bruising using the falling bolt method

The harvest time provided the most consistent and marked effects on storage quality across all varieties. Tubers from field trial 2 were subjected to the same method applied on field trial 1, impacted at the stolon end of the potatoes. However, a higher percentage of severe bruising and skin damage were found when samples were cooled down before the assessment using the falling bolt, being damaged with an energy of impact of 0.6 J and incubation lasting for 48 h at 33 °C (data not shown). Adaptations to the

bruising protocol were needed which were using lower energy impact (0.3 J) and shorter incubation for 20 h at 25 °C and 95%+ RH. Even after adaptations to the protocol, a high incidence of bruising was found compared with field trial 1 as shown in figure 5.2.

At the first harvest time, a higher incidence of severe bruising has been found in RB (93%), followed by MP (61%) and LR (52%). At the second harvest, similar results were found for the three varieties. The higher incidence was found in RB (88%), showed a decrease in the incidence of severe bruising by 5%, followed by MP (85%) with an increase of 24% and LR (82%) with an increase of 29% respective to H1 as shown in figure 5.2 and Table 5.2 (periods of comparison H).

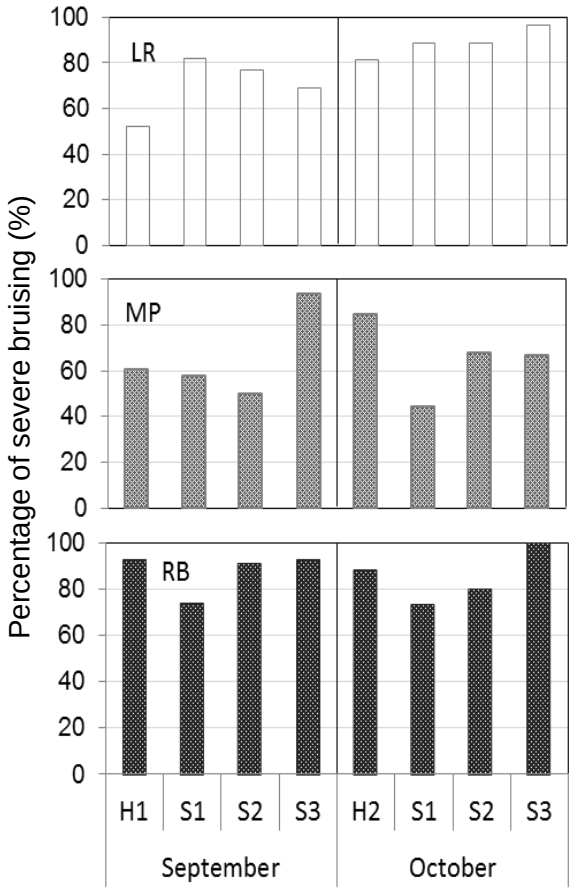


Figure 5.2 Effect of variety, harvest and storage time on the percentage of severe bruising (%) following damage with the falling bolt in potatoes from crops harvested in September (H1) and October (H2) and stored until January (S1), March (S2) and May (S3), field trial 2. Values show percentage of severe bruising (%) (n>21).

Table 5.2 Comparisons of the percentage of severe bruising in potatoes from crops harvested (H) in October (H2) compared to September (H1) and both stored until January (S1-H2S1 to H1S1), March (S2-H2S2 to H1S1) and May (S3-H2S3 to H1S3), field trial 2.

| | | Period of comparison (H2 to H1) | | |
|---|---|---|---|---|
| **Variety** | **H** | **S1** | **S2** | **S3** |
| **LR** | +29 | +7 | +12 | +28 |
| **MP** | +24 | -13 | +18 | -27 |
| **RB** | -5 | -1 | -11 | +7 |

The results from stored potatoes showed that the magnitude and direction of changes in bruising severity could not be predicted for all varieties as shown in table 5.2 and 5.3.

Different trends were found in the stored varieties harvested in September. LR increased the incidence of severe bruising by 30% at S1 following slight decreases along medium (S2) and long storage (S3). Over the short (S1) and medium (S2) period of storage, MP and RB presented a lower incidence in severe bruising compared to harvest time following an increased incidence at S3 to +33% in MP and no changes for RB.

Table 5.3 Percentage change in the incidence of severe bruising when compared the storage periods (S1 January, S2 March and S3 May) to the respective harvests (September and October)

| **Harvest** | **September (H1)** | | | **October (H2)** | | |
|---|---|---|---|---|---|---|
| **Variety/Storage** | **S1** | **S2** | **S3** | **S1** | **S2** | **S3** |
| **LR** | +30 | +25 | +17 | +7 | +7 | +15 |
| **MP** | -3 | -11 | +33 | -40 | -17 | -18 |
| **RB** | -19 | -2 | 0 | -15 | -8 | +12 |

Potatoes harvested in October (H2) showed different trends than potatoes harvested in September (H1) upon storage. LR presented a slight increase along storage (up to 15% at S3). MP presented unexpectedly lower incidence of bruising upon storage, with a reduction of 40% with short storage (S1) and 17 and 18% at medium (S2) and long (S3) term storage respectively compared to freshly harvested tubers. A lower incidence of bruising was also observed for RB at S1 and S2 but the incidence increased by 12% at S3 compared with incidence at harvest time (October).

When comparing the storage periods between samples harvested early (H1) and late (H2), more bruising for the variety LR was associated with late harvest (table 5.3). Late harvest (H2) occasionally affected the incidence of bruising in MP where short storage (S1) and long storage (S3) presented lower incidence of bruising in potatoes. RB presented a lower incidence of bruising in potatoes harvested late and stored for short (S1) and medium (S2) periods, what may be of commercial relevance for up to medium term storage.

When assessing the slight and severe bruising of the varieties along harvests and storage, similar trends were found, although the total incidence of bruising was greater than 85% as shown in figure 5.3. It was thought that the use of this type of bruising assessment for incidence of slight and severe bruising did not allow for an accurate comparison between samples. For this reason, the bruising index was used for comparison (Section 5.2.2.3).

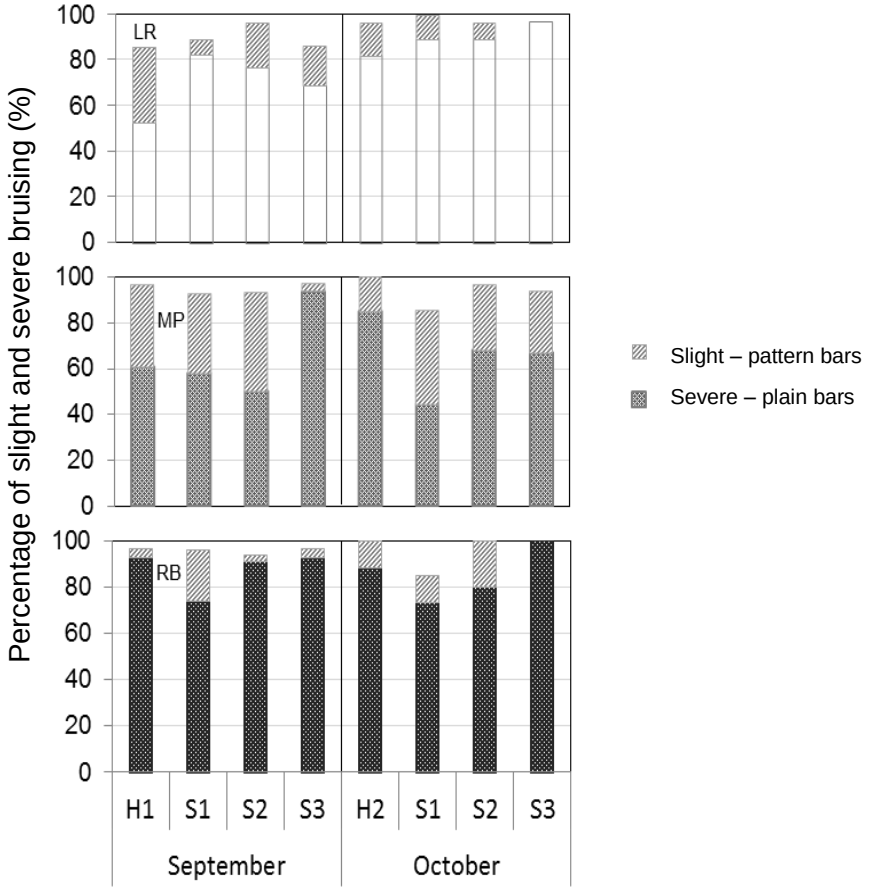


Figure 5.3 Effect of variety, harvest and storage time on percentage of slight (pattern bars) and severe bruising (plain bars) (%) following damage with the falling bolt in potatoes from crops harvested in September and October, stored until January (S1), March (S2) and May (S3), field trial 2. Values show percentage of severe bruising (%) (n>21).

### 5.2.2.2 Damage

The harvest and storage affected the incidence of breaking damage on the external tissue following the assessment of bruising using the falling bolt method and incubation. Varietal differences on the sensitivity of external tissue towards bolt damage are shown in figure 5.4. Higher incidences were found in the variety LR at H1 (30%), followed by RB and MP at H2 (18 and 15% respectively). The incidence of damage to the skin diminished along storage for all varieties and harvests studied.

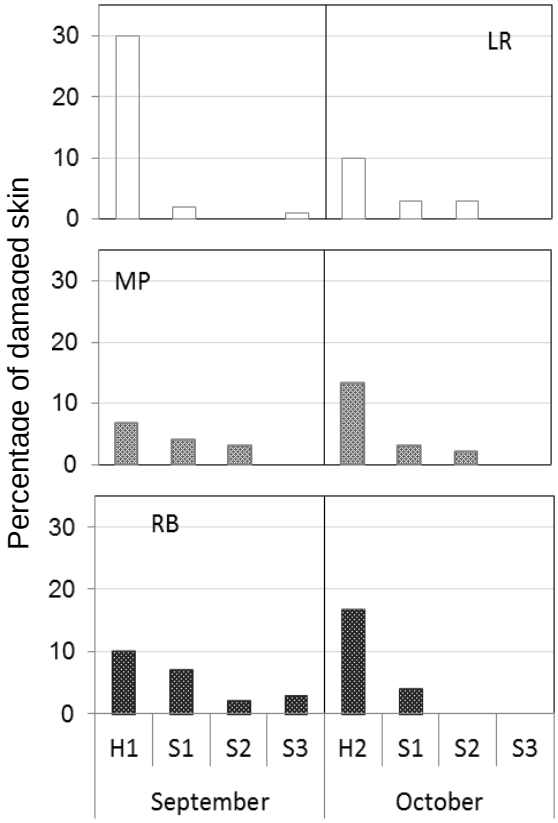


Figure 5.4 Effect of variety, harvest and storage time on percentage of damaged skin following damage with the falling bolt in potatoes from crops harvested in September and October, stored until January (S1), March (S2) and May (S3), field trial 2, (n=30).

### 5.2.2.3 Bruising Index

After the impact energy of 0.3 J and incubation for 20 h at 25 ºC, potatoes were classified according to bruising index (BI). Differences in trends for the three varieties studied were found and results are shown in figure 5.5.

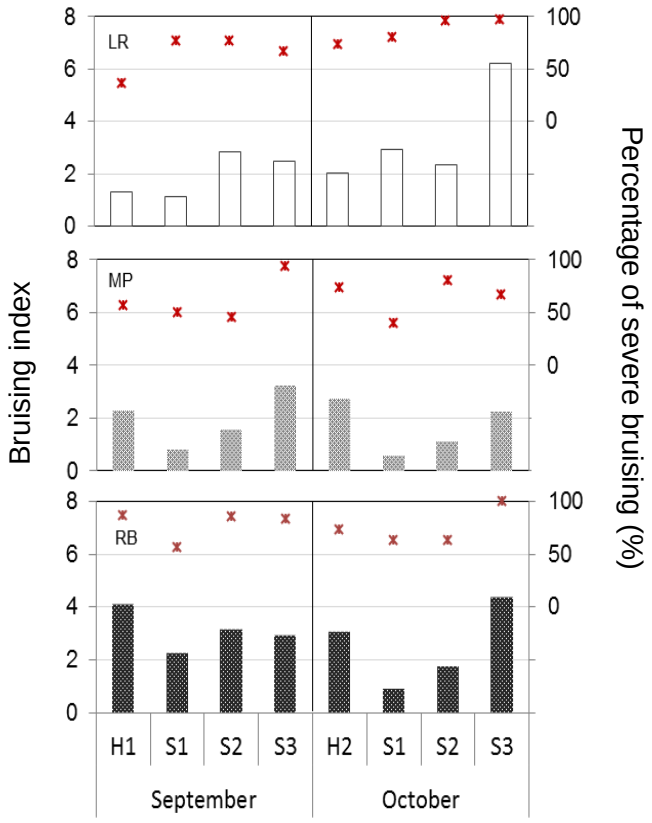


Figure 5.5 Effect of variety, harvest and storage time on bruising index (scale 1-10) following damage with the falling bolt in potatoes from crops harvested in September (H1) and October (H2), stored until January (S1), March (S2) and May (S3), field trial 2. Value bars show means (n>21) and scatter shows percentage of severe bruising (%) (n>21).

At harvest time, similar trends in the incidence of severe bruising were found. A higher BI was found in RB (4.07), followed by MP (2.29) and LR (1.32). At the second harvest, a higher incidence was found in RB (3.07), which showed a decrease in BI of 1.01, followed by MP (2.73), with increase of 0.44, and LR (2.03), with increase of 0.72 respective to H1 as shown in table 5.4 (periods of comparison H).

Table 5.4 Changes in bruising index of storage periods when comparing tubers harvested (H) in October (H2) to September (H1) and both stored until January (S1-H2S1 to H1S1), March (S2-H2S2 to H1S1) and May (S3-H2S3 to H1S3), field trial 2.

| | Periods of comparison (H2-H1) | | | |
|---|---|---|---|---|
| **Variety** | **H** | **S1** | **S2** | **S3** |
| **LR** | +0.7 | +1.8 | -0.5 | +3.7 |
| **MP** | +0.4 | -0.2 | -0.5 | -1.0 |
| **RB** | -1.0 | -1.4 | -1.4 | +1.4 |

Similar trends were found when comparing BI with the incidence of severe bruising in stored potatoes. The exception was RB at long-term storage (S3), which presented no changes in the incidence of severe bruising when compared to harvested time in September and showed lower BI at this period.

Comparisons of stored tubers with the respective fresh harvested in September and October are shown in table 5.4. LR presented an increase in the incidence of severe bruising for both harvests along the storage time showing a higher incidence in potatoes harvested late, except at medium storage time (S2, -0.5). MP presented less bruising when harvested late in all stored periods studied and RB presented less bruising when harvested later up to medium term storage (S2), with increase in BI at long-term storage harvested in October (H2 S3).

Table 5.5 Percentage change in the incidence of severe bruising when compared the storage periods (S1 January, S2 March and S3 May) to the respective harvests (September and October).

| Harvest | September (H1) | | | October (H2) | | |
|---|---|---|---|---|---|---|
| Variety/Storage | S1 | S2 | S3 | S1 | S2 | S3 |
| **LR** | +0.2 | +1.5 | +1.2 | +0.9 | +0.3 | +4.2 |
| **MP** | -1.5 | -0.7 | +0.9 | -2.2 | -1.6 | -0.5 |
| **RB** | -1.8 | -0.9 | -1.1 | -2.2 | -1.3 | +1.3 |

The differences in assessment of severe bruising and BI lead to different trends upon storage (tables 5.4 and 5.5). Negative value in BI index was found comparing LR H2S2 to LR H1S2 (-0.5) whereas percentage of severe bruising was positive (+7) at the same period. Exploring the factors involved in calculating BI showed that this difference was dependent on the three factors used to calculate BI: lower depth (H1S2 4.80 and H2S2 4.38), width (H1S2 1.08 and H2S2 1.03) and lower colour formation (H1S2 1.54 and H2S2 1.52) in bruised tissues.

Regardless of MP showing a negative value for BI of H2 when comparing to H1 upon medium period storage (S2) (-0.5) and positive when comparing the same period of incidence of severe bruising (+18%), the difference in results were related to lower depth (H1S2 3.9 and H2S2 3.82), width (H1S2 0.93 and H2S2 0.88) but not colour (H1S2 1.43 and H2S2 1.76) of bruised tissue used to calculate BI.

However, strong positive correlation with results from incidence of severe bruising and BI was found for RB (R=0.82) and for MP (R=0.68) and moderate correlation for LR (R=0.37).

### 5.2.2.4 Spectrophometric assessment of oxidative potential

There was a significant difference ($p<0.05$) among the three varieties studied in the oxidative potential (OP). On average, higher values of absorbance was found for RB, followed by LR and MP as shown in figure 5.6.

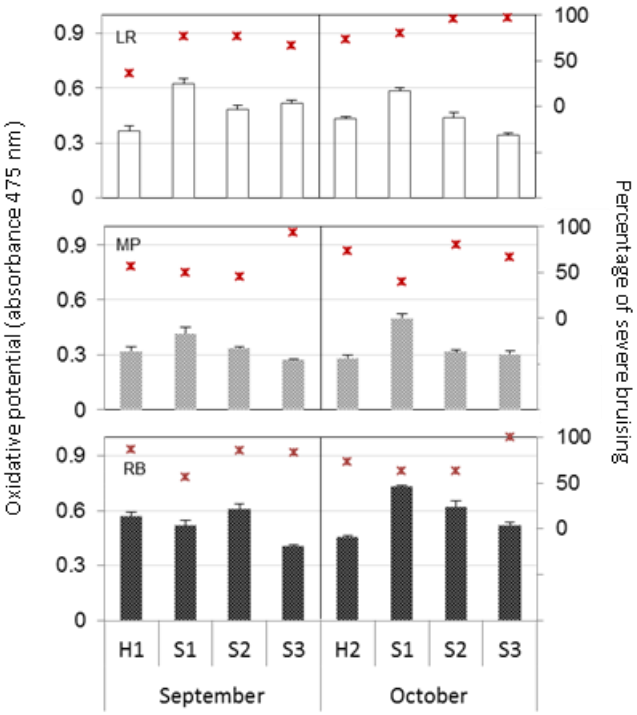


Figure 5.6 Effect of variety, harvest and storage time on oxidative potential following 20 hours oxidation (bars) and percentage of severe bruising (%) (scatter) from crops harvested in September and October, stored until January (S1), March (S2) and May (S3), field trial 2. Value bars show means (n=3), error bars are SE and scatter shows percentage of severe bruising (%) (n>21).

Significant difference between tubers harvested in September and October was found between harvest only for the variety RB ($p<0.01$) as shown in table 5.6 (interaction between harvests).

Table 5.6 Analysis of variance using a factorial 2-way ANOVA to compare the oxidative potential (absorbance 475 nm) from each individual variety of tubers harvested in September (H1) and October (H2) and stored until January (S1), March (S2) and May (S3). Significance codes: <0.001 '***' <0.01 '**' <0.05 '*' >0.05 'ns'.

| Varieties/ interaction | | Interaction Along storage with harvest | | | Interaction Between storages | |
|---|---|---|---|---|---|---|
| | | S1 | S2 | S3 | S1-S2 | S2-S3 |
| **LR** | H1 | *** | *** | *** | *** | ns |
| | H2 | *** | ns | ns | *** | *** |
| **Between harvests/storages** | ns | ns | ns | *** | | |
| **MP** | H1 | * | ns | ns | ns | ns |
| | H2 | *** | ns | ns | *** | ns |
| **Between harvests/storages** | ns | *** | ns | ns | | |
| **RB** | H1 | ns | * | ns | ns | *** |
| | H2 | *** | *** | ns | ns | *** |
| **Between harvests/storages** | ** | *** | ns | ns | | |

Short storage increased significantly ($p<0.05$) the OP for all varieties comparing with respective harvest time, with the exception for RB H1 S1. On average the oxidative potential tended to decrease with the longer storages periods.

Comparing periods of tubers harvested in September and October (table 5.6 – between harvests/storages), higher and significant different ($p<0.001$) OP was found in potatoes harvested in October upon short storage period (S1) for MP and RB. LR presented higher ($p<0.001$) OP for long period storage of tubers (S3), being the highest in potatoes harvested in September. A similar increase in the incidence of severe bruising was not observed at these specific points.

A strong negative correlation was found for MP when contrasting results for incidence of severe bruising and OP (R= -0.60), weak negative correlation for RB (R=-0.27) and no correlation LR (R=0.01). When compared BI and OP, very strong negative correlation was found for MP (R=-0.71), but moderate negative for RB (R=-0.31) and weak for LR (R=-0.21).

### 5.2.3 Physical properties

#### 5.2.3.1 Weight

The purpose of analysing weight of tubers was to find the difference in yield between potatoes harvested in September (H1) and October (H2), the association of weight and bruising susceptibility and to access differences in sampling.

Among varieties, analysis of variances showed significant difference in weight between RB and MP ($p<0.001$). Analysing harvests, significant increase in weight was found for LR ($p<0.01$) when harvested in October while MP and RB showed decrease in weight at the same period, being significant only for MP ($p<0.001$) as shown in figure 5.7 and table 5.7. This decrease in weight was unexpected as potatoes were grown randomly to minimise effects of field treatments such as soil and temperature.

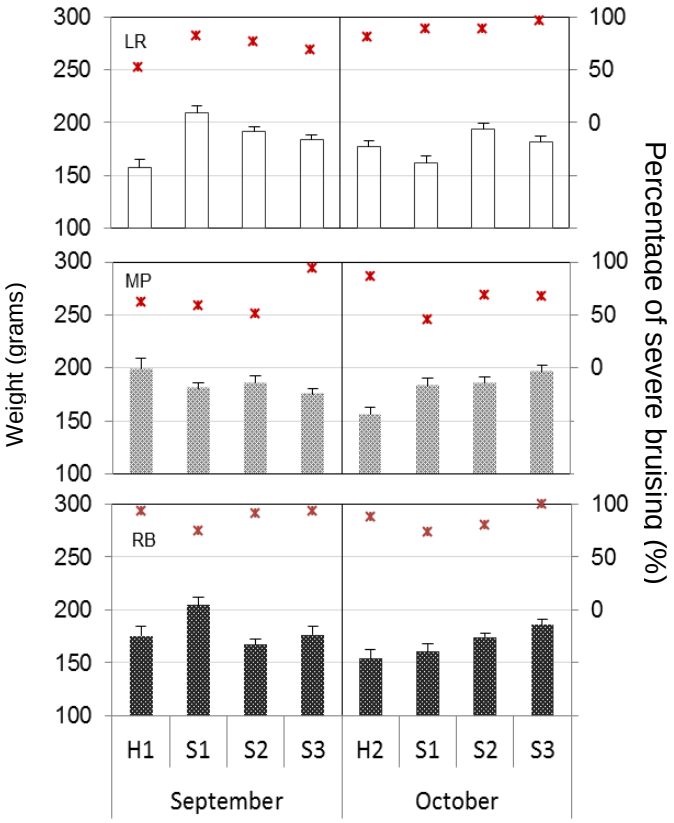


Figure 5.7 Effect of variety, harvest and storage time on weight of samples in grams (bars) and percentage of severe bruising (%) (scatter) from crops harvested in September and October, stored until January (S1), March (S2) and May (S3), field trial 2. Value bars show means (n=30), error bars are SE and scatter shows percentage of severe bruising (%) (n>21).

On average, differences were found in stored samples compared to harvest time, significant only at medium term storage (S2) for MP harvested in October ($p<0.05$) and long term storage (S3) for LR, MP and RB harvested in October ($p<0.01$) (table 5.7).

Weak correlations were found between weight and incidence of severe bruising ($R<0.27$) and no correlation for bruising index ($R<0.11$).

Table 5.7 Analysis of variance using a factorial 2-way ANOVA to compare the weight (grams) from each individual variety of tubers harvested in September (H1) and October (H2) and stored until January (S1), March (S2) and May (S3). Significance codes: <0.001 '***' <0.01 '**' <0.05 '*' >0.05 'ns'.

| Varieties/ interaction | | Interaction Along storage with harvest | | | Interaction Between storages | |
|---|---|---|---|---|---|---|
| | | S1 | S2 | S3 | S1-S2 | S2-S3 |
| **LR** | H1 | ns | ns | ns | ** | ns |
| | H2 | ns | ns | * | ns | ns |
| **Between harvests/storages** | ** | ** | ns | ns | | |
| **MP** | H1 | ns | ns | ns | ns | ns |
| | H2 | ns | * | ** | ns | ns |
| **Between harvests/storages** | *** | ns | ns | ns | | |
| **RB** | H1 | ns | ns | ns | ** | ns |
| | H2 | ns | ns | * | ns | ns |
| **Between harvests/storages** | ns | ** | ns | ns | | |

### 5.2.3.2 Specific gravity

The purpose of analysing specific gravity (SG) was to investigate the association of SG and bruising susceptibility. Significant differences among all varieties studied was found ($p<0.05$). The results from H1 and H2 showed higher specific gravity in LR followed MP and RB (figure 5.8). No significant difference ($p>0.05$) was found when comparing H1 to H2 for the varieties studied as shown in table 5.8 (Interaction between harvests (H)).

Although increments in SG at S1 were observed for all varieties compared to the respective harvest time (except MP H1S1), no significant difference was found at this time point ($p>0.05$) (table 5.8).

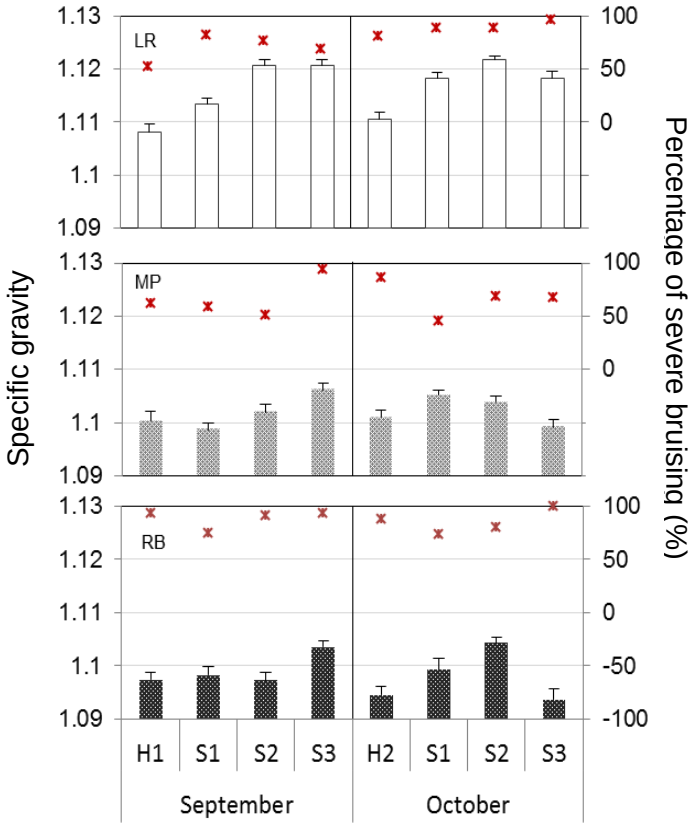


Figure 5.8 Effect of variety, harvest and storage time on the specific gravity of samples (bars) and percentage of severe bruising (%) (scatter) from crops harvested in September and October, stored until January (S1), March (S2) and May (S3), field trial 2. Value bars show means (n=30), error bars are SE and scatter shows percentage of severe bruising (%) (n>21).

The moisture loss thought skin evaporation was more prominent at mid-point storage (S2), for RB and LR when harvested late (October) showing a significant increase ($p<0.001$) in SG compared to the respective harvest period.

Following a long storage period (S3), there was a decrease in SG observed in potatoes from late harvest. The reduction in SG at H2S3 was related to a higher bruising percentage in LR and RB compared to H1S3 but not for MP.

Weak and negligible correlations were found for all the varieties when the SG was compared to the incidence of severe bruising (R <0.25) and BI (R <0.19). These results are in accordance to previous research by Baritelle and Hyde (2003) on fresh tubers and Workman and Holm (1984) on stored tubers. These observations indicated that other factors may become more important than the SG in determining bruising.

Table 5.8 Analysis of variance using a factorial 2-way ANOVA to compare the specific gravity from each individual variety of tubers harvested in September (H1) and October (H2) and stored until January (S1), March (S2) and May (S3). Significance codes: <0.001 '***' <0.01 '**' <0.05 '*' >0.05 'ns'.

| Varieties/ interaction | | Interaction Along storage with harvest | | | Interaction Between storage | |
|---|---|---|---|---|---|---|
| | H | S1 | S2 | S3 | S1-S2 | S2-S3 |
| **LR** | H1 | ns | ns | * | ns | ns |
| | H2 | ns | *** | ns | ns | *** |
| **Between harvests/storages** | ns | ns | * | *** | | |
| **MP** | H1 | ns | ns | ns | ns | ns |
| | H2 | ns | ns | ns | ns | ns |
| **Between harvests/storages** | ns | * | ns | ** | | |
| **RB** | H1 | ns | ns | ns | ns | ns |
| | H2 | ns | *** | ns | * | *** |
| **Between harvests/storages** | ns | ns | * | *** | | |

## 5.2.4 Mechanical properties

### 5.2.4.1 Energy required to break the potato skin tissue

In general, the energy required to break the skin decreased with harvest time and increased with storage for all varieties as shown in figure 5.9. Significant differences were found amongst the varieties studied ($p<0.01$).

RB, the variety that bruised more, required on average lower energy to break the skin tissue, followed by MP and LR. Significant differences were found comparing harvest to storage period ($p<0.01$) for all varieties, with exception of MP H1S2 compared to MP H1 as shown in table 5.9. Comparing the storage period, the only significant difference was found between S1 and S2 for MP harvested in September ($p<0.001$) (table 5.9).

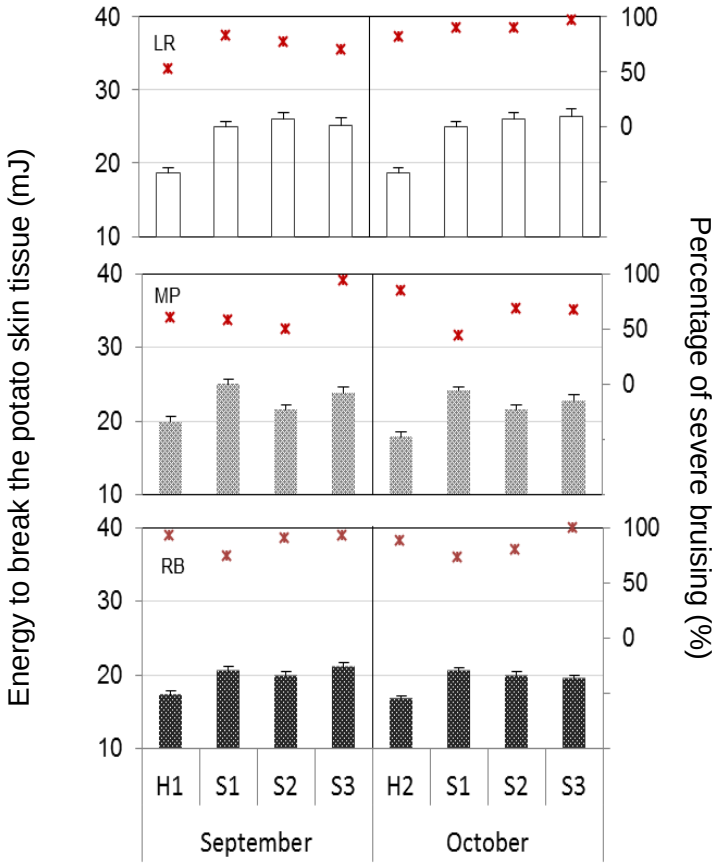


Figure 5.9 Effect of variety, harvest and storage time on the energy to break the potato skin tissue (mJ) (bars) and percentage of severe bruising (%) (scatter) from crops harvested in September (H1) and October (H2), stored until January (S1), March (S2) and May (S3), field trial 2. Value bars show means (n=30), error bars are SE and scatter shows percentage of severe bruising (%) (n>21).

Table 5.9 Analysis of variance using a factorial 2-way ANOVA to compare the energy to break the tissue from each individual variety of tubers harvested in September (H1) and October (H2) and stored until January (S1), March (S2) and May (S3). Significance codes: <0.001 '***' <0.01 '**' <0.05 '*' >0.05 'ns'.

| Varieties/ interaction | | Interaction Along storage with harvest | | | Interaction Between storages | |
|---|---|---|---|---|---|---|
| | | S1 | S2 | S3 | S1-S2 | S2-S3 |
| LR | H1 | ** | ** | *** | ns | ns |
| | H2 | *** | *** | *** | ns | ns |
| Between harvests/storages | | ns | ns | ns | ns | |
| MP | H1 | *** | ns | ** | ** | ns |
| | H2 | *** | ** | *** | ns | ns |
| Between harvests/storages | | ns | ns | ns | ns | |
| RB | H1 | ** | * | *** | ns | ns |
| | H2 | *** | *** | ** | ns | ns |
| Between harvests/storages | | ns | ns | ns | ns | |

The skin strength was measured by the force (N) at the point when the tissue breaks and the deformability were measured by the distance (mm) to rupture the tissue. The force to break the skin diminished for all varieties along harvest. In stored tubers harvested in September (H1) the force to break the skin diminished until S2 and increased at S3. In stored LR and MP tubers harvested in October (H2), the force to break skin increased at S1 followed by decrease at S2. Small changes in force to break the skin along storage of RB tubers were observed as shown in figure 5.10.

From the results, the distance to rupture the skin tissue, LR presented a higher degree of deformability, followed by MP and RB as shown in figure 5.11. An increase in distance to rupture the skin was observed for LR along the harvest period whereas no changes were observed for the varieties MP

and RB. Substantial increase was observed when comparing the fresh to stored tubers but no great differences were observed when comparing the storage periods.

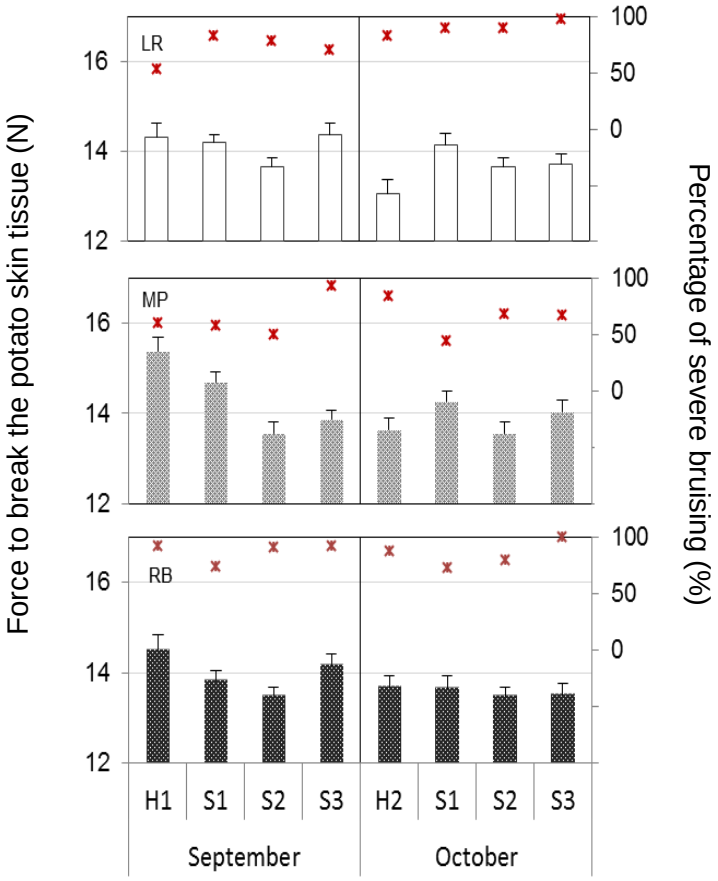


Figure 5.10 Effect of variety, harvest and storage time on the force to break the potato skin tissue (N) (bars) and percentage of severe bruising (%) (scatter) from crops harvested in September (H1) and October (H2), stored until January (S1), March (S2) and May (S3), field trial 2. Value bars show means (n=30), error bars are SE and scatter shows percentage of severe bruising (%) (n>21).

It is usually desired that at harvest time the tubers have high degree of mechanical strength to protect the tissue from damage, such as bruising, during transport and handling. The results from this research indicated that skin tissue loses a degree of mechanical strength along harvest and it was observed that LR becomes more deformable when harvested in October

than in September but it was not observed in MP and RB. However, all varieties lost strength and the skin became upon storage. During storage period all varieties lost strength and the skin tissue became softer.

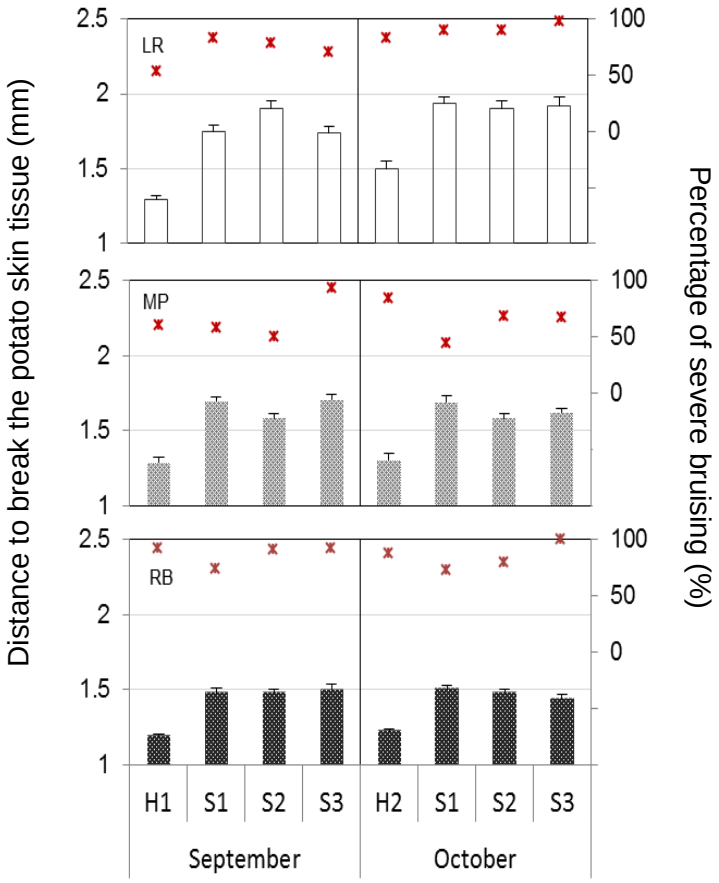


Figure 5.11 Effect of variety, harvest and storage time on the distance to break the potato skin tissue (mm) (bars) and percentage of severe bruising (%) (scatter) from crops harvested in September (H1) and October (H2), stored until January (S1), March (S2) and May (S3), field trial 2. Value bars show means (n=30), error bars are SE and scatter shows percentage of severe bruising (%) (n>21).

A moderate correlation was found between incidence of severe bruising and energy to break the skin tissue for LR (R=0.36) and no correlation was found for MP (R= -0.11) and RB (R= -0.09). When comparing the energy to break the skin tissue to bruising index (BI), weak correlations were found for all varieties: LR (R= 0.23), MP (R= - 0.25) and RB (R= -0.24).

The combined results show that the softer the skin become, the more susceptible they were to bruise for LR but the opposite for MP and RB as negative correlation was found. This means that RB and MP may be more dependent on biochemical apparatus to bruise.

#### 5.2.4.2 Energy required to break the potato cortex tissue

The energy required to break the cortex tissue was significantly different between the varieties studied ($p<0.05$), being lower for RB, followed by MP and LR. On average a slight decrease in the energy required to break the cortex was observed between harvests for the varieties MP and RB, and an increase for LR as shown in figure 5.12. Over the period of storage, increases in the energy to break the cortex tissue were measured for all varieties. MP showed significantly different variances along all storage points comparing storage to the respective harvests (H1 or H2) but LR and RB were significant different only comparing H1 to H1S3 and H2 to H2S2 as shown in table 5.10.

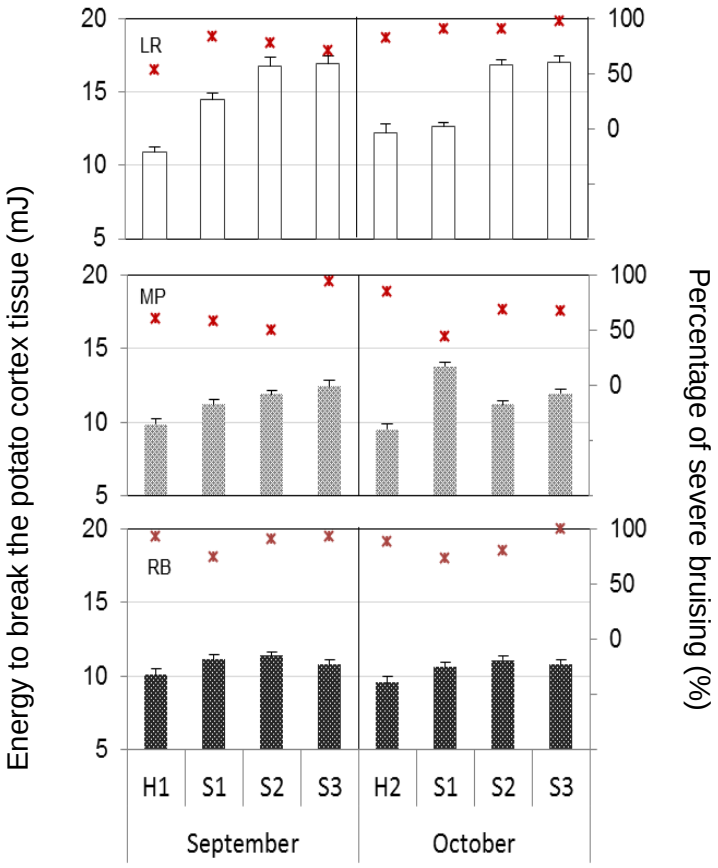


Figure 5.12. Effect of variety, harvest and storage time on the energy required to break the potato cortex tissue (mJ) (bars) and percentage of severe bruising (%) (scatter) from crops harvested in September and October, stored until January (S1), March (S2) and May (S3), field trial 2. Value bars show means (n=30), error bars are SE and scatter shows percentage of severe bruising (%) (n>21).

The underlying flesh tissue of the tuber was substantially weaker and softer compared to the skin measurements. The changes in energy to break cortex tissue during storage were strongly dependent of the distance and force, to penetrate into tissue showed increase along storage period as presented in figures 5.13 and 5.14.

Table 5.10 Analysis of variance using a factorial 2-way ANOVA to compare the energy to break the tissue from each individual variety of tubers harvested in September (H1) and October (H2) and stored until January (S1), March (S2) and May (S3). Significance codes: <0.001 '***' <0.01 '**' <0.05 '*' >0.05 'ns'.

| Varieties/ interaction | | Interaction storage with harvest | | | Interaction Between storages | |
|---|---|---|---|---|---|---|
| | H | S1 | S2 | S3 | S1-S2 | S2-S3 |
| LR | H1 | ns | ns | * | ns | ns |
| | H2 | ns | * | ns | ns | ns |
| Between harvests/storages | | ns | ns | ns | ns | |
| MP | H1 | * | *** | **** | ns | ns |
| | H2 | *** | ** | *** | *** | ns |
| Between harvests/storages | | ns | *** | ns | ns | |
| RB | H1 | ns | ns | * | ns | ns |
| | H2 | ns | * | ns | ns | ns |
| Between harvests/storages | | ns | ns | ns | ns | |

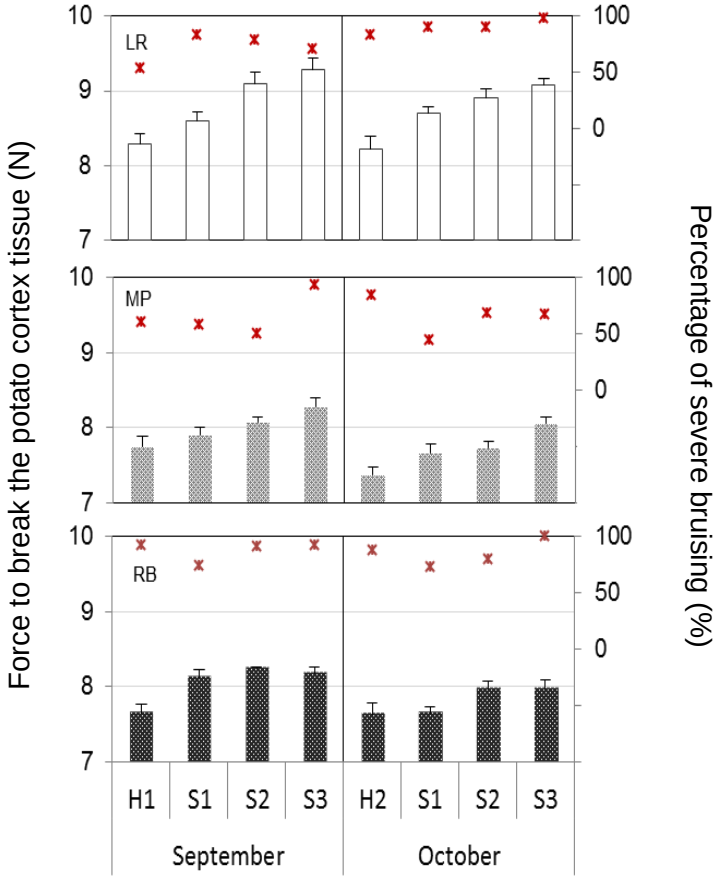


Figure 5.13 Effect of variety, harvest and storage time on the force to break the potato cortex tissue (N) (bars) and percentage of severe bruising (%) (scatter) from crops harvested in September and October, stored until January (S1), March (S2) and May (S3), field trial 2. Value bars show means (n=30), error bars are SE and scatter shows percentage of severe bruising (%) (n>21).

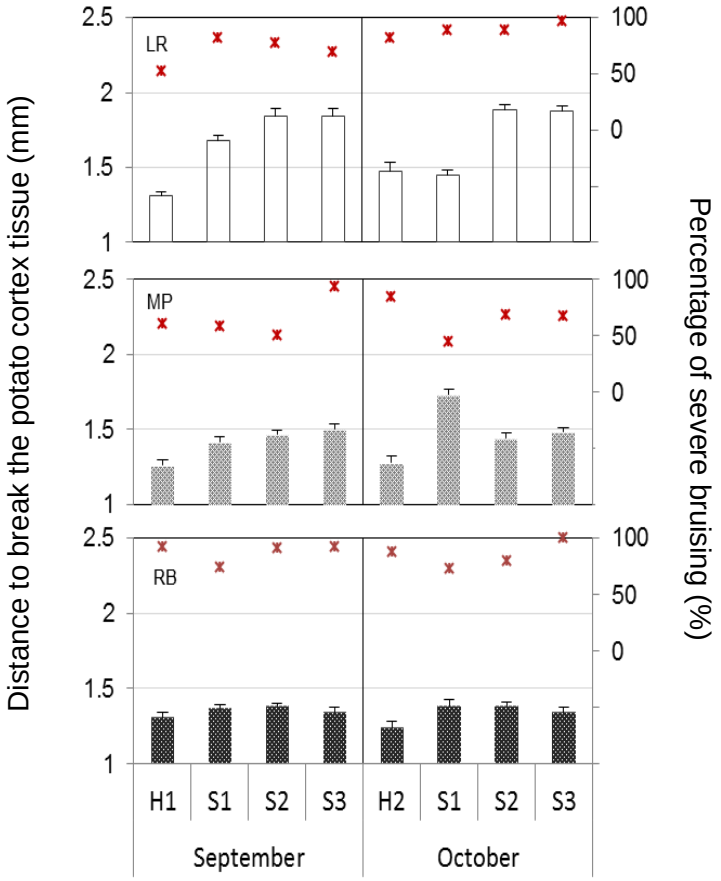


Figure 5.14 Effect of variety, harvest and storage time on the distance to break the potato cortex tissue (mm) (bars) and percentage of severe bruising (%) (scatter) from crops harvested in September and October, stored until January (S1), March (S2) and May (S3), field trial 2. Value bars show means (n=30), error bars are SE and scatter shows percentage of severe bruising (%) (n>21).

The results suggest that the energy required to break the cortex tissue and incidence of severe bruising showed a weak correlation for LR (R=0.20) and no correlation for RB (R = -0.03) and MP (R= -0.13). The correlations with BI values showed weak correlation for LR (R= 0.26) and MP (R= -0.17) and no correlation for RB (R= - 0.05).

## 5.2.5 Phenolic composition

### 5.2.5.1 Phenolic acids

The concentration of free phenolic acids present in the methanolic extracts assayed are shown in figures 5.15 and 5.16. Different profiles of the compounds were found among the varieties studied, with LR presenting the higher amount of free phenolics, followed by RB and MP.

Of the hydrocinnamic acids, 5-*O*-caffeoylquinic acid (5-CQA) was most abundant for all varieties, constituting between 64 to 86% of the total phenolics analysed. The amount of 5-CQA was significant different among the varieties studies ($p<0.001$).

The varieties presented different metabolism profiles of 5-CQA. The content of 5-CQA in MP did not change significantly comparing harvests, storage periods to harvests and periods between storage, with the only exception for the short storage H1S1 compared to H1 and H1S2 ($p<0.001$) (table 5.11).

LR presented significant decreases ($p<0.001$) in the content of 5-CQA when harvested late (October H2) compared to early (September H1). When comparing storage periods to the respective harvest, significantly different ($p<0.01$) results were found, except H2S3 compared to H2. Comparing the storage periods from both harvests, significantly different amounts of 5-CQA were found only in tubers stored until March S2 ($p<0.001$) as shown in table 5.11.

RB showed a significant increase ($p>0.001$) in 5-CQA content during the harvest period. Significantly increase ($p<0.001$) in the content of 5-CQA was found in tubers harvested early upon storage and significant decreases ($p<0.001$) in tubers harvested late, with exception of RB H2S2. Significantly different amounts of 5-CQA was found in tubers stored until January (H1) and March (S2) ($p<0.001$) when compared storage periods of both harvests (table 5.11).

The observations made for CQA in stored tubers harvested in September and October (MP S1, LR S2 and RB S1 and S2) were not associated with changes in the incidence of bruising. From this observation, no consistent effect of harvest time in the amount of 5-CQA was found to predict bruising in stored tubers.

The chlorogenic acid isomer cryptochlorogenic acid (4-CQA) was more abundant than neochlorogenic acid (3-CQA) in all varieties and ranged from 0.9-10.1% of the total chlorogenic acids, whereas 3-CQA comprised of 0.3-5.9% of the total. Both 3-CQA and 4-CQA presented higher levels at stored tubers compared to the respective harvests in September or October.

A moderate positive correlation ($R=0.52$) was observed between the variations of 5-CGA with the accumulation 3- and 4-CGA isomers. The positive correlation could improve the knowledge of isomers as occurring naturally and not being artefacts formed during extraction and isolation. This issue was noted by Molgaard and Ravn (1988).

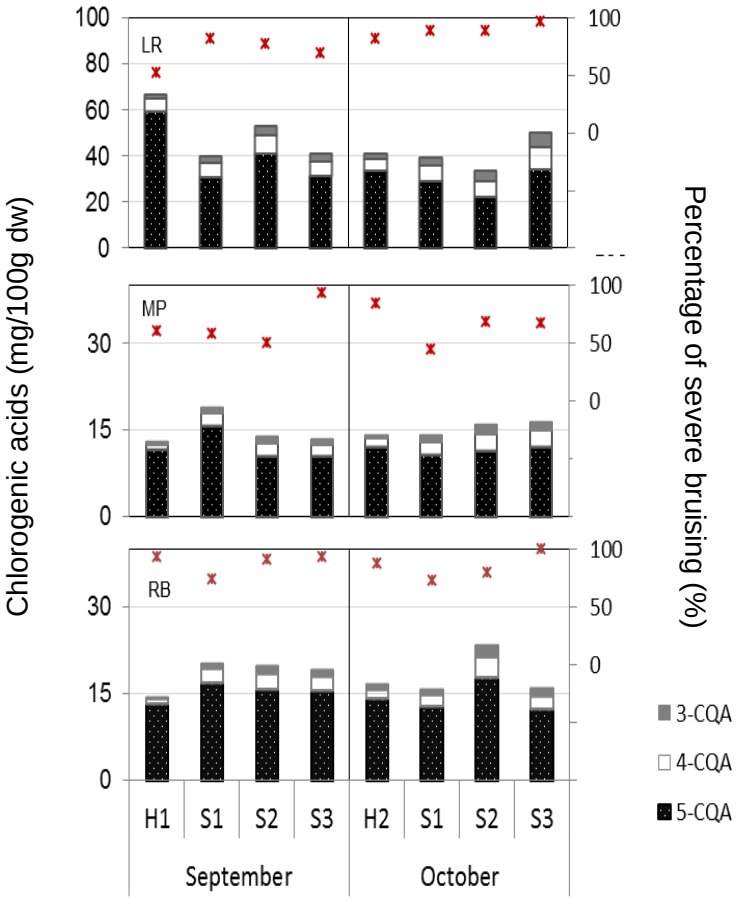


Figure 5.15 Effect of variety, harvest and storage time on chlorogenic acids (3-, 4- and 5- CQA) of lyophilized cortex (mg/100 g dw) (bars) and percentage of severe bruising (%) (scatter) from crops harvested in September (H1) and October (H2), stored until January (S1), March (S2) and May (S3), field trial 2. Value bars show means (n=3), error bars are SE and scatter shows percentage of severe bruising (%) (n>21).

Table 5.11 Analysis of variance using a factorial 2-way ANOVA to compare the 3-, 4-, and 5- CQA contents from each individual variety of tubers harvested in September (H1) and October (H2) and stored until January (S1), March (S2) and May (S3). Significance codes: <0.001 '***' <0.01 '**' <0.05 '*' >0.05 'ns'.

| Varieties/ interaction | Interaction storage with harvest | | | | Interaction Between storages | |
|---|---|---|---|---|---|---|
| | H | S1 | S2 | S3 | S1-S2 | S2-S3 |
| LR 3-CQA | H1 | *** | *** | *** | *** | *** |
| | H2 | *** | *** | *** | *** | *** |
| Between harvests/storages | *** | *** | ns | *** | | |
| LR 4-CQA | H1 | ns | *** | ns | *** | *** |
| | H2 | *** | *** | *** | ns | ns |
| Between harvests/storages | *** | * | *** | *** | | |
| LR 5-CQA | H1 | *** | *** | *** | *** | *** |
| | H2 | * | *** | ns | *** | *** |
| Between harvests/storages | *** | ns | *** | ns | | |

| Varieties/ interaction | Interaction Storage with harvest | | | | Interaction Between storages | |
|---|---|---|---|---|---|---|
| | H | S1 | S2 | S3 | S1-S2 | S2-S3 |
| MP 3-CQA | H1 | *** | *** | *** | ns | ns |
| | H2 | *** | *** | *** | *** | ns |
| Between harvests/storages | ns | ns | *** | *** | | |
| MP 4-CQA | H1 | *** | *** | *** | ns | ns |
| | H2 | *** | *** | *** | * | ns |
| Between harvests/storages | ** | ns | ** | *** | | |
| MP 5-CQA | H1 | *** | ns | ns | *** | ns |
| | H2 | ns | ns | ns | ns | ns |
| Between harvests/storages | ns | *** | ns | ns | | |

| Varieties/ interaction | Interaction storage with harvest | | | | Interaction Between storages | |
|---|---|---|---|---|---|---|
| | H | S1 | S2 | S3 | S1-S2 | S2-S3 |
| RB 3-CQA | H1 | *** | *** | *** | *** | * |
| | H2 | * | *** | *** | *** | *** |
| Between harvests/storages | *** | ns | *** | * | | |
| RB 4-CQA | H1 | *** | *** | *** | * | *** |
| | H2 | *** | *** | *** | *** | *** |
| Between harvests/storages | *** | *** | *** | ns | | |
| RB 5-CQA | H1 | *** | *** | *** | *** | *** |
| | H2 | *** | *** | *** | *** | *** |
| Between harvests/storages | *** | *** | *** | ns | | |

The minor compounds, namelly caffeic acid (CA), vanillic acid (VA), ferulic acid (FA) and *p*-coumaric (*p*Cou) were found in all varieties during the periods analysed and ranged from 0.9 to 3% of the total compounds analysed. Among the minor compound, CA presented the higher concentration for all varieties as shown in figure 5.16. Lower concentration of the minor compounds was observed in tubers stored for long period (S3), with exception of RBH1S3.

Different profiles among the varieties were found. On average, LR and RB presented higher concentration of FA than MP. Amounts of FA were higher when potatoes were harvested in October. Similar concentrations of VA were found for all varieties, where higher amount were observed at H2 and S2 for the varieties LR and MP and H1 and H2S1 for RB. Higher concentrations of CA at H2 were found for MP and RB and H1 for LR. The compound *p*Cou was the only one with higher concentrations at H1 for all varieties but changes along storage time were observed.

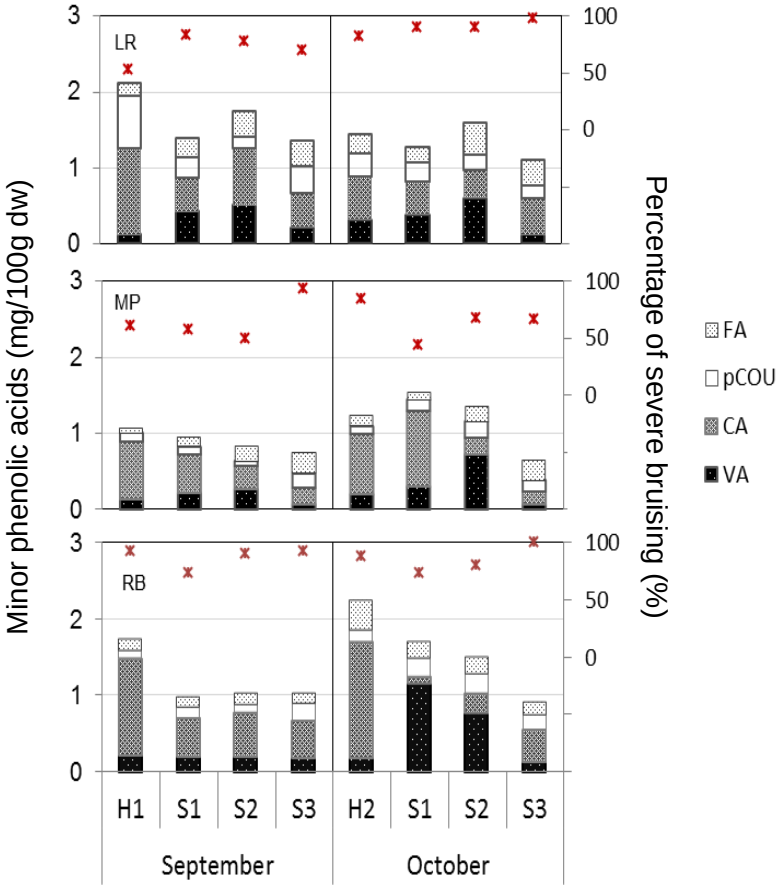


Figure 5.16 Effect of variety, harvest and storage time on minor phenolic acids (FA, *p*COU, CA, VA) of lyophilized cortex (mg/100 g dw) (bars) and percentage of severe bruising (%) (scatter) from crops harvested in September (H1) and October (H2), stored until January (S1), March (S2) and May (S3), field trial 2. Value bars show means (n=3), error bars are SE and scatter shows percentage of severe bruising (%) (n>21).

The results from three caffeoylquinic acids quantitated (CQA's) showed a strong negative correlation with incidence of severe bruising for LR (R=-0.75), moderate positive for RB (R= 0.69) and no correlation for MP (R= 0.03). Correlations of CQA's with bruising index were moderate for RB (R=0.40) and not correlated for LR and MP (R= <0.04).

Among the minor compounds, CA showed a very strong (R=-0.71), a strong (R=-0.52) and a weak (R= -0.29) negative correlations with severe bruising

for LR, RB, MP respectively. Very strong negative correlation was found between CA and BI for MP, but no correlation was found for the other varieties.

Some authors have attempted to correlate the amount of chlorogenic acids with oxidative potential. Dean *et al*. (1993) found a decrease in chlorogenic and caffeic acids during storage when overall oxidation potential increased and suggested the amount of the organic acids may not be critical regards to bruising susceptibility. Stevens and Davelaar (1996) suggested attention to chlorogenic acid as substrate for PPO because most cultivars have very low chlorogenic acid content leading to low correlation with OP. However, Delgado *et al.* (2001) found conflicting data suggesting that chlorogenic acid content increased during storage and gave a significant correlation with bruising discolouration. In this work weak correlation between CQAs and oxidative potential was found for LR (R= -0.29) and no correlation for RB (R= -0.13) and MP (R= -0.02).

#### 5.2.5.2 Tyrosine

Significant variations of the amount of free tyrosine was observed among all varieties ($p<0.001$). The concentration of free tyrosine in the tuber cortices were on average higher for RB, the most susceptible cultivar to bruise, followed by MP and LR as shown on figure 5.17.

Significant increases in tyrosine levels ($p<0.001$) during growth was observed for all varieties (table 5.12) LR and RB showed increases of 21%

on the tyrosine content at H2 compared to H1 whereas MP showed increase of 46%.

A linear increase along storage periods was found for all varieties with the only exception for MP H2 which presented a linear decrease along the storage period. In tubers harvested early, a marked significant increase ($p<0.001$) was found between S1 and S2 for all varieties. Slightly higher amounts of tyrosine was observed in stored potatoes harvested late (H2) and a significant increase was found only for LR between all storage periods.

Comparisons of stored periods from tubers harvested early and late were significant different ($p<0.001$) as shown in table 5.12, with exception of MP at S2 and S3. This exception was due the decrease of the content of tyrosine observed along storage of MP tubers harvested late (H2).

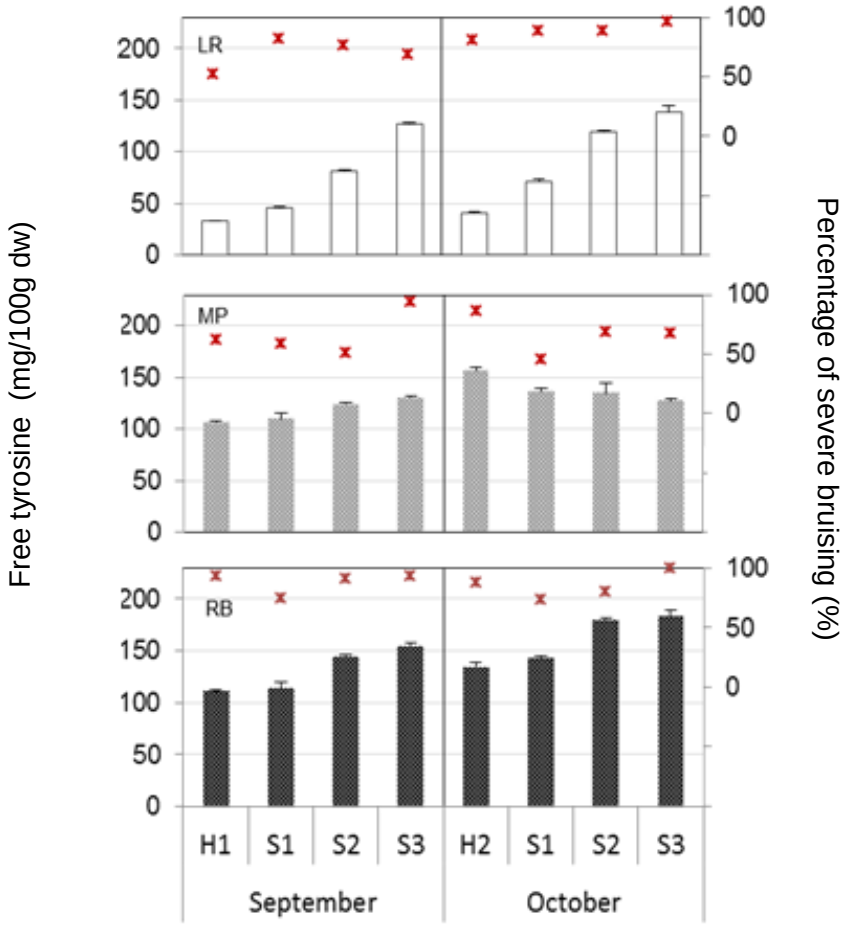


Figure 5.17 Effect of variety, harvest and storage time on free tyrosine of lyophilized cortex (mg/100 g dw) (bars) and percentage of severe bruising (%) (scatter) from crops harvested in September and October, stored until January (S1), March (S2) and May (S3), field trial 2. Value bars show means (n=3), error bars are SE and scatter shows percentage of severe bruising (%) (n=>21).

Table 5.12 Analysis of variance using a factorial 2-way ANOVA to compare the free tyrosine content from each individual variety of tubers harvested in September (H1) and October (H2) and stored until January (S1), March (S2) and May (S3). Significance codes: <0.001 '***' <0.01 '**' <0.05 '*' >0.05 'ns'.

| Varieties/ interaction | | Interaction storage with harvest | | | Interaction Between storages | |
|---|---|---|---|---|---|---|
| | | S1 | S2 | S3 | S1-S2 | S2-S3 |
| LR | H1 | *** | *** | *** | *** | *** |
| | H2 | *** | *** | *** | *** | *** |
| Between harvests/storages | | *** | *** | *** | *** | |
| MP | H1 | ns | *** | *** | * | ns |
| | H2 | *** | *** | *** | ns | ns |
| Between harvests/storages | | *** | *** | ns | ns | |
| RB | H1 | ns | ***` | *** | *** | ns |
| | H2 | ns | *** | *** | ns | ns |
| Between harvests/storages | | *** | *** | *** | *** | |

Weak correlation coefficients (R) between the incidence of severe bruising and free tyrosine was found for LR and MP (R=0.2) and no correlation found for RB (R=0.1). Bruising index and oxidative potential correlated with free tyrosine for LR (R=0.50 and -0.35 respectively) and a weak positive correlate with severe bruising for MP (R=0.22). No correlations were found for RB (R <0.04).

The results clearly indicate that the biochemical potential to synthesise bruising pigments was not an indicator of the bruising incidence of potato tubers, supporting the previously published experiments of McGarry *et al*. (1996) and Stevens and Davelaar (1997).

### 5.2.6 Cell wall composition

#### 5.2.6.1 Immunofluorescence localization of cell wall polymers

Cortical tissue were investigated by immunofluorescence microscopy using specific antibodies JIM 5 and JIM 7 that recognise methyl-unesterified and methyl-esterified homogalacturonan domain of pectic polysaccharides respectively.

Immunofluorescence micrographs from LR, MP and RB from crops at two different harvest times (September and October) are shown in figure 5.18.

Tubers from LR showed loss of methyl esterified homogalacturan in cortex tissue when harvested in October (H2) compared to September (H1). However, there was an increase observed in methyl unesterified homogalacturan in cortex tissue harvested in October (H2) compared to the September harvest (H1). This can be associated with lower levels of pectin methyl esterification in cortex cell walls, leading to increased ionic interactions with calcium in later harvest (H2). Similar observation was made for RB, where there were highly methylated homogalacturan in H1 compared to H2 which showed loss in methylation.

MP presented loss of partially methylesteriefied pectin epitopes (JIM 7) comparing potatoes harvested H1 to H2 but it was not associated with an increase of unmethylated pectin epitopes (JIM5).

No difference was observed between harvests when the force used to break the cortex tissue was measured.

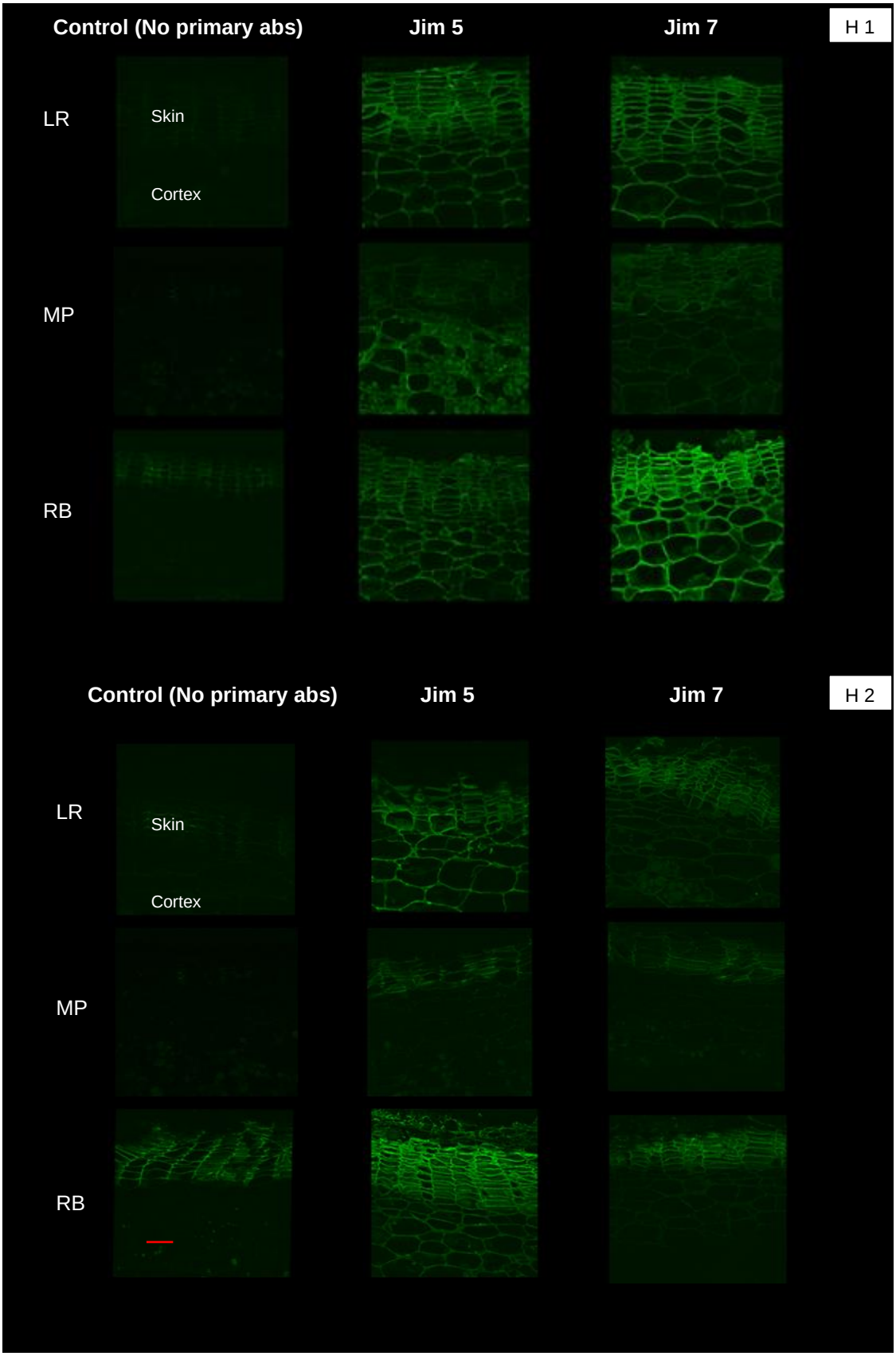


Figure 5.18 Fluorescent microscopy of potato cortex sections labelled with JIM5 or JIM7. Control indicates no primary antibody. Magnification 20X, scale bar=50 µm

#### 5.2.6.2 Cell wall composition of potatoes harvested in September and October and stored until May

Changes in the chemical composition of the cell walls of raw material over the harvest period and with long term storage were studied. The monosaccharide concentration was characterised after sequential hydrolysis (0.1 and 2 M TFA) of cell wall material (CWM). This compositional analysis was performed in order to gain information about how the cell wall composition of cortex cells influences the mechanical properties of the tuber and therefore the bruising.

At first glance, the chemical composition did not show a large variation among varieties (see table 5.13). However, the levels of the sugars galacturonic acid and glucuronic acid were statistically significant ($p<0.01$) for all varieties. The content of rhamnose, arabinose and xylose were significantly different comparing LR to MP and RB ($p<0.001$), but no significantly difference was observed between MP and RB. Galactose content of RB was significant different ($p<0.001$) between LR and MP, but no significant difference ($p<0.05$) between MP and LR. The yield comprises between 0.26 and 1.02% of the dry matter. Because glucose is not a pectic sugar it was omitted from table 5.13. The question addressed was if a systematic trend could be discovered among harvests and storage.

An increase in uronic acid (galacturonic acid + glucuroic acid) was observed for MP and RB along harvests and with storage for both harvests. LR

presented the opposite trend, with a decrease in the content along harvests and the respective storage.

Potatoes harvested late (H2) presented significant decreases in arabinose ($p<0.001$) and significant increases in galactose ($p<0.001$) for all varieties. Upon storage, the content of arabinose showed a significant decrease and the content of galactose showed a significant increase for all varieties studied for both harvests (H1 and H2), with exception for RB H2 compared to RB H2S3, where it was observed an overall decrease in the content of galactose but results were not significant different ($p>0.05$).

The rhamnose content showed a significant increase for all varieties ($p<0.001$) along harvests. Different patterns were observed upon storage in the content of rhamnose. LR showed a significant increase for both harvests ($p<0.001$). MP presented a significant increase in potatoes harvested early and an overall decrease but not significant ($p>0.05$) with storage. RB showed significant decreases for both harvests in stored samples ($p<0.001$).

The xylose showed a different pattern among the varieties studied. A significant increase ($p<0.001$) along harvest was observed for LR and RB and decrease ($p<0.001$) for MP. It was observed that storage of both harvests increased the content of xylose in LR significantly ($p<0.001$) and decreased the content in tubers harvested in September for MP and October for RB significantly ($p<0.001$).

Table 5.13 Monosaccharide concentration (rhamnose (Rha), arabinose (Ara), galactose (Gal), Xyose (Xyl), galacturonic acid (GalA) and glucuronic acid (GluA) from CWM of cortex tissue of the cultivars LR, MP and RB from tubers harvested in September (H1) and October (H2) 2011, and stored until May 2012 (S3) after sequential hydrolysis with 0.1M and 2M TFA. Concentrations are expressed per %mol. Values represent average ± SEM (n=4).

| **Monosaccharides (%mol)** | **LR H1** | **LR H1S3** | **LR H2** | **LR H2S3** |
|---|---|---|---|---|
| **Rha** | 0.7 ± 0.0 | 1.2 ± 0.1 | 1.0 ± 0.0 | 1.7 ± 0.1 |
| **Ara** | 36.4 ± 0.1 | 22.3 ± 0.6 | 31.5 ± 0.5 | 17.6 ± 0.0 |
| **Gal** | 20.5 ± 0.0 | 30.8 ± 0.2 | 26.5 ± 0.3 | 33.3 ± 0.4 |
| **Xyl** | 14.1 ± 0.2 | 25.1 ± 0.7 | 17.5 ± 0.3 | 36.5 ± 0.1 |
| **GalA** | 21.4 ± 0.3 | 17.6 ± 0.2 | 16.7 ± 0.2 | 8.2 ± 0.0 |
| **GluA** | 6.9 ± 0.2 | 3.1 ± 0.1 | 6.9 ± 0.0 | 4.5 ± 0.1 |
| **Monosaccharides (%mol)** | **MP H1** | **MP H1S3** | **MP H2** | **MP H2S3** |
| **Rha** | 0.5 ± 0.1 | 1.0 ± 0.1 | 1.1 ± 0.1 | 0.9 ± 0.1 |
| **Ara** | 31.1 ± 0.7 | 19.8 ± 0.6 | 24.1 ± 2.7 | 17.5 ± 0.1 |
| **Gal** | 19.4 ± 0.6 | 31.1 ± 1.3 | 29.4 ± 1.7 | 34.0 ± 0.2 |
| **Xyl** | 25.8 ± 0.8 | 11.7 ± 0.5 | 11.7 ± 2.4 | 13.2 ± 0.4 |
| **GalA** | 17.5 ± 0.1 | 30.0 ± 1.7 | 26.6 ± 3.6 | 28.8 ± 0.4 |
| **GluA** | 5.8 ± 0.4 | 6.5 ± 0.1 | 7.1 ± 0.1 | 5.6 ± 0.2 |
| **Monosaccharides (%mol)** | **RB H1** | **RB H1S3** | **RB H2** | **RBH2S3** |
| **Rha** | 0.9 ± 0.1 | 0.6 ± 0.0 | 1.3 ± 0.0 | 0.8 ± 0.0 |
| **Ara** | 35.4 ± 1.0 | 21.4 ± 0.2 | 26.1 ± 0.5 | 20.0 ± 1.0 |
| **Gal** | 27.4 ± 1.1 | 36.0 ± 0.5 | 32.7 ± 0.8 | 30.1 ± 3.0 |
| **Xyl** | 14.8 ± 0.8 | 12.5 ± 1.2 | 17.6 ± 2.4 | 12.2± 2.9 |
| **GalA** | 17.7 ± 0.8 | 25.3 ± 0.6 | 17.9 ± 1.9 | 31.2 ± 1.2 |
| **GluA** | 3.8 ± 0.1 | 4.3 ± 0.2 | 4.4 ± 0.3 | 5.7 ± 0.3 |

The backbone of pectin consists in a linear polygalacturonic chain interspersed with (1→2)-linked *α-L* –rhamnopyranosyl residues, causing kinks in the chain. An increasing amount of rhamnose residues in the polygalacturonic chain goes at the expense of its linearity. Therefore, the ratio of the molar amount of uronic acids (galacturonic acids + glucuronic acid) over the molar amount of Rha (UA/rhamnose) is considered to

represent the measure for the linearity of the cell wall (van Dijk *et al*., 2002). Covalently attached to this rhamnopyranosyl backbone, primarily thought the rhamnopyranosyl residues are side chains mainly consisting of neutral oligo- and polysaccharides. The ratio of the amount of the main pectic sugars arabinose and galactose over the uronic acids (galacturonic acid + glucuronic acid) represents a measure for the side-chain extent. Because linearity and side-chain extent are important characteristics of pectin, these values are included in table 5.14.

Table 5.14 Neutral sugars (rhamose+arabinose+galactose (Rham+Ara+Gal)), branching (molar ratio of arabinose + galactose to uronic acids (Ara+Gal/UA)) and number of side chains (uronic acids (UA)/rhamnose) in CWM of the cultivars LR, MP and RB harvested in September (H1) and October (H2) 2011 and stored until May 2012 (S3) after sequential hydrolysis with 0.1 M and 2 M TFA.

| | **Varieties and time** | | | |
|---|---|---|---|---|
| | **LR H1** | **LR H1S3** | **LR H2** | **LR H2S3** |
| **Neutral sugars (Ara+Gal+Rha)** | 57.6 | 54.2 | 59.0 | 52.6 |
| **Molar ratio (Ara+Gal/UA)** | 2.0:1.0 | 2.6:1.0 | 2.5:1.0 | 4.0:1.0 |
| **UA/Rhamnose** | 82.0:1.0 | 44.4 | 58.4:1.0 | 30.8:1.0 |
| | **MP H1** | **MP H1S3** | **MP H2** | **MP H2S3** |
| **Neutral sugars (Ara+Gal+Rha)** | 51.0 | 51.9 | 54.6 | 52.4 |
| **Molar ratio (Ara+Gal/UA)** | 2.2:1.0 | 1.4:1.0 | 1.6:1.0 | 1.5:1.0 |
| **UA/Rhamnose** | 112.2:1.0 | 53.4:1.0 | 50.1:1.0 | 59.2:1.0 |
| | **RB H1** | **RB H1S3** | **RB H2** | **RBH2S3** |
| **Neutral sugars (Ara+Gal+Rha)** | 63.8 | 58.0 | 60.1 | 50.9 |
| **Molar ratio (Ara+Gal/UA)** | 2.9:1.0 | 1.9:1.0 | 2.6:1.0 | 1.4:1.0 |
| **UA/Rhamnose** | 66.7:1.0 | 95.7:1.0 | 44.4:1.0 | 66.0:1.0 |

On the basis of the data presented in table 5.14 it is suggested that the pectin moiety within CWM changes along harvest periods and upon storage. The LR pectin becomes less linear (the ratio of UA/Rha decreases) and more branched (the ratio of arabinose + galactose /UA increases) along harvest period and upon storage. MP and RB pectins become less linear along harvest and along storage (the ratio of UA/rhamnose decreases), with exception of tubers harvested in October (H2) when stored, and less branched along harvest and upon storage (the ratio of arabinose + galactose/UA decreases).

Moderate negative correlation (R=0.34) and strong positive correlation (R=0.40) were found for arabinose and galactose content with severe bruising of all varieties respectively. However, negligible relationship was found (R<0.16) when correlating the degree of branching and molar ratio with severe bruising incidence of all varieties.

### 5.2.7 Relationship between analyses

Correlations of severe bruising, bruising index and oxidative potential with physical/compositional aspects of the crop during harvests and upon storage were analyzed and summarized in table 5.15, indicated as R values.

Table 5.15 The relationships (R) and significance P value of severe bruising (SB), bruising index (BI) and oxidative potential (OP) with physical, mechanical and compositional aspects of the varieties LR, MP and RB, field trial 2.

| | **Severe bruising** | | | | | |
|---|---|---|---|---|---|---|
| **Variety and correlation sample size** | **LR (n=8)** | | **MP (n=8)** | | **RB (n=8)** | |
| **Assessment, sample size / R and P value** | **R** | **P value** | **R** | **P value** | **R** | **P value** |
| BI (n=3) | **0.37** | 0.37 | **0.68** | 0.07 | **0.82** | 0.01 |
| OP (n=3) | 0.01 | 0.98 | -0.60 | 0.11 | -0.27 | 0.52 |
| Weight (n=30) | 0.13 | 0.76 | -0.27 | 0.52 | -0.01 | 0.98 |
| SG (n=30) | 0.25 | 0.63 | 0.04 | 0.92 | 0.15 | 0.72 |
| Energy to break the skin tissue (n=30) | 0.36 | 0.38 | -0.09 | 0.83 | -0.11 | 0.79 |
| Force to break the skin tissue (n=30) | -0.21 | 0.62 | -0.09 | 0.83 | 0.04 | 0.93 |
| Distance to break the skin tissue (n=30 | **0.24** | 0.57 | -0.17 | 0.68 | -0.14 | 0.74 |
| Energy to break the cortex tissue (n=30) | 0.20 | 0.63 | -0.13 | 0.76 | -0.03 | 0.94 |
| Force to break the cortex tissue (n=30) | 0.09 | 0.83 | 0.01 | 0.98 | 0.02 | 0.96 |
| Distance to break the cortex tissue (n=300 | **0.24** | 0.57 | -0.17 | 0.68 | -0.14 | 0.74 |
| Tyrosine (n=3) | 0.18 | 0.67 | **0.22** | 0.60 | 0.09 | 0.83 |
| Chlorogenic acids (n=3) | **-0.75** | **0.03** | -0.03 | 0.94 | **0.69** | 0.06 |
| Phenolic acids (n=3) | **-0.78** | **0.02** | -0.04 | 0.92 | **0.54** | 0.17 |
| 5-CQA (n=3) | **-0.78** | **0.02** | 0.07 | 0.87 | 0.34 | 0.41 |
| CA (n=3) | **-0.71** | 0.05 | **-0.29** | 0.49 | **-0.52** | 0.17 |
| | **Bruising Index (BI)** | | | | | |
| OP (n=3) | **0.21** | 0.61 | **-0.71** | **0.05** | **-0.31** | 0.45 |
| Weight (n=30) | -0.01 | 0.98 | -0.06 | 0.89 | -0.11 | 0.80 |
| SG (n=30) | 0.19 | 0.65 | 0.00 | 1.00 | 0.18 | 0.67 |
| Energy to break the skin tissue (n=30) | 0.23 | 0.58 | -0.25 | 0.55 | -0.24 | 0.57 |
| Force to break the skin tissue (n=30) | -0.05 | 0.91 | -0.01 | 0.98 | 0.10 | 0.81 |
| Distance to break the skin tissue (n=30 | **0.23** | 0.58 | **-0.24** | 0.57 | **-0.23** | 0.58 |
| Energy to break the cortex tissue (n=30) | 0.26 | 0.53 | -0.17 | 0.69 | -0.05 | 0.91 |
| Force to break the cortex tissue (n=30) | 0.28 | 0.50 | 0.05 | 0.91 | 0.01 | 0.98 |
| Distance to break the cortex tissue (n=300 | **0.23** | 0.58 | **-0.25** | 0.55 | **-0.23** | 0.58 |
| Tyrosine (n=3) | 0.50 | 0.21 | 0.05 | 0.91 | -0.00 | 1.00 |
| Chlorogenic acids (n=3) | -0.09 | 0.83 | 0.04 | 0.92 | 0.40 | 0.33 |
| Phenolic acids (n=3) | -0.09 | 0.83 | 0.03 | 0.94 | 0.27 | 0.52 |
| 5-CQA (n=3) | -0.15 | 0.72 | 0.02 | 0.96 | 0.15 | 0.72 |
| CA (n=3) | -0.12 | 0.78 | **-0.72** | 0.04 | -0.00 | 1.00 |
| | **Oxidative potential (OP)** | | | | | |
| Energy to break the skin tissue (n=30) | 0.11 | 0.80 | 0.33 | 0.42 | **0.03** | 0.94 |
| Force to break skin tissue (n=30) | 0.13 | 0.76 | 0.09 | 0.83 | -0.11 | 0.80 |
| Distance to break the skin tissue (n=30 | 0.11 | 0.80 | 0.18 | 0.67 | 0.07 | 0.87 |
| Energy to break the cortex tissue (n=30) | 0.00 | 1.00 | 0.32 | 0.44 | 0.08 | 0.85 |
| Force to break the cortex tissue (n=30) | 0.00 | 1.00 | -0.04 | 0.93 | -0.07 | 0.87 |
| Distance to break the cortex tissue (n=300 | 0.00 | 1.00 | **0.44** | 0.28 | **0.34** | 0.41 |
| Tyrosine (n=3) | -0.35 | 0.47 | -0.05 | 0.91 | 0.00 | 1.00 |
| Chlorogenic acids (n=3) | -0.29 | 0.48 | -0.02 | 0.96 | -0.13 | 0.76 |
| Phenolic acids (n=3) | -0.29 | 0.48 | -0.02 | 0.96 | -0.07 | 0.87 |
| 5-CQA (n=3) | -0.30 | 0.47 | -0.01 | 0.98 | -0.09 | 0.83 |
| CA (n=3) | -0.36 | 0.38 | 0.40 | 0.33 | 0.10 | 0.81 |

Although Person's coefficient (R) indicates some correlations, most of them were not statistically significant ($p>0.05$), what reflect a very small correlation sample size (n=8). So, the purpose of this exercise was to establish general trends between physical/composition aspects and the indicators of severe bruising.

Severe bruising incidence and bruising index (BI) showed a moderate to very strongly correlation for all varieties studied which indicates that severe bruising assessment, which considers only the depth of bruised tissue, is a good assessment method to measure the incidence of bruising in crops.

In LR, the positive strong correlation between tyrosine content with BI (R=0.50) could indicated that these components may have contributed to the pigment formation as previously reported (Sabba and Dean, 1994; McNabay, 1999; Strehmel *et al*., 2010a). However, the negative relationship between tyrosine and chlorogenic acids with OP (R=-0.35 and R=-0.29 respectively) suggests that for LR there is no association of the content of substrates for in the pigment formation, but in fact other factors are involved in the *in vitro* assay i.e. the activity of the enzyme and presence of ascorbic acid, which could prevent formation of pigment. Also, the strong negative correlation between incidence of severe bruising and phenolic acids (R=-0.78) was not expected once higher content of phenolics was found during 2012 season compared to field trial 1 as 2012 was a hot and dry season. Weak positive correlation was observed between the distance to break the skin and cortex (measure of deformability) with severe bruising and BI

(R=0.24 and 0.23 respectively). Although the correlations of bruising with mechanical parameters were weak, small changes on the mechanical properties may represent increase in susceptibility of LR as this variety presented higher specific gravity which indicates higher DM content, leading to more absorbance of the impact energy. Interesting is the fact that a positive correlation with deformability of skin and cortex was found for LR and negative for MP and RB.

MP has different correlations than LR, there was no a single measurement strongly correlated to bruising. A weak correlation was found between severe bruising and tyrosine content (R=0.22). In fact, MP presented a lower concentration of phenolic acids than LR and RB and may be below the threshold for bruising as cited in chapter 4. A negative weak correlation between of the distance to break the skin and cortex to BI was observed (R=-0.24 and R=-0.25 respectively).

RB showed a strong correlation between incidence of severe bruising and concentration of phenolic acids (R=0.54), indicating that for RB, the variety that bruises more, the phenolic acids may act in participating on bruising. The negative correlation observed between softening of skin and cortex with severe bruising and BI (R=-0.23 and R=-0.25 respectively) indicate that the biochemical apparatus is the parameter more detrimental for bruising in this variety.

Further correlation analyses were conducted to investigate the overall relationships. The results for the three varieties studied under two harvests

and three periods of storage are summarized in PCA bi-plot (figure 5.19).

The labels are indicated in table 5.16.

Table 5.16 Labels in the PCA graphs from LR, MP an RB crops harvested in September and October, stored until January (S1), March (S2) and May (S3), field trial 2.

| | Variety | LR | | | MP | | | RB | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Letter** | **Assessment** | **No.** | **Label** | | **No.** | **Label** | | **No.** | **Label** | |
| A | Severe Bruising | 1 | September | H1 | 9 | September | H1 | 17 | September | H1 |
| B | Optical density | 2 | | S1 | 10 | | S1 | 18 | | S1 |
| C | Energy to break the skin tissue | 3 | | S2 | 11 | | S2 | 19 | | S2 |
| D | Force to break the skin tissue | 4 | | S3 | 12 | | S3 | 20 | | S3 |
| E | Distance to break the skin tissue | | | | | | | | | |
| F | Energy to break the cortex tissue | 5 | October | H2 | 13 | October | H2 | 21 | October | H2 |
| G | Force to break the cortex tissue | 6 | | S1 | 14 | | S1 | 22 | | S1 |
| H | Distance to break the cortex tissue | 7 | | S2 | 15 | | S2 | 23 | | S2 |
| I | Vanillic acid | 8 | | S3 | 16 | | S3 | 24 | | S3 |
| J | Caffeic acid | | | | | | | | | |
| K | *p* Coumaric acid | | | | | | | | | |
| L | Ferulic acid | | | | | | | | | |
| M | 3-CQA | | | | | | | | | |
| N | 4-CQA | | | | | | | | | |
| O | 5-CQA | | | | | | | | | |
| P | Tyrosine | | | | | | | | | |
| Q | Bruising index | | | | | | | | | |

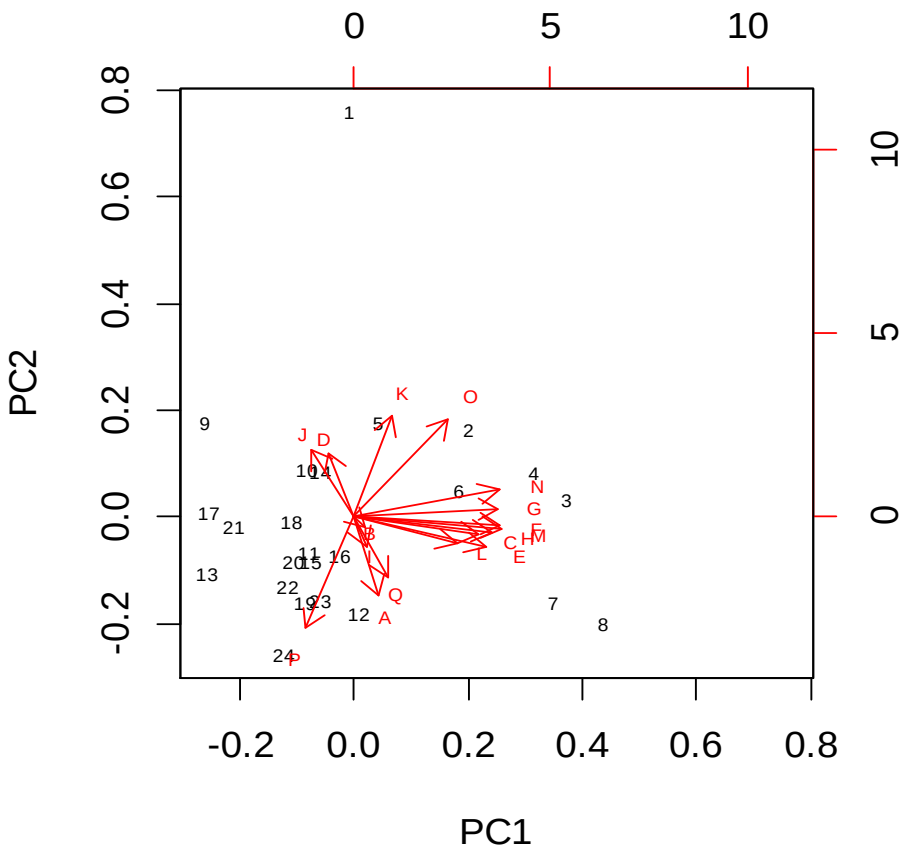


Figure 5.19 PCA bi-plot of data from potatoes harvested in September and October, stored until January (S1), March (S2) and May (S3), field trial 2. PC1 explains 41% of variance and PC2 15%.

The relationships show in the PCA considers levels of components and changes among varieties. This analysis generates a substantial number of correlations and the model of PCA explained 56% of the data variance. The two components allowed discrimination of varieties. Investigation into the relative contribution (loadings) of individual variables in the PC1 dimension highlighted components with a significant impact on bruising. All of these variables correspond to 3 and 4-CQA, work and force to break the cortex and the distance to break skin and cortex. The measurements of bruising (SB, BI and OP) were strongly correlated and all of them correlated well with

tyrosine content. The force to break skin and caffeic acid content were perpendicular related to severe bruising, bruising index and OP, suggesting strongly negative correlation between these factors. A positive correlation was found between the distance to break tissues (cortex and skin) and bruising.

## 5.3 Discussion

Relationships between bruising and tuber properties are reconsidered and the strength of evidence for their roles evaluated. The relevance of tuber maturity status at harvest is given particular attention because there is a strong evidence that it is of major importance in the quality of stored tubers. A more quantitative approach to its characterisation was required for the information to be of practical value. Its relationship with other tuber properties such as weight, specific gravity, mechanical properties, cell wall composition, phenolic acids and tyrosine content was examined together with the potential for these as mechanisms underlying external factors.

All varieties were mature at the harvest time based on the field indicator of the decline of canopy. Due to the early senescence in this field trial, a high incidence of bruising was found. In this field trial, a slight reduction in bruising was found only for RB (5%) when compared the tubers harvested in October (43 days after full senescence) to tubers harvested in September (34 days after full senescence). The incidence of severe bruising was considered a good predicative of bruising susceptibility as correlated well with BI.

The cultivars LR and MP are considered to represent less susceptible cultivars compared to RB with regard to bruising. However in this research when tubers were harvested late (October) the incidence of severe bruising was similar for all varieties (82-88%). During tuber growth an increase in bruising incidence for LR and MP and a slight decrease for RB were observed. However, bruising in stored samples was influenced by harvest time. It was observed there was more bruising in the variety LR when harvested late. The opposite was found for the varieties MP and RB. In general, a lower incidence of bruising was observed for MP and RB tubers when harvested late. The variations in the incidence of severe bruising did not reflect with concomitant increase/decrease in tyrosine content or phenolic acids. So, the composition of these phenolics at harvest can not be used as predictive of the quality for stored samples.

An increase in specific gravity along harvests and storage periods was observed. The increase in SG was more prominent at medium period storage (S2), where RB and LR were harvested late (October) showed a significant increase in SG compared to the respective harvest period. At this storage period (S2) this is likely to be due to moisture loss through evaporation. Tubers harvested late presented a decrease in SG at long term storage (S3), possibly due to a higher rate of respiration and starch break down in these tubers.

Corsini *et al.* (1999) found that RB stored for 4 months at 7°C presented moisture losses resulting in low tuber turgidity and this increased bruising susceptibility. In this research a weak correlation (R=0.25) was found only for LR comparing SG and severe bruising and no correlation for the other

varieties. Although varietal differences were found in physical aspects such as weight and SG, these factors were not strongly correlated with the type of assessment used in this research but may be relevant in assessment of bruising that simulate handling tubers, i.e. barrel or dropping tubers from a specific height, as it considers the potential energy that will be dissipated through the skin and subjacent tissue.

Respiration and evaporation during storage also influenced the tissue properties. Tissues become more deformable along the storage period which may have prevented damage of the skin with the falling bolt method during bruising assessment. The cell walls of potatoes have an important role in maintaining the freshness of potato tubers during storage (Jarvis *et al*., 2003). The combined results showed that the more deformable the LR tissues became, the more susceptible to bruise but the opposite was found for MP and RB. However, MP and RB tubers required less force to break the tissue. This means that bruising in LR tubers was directly linked to deformability of the tissue but RB and MP were dependent of other factors.

One of these factors can be the tyrosine content of MP and RB which was~ 2X higher than LR. However, the content of tyrosine, the main substrate of the discolouration reaction, was not strongly correlated with bruising for these varieties separately, but a negative correlation was found analysing all results together with PCA. These results are in disagreement with previous *in vitro* assays reported by Sabba and Dean (1994) and Corsine *et al.* (1992) which suggested that tyrosine could be responsible for an increase in susceptibility of the tuber to bruise during cold storage at 4°C and 6°C respectively. Cold storage may lead to more membrane damage.

The changes in the pectic composition of the cell wall were dependent on variety. Major changes in pectin composition were observed in the content of pectic side chain of RGI. LR had showed more side chains with more/longer neutral sugars on the pectin backbone than MP and RB, which became less branched along harvest and storage. This varietal difference observed in LR may not have contributed to the tensile strength of cell walls as previously supported by Skjøt *et al*. (2002); Oomen *et al*. (2002); Ulvskov *et al*.(2005); Ryden *et al*.(2003); Caffall and Mohnen (2009) and Orfila *et al*. (2012). However, the differences on the amounts of neutral sugars (arabinose + galactose + rhamnose) among varieties were small (LR: 53-59%; MP 51-55% and RB 51-64% Mol of CWM). On the other hand, it was expected increase in the strength of the tissue in tubers harvested in October due the loss of methyl esterification in pectin cortex observed for all varieties, which may ionically interact with calcium. In fact, the force to break cortex tissue slight diminished along harvest but was not statistically significant ($p<0.05$).

As discussed above, it was shown that:

(i) The variety LR presented a higher deformability concomitant with lower tyrosine and higher phenolic acids content compared to the other varieties but substrates to PPO do not appear to the main fact for pigment formation.

(ii) RB presented the lower deformability and higher tyrosine content to the other varieties, and this was associated with high incidence of bruising in RB for both harvests.

(iii) Bruising in MP depends of both, this variety has strong mechanical properties which may protected tubers from impact mechanical and has intermediate levels of substrates for PPO (phenolics and tyrosine).

On the basis of this information it is possible to conclude that the sum of factors determinate the bruising incidence. However, the following considerations have to be kept in mind:

(i) Tyrosine and chlorogenic acid were not predicative of bruising at harvest for stored potatoes, only if considered together.

(ii) Tyrosine content accumulates with harvest and storage.

(iii) Tissue deformability increases along harvest and storage.

(iv) SG and weight of tubers have no association with bruising.

In conclusion, during harvest and storage periods substantial changes in the bruising, texture, cell wall and biochemical composition were observed.

These changes in stored samples are the consequence of varietal differences and harvest time. It was observed that more bruising occurred in stored tubers from LR harvested in October whereas MP and RB tend to bruise less in stored tubers harvested in October. This showed that tuber maturity at harvest was the predominant factor influencing bruising, both at harvest and thoughout storage.

# 6 Effect of Nitrogen on bruising along harvest time – field trial 3

## 6.1 Introduction

The aim of this chapter is to investigate the effect of nitrogen (N) application on the incidence of bruising in the variety Lady Rosetta (LR). This variety was selected for this field trial because it presents higher content of dry matter compared to other varieties which is ideal for crisps production.

The use of fertilizer N is a common agricultural practice, generally needed because of its mobility in soils and the large amounts needed by plants. Knowledge of the residual soil N, rate and amount of N mineralized from soil organic sources, and individual crop needs are all required to optimize N fertilizer recommendations. Recommendations based on these factors have the potential for improving N fertilizer efficiencies, as well as increasing production with indeterminate potato varieties (Westerman and Klienkopf, 1985). The current N recommendations in the UK are based on guidelines set out in DEFRAs Fertiliser Manual, where a soil N supply (SNS) index is calculated on soil type, winter rainfall and previous crop (DEFRAs RB209).

Supplementing crops with N can have a significant effect on a number of physiological processes in potatoes, as influence on crop senescence, skin set and dry matter (Pringle *et al.,* 2009). The leaf area of the potato plant is considerably dependent upon N supply, both the number and size being increased by increased N. Number because of the stimulation of growth of

both apical and lateral meristems, the lateral meristems leading to more branches and size by the stimulation of cell division, leading to a greater number of cells in the leaf (Burton, 1989).

According to personal communication with producers, the higher the dry matter content, the higher is the incidence of bruising. When dry matter of the tubers reaches about 19-20%, farmers plan the harvest because they have observed that within a few days the dry matter rises quickly and potatoes bruise more. Crops where N is over-applied, either initially or later through top dressing, will take longer to reach maturity, will have reduction of potato yield and delay the achievement of the dry matter content. The haulm can be more difficult to desiccate and it can also take longer to set tuber skins (Sun *et al.*, 2012), adversely affecting processing quality.

Early literature (De Bruyn, 1929) summarized by Mondy and Koch (1978) suggested that the incidence of bruising increased with application of N fertilizer. This effect has been recorded as an increase from 12 to 24% for an increase in N application from 30 to 100 kg/ha. (Koblet, *et al*. 1948 in McGarry, 1996). In contrast, the authors Kunkel and Dow (1961) observed that increasing N fertilizer from 100 kg/ha to 290 kg/ha was associated with a decrease in susceptibility to bruising using a falling bolt test. Rogers-Lewis (1980) observed no effect of additional N fertilizer on bruising incidence using a pendulum to damage the tubers, but recorded a decrease in incidence with additional N in one of three years experiments, when harvesting operations were used to inflict damage. Silva *et al.* (1991) also reported no effect of N on the incidence of bruising resulting from harvesting operations or from subsequent impacts in a rotating drum.

It was found by Hole (1997) that timing of harvest can affect the incidence of bruising. With advances in crop development, tubers treated with N become less susceptible to bruising, whereby the symptoms change from visible tissue fracture (damage) and brown discolouration to less obvious fracture and grey/black discolouration. This association may be due to additional N slowing the rate of crop development and maturity.

The influence of N fertilizing practices on impact sensitivity, especially resistance to bruise was suggested also by Baritelle *et al. (*2000). Additionally, soil type can interact with the effects of the N fertilizing pattern. The split 56 kg:ha preplant and 56 kg:ha postplant N treatment in Russet Burbank gave significantly higher bruise threshold in the higher permeability soil and highest bruise resistance in both soils (higher is better).

Considering that bruising is a significant problem for crisps production, in the recent years research has been carried out focusing on the study of bruising potato cultivars and genetic modification to develop resistant varieties, however, little attention has been paid to the application of N despite the fact that the application of N is a common agricultural practices that may have a relevant impact on the bruising susceptibility. There is also little understanding of the physicochemical factors that may determine bruise development. Therefore the objective of the present research was to investigate the influence of application of N on bruising incidence, and to investigate the relationship between observed bruising and physicochemical factors such as specific gravity, phenolic acids tyrosine content, mechanical properties and cell wall composition of the LR.

### 6.1.1 Aim

The aim of this project was to determine whether N fertilizer application influences bruising susceptibility and how it affects the biochemical and physical parameters normally associated with bruising in LR. This will enhance understanding of the crop response to N application leading to industry standards for the production of crisps.

On the 3rd field trial, tubers from the variety LR treated with N 200 kg/ha and control (not-treated) were studied. According to the Potato Council independent variety trials, LR showed a bruising susceptibility score of 6, in ratings ranging from 0 (most susceptible) to 9 (least susceptible) (Carnegie et al., 2005; BPVD, 2012). Details about how independent trial was calculated are shown on Chapter 1, section 1.5.1.

LR plants were grown at Cambridge University Farm (CUF), planted on 23 April 2013 and harvested at four time points, as indicated in table 2.3. Trials were randomised with two factors (harvest and N) with six replicate plots. Ten tubers per plot were collected and sent to Leeds on harvest day.

### 6.1.2 Hypotheses

The hypotheses tested are:

1) Specific gravity is higher in tubers supplied with N.

2) Potatoes supplied with N fertilizer show more bruising along harvest.

3) Phenolic substrates increase in content along harvest and are higher in N treated tubers.

4) Tubers treated with N present changes in the mechanical properties of the tuber and the cell wall composition of cortex cells.

### 6.1.3 Objectives

1) To determine if application of N fertilizer produces tubers with more potential to bruise by comparing the bruising susceptibility of treated and non-treated tubers using the falling bolt method.

2) To identify if N application affect the mechanical properties of tubers and if this could be associated with resistance/susceptibility to bruising.

3) To improve the potential for using physical measurements as dry matter as indicators of bruising on the variety LR.

4) To improve the understanding of the biochemical apparatus for bruising along harvest date and the correlation with the incidence of bruising.

## 6.2 Results

### 6.2.1 Field phase

#### 6.2.1.1 Meteorological data

Rainfall and mean temperature of the air and soil are presented in figure 6.1. 2013 season was characterized by being mild in temperature, but had a 7

days period starting in mid-July 2013 when temperatures reached a maximum of over 26°C each day with radiation receipts in July and August being 10 -15% above field trial 1 and 2 average. Reference $Et_0$ (Evapotranspiration (ET) - is the sum of soil water evaporation (E) and plant transpiration (T)) was consequently greater in July, with mean daily $ET_0$ 3.94 mm/day and only 3.30 in June and in August. $ET_0$ attained a peak of 5.9 mm/day in mid-July but generally $ET_0$ was lower than typical for the temperatures since windruns were small and relative humidity's high which restricted $ET_0$. August was slight wetter than average.

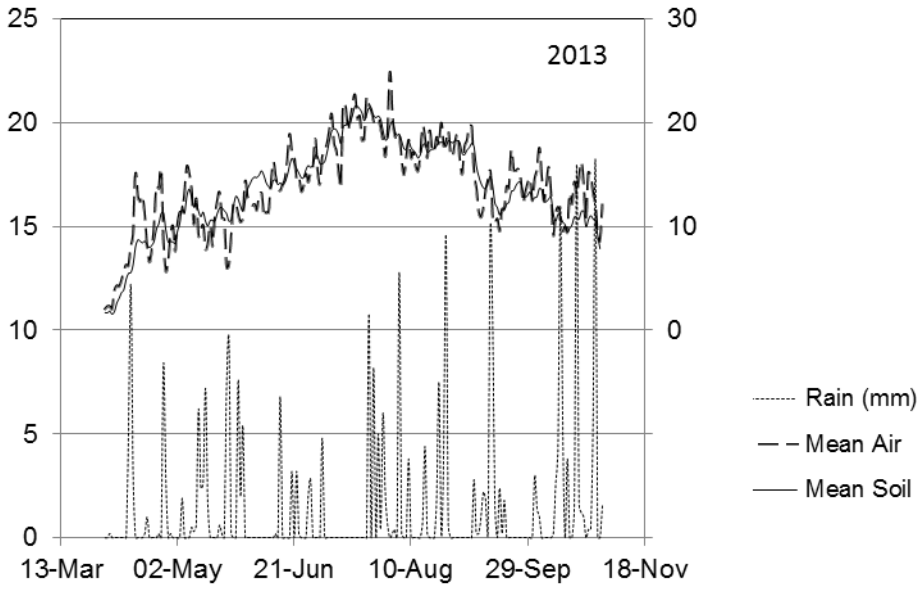


Figure 6.1 Rainfall (mm) and mean temperature of the air and soil (secondary axis) in field trial 3, 2013.

### 6.2.1.2 Green canopy cover (%)

Measurements of decline of canopy was taken as key indicators of the effect of crop treated with 200 kg/ha of N and no treated on the physiological characteristics. The data clearly indicate that soil treatment with N significantly affected crop maturity at harvest, shown in table 6.1.

Table 6.1 Senescence measured by the green canopy cover (%) of LR tubers no treated and treated with N 200 kg/ha, harvested in July (H1), August (H2 and H3) and September (H4).

| Harvest | No treatment | N200 |
|---|---|---|
| **H1** | 93 | 99 |
| **H2** | 66 | 91 |
| **H3** | 22 | 67 |
| **H4** | 2 | 19 |

Delay in senescence was observed in LR tubers supplied with N along harvest times.

## 6.2.2 Bruising assessment

### 6.2.2.1 Assessment of severe bruising using falling bolt

Application of N reduced bruising incidence of the variety LR when tubers were harvested earlier than 112 days after planting (H1 and H2), shown in figure 6.2. After this period, tubers grown in fertilized soil showed higher incidence by 5 and 10 % when harvested in late August (H3) and early September (H4), respectively compared to unfertilized.

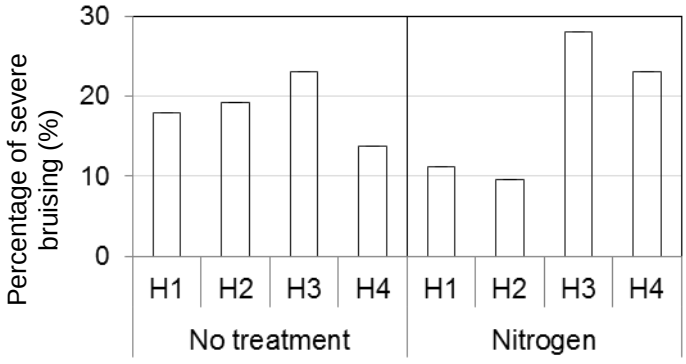


Figure 6.2 Effect of harvest and N treatment (200 kg/ha) on the incidence of severe bruising following damage with the falling bolt in LR tubers harvested in July (H1), August (H2 and H3) and September (H4), field trial 3. Values show incidence of severe bruising (n>21).

When analysing slight and severe bruising (presented in figure 6.3), tubers grown on treated soil presented higher incidence of bruising from the second harvest (112 days after planted) and similar incidence of slight plus severe bruising when harvested in September (H4).

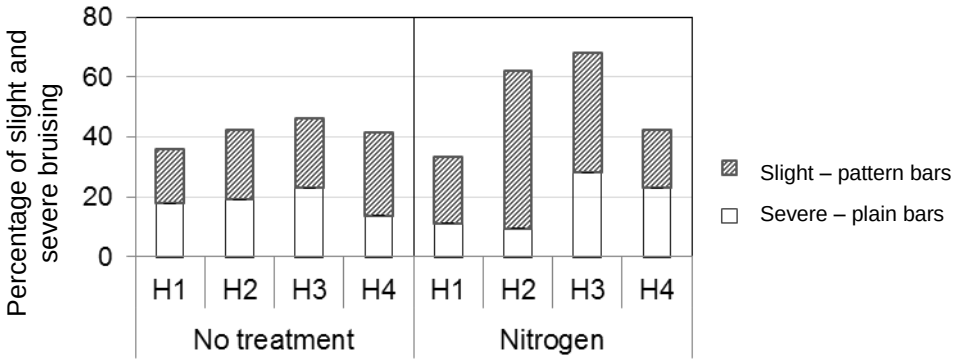


Figure 6.3 Effect of harvest and N treatment (200 kg/ha) on the incidence of slight and severe bruising following damage with the falling bolt in LR tubers harvested in July (H1), August (H2 and H3) and September (H4), field trial 3. Values show incidence of severe bruising (n>21).

#### 6.2.2.2 Damage

The incidence of damaged skin was higher in tubers from N treated soil along all harvest times. As N application delays crop maturity, skin set was probably delayed and consequently tubers were probably less resistant to the impact, contrary to observed by Hole (1997) and Baritelle *et al.* (2000). Results are presented in figure 6.4.

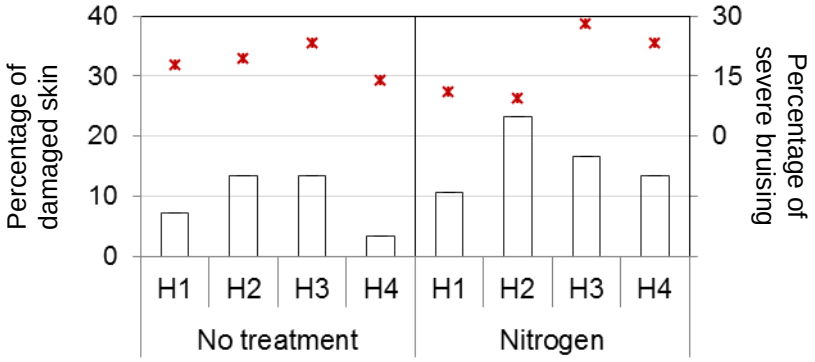


Figure 6.4 Effect of harvest and N treatment (200 kg/ha) on the incidence of skin damage following damage with the falling bolt (bars) and percentage of severe bruising (scatter) in tubers from the variety LR harvested in July (H1), August (H2 and H3) and September (H4), field trial 3. Value bars show incidence of damaged tissue (n=30), and scatter shows incidence of severe bruising (n>21).

#### 6.2.2.3 Bruising Index

When including the area of impact and colour formation as part of the measurement of bruising, on the assessment of bruising index, results were slightly different than assessment of severe bruising, as shown in figure 6.5. Tubes from treated soil presented lower BI than control until late August (H3) and higher incidence when harvested in September (H4) compared to control.

Comparing the results from H4, the bruised tissue from control tubers presented lower depth and width but slight higher colour formation.

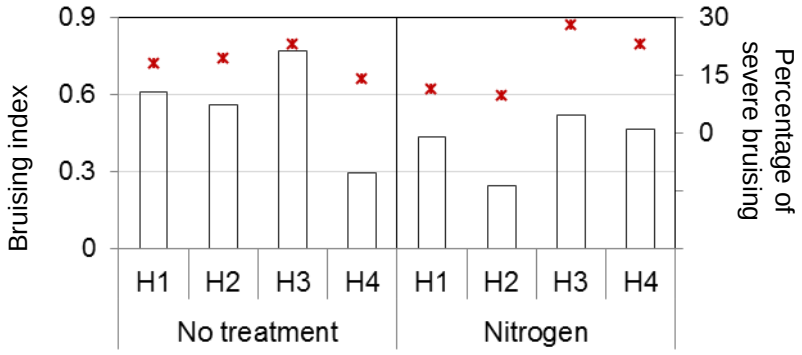


Figure 6.5 Effect of harvest and N treatment (200 kg/ha) on the bruising index (bars) and percentage of severe bruising (scatter) in tubers from the variety LR harvested in July (H1), August (H2 and H3) and September (H4), field trial 3. Values show means (bars) and incidence of severe bruising (scatter), (n>21).

### 6.2.2.4 Spectrophometric assessment of oxidative potential

Absorbance of extracts from samples treated and untreated left to oxidize for 20 hours are shown in figure 6.6. Although average changes were observed along harvest times, no significantly different results were found on oxidative potential when comparing treatments at each time point studied and when compared harvests (p>0.05).

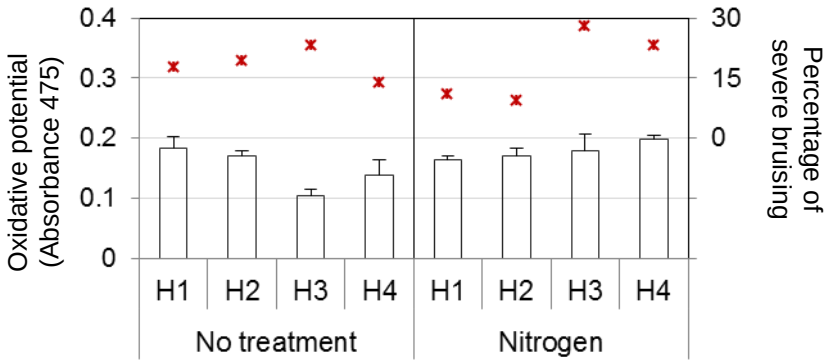


Figure 6.6 Effect of harvest and N treatment (200 kg/ha) on the oxidative potential (scale 0-1) following 20 h oxidation (bars) and percentage of severe bruising (scatter) in tubers from the variety LR harvested in July (H1), August (H2 and H3) and September (H4), field trial 3. Value bars show means (n=3), error bars are SE and scatter shows incidence of severe bruising (n>21).

### 6.2.3 Physical properties

#### 6.2.3.1 Weight

Significant differences were found in the yield of tubers treated and untreated at the 3$^{th}$ and the 4$^{th}$ harvest ($p<0.01$), where tubers treated with N presented ~% 35 more weight than untreated as shown in figure 6.7. These results are in accordance to previous studies of Westerman and Klienkopf (1985), which stated that mean tuber yields were greatly increased by N fertilizer treatments.

Among harvests, a significant increase was found in treated tubers between H2 and H3 ($p<0.01$). Increases were observed in the weight of control tubers but no significant differences were found when comparing the weight of tubers along harvest time.

When contrasting the incidence of severe bruising and weight of tubers, no correlation was found for control ($R= 0.01$) and very strong positive correlation for treated tubers ($R=0.84$). When contrasting the weight of tubers with BI, no correlation was found for control ($R=-0.09$) and weak positive correlation was found for treated tubers ($R=0.26$).

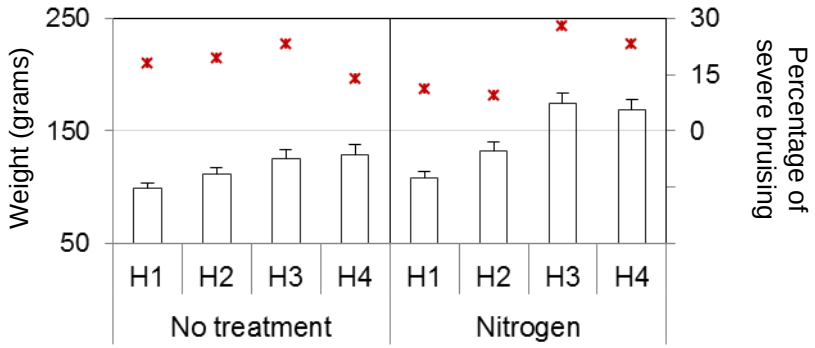


Figure 6.7 Effect of harvest and N treatment (200 kg/ha) on the weight (grams) (bars) and percentage of severe bruising (scatter) in tubers from the variety LR harvested in July (H1), August (H2 and H3) and September (H4), field trial 3. Value bars show means (n=30), error bars are SE and scatter shows incidence of severe bruising (n>21).

### 6.2.3.2 Specific gravity

The purpose of analysing specific gravity (SG) of tubers was to find the association of SG and bruising susceptibility, as SG correlates directly with dry matter.

Significant differences were found in the SG between tubers treated and untreated at the 3$^{th}$ harvest ($p<0.01$), where tubers treated with N presented higher SG than untreated, shown in figure 6.8. Among harvest, significant increase was found only in treated tubers between H1-H2, H2- H3 and significant decrease between H3 and H4 ($p<0.05$) for both samples (treated/untreated).The decrease in dry matter may be due increase in turgor at the last harvest due high rainfall by end of August.

When contrasting the incidence of severe bruising and the SG of tubers, very strong positive correlations were found for control (R= 0.76) and for treated tubers (R=0.70). Contrasting the SG of tubers with BI, strong positive

correlation was found for control (R=0.49) and no correlation for treated tubers (R=0.16).

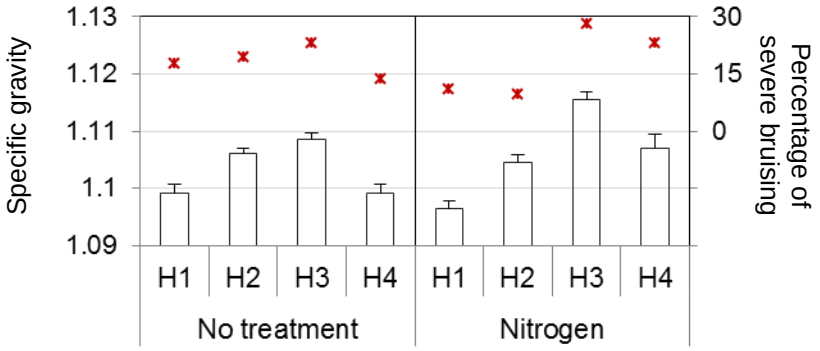


Figure 6.8 Effect of harvest and N treatment (200 kg/ha) on the specific gravity (bars) and percentage of severe bruising (scatter) in tubers from the variety LR harvested in July (H1), August (H2 and H3) and September (H4), field trial 3. Value bars show means (n=30), error bars are SE and scatter shows incidence of severe bruising (n>21).

## 6.2.4 Mechanical properties

### 6.2.4.1 Energy required to break the potato skin tissue

In general, the energy required to break the skin decreased with harvest in treated and untreated tubers as shown in figure 6.9. Significant differences ($p<0.05$) were found among the samples studied at H2 and H3.

Untreated samples presented significant decrease ($p<0.05$) from H1 to H2 and H2 to H3 but not from H3 to H4 ($p>0.05$). Treated samples presented significant decrease ($p<0.01$) comparing H2 to H3 only.

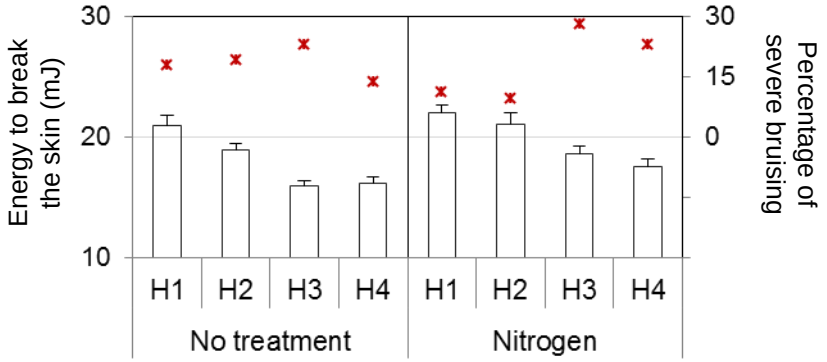


Figure 6.9 Effect of harvest and N treatment (200 kg/ha) on the energy (mJ) required to break the skin tissue (bars) and percentage of severe bruising (scatter) in tubers from the variety LR harvested in July (H1), August (H2 and H3) and September (H4), field trial 3. Value bars show means (n=30), error bars are SE and scatter shows incidence of severe bruising (n>21).

When contrasting the incidence of severe bruising and the energy to break skin tissue, no correlation was found for control (R= -0.01) and very strong negative correlations was found for treated tubers (R=-0.77). Contrasting the energy to break the skin tissue with BI, negligible (R=0.12) and weak negative correlation ( R=-0.21) were found for control and treated tubers respectively.

It was observed that on average skin tissue strength (measured by force) decreased till the 3$^{th}$ harvest (except untreated H2) and increased at the 4$^{th}$ harvest but deformability (distance) to rupture skin slightly decreased along time for both samples as shown in figure 6.10 and 6.11.

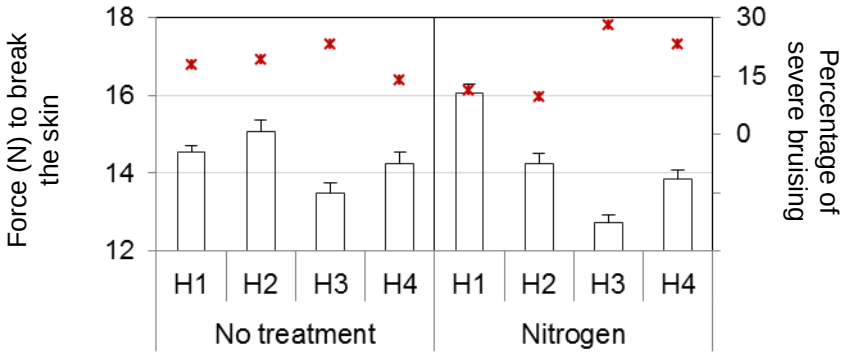


Figure 6.10 Effect of harvest and N treatment (200 kg/ha) on the force (N) required to break the skin tissue (bars) and percentage of severe bruising (scatter) in tubers from the variety LR harvested in July (H1), August (H2 and H3) and September (H4), field trial 3. Value bars show means (n=30), error bars are SE and scatter shows incidence of severe bruising (n>21).

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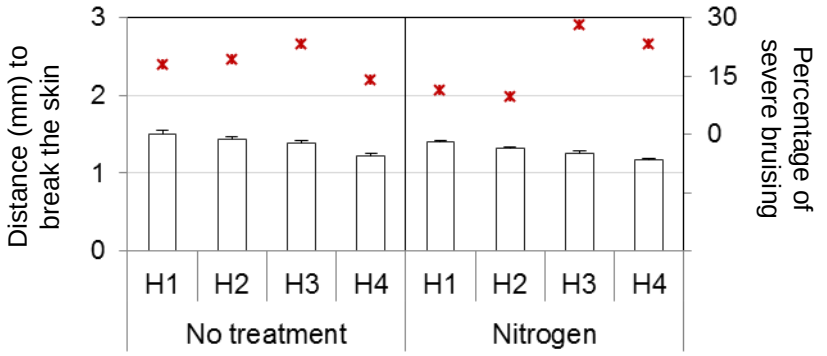


Figure 6.11 Effect of harvest and N treatment (200 kg/ha) on the distance (mm) required to break the skin tissue (bars) and percentage of severe bruising (scatter) in tubers from the variety LR harvested in July (H1), August (H2 and H3) and September (H4), field trial 3. Value bars show means (n=30), error bars are SE and scatter shows incidence of severe bruising (n>21).

### 6.2.4.2 Energy required to break the potato cortex tissue

Results of the energy required to break the cortex tissue were no significantly different between the tubers treated and untreated and among harvests, shown in figure 6.12.

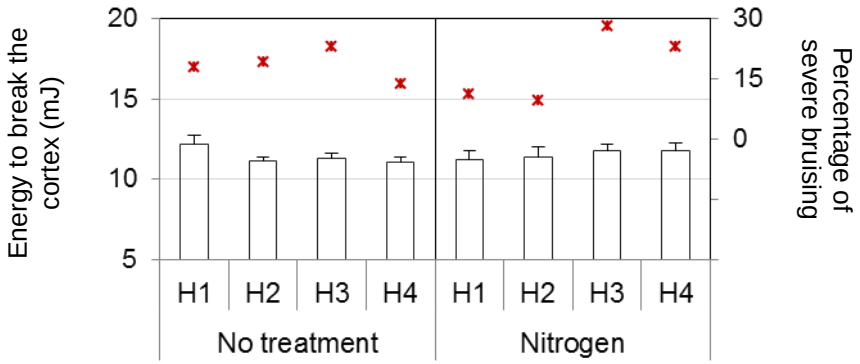


Figure 6.12 Effect of harvest and N treatment (200 kg/ha) on the energy (mJ) required to break the cortex tissue (bars) and percentage of severe bruising (scatter) in tubers from the variety LR harvested in July (H1), August (H2 and H3) and September (H4), field trial 3. Value bars show means (n=30), error bars are SE and scatter shows incidence of severe bruising (n>21).

An increase in force to break the cortex tissue at the 4$^{th}$ harvest for both treatments was observed (figure 6.13). The increase in force was associated with slight lower deformability of the cortex, observed in figure 6.14 by the distance reached with the probe at rupture point.

Results from energy to break the cortex tissue and the incidence of severe bruising showed no correlation for control (R=0.01) and very strong positive correlation for N treated tubers (R = 0.88). When correlating the energy to break the cortex tissue with BI, weak and moderate positive correlations were found for control (R= 0.12) and treated tubers (R= 0.35).

According to the results from previous trials, it was expected that force to break the cortex tissue diminish along harvests and distance diminish when tubers were harvested until the end of September. The variation found in force at the 4$^{th}$ harvest may be linked with more turgor as the tubers were harvested after a wetter month (August 2013) than other trials.

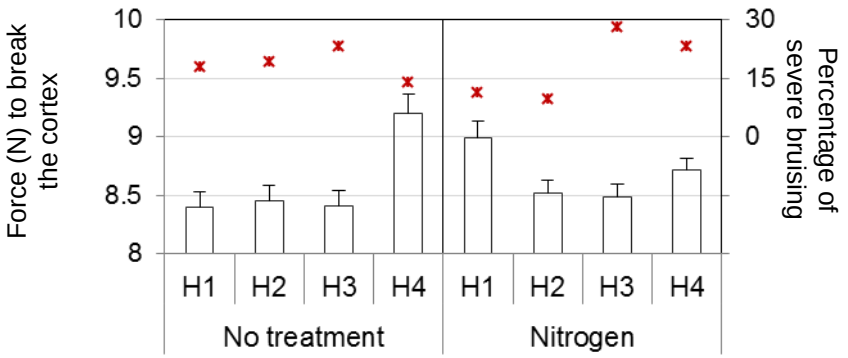


Figure 6.13 Effect of harvest and N treatment (200 kg/ha) on the force (N) required to break the cortex tissue (bars) and percentage of severe bruising (scatter) in tubers from the variety LR harvested in July (H1), August (H2 and H3) and September (H4), field trial 3. Value bars show means (n=30), error bars are SE and scatter shows incidence of severe bruising (n>21).

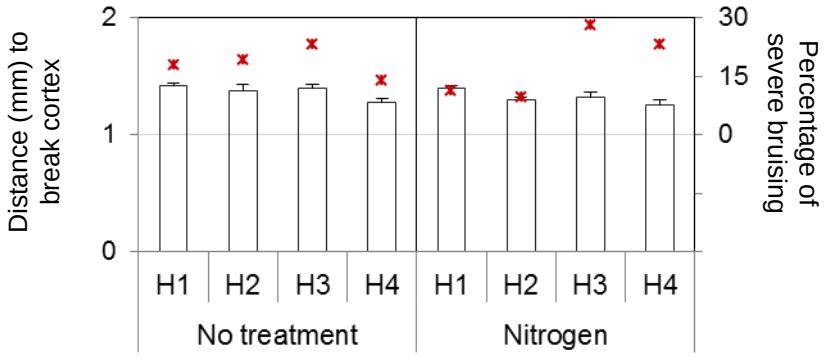


Figure 6.14 Effect of harvest and N treatment (200 kg/ha) on the distance (mm) required to break the cortex tissue (bars) and percentage of severe bruising (scatter) in tubers from the variety LR harvested in July (H1), August (H2 and H3) and September (H4), field trial 3. Value bars show means (n=30), error bars are SE and scatter shows incidence of severe bruising (n>21).

## 6.2.5 Phenolic composition

### 6.2.5.1 Phenolic acids

Chlorogenic acid (5-CQA), the most abundant compound found, increased in content up to the 3rd harvest time and diminished from the 3rd to the 4th harvest for both samples. The levels and changes along harvest were similar

with results from the field trial 1. This reinforces the evidences that phenolic acids may act as protective in bruising when levels are low. Significant increases in phenolic content ($p<0.05$) were found from H2 to H3 and decrease from H3 to H4 in tubers untreated, but no significantly difference were found in the treated samples ($p>0.05$) in 5-CQA content, shown in figure 6.15. Tubers treated with N showed slight higher content of 5-CQA but significantly higher amounts ($p<0.001$) were only found at the 4$^{th}$ harvest.

The chlorogenic acid isomer cryptochlorogenic acid (4-CQA) and neochlorogenic acid (3-CQA) contents were significantly different ($p<0.05$) at the 2$^{nd}$ and the 3$^{rd}$ harvest, presenting slight higher contents in tubers from untreated plants.

Strong correlations were found when contrasting incidence of severe bruising and chlorogenic acids for control (R=0.78) and treated tubers (R=0.60). When correlating chlorogenic acids with BI, weak and moderate positive correlations were found for control (R= 0.12) and treated tubers (R=0.35) respectively.

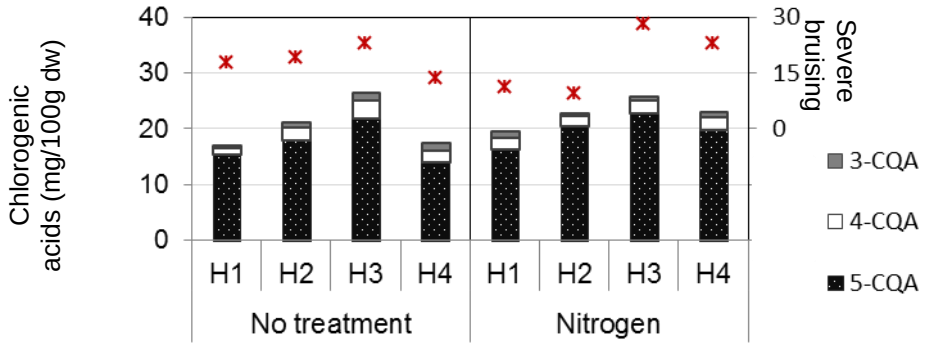


Figure 6.15 Effect of harvest and N treatment (200 kg/ha) on the chlorogenic acids (3-, 4- and 5- CQA) of lyophilized cortex (mg/100 g dw) (bars) and percentage of severe bruising (scatter) in tubers from the variety LR harvested in July (H1), August (H2 and H3) and September (H4), field trial 3. Value bars show means (n=3), error bars are SE and scatter shows incidence of severe bruising (n>21).

Among the minor phenolic acids detected, caffeic acid (CA) was detected in both samples and the content ranged from 0.43-0.70 mg/100 g dw (figure 6.16). There was not a clear trend along harvests when considering CA content in treated or untreated tubers.

A trend was found for the compounds vanillic acid (VA), ferulic acid (FA) and *p*-coumaric (pCou), with general increase up to the 3$^{rd}$ harvest followed by decrease at the 4$^{th}$ harvest.

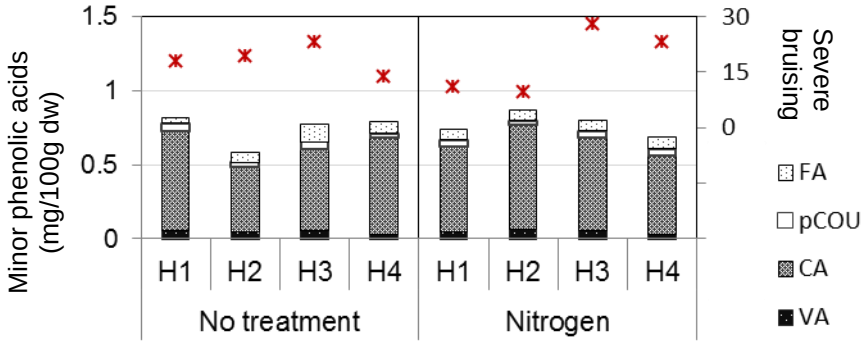


Figure 6.16 Effect of harvest and N treatment (200 kg/ha) on the minor phenolic acids (FA, pCOU, CA, VA) (mg/100 g dw) of lyophilized cortex (bars) and percentage of severe bruising (scatter) in tubers from the variety LR harvested in July (H1), August (H2 and H3) and September (H4), field trial 3. Value bars show means (n=3), error bars are SE and scatter shows incidence of severe bruising (n>21).

### 6.2.5.2 Tyrosine

Significant variations of free tyrosine content ($p<0.001$) among treated samples with N and untreated were found at the 2nd, 3rd and 4th harvest, being higher for untreated samples at the second and third harvests as shown on figure 6.17.

Significantly different contents ($p<0.001$) were observed among harvests for both samples, treated and untreated, with lower significance level ($p<0.05$) between H1 to H2 in treated samples.

Moderate and strong positive correlations were found when contrasting the incidence of severe bruising and free tyrosine levels for control (R=0.38) and treated tubers (R=0.65). When tyrosine levels and BI were correlated, weak and moderate positive correlations were found for control (R= 0.23) for treated tubers (R=0.31) respectively.

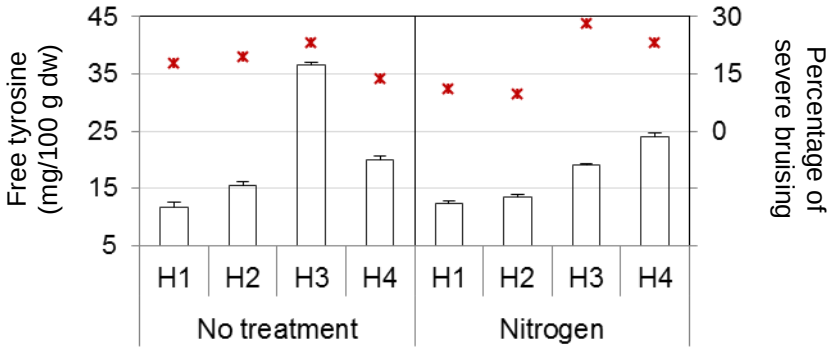


Figure 6.17 Effect of harvest and N treatment (200 kg/ha) on the free tyrosine content (mg/100 g dw) of lyophilized cortex (bars) and percentage of severe bruising (scatter) in tubers from the variety LR harvested in July (H1), August (H2 and H3) and September (H4), field trial 3. Value bars show means (n=3), error bars are SE and scatter shows incidence of severe bruising (n>21).

## 6.2.6 Cell wall composition

### 6.2.6.1 Analysis of Cell Wall Material (CWM)

The monosaccharide content of potatoes from the 3$^{rd}$ and the 4$^{th}$ harvest are summarised in table 6.2. Concentrations are expressed in %mol. The yield of extractions comprised between 0.17 and 0.46% of the dry mater.

Fucose and mannose were not detected in the chromatograms from the potato cell wall hydrolysates. Results for glucose were omitted as it was not from a pectic source.

Among the monosaccharides studied, the higher proportion comprised of arabinose and galacturonic acid, indicating that pectin of LR from field trial 3 had less galactose (12.7-16.5% mol) than field trial 1 and 2, averaging from 28.5-30.5% mol and 27.1-32.1% mol respectively.

Comparing untreated and treated results, untreated samples presented significantly lower content ($p<0.001$) of rhamnose and galactose and significant higher content ($p<0.001$) of xylose than treated samples at the 3$^{rd}$ harvest. At the 4$^{th}$ harvest, no significant differences ($p>0.05$) were found on the content of sugars analysed when compared to control, although average higher content of rhamnose was found in treated samples with significant decrease ($p<0.05$) in samples harvested late (H4) (treated samples).

A trend was found in the content of uronic acids, where the concentration of galacturonic acid diminished comparing H3 to H4 for both samples (control and treated) and glucuronic acid concentrations increased for both samples, but it was only significant for GluA untreated samples and GalA treated samples ($p<0.01$).

Table 6.2 Monosaccharide composition of cell walls from tubers (rhamnose (Rha), arabinose (Ara), galactose (Gal), xylose (Xyl), galacturonic acid (GalA) and glucuronic acid (GluA) from CWM of the cortex tissue from LR tubers not treated (control) and treated with N (200 kg/ha) harvested in late August (H3) and early September (H4), field trial 3. Concentrations are expressed per %mol. Values represent average ± SEM (n=3).

| | HARVEST 3 | |
|---|---|---|
| **Monosaccharides** | **No treatment** | **200 kg/ha** |
| **Rha** | 0.6 ± 0.2 | 4.8 ± 0.5 |
| **Ara** | 30.5 ± 0.3 | 28.5 ± 0.8 |
| **Gal** | 12.7 ± 0.5 | 16.5 ± 0.1 |
| **Xyl** | 18.9 ± 1.6 | 10.9 ± 0.6 |
| **GalA** | 31.7 ± 1.0 | 32.1 ± 1.5 |
| **GluA** | 5.5 ± 1.1 | 7.2 ± 0.3 |
| | **HARVEST4** | |
| **Monosaccharides** | **No treatment** | **200 kg/ha** |
| **Rha** | 0.9 ± 0.2 | 1.8 ± 0.4 |
| **Ara** | 30.3 ± 1.3 | 28.7 ± 0.3 |
| **Gal** | 13.8 ± 0.2 | 14.3 ± 0.2 |
| **Xyl** | 15.9 ± 0.7 | 17.9 ± 0.3 |
| **GalA** | 28.7 ± 1.0 | 27.1 ± 0.2 |
| **GluA** | 10.5 ± 0.9 | 10.3 ± 1.4 |

The molar ratio of arabinose + galactose to uronic acids was calculated for the cultivars and presented in table 6.3. A similar molar ratio of arabinose + galactose to uronic acids was observed in untreated and treated samples (1.1-1.2), which indicated that there are no differences in the number or length of neutral sugar side chains.

The molar ratio of uronic acids to rhamnose (the measure of linearity of the cell wall pectin) was lower in tubers supplied with N, being less linear than untreated tubers. Comparing results from H3 and H4, untreated tubers showed decrease in pectin linearity and treated tubers increased linearity.

These differences are possibly due the delay in maturity of tubes supplied with N.

Table 6.3 Neutral sugars (rhamose+arabinose+galactose), branching (molar ratio of arabinose + galactose to uronic acids) and number of side chains (UA/Rhamnose) in CWM of the cultivar LR untreated and treated with N (200 kg/ha), harvested in late August (H3) and September (H4), field trial,3 after sequential with 0.1 M and 2 M TFA.

| | HARVEST 3 | |
|---|---|---|
| **Ratios** | **No treatment** | **200 kg/ha** |
| **Neutral sugars (Rha+Ara+Gal)** | 43.8 | 49.8 |
| **Molar ratio (Ara+Gal/UA)** | 1.2 | 1.1 |
| **UA/Rhamnose** | 68.8 | 9.3 |
| | **HARVEST 4** | |
| | **No treatment** | **200 kg/ha** |
| **Neutral sugars (Rha+Ara+Gal)** | 45.0 | 44.7 |
| **Molar ratio (Ara+Gal/UA)** | 1.1 | 1.2 |
| **UA/Rhamnose** | 50.9 | 23.7 |

### 6.2.7 Relationship between analyses

Correlations from the analyses and incidence of severe bruising, bruising index and oxidative potential are summarized in table 6.4, indicated as R values.

Table 6.4 The relationships (R) and significance P value of severe bruising (SB), bruising index (BI) and oxidative potential (OP) with physical, mechanical and compositional aspects of the varieties LR treated with N 200 kg/ha (N200), not treated and the combined results from treated and not treated, field trial 3.

| Treatment and correlation sample size | Severe bruising (SB) | | | | | |
|---|---|---|---|---|---|---|
| | No treatment (n=4) | | N200 (n=4) | | No treatment + N200 (n=8) | |
| **Assessment , sample size/ R and P value** | **R** | **P value** | **R** | **P value** | **R** | **P value** |
| BI (n=3) | **0.92** | 0.08 | **0.64** | 0.36 | 0.42 | 0.30 |
| OP (n=3) | -0.15 | 0.85 | **0.43** | 0.57 | 0.00 | 1.00 |
| Energy to break the skin tissue (n=30) | -0.01 | 0.99 | **-0.77** | 0.23 | -0.29 | 0.49 |
| Force to break the skin tissue (n=30) | -0.26 | 0.74 | 0.13 | 0.87 | -0.25 | 0.55 |
| Distance to break the skin tissue (n=30) | 0.10 | 0.90 | -0.45 | 0.55 | -0.14 | 0.74 |
| Energy to break the cortex tissue (n=30) | 0.01 | 0.99 | **0.88** | 0.12 | 0.19 | 0.65 |
| Force to break the cortex tissue (n=30) | -0.26 | 0.86 | 0.13 | 0.87 | 0.03 | 0.94 |
| Distance to break the cortex tissue (n=30) | 0.14 | 0.86 | -0.11 | 0.89 | -0.02 | 0.96 |
| Weight (n=30) | 0.01 | 0.99 | 0.84 | 0.16 | 0.35 | 0.39 |
| SG (n=30) | **0.76** | 0.24 | **0.70** | 0.30 | 0.64 | 0.09 |
| Tyrosine | **0.38** | 0.62 | **0.65** | 0.35 | 0.29 | 0.48 |
| Phenolic acids (n=3) | **0.78** | 0.22 | **0.57** | 0.43 | 0.35 | 0.39 |
| Chlorogenic acids (n=3) | **0.78** | 0.22 | **0.60** | 0.40 | 0.36 | 0.38 |
| 5-CQA (n=3) | **0.91** | 0.09 | **0.44** | 0.56 | 0.34 | 0.41 |
| CA (n=3) | -0.26 | 0.74 | -0.16 | 0.84 | -0.14 | 0.74 |
| | **Bruising Index (BI)** | | | | | |
| OP (n=3) | -0.05 | 0.95 | 0.14 | 0.86 | 0.10 | 0.81 |
| Energy to break the skin tissue (n=30) | 0.12 | 0.88 | -0.21 | 0.79 | -0.08 | 0.85 |
| Force to break the skin tissue (n=30) | -0.06 | 0.94 | 0.05 | 0.95 | -0.09 | 0.83 |
| Distance to break the skin tissue (n=30) | 0.26 | 0.74 | -0.13 | 0.87 | 0.01 | 0.98 |
| Energy to break the cortex tissue (n=30) | 0.12 | 0.88 | **0.35** | 0.65 | 0.08 | 0.85 |
| Force to break the cortex tissue (n=30) | -0.06 | 0.94 | 0.05 | 0.95 | 0.00 | 1.00 |
| Distance to break the cortex tissue (n=30) | **0.38** | 0.62 | 0.00 | 1.00 | 0.01 | 0.98 |
| Weight (n=30) | -0.09 | 0.91 | 0.26 | 0.74 | -0.03 | 0.94 |
| SG (n=30) | **0.49** | 0.51 | 0.16 | 0.84 | 0.10 | 0.81 |
| Tyrosine (n=3) | 0.23 | 0.77 | 0.31 | 0.69 | 0.29 | 0.49 |
| Phenolic acids (n=3) | 0.12 | 0.88 | 0.35 | 0.65 | 0.12 | 0.12 |
| Chlorogenic acids (n=3) | **0.53** | 0.47 | 0.08 | 0.92 | 0.13 | 0.78 |
| 5-CQA (n=3) | **0.71** | 0.29 | 0.02 | 0.98 | 0.09 | 0.83 |
| CA (n=3) | -0.10 | 0.90 | **-0.49** | 0.51 | -0.21 | 0.62 |
| | **Oxidative Potential (OP)** | | | | | |
| Weight (n=30) | **-0.73** | 0.27 | **0.62** | 0.38 | 0.07 | 0.87 |
| SG (n=30) | -0.29 | 0.71 | 0.22 | 0.78 | 0.00 | 1.00 |
| Tyrosine (n=3) | **0.93** | 0.07 | **-0.95** | **0.05** | -0.42 | 0.30 |
| Phenolic acids (n=3) | -0.54 | 0.46 | 0.19 | 0.81 | -0.05 | 0.91 |
| Chlorogenic acids (n=3) | -0.53 | 0.47 | 0.21 | 0.79 | 0.00 | 1.00 |
| 5-CQA (n=3) | -0.36 | 0.64 | 0.14 | 0.86 | 0.00 | 1.00 |
| CA (n=3) | -0.26 | 0.74 | -0.16 | 0.84 | 0.00 | 1.00 |

Although Person's coefficient (R) indicates some correlations, most of them were not statistically significant ($p>0.05$), what reflect a very small correlation sample size (n=4). Analysis of both treated and no treated tubers together not increased the strength of the correlations and the significance, although had larger correlation sample size (n=8). So, the purpose of the analyze of

correlations was to establish general trends between the physical/composition aspects and the incidence of bruising.

Severe bruising incidence and bruising index (BI) were strongly correlated for treated tubers (R=0.64) and very strong for control tubers (R= 0.92).

Some correlations were found between mechanical properties and bruising incidence, where very strong negative correlation was observed between the energy to break the skin tissue and severe bruising (R=-0.77), and very strong positive between energy to break cortex tissue and severe bruising in treated samples. Control samples had no correlations between the same factors.

Very strong correlation for both samples were found between SG and SB (R>0.70), but it decreased in strength when comparing SG with BI, being strong positive for untreated tubers (R=0.49) and negligible (R=0.16) for treated tubers.

Moderate positive and strong positive correlation was found between tyrosine content and SB (for untreated R=0.38 and treated R=0.65 samples respectively). Very strong correlation was found between tyrosine content and OP, being positive for untreated (R=0.93) and negative for treated samples (R=-0.95).

Chlorogenic acid had strong and very strong positive correlation with severe bruising (for treated R=0.60 and untreated R=0.93 samples respectively). Strong positive association was found with chlorogenic acids and OP (R= 0.53) for untreated samples but not for treated samples.

A further analysis of the relationship between all variables was conduced by principal component analysis (PCA). The results for LR outgrown in soil treated or untreated with N (field trial 3) are summarized in the PCA bi-plot (figure 6.18). The labels are indicated in table 6.5. These analyses generated substantial number of correlations and the model of PCA explained about 63% of the data variance. No discrimination of varieties was found in the principal components. High loadings on PC1 suggest that variables can be represented by PC1. The relative contribution (loadings) of individual variables in the PC1 dimension highlighted the components 3- and 4-CQA, tyrosine (TYR), ferulic acid (FA), severe bruising (SB) and bruising index (BI) and not much contribution from mechanical properties of skin (Energy to break the skin (ES) and the cortex (EC), force to break the skin (FS) and the cortex (FC) and distance to break the skin (DS) and the cortex (DC)).

The oxidative potential (OP) was negatively correlated with tyrosine content, which may suggests that in LR no dependence of substrate content tyrosine for bruising formation.

Severe bruising, 5-CQA and *p*-Coumaric (*p*Cou) dimensions suggests strong correlation on this study and the phenolic acid caffeic acid was strongly negative correlated to severe bruising incidence, providing evidence of a useful link between bruising and these phenolic acids in LR.

The force required to break the skin (FS) and cortex (FC) correlated with measurements of bruising (severe bruising, BI ) negatively, meaning that more force to break the skin tissue resulted in less bruising. On the other

hand, the distance to break the tissue (DS and DC) was positively correlated with bruising measures (SB and BI) on PC1, where more deformable tuber presented more bruising.

Table 6.5 Labels in the PCA graphs from LR treated with N 200 kg/ha and no treated harvested in July (H1), August (H2 and H3) and September (H4), field trial 3.

| | | | | No treatment | | N200 kg/ha | |
|---|---|---|---|---|---|---|---|
| Letter | Assessment | Letter | Assessment | No. | Harvest | No. | Harvest |
| A | Severe Bruising | J | Caffeic acid | 1 | H1 | 5 | H1 |
| B | Optical density | K | *p* Coumaric acid | 2 | H2 | 6 | H2 |
| C | Energy to break the skin tissue | L | Ferulic acid | 3 | H3 | 7 | H3 |
| D | Force to break the skin tissue | M | 3-CQA | 4 | H4 | 8 | H4 |
| E | Distance to break the skin tissue | N | 4-CQA | | | | |
| F | Energy to break the cortex tissue | O | 5-CQA | | | | |
| G | Force to break the cortex tissue | P | Tyrosine | | | | |
| H | Distance to break the cortex tissue | Q | Bruising index | | | | |
| I | Vanillic acid | | | | | | |

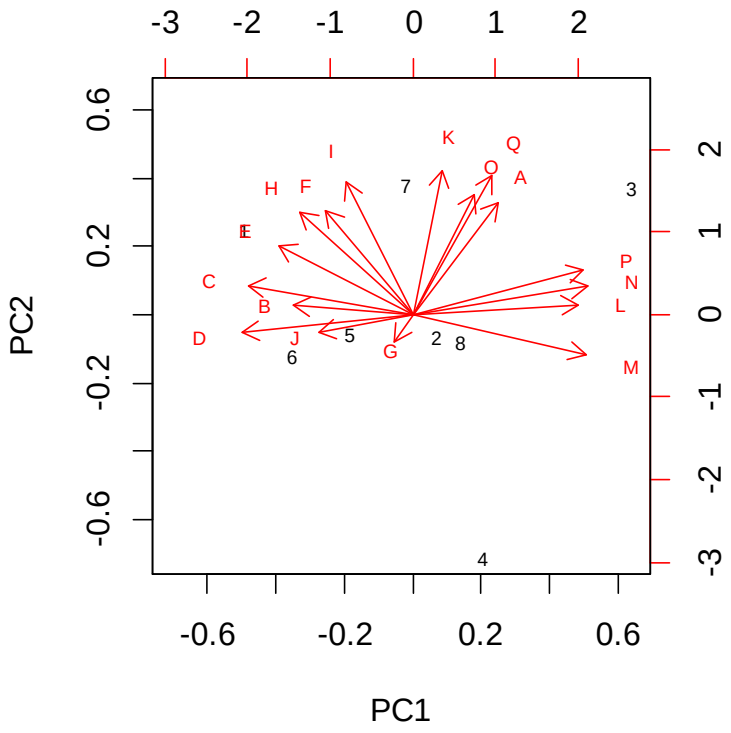


Figure 6.18 PCA bi-plot of data from LR treated with N 200 kg/ha and no treated harvested in July (H1), August (H2 and H3) and September (H4), field trial 3. PC1 explains 43% of variance and PC2 20%.

## 6.3 Discussion

Application of N to the soil led to increased levels of bruising, but only in later harvests. It was observed also that higher percentage of slight bruising and higher level of damaged skin in tubers with applied N to the soil compared to the control.

N application increased weight and specific gravity, due maintenance of plant canopy in tubers for longer period, having a continued tuber growth through season. Weight and SG do not affect bruising if potatoes are harvested early September. The increase in SG upon application of N to soil

was associated with higher bruising at later harvests. This association may be due to the high content of dry matter affecting cellular stability potential for physical damage to membranes when cells are deformed by impact as observed by Wulkow (2009) and Urbany *et al*. (2011). In fact, the incidence of severe bruising diminished at the H4 compared to H3 harvest which coincide with lower specific gravity for both samples (treated and untreated). According to Ross *et al.* (2011a) the dry matter increases over time and reaches a peak 4-6 weeks before defoliation. For control, defoliation (the measure of maturity) was practically reached at the H4, presenting 2% of green canopy coverage but plants from tubers treated were still with 19% coverage and yet presented decrease in specific gravity.

The higher incidence of bruising when harvested in later in the season was also associated with high deformability, most particularly skin, and a strong correlation between mechanical properties and bruising was found. N application affected linearity of pectin, being more branched (less linear) than control. Slightly higher force and distance to break the cortex tissue in treated tubers was observed at the harvest periods studied (H3 and H4).

Increases in chlorogenic acids were observed until later August following by decrease in early September. The trend observed and the levels of chlorogenic acids were similar to the first trial. Levels of chlorogenic acids were strongly associated with the incidence of severe bruising in this trial, what was interesting as the content of phenolic acids was relatively lower than 2012 season where negative correlation was found. Treatment affected levels of phenolics but not affect tyrosine levels considerably.

Tyrosine levels were variable along the harvest for both nitrogen treated and control tubers. Although treatment has not affected the levels of tyrosine compared to the control, strong correlation with bruising was found only in treated tubers.

In conclusion, N application increases weight and specific gravity, and does not affect bruising if potatoes are harvested early (September), however, higher incidence of bruising is observed in tubers from treated soils in later harvests.

# 7 General discussion

With increasing quality standards demanded by the markets and increased demand for food due to an increasing population, particularly in developing countries, efforts must be maintained to minimize tuber bruising. Great hopes are attached to the constant improvement of agricultural practices to avoid losses and genetic engineering in terms of the breeding of cultivars less susceptible to damage.

The main aim of this study was: 1) to investigate the effect of defoliation, harvest time, storage and application of nitrogen on bruising incidence in three UK varieties of potatoes; 2) to investigate the physical and biochemical factors that may be associated with bruising.

**7.1 Method development-** HPLC has been widely used to identify phenolics in food studies and parameters for the optimal quantification of individual components were tested.

Extraction of phenolics was efficient after 3 sequential extractions with 50% MeOH acidified with 2.5% metaphosphoric acid using vortex. The HPLC analysis of phenolic acids and tyrosine demonstrated good resolution, good sensitivity and peak sharpness for the components in both procedures.

For the extraction of monosaccharides from the potato cell wall, the goal of sequential hydrolysis was the liberation of different pectin components, preventing degradation under the hydrolysis condition. A possible sequence using stronger acid (e.g. sulphuric acid ($H_2SO_4$) or hydrochloric acid (HCl))

would be useful to analyse any remaining pectin, hemicelluloses and cellulose components. To the author's knowledge, this is the first time that potato cell wall monosaccharides have been hydrolysed with sequential chemical hydrolysis using TFA.

Analytical validation of the HPAEC - PAD method for analysis of monosaccharides demonstrated good precision (R.S.D. < 6.4%) and accuracy (R.E.± <-7%), which had excellent sensitivity in the low µg/mL ranges (47-93 µg/mL). Notably in the results greater amounts of arabinose than galactose were observed in potato pectin isolated from cortex tissue, contrary to other reports (van Marle *et al*., 1997, Obro *et al*., 2000, Orfila *et al*., 2012). This arabinose-rich fraction may have been destroyed during standard hydrolysis. The present study showed for the first time that potatoes contain a fraction of pectin (RG-I) that is very rich in arabinose. Arabinan rich polysaccharides have been attributed to many cell wall functions, including cell adhesion (Orfila *et al*., 2000) and cell wall porosity (Mohnen, 2008).

In the embedding procedure for immunolocation, a longer incubation time for infiltration was effective for potato cell wall embedding. Elimination of autofluorescence in the phenolics in the cell wall with toluidine blue is recommended. There are very few reports of the ultrastructure of potato tuber tissue (Bush and McCain, 1999; Sørensen *et al*., 2000; Oomen *et al*., 2002) and this may be due to the difficulties associated with obtaining good quality sections.

**Bruising assessment** - Due to many terminologies and ranges of subjective bruise scores being used to characterise bruising, it is difficult to compare results when researchers have used different terminologies to describe it. In the experiment by using a high speed camera to assess rebound height, it was observed that the bolt impacted the tuber from different angles and often more than once, showing that the transfer of energy was not homogeneous. Although the use of plates to calculate the energy absorbed (Jiménez-Jiménez *et al*., 2013) is a more accurate method to measure the impact energy, this method does not consider the impact surface. To improve this method, adaptation using a pendulum would be recommended for better control of the impact zone. This research followed previous studies from Stalham (2008) using a known mass dropped from an equivalent height using a simple tube in an attempt to compare results with previous studies. However, unexpectedly, great seasonal changes were observed during the three different trials, which culminated in the method being modified as shown for LR along the three field trials in table 7.1.

Table 7.1 Effect of season on percentage of severe bruising (%) following damage using a falling bolt method 1 (bolt damaged the side of potatoes at temperature <10 °C following incubation for 48 h at 33 °C) and method 2 (bolt damaged the stolon of potatoes at room temperature following incubation for 20 h at 25 °C) in LR tubers harvested around 150 days after planted in field trial 1 (2010), field trial 2 (2011) and field trial 3 (2013).

| Field trial | Year | Method | Days after planting | Percentage of severe bruising |
|---|---|---|---|---|
| 1 | 2010 | 1 | 150 | 50.00 |
| 2 | 2011 | 2 | 157 | 52.38 |
| 3 | 2013 | 2 | 143 | 13.79 |

Bruising was observed in LR in all seasons but in field trial 2 LR bruised worse as the tubers were not cooled down before bruising damage as in field trial 1, resulting in more bruising.

This study aimed to establish the relationship between susceptibility to bruising measured by oxidative potential and severe bruising following tuber damage. In general, oxidative potential was well correlated with tyrosine levels and the results were in accordance with the authors Sabba and Dean (1994); Kim and Dean (1998); Stevens and Davelaar (1997) and McNabnay *et al*.(1999). However, the oxidative potential did not always reflect the changes in severe bruising. Correlation was found only when analysing the varieties together with PCA in field trial 2.

**7.2 Varietal differences-** During field trials 1 and 2, three varieties were investigated simultaneously and it was apparent that the varieties responded differently to the test conditions of the field trial. RB presented the highest incidence of severe bruising in field trials 1 and 2 during early to late harvests. This suggests that RB probably reached its maturation stage earlier than the other two varieties. Interestingly, in very late harvests (e.g. H4 in field trial 1), RB appeared to bruise less, with LR being the most susceptible cultivar. This suggests that there is a peak time for bruising, which was reached by RB around September, and by LR around October. LR presented the highest incidence of bruising during storage, particularly for potatoes harvested later (October), and this was associated with increased deformability of tubers. MP presented a lower incidence of severe bruising compared to RB and LR in field trials 1 and 2. This suggests that

MP may mature at a later stage than the other two varieties. All varieties bruised significantly when stored for a long time.

It is interesting that RB required the lowest amount of energy to break the skin and cortex tissues, and also presented the highest susceptibility to bruising. The cellular arrangement of the cells of RB may be a contributing factor. However, these mechanical properties showed no statistical correlation to bruising incidence when varieties were analysed separately, indicating that other factors are also important for this variety. In this variety, tyrosine levels were the highest and the levels correlated with bruising, indicating a biochemical readiness to bruise. LR, however, required higher energy to break the tissue and showed low levels of tyrosine but presented major changes in deformability at late harvest and along storage, which may lead to ideal physical conditions to initiate bruising. Also, LR has a higher specific gravity which in flaccid/soft cells may influences impact susceptibility as reported by Wulkow (2009). MP presented intermediate mechanical properties and phenolic substrate levels. Overall, these observations suggest that at early stages of tuber maturation, mechanical properties may be important at protecting tubers, but this is overridden at later stages by high phenolic/tyrosine content in mature tubers which promote tuber bruising regardless of mechanical properties.

**7.3 Specific gravity**- Specific gravities were distinct between varieties. The variety MP was quite different to LR and RB, having lower SG than the other varieties and low severe bruising incidence. Within all varieties, the tuber-specific gravity increased with harvest and storage but the incidence of severe bruising was highly variable during harvest and during the first

months of storage. Therefore, there was no overall relationship between bruising and SG in any experiment in the project that be regarded as causal, even though there was a significant positive correlation between bruising and SG in the 3rd field trial upon application of nitrogen to soil, and that was associated with higher bruising at later harvests.

**7.4 Phenolics** – The phenolic compounds were measured in order to investigate if harvest, storage, defoliation and nitrogen had an effect on the composition of these compounds in the potato tubers and their relationship with bruising. The results showed a positive correlation between percentage of severe bruising in field trial 1 and 2 for RB, but only in field trial 1 for MP. LR showed different results, where the amounts of phenolic acids were higher in the hotter season (field trial 2) and it was negatively correlated with bruising. However, in field trial 3, a positive correlation was found. This findings indicates that there may not be a threshold in the amounts of phenolics required for bruising, as it was observed for tyrosine by Corsini *et al.* (1992).

There was no a marked accumulation of phenolic acids observed along storage compared to the amounts at the harvest time. It is known that several types of stress such as temperature, mechanical injuries and sprouting might affect the chemical composition of tubers (Lisinka and Leszcynski, 1989) and in particular, the phenolic compounds are accumulated upon wounding and biotic stress (Ramamurthy *et al*., 1992). One possible explanation of this finding is that the content of phenolic acids was high at harvest time due to a hotter season. The varieties did not show a clear tendency of phenolic metabolism during storage.

Another interesting finding was that defoliation induced either an increase or decrease in the amounts of phenolic acids and increased the tyrosine content which can be caused by the mechanical and chemical stress during this agricultural practice. However, in this case the damage is systemic since the damage is not caused directly to the tubers. One possible explanation is the activity of the phenylalanine ammonia-lyase (PAL) because it was postulated by Jones (1984) that this enzyme can be induced by wounding and it stimulated the synthesis of phenolic compounds as a systemic defence. Also, as a response to wounding the increase in chorismate mutase levels which stimulate the synthesis of tyrosine was observed by Kuroki and Conn (1988).

Although accumulation of tyrosine and alteration of the metabolism of phenolic acids were observed, the variations in the incidence of severe bruising did not always reflect the levels of tyrosine or phenolic acids. RB was the only variety where tyrosine levels were associated with bruising. The results were in accordance to Strehmel *et al*. (2010a) which stated that bruising susceptibility were not mediated by precursor accumulation or limitation. So, composition of these phenolics substrates at harvest could not be used as a predictor of bruising susceptibility for stored samples.

**7.5 Mechanical properties**- The harvest time resulted in an important drop in energy, force and distance required to break the skin and the cortex tissue of the varieties studied. This suggests an important role for skin and cortex in the bruising of the potato. Having very little starch, the mechanical properties of the skin and cortex are very much influenced by the turgor and cell wall properties. However, defoliation of plants which could lead to an

decrease in turgor of cells did not affect the energy to break the skin which was unexpected. The association between mechanical properties and bruising was most apparent in MP and LR during harvest and LR during storage. The variety RB was less deformable requiring less force and energy to break the skin and the cortex tissues. Tuber mechanical properties were mostly affected by storage whereby the tubers required more energy to break the tissue, became more deformable and more prone to bruising.

RB epidermal cells appear very neatly stacked between varieties. This stacking created lines of weakness between cells, and if significant pressure is applied cells can shear or grow apart (Wiltshire et al., 2005). LR and MP were less prone to microscopic cracking with the “brick wall type” cell arrangements that contain fewer lines of weakness. Therefore, the data from the trials suggest that there is a major mechanical contribution to tissue resilience since bruises became universally deeper in the variety RB. The general increase in deformability over storage may be related to starch utilisation by the tuber, the moisture loss observed with increases in SG along storage and it could also be attributed to the irrevocable loss in the integrity of cellular membranes along the storage period.

Texture measurements indicate that the mechanical properties of the skin could predict bruise susceptibility among varieties. The potential biochemical apparatus to bruise and resistance to breakage of the tissue could act synergistically as the further the needle goes though the tissue, the more breakage of the membranes of surrounding cells occurs, releasing biochemical components within the cells.

**7.6 Cell wall properties**- When pectin presented longer or more side chains, higher energy and deformability of the cortex tissue was observed within the varieties and harvests. Losses in the linearity of pectin of the LR along storage were observed, implying an decrease in the cell–cell adhesion, particularly in the pectin-rich middle lamella between cells due the decrease of calcium-bridges. However, no direct relationship between mechanical properties and pectin linearity or branching was found with harvest, storage and defoliation. There was a decrease in methylation of pectin along harvest time, which is expected during cell wall maturation. However, the changes were not significant enough to explain differences in bruising incidence between varieties.

Other analyses would be recommended for better understanding of the pectin properties such as acetylation of galacturonic acid which impairs calcium crosslinks between HG chains (Renard and Jarvis, 1999). The fractions of cell wall polymers such as hemicelluloses, which has a contribution to strengthening the cell wall by interaction with cellulose (Scheller and Ulvskov, 2010) and cellulose which has side chains of RG-I galactan and arabinan (Zykwinska *et al*. 2007) would also bring some more details of the structure. A micro-penetration technique described by Hiller *et al*. (1996) for mechanical testing of cell walls would be recommended to pick up subtle changes.

**7.7 Environmental influences-** The 2012 season (trial 2) was characterised by appreciably low rainfall (<0.8 mm) during tuber growth (before end of May) and the temperature of the air and soil was slightly higher than average years. The hot and dry conditions during tuber development were associated

with early plant senescence and bruising incidence was considerably worse in 2012. These conditions lead to an increase in bruising. Earlier harvest during hot seasons is recommended.

**7.8 Storage**- Incidence of severe bruising of the varieties studied was highly variable during the first months of storage for both harvests period. Short storage (until January) was not associated with increased bruising incidence in any of the varieties and does not appear to be detrimental to bruising. This observation is useful for the industry although as mentioned in the introduction, post-Christmas is a period of weaker demand.

Longer storage (until March) did significantly increase the bruising incidence for all varieties, particularly LR harvested late. Storage increased the deformability of the cortex in all varieties, most particularly LR which bruised the most during storage. Both phenolic acids and tyrosine levels increased during storage, although the highest levels were not found in LR, but in RB. This indicates that tyrosine is not always the predicting factor for bruising. The practical recommendation for storage of tubes is to harvest LR in September and MP and RB in October to grant lower incidence of bruising along storage.

**7.9 Defoliation-** A higher incidence of bruising was found in defoliated samples harvested 24 and 38 days after defoliation. This supports observations from earlier studies by Stalham (2008) which showed higher incidence of bruising when potatoes where harvested 3 to 5 weeks after defoliation. However, tubers from defoliated plants presented a lower incidence of bruising than undefoliated at H4 harvest (49 days after

defoliation). These results were surprising since one would expect an increase in bruising associated with the stress of defoliation, notably by the increase of endogenous level of free tyrosine in LR and RB tubers from defoliated plants.

It suggests that defoliation promotes skin setting which may be protective. However, no significant effects of defoliation on mechanical properties or tissue ultrastructure were observed in this study. The reduced bruising at later harvest times may be associated with a halting of tuber maturation due to forced defoliation. Defoliation did decrease tuber weight significantly.

**7.10 Nitrogen**- Nitrogen application to the soil delayed the maturity of the tubers and led to increased levels of bruising, but only in later harvests. The treatment affected mechanical properties of the tubers, most particularly skin, and tubers from treated soils showed a strong correlation between mechanical properties and bruising. Nitrogen application to the soil did not affect tyrosine levels considerably. Nitrogen application increases weight and specific gravity.

# 8 Conclusion and recommendations

The study demonstrated that the bruising susceptibility is due to multiple factors that are dependent on the variety.

RB presented the highest incidence of bruising at early harvests (September), and that was associated with low mechanical strength and deformability, and higher tyrosine content. RB benefited from early harvest and short storage. LR presented higher incidence of bruising when harvested in later season (October) and was significantly affected by storage. This was associated with higher deformability and levels of phenolics (but not necessarily tyrosine). However, MP appeared to show moderate bruising until later harvests (October). It appeared to have tissue with strong mechanical properties that protected tubers from impact and intermediate levels of phenolics and tyrosine.

An increase in bruising in stored samples is associated with higher specific gravity, higher tissue deformability and higher phenolic acid and tyrosine levels. However, the tyrosine levels or specific gravity were not always associated with a highest bruising incidence. It is noteworthy, that the mechanical properties of the tissue of potatoes are very important factors with regard to bruising.

As a practical recommendation, the best period to harvest to prevent bruising of fresh and stored for LR is in September and for MP in October. Harvest in September is recommended for fresh market of RB and in October for storage. Tubers from defoliated plants presented less bruising

when tubers were left in the ground for longer periods (>49 days), but it requires evaluation of the pros and cons as defoliation affects tuber yield significantly. Application of nitrogen for LR harvest in late July/ early August is effective in producing potatoes with high weight and lower bruising susceptibility.

In conclusion, while general trends were observed, the factors determining bruising seem to be dependent upon variety and the maturity of the tubers at harvest. Further research to identify factors associated with senescence and tuber maturation is recommended.

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