Aroma profiles and non-destructive determination of quality parameters of Japanese plums (*Prunus salicina* Lindl.).

by

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DECLARATION

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the authorship owner thereof and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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SUMMARY

Aroma profiles and non-destructive determination of quality parameters of Japanese plums (*Prunus salicina* Lindl.).

Plums with good taste, aroma and eating quality lead to repeat purchases and sustained demand. Taste includes non-volatile compounds, e.g. sugars and acids, and has been well researched to meet the consumers' preferences. Plum aroma, however, has not enjoyed the same attention. Limited literature is available on the aroma of Japanese plums and none could be found on the effects of relatively long cold storage on the profiles. The main aim of this study is to investigate the changes in aroma compounds of Japanese plums throughout maturation and ripening and the effects of commercial cold storage regimes. Near infra-red (NIR) spectroscopy was also evaluated as a non-destructive method to determine plum quality parameters aimed at minimising sample variability.

In Paper 1, NIR spectroscopy was used to develop prediction models for total soluble solid (TSS), total acidity (TA), sugar-to-acid ratio, firmness and weight in three cultivars (Pioneer, Laetitia and Angeleno) and a multi-cultivar model. Samples were collected for seven consecutive weeks and repeated over two seasons. TSS results showed excellent predictability (R^2 = 0.817-0.955; RMSEP= 0.453-0.610 % Brix) but the TA models did not perform well. The sugar-to-acid ratio models had results comparable to that of TSS. Both the firmness and weight models had acceptable results. The models of 'Pioneer' and 'Laetitia' had a better predictability capacity than the 'Angeleno' model. Although the multi-cultivar models outperformed the single cultivar models on R^2 values it had higher prediction errors. The robustness of all the TSS, TA and firmness models is high in terms of seasonality, range and cultivar.

Papers 2 and 3, the main focus of the study, are concerned with the aroma profile dynamics of Japanese plums. HS-SPME was used in both papers to extract the aroma compounds followed by GC-TOFMS for separation and identification. In Paper 2, the aroma volatile compounds of three cultivars (Pioneer, Laetitia and Angeleno) were determined for a seven week period including samples from three maturity stages (immature, harvest and tree-ripe). A total of 35 compounds were identified of which ten were generic. Each cultivar had five unique compounds resulting in different aroma profiles for each of the maturity stages and distinct separation patterns using discriminant analysis.

The study was extended in Paper 3 where the aroma volatile compounds of six cultivars (Pioneer, Sapphire, Laetitia, Songold, Larry Anne and Angeleno) and one plumcot (Flavor King) were determined at three functional stages (commercial harvest, tree-ripe fruit and cold stored fruit). A total of 62 compounds were identified and classified into three groups ('unique' (31), 'generic' (11) and 'frequent' (20)) based on

their frequency of occurrence. The aroma profiles of ‘Larry Anne’ and ‘Flavor King’ are the most affected by cold storage conditions and ‘Pioneer’ appears to be the least affected. All the cultivars have significantly different aroma profiles at all three of the functional stages with ‘Sapphire’, ‘Larry Anne’ and ‘Flavor King’ showing the largest differences. ‘Flavor King’, a plumcot, presented a ripe aroma profile that was much diverged from that of the true plums.

OPSOMMING

Aromaprofiele en nie-destruktiewe bepaling van kwaliteitsparameters van Japanese pruime (*Prunus salicina* Lindl.).

Pruime met ‘n goeie smaak, aroma en eetkwaliteit lei tot herhaalde verkope en volhoubare aanvraag. Smaak sluit die nie-vlugtige stowwe (suikers en sure) in en is goed nagevors om die verbruikersvoorkeure te bevredig. Pruim aroma het egter nie dieselfde aandag geniet nie. Daar is beperkte literatuur beskikbaar wat handel oor die aroma van Japanese pruime en geen kon gevind word oor die effekte van lang koelopberging op die aromaprofiele nie. Die hoof doel van hierdie studie is om die veranderinge in die aromatiese komponente van Japanese pruime te ondersoek tydens die volwassewording- en rypwordingsprosesse asook die effekte van kommersiële koelopberging. Naby infrarooi (NIR) spektroskopie is ook geevalueer as ‘n nie-destruktiewe manier om pruim kwaliteitsparameters te bepaal met die doel om monstervariasie te beperk.

In Artikel 1 is NIR spektroskopie gebruik om voorspellingsmodelle vir totale oplosbare suikers (TOS), totale suur (TS), suiker-tot-suur verhouding, fermheid en gewig te bepaal in drie kultivars (Pioneer, Laetitia en Angeleno) asook ‘n multi-kultivar model. Monsters is vir sewe opeenvolgende weke versamel en herhaal oor twee seisoene. TOS resultate toon uitstekende voorspelbaarheid (R^2 = 0.817-0.955; RMSEP= 0.453-0.610 % Brix) maar TS modelle het egter nie so goed gevaar nie. Die suiker-tot-suur verhoudingsmodelle se resultate was vergelykbaar met die van TOS. Beide die fermheid- en gewigsmodelle het aanvaarbare resultate opgelewer. Die modelle vir ‘Pioneer’ en ‘Laetitia’ het ‘n beter voorspelbaarheidskapasiteit getoon as die van ‘Angeleno’. Alhoewel die multi-kultivar model beter presteer het as die enkel kultivar modelle op die R^2-waardes was daar meer voorspellingsfoute. Hoë robuustheid is gevind i.t.v. seisoene, datagrense en kultivar vir al die TOS, TA en fermheidsmodelle.

Artikels 2 en 3, die fokuspunt van die studie, handel oor die dinamika van die aromaprofiel van Japanese pruime. HS-SPME is in beide artikels gebruik on die aromatiese verbindings te ekstraeer gevolg deur GC-TOFMS vir skeiding en identifikasie. In Artikel 2 is die aromatiese stowwe van drie kultivars (Pioneer, Laetitia en Angeleno) bepaal vir sewe opeenvolgende weke en sluit monsters van drie volwassenheidsstadiums in (onvolwasse, oes en boom-rypgemaakte pruime). ‘n Totaal van 35 verbindings is geidentifiseer waarvan tien as generies beskou kan word. Elke kultivar het vyf unieke komponente gehad en het gelei tot verskillende aromaprofiele vir elk van die volwassenheidsstadiums en diverse skeidingspatrone tydens die gebruik van diskriminant analise.

Die studie is uitgebrei in Artikel 3 waartydens die aromatiese vlugtige stowwe van ses kultivars (Pioneer, Sapphire, Laetitia, Songold, Larry Anne en Angeleno) en een plumcot (Flavor King) bepaal is tydens drie funksionele stadiums (oes, boom-rypgemaak en koelopgebergde pruime). ‘n Totaal van 62 verbindings is geidentifiseer en in drie groepe geklassifiseer (‘uniek’ (31), ‘generies’(11) en ‘gereeld’ (20)) gebaseer op voorkomsfrekwensie. Die aromaprofiele van ‘Larry Anne’ en ‘Flavor King’ is die meeste deur die koelopberging geaffekteer en ‘Pioneer’ die minste. Al die kultivars het kenmerkend verskil t.o.v. hul aromaprofiele in al drie die funksionele groepe en ‘Sapphire’, ‘Larry Anne’ en ‘Flavor King’ het die grootste verskille getoon. ‘Flavor King’, die plumcot, het ook ‘n ryp aromaprofiel gehad wat baie van die van die egte pruime verskil het.

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TABLE OF CONTENTS

This dissertation presents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters, therefore, has been unavoidable.

GENERAL INTRODUCTION

Plum quality is traditionally determined by a combination of external (size, colour, visible physiological defects) and internal characteristics (flesh firmness, sugar and acid content) that are well researched to meet the consumers' preferences (Crisosto and Bowerman, 2003; Crisosto and Crisosto, 2005). Aroma volatiles released during the eating process give a distinctive flavour to a specific fruit type and studies indicate that plum consumer inclinations and repeat purchases are also based on flavour (Leumann et al., 2004; Schotsmans and Prange, 2006). Understanding the aroma profile dynamics of plums during maturation, ripening and post harvest manipulations can assist in enabling a grower to produce fruit with superior quality and flavour. Plum export countries that are distant from the market are also interested in the post harvest storability of plums and as a result of consumer complaints concerning a lower intensity of flavour in exported plums compared to local market plums, a growing interest is observed in studying the behaviour of flavour compounds during cold storage. South Africa is such a country with annual export figures (2009) of close to 9 million cartons (5.25 kg equivalent cartons) comprising 35 different plum cultivars and an annual supply window of six months (October to April) shipping mostly to markets in the European Union and the United Kingdom (PPECB Information portal: http://info.ppecb.com). Fruit are in transit for up to 42 days and to ensure quality the plums are harvested relatively unripe (physiologically mature but unripe) and stored at low temperatures. Knowledge of the behavior of plum aroma volatiles under such conditions can serve as a basis for improving storage protocols to deliver fruit with good flavour.

Post harvest studies are plagued by sample variation as numerous pre harvest conditions (climatic conditions e.g. winter chilling, soil type, bearing position of fruit on the tree, age of the tree, irrigation and fertilization schedules, etc.) can potentially influence the development of the individual fruit on the tree. Thus, a pool of fruit taken from one tree or orchard has huge variation that may mask or enhance the parameters of interest and complicate the interpretation of the data. To avoid results based on possible correlations when investigating the flavour of plums one ideally needs to minimise this variation and analyse fruit with similar non-volatile composition (predominantly sugar and acid content) in order to study the dynamics of only one variable viz. the aroma volatiles. The traditional methods to determine non-volatile components of plums involve milling or juicing of the fruit and ultimately result in the destruction of the fruit sample prior to performing any volatile analysis making it cumbersome to determine the non-volatile and volatile components within the same sample. The first part of this study (Paper 1) focuses on using and assessing near infrared (NIR) spectroscopy as a non-destructive alternative in determining sugar and acid levels in plums in order to minimise variability when selecting samples for the aroma volatile study. Thus, a 'method development step' to assist in minimising sample variability in terms of non-volatiles. After the pilot analysis (data not shown) of the NIR spectra of fruit ranging from immature to over-ripe the sugar level prediction models looked very promising. In Paper 1 we continued with a more

comprehensive study wherein we evaluate three cultivars over a period of seven consecutive weeks including five different quality parameters (sugar levels, acid levels, sugar-to-acid ratio, flesh firmness and fresh weight) over two seasons. Data are also pooled to compare the cultivar specific models to those of a multi-cultivar model and the robustness of all the models are tested in terms of cultivar, seasonality and range. On a production scale there are also obvious benefits to finding an accurate and robust non-destructive way to predict quality parameters such as flesh firmness and sugar levels as it can serve as a way to determine optimum harvest dates (Guerra and Casquero, 2008; Crisosto, 1994, Valero et al., 2007) or assist as an automated grading system in a packing line to sort plums of different quality (Kawano, 1994). Thus, investigating the use of NIR spectroscopy to predict quality parameters has potential value to both the aroma profile researcher and the large scale plum producer.

The main part of this dissertation (Papers 2 and 3) is concerned with the aroma volatile components found in Japanese plums cultivated under South African conditions intended for the export market. As very little is known about the dynamics of volatiles in Japanese plums during maturation and ripening the first investigation (Paper 2) aims to study the changes in the aroma profiles of three Japanese plum cultivars (Pioneer, Laetitia and Angeleno) over a period of seven consecutive weeks capturing a contrast of immature fruit, ripe fruit, fruit from a commercial harvest and fruit left to ripen on the tree. With our choice in cultivars we strive to include cultivars of economical value to the South African market as well as cultivars ripening throughout the plum season. Pioneer is the first cultivar to be harvested, Laetitia a high volume cultivar harvested in mid-season and Angeleno being a late-season cultivar in the South African plum season. Thus, the intention of Paper 2 is to trace the pre harvest aroma profiles of the cultivars throughout fruit maturation and ripening but before the onset of decay.

Paper 3 is an extension of Paper 2 wherein we direct our investigation at the post harvest behaviour of plum aroma volatiles during cold storage and comparing it to that of harvested fruit (mature but unripe) and fruit left to ripen on the tree. We attempt to investigate the export practices by exposing commercially harvested fruit to cold storage protocols similar to those currently enforced by the Perishable Products Export Control Board (PPECB) of South Africa. We extended the range of cultivars compared to Paper 2 by including three more plum (Japanese) and one plumcot cultivars. The three additional plum cultivars were again chosen based on their economical value to the export trade. The plumcot (‘Flavor King’) was included to provide further contrast in terms of flavour as it is well known for its strong plum aroma. Thus, Paper 3 strives to identify possible similarities and differences in the behavior of the aroma volatiles in plums that are harvested when mature but unripe, exposed to long term cold storage and then ripened, compared to plums that were left to ripen on the tree.

When extracting and analysing aroma volatiles there are numerous methods that can be used (Crouzet et al., 1990). As each method favours the extraction of certain compounds it is almost impossible to

compare results obtained from different methods. We used Headspace Solid-Phase Microextraction (HS-SPME) (Zhang and Pawliszyn, 1993) with identical conditions in all our extractions coupled with gas chromatography to separate the compounds and time-of-flight-mass-spectroscopy (TOF-MS) for identification. This extraction method is rapid and accurate in assessing the aroma volatiles of many food and fruit types (Kataoka et al., 2000). Sample preparation and the experimental conditions used during the HS-SPME were selected after a series of method development steps to optimise extraction time, temperature and minimise enzymatic oxidation of phenolics (flesh browning) and the effects of deep-freezing and thawing on the aroma profile of the samples (Appendix 1). Method development steps also included the use of a full factorial experimental design that identified extraction temperature, time and sample dilution as critical variables and the further use of a central composite design to optimise the levels of these variables (data not shown). The effects of flesh browning and deep-freezing were described in the literature (Ismail et al., 1981a or b; Etiévant et al., 1986) and we conducted experiments to identify the extent thereof in our laboratory using PCA to illustrate possible differences in treated and untreated samples (Appendix 1), this was used to shape our sample preparation step.

Although this dissertation covers two seemingly different topics, viz. NIR spectroscopy to determine quality parameters (Paper 1) and aroma dynamics in plum development, ripening and storage (Papers 2 and 3), the literature review will only focus on the aroma topic. The decision to omit a review on the use of NIR spectroscopy in the measurement of fruit quality was taken based on the relatively recent publication of a comprehensive and thorough review article by Nicolaï et al. (2007).

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LITERATURE REVIEW

Plum fruit development and aroma volatiles.

1. Introduction

Traditionally the fruit consumer selects fresh produce based on appearance, i.e. colour, shape, absence of visual defects and injury, but satisfaction and repeat purchase are mainly determined by flavour (Schotsmans and Prange, 2006). Flavour has two types of components, those giving rise to the taste sensation and those responsible for the aromatic character (Williams and Ismail, 1981). Taste components include non-volatile chemicals such as sugars (sucrose, glucose, fructose, etc), acids (malic acid, etc) and polyphenolic material that give rise to taste sensations such as sweet, sour, astringent and bitter. Aroma volatiles on the other hand, create that characteristic aroma of a fruit and include some key compound(s) that distinguish them from each other (Grab, 2007). In sensory evaluation such as consumer studies, taste and aroma cannot be separated but in order to study and identify the aroma volatile components produced by a fruit it is necessary to do this. Fruit release aroma volatiles from a large range of chemical groups including esters, lactones, alcohols, acids, aldehydes, ketones, acetals, hydrocarbons, phenols, ethers and heterocyclic oxygen compounds (Kays and Paull, 2004) and each compound has an odour threshold level above which it is detectable to the human palate, thus the mere presence of a compound does not mean it is contributing to the aroma. It is often not a single compound that represents a characteristic fruit flavour, but rather a combination of compounds working in synergy (Williams and Ismail, 1981; Schotsmans and Prange, 2006) and is often referred to as “character impacting compounds”. Kays and Paull, (2004) present an overview of the main volatiles for a range of fruit and vegetables.

Aroma volatile production is a dynamic process and the pattern of volatile constituents, both qualitative and quantitative, can vary greatly during fruit maturation (Agozzino et al., 2007), ripening (Yahia et al., 1990; Visai and Vanoli, 1997) and storage (Aubert et al., 2010; Chen et al., 2006; Raffo et al., 2008). As aroma volatiles belong to different chemical groups there are several biochemical pathways involved in their biosynthesis. The precursors to most of the volatiles are amino acids, membrane lipids, fatty acids and carbohydrates (Dixon and Hewett, 2000; Song and Bangerth, 2003; Fellman et al., 2000). Knowledge of the metabolism and degradation of these chemicals under different conditions aids in understanding the mechanisms responsible for the dynamics of aroma volatiles. For example, aroma development in apples is mediated by ethylene production during climacteric ripening. If this process is delayed or compromised the production of volatile esters is inhibited (Fan et al., 1998). Research further suggests that continuous ethylene presence at a high level is required for the successful development of the aroma

profile (Fan et al., 1998). The implications of this include the risk that fruit harvested before they have produced sufficient levels of ethylene and/or are exposed to different storage conditions may fail to develop the desired aroma profile post harvest.

In 1990, Crouzet et al. (1990) published a book chapter on the volatile constituents of stone fruit including a lengthy review on plum volatiles. This current literature review aims to summarise the publications on plum flavour components and extend the review of Crouzet et al. (1990) to include the research done in this field over the last 20 years.

2. Plum characteristics

Plums belong to the family Rosaceae, genus *Prunus* that also includes other stone fruit such as peaches, apricots, nectarines and cherries. Based on a survey done by Blažek (2007) on the genetic resources used in plum breeding the author commented that plums constitute the most numerous and diverse group of fruit tree species with over 6000 cultivars referable to more than 20 species differing in their geographical origin, chromosome numbers and climatic demands. However, only a few *Prunus* species comprising plum fruit are of commercial importance namely *Prunus domestica* L. (European plum or Garden plum), *Prunus salicina* Lindl. (Japanese plum) and to a lesser degree *Prunus cerasifera* Ehrh. (Myrobalan or cherry plum) that is mainly cultivated as a rootstock (Blažek, 2007). Hybrids derived from crossings between these and other stone fruit, especially apricots, are also becoming increasingly popular. In the classification of these interspecific hybrids it is necessary to discuss the differences between plumcots, pluots and apriums. Plumcots are hybrids bred from Japanese plums and apricots (*P. salicina* x *P. armeniaca*) and are described as sweet and having a complex but excellent flavour (Blažek, 2007). 'Flavor King' is such a plumcot that has, over the last decade become, a valued cultivar for commercial growing and export in South Africa. The plumcots gave rise to the pluots and apriums by crossing them back with either Japanese plums or apricots. Pluots are derived from crosses like *P. salicina* x *P. armeniaca* x *P. salicina* and are thus more closely related to plums than to apricots (Blažek, 2007). Apriums originate from *P. salicina* x *P. armeniaca* x *P. armeniaca* and resemble apricots more than plums (Fideghelli, 2002).

Plums, like most stone fruit or 'drupes', are round or oval shaped fruit with a characteristic lignified endocarp, a fleshy mesocarp and a thin exocarp (peel) (Brady, 1993). Exocarp colour is traditionally light and dark shades of purple ranging from pink to almost black, but the yellow cultivars, such as Songold, are also popular. Mesocarp colours include yellow, red and white. The fruit have a triphasic pattern of development resulting in a double sigmoidal growth curve that is well described by Tukey (1936). Stage I of the development is recognised by rapid cell division and an increase in the volume of the pericarp, stage II (pit-hardening) is a period of quiescence in the pericarp and rapid development of the embryo and in stage III the endocarp completes its development and the pericarp resumes a rapid increase in

volume mainly due to cell expansion (Tukey, 1936). An incomplete or suppressed stage II is often present in early maturing cultivars with a low requirement for winter chill and the endocarp closure may not be complete resulting in the 'split-pit' syndrome (Brady, 1993).

Fruit maturation is the time between final growth and the beginning of ripening and senescence (Crisosto, 1994) with maturity as the endpoint of maturation. Stone fruit maturation and ripening are accompanied by substantial physical and biochemical changes. The visible and external changes include changes in peel colour (rapid disappearance of ground colour) and increase in size as the fruit nears maturity (Abdi et al., 1997). Internally the sugars accumulate rapidly during ripening, sucrose being the main sugar (Brady, 1993) but glucose, fructose and sorbitol are also important with considerable variation between cultivars in sugar content and in the proportions of the four major sugars (Vitanov et al., 1988). A study done by Crisosto et al. (2007) on 12 plum and four pluot cultivars indicated a general trend that the ripe fruit of early season cultivars had lower sugar levels than late season cultivars. Acid levels decrease during maturation (Abdi et al., 1997) due to their utilization as respiratory substrates (Tucker, 1993). Malic acid is the most common organic acid found in Japanese plums (Taylor, 1993a). Crisosto et al. (2007) commented that titratable acids in various ripe plum cultivars did not relate to time of season meaning that early and late season plums exhibit similar acid levels. Sugar-acid ratios are also thought to be important especially in consumer acceptance studies. Flesh firmness in plums starts to decrease after pit-hardening and continues until the fruit reach full colour (Abdi et al., 1997). The mechanisms responsible for the softening of the flesh include the enzymatic modification of the cell wall architecture whereby polygalaturonase depolymerises and solubilises the pectin and as a result the cell wall becomes increasingly hydrated as the cohesion of the pectin gel changes causing a change in the flesh firmness and texture (Brummell and Harpster, 2001; Harker and Maindonald, 1994; Giovannoni, 2001). Many of the changes associated with maturation have been identified and are currently used as maturity indices to aid growers in selecting an optimum harvest date for their crops to ensure minimum acceptable eating quality and long storage life (Crisosto, 1994). Abdi et al. (1997) however, warn against the reliability of these parameters to judge harvest maturity of all cultivars and suggest that the parameters must be cultivar specific.

In spite of the changes discussed above fruit types are further divided into two broad groups based on their ripening behavior, namely climacteric and non-climacteric (Biale, 1964). This categorisation is based on the fruits' ability to exhibit a peak in respiration rate and ethylene production during ripening. Typically climacteric fruit, such as plums, have a surge in respiration and ethylene biosynthesis during ripening that is absent from non-climacteric fruit (Giovannoni, 2001). Ethylene is further necessary for the mediation and completion of many of the physiological and biochemical changes during ripening (Lelievre et al., 1997) by means of complex biochemical and molecular pathways. Some plum cultivars however, portray a ripening behaviour that is atypical of true climacteric in the sense that they produce an ethylene peak

much later in the ripening process and the peak is 15-500 times smaller than expected. These plums also have a reduced respiratory climacteric and were termed 'suppressed climacteric' by Abdi et al. (1997). Examples of such cultivars include Shiro and Rubyred (Abdi et al., 1997) and Songold and Angeleno (Kruger, et al. 2003).

South Africa has an active Japanese plum breeding and production sector with annual export figures (2009) of close to 9 million cartons (5.25 kg equivalent cartons) comprising 35 different plum cultivars and an annual supply window of six months (October to April)) (PPECB Information portal: http://info.ppecb.com). Due to the short storage life, plums exported from South Africa to European markets are harvested relatively unripe but ripen whilst in transit for up to 42 days in temperature controlled containers at sea. To prevent chilling injuries such as internal browning and gel breakdown (Taylor et al., 1993b) most plum cultivars are stored under a dual temperature regime whereby the fruit are first stored at -0.5°C for 8 to10 days followed by an increase in temperature to 7.5°C for a further 5 to 7 days after which the temperature is dropped again to -0.5°C for up to 25 days. The protocol is flexible as some cultivars are more susceptible to the disorders than others. With plum consumer preference now shifting towards flavour and taste (SASPA/Richmond Towers, UK consumer research de-brief, 2006) it has become important to analyse the aroma profiles of Japanese plums including the possible effects of cold storage to ensure that the flavour persists throughout the marketing operation.

3. Plum aroma in general

Aroma volatile profiles of stone fruit such as peach, nectarine and apricot are well researched and documented, but plum aroma seems to have had much less interest. Only 22 publications and one review article could be found dating from 1974 to present. The publications cover 23 different cultivars from four plum species and seven plumcot cultivars (*P. salicina* x *P. armeniaca*). The review article by Crouzet et al. (1990) is a thorough discussion of plum aroma related literature (15 publications) from 1974 up to 1990 and contains a table illustrating the relationship between the different plum cultivars studied for their aroma. We have reworked and updated this table to now include the plum aroma literature published from 1974 to 2010 (Table 1, dashed lines indicate new information for the time period 1990 to 2010). As stated by Crouzet et al. (1990), the first chemical investigation on plum aroma was published by Forrey and Flath in 1974 and concerned the species *P. salicina*, cultivar Santa Rosa. Subsequent to this another four publications appeared in the 1970's (Moutounet et al., 1975; Ismail et al., 1977; Kereselidze and Mikeladze, 1977; Moutounet, 1978) mostly concerned with *P. domestica* cultivars except for one study on 'Bullac' plum (*P. institia*). Most of the plum aroma investigations took place in the 1980's with 10 publications (Ismail et al., 1980a,b,c; Ismail et al., 1981a,b; Williams and Ismail, 1981; Vernin et al., 1985; Etiévant et al., 1986; Dirninger-Rigo, 1987; Le Quéré et al., 1987) predominantly from two institutions, namely Long Ashton Research Station (University of Bristol, U.K.) and the Institut National de la Recherché Agronomique (INRA) in Toulouse, Dijon and Colmar (France). The research done in the

1980's also focused primarily on cultivars from two *P. domestica* subspecies. Interestingly, four of the five publications that followed in the 1990's did not include any *P. domestica* or *P. insititia* cultivars, but the focus shifted back to *P. salicina* (Gómez and Ledbetter, 1993 and 1994) and an additional plum Prunus species, namely *P. simonii* (Gómez and Ledbetter, 1994). Another *P. domestica* subspecies, *P. domestica syriaca*, was also described for the first time (Krammer et al., 1991) concerned with glycoconjugates as plum flavour precursors. This time period also saw the first documentation of the aroma profiles of hybrid species. Plumcot aroma of seven cultivars was described for the first time (Gómez and Ledbetter, 1993 and 1997) as well as six cultivars from the *P. salicina* x *P. americana* true plum crossings (Horvat et al., 1992). We could only find two publications in the last decade relating to plum aroma, namely that of a prune cultivar (Sabaraz et al., 2000) from *P. domestica domestica* subspecies and six *P. salicina* cultivars (Lozano et al., 2009). In retrospect, Table 1 suggests that although the research done on plum aroma started on a single cultivar of the Japanese plum (P*. salicina)* 35 years ago, it continued mainly on the European plum (*P. domestica*) cultivars for the first 12 years before it again included more Japanese plum cultivars, resulting in the publications of the last 17 years predominantly focusing on Japanese cultivars and their hybrids with either other plum or apricot species.

The methodology of an aroma volatile investigation typically requires four steps starting with sample/substrate preparation (if whole fruit is used this step is obsolete/reduced), step two: extraction of the volatile compounds, step three: separation of the volatiles (generally done by gas-chromatography) and finally step four: identification of the volatiles (generally done by mass-spectroscopy). The sample preparation step and especially the extraction step define the nature of the chemical groups that will be separated and identified, making it almost impossible to compare results from studies not using the same methodology (Crouzet et al., 1990). The plum literature warns against two specific pitfalls that can alter the aroma profiles during the sample preparation and extraction steps. The first, a common problem in many fruit types, is flesh browning that is a result of enzymatic oxidation of phenolic compounds into quinones (Mayer and Harel, 1981; Lee, 1991; Nicolas et al., 1994). This reaction is catalysed by polyphenoloxidase in the presence of oxygen (Vamos-Vigyazo, 1981; Eskin, 1990). Thus, during sample preparation when plums are cut, destoned and milled and the flesh is exposed to oxygen containing air the risk of deterioration is high (Ismail et al., 1981a or b ; Etiévant et al., 1986; Dirninger-Rigo, 1987). As Crouzet et al. (1990) explained, these authors avoided flesh browning by using various methods such as inclusion of ascorbic acid as an oxygen trap (Ismail et al., 1980b; Dirninger-Rigo, 1987), adding sulphur dioxide to combine with the phenolic substrates (Dirninger-Rigo, 1987) and methanol mediated deactivation of the responsible proteins (Etiévant et al., 1986; Dirninger-Rigo, 1987). The second possible pitfall is illustrated by the study of Etiévant et al. (1986) that showed that the aroma profile of plums changes once exposed to deep-freezing (-30°C) and thawing. Samples exposed to deep-freezing and thawing showed an increase in C_6-alcohols, aldehydes and terpenes possibly due to cell structure disruption and consequent decompartmentation leading to abnormal exposure of enzymes and

substrates (Etiévant et al., 1986). In contrast, esters decreased after deep-freezing and thawing possibly indicating that the process favours the activity of esterase or, more probably, the denaturation of inhibition of enzymes involved in the biosynthesis of esters (Etiévant et al., 1986). Due to the complexity of the mechanisms it cannot be prevented or treated chemically and the best way to preclude this is to avoid deep-freezing and thawing by analysing all samples fresh. In a study on peaches, Raffo et al. (2008) warn against formation of C_6-aldehydes originating from increased lipoxygenase activity associated with the crushing of fruit and suggest an enzyme deactivation step with saturated $(NH_4)_2SO_4$ during sample homogenisation to prevent inflated aldehyde levels in the results.

The review by Crouzet et al. (1990) also contains a lengthy list of 274 volatile constituents (with references for each) identified in plum extracts. These compounds belong to more than 15 different chemical groups illustrating the complexity of plum aroma. From this list and a table indicating the relative percentages of major volatile compounds in plum literature, Crouzet et al. (1990) deducted that esters are qualitatively more important than any other class of compound in plums. Quantitatively they rate alcohols or esters the major components of plum aromatic extraction (Crouzet et al., 1990) but also stated that none of the alcohols or esters by themselves exhibit a plum-like flavour and that authors have tried to identify other substances as potential contributors to plum aroma. This apparent importance of ester and alcohols in plum aroma profiles continues to dominate the literature published post 1990 (Gómez and Ledbetter, 1994, Horvat et al., 1992; Lozano et al., 2000) but several authors now also highlight and associate plum aroma with the presence of lactones (γ-decalactone, γ-dodecalactone) and some C_6-compounds (hexanal and 2-hexenal) (Gómez and Ledbetter, 1994, Horvat et al., 1992).

As each aroma compound has an odour threshold level above which it is detectable to the human palate the mere presence of a compound does not mean it is contributing to the aroma. It is often not a single compound that represents a characteristic flavour, but rather a combination of compounds working in synergy (Williams and Ismail, 1981). This makes it difficult to comment on the character impacting compounds without a thorough sensory assessment of the compounds. Williams and Ismail (1981) describes four methods to evaluate the sensory impact of a compound on the specific plum flavour of two *P. domestica* cultivars, Victoria and Marjorie's Seedlings. In the first and most detailed method, they sniffed the exit of the gas-chromatographic column and identified three chromatographic regions associated with a plum-like flavour by giving a descriptive evaluation. Comparing this to the mass-spectrometric data that identified the compounds present in the regions they were able to group and name compounds responsible for fresh plum aroma. They found two regions resembling fresh plum odour with linalool, benzaldehyde and ethyl nonanoate peaks present in the one region and a methyl cinnamate and γ-dodecalactone combination in the other. The third region of plum aromatic importance was more reminiscent of cooked plums and included peaks of γ-octalactone, 2-phenylethanol and damascenone. Further chemical separation techniques focused around these regions enabled them (Williams and Ismail,

1981) to identify that removing linalool and the two lactones from the regions caused the plum-like odour to disappear. However from descriptions of linalool and the lactones on their own it seems most unlikely that the plum aroma is due entirely to these compounds and they tentatively concluded that the aroma arising from the regions must be the result of a fortuitous co-elution of compounds producing a plum-like odour in synergy (Williams and Ismail, 1981). They also commented on other compounds besides those found in the three regions as being important to the overall plum flavour. These included nonanal found in epicuticular wax of plums and in synergy with benzaldehyde and benzyl acetate is believed to play a role in the aroma of canned plums. Hexanols and hexanals with their 'green' aromas were also named as important in plum aroma particularly in the preparation of juices (Williams and Ismail, 1981).

The next three methods used by Williams and Ismail (1981) to identify possible character impacting compounds in plums are non-descriptive and more of a calculative approach to establish the true significance of the compounds identified in the first method. They used threshold values and odour units to calculate the relative importance of the compounds to the aroma of the extracts. A compound with a relatively low threshold value (done in water and in a sugar-acid solution) and a high odour unit is believed to be of higher importance and significance to the overall aroma. Their results indicated that benzaldehyde, linalool, γ-dodecalactone, γ-octalactone are potentially more important to the plum aroma than ethyl nonoate, methyl cinnamate, 2-phenylethanol and damascenone (Williams and Ismali, 1981). The exceptionally high odour units of nonanal, ethyl butyrate and n-hexanol also points to an important contributions to plum aroma (Williams and Ismail, 1981).

Another non-descriptive method used and described by Williams and Ismail (1981) is concerned with the relationship between the concentration of a compound (peak height) and the flavour character, i.e. plotting increasing plum-like aroma against increasing relative peak heights of a compound and determining the slope or regression coefficient of the relationship. A positive relationship (slope) is seen to be indicative of a compound that is more likely to contribute to the plum flavour. Williams and Ismail (1981) found that four compounds, methyl cinnamate, benzaldehyde, γ-decalactone and linalool all had positive relationships whereas nonanal and hexanol had negative slopes suggesting that the latter two compounds are less likely to relate to plum flavour.

The last sensory evaluation method used by Williams and Ismail (1981) to investigate the character impacting compounds in plums involved assessing a compound's potential importance by scoring its plum aroma individually and in combinations (mixtures) in a sugar-acid solution. The authors are of the opinion that the only reliable method of evaluating the true significance of a compound to a product is to add it, both alone and in combination with other compounds in varying concentrations, to either the product itself or a medium reminiscent of the non-volatile portion of the product and to assess its aroma and flavour. The results from their experiments showed that when tested singly only the γ-decalactone, irrespective of

concentration, gave a high score for being plum-like. The binary mixtures of γ-decalactone and methyl cinnamate also consistently gave high scores. Particular concentrations of the benzaldehyde-linalool and linalool-ethyl nonanote mixtures gave scores approaching and passing that of the thawed plum juice standard (Williams and Ismail, 1981). The mixtures containing the compounds identified in the two chromatic regions associated with fresh plum aroma, as discussed earlier, produced consistently higher scores for similarity to fresh plums and lower scores for cooked plums. In conclusion this last method helped in establishing that linalool, benzaldehyde, γ-decalactone and methyl cinnamate do contribute to plum aroma.

More recent work done by Crisosto et al. (2007) aimed to segregate plum and pluot cultivars according to their organoleptic characteristics. This type of study excludes the time-consuming and labour intensive chemical analysis and identification of aroma compounds but makes use of taste panels and statistics to classify different cultivars according to sensory variables such as sweetness, sourness, aroma and plum flavour. Their results segregated 12 plum cultivars into three groups namely, tart plums ('Earliqueen', 'Purple Majesty' 'Black Amber' 'Simka', 'Betty Anne' and 'Flavorich'), plums high in aroma ('Royal Zee', 'Joanna Red', 'Fortune' and 'Flavorosa') and plums high in flavour and sweetness ('Catalina', 'Dapple Dandy', 'October Sun', 'Hiromi Red', 'Friar' and 'Flavor Grenade'). The authors further analysed their results by investigating the relationship between these sensory attributes and the non-volatile chemical composition (sugar and acid levels) of the cultivars. This indicated that the perceptions of sweetness, flavour intensity and aroma correlated significantly with the sugar (soluble solutes) concentration but not with the acid concentration. Sugar-acid ratios significantly correlated only with sweetness and plum flavour intensity. Sourness and acidity were never significantly correlating with any of the chemical or sensory attributes (Crisosto et al., 2007). They concluded by advising that plums and pluot cultivars with a sugar level exceeding 12.0%, grouped in their organoleptic class and delivered to the consumer close to their 'ready to eat' stage will assure satisfaction of a high percentage of consumers, help match consumer preference, enhance consistent flavour delivery and ultimately increase plum consumption. More modern studies such as this aim to use non-volatile characteristics that are relatively quick and simple to determine to indirectly comment on the volatile composition of the fruit and at the same time incorporate the consumer preferences.

4. Plum aroma dynamics

The biosynthesis and degradation of aroma volatiles are processes mediated by many internal and external factors. Some of these factors have been identified for plums and their ability to cause variation has been studied. As mentioned before it is impossible to compare aroma dynamics across literature if different sample preparation and extraction methods are used, but discussing the findings of single studies remains useful. The next section will aim to address some of the factors that can cause change and variation in the general plum aroma profile.

Cultivar

Although fruit of equal ripeness and belonging to the same fruit type have a similar general flavour associated with that fruit type it is undeniable that different cultivars of the same fruit type have distinctly different flavours. These differences and similarities are due to genetic differences and thus of great importance to fruit breeders. With over 6000 plum cultivars present it is surprising that the aroma volatile profiles of only 25 cultivars could be found in the literature. The first study on plum aroma conducted by Forrey and Flath (1974) listed 53 compounds extracted from 'Santa Rosa' plums. Acetate esters were found to be predominant and appreciable quantities of higher γ-lactones were also present (Forrey and Flath, 1974). It is, however, only in studies using the same methodology but conducted on multiple cultivars that one can estimate the influence of cultivar. Such a study done by Ismail et al. (1981a) on four *P. domestica domestica* cultivars (Marjorie's Seedling, Merton Gem, NA 10 and Victoria) identified the same 33 individual aroma compounds in all four cultivars with only quantitative differences. The extracts were dominated by hexanol and nonanol and relatively high levels of 3- methylbutanol and linalool were also present in all but the cultivar NA 10, but only linalool, benzaldehyde, methyl cinnamated and γ-decalactone were present in chromatogramic regions associated with plum-like aroma (Ismail et al., 1981a). In this study the four cultivars were also given a sensory rating by panelists in respect to their plum aroma resulting in 'Merton Gem' considered to be the most plum-like followed by 'Victoria' and 'Marjorie's Seedling, 'NA 10' being the least plum-like in aroma. When comparing the sensory results to the four compounds mentioned earlier believed to be important in plum aroma, it followed the same trend (Ismail et al., 1981a). Interestingly hexanol, with its 'green' aroma followed the opposite trend being largest in 'NA 10' which had the poorest aroma and smallest in 'Merton Gem' which had the most plum-like aroma. This made the authors conclude that the cultivar specific variation in hexanol and its negative effect on plum aroma may overpower any positive characteristics imparted by linalool, benzaldehyde, methyl cinnamate and γ-decalactone. A similar study done previously by Ismail and coworkers (Ismail et al., 1980c) on 'Victoria' and 'Golden Egg' showed that these two cultivars differ in their sensory assessment with 'Victoria' described as more almond-like and 'Golden Egg' more woody. These olfactory differences were believed to be due to cultivar related differences in benzaldehyde (higher in 'Victora') and nonanal (higher in 'Golden Egg') (Ismail et al., 1980c).

In the case of *P. domestica insitita* plum cultivars, Crouzet et al. (1990) mention in their review that 'Bullace' plums (as studied by Kereselidze and Mikeladze, 1997) were rich in α- and β-ionone and that together with other esters these compounds could be cultivar specific as they had not been identified in other cultivars at that time. We now know that this is no longer the case as β-ionone has been subsequently identified in two *P. salicina* cultivars ('Black Amber' and 'Friar') and one *P. simonii* cultivar ('PI 91527') by Gómez and Ledbetter (1994) and six cultivars from *P. salicina* x *P. americana* true plum hybrids (Horvat et al., 1992). α-Ionone has also been positively identified in the *P. simonii* cultivar, 'PI 91527' by Gómez and Ledbetter (1994). Another *P. domestica insitita* plum cultivar, Mirabelle, was of

great interest in the latter half of the 1980's when its aroma profile was studied by four different groups of researchers (Vernin et al., 1985; Etiévant et al., 1986; Dirninger-Rigo, 1987; Le Quéré et al., 1987). According to Etiévant et al. (1986) 'Mirabelle' plums can be easily distinguished from other plum cultivars by their very specific pleasant aroma and they are widely used in the canning industry and brandy making. Etiévant and co-workers identified 130 components in 'Mirabelle' with 48% of them belonging to the ester chemical group. They claim that 'Mirabelle' is different from other *P. domestica* subspecies cultivars due to the absence or low concentrations of terpene alcohols such as linalool, α-terpineol and geraniol which have also not been reported in other cultivars belonging to the same subspecies (Kereselidze and Mikeladze, 1977), but have been documented as major constituents of a large number of different *P. domestica domestica* cultivars (Ismail et al., 1980a, 1981a and b). Other compounds absent in 'Mirabelle' plums but present in other subspecies include verbenone and methyl cinnamate (Ismail et al., 1981b) and nonanal (Ismail et al., 1977). Furthermore, 'Mirabelle' seems to differ also from cultivars within the same subspecies, such as Bullace plums (Kereselidze and Mikeladze, 1977), in the absence of β-damascenone and α- and β-ionones in its extracts. The absence of some of these compounds and the presence of compounds such as δ-lactones and methylnaphthalene, seemingly unique to 'Mirabelle', may explain the very characteristic aroma of this cultivar (Etiévant et al., 1986).

Other examples of studies comparing cultivars are that of Horvat et al. (1992) and Lozano et al. (2009). Horvat and co-workers (1992) investigated the aroma volatiles of six relatively new developed cultivars originating from hybrids between *P. salicina* and *P.* a*mericana*. They list 36 compounds found in these cultivars but only give the relative percentages of the eight major compounds. Although 34 of the 36 compounds have been isolated by other authors (Crouzet et al., 1990) it is still clear that there are considerable differences amongst the cultivars. Lozano et al. (2009) studied six *P. salicina* cultivars and commented that of the 40 compounds identified not all of the compounds were present in all of the cultivars and, where they do occur, they were not present in the same quantities. They further found that 'Fortune' is the cultivar with the greatest volatile content, substantially different from the others ('Suplumsix' ('Angeleno'), 'Black Amber', 'Larry Anne', 'Suplumeleven' and 'Songold') and containing the most esters. Interestingly, they showed that the two suppressed climacteric cultivars, 'Suplumsix' ('Angeleno') and 'Songold' were the cultivars with the lowest volatile content (Lozano et al., 2009). Analysis of variance of the volatile fractions also revealed significant differences among the cultivars in 15 variables of which four esters clearly differentiate 'Fortune' from the others, 2-methyl-3-buten-2-ol puts 'Larry Anne' in a category of its own and 2-hexanyl butanoate and 2-hexenyl hexanoate distinguish 'Suplumeleven' (Lozano et al., 2009). This again illustrates the differences in the aroma profiles found amongst cultivars.

In a study done by Gómez and Ledbetter (1994), they did a direct comparison (using similar methodology) between the aroma profiles of two plum Prunus species (*P. salicina* and *P. simonii)* using

two *P. salicina* cultivars ('Blackamber' and 'Friar') and one *P. simonii* cultivar ('PI 91527'). They concluded that the two *P. salicina* cultivars had very similar profiles that were quite different from that of *P. simonii* suggesting that aroma profile differences may be influenced on species level rather than cultivar level. Of the 60 quantified compounds identified in this study, 23 (38%) compounds (representing all of the chemical classes found) were present only in *P. simonii*. Esters made up the bulk (52%) of the unique compounds and were also quantitatively higher. They are thought to contribute to the more intense flavour of the *P. simonii* compared to the Japanese cultivars (Gómez and Ledbetter, 1994). These species also exhibited considerable differences when the odour units of some of the important compounds were calculated. Although the compounds with the highest odour units (β-ionone, nonanal and hexyl acetate) were similar for all three cultivars, the odour units of β-ionone and hexyl acetate were significantly higher in *P. simonii* (Gómez and Ledbetter, 1994) illustrating again the species-specific, rather than cultivar-specific, differences amongst plums.

Maturity

The only in-depth study done on the influence of maturity on the aroma volatile profile of true plums comes from a Russian research thesis by Dirninger-Rigo in 1987. Crouzet et al. (1990) gave a concise summary of this thesis in their review article and highlighted the dynamics of different chemical groups as the plums mature: Dirninger-Rigo identified 56 different compounds in three maturity groups (half-ripe, ripe and over-ripe) of 'Mirabelle' plums. Hydrocarbons and aldehydes mostly decreased with increasing maturity and it was suggested that this facilitates the decrease in 'green' and 'fresh' aromas as estimated by sniffing the chromatographic effluent. The observed development of esters with advancing maturity was, however, not so simple. Mixed patterns were observed including increasing (butyl butanoate, dec-4-enoate, hexyl hexanoate, 2-methyl butanoate, propanoate and methyl nicotinate), decreasing (methyl hexanoate, ethyl octanoate, butyl propanoate and *cis*-hex-3-enyl butanoate) and initially increasing until normal ripeness and then stabilising aroma volatile levels (methyl octanoate, dec-4-enoate, butyl 3-hydroxy butanoate and 3-methyl buthyl butanoate) (Dirninger-Rigo, 1987). Conversely, most of the terpene alcohols increased with increased maturity with the exception of linalool that first increased until normal ripeness and then decreased rapidly in the over ripe samples, suggesting that linalool may be more interesting in order to evaluate maturity than for sensory impact. The chemical group with the most drastic modification with maturity was the lactones of which the concentration was 77 times higher in over-ripe plums than in half-ripe fruit (Dirninger-Rigo, 1987). The odours of especially γ-octa-, nona- and decalactones intensified as the samples increased in ripeness as detected by the sniffing technique.

The only other study that could be found on the dynamics of aroma volatiles and maturity was that of Gómez and Ledbetter (1997) who characterised and compared a plumcot ('P251-002') and an apricot ('P305-175') cultivar at three maturity stages, viz. 'mature green', 'commercial ripe' and 'tree ripe'. Although the paper mainly focused on comparisons between the two fruit types rather than the changes

within the maturation of each cultivar, patterns could still be recognised by studying the table that listed the concentrations of the compounds at the different stages. From this it was clear that of the 38 compounds identified for the plumcot most decreased as maturity and ripeness increased. Some exceptions include increases in ketones (3- methyl-2-pentanone, geranyl acetone, β-ionone), esters (ethyl 3-methylpentanoate, hexyl butanoate, methyl salicylate) and especially lactones (γ-deca- and dodecalactones) (Gómez and Ledbetter, 1997). The significant increase in lactones coinciding with increase in ripeness is also highlighted and again linked to increasing flavour in both apricot and plumcot cultivars.

Processing and preserving

Apart from fresh consumption, plums are also enjoyed in several processed forms. Some *P. domestica* cultivars (e.g. prunes d'Ente and prunes d'Agen) are conventionally preserved as prunes after dehydration down to 18 – 28% water in a heated and ventilated tunnel (Crouzet et al., 1990). Other processing practices include preserving in cans, jams or fermenting plums into a brandy. The influences of these processing practices on the aroma profiles are included in great detail in the review article by Crouzet et al. (1990) and as this dissertation is concerned with the aroma volatiles in fresh plums it will not be discussed in this review.

Most if not all of the processing practices mentioned above include the disruption and decompartmentation of the cell structure through heating or crushing of the fruit. This disturbance in the cell contents may lead to the enzymatic hydrolysis of glycosidically bound aroma compounds which will then become volatile and add to the aroma profile of the fruit (Williams, 1993). The hydrolysis reaction and release of previously bound and odourless compounds is a direct result of contact between glycosides and the glycosidase complex which includes a β-glucosidase activity (Heidlas et al., 1984). Another mechanism that may lead to the formation and release of volatile aroma compounds is acid hydrolysis that is triggered by a decrease in pH (Williams, 1993) usually associated with juicing of fruit. Krammer et al. (1991) describe the glycosidically bound aroma compounds released from 'Nancy' plums (*P. domestica,* ssp. *syriaca*) after simultaneous enzyme catalysis extraction using emulsion (β-glucosidase). They list 31 enzymatically released aglycones and claim that they mainly fall into three categories biogenetically derived from fatty acids, phenylpropanoid and terpene metabolism. They further pay special attention to the monoterpene diols and C_{13}-norisoprenoids found in the three categories. Some of the compounds they discuss have been mentioned before as potentially important in plum aroma, e.g. linalool, α-terpineol, geraniol, benzaldehyde and damascenone derivatives (Krammer et al., 1991). The identification and further study of these glycosidically bound compounds is important for two reasons, a) they contribute to the understanding of flavour biogenesis during fruit ripening and b) more practically, to predict the flavour of fruit product such as juices and wines (Stahl-Biskup et al., 1993). The presence and influence of these aroma volatile precursors can also be of importance in the study of fresh

plum aroma as the sample preparation and/or extraction steps may often involve mechanisms that cause cell disruption and thus favour the release of glycosidically bound compounds that impact on the aroma profile results.

Cold-storage

To prolong the post harvest life-span of fresh plums they are stored at low temperatures until consumption. This storage period can vary in length and is often up to 42 days in the case of export countries (such as South Africa) that are far away from their markets and relying on sea freight to deliver their fresh produce. The effects of such prolonged exposure to low temperatures on quality parameters such as flesh firmness and non-volatile compounds (sugar and acid content) have been studied and documented for plums (Taylor et al., 1993a; Kreck et al., 2005; Robertson et al., 1991). The effects on the aroma profile i.e. individual aromatic compounds, however, have been studied in stone fruit such as apricots (Aubert et al., 2010) and peaches (Robertson et al., 1990; Raffo et al., 2008), but to date no literature could be found for plums. In the study on two peach cultivars, Raffo and co-workers found that after one week of cold storage (1 ℃ plus one day of shelf-life at 15 ℃) the lactone levels (especially γ- and δ-decalactones) increased drastically by an average of 95% and 83% for white-fleshed and yellow-fleshed peaches, respectively. After two weeks, however, the levels had decreased again and were similar to those found in the fresh samples. They suggested that cold storage significantly reduced the fruits' ability to perform lactone accumulation and, consequently, to develop its aroma after it was exposed to ripening temperature (Raffo et al., 2008). This initial rise and then sharp decline in peach lactone levels during cold storage was also observed by Robertson et al. (1990) although the pattern was shifted by a week with the increase and decline documented at the end of two weeks of storage at 0 ℃. A similar effect, although less marked, was also observed for C_{13}-norisoprenoids (Raffo et al., 2008). Furthermore the C_6 aldehydes, hexanal and (*E*)-2-hexenal, also seemed to decrease during prolonged cold storage (Robertson et al., 1990). In both studies (Robertson et al., 1990; Raffo et al., 2008) linalool was found in relatively high levels at harvest and decreased during storage. Although the above mentioned patterns were observed in peaches the aroma volatiles that showed decreasing patterns have also been detected in plums and identified as important in producing the characteristic plum flavour (Crouzet et al., 1990; Williams and Ismail, 1981) and may thus show similar behaviour in plums stored at low temperatures and ultimately impact on the flavour.

Other pre- or postharvest treatments, usually associated with cold storage, that are also aimed at delaying ripening and senescence and that have been studied for plums include the use of polyamine sprays (Khan et al., 2008; Serrano et al., 2003; Pérez-Vicente et al., 2002), 1-methylcyclopropene (Shao et al., 2010; Khan and Singh, 2009; Alves et al., 2010), controlled atmosphere (Folchi et al., 1994; Ke et al., 1991), polyethylene bags with potassium permanganate (Hao et al., 2006) and heat treatment (Serrano et al., 2004). Again, all of these studies address only quality parameters such as firmness, sugar

and acid contents and did not investigate the possible effects on individual aroma volatile compounds. Some of these papers comment on the sensory changes that are associated with low temperature storage by means of taste panels, but no research could be found on the actual aroma volatile compound dynamics.

5. Conclusion

The two components of plum flavour, namely taste and aroma have not been studied to the same extent. Studies on taste are numerous and mostly concentrated around the investigation of the non-volatile sugar and acid content associated with quality parameters. The aroma aspect of plums has not enjoyed the same interest and only a few publications exist compared to many more published for other stone fruit such as peaches and apricots. The publications that do exist focus mainly on identifying and listing the aroma compounds found in different plum species and cultivars. Apart from a single study, the influences of factors such as seasonality, pre-harvest practices, maturity, ripening and cold storage have not been researched well and studies linking aroma volatile components to consumer preferences could not be found. It is focus areas such as these that can provide information to improve production and export practices of plums and aim to increase and protect market share by delivering produce that is not just approved but also preferred by the consumer.

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7. References

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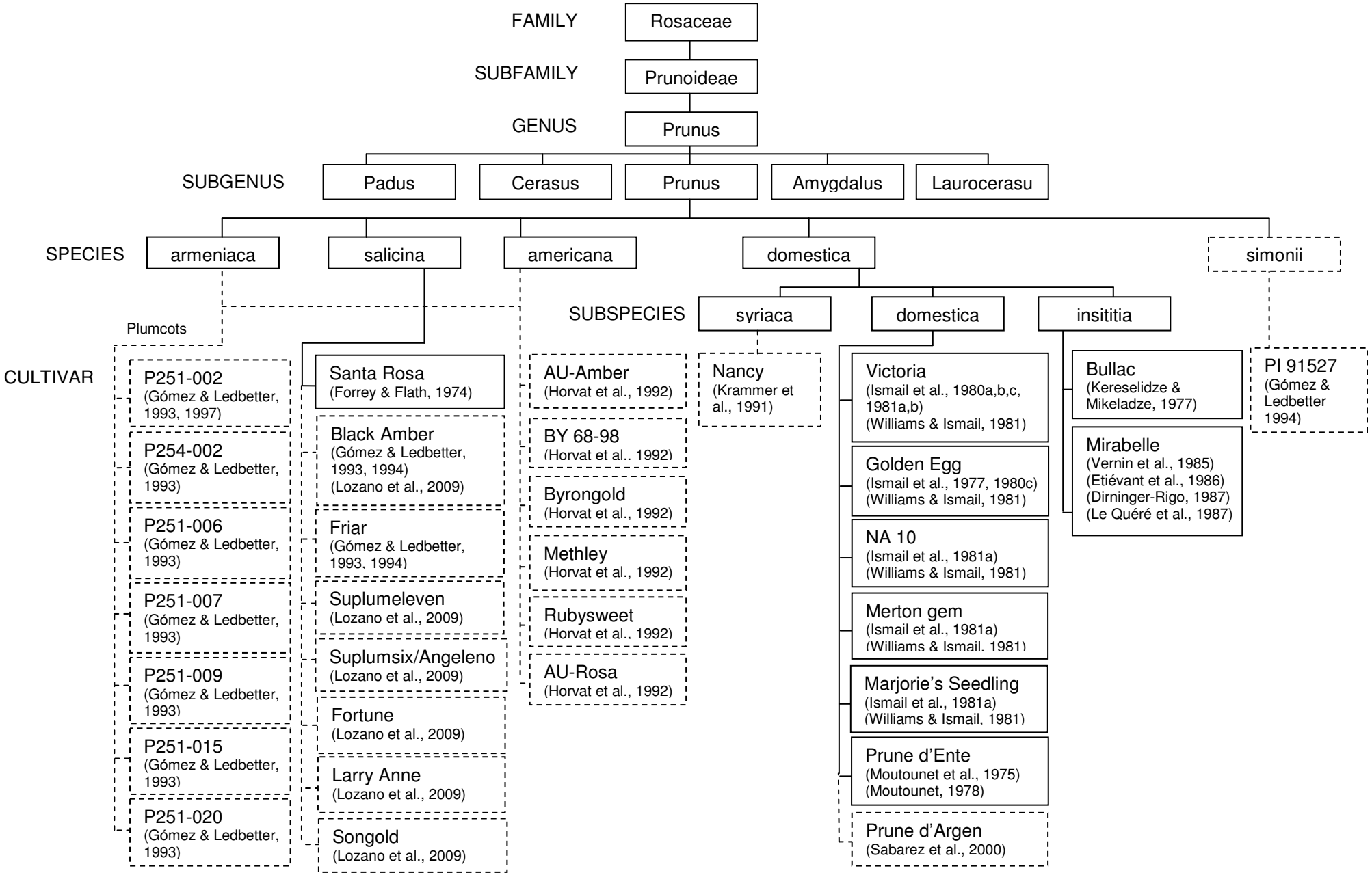
Table 1: Relationship between the different plum and plumcot cultivars studied for their aroma reported in the literature (1974 – 2010). Redrawn and updated from Crouzet et al. (1990). - - - - indicates updated information.



PAPER 1

Robust prediction models for quality parameters in Japanese plums (*Prunus salicina* L.) using NIR spectroscopy.

Abstract

Fourier-transformed near infrared (FT-NIR) reflectance spectroscopy was used over a spectral range of 800-2700 nm to develop multivariate prediction models for total soluble solid (TSS), total acidity (TA), sugar-to-acid ratio, firmness and weight in three South African plum cultivars (Pioneer, Laetitia and Angeleno) and a multi-cultivar model. Samples were collected for seven weeks throughout the ripening period and repeated over two seasons. The validation results had mixed success with TSS (R^2 = 0.817-0.955; RMSEP= 0.453-0.610 % Brix), TA (R^2 =0.608-0.830; RMSEP=0.110-0.194% malic acid), sugar-to-acid ratio (R^2 = 0.718-0.896; RMSEP= 0.608-1.590), firmness (R^2 = 0.623-0.791; RMSEP= 12.459-22.760 N) and weight (R^2 = 0.577-0.817; RMSEP= 7.700-12.800 g). The cultivar-specific models of 'Pioneer' and 'Laetitia' had a better predictability capacity than the 'Angeleno' model on all parameters. Although the multi-cultivar model for TSS, TA and sugar-to-acid ratio outperformed the single cultivar models on R^2 values, they had higher prediction errors. The robustness of all the TSS, TA and firmness models is high in terms of seasonality, range and cultivar.

Key words:

firmness, near infrared (NIR) spectroscopy, robustness, prediction models, TA, TSS, weight

Note:

This chapter has been published in *Postharvest Biology and Technology (2010), Vol 58, Iss 3, pp 176-184.* Since publication we have extended the paper to include data and a discussion on model robustness in terms of cultivar. Additional data can be found in Table 6 and the discussion in the latter part of section 3.6 Model robustness.

Introduction

Japanese plums (*Prunus salicina* L.) have been bred and cultivated in South Africa for many decades. With more than eight million cartons of 35 different cultivars exported annually, plums are one of the most diverse fruit tree species traded internationally. As plums have a relatively short shelf life South Africa is geographically placed at a disadvantage when exporting plums to European markets. As sea freight is the most economical mode of transporting fresh fruit from South Africa plums are harvested relatively unripe but physiologically mature and ripen while in transit for up to 42 days. This makes determining and monitoring of quality parameters in the orchard, pack house and delivery points crucial in producing a product that is acceptable to the end user.

Consumer acceptance studies have shown that total soluble solid (TSS) concentration and titratable acidity (TA) are two important quality parameters in plums (Crisosto and Bowerman, 2003; Crisosto and Crisosto, 2005). Fruit firmness has been proven to indicate an acceptable shelf life (Valero et al., 2007) and can be used successfully to determine the optimum harvest date of plums (Guerra and Casquero, 2008). The weight of fruit is not directly associated with fruit quality or consumer acceptance, but can be an indication of water loss and shrivel that have a negative impact on fruit appearance.

Currently all the quality parameters are determined using destructive measures viz. paring and crushing to determine firmness and juicing to measure TSS and TA. As this makes it impossible to test every unit of fruit a statistically determined subset of a batch is tested and the results are taken as representative of the entire batch. Large variability can exist between individual fruit as a result of pre-harvest factors (climatic conditions e.g. winter chilling, soil type, bearing position of fruit on the tree, age of the tree, irrigation and fertilization schedules, etc) or post-harvest factors (time of harvest, pre-cooling, handling and storage practices, etc) and with numerous quality parameters to test it can be difficult to accurately assess the quality of the entire batch of fruit. The South African plum industry can benefit from nondestructive technology that rapidly and accurately predicts the quality parameters of individual fruit.

Near Infrared (NIR) spectroscopy can possibly serve as a non-invasive technique to determine quality in plums as it interacts with molecular groups associated with quality parameters such as sugars (C-H group), acids and moisture (O-H group), and scattering from microstructures (Abu-Khalaf and Bennedsen, 2002; Nicolaï et al., 2007) can indirectly indicate physical parameters. Most of the NIR absorption bands associated with these groups are overtone or combination bands of the fundamental absorption bands in the infrared region which are due to vibrational and rotational transitions (Nicolaï et al., 2007). Exposing intact fruit samples to NIR spectroscopy will produce an absorption pattern of the chemicals present in the fruit in a rapid and non-destructive way. These spectra can then be manipulated using multivariate data analysis techniques to develop prediction models for each measured variable. Although the initial model

building will require reference data based on the traditional destructive methods a robust model can thereafter be used to predict the quality parameters nondestructively.

When considering a prediction model it is important to take note of the type of validation method that was used. Many different validation methods are available and the choice is often driven by logistics or cost. However, there is no better validation than testing on an entirely independent data set (external validation). NIR spectroscopy prediction models (using different validation methods) have been reported for numerous fruit and vegetable types focusing on TSS, TA and firmness as the predicted quality parameter. Some of these include models for apricots (Bureau et al., 2009; Camps and Christen, 2009), tomatoes (Flores et al., 2009), loquats (Fu et al., 2009), apples (Lui et al., 2007; Peirs et al., 2005; Paz et al., 2009), mangos (Schmilovitch et al., 2000), pears (Lui et al., 2008; Cavaco et al., 2009), kiwi fruit (McGlone and Kawano, 1998), watermelons (Ito et al., 2002), peaches (Ma et al., 2007), nectarines (Peréz-Marín et al., 2009), prunes (Slaughter et al., 2003) and plums (Paz et al., 2008; Onda et al., 1994; Abu-Khalaf and Bennedsen, 2002).

This study aims to determine if NIR spectroscopy can be used as a non-destructive alternative for the accurate prediction of quality parameters such as TSS, TA, sugar-to-acid ratio, firmness and weight in three South African plum cultivars harvested at different stages of ripeness over two seasons.

2. Material and Methods

2.1 Plum fruit selection:

Fruit from three plum (*Prunus salicina* L.) cultivars grown near Stellenbosch (Western Cape, South Africa) were used in this study. The cultivars selected were Pioneer, Laetitia and Angeleno. Eighty fruit were collected weekly over a seven week period starting three weeks prior to the expected commercial harvest date and continuing for three weeks thereafter. Fruit of similar size and colour were selected from the middle of the canopy approximately 1.5 m above the orchard floor. Non-destructive NIR measurements were taken on the same day as harvest and the destructive measurements were done within 36 hours after harvest. Fruit were stored at ambient temperature and not exposed to any postharvest treatments prior to processing. The study was conducted over two plum seasons (2007 and 2008) with total fruit numbers of 1200 for ‘Pioneer’ (8 harvest weeks in 2008), 1120 for ‘Laetitia’ and 1040 for ‘Angeleno’ (6 harvest weeks in 2008).

2.2 Non-destructive near-infrared spectrum collection

FT-NIR spectra were obtained using a multi-purpose analyser (MPA) spectrometer (Bruker Optics, Ettlingen, Germany) fitted with a solid probe fiber optics module containing a high sensitivity, thermoelectrically cooled InGaAs detector with a tungsten lamp as the NIR source. For each plum the probe (5 mm diameter with roughly 100 optic fibers) was directed onto the skin of two opposite sides of

the intact fruit and an absorbance spectrum covering a wavelength range of 800–2700 nm (resolution of 8 nm, scanner velocity of 10 KHz) was captured through reflectance geometry. For each spectrum the average of 16 scans with a resolution of 8 nm was used. A white Spectralon tile was used as a 100% reflective background reference.

2.3 Determination of fruit quality parameters (reference data)

The reference data were collected using the conventional destructive methods. Fruit weight was determined in grams using a calibrated balance (GÜSS GS20 FTA, Cape Town, South Africa). Flesh firmness was measured in kilograms on two opposite, pared sides of the fruit after exposing the flesh to an electronic penetrometer (GÜSS GS20 FTA, Cape Town, South Africa) fitted with an 11.0 mm tip. All values were converted to Newton by multiplying with 9.81. To determine the total soluble solids (TSS) the fruit were juiced individually using a commercial fruit blender. A drop of juice from each fruit was placed onto a temperature-controlled, digital refractometer (Palette PR-32 ATAGO, Bellevue, USA) which measured the TSS levels in % Brix. Total acid (TA) was expressed as % malic acid by titrating a 10 g aliquot of the individual plum juice with 0.1 M NaOH to a pH end-point of 8.2 using an automated titrator (Metrohm AG 760, Herisau, Switzerland). In cases where the fruit were very small and did not produce enough juice (<10 g) the juice of up to three plums was pooled, measured and given the same TA value. Data from the three cultivars were pooled to create reference data for the multi-cultivar model. The mean and standard deviation values were determined for each quality parameter (Table 1).

2.4 Chemometric data analysis

OPUS version 6.1 (Bruker Optics, Ettlingen, Germany) chemometric software was use to perform all the multivariate calculations. Spectral parameters were selected using the “Optimize” function of the software which checks common wavelength frequency regions in combination with several data preprocessing methods. The software then yields a list of the possible parameter combinations and the resulting RMSECV value and number of latent variables. From this we selected the method that presented the best all-round performance (in terms of frequency region, number of latent variables and error) for each model (Table 1). Only the informative frequency regions for each spectrum were retained from the initial wavelength interval of 800 – 2700 nm and used in further calculations. The partial least square (PLS) regression method (including mean centering) was applied to the transformed data to create prediction models for each of the quality parameters. Outliers were quantified and removed by deriving a threshold value using the Mahalanobis distance of each calibration spectrum. To construct calibration models with high robustness we combined all data for each cultivar (2007 and 2008 seasons) and split it into two equal, unique subsets. One subset was used to build the calibration model before testing it internally via cross validation (leaving out 10 samples) to determine the complexity by using the number of latent variables (LV’s) that presented the lowest RMSECV. The second subset was then used to do an external validation of the calibration model using the complexity as calculated by the cross validation. To illustrate

the robustness in terms of seasonality the data were split into the two seasons (2007 and 2008). Data from one season were used as a calibration set and tested internally via cross validation to determine complexity. This was followed by an external validation using data from the other season. Robustness in terms of range was illustrated by reducing the sample collection period from the initial 7 weeks (W1-W7) to only 3 weeks (W3-W5) including the week of commercial harvest (W4) and the two flanking weeks. Using less data in this way reduces the range of each of the variables when compared to the initial model. Again each model was tested using a cross validation and complexity was determined by the lowest RMSECV. External validation was done twice for each model, firstly using a reduced validation set also only containing data from W3-W5 ("reduced validation") and then secondly using the full validation set containing data from W1-W7 ("full validation"). In all cases the spectra from the three cultivars were also pooled to create spectral data for the multi-cultivar model. When investigating the effect of cultivar, multi-cultivar models, containing two or more cultivars, were constructed by pooling the calibration data for the respective cultivars and validating externally using the single cultivar data sets after a cross validation was performed to determine model complexity.

Model performance is described by the following statistical terms:

- **b-coefficient:** This value represents the "b" in the regression line $\hat{Y}_i = a + b_1X_{1i}$ that is calculated using the least square method. This value can be seen as an indication of the accuracy of the model. The closer the value is to 1, the more accurate the model will be. This is also referred to as the regression slope.
- **R^2:** The coefficient of determination (R^2) gives the variance present in the true values which is reproduced in the prediction. R^2 approaches 1 as the predicted values approach the true values. This value indicates the precision of the model. R^2 will be negative when the residuals are larger than the variance in the true values.
- **RMSECV** (Root Mean Square Error of Cross Validation): Indicates the modeling error or calibration variance, thus the imprecision (quality) of the calibration model when tested internally. The smaller the value the better the model.
- **RMSEE** (Root Mean Square Error of Estimation): Indicates the modeling error or calibration variance, thus the imprecision (quality) of the calibration model when tested externally. The smaller the value the better the model.
- **RMSEP** (Root Mean Square Error of Prediction): Indicates the prediction error or validation variance, thus the imprecision (quality) of the validation model. The smaller the value the better the model.
- **Number of latent variables (LV's):** The number of PLS eigen-vectors (factors) used to explain the model. This number also indicates the complexity of the model and is based on the minimum RMSECV.

3. Results and Discussion

3.1 Spectrum description:

The typical NIR spectra for the three cultivars are presented in Fig 1. The spectra are very comparable to the plum profiles presented by Paz et al. (2008) and to those of other fruit such as apricot (Bureau et al., 2008), loquats (Fu et al., 2009), apples (Lui and Ying, 2005) and tomatoes (Flores et al., 2009). The spectra for the respective cultivars appear remarkably similar and all show six broad absorption peaks around the 970, 1190, 1450, 1790, and 2380 nm regions (see arrows in Fig 1). Four of these regions (970, 1190, 1450 and 1940 nm) coincide with the NIR absorbance bands of pure water as described by Rambla et al. (1997) and are due to the O-H stretching overtone at these wavelengths (Polessello and Giangiacomo, 1981). Peaks at 970 and 1190 nm also agree with the second and third C-H overtone regions associated with sugar solutions (Osborne et al., 1993). The peak at 1790 nm overlaps with the first C-H overtone region that is also sugar related. The slight peak at 2380 nm falls within the combinations region associated with the C-H and C-H-combinations grouping. In general the absorbance patterns seen here can be loosely related to the functional groups associated with water and sugar; this is not surprising as plums, like most fruit, consist of 80-90% water and show a rising sugar content throughout ripening.

3.2 Total soluble solids (TSS) prediction model:

The cultivar-specific calibration models for all three cultivars indicated a high correlation between the NIR spectra and the measured TSS values with values: 'Pioneer' (R^2 = 0.966, RMSEE = 0.487%Brix), 'Laetitia' (R^2 = 0.918, RMSEE = 0.411%Brix) and 'Angeleno' (R^2 = 0.849, RMSEE = 0.493%Brix) (Table 2). Model complexity (number of latent variables used) and RMSECV, determined using cross validation, are also presented in Table 2. The high model precision indicates that the majority of the variance presented in the measured values is reproduced in the prediction model. After external validation (using an independent data set) the correlations remained similar for all three cultivars values: 'Pioneer' (R^2 = 0.959, RMSEP = 0.520%Brix), 'Laetitia' (R^2 = 0.905, RMSEP = 0.453%Brix) and 'Angeleno' (R^2 = 0.817, RMSEP = 0.569%Brix) (Table 2 and Fig. 2). The b-coefficient for each model (Table 2) indicates an accurate correlation between the tested and predicted values. When compared to other plum cultivar-specific models reported in the literature (Table 3) 'Pioneer' and 'Laetitia' presents some of the highest R^2 values and 'Angeleno' performs similar to a study done on 'Late Royal' plums (Paz et al., 2008). When compared to other stone fruit types similar high prediction capability is seen in TSS models for 'Jinhua' peaches (R^2 = 0.99, RMSEP = 0.939%Brix) (Ma et al., 2007), 'Bergarouge' apricots (R^2 = 0.96, RMSEP = 1.0%Brix) (Camps and Christen, 2009), 'Ravenna' cherries (R^2 =0.97%, RMSEP =0.49%Brix) (Carlini et al., 2000) and 'Sweet Lady' nectarines (R^2 = 0.89, RMSEP = 0.75-0.81%Brix) (Peréz-Marín et al., 2009).

When all the samples from the three cultivars are pooled and a multi-cultivar TSS model is developed, the predictability is very similar to that of the 'Pioneer' model with R^2 = 0.946 and RMSEE = 0.610%Brix.

(Table 2 and Fig. 2). In this study the multi-cultivar TSS model have an equal or better precision than the single-cultivar models (although the RMSEP values increased), this is in contrast to literature that suggests that a single-cultivar model predicts TSS better and more reliably than a multi-cultivar model (Golic and Walsh, 2006). Other multi-cultivar models (Paz et al., 2008; Abu-Khalaf and Bennedsen, 2002; Golic and Walsh, 2006) had a predictability capacity lower than the current study (Table 3), but these studies based their results on single sampling dates or a maximum of three sampling dates within a three week period. It is envisaged that a longer sampling time (range) and inclusion of more than one season, as used in our study, may result in better model performance.

3.3 Prediction of TA (malic acid)

The single-cultivar TA (expressed as % malic acid content) calibration models did not perform as well as the TSS models: 'Pioneer' (R^2 = 0.707, RMSEE = 0.149%), 'Laetitia' (R^2 = 0.830, RMSEE = 0.147%) and 'Angeleno' (R^2 = 0.737, RMSEE = 0.094%) (Table 2). After external validation the precision of all the models decreased: 'Pioneer' (R^2 = 0.618, RMSEP = 0.176%), 'Laetitia' (R^2 = 0.785, RMSEP = 0.160%) and 'Angeleno' (R^2 = 0.608%, RMSEP = 0.110%) (Table 2 and Fig 3). However, when the cultivars are combined into one model the performance increased significantly for both the calibration (R^2 = 0.858, RMSEE = 0.181%) and validation models (R^2 = 0.830, RMSEP = 0.194%) although the error of prediction is highest (Table 2 and Fig. 3).

Published work on NIR calibration models for TA prediction in intact fruit are few compared to TSS and firmness prediction models and are characterised by low concentrations with narrow ranges. Nicolaï et al. (2007) explains this scarcity by stating that the NIR spectrum of fruit and vegetables is dominated by water absorption bands and that the typical low acid concentration (compared to sugar) found in fruit cannot be measured well. Similarly, in a plum study done by Abu-Khalaf and Bennedsen (2002) the TA levels of 'Reine Claude' and 'Blackamber' were measured, but the acidity modeling is stated as not satisfactory with very low validation correlation coefficients. Although low predictability has also been reported for mangos (R^2 = 0.393) (Schmilovitch et al., 2000) and loquats (R^2 = 0.374 – 0.601) (Fu et al., 2009), acceptable TA prediction models have been developed for apricots (R^2 = 0.88) (Bureau et al., 2009), apples (R^2 =0.72) (Lui and Ying 2005; Lammertyn et al., 1998), strawberries (Shao and He, 2008), tomatoes (Pedro and Ferreira, 2007) and mandarins (Gomez et al., 2006).

3.4 Prediction of the sugar-to- acid ratio

Some producers and exporters of fruit prefer to use the ratio of sugar-to-acid as an indication of fruit eating quality rather than just one of the two parameters. This is supported by the notion that the ripening process of plums is characterised by an increase in sugar levels and a decrease in acidity as the fruit matures and that sugars and acids act in synergy to create a specific taste. Modeling the predictability of the sugar-to-acid ratio does not entail any new measurement, but is simply correlating the NIR spectra of

each sample with the value obtained from dividing the TSS value by the TA value for each sample. Thus, the model is based on the mathematical manipulation of the data.

In this study the single-cultivar calibration models were acceptable: 'Pioneer' (R^2 = 0.838, RMSEE = 0.782), 'Laetitia' (R^2 = 0.895, RMSEE = 0.551), and 'Angeleno' (R^2 = 0.806, RMSEE = 1.33), but the multi-cultivar model greatly improves the precision with R^2 = 0.907, RMSEE = 1.340 (Table 2 and Fig 4) with a sharp increase in the margin of error for 'Pioneer' and 'Laetitia'. A similar pattern was observed when the models were validated using the same number of independent samples: 'Pioneer' (R^2 = 0.816, RMSEP = 0.888), 'Laetitia' (R^2 = 0.887, RMSEP = 0.608), and 'Angeleno' (R^2 = 0.718, RMSEP = 1.590) and the multi-cultivar model performing better with R^2 = 0.896, but an increased error with RMSEP = 1.400 (Table 2 and Fig. 4) for 'Pioneer' and 'Laetitia'. No other studies attempted to model the sugar-to-acid ratio.

3.5 Prediction of Firmness

NIR spectroscopy cannot determine fruit firmness directly, but it can give a measurement of the NIR light scattering properties of tissue, such as the cell wall, associated with fruit firmness (Nicolaï et al., 2007). During fruit ripening the cell wall structure changes in terms of the composition of pectins, cellulose and hemicellulose and the scattering and absorption of these carbohydrates can be determined when exposed to NIR light. Carbohydrate absorption bands are well documented for a number of wave lengths between 700 and 1100 nm (Williams and Norris, 1987) with a pectin absorption band identified in the 980 nm region. In a study on pear firmness prediction Cavaco et al. (2009) list at least six physiological mechanisms that occur during the ripening process and discuss the effects they have on the correlation between firmness and reflectance at different wavelength regions. This indicates that the indirect measurement of a physical parameter, such as firmness, is influenced by many factors and it is understandable that some authors have expressed difficulty in interpreting results (Paz et al., 2008; Peréz-Marín et al., 2009).

In our study, the firmness calibration models for 'Pioneer' and 'Laetitia' performed well with R^2 values of 0.832 and 0.814 and RMSEE equal to 21.090 N and 14.028 N, respectively. The 'Angeleno' and multi-cultivar models reflected lower calibration results with R^2 values of 0.638 and 0.707 and RMSEE equal to 12.02 N and 20.209 N, respectively (Table 2 and Fig 5). The external validation of the models resulted in a decrease in the prediction capability of all models including the multi-cultivar model: 'Pioneer' (R^2 = 0.791, RMSEP = 22.760 N), 'Laetitia' (R^2 = 0.766, RMSEP = 15.790 N), 'Angeleno' (R^2 = 0.623, RMSEP = 12.459 N) and the multi-cultivar model (R^2 = 0.637, RMSEP = 22.367 N) (Table 2 and Fig. 5). Only two other studies could be found on the use of NIR spectroscopy to predict firmness in plums, Paz et al. (2008) reported a model for the cultivar Late Royal with a low predictability capacity of (R^2 = 0.52, RMSEP = 2.54 N) compared to that reported by Onda et al. (1994) for a Japanese cultivar, Ooishi Wase (R^2 =

0.83, RMSEP = 2.06 N). Models for other fruit types give better results with good predictability by Fan et al. (2009) for 'Red 'Fuji' apples (R^2 = 0.81, RMSEP = 0.534 kg/cm2), Gomez et al. (2006) for 'Satsuma' mandarin (R^2 = 0.83, RMSEP = 8.53 N), Lui et al. (2008) for 'Fengshui' pears (R^2 = 0.85, RMSEP = 1.232 N) and Peréz-Marín et al. (2009) for 'Sweet Lady' nectarines (R^2 = 0.86, RMSEP = 12.71 N). Some literature stresses that the instrument and method used to measure the reference data and the size of the wave length interval that is measured can influence the quality of the results (Sohn and Cho, 2000).

3.6 Prediction of weight

The calibration results for both 'Pioneer' and 'Laetitia' indicated acceptable predictability with (R^2 = 0.862, RMSEE = 6.8g) and (R^2 = 0.855, RMSEE = 10.1g) for the respective cultivars. The calibration model for 'Angeleno' was less successful with (R^2 = 0.691, RMSEE = 7.2g) (Table 2 and Fig. 6). The multi-cultivar model suggested a fair prediction capability with R^2 = 0.813 but an increased RMSEE of 11.0 g). When validated using independent samples the predictability decreased in all models, especially in the 'Angeleno' model: 'Pioneer' (R^2 = 0.817, RMSEP =7.70g), 'Laetitia' (R^2 = 0.805, RMSEP = 11.9g), 'Angeleno' (R^2 = 0.577, RMSEP = 8.65g) and the multi-cultivar model (R^2 = 0.751, RMSEP = 12.8g) (Table 2 and Fig. 6).

Weight is another physical parameter that cannot be measured directly by NIR spectroscopy, but can be quantified indirectly by measuring fruit water content. Stage III of the double sigmoidal pattern of stone fruit development is characterised by the rapid increase in fruit volume predominantly due to cell expansion through the accumulation of moisture (Brady, 1993). This increase in water content as the fruit ripens contributes most significantly to the weight of the fruit. Only one other study could be found on predicting fruit weight using NIR spectroscopy; Peréz-Marín et al. (2009) developed a calibration model for 'Sweet Lady' nectarines with excellent predictability of (R^2 = 0.97, RMSEP = 6.76g). The authors also link the results to a direct measurement of fruit water content as portrayed by water bands present in the 950 and 1400 nm wavelength regions.

3.7 Model robustness

The degree of validity that a model has in predicting future spectroscopic measurements describes its robustness (Thomas and Ge, 2000). This validity depends on how well the calibration set represents the composition of the new or future data (Peirs et al., 2003). In horticultural studies one of the major causes of low robustness is the high biological variability that can exist among individual samples as described earlier. The best way to develop robust models for biological products is to acquire calibration data over a sufficient period of time to span an appropriate range of instrumental and environmental conditions, however, the disadvantage of this is the inability to know what constitutes a sufficient time period (Peirs et al., 2003). This study includes a relatively large amount of biological variability as each cultivar comprises of eighty weekly samples taken over seven consecutive weeks, over two seasons.

The robustness of the models was illustrated by looking at three aspects: seasonality, range and cultivar. To investigate the importance of including data from more than one season into the calibration model, the original data were split into the two seasons (2007 and 2008) and a calibration model was constructed using data from one season and validating it externally using the data from the other season. This resulted in 2 sets of models (e.g. 2007 calibration–2008 validation, and 2008 calibration-2007 validation models) that can be compared to the original model containing data from both seasons. See Table 4 for results on TSS, TA and firmness models. The results clearly show that models containing data from only a single season have a lower predictability and a higher margin of error. This effect was not as significant in the TSS models as in the TA and firmness models where some R^2 values became negative and RMSEP values doubled. The sharp decline in robustness is due to the lack of biological variability associated with the data sets from a single season. This illustrates that the original calibration models constructed in this study are robust in terms of seasonality as they comprise data from more than one season.

The second aspect used in investigating the model robustness involves the reduction of the range of the variables. To achieve this the original data set (W1-W7) was reduced in terms of time period to include only the data from the three consecutive weeks (W3-W5) spanning the week of commercial harvest (W4). As mentioned earlier, the external validations were done using a data set with reduced range and a data set containing data from the full seven weeks. See Table 5 for results on TSS, TA and firmness models. The results show that the reduced calibration models are almost always poorer than the original calibration models, but more importantly they also indicate that when the reduced models are validated using samples from the full validation set the predictability drops and the RMSEP increases drastically. The sharp decline in the model performance is due to the fact that the model is now forced to predict values from samples that fall outside of the range of the calibration model. This suggests that the original calibration models constructed in this study are robust in terms of range as they contain data from a wide time period increasing their biological variability. Interestingly, when considering the relatively constant performance of the multi-cultivar models compared to the single cultivar models when testing the robustness in the above cases, it is evident that including more cultivars into a calibration model can increase the biological variability and range of a model with limited data and will thus increase the robustness and predictability of future samples.

This brings us to the last aspect of robustness, namely cultivar. The models were tested in terms of cultivar by validating them (single cultivar, and multi-cultivar - including both two and three cultivars) using data from the single cultivars, e.g. comparing the Laetitia calibration model when validated using Laetitia data, Pioneer data and Angeleno data, or comparing a Pioneer-Laetitia calibration model when validating using the single cultivar data sets. Table 6 contains the results from the different validations using TSS as a variable. This exercise shows that, as expected, a model performs at its best when tested using data

from the same cultivar and worst when validated using a data set that does not contain any samples from that specific cultivar, e.g. Laetitia model performs highest when used on the Laetitia only validation set (R^2 = 0.905; RMSEP = 0.453% Brix) and worst when validated with Pioneer only (R^2 = -0.852; RMSEP = 1.99% Brix) and Angeleno only (R^2 = 0.542; RMSEP = 0.991% Brix) data. The calibration models containing two cultivars perform very well against validation sets containing the same cultivar and poorer against validation sets containing samples of a different cultivar. E.g. Pioneer–Laetitia model and Laetitia-Angeleno model show validation results of (R^2 = 0.895; RMSEP = 0.482% Brix) and (R^2 = 0.887; RMSEP = 0.500% Brix), respectively when validated with Laetitia only data but the Pioneer-Angeleno model has a much lower performance of (R^2 = 0.697; RMSEP = 0.806% Brix) when tested against the same data set. This drop in predictability is due to the fact that the Pioneer-Angeleno model does not contain any Laetitia samples and thus does not have the biological variability to successfully predict future Laetitia samples. This drop in predictability is, however, rectified when the Laetitia data are included in the Pioneer-Angeleno model to form the "All cultivars" model with results of R^2 = 0.886 and RMSEP = 0.501% Brix when validated using the Laetitia only samples. It is however important to note that although the multi-cultivar models produce R^2 values equal to that of the single cultivar models, the error (RMSEP) is always higher and therefore we suggest that single cultivar models are best in terms of robust predictability.

In summary we illustrated that models will be more robust if the calibration data comprise samples from the appropriate cultivar, taken over a wide period of time in any given season and over more than one season. This will ensure that the model contains enough biological variability to make accurate, reliable predictions.

4. Conclusion

The results show that non-destructive NIR spectroscopy can be used to develop relatively accurate prediction models for internal quality traits of South African plums such as TSS, TA, sugar-acid ratio and firmness. The quality of the model performance was cultivar specific with 'Pioneer' and 'Laetitia' models presenting a high predictability for all the quality parameters tested and fair predictability for TA content. The models for 'Angeleno' delivered only acceptable predictability for the TSS measurement. Although the multi-cultivar models often outperformed the single-cultivar models in terms of R^2 values, it is still suggested that a single-cultivar model should be developed rather than a multi-cultivar model as all the single-cultivar models have smaller prediction errors. However, when calibration models are low in robustness the inclusion of more than one cultivar can boost the predictability as it often increases its range. The TSS, TA and firmness models for all the cultivars show a high degree of robustness over the two seasons as well as when reduced in terms of range. The cultivar(s) used in the construction of the calibration model is of utmost importance to maintain good predictability. These findings can be utilised by the South African plum industry to develop a grading/sorting system that is based on the rapid and accurate prediction of various quality parameters of each unit of fruit.

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Table 1: Mean, standard deviation (SD), range, frequency regions and pre-processing methods used for the calibration and validation subsets of 'Pioneer' (*n* = 1200), 'Laetitia' (*n* = 1120), 'Angeleno' (*n* = 1040) and all cultivars grouped together (*n* = 3360). *n* = total number of samples. *Standard Normal Variate correction.

Cultivars, parameters and data sets			Mean (±SD)	Range	Informative frequency regions (nm)	Pre-processing method
TSS (%Brix)	**Pioneer**	Cal	10.34 (2.72)	5.7-18.3	1820.4 - 800.4	Multiplicative Scattering Correction
		Val	10.21 (2.70)	5.8-18.9		
	Laetitia	Cal	10.10 (1.44)	7.0-14.0	1820.4 - 1310.2 1140.6 - 800.4	Multiplicative Scattering Correction
		Val	10.12 (1.53)	7.1-14.6		
	Angeleno	Cal	14.01 (1.37)	10.3-17.8	1650.3 - 1310.2 970.5 - 800.4	Min-Max normalization
		Val	13.98 (1.44)	10.5-17.6		
	All cultivars	Cal	11.36 (2.64)	5.6-22.3	1820.4 - 800.4	First derivative and vector normalization (SNV*)
		Val	11.35 (2.67)	5.7-18.3		
TA (%malic acid)	**Pioneer**	Cal	1.80 (0.29)	0.89-2.54	1820.4 - 1479.7 1310.6 - 800.4	Multiplicative Scattering Correction
		Val	1.79 (0.29)	0.95-2.51		
	Laetitia	Cal	1.97 (0.39)	1.05-2.77	1820.4 - 1649.8 1310.6 - 970.0	Vector normalization (SNV*)
		Val	1.94 (0.35)	0.89-2.73		
	Angeleno	Cal	1.07 (0.19)	0.64-1.74	1820.4 - 1479.7 1310.6 - 1140.1 970.5 - 800.4	Vector normalization (SNV*)
		Val	1.09 (0.48)	0.67-1.69		
	All cultivars	Cal	1.63 (0.49)	0.67-2.73	1820.4 - 1649.8 1310.6 - 800.4	Multiplicative Scattering Correction
		Val	1.23 (0.52)	0.64-2.77		
Sugar-to-Acid ratio	**Pioneer**	Cal	5.98 (2.26)	3.23-13.44	2499.6 - 1989.4 1820.4 - 1479.7 1140.6 - 800.4	Straight line subtraction
		Val	5.95 (2.25)	3.05-12.48		
	Laetitia	Cal	5.46 (1.85)	1.79-12.87	1820.4 - 1649.8 1480.2 - 800.4	Multiplicative Scattering Correction
		Val	5.53 (1.88)	2.98-11.50		
	Angeleno	Cal	13.65 (3.16)	6.89-22.17	1650.3 - 1310.2 970.5 - 800.4	Min-Max normalization
		Val	13.6 0(3.21)	7.63-22.68		
	All cultivars	Cal	8.18 (4.42)	2.98-22.68	1650.3 - 1479.7 1310.6 - 1140.1 970.5 - 800.4	Vector normalization (SNV*)
		Val	8.17 (4.40)	1.79-22.17		
Weight (g)	**Pioneer**	Cal	54.11 (18.49)	22-96	1820.4 - 1649.8 1140.6 - 800.4	Vector normalization (SNV*)
		Val	53.93 (18.06)	20-95		
	Laetitia	Cal	89.27 (27.21)	44-165	1820.4 - 1649.8 1310.6 - 800.4	Multiplicative Scattering Correction
		Val	89.63 (28.04)	42-175		
	Angeleno	Cal	80.31 (13.32)	47-114	1820.4 - 1649.8 1310.6 - 800.4	Constant offset elimination
		Val	80.71 (13.44)	53-117		
	All cultivars	Cal	73.94 (25.59)	20-162	1820.4 - 1649.8 1310.6 - 800.4	Multiplicative Scattering Correction
		Val	74.12 (25.95)	22-161		
Firmness (N)	**Pioneer**	Cal	90.7 (71.9)	5.0-195.2	1820.4 - 1479.7 1140.6 - 800.4	None
		Val	90.0 (68.3)	5.7-194.2		
	Laetitia	Cal	91.3 (32.6)	10.1-166.7	1820.4 - 1479.7 1310.6 - 800.4	Constant offset elimination
		Val	92.2 (33.0)	10.9-175.5		
	Angeleno	Cal	94.5 (21.9)	42.0-159.6	1820.4 - 1140.1 970.5 - 800.4	Vector normalization (SNV*)
		Val	92.6 (21.1)	28.6-148.1		
	All cultivars	Cal	92.1 (47.9)	5.0-196.2	1820.4 - 1649.8 1140.6 - 800.4	Vector normalization (SNV*)
		Val	91.5 (45.9)	7.6-196.2		

Table 2: Model performance for each quality parameter of 'Pioneer' (*n* = 1200), 'Laetitia' (*n* = 1120), 'Angeleno' (*n* = 1040) and all cultivars grouped together (*n* = 3360). *n* = total number of samples.

Cultivars and quality parameters		LVs	RMSECV	Calibration Model		Validation model		
				R^2	RMSEE	R^2	RMSEP	b-coefficient
TSS (% Brix)	**Pioneer**	10	0.541	0.966	0.487	0.959	0.520	0.946
	Laetitia	10	0.463	0.918	0.411	0.905	0.453	0.913
	Angeleno	10	0.565	0.849	0.493	0.817	0.569	0.806
	All cultivars	12	0.598	0.958	0.537	0.946	0.610	0.942
TA (% malic acid)	**Pioneer**	8	0.171	0.707	0.149	0.618	0.176	0.605
	Laetitia	11	0.175	0.830	0.147	0.785	0.160	0.853
	Angeleno	10	0.111	0.737	0.094	0.608	0.110	0.672
	All cultivars	10	0.204	0.858	0.181	0.830	0.194	0.863
TSS:TA	**Pioneer**	8	0.848	0.838	0.782	0.816	0.888	0.760
	Laetitia	10	0.637	0.895	0.551	0.887	0.608	0.871
	Angeleno	10	1.540	0.806	1.33	0.718	1.590	0.756
	All cultivars	11	1.490	0.907	1.340	0.896	1.400	0.914
Firmness (N)	**Pioneer**	10	24.231	0.832	21.090	0.791	22.760	0.806
	Laetitia	11	15.790	0.814	14.028	0.766	15.790	0.798
	Angeleno	9	13.087	0.638	12.202	0.623	12.459	0.617
	All cultivars	11	23.152	0.707	20.209	0.637	22.367	0.675
Weight (g)	**Pioneer**	8	8.190	0.862	6.800	0.817	7.700	0.828
	Laetitia	7	11.600	0.855	10.100	0.805	11.900	0.838
	Angeleno	10	8.38	0.691	7.200	0.577	8.650	0.604
	All cultivars	12	13.000	0.813	11.000	0.751	12.800	0.786

Table 3: Performance of published TSS prediction models for plums (single and multi-cultivar models).

Plum cultivar	R^2	RMSEP or SEP (%Brix)*	Reference
Pioneer	0.96	0.55	This study
Pioneer, Laetitia and Angeleno	0.95	0.61	This study
Ooishi Wase	0.92	0.41	Onda et al., 1994.
Laetitia	0.91	0.45	This study
Amber Jewel, Autumn Giant, Black Beauty, Queen Rosa and Santa Rosa	>0.90	<0.64	Golic and Walsh, 2006.
Autumn Giant	0.89	0.69	Walsh et al., 2007.
Late Royal	0.83	0.77	Paz et al., 2008.
Angeleno	0.82	0.57	This study
Reine Claude and Blackamber	0.80	1.56	Abu-Khalaf and Bennedsen, 2002.
African Pride, Black Diamond, Fortune, Laetitia, Larry Anne, Late Royal, Prime Time, Sapphire and Songold	0.72	0.86	Paz et al., 2008.
Fortune	0.71	0.48	Paz et al., 2008.
Not specified	0.71	0.47	Walsh et al., 2004.

*Assuming that $Bias^2 \approx 0$ and therefore RMSEP = SEP

Table 4: Model performance when splitting data into the two seasons (2007 and 2008) and using both sets respectively for validation and calibration. "Original" models represent the models were 2007 and 2008 data were pooled.

Cultivars, quality parameters and models			LV's	RMSECV	Calibration Model		Validation Model		
					R^2	RMSEE	R^2	RMSEP	b-coefficient
TSS (% Brix)	**Pioneer**	**Original**	10	0.541	0.966	0.487	0.959	0.520	0.946
		2007 Cal/ 2008 Val	9	0.535	0.964	0.506	0.897	0.710	0.951
		2008 Cal /2007 Val	9	0.556	0.946	0.518	0.923	0.732	0.810
	Laetitia	**Original**	10	0.463	0.918	0.411	0.905	0.453	0.913
		2007 Cal/ 2008 Val	8	0.451	0.919	0.425	0.527	0.801	0.557
		2008 Cal /2007 Val	9	0.454	0.872	0.419	0.768	0.717	0.665
	Angeleno	**Original**	10	0.565	0.849	0.493	0.817	0.569	0.806
		2007 Cal /2008 Val	10	0.566	0.865	0.497	0.745	0.628	0.825
		2008 Cal /2007 Val	8	0.566	0.808	0.540	0.759	0.656	0.693
	Multi - cultivar	**Original**	12	0.598	0.958	0.537	0.946	0.610	0.942
		2007 Cal /2008 Val	12	0.558	0.955	0.502	0.938	0.669	0.898
		2008 Cal /2007 Val	12	0.593	0.960	0.539	0.919	0.674	0.898
TA (% malic acid)	**Pioneer**	**Original**	8	0.171	0.707	0.149	0.618	0.176	0.605
		2007 Cal /2008 Val	9	0.148	0.734	0.148	0.073	0.232	0.210
		2008 Cal /2007 Val	8	0.168	0.638	0.145	0.025	0.289	0.223
	Laetitia	**Original**	11	0.175	0.830	0.147	0.785	0.160	0.853
		2007 Cal /2008 Val	8	0.159	0.845	0.146	0.276	0.263	0.498
		2008 Cal /2007 Val	8	0.161	0.766	0.150	-0.056	0.379	0.328
	Angeleno	**Original**	10	0.111	0.737	0.094	0.608	0.110	0.672
		2007 Cal /2008 Val	9	0.114	0.729	0.104	-0.044	0.162	0.252
		2008 Cal /2007 Val	9	0.096	0.686	0.088	0.004	0.202	0.130
	Multi - cultivar	**Original**	10	0.204	0.858	0.181	0.830	0.194	0.863
		2007 Cal /2008 Val	11	0.172	0.896	0.156	0.699	0.254	0.733
		2008 Cal/2007 Val	10	0.186	0.871	0.167	0.523	0.334	0.443
Firmness (N)	**Pioneer**	**Original**	10	24.231	0.832	21.090	0.791	22.760	0.806
		2007 Cal /2008 Val	10	18.541	0.894	16.730	0.199	43.948	0.331
		2008 Cal /2007 Val	8	23.616	0.844	19.326	0.466	37.867	0.366
	Laetitia	**Original**	11	15.790	0.814	14.028	0.766	15.790	0.798
		2007 Cal /2008 Val	9	12.750	0.862	11.968	0.032	31.098	0.222
		2008 Cal /2007 Val	8	14.617	0.803	13.930	-0.630	41.300	0.071
	Angeleno	**Original**	9	13.087	0.638	12.202	0.623	12.459	0.617
		2007 Cal /2008 Val	8	12.263	0.646	11.576	0.281	16.971	0.298
		2008 Cal /2007 Val	8	13.538	0.551	12.851	-0.975	27.752	0.104
	Multi - cultivar	**Original**	11	23.152	0.707	20.209	0.637	22.367	0.675
		2007 Cal /2008 Val	11	19.326	0.788	17.680	0.264	30.705	0.339
		2008 Cal /2007 Val	10	21.190	0.729	19.1230	0.204	32.770	0.244

Table 5: Model performance when reducing the original data to only three weeks (W3-W5). "Original" represents the models with no reduction of data (W1-W7). "Red" indicates that the validation data set was reduced to three weeks and "Full" indicates that the validation data set was unchanged using samples from all seven weeks.

Cultivars, quality parameters and models			LV's	RMSECV	Calibration Model		Validation Model		
					R^2	RMSEE	R^2	RMSEP	b-coefficient
TSS (% Brix)	Pioneer	Original	10	0.541	0.966	0.487	0.959	0.520	0.946
		W3 - W5 Red	9	0.420	0.966	0.363	0.940	0.445	0.947
		W3 - W5 Full					0.941	0.655	0.866
	Laetitia	Original	10	0.463	0.918	0.411	0.905	0.453	0.913
		W3 - W5 Red	6	0.456	0.837	0.435	0.819	0.451	0.822
		W3 - W5 Full					0.731	0.760	0.662
	Angeleno	Original	10	0.565	0.849	0.493	0.817	0.569	0.806
		W3 - W5 Red	8	1.380	0.615	1.220	0.591	1.27	0.614
		W3 - W5 Full					0.408	1.63	0.428
	Multi - cultivar	Original	12	0.598	0.958	0.537	0.946	0.610	0.942
		W3 - W5 Red	11	0.540	0.963	0.499	0.956	0.521	0.953
		W3 - W5 Full					0.938	0.657	0.916
TA (% malic acid)	Pioneer	Original	8	0.171	0.707	0.149	0.618	0.176	0.605
		W3 - W5 Red	5	0.142	0.651	0.133	0.564	0.142	0.577
		W3 - W5 Full					-0.429	0.333	0.152
	Laetitia	Original	11	0.175	0.830	0.147	0.785	0.160	0.853
		W3 - W5 Red	8	0.152	0.675	0.132	0.643	0.135	0.637
		W3 - W5 Full					0.645	0.205	0.542
	Angeleno	Original	10	0.111	0.737	0.094	0.608	0.110	0.672
		W3 - W5 Red	8	0.085	0.609	0.073	0.365	0.091	0.441
		W3 - W5 Full					0.183	0.161	0.179
	Multi - cultivar	Original	10	0.204	0.858	0.181	0.830	0.194	0.863
		W3 - W5 Red	12	0.188	0.908	0.146	0.848	1.85	0.884
		W3 - W5 Full					0.683	0.265	0.676
Firmness (N)	Pioneer	Original	10	24.231	0.832	21.090	0.791	22.760	0.806
		W3 - W5 Red	9	18.247	0.743	15.402	0.686	17.756	0.684
		W3 - W5 Full					0.307	42.77	0.305
	Laetitia	Original	11	15.790	0.814	14.028	0.766	15.790	0.798
		W3 - W5 Red	8	14.028	0.546	12.753	0.439	13.734	0.484
		W3 - W5 Full					0.485	23.446	0.413
	Angeleno	Original	9	13.087	0.638	12.202	0.623	12.459	0.617
		W3 - W5 Red	8	13.54	0.624	12.263	0.613	12.164	0.653
		W3 - W5 Full					0.432	15.500	0.488
	Multi - cultivar	Original	11	23.152	0.707	20.209	0.637	22.367	0.675
		W3 - W5 Red	12	17.854	0.585	15.206	0.530	16.383	0.545
		W3 - W5 Full					0.360	29.136	0.296

Table 6: The model performance of single and multi-cultivar calibration models for TSS when validated using data from the single cultivars only.

Cultivars	CALIBRATION MODEL				VALIDATION MODELS					
					Pioneer		Laetitia		Angeleno	
	LVs	RMSECV	R^2	RMSEE	R^2	RMSEP	R^2	RMSEP	R^2	RMSEP
Pioneer	10	0.541	0.966	0.487	0.959	0.520	-0.852	1.990	0.217	1.117
Laetitia	10	0.463	0.918	0.411	0.865	0.996	0.905	0.453	0.428	0.989
Angeleno	10	0.565	0.849	0.493	0.859	1.010	0.542	0.991	0.817	0.569
Pioneer - Laetitia	14	0.531	0.956	0.458	0.951	0.599	0.895	0.482	0.461	0.959
Pioneer - Angeleno	11	0.616	0.965	0.528	0.944	0.638	0.697	0.806	0.817	0.598
Laetitia - Angeleno	12	0.560	0.959	0.485	0.904	0.839	0.887	0.500	0.829	0.557
All cultivars	12	0.598	0.958	0.537	0.947	0.618	0.886	0.501	0.831	0.576

Figure 1: Typical NIR spectra for ‘Pioneer’, ‘Laetitia’ and ‘Angeleno’ fruit taken at the start of fruit ripening.

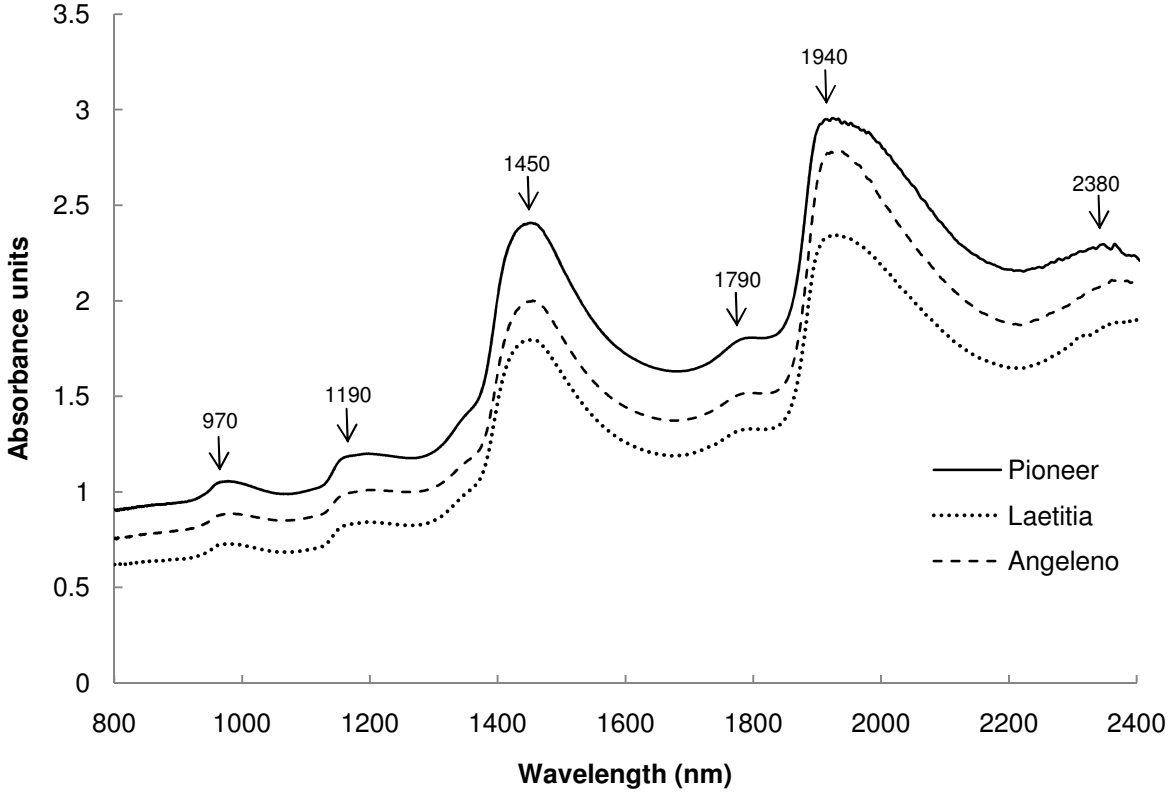


Figure 2: Non-destructive NIR spectroscopy prediction results for TSS (%Brix) plotted against the destructively acquired reference data for 'Pioneer', 'Laetitia', 'Angeleno' and all the cultivars combined.

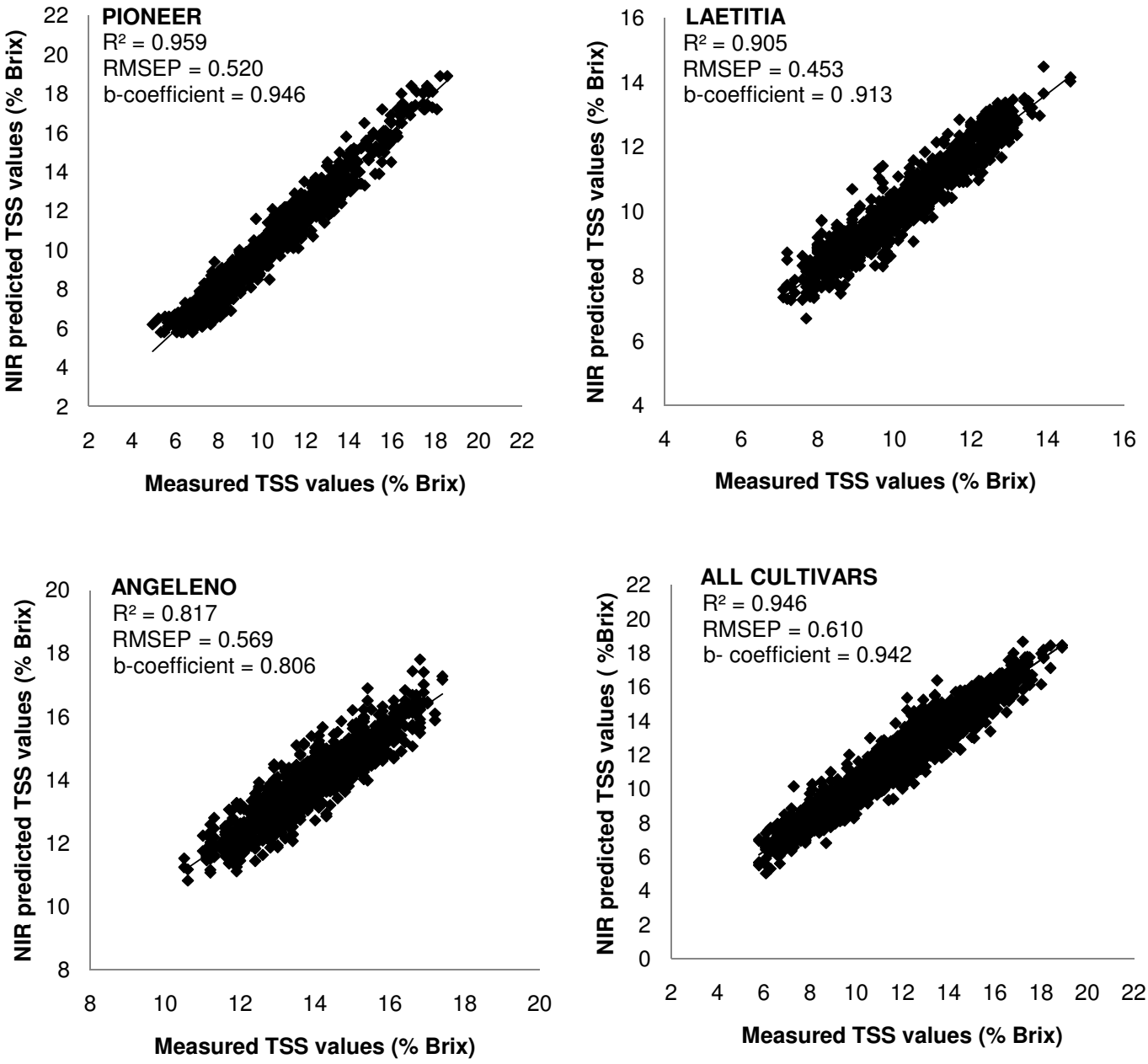


Figure 3: Non-destructive NIR spectroscopy prediction results for TA (%malic acid) plotted against the destructively acquired reference data for 'Pioneer', 'Laetitia', 'Angeleno' and all the cultivars combined.

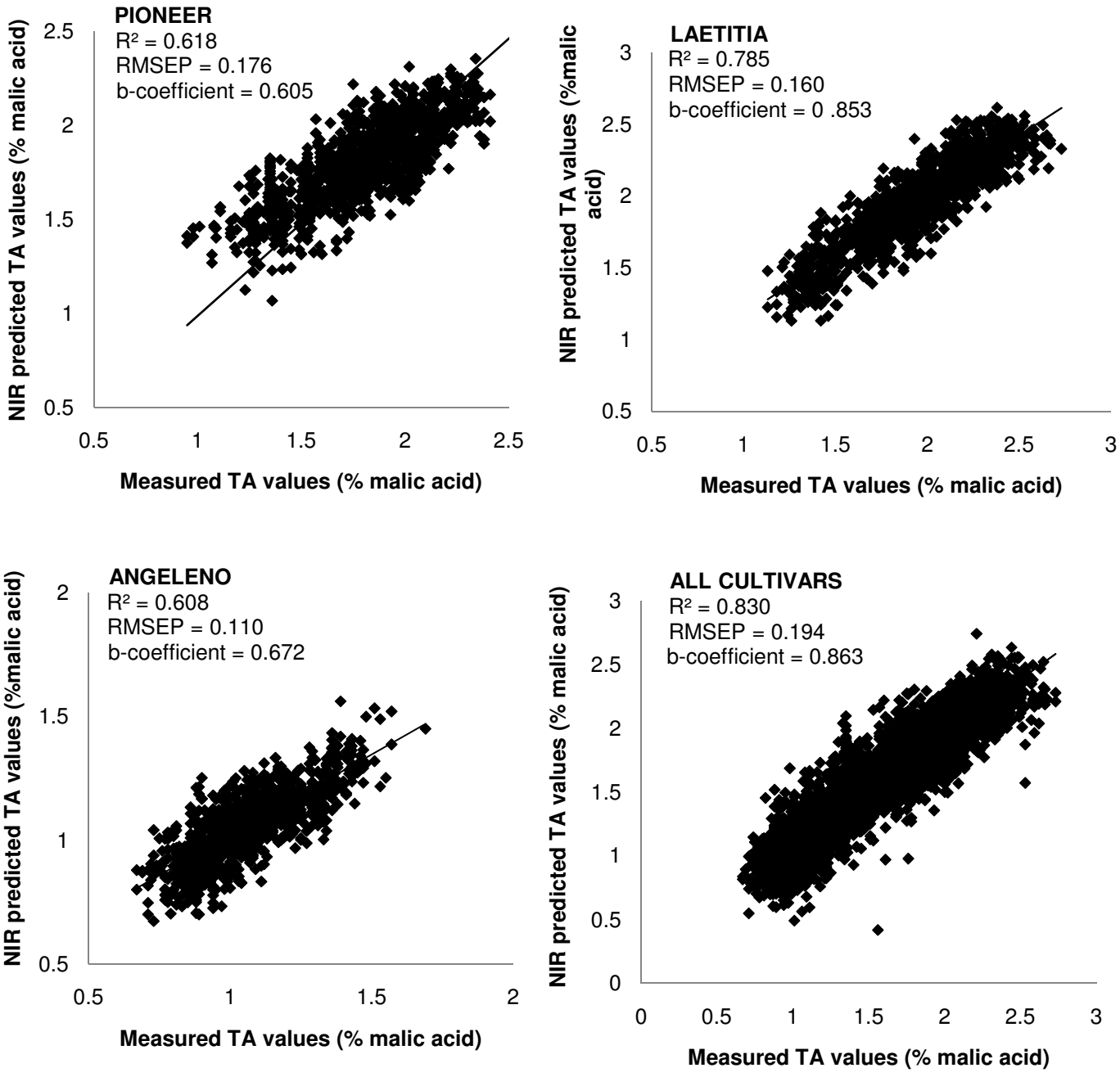


Figure 4: Non-destructive NIR spectroscopy prediction results for TSS:TA plotted against the destructively acquired reference data for 'Pioneer', 'Laetitia', 'Angeleno' and all the cultivars combined.

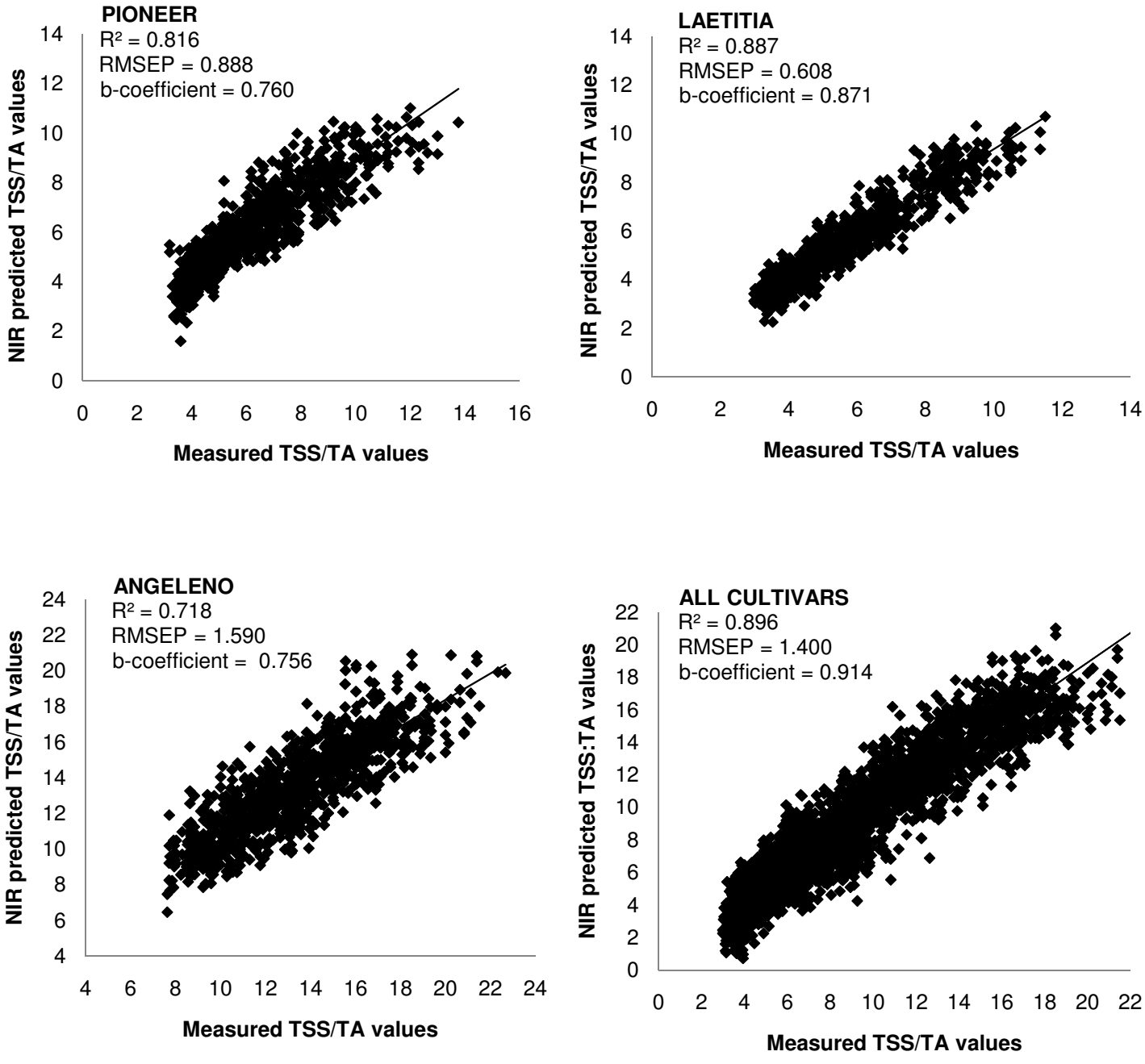


Figure 5: Non-destructive NIR spectroscopy prediction results for firmness (N) plotted against the destructively acquired reference data for 'Pioneer', 'Laetitia', 'Angeleno' and all the cultivars combined.

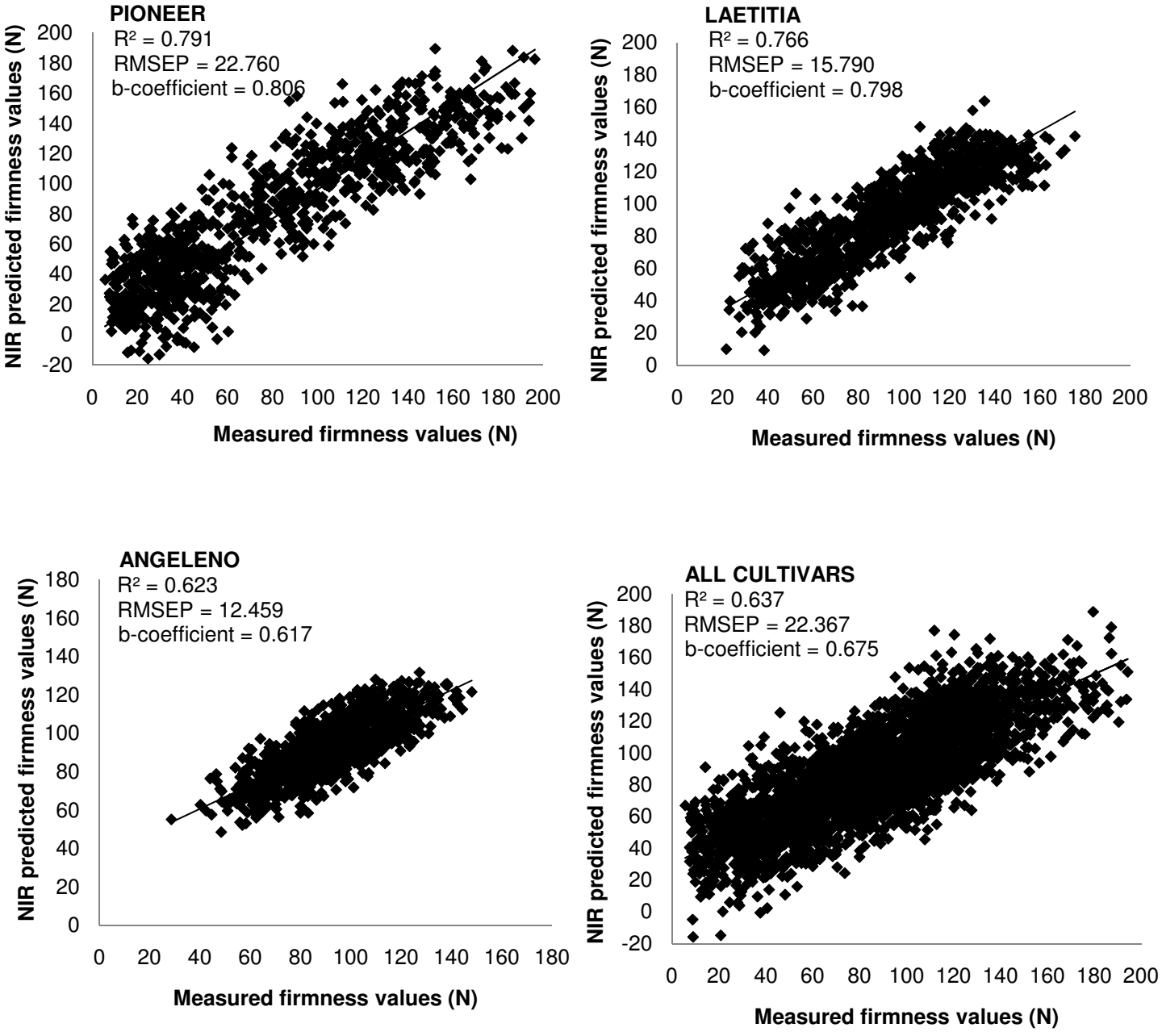
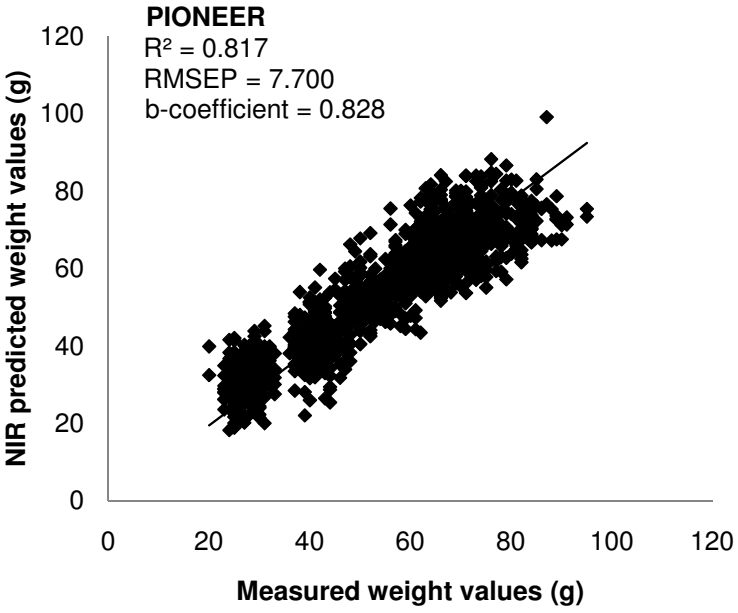
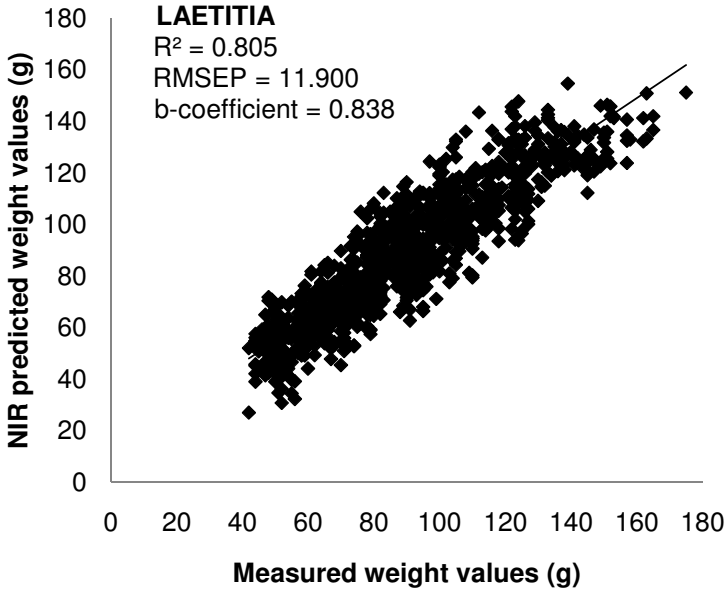
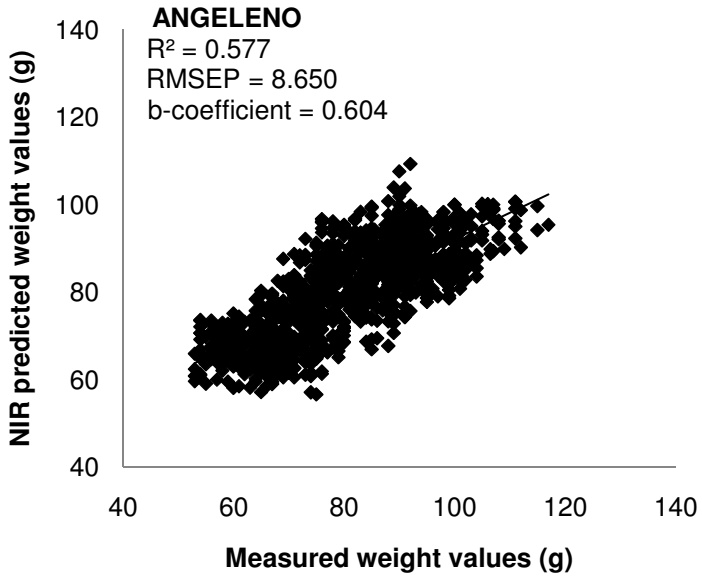
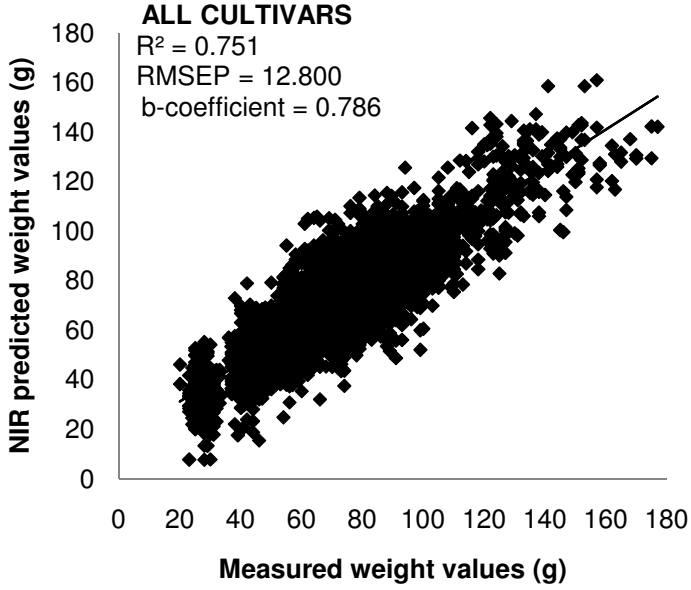


Figure 6: Non-destructive NIR spectroscopy prediction results for weight (g) plotted against the reference data for 'Pioneer', 'Laetitia', 'Angeleno' and all the cultivars combined.









PAPER 2

Aroma volatile dynamics during fruit maturation and ripening of three Japanese plum cultivars (*Prunus salicina* Lindl.).

Abstract

The aroma volatile compounds of three commercial Japanese plum cultivars (Pioneer, Laetitia and Angeleno) were determined for a seven week period to include samples from three different maturity stages (immature, harvest and tree-ripe) over two fruiting seasons. HS-SPME was used for extraction coupled with GC-TOFMS for separation and identification. A total of 35 different compounds was identified with 10 of the compounds found to be generic amongst the three cultivars, viz. hexanal, 2-hexenal, hotreinol, linalool, trans-linalool oxide, cis-linalool oxide, p-menth-1-en-9-al, β-damascenone, 2-bornene and α-terpineol. Each cultivar had five unique compounds resulting in different aroma profiles for each of the maturity stages and distinct separation patterns using discriminant analysis. The compounds contributing most to the distinctness of the maturity stages within a cultivar were identified and found to be different from the compounds identified as important for separating the cultivars.

Key words:

Aroma, volatile compounds, plums, SPME, GC-TOFMS, maturation, ripening

1. Introduction

Plums belong to the family Rosaceae, genus *Prunus* that also includes other stone fruit such as peaches, apricots, nectarines and cherries. When considering the amount of research done on the aroma profiles of the different plum species most of the studies were done prior to 1990 and concentrated on the European plum, *P. domestica*, with aroma descriptions of more than 10 different cultivars (Crouzet et al., 1990). However, the very first publication on plum aroma was done in 1974 by Forrey and Flath on the Japanese plum, *P. salicina* cv. Santa Rosa. Since then only two studies on Japanese plums have been published namely that of Gómez and Ledbetter (1994), describing the aroma constituents of the cultivars Black Amber and Friar and more recently, Lozano et al. (2009) identifying volatile constituents in six Japanese plum cultivars.

Plum fruit are valued by consumers for their colour, palatability and aromatic characteristics. The eating experience is based on both taste and flavour in which non-volatiles such as sugars and acids are mainly responsible for the former and volatile aromatic compounds for the latter (Williams and Ismail, 1981). Like most stone fruit, the physical (firmness, weight, appearance) and non-volatile (sugar, acid) components of plums have been studied well and these are often used as guidelines to establish optimum harvest dates and export grades. However, the flavour components that are responsible for the characteristic plum flavour are not as widely researched as for apricots, peaches and nectarines. The production of aroma volatiles is dynamic and the pattern of volatile constituents, both qualitative and quantitative, can vary greatly during fruit maturation (Agozzino et al., 2007). As each compound has an odour threshold level above which it is detectable to the human palate the mere presence of a compound does not mean it is contributing to the aroma. It is often not a single compound that represents a characteristic flavour, but rather a combination of compounds working in synergy (Williams and Ismail, 1981). This makes it difficult to comment on the character impacting compounds without a thorough olfactory assessment of the compounds.

South Africa has an active Japanese plum breeding and production sector with annual export figures of close to 9 million cartons (5.25 kg equivalent cartons) comprising 35 different plum cultivars. With plum consumer preference now shifting towards flavour and taste (SASPA/Richmond Towers, UK consumer research de-brief, 2006) it has become important to further analyse the aroma profiles of Japanese plums. Thus, the aim of this study is to investigate the aroma profiles of three Japanese plum cultivars (Pioneer, Laetitia and Angeleno) produced under South African conditions and to describe the influence of fruit maturity by comparing immature, harvested and tree ripened fruit within and across the cultivars.

2. Material and Methods

2.1 Fruit selection and harvesting conditions

Fruit from three plum (*Prunus salicina* Lindl.) cultivars grown near Stellenbosch (Western Cape, South Africa) were used in this study. The cultivars selected were Pioneer, Laetitia and Angeleno. Fruit were collected from commercial orchards trained to a flat trellis system. Fruit collection occurred weekly over a seven week period starting three weeks prior to the expected commercial harvest date and continuing for three weeks thereafter. If the fruit had not reached a firmness of below 29.43 N (3 kg) within the seven weeks a further picking date was included. Fruit of similar size and colour were selected from the middle of the canopy approximately 1.5 m above the orchard floor. Fruit were transported to our laboratory, stored at ambient temperature and processed within 24 hours of harvest. No fruit were exposed to any postharvest treatments or cold storage prior to processing. The study was conducted over two plum harvest seasons (2008 and 2009) with total fruit numbers of 83 for 'Pioneer', 90 for 'Laetitia' and 84 for 'Angeleno' (6 harvest weeks in 2008) (Table 1).

2.2 Determination of fruit quality parameters

Fruit quality parameters (firmness, weight, total soluble solids and total acid content) were determined using the conventional destructive methods. Fruit weight was determined in grams using a calibrated balance (GÜSS GS20 FTA, Cape Town, South Africa). Flesh firmness was measured in kilograms on two opposite pared sides of the fruit with an electronic penetrometer (GÜSS GS20 FTA, Cape Town, South Africa) fitted with an 11.0 mm tip. All values were converted to Newton by multiplying with 9.81. To determine the total soluble solids (TSS) the fruit were destoned and juiced individually using a commercial fruit blender (Kenwood). A drop of juice from each fruit was placed onto a temperature-controlled, digital refractometer (Palette PR-32 ATAGO, Bellevue, USA) which measured the TSS levels in % Brix. Total acid (TA) was expressed as % malic acid by titrating a 10 g aliquot of the individual plum juice with 0.1 M NaOH to a pH end-point of 8.2 using an automated titrator (Metrohm AG 760, Herisau, Switzerland). In cases where the fruit were very small and did not produce enough juice (<10 g) the juice of up to three plums were pooled, measured and given the same TA value. The range, mean and standard deviation values were determined for each quality parameter (Table 1).

2.3 Aroma volatile sample preparation

Aroma analysis were carried out on fresh plum puree obtained from individual fruit (flesh and peel) after destoning and blending using a commercial fruit blender (Kenwood). Plums are prone to enzymatic oxidation and the consequent browning of the flesh can cause a deterioration of the aroma (Ismail et al., 1981; Etiévant et al., 1986). Etiévant et al. (1986) also showed that frozen plum samples have a different aroma profile to fresh samples. Both of these profile altering conditions were tested in our laboratory and found to be true (Appendix 1). To counter this, all samples were processed fresh and treated with ascorbic acid to a final concentration of 0.02% immediately after blending by adding 4 ml of the plum

puree into a 10 ml SPME glass bottle already containing a 1 ml solution of ascorbic acid (Sigma, St. Louis, MO, USA). The solution was capped and mixed immediately. As an internal standard, 2-octanol (Sigma, St. Louis, MO, USA) was added to a final concentration of 50 nl/l. To enhance the release of the volatiles from the mixture the "salting-out" effect (Lachenmeier et al., 2006) was created by adding 1 g NaCl (Sigma, St. Louis, MO, USA) to each sample. Each sampling incident comprised six samples representing six individual fruit.

2.4 HS-SPME conditions

All the head-space solid-phase microextractions (HS-SPME) were performed using a PAL Combi autosampler (CTC Analytics, Zwingen, Switzerland) attached to the injector port of the gas chromatograph. Pilot experiments identified the 75 µm poly(dimethylsiloxane) carboxen (PDMS/CAR) SPME fiber (Supelco, USA) to be superior to the 100 µm poly(dimethylsiloxane) (PDMS) SPME fiber (Supelco, USA) for extracting plum volatiles from the head space (Appendix 1). The PDMS/CAR fiber was preconditioned, as prescribed by the manufacturer, at 250°C for 1 hour. SPME sampling conditions were determined during preliminary experiments using a full factorial experimental design (data not shown). From this an extraction time and temperature of 15 min and 85°C produced the highest peak areas. The extracted volatiles were thermally desorbed from the fiber for 90 seconds at 250°C into the glass-lined, splitless injector port of the GC.

2.5 GC-TOFMS conditions and compound identification

Separation and identification of the aroma volatiles were carried out on an Agilent 6890N gas chromatograph (GC) (Agilent Technologies, Palo Alto, California, USA) directly linked to a Waters GCT Premier time-of-flight mass spectrometer (TOFMS) (Micromass, Manchester, UK) and governed by the MassLynx V4.1 software (Waters Laboratory Informatics, 2006). Separation of the volatiles was achieved using a capillary column (BP5, 30 m x 0.25 mm id., 0.25 µm film thickness) (J&W Scientific, Folscom, CA, USA). The carrier gas was helium at a constant flow rate of 1 ml/min. The column temperature program was initially set at 40°C for 5 min, then raised to 150°C at a rate of 5°C/min followed by a further increase up to 280°C at 10°C/min where it was held for 3 min. The total time of a single run was about 35 min. The transfer line to the TOFMS was maintained at 250°C. Aroma volatile detection was performed by TOFMS using electron impact ionization and mass spectra were collected at a rate of 40 spectra over a range of m/z 30 – 350. The ionization energy was 70 eV.

Compounds were primarily identified by comparing their mass spectra to that of the NIST/EPA/NIH mass spectral library using the NIST Mass Spectral Search Program Version 2.0d. Where possible, an additional identification/confirmation was performed by matching the retention time and mass spectrum of commercially acquired standard solutions. Once identified the peaks were reconstructed using only the corresponding spectral masses (m/z) and then integrated by means of the QuantLynx software program

(Waters Laboratory Informatics, 2006). The peak area for each compound was "corrected" by dividing it by the peak area of the internal standard of the same run, this enabled inter-chromatogram comparisons.

The repeatability of the HS-SPME-GC-TOFMS was assessed by comparing the peak areas of six identical samples run back-to-back. The standard deviations of 13 different peaks were determined and expressed as a percentage of the mean. Although the repeatability varied for the different compounds it was on average between 6 and 7% of the mean (Appendix 1).

2.6 Data classifications and statistical analysis

Firmness was used as an indicator of fruit maturity as this is the primary parameter used by the South African export authorities. The data from each cultivar were sorted according to decreasing firmness and then categorised into three maturity classes: immature, harvested and tree-ripened fruit. Immature fruit had a minimum firmness of >93.2 N (>9.5 kg) for 'Pioneer', >83.4 N (>8.5 kg) for 'Laetitia' and >78.5 N (>8.0 kg) for 'Angeleno' and samples consisted of unripe fruit. Harvested fruit consisted of a firmness range of 83.4 - 45.9 N (8.5 - 4.68 kg) for 'Pioneer', 78.5 - 45.9 N (8.0 – 4.68 kg) for 'Laetitia' and 76.5 - 39.2 N (7.80 – 4.00 kg) for 'Angeleno'. These ranges were established based on the export standards and requirements for South African plums. Harvest fruit are considered physiologically mature although not "ripe-and-ready-to-eat". Plums are harvested relatively unripe and ripen whilst in transit to Europe for up to 42 days in temperature controlled containers at sea. Tree ripened fruit consisted of samples with a firmness <39.2 N (<4.0 kg) for 'Pioneer', <29.4 N (<3.0 kg) for 'Laetitia' and <39.2 N (<4.0 kg) for 'Angeleno' and are characterised as "ripe-and-ready-to-eat". The high standard deviations obtained in most of the classes are expected not only because biological variability can be high amongst maturing fruit, but also due to some of the classes, especially the "immature" class, consisting of many samples taken over up to four consecutive weeks and thus depicting the ripening process within the class.

Discriminant Analysis (DA) was performed on the three maturity classes within each cultivar to establish if the classes are indeed distinctly different in terms of their aroma profiles and to explain the classes using the stepwise method identifying the aroma volatiles that contribute significantly to the classes. This will aid in the understanding of the dynamics of the aroma volatiles as the fruit matures and ripens on the tree. Inter-cultivar DA was also performed on every class to identify the aroma profile associated most with each cultivar. All calculations and modeling were performed using XLSTAT Version 2010.4.01. The results of the DA are described using the following statistical terms, figures and tables (Microsoft Windows Help for XLSTAT Version 5.1.2600.5512):

- **Discriminant factors**: For the three maturity groups there are only two discriminant factors (F1 and F2) that will describe 100% of the variance. (The maximum number of factors is equal to k-1,

when n>p>k, where n is the number of observations, p the number of explanatory variables, and k the number of groups.)

- **Observations chart (figure):** This figure represents each of the observations on the factors axes. It allows confirming that the groups are well discriminated on the factor axes extracted from the original explanatory variables.
- **Variables chart (figure):** This figure shows how the initial variables are correlated with the two factors and aims to describe the factor axes.
  Note that observation and variable charts are usually discussed together by overlaying the four quadrants. (Quadrants are numbered clockwise starting with 1st quadrant in the upper right-hand corner).
- **Confusion matrix (table):** This table summarises the reclassification of the observations, and allows to quickly assess the percentage of well classified observations, which is the ratio of the number of observations that have been well classified over the total number of observations.
- **Variable selection table**: This table represents a summary of the variables that contribute most to the groups and are determined using a forwards stepwise analysis to build a model of discrimination. This table also shows the statistics used to evaluate the goodness of fit of the model (determination coefficient for the model (Partial R^2), F-ratio test (F statistic), and p-value at a significance level of 0.05 (Pr>F)).

3. Results

3.1 Quality parameters:

The physical characteristics of both 'Pioneer' and 'Laetitia', as described in Table 1, followed the general trends that one would expect during the maturation and ripening of most fruit. Fruit weight increased as the fruit expanded, firmness decreased as the fruit softened and sugar and acid levels displayed inverse movement with sugar levels increasing and acid levels decreasing creating an increase in the sugar-acid ratio towards the end of the ripening process. 'Angeleno', however, followed these trends in a subdued fashion with virtually no weight increase, a proportionally small decrease in firmness and sugar levels showing only a small increase. Acid levels, to the contrary, decreased similarly to the other cultivars but had already low levels in the immature stage resulting in significantly low levels in the ripe fruit causing the sugar-acid ratio to be much higher than that of the other two cultivars.

3.2 General plum aroma:

The results identified a total of 35 different aroma compounds for the three plum cultivars (Table 2). From this, ten compounds (with relatively large peak areas) were found in all three cultivars: hexanal, 2-hexenal, *trans*-linalool oxide, *cis*-linalool oxide, linalool, hotreinol, α-terpineol, p-menth-1-en-9-al, 2-bornene and β-damascenone. Seven of the ten compounds showed a decreasing trend towards ripening in all three of the cultivars. Hotreinol levels in 'Pioneer' also decreased during ripening but in 'Laetitia' and

especially ‘Angeleno’ there was an increasing trend. 2-Hexanal seemed to stay relatively stable throughout the ripening process for all the cultivars. β-Damascenone showed a similar pattern in all three cultivars where the levels increased from the immature to the harvest stage, but then decreased towards tree ripening to levels equal (‘Pioneer’) or below (‘Laetitia’ and ‘Angeleno’) the levels initially found for the immature stage. However, none of the ten compounds disappeared below the detection level in any of the maturity classes (Table 2).

DA showed that the three cultivars could be separated using only these ten compounds (Fig. 1) with the stepwise model indicating that nine of the compounds (bar 2-bornene) contributed significantly to the separation of the cultivars (Table 3(a)). Factor 1, in Fig 1, seemed to describe the differences between the ‘Pioneer’ group and the ‘Laetitia’-‘Angeleno’ group and factor 2 separating the ‘Laetitia’ and ‘Angeleno’ groups. The DA model predicted 95.42% of the samples correctly into the three maturity classes with most of the confusion between ‘Pioneer’ and ‘Laetitia’ samples (Table 3(b)). The results imply that each of the cultivars has a unique combination of the ten ‘generic’ compounds with little similarity in the levels throughout ripening.

3.3 ‘Pioneer’ aroma profiles:

Of the total 35 compounds identified, only 20 were detected in ‘Pioneer’ samples throughout ripening and the following five compounds were found to be unique: limonene oxide, β-cymene, α-terpinene, unidentified 1 and 2H-pyran,3,6.dihydro-4-methyl-2-(2-methyl-1-propenyl) (Table 2). All of the unique compounds were more associated with the immature stage and relatively low levels were detected in the riper fruit. The immature stage was further characterised by the presence of 14 of the 20 compounds having their highest recorded levels. These levels dropped sharply as the fruit reached the harvest stage with 13 of the compounds losing between 61 and 86% of their initial level. Once ripe, the fruit showed a further reduction in these compounds with only linalool, its two oxides (*trans*- and *cis*-linalool oxide), hotreinol, α-terpineol and p-menth-1-en-9-al maintaining a level above 40% of their harvest level. Only hexanal and 4-(2,4,4-trimetyl-cyclohexa-1,5-dienyl)but-3-en-2-one showed an increasing trend throughout ripening with hexanal reaching levels of almost 3.5 fold that of the immature stage when ripe. Harvest fruit were characterised by high levels of β-damascenone and intermediate levels of most of the other compounds. Relatively high levels of 2-hexanal were present in all three the maturity stages with only a small increase during harvest. α- and β-Ionone also remained relatively constant in all the samples but at levels much lower compared to 2-hexanal (Table 2).

Although DA, done using the 20 compounds identified for ‘Pioneer’, resulted in the separation of the three groups, a clear transition from immature to ripe is seen on the observation chart (Fig. 2(a)) where factor 1 describes all three the maturity classes and factor 2 distinguishes vertically between the ‘harvest-group’ and the ‘immature-ripe group’. The confusion matrix had a total of 90.12% correctly predicted samples

with most of the miss-classifications made in the immature class (Table 4(b)). When the observation and variables charts were studied together, it confirmed the profiles described above with most of the compounds found in the 2nd quarter similar to where the immature samples occurred in the observations chart (Fig 2(a) and (b)). β-Damascenone and 2-hexenal dominated the 1st and 4th quarters parallel to the harvest group and hexanal and 4-(2,4,4-trimetyl-cyclohexa-1,5-dienyl) but-3-en-2-one are the only two compounds found in the 3rd quarter corresponding with the tree ripe fruit observations. The stepwise DA identified 11 of the 20 compounds as having a high contribution to the separation of the maturity classes (Table 4(a)). When using only these 11 compounds to reconstruct the observation chart there was very little difference in the separation pattern (Fig 2(c)) indicating that the 11 compounds chosen by the stepwise method were mainly responsible for creating the variance amongst the maturity classes with the correctly predicted samples dropping only slightly to 86.42% (Table 4(c)).

When the two seasons were analysed separately the aroma profiles were different (see Appendix 2, Section A for DA of individual seasons), although all the compounds were present in both seasons only five compounds (β-cymene, β-damascenone, 2-bornene, limonene oxide and D-limonene) were chosen by the stepwise method for 2008 and three different compounds (hotreinol, hexanal and β-ionone) for the 2009 season. When analysed together, almost all the compounds selected in the individual seasons were selected again together with 6 additional compounds (see [a] and [b] notation in Table 4(a)).

3.4 'Laetitia' aroma profiles:

Twenty-two of the total of 35 compounds were detected in the 'Laetitia' samples throughout ripening (Table 2). Five of the compounds were only found in 'Laetitia' fruit: phenol, menthol, unidentified 2, unidentified 3 and unidentified 4. The five unique compounds showed increasing levels as the fruit matured, reaching their highest levels in the harvest stage and then dropping off with only menthol, unidentified 3 and unidentified 4 still present in the tree ripe stage. The general profile of 'Laetitia' indicated that most compounds (15 of the 22) reached their highest levels when close to harvest then dropped sharply as the fruit ripened on the tree. This differed from 'Pioneer' where it was found that the immature stage had the most compounds with high levels and that the levels had already dropped off significantly as the fruit approached harvest. More specifically, the harvest samples of 'Laetitia' can be characterised as containing high levels of hexanal, β-damascenone, benzaldehyde, phenol, menthol and unidentified 3. Although the immature stage had all the compounds present, it showed high levels of only α-terpineol, p-menth-1-en-9-al and 2-bornene. Once ripe 'Laetitia' developed exceptionally high amounts of 1-hexanol with levels more than 400% than what was present at the immature stage. Furthermore, linalool and hotreinol also increased more than 3 fold as the fruit ripened. 2-Hexenal and β-ionone, similarly to 'Pioneer', were found in stable amounts throughout development although 2-hexanal had a level almost 91 times that of β-ionone (Table 2).

The aroma profiles for the three maturity stages corresponded to the results obtained from the DA. The analysis showed the three stages as distinct (Fig 3(a)) and the variables matched up to the stages as described above (Fig 3(b)) with quadrant 2 describing the ripe fruit, quadrant 3 the immature fruit and quadrant 4 the harvest samples. The confusion matrix indicated that the model identified 91.35% of the samples correctly with the largest miss-classifications made when having to sort the immature and harvest samples (Table 5(b)). The stepwise discriminant procedure identified nine of the 22 compounds to be significantly associated with the separation pattern observed for the maturity stages (Table 5(a)) with 1-hexanol as the main compound. When the DA was repeated using only the nine compounds selected by the stepwise procedure, the observations chart became only slightly less distinct (Fig 3(c)) and the percentage of correctly predicted samples declined to 87.65% (Table 5(c)). This emphasises that the nine chosen compounds were the main volatiles responsible for the dynamics of the ripening process.

The aroma profiles for the individual seasons did show some differences but both years indicated the high levels of 1-hexanol in the ripe samples and the high levels of β-damascenone in the harvest samples. See Appendix 2, Section B for the DA results for the individual seasons. In the 2008 season eight compounds were identified as contributing to the separation of the classes compared to only six in 2009 with 1-hexanol, β-damascenone and *cis*-linalool oxide chosen in both years. Almost all the compounds identified as important in the individual seasons were chosen again when the data were combined with the addition of hexanal and linalool (see [a] and [b] notation in Table 5(a)). The separations made by the discriminate models were identical for the two seasons and the combined data with all three groups distinctly apart but the immature and harvest samples grouped closer together compared to the ripe samples. This suggested that the aroma profile of 'Laetitia' fruit changed rapidly over a short period as the fruit reaches optimal ripeness.

3.5 'Angeleno' aroma profiles:

The aroma profile of 'Angeleno' consists of 23 of the 35 compounds with five exclusive compounds; 2H-pyran,2-ethenyltetrahydro-2,6,6tri-methyl, ocimenol, benzyl acetate, unidentified 5 and p-cymen-8-ol (Table 2). Of these, only unidentified 5 had relatively high levels in the ripe stage, the others all peaked in the immature and/or harvest stages and decreased to very low levels in the ripe stage with p-cymen-8-ol not detected in the ripe stage. All 23 compounds were present in the early stages of development with nine compounds peaking in the immature stage and six compounds peaking in the harvest stage. After the harvest stage the levels of most compounds decreased drastically with most compounds losing between 60 and 70% of their initial level with hexenal, p-cymen-8-ol, and bisabolol oxide B no longer detected in the ripe stage. The immature stage was essentially characterised by high levels of benzaldehyde, D-limonene, *trans*- and *cis*-linalool oxide, α-terpineol, 2-bornene and α-ionone while the harvest samples were rich in 2-hexen-4-olide, linalool and β-damascenone. Similarly to the ripe 'Laetitia' samples exceedingly high levels of 1-hexanol (410% more) and hotreinol (2.3% more) were present in the

ripe samples compared to the immature samples. Consistently high levels of 2-hexanal were present throughout the ripening process with lower, but equally consistent, levels of hexanal, linalool and unidentified 5 (Table 2).

Again the aroma profiles identified by comparing the levels that were measured in each stage were comparable to the observations and variables charts resulting from a DA (Fig 4(a) and (b)). Of the three cultivars 'Angeleno' showed the least discrete separation between the maturity classes with a pattern similar to 'Pioneer' where a clear transition from the one stage to the next was visible. Quadrant 1 was associated with immature fruit gradually moving into quadrant 2 and 3 where most of the harvest samples were situated. The ripe samples had a better separation with most of the samples found in quadrant 4. The aroma compound arrangement into the four quadrants was similar to the description above with most compounds divided between the immature and harvest samples and the ripe fruit depicting a clear association with 1-hexanol and hotreinol. Six compounds were selected by the stepwise method to be of most importance with 1-hexanol as the main contributor to the separation pattern (Table 6(a)). Surprisingly, hotreinol was not chosen to be of importance but rather α-ionone. The confusion matrixes (Table 6 (b) and (c)) also reflected the relatively poor separation (when compared to 'Pioneer' and 'Laetitia') with a success rate below 90% when all the compounds were used and a further decrease to 85.90% when only the six chosen compounds are used. Although lower, the separations were still distinct and clear differences could be observed between the aroma profiles of immature, harvest and ripe samples.

When the seasons were analysed individually the separation patterns were clearly different (see Appendix 2, Section C for results of individual seasons). The 2008 season had a pattern similar to that of 'Laetitia' with the immature and harvest samples grouped together and the ripe samples clearly separated along the x-axis. The 2009 season showed separations similar to that of 'Pioneer' with the classes grouped closer together. However, in both years 1-hexanol was identified as a compound significantly responsible for the separation of the ripe samples.

3.6 Comparing the different maturity classes of the cultivars:

The data were further analysed by performing an inter-cultivar DA on each of the maturity classes. The aim here was to identify, if possible, at what stage of the development the aroma profiles of the different cultivars started to diverge significantly from each other and to identify the compounds contributing most to such a divergence. Thus, no new data were added but instead the classes within each DA now consisted of the three cultivars with samples from similar developmental stages.

The DA results clearly showed that the aroma profiles of the three cultivars already differ significantly at the immature stage with well separated groups (Fig 5(a)) and no misinterpretations in the cross validation

(Table 7(b). The variables chart (Fig 5(b)) evidently depicted the unique compounds of each cultivar parallel to the observations for that specific cultivar. However, each cultivar also had associations with several non-unique compounds that were identifiable by the grouping of the compounds in the different quadrants of the variables chart compared to the observations chart. For example, immature 'Pioneer' samples found in quadrant 4 of the observations chart had the most compounds represented in quadrant 4 of the variables chart with relatively high levels of especially hotreinol, 2-hexenal 2-bornene and *cis*-and *trans*-linalool oxide. 'Laetitia' (grouped along the y-axis separating quadrant 2 and 3) was also characterised by the presence of β-ionone, 4-(2,4,4-trimetyl-cyclohexa-1,5-dienyl)but-3-en-2-one and bisabolol oxide B while 'Angeleno' immature samples (quadrant 1) had the highest levels of hexanal, α-ionone and 1-hexanol. The stepwise method identified 15 compounds as contributing to the alignment of the samples (Table 7(a)) with α-ionone and β-cymene providing the most variability. Interestingly this list of compounds contained all of the unique compounds found in 'Angeleno', three of the five found in 'Pioneer' but none belonging to 'Laetitia'. The grouping of the cultivars became less distinct and more closely associated when only the 15 compounds identified by the stepwise method (Fig 5(c)) were used in a DA with the percentage correctly predicted samples dropping to 98.75% (Table 7(c)).

The DA results for the harvest and tree ripe stage were similar to that found for the immature stage with very clearly separated cultivars (especially in the ripe stage) and the aroma compounds associated with each cultivar could be traced by comparing the observations and variables charts. See Fig 6(a) and (b) for harvest stage and Fig 7(a) and (b) for the ripe stage. Again the aroma profiles for each cultivar were similar to those described by the results from the DA done on the individual cultivars with each cultivar having an increasingly different profile as the fruit progress through maturation. The confusion matrixes indicated that 100% of all the samples could be predicted correctly in both of the harvest (Table 8(b)) and ripe (Table 9(b)) stages confirming the clarity of the separations and the significant differences in the aroma profiles (Table 8(a) and 9(a)). The stepwise method identified 15 compounds of importance for the harvest stage and nine for the ripe stage. In the harvest stage β-cymene was recognised again, similarly to the immature stage, as the compound with the biggest contribution followed by 2-bornene and D-limonene (Table 8(a)). The selection made for the ripe stage (Table 9(a)) interestingly revealed that the first four compounds (2-hexanal, β-ionone, unidentified 3 and 2H-pyran,3,6.dihydro-4-methyl-2-(2-methyl-1-propenyl) had similar importance (partial R^2 values).

4. Discussion and Conclusion

Studies on the aroma of plums, especially Japanese plums, are not as widely documented as for other stone fruit such as apricots and peaches. Although plum aroma has been described in general and close to 300 different compounds have been identified from plum extracts (Crouzet et al., 1990) to date no references could be found describing the aroma profiles of 'Pioneer' and 'Laetitia'. Only one (Lozano et al., 2009) aroma study on 'Angeleno' (also called 'Suplumsix') plums was located describing the aroma

volatiles found in ripe fruit. Limited English literature is available on the changes of the aroma profile during maturation and to include the work published in languages other than English interpretations and summaries made by other authors, e.g. the description of Dirninger-Rigo's study (1987) on the aroma of half-ripe, ripe and overripe Mirabelle plums as presented by Crouzet et al. (1990) were used. Only one other reference could be found describing the development of volatile compounds during fruit maturation including results from a plumcot (apricot x plum hybrid) (Gomez and Ledbetter, 1997). This scarcity in comparative plum literature necessitated a wider discussion on stone fruit and other fruit types.

Of the 35 compounds identified within the three cultivars, ten compounds were present in all the maturity stages of all cultivars. These compounds also accounted for about 50% of the total amount of volatiles measured for each cultivar. This similarity in occurrence may propose that these ten compounds are responsible for the general plum flavour. Most of these 'generic' compounds, with the exception of 2-bornene, p-menth-1-en-9-al and hotreinol, were described in previous plum studies (summary in Crouzet et al., 1990) and linalool in particular, was identified to have a fresh plum aroma (Williams and Ismail, 1981).

Results from all three the cultivars indicated that the number of different aroma compounds decreases during maturation with many compounds present in the immature fruit and only a few maintaining their levels as the fruit ripens. Only the two aldehydes, hexanal and 2-hexenal, were present in relatively high levels throughout the ripening process of all three cultivars. This is contradictory to several publications claiming that both these aroma compounds have a strong, green flavour (Guichard et al., 1990) associated with immature fruit and diminished in concentration as ripening proceeds (Gómez and Ledbetter, 1997) rather than remaining relatively constant as found in this study.

The terpene alcohol linalool is also widely described in plum literature and is often identified as having a plum-like aroma (Williams and Ismail, 1981). However Dirninger-Rigo (1987) (as summarised by Crouzet et al., 1990) concluded that the decrease found in linalool levels in 'Mirabelle' plums during ripening is more interesting in order to evaluate the degree of maturity than for sensory impact. Decreasing levels of linalool had also been described in the maturation of peaches (Chapman et al., 1991; Robertson et al., 1990). In the cultivars included in this study we report both an increase ('Laetitia') and a decrease ('Pioneer' and 'Angeleno') in linalool as the fruit ripened. Guillot et al. (2006) investigated linalool in six different apricot varieties and also reported varying levels in the ripe fruit. The two linalool oxides (*trans* and *cis*) that were always present in the analysis of the three cultivars have also been reported in ripe plums and peaches (Williams and Ismail, 1981). Another terpene alcohol, α-terpeniol, found in the list of ten 'general' plum compounds showed a steep decrease during ripening in all of the cultivars; this is similar to findings by Gómez and Ledbetter (1997) although they did report that the apricot appeared to have much higher concentrations of α-terpeniol than the plumcot. β-Damascenone is widely documented

in plums and has been linked to the aroma of cooked plums (Williams and Ismail, 1981) and also described as honey-like where it is believed to be the most important contributor to the lime tree honey aroma (Soria et al., 2009). β-Damascenone was found to be highest in the harvest samples of all three the cultivars investigated in this study and might thus be an indicator of physiological maturity rather than ripeness as harvest fruit are mature but not yet ripe.

As mentioned earlier, p-menth-1-en-9-al and hotreinol have not been recorded in plum extracts before but are common volatiles found in citrus honey (Soria et al., 2009). Hotreinol is also present in a variety of fruit types such as nectarine cultivars (Engel et al., 1988), grapes (Williams et al., 1982), passion fruit (Engel and Tressl, 1983) and papaya (Schreier et al., 1985). Both these compounds are found in decreasing amounts in the three cultivars with the exception of 'Laetitia' and 'Angeleno" where hotreinol was found to increase with maturity. The compound 2-bornene that is also one of the ten 'generic' plum volatiles has not been described in any fruit types but traces of it has been found in the leaf oils of the Mei Pan tree (Yu-Jing et al., 1987).

In conclusion it is also relevant to note that the compounds identified as contributing to the separation of the different maturity classes within each cultivar were not necessarily assigned as important in the separation of the different cultivars. It was also not always the compounds unique to a cultivar that were responsible for the separation pattern seen for the three cultivars. Moreover, it was not essentially the compounds with high levels that were significantly contributing to the distinctness of a cultivar. Bearing all of this in mind it is clear that although the general plum flavour is present in all the cultivars, each cultivar had a complex aromatic composition already present in immature fruit. This dynamic continued throughout maturation and ripening and resulted in fruit with distinctly different aroma profiles. However, inter-cultivar comparisons did suggest that the three cultivars investigated in this study were more similar during the immature stage with increasing divergence towards maturity.

Crouzet et al. (1990), conclude their review of plum aroma by suggesting that esters, alcohols and aldehydes may be the major constituents of fresh plums. Our study contributes to underlining the importance of aldehydes and especially terpene alcohols but, interestingly, very few esters were identified. This is contradictory to all the plum literature published to date as all report the presence of numerous esters (Crouzet et al., 1990; Gómez and Ledbetter, 1994; Williams and Ismail, 1981; Etievant et al., 1986). In the aroma study on six Japanese plums by Lozano and co-workers, (2009) they identified 16 esters of which eight were found in 'Angeleno' ('Suplumsix'), in contrast we detected only one, benzyl acetate, which was not detected by them. Our study does in no way advocate that there are no/fewer esters present in the cultivars investigated, but suggests that future work should include repeating the extractions but perhaps using a different SPME fiber or extraction method more conducive to the extraction of esters. Similarly, future work may also include more sensitive methods for detecting

lactones in these cultivars as they may be markers for ripeness in plums as several authors have reported lactones present in ripe plums (Horvat et al., 1992, Gómez and Ledbetter, 1994) but absent in mature, unripe plums (Gómez and Ledbetter, 1997). Applications of this study should include relating the aroma profiles to consumer preferences and sniffing panels to identify possible critical impacting compounds and investigating the dynamics thereof during long cold storage.

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Table 1: Range, mean and standard deviation (STDev) of the quality parameters determined for 'Pioneer', 'Laetitia' and 'Angeleno' over two seasons. (To convert Newton to kilogram, divide by 9.81)

Non-volatile parameters		'Pioneer'			'Laetitia'			'Angeleno'		
		Immature *n* = 34	**Harvest** *n* = 29	**Ripe** *n* = 20	**Immature** *n* = 42	**Harvest** *n* = 38	**Ripe** *n* = 10	**Immature** *n* = 34	**Harvest** *n* = 38	**Ripe** *n* = 12
Firmness (N)	Range	100.4 - 196.2	46.5 - 83.4	12.8 - 35.9	84.4 - 162.9	50.0 - 75.4	13.3 - 23.8	78.8 - 122.3	48.0 - 76.4	19.8 - 37.9
	Mean (±STDev)	*151.40 (±31.54)*	*66.49 (±11.51)*	*21.25 (±6.99)*	*115.82 (±18.12)*	*63.26 (±7.57)*	*16.64 (±3.30)*	*96.88 (±12.97)*	*62.50 (±7.68)*	*29.69 (±6.37)*
Weight (g)	Range	25 - 51	37 - 80	57 - 82	46 - 101	73 - 107	99 - 165	56 - 110	73 - 103	62 - 104
	Mean (±STDev)	*35.60 (±8.13)*	*60.31 (±10.53)*	*68.55 (±8.75)*	*72.44 (±16.00)*	*91.39 (±9.26)*	*118.92 (±18.74)*	*80.43 (±12.03)*	*89.63 (±7.96)*	*79.64 (±11.99)*
Sugar (% Brix)	Range	6.2 - 11.7	7.2 - 12.7	8.6 - 14.9	7.7 - 12.5	8.4 - 13.7	8.8 - 15.7	10.5 - 16.0	12.6 - 16.9	13.1 - 16.5
	Mean (±STDev)	*8.22 (±1.14)*	*10.55 (±1.20)*	*11.55 (±1.98)*	*9.87 (±1.50)*	*11.20 (±1.69)*	*12.73 (±2.55)*	*13.44 (±1.03)*	*14.76 (±0.91)*	*15.32 (±0.92)*
Acid (% malic acid)	Range	1.61 - 2.27	1.36 - 2.17	0.98 - 1.97	1.79 - 2.68	0.80 - 1.86	0.70 - 1.98	0.99 - 1.53	0.60 - 1.16	0.58 - 0.80
	Mean (±STDev)	*1.93 (±0.18)*	*1.86 (±0.21)*	*1.37 (±0.26)*	*2.17 (±0.19)*	*1.63 (±0.20)*	*1.21 (±0.48)*	*1.16 (±0.12)*	*0.91 (±0.16)*	*0.68 (±0.07)*
Sugar:Acid	Range	3.32 - 6.13	3.56 – 7.79	5.84 - 12.48	3.25 - 6.51	4.81 - 12.88	5.05 - 21.57	8.37 - 16.16	11.42 - 25.67	20.63 – 26.72
	Mean (±STDev)	*4.27 (±0.62)*	*5.73 (±0.84)*	*8.62 (±1.74)*	*4.59 (±0.93)*	*6.82 (±1.66)*	*12.79 (±6.66)*	*11.78 (±1.70)*	*16.74 (±3.56)*	*22.57 (±1.87)*

Table 2: Range, mean and standard deviation (STDev) of the aroma volatile variables determined for 'Pioneer', 'Laetitia' and 'Angeleno' over two seasons. The retention time (RT) order represents the chronological order in which the compounds were separated, starting with hexanal at ±5.3 min and ending with bisabalol oxide B at ±29.8 min. * indicates compounds identified via the NIST library and ** indicates identification via NIST library and confirmation using commercial standards. ND = Not detected. Abb = Abbreviation.

RT order	Abb	Volatile compound name		'Pioneer'			'Laetitia'			'Angeleno'		
				Immature *n* = 34	**Harvest** *n* = 29	**Ripe** *n* = 20	**Immature** *n* = 42	**Harvest** *n* = 38	**Ripe** *n* = 10	**Immature** *n* = 34	**Harvest** *n* = 38	**Ripe** *n* = 12
1	Hexa	Hexanal**	Range	0.032-0.729	0.080-1.423	ND - 2.064	0.011-0.320	0.012-0.538	ND-0.124	0.024-0.396	0.114-0.593	0.043-0.446
			Mean (±STDev)	*0.178 (± 0.135)*	*0.293 (±0.274)*	*0.580 (± 0.592)*	*0.095 (±0.085)*	*0.142 (±0.128)*	*0.034 (±0.038)*	*0.213 (±0.118)*	*0.285 (±0.132)*	*0.211 (±0.170)*
2	Hexe	2-Hexenal**	Range	1.232-5.272	1.209-5.747	1.302-4.182	0.296-3.838	0.335-2.137	0.082-3.183	0.533-2.646	0.426-2.280	0.430-1.149
			Mean (±STDev)	*2.963 (±1.133)*	*3.353 (±1.179)*	*2.757 (±0.823)*	*1.044 (±0.791)*	*0.952 (±0.501)*	*1.250 (±0.812)*	*1.297 (±0.469)*	*1.114 (±0.456)*	*0.837 (±0.217)*
3	Hex	1-Hexanol**	Range		ND		ND - 0.032	ND - 0.060	0.143-5.143	0.002-0.137	0.007-0.311	0.131-1.360
			Mean (±STDev)				*0.005 (±0.009)*	*0.020 (±0.018)*	*2.053 (±1.446)*	*0.035 (±0.032)*	*0.079 (±0.071)*	*0.679 (±0.414)*
4	Eth	2-Ethylfuran*	Range		ND		ND - 0.331	ND - 0.205	ND - 0.036	ND - 0.183	ND - 0.159	ND - 0.009
			Mean (±STDev)				*0.057 (±0.078)*	*0.061 (±0.076)*	*0.011 (±0.015)*	*0.042 (±0.056)*	*0.045 (±0.050)*	*0.003 (±0.003)*
5	Benz	Benzaldehyde**	Range		ND		ND - 0.074	ND - 0.091	ND - 0.026	ND - 0.076	ND - 0.046	ND - 0.010
			Mean (±STDev)				*0.017 (±0.023)*	*0.028 (±0.030)*	*0.010 (±0.011)*	*0.016 (±0.021)*	*0.011 (±0.014)*	*0.004 (±0.004)*
6	Pyran2	2H-Pyran,2-ethenyltetrahydro - 2,6,6 tri-methyl*	Range		ND			ND		ND - 0.026	ND - 0.099	ND - 0.010
			Mean (±STDev)							*0.008 (±0.010)*	*0.012 (±0.024)*	*0.001 (±0.003)*
7	Hexen	2-Hexen-4-olide*	Range		ND		ND - 0.437	ND - 0.261	ND - 0.034	ND - 0.242	ND - 0.549	ND
			Mean (±STDev)				*0.070 (±0.102)*	*0.078 (±0.089)*	*0.007 (±0.013)*	*0.027 (±0.060)*	*0.061 (±0.118)*	
8	Phe	Phenol*	Range		ND		ND - 0.476	ND - 0.539	ND		ND	
			Mean (±STDev)				*0.013 (±0.074)*	*0.045 (±0.133)*				
9	U2	Unidentified 2	Range		ND		ND - 0.534	ND - 0.259	ND		ND	
			Mean (±STDev)				*0.049 (±0.103)*	*0.047 (±0.065)*				

RT order	Abb	Volatile compound name		'Pioneer'			'Laetitia'			'Angeleno'		
				Immature *n* = 34	**Harvest** *n* = 29	**Ripe** *n* = 20	**Immature** *n* = 42	**Harvest** *n* = 38	**Ripe** *n* = 10	**Immature** *n* = 34	**Harvest** *n* = 38	**Ripe** *n* = 12
10	Limox	Limonene oxide **	Range	0.001-0.076	0.006-0.029	ND - 0.010		ND			ND	
			Mean (±STDev)	*0.021 (±0.013)*	*0.013 (±0.006)*	*0.004 (±0.003)*						
11	Cym	β-Cymene**	Range	0.006-0.065	0.003-0.020	ND - 0.002		ND			ND	
			Mean (±STDev)	*0.027 (±0.015)*	*0.009 (±0.004)*	*0.001 (±0.001)*						
12	Lim	D-Limonene**	Range	0.005-0.122	0.001-0.014	ND - 0.008		ND		0.009- .043	ND - 0.039	ND - 0.015
			Mean (±STDev)	*0.029 (±0.024)*	*0.007 (±0.003)*	*0.002 (±0.002)*				*0.021 (±0.007)*	*0.014 (±0.008)*	*0.003 (±0.005)*
13	Car	3-Carene **	Range	0.002- .087	0.001-0.009	ND - 0.006		ND		ND - 0.015	ND - 0.020	ND - 0.003
			Mean (±STDev)	*0.014 (±0.016)*	*0.004 (±0.002)*	*0.001 (±0.001)*				*0.004 (±0.004)*	*0.004 (±0.005)*	*0.001 (±0.001)*
14	aTerp	α-Terpinene*	Range	0.002-0.035	0.001-0.007	ND - 0.003		ND			ND	
			Mean (±STDev)	*0.010 (±0.007)*	*0.003 (±0.002)*	*0.001 (±0.001)*						
15	tLinox	*trans* Linalool oxide**	Range	0.004-1.845	0.029-0.300	0.005-0.467	0.023-0.380	0.019-0.301	0.001-0.165	0.015-0.155	0.005-0.180	0.002-0.021
			Mean (±STDev)	*0.239 (±0.308)*	*0.093 (±0.062)*	*0.094 (±0.110)*	*0.139 (±0.074)*	*0.121 (±0.083)*	*0.057 (±0.048)*	*0.068 (±0.041)*	*0.046 (±0.043)*	*0.011 (±0.007)*
16	cLinox	*cis* Linalool oxide**	Range	0.019-0.980	0.013-0.081	0.002-0.101	0.013-0.116	0.007-0.105	ND - 0.041	0.012-0.063	0.003-0.057	0.001-0.009
			Mean (±STDev)	*0.113 (±0.162)*	*0.034 (±0.017)*	*0.021 (±0.022)*	*0.049 (±0.025)*	*0.043 (±0.030)*	*0.014 (±0.013)*	*0.032 (±0.015)*	*0.018 (±0.012)*	*0.004 (±0.002)*
17	U1	Unidentified 1	Range	0.009-0.121	0.003-0.043	ND - 0.022		ND			ND	
			Mean (±STDev)	*0.040 (±0.027)*	*0.013 (±0.010)*	*0.003 (±0.005)*						
18	Lina	Linalool**	Range	0.029-1.708	0.014-0.177	0.005-0.094	0.002-0.055	ND - 0.049	ND - 0.431	0.058-0.259	0.043-0.531	0.055-0.100
			Mean (±STDev)	*0.262 (±0.321)*	*0.046 (±0.035)*	*0.024 (±0.023)*	*0.013 (±0.011)*	*0.012 (±0.010)*	*0.044 (±0.122)*	*0.116 (±0.052)*	*0.136 (±0.087)*	*0.080 (±0.015)*
19	Hot	Hotrienol*	Range	0.052-1.081	0.052-0.372	0.022-0.432	ND - 0.010	ND - 0.010	ND - 0.029	ND - 0.022	ND - 0.030	ND - 0.121
			Mean (±STDev)	*0.473 (±0.267)*	*0.182 (±0.102)*	*0.132 (±0.117)*	*0.003 (±0.003)*	*0.003 (±0.003)*	*0.007 (±0.010)*	*0.006 (±0.007)*	*0.010 (±0.010)*	*0.043 (±0.044)*
20	Oci	Ocimenol*	Range		ND			ND		ND - 0.045	ND - 0.030	ND - 0.006
			Mean (±STDev)							*0.010 (±0.012)*	*0.008 (±0.010)*	*0.002 (±0.003)*
21	Menth	Menthol*	Range		ND		0.002-0.054	ND - 0.060	ND - 0.020		ND	
			Mean (±STDev)				*0.008 (±0.008)*	*0.011 (±0.014)*	*0.006 (±0.005)*			
22	Benzyl	Benzylacetate**	Range		ND			ND		ND - 0.013	ND - 0.009	ND - 0.003
			Mean (±STDev)							*0.003 (±0.004)*	*0.003 (±0.003)*	*0.001 (±0.001)*

RT order	Abb	Volatile compound name		'Pioneer'			'Laetitia'			'Angeleno'		
				Immature *n* = 34	**Harvest** *n* = 29	**Ripe** *n* = 20	**Immature** *n* = 42	**Harvest** *n* = 38	**Ripe** *n* = 10	**Immature** *n* = 34	**Harvest** *n* = 38	**Ripe** *n* = 12
23	Pyran3	2H-Pyran,3,6.dihydro-4-methyl-2-(2-methyl-1-propenyl)*	Range	0.015-0.436	0.011-0.064	ND-0.024	ND			ND		
			Mean (±STDev)	*0.106 (±0.085)*	*0.025 (±0.012)*	*0.009 (±0.006)*						
24	U5	Unidentified 5	Range	ND			ND			0.011-0.128	0.004-0.120	0.008-0.163
			Mean (±STDev)							*0.046 (±0.028)*	*0.039 (±0.028)*	*0.034 (±0.044)*
25	Cymol	p-Cymen-8-ol*	Range	ND			ND			ND - 0.012	ND - 0.006	ND - 0.001
			Mean (±STDev)							*0.003 (±0.004)*	*0.002 (±0.002)*	*ND*
26	aTerpol	α-Terpineol**	Range	0.033-2.524	0.011-0.127	0.004-0.309	0.006-0.255	0.004-0.096	ND - 0.040	0.028-0.309	0.012-0.215	0.011-0.038
			Mean (±STDev)	*0.391 (±0.462)*	*0.055 (±0.036)*	*0.043 (±0.074)*	*0.057 (±0.048)*	*0.038 (±0.027)*	*0.014 (±0.014)*	*0.130 (±0.086)*	*0.080 (±0.050)*	*0.026 (±0.010)*
27	pMenth	p-Menth-1-en-9-al*	Range	0.042-0.943	0.028-0.278	0.007-0.571	0.024-0.229	0.015-0.140	0.011-0.054	ND - 0.436	0.025-0.287	0.009-0.141
			Mean (±STDev)	*0.266 (±0.174)*	*0.103 (±0.062)*	*0.077 (±0.129)*	*0.081 (±0.046)*	*0.063 (±0.033)*	*0.031 (±0.013)*	*0.178 (±0.111)*	*0.129 (±0.067)*	*0.071 (±0.042)*
28	Bor	2-Bornene*	Range	0.007-0.766	0.005-0.049	0.001-0.013	0.008-0.089	0.007-0.036	ND - 0.010	ND - 0.018	ND - 0.013	ND - 0.002
			Mean (±STDev)	*0.070 (±0.130)*	*0.018 (±0.012)*	*0.004 (±0.004)*	*0.028 (±0.018)*	*0.019 (±0.007)*	*0.004 (±0.003)*	*0.005 (±0.006)*	*0.003 (±0.004)*	*0.001 (±0.001)*
29	U3	Unidentified 3	Range	ND			0.006-0.347	0.006-0.460	0.003-0.119	ND		
			Mean (±STDev)				*0.052 (±0.068)*	*0.106 (±0.121)*	*0.057 (±0.051)*			
30	aIon	α-Ionone**	Range	0.023-0.136	0.003-0.178	0.003-0.299	ND			0.066-0.390	0.037-0.183	0.021-0.046
			Mean (±STDev)	*0.053 (±0.025)*	*0.060 (±0.046)*	*0.046 (±0.074)*				*0.148 (±0.062)*	*0.089 (±0.038)*	*0.031 (±0.007)*
31	bDam	β- Damascenone**	Range	0.020-0.146	0.031-0.300	0.012-0.182	0.061-0.462	0.075-0.463	0.013-0.181	0.012-0.329	0.055-0.344	0.025-0.175
			Mean (±STDev)	*0.066 (±0.028)*	*0.099 (±0.067)*	*0.068 (±0.047)*	*0.152 (±0.091)*	*0.219 (±0.113)*	*0.056 (±0.058)*	*0.138 (±0.076)*	*0.151 (±0.063)*	*0.061 (±0.042)*
32	U4	Unidentified 4	Range	ND			0.008-0.181	0.006-0.202	ND - 0.136	ND		
			Mean (±STDev)				*0.043 (±0.042)*	*0.070 (±0.055)*	*0.062 (±0.049)*			
33	Trimeth	4-(2,4,4-Trimetyl-cyclohexa-1,5-dienyl) but-3-en-2-one*	Range	0.006-0.067	0.003-0.037	0.001-0.284	0.008-0.109	0.007-0.076	ND - 0.017	ND		
			Mean (±STDev)	*0.017 (±0.012)*	*0.013 (±0.009)*	*0.028 (±0.069)*	*0.029 (±0.023)*	*0.025 (±0.016)*	*0.005 (±0.006)*			
34	bIon	β- Ionone**	Range	0.002-0.015	0.002-0.021	0.001-0.031	0.002-0.043	0.002-0.027	ND-0.046	ND		
			Mean (±STDev)	*0.005 (±0.003)*	*0.005 (±0.003)*	*0.005 (±0.007)*	*0.011 (±0.011)*	*0.011 (±0.006)*	*0.016 (±0.015)*			
35	Bisa	Bisabalol oxide B*	Range	ND			0.003-0.043	0.002-0.029	ND - 0.004	ND - 0.006	ND - 0.005	ND
			Mean (±STDev)				*0.013 (±0.008)*	*0.012 (±0.007)*	*0.001 (±0.002)*	*0.003 (±0.002)*	*0.002 (±0.002)*	

Table 3: (a) A summary of the variables selected by a stepwise DA performed on ten "generic" aroma compounds found in **'Pioneer', 'Laetitia'** and **'Angeleno'**. (See Table 2 for explanation of compound abbreviations). (b) Confusion matrix indicating the percentage correctly predicted samples.

(a)

Variable IN/OUT	Status	Partial R^2	F statistic	Probability > F
Hexe	IN	0.572	158.498	< 0.0001
Hot	IN	0.187	27.199	< 0.0001
Lina	IN	0.253	39.774	< 0.0001
tLinox	IN	0.303	50.811	< 0.0001
pMenth	IN	0.245	37.842	< 0.0001
Hexa	IN	0.079	10.011	< 0.0001
bDam	IN	0.065	7.984	0.0001
aTerpol	IN	0.044	5.320	0.006
cLinox	IN	0.080	9.938	< 0.0001

(b)

from \ to	Angeleno	Laetitia	Pioneer	Total	% correct
Angeleno	75	3	0	78	96.15%
Laetitia	1	78	2	81	96.30%
Pioneer	1	4	76	81	93.83%
Total	77	85	78	240	95.42%

Table 4: DA results for the three maturity classes of **'Pioneer'**. (a) Variable selection table using the stepwise method' (see Table 2 for explanation of compound abbreviations). Confusion matrixes indicating the percentage correctly predicted samples for the three classes using all the compounds (b) and using only the 11 compounds identified by the stepwise method (c).

(a)

Variable IN/OUT	Status	Partial R^2	F statistic	Probability > F
Cym[a]	IN	0.541	47.174	< 0.0001
bDam[a]	IN	0.111	4.787	0.011
Hexa[b]	IN	0.093	3.917	0.024
Hexe	IN	0.086	3.526	0.034
tLinox	IN	0.141	6.057	0.004
Limox[a]	IN	0.179	7.937	0.001
Lim[a]	IN	0.140	5.874	0.004
cLinox	IN	0.091	3.553	0.034
bIon[b]	IN	0.106	4.146	0.020
pMenth	IN	0.115	4.463	0.015
Car	IN	0.091	3.395	0.039
Lim	OUT	0.046	1.629	0.204

[a] Variable also selected for 2008 season
[b] Variable also selected for 2009 season

(b)

from \ to	Harvest	Immature	Tree ripened	Total	% correct
Harvest	26	1	0	27	96.30%
Immature	5	29	0	34	85.29%
Tree ripened	2	0	18	20	90.00%
Total	33	30	18	81	90.12%

(c)

from \ to	Harvest	Immature	Tree ripened	Total	% correct
Harvest	25	2	0	27	92.59%
Immature	5	28	1	34	82.35%
Tree ripened	3	0	17	20	85.00%
Total	33	30	18	81	86.42%

Table 5: DA results for the three maturity classes of **'Laetitia'**. (a) Variable selection table using the stepwise method' (see Table 2 for explanation of compound abbreviations). Confusion matrixes indicating the percentage correctly predicted samples for the three classes using all the compounds (b) and using only the nine compounds identified by the stepwise method (c).

(a)

Variable IN/OUT	Status	Partial R^2	F statistic	Probability > F
Hex[ab]	IN	0.649	72.190	< 0.0001
Bor[b]	IN	0.152	6.875	0.002
bDam[ab]	IN	0.185	8.608	0.0001
U4[a]	IN	0.147	6.454	0.003
cLinox[ab]	IN	0.195	8.966	0.0001
Bor	OUT	0.022	0.844	0.434
Bisa[a]	IN	0.163	7.204	0.001
Hexa	IN	0.105	4.279	0.017
Trimeth[b]	IN	0.105	4.213	0.019
Lina	IN	0.101	3.988	0.023

[a] Variable also selected for 2008 season
[b] Variable also selected for 2009 season

(b)

from \ to	Harvest	Immature	Tree ripened	Total	% correct
Harvest	25	3	0	28	89.29%
Immature	3	38	0	41	92.68%
Tree ripened	1	0	11	12	91.67%
Total	29	41	11	81	91.36%

(c)

from \ to	Harvest	Immature	Tree ripened	Total	% correct
Harvest	24	4	0	28	85.71%
Immature	5	36	0	41	87.80%
Tree ripened	1	0	11	12	91.67%
Total	30	40	11	81	87.65%

Table 6: DA results for the three maturity classes of **'Angeleno'**. (a) Variable selection table using the stepwise method' (see Table 2 for explanation of compound abbreviations). Confusion matrixes indicating the percentage correctly predicted samples for the three classes using all the compounds (b) and using only the six compounds identified by the stepwise method (c).

(a)

Variable IN/OUT	Status	Partial R^2	F statistic	Probability > F
Hex[ab]	IN	0.660	72.709	< 0.0001
alon	IN	0.287	14.922	< 0.0001
Hexe[b]	IN	0.132	5.535	0.006
Eth	IN	0.147	6.226	0.003
Bisa[b]	IN	0.098	3.866	0.025
Pyran2[b]	IN	0.097	3.771	0.028

[a] Variable also selected for 2008 season
[b] Variable also selected for 2009 season

(b)

from \ to	Harvest	Immature	Tree ripened	Total	% correct
Harvest	30	2	0	32	93.75%
Immature	4	31	0	35	88.57%
Tree ripened	2	0	9	11	81.82%
Total	36	33	9	78	89.74%

(c)

from \ to	Harvest	Immature	Tree ripened	Total	% correct
Harvest	32	0	0	32	100.00%
Immature	8	27	0	35	77.14%
Tree ripened	3	0	8	11	72.73%
Total	43	27	8	78	85.90%

Table 7: DA results for the **'immature class'** of all three cultivars'. (a) Variable selection table using the stepwise method' (see Table 2 for explanation of compound abbreviations). Confusion matrixes indicating the percentage correctly predicted samples for the three classes using all the compounds (b) and using only the 15 compounds identified by the stepwise method (c).

(a)

Variable IN/OUT	Status	Partial R^2	Fstatistic	Probability> F
aIon	IN	0.732	146.430	< 0.0001
Cym	IN	0.696	121.483	< 0.0001
Bisa	IN	0.312	23.756	< 0.0001
Hexe	IN	0.280	20.204	< 0.0001
aTerpol	IN	0.216	14.174	< 0.0001
Pyran2	IN	0.197	12.491	< 0.0001
Benzyl	IN	0.362	28.610	< 0.0001
Limox	IN	0.164	9.817	0.0001
Oci	IN	0.155	9.082	0.0002
Cymol	IN	0.187	11.259	< 0.0001
Lina	IN	0.123	6.801	0.002
U5	IN	0.117	6.388	0.002
Hot	IN	0.092	4.807	0.010
Cym	OUT	0.032	1.592	0.209
Hex	IN	0.080	4.121	0.019
U1	IN	0.069	3.468	0.035

(b)

from \ to	Angeleno	Laetitia	Pioneer	Total	% correct
Angeleno	35	0	0	35	100.00%
Laetitia	0	41	0	41	100.00%
Pioneer	0	0	34	34	100.00%
Total	35	41	34	110	100.00%

(c)

from \ to	Angeleno	Laetitia	Pioneer	Total	% correct
Angeleno	77	1	0	78	98.72%
Laetitia	1	80	0	81	98.77%
Pioneer	1	0	80	81	98.77%
Total	79	81	80	240	98.75%

Table 8: DA results for the **'harvest class'** of all three cultivars'. (a) Variable selection table using the stepwise method' (see Table 2 for explanation of compound abbreviations). Confusion matrixes indicating the percentage correctly predicted samples for the three classes using all the compounds (b) and using only the 15 compounds identified by the stepwise method (c).

(a)

Variable IN/OUT	Status	Partial R^2	F statistic	Probability > F
Cym	IN	0.779	147.804	< 0.0001
Bor	IN	0.626	69.475	< 0.0001
Lim	IN	0.597	60.736	< 0.0001
Hexe	IN	0.420	29.288	< 0.0001
Hex	IN	0.217	11.093	< 0.0001
U1	IN	0.196	9.653	0.000
aIon	IN	0.172	8.116	0.001
bIon	IN	0.139	6.235	0.003
Hexa	IN	0.128	5.594	0.005
Limox	IN	0.188	8.707	0.000
Hot	IN	0.240	11.714	< 0.0001
Pyran3	IN	0.153	6.571	0.002
U4	IN	0.136	5.686	0.005
cLinox	IN	0.082	3.184	0.047
pMenth	IN	0.183	7.822	0.001

(b)

from \ to	Angeleno	Laetitia	Pioneer	Total	% correct
Angeleno	32	0	0	32	100.00%
Laetitia	0	28	0	28	100.00%
Pioneer	0	0	27	27	100.00%
Total	32	28	27	87	100.00%

(c)

from \ to	Angeleno	Laetitia	Pioneer	Total	% correct
Angeleno	32	0	0	32	100.00%
Laetitia	0	28	0	28	100.00%
Pioneer	0	0	27	27	100.00%
Total	32	28	27	87	100.00%

Table 9: DA results for the **'tree ripe'** class of all three cultivars'. (a) Variable selection table using the stepwise method' (see Table 2 for explanation of compound abbreviations). Confusion matrixes indicating the percentage correctly predicted samples for the three classes using all the compounds (b) and using only the nine compounds identified by the stepwise method (c).

(a)

Variable IN/OUT	Status	Partial R^2	F statistic	Probability > F
Hexe	IN	0.609	31.101	< 0.0001
bIon	IN	0.560	24.798	< 0.0001
U3	IN	0.626	31.827	< 0.0001
Pyran3	IN	0.520	20.072	< 0.0001
aIon	IN	0.377	10.903	0.0002
Oci	IN	0.191	4.124	0.025
Car	IN	0.358	9.477	0.001
pMenth	IN	0.251	5.527	0.009
Trimeth	IN	0.277	6.126	0.006

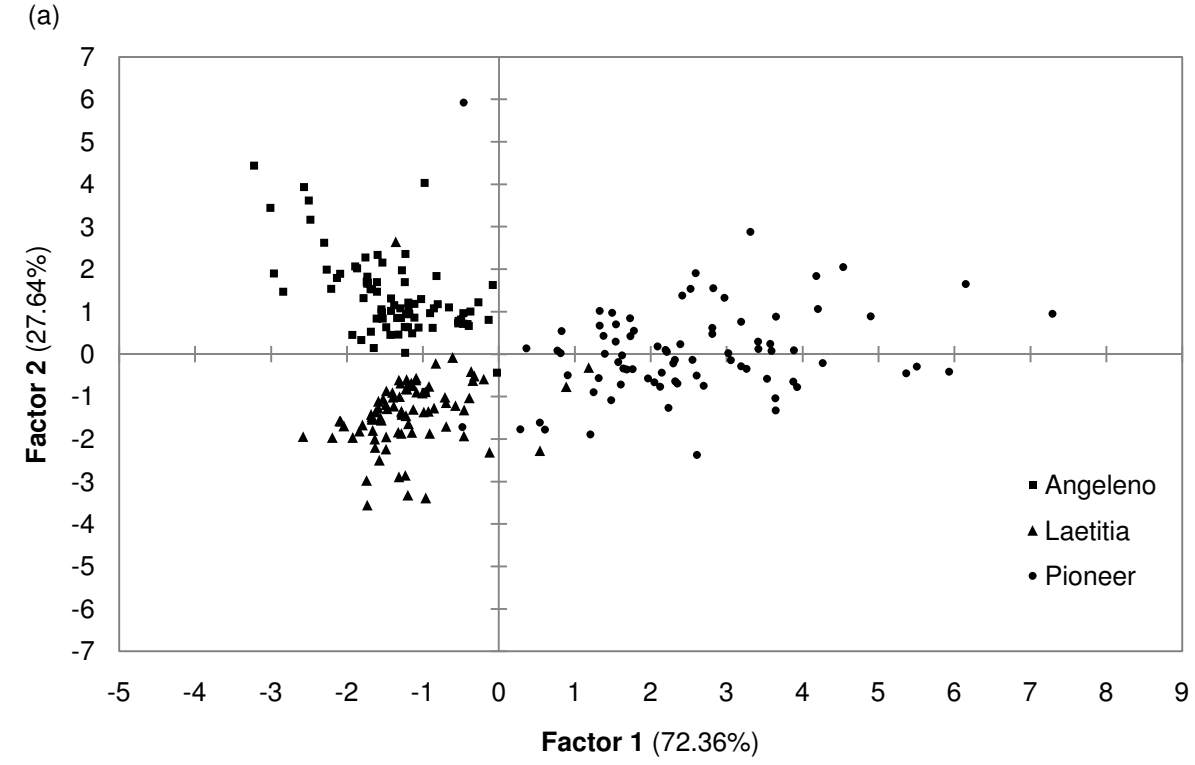
(b)

from \ to	Angeleno	Laetitia	Pioneer	Total	% correct
Angeleno	11	0	0	11	100.00%
Laetitia	0	12	0	12	100.00%
Pioneer	0	0	20	20	100.00%
Total	11	12	20	43	100.00%

(c)

from \ to	Angeleno	Laetitia	Pioneer	Total	% correct
Angeleno	11	0	0	11	100.00%
Laetitia	0	12	0	12	100.00%
Pioneer	0	0	20	20	100.00%
Total	11	12	20	43	100.00%

Figure 1: (a) Observations chart and (b) Variables chart for **'Pioneer', 'Laetitia'** and **'Angeleno'** using only the ten "generic" components as variables in a discriminant analysis. Percentages indicate the corresponding percentage of variance described by each factor. (See Table 2 for explanation of compound abbreviations).



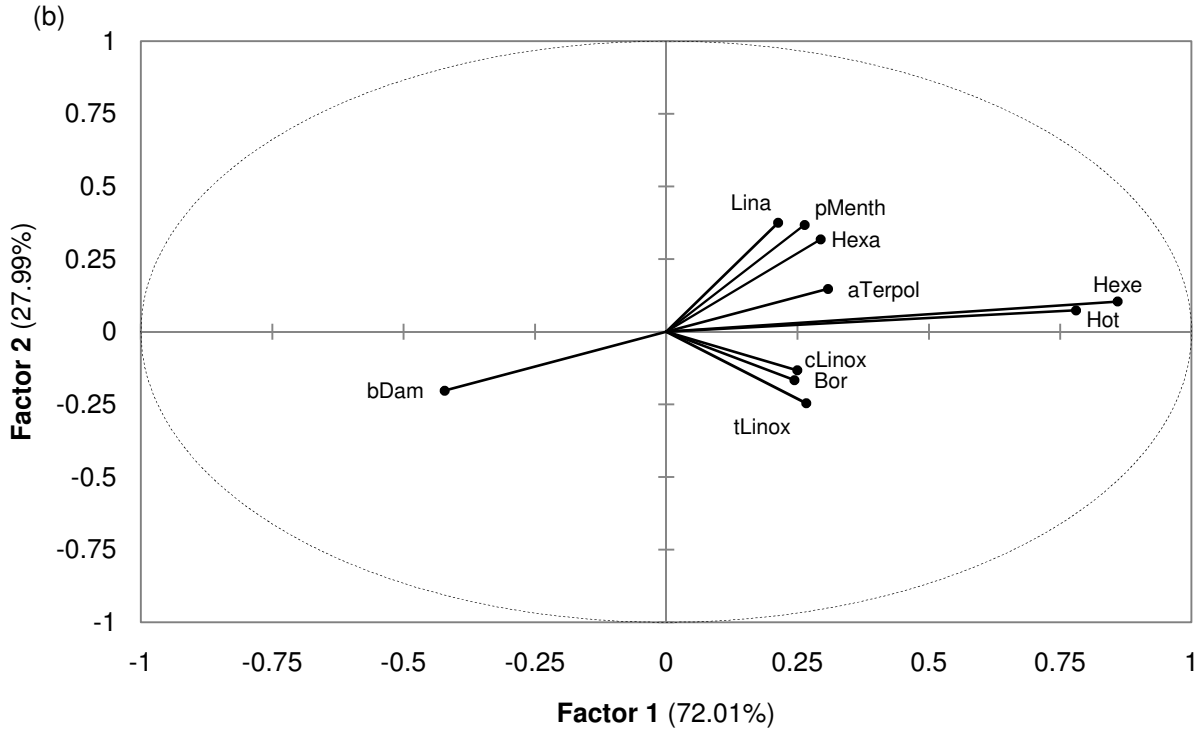


Figure 2: Observations and Variables charts for **'Pioneer'** using all the compounds ((a) and (b)) and using only the compounds identified in the stepwise method of a DA ((c) and (d)). Percentages indicate the corresponding percentage of variance described by each factor. (See Table 2 for explanation of compound abbreviations).

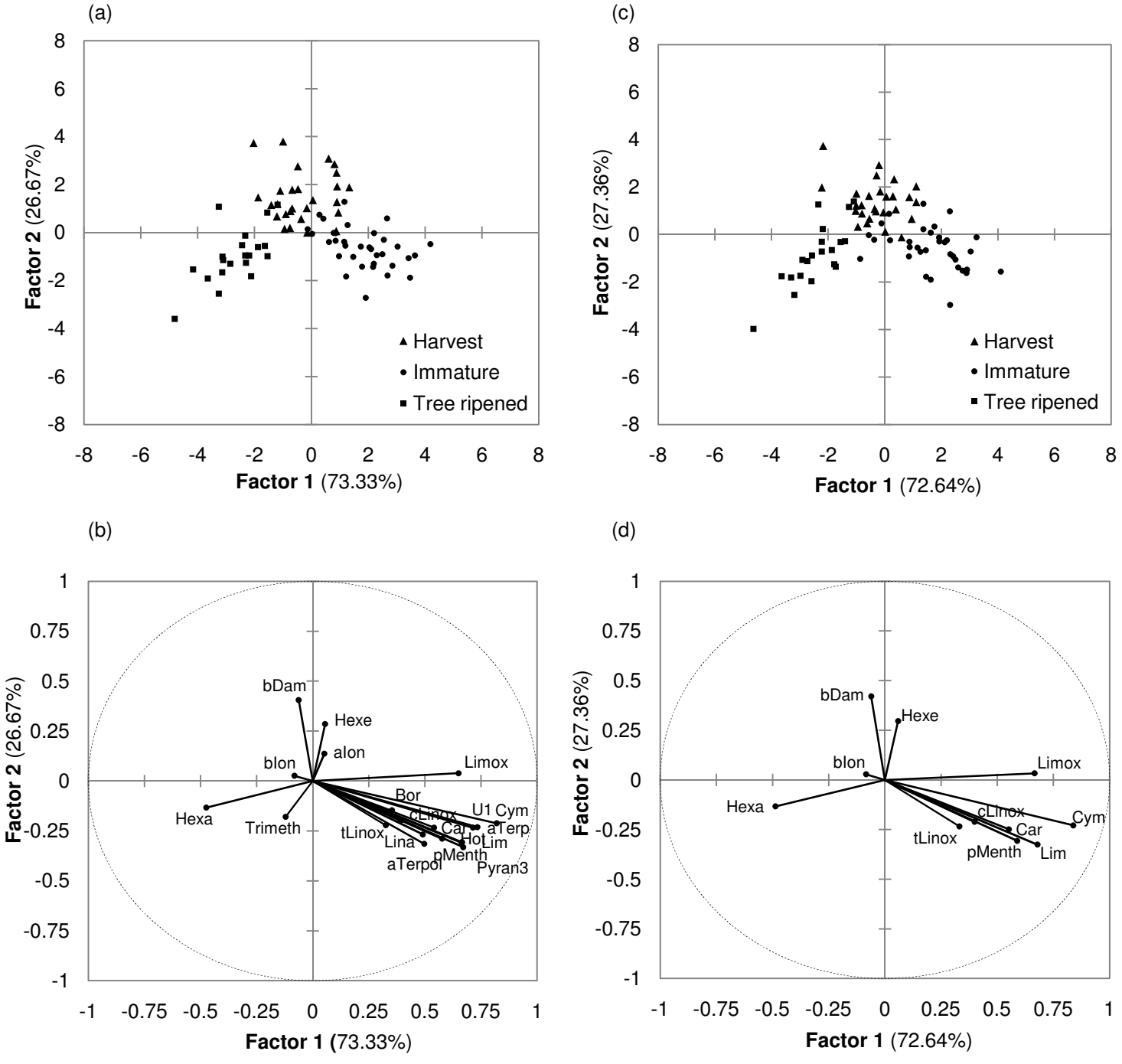


Figure 3: Observations and Variables charts for **'Laetitia'** using all the compounds ((a) and (b)) and using only the compounds identified in the stepwise method of a DA ((c) and (d)). Percentages indicate the corresponding percentage of variance described by each factor. (See Table 2 for explanation of compound abbreviations).

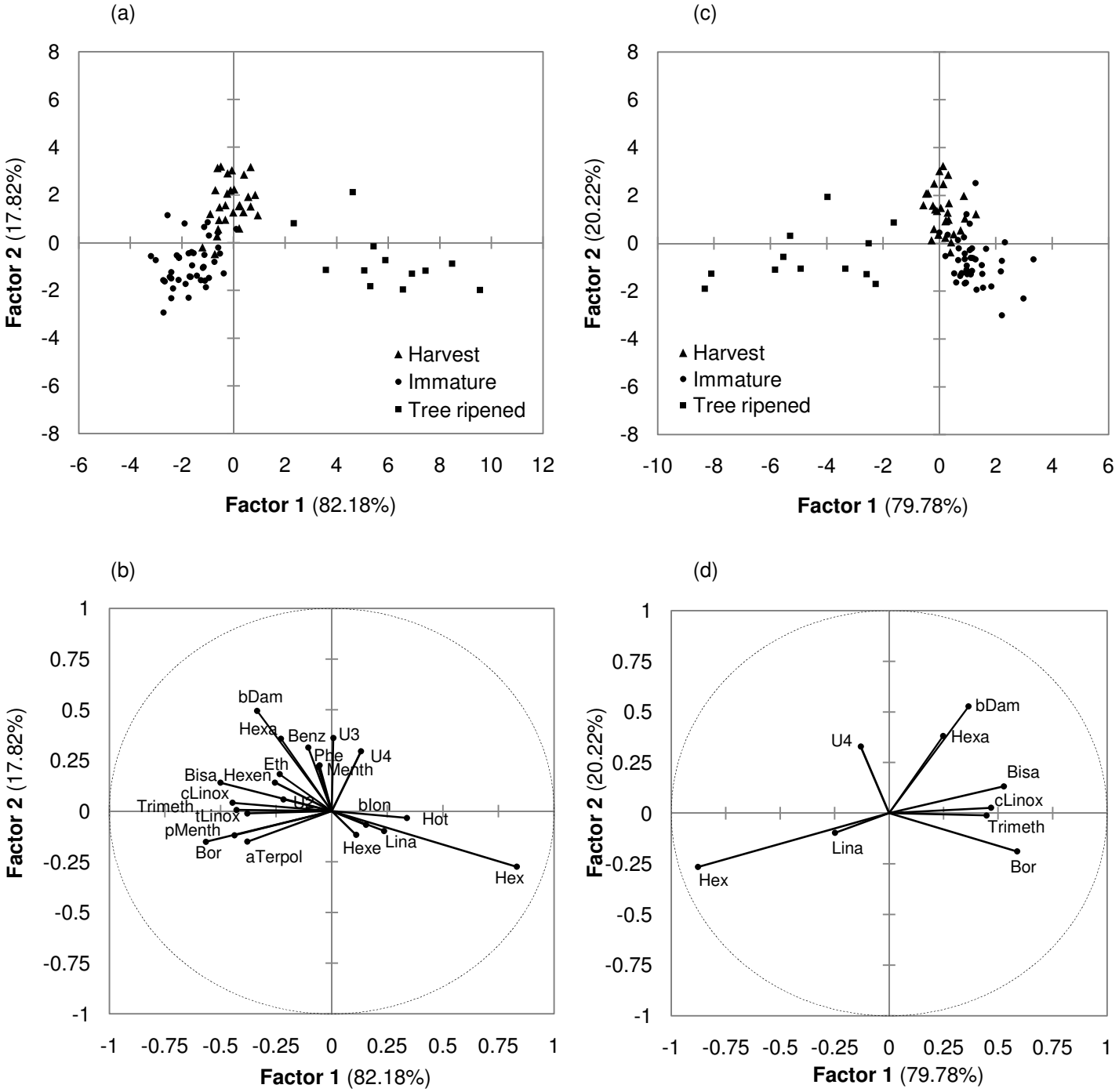


Figure 4: Observations and Variables charts for '**Angeleno**' using all the compounds ((a) and (b)) and using only the compounds identified in the stepwise method of a DA ((c) and (d)). Percentages indicate the corresponding percentage of variance described by each factor. (See Table 2 for explanation of compound abbreviations).

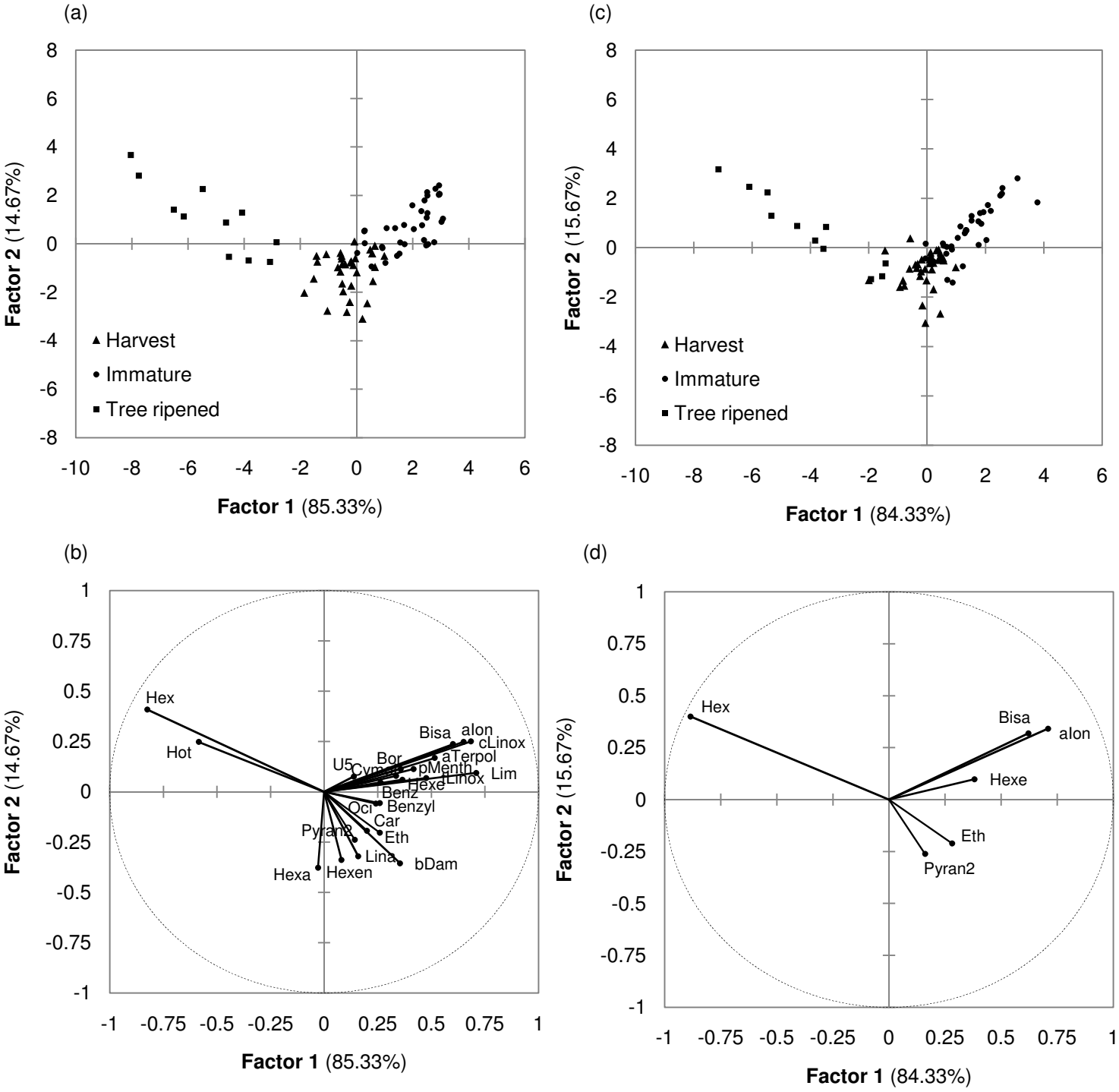


Figure 5: Observations and Variables charts for **'immature classes'** of all the three cultivars using all the compounds ((a) and (b)) and using only the compounds identified in the stepwise method of a DA ((c) and (d)). Percentages indicate the corresponding percentage of variance described by each factor. (See Table 2 for explanation of compound abbreviations).

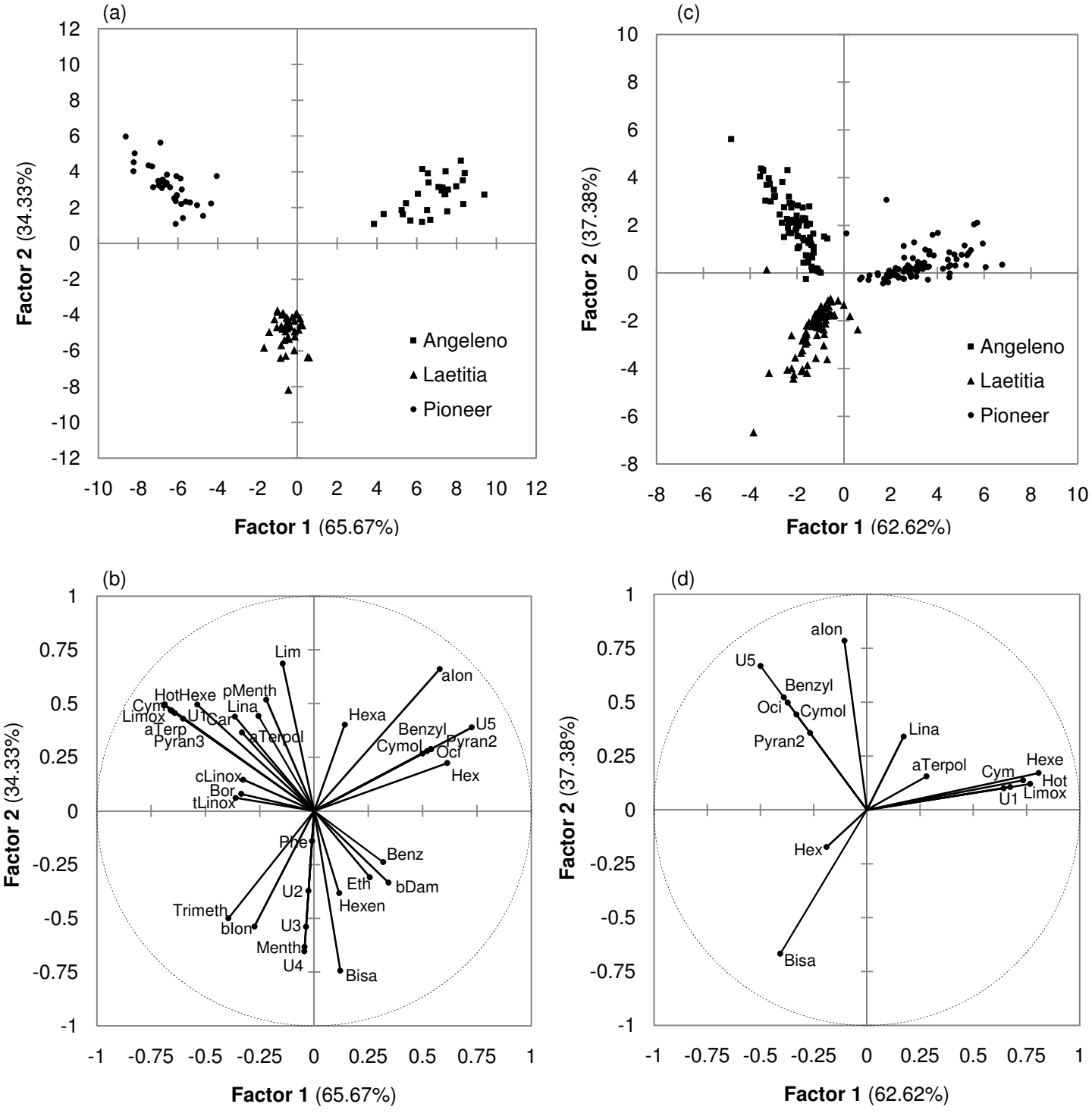


Figure 6: Observations and Variables charts for **'harvest classes'** of all the three cultivars using all the compounds ((a) and (b)) and using only the compounds identified in the stepwise method of a DA ((c) and (d)). Percentages indicate the corresponding percentage of variance described by each factor. (See Table 2 for explanation of compound abbreviations).

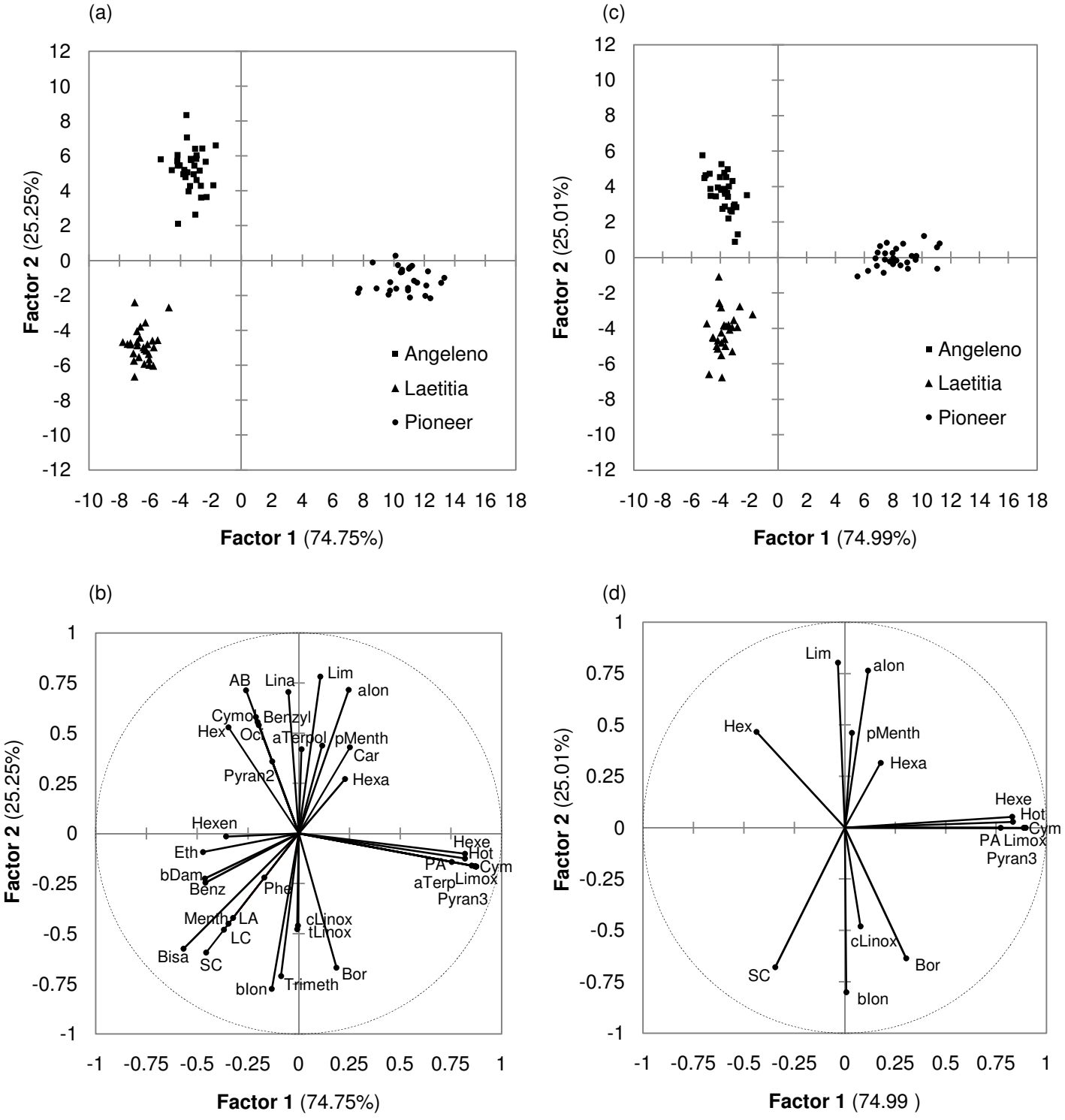
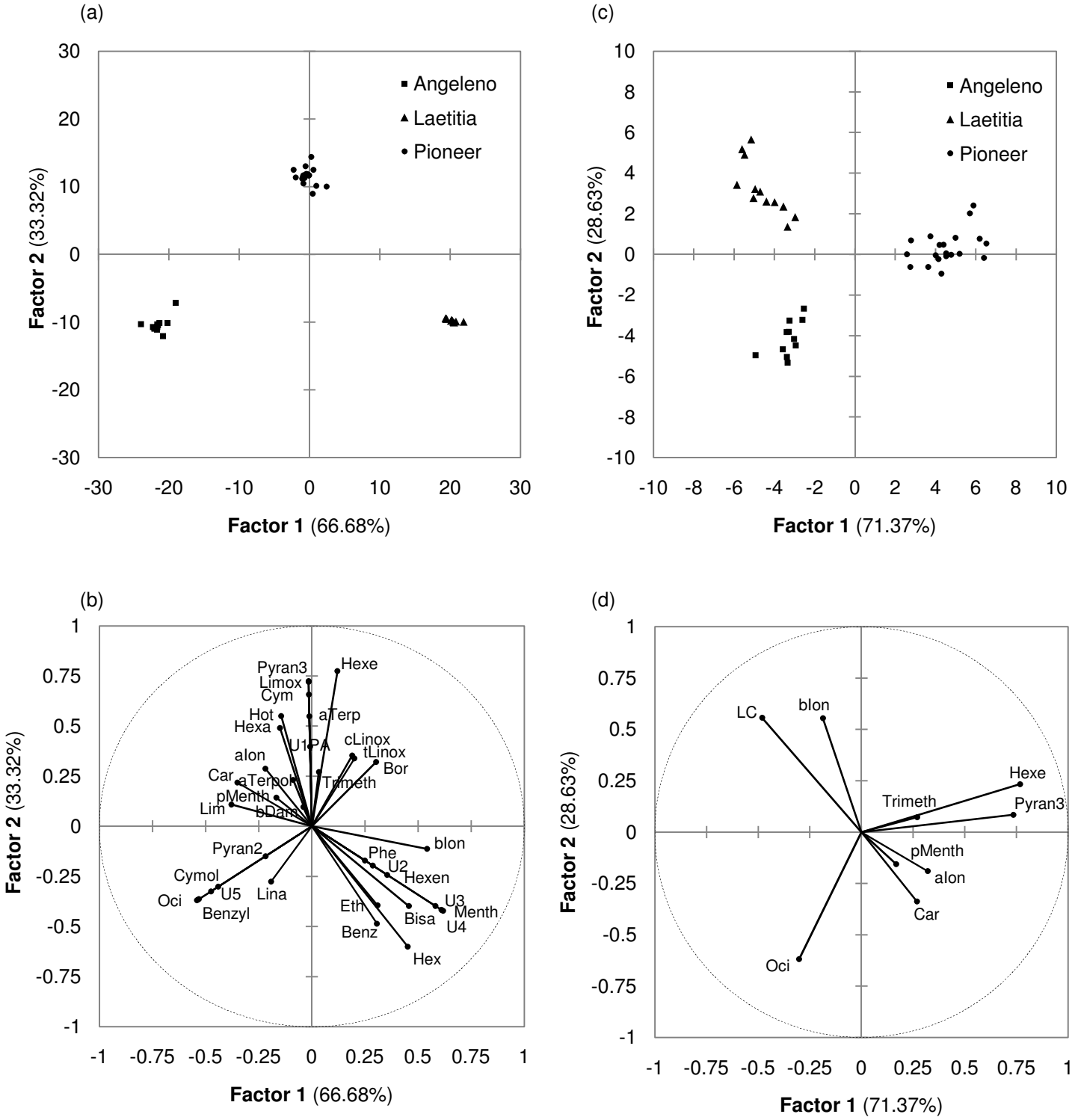


Figure 7: Observations and Variables charts for **'tree ripe classes'** of all the three cultivars using all the compounds ((a) and (b)) and using only the compounds identified in the stepwise method of a DA ((c) and (d)). Percentages indicate the corresponding percentage of variance described by each factor. (See Table 2 for explanation of compound abbreviations).



PAPER 3

The effects of ripening and cold storage on the aroma profiles of six Japanese plum cultivars (*Prunus salicina* Lindl.) and one interspecific plum-apricot cultivar.

Abstract

The aroma volatile compounds of six commercial Japanese plum cultivars (Pioneer, Sapphire, Laetitia, Songold, Larry Anne and Angeleno) and one plumcot (Flavor King) were determined at three functional stages: commercial harvest, tree-ripe fruit and fruit stored under commercial cold storage and ripening conditions. Data were collected over two plum seasons. HS-SPME was used for extraction coupled with GC-TOFMS for separation and identification of the aroma compounds. A total of 62 compounds were identified and classified into three groups ('unique' (31 compounds), 'generic' (11 compounds) and 'frequent' (20 compounds)) based on their frequency of occurrence. Discriminant analysis was used to determine the distinctness of the functional groups within each cultivar and to identify the main compounds contributing to the patterns. Results showed that the aroma profiles of 'Larry Anne' and 'Flavor King' are the most affected by cold storage conditions and 'Pioneer' appears to be the least affected. Inter-cultivar analysis indicated that all the cultivars have significantly different aroma profiles at all three of the functional stages with 'Sapphire', 'Larry Anne' and 'Flavor King' showing the largest differences. 'Flavor King', a plumcot, also presented a ripe aroma profile that was much diverged from that of the true plums.

Key words:

Aroma, volatile compounds, plums, SPME, GC-TOFMS, cold storage, harvest, tree-ripe

1. Introduction

Plums belong to the family Rosaceae, genus *Prunus* that also includes other stone fruit such as peaches, apricots, nectarines and cherries. It is a soft fruit valued by consumers for its colour, palatability and aromatic characteristics. The eating experience is based on both taste and flavour in which non-volatiles such as sugars and acids are mainly responsible for the former and volatile aromatic compounds for the latter (Williams and Ismail, 1981). Like most stone fruit, the physical (firmness, weight, appearance) and non-volatile (sugar, acid) components of plums have been studied well and are used as guidelines to establish optimum harvest dates and export grades. However, the flavour components that are responsible for the characteristic plum flavour are not as widely researched as for apricots, peaches and nectarines. The production of aroma volatiles is dynamic and the pattern of volatile constituents, both qualitative and quantitative, can vary greatly during fruit maturation and ripening (Agozzino et al., 2007; Paper 2). The effects of prolonged exposure to low temperatures i.e. cold storage on the aroma profile of stone fruit have been identified and documented for apricots (Aubert et al., 2010) and peaches (Raffo et al., 2008), but to date no literature could be found for plums.

South Africa has an active Japanese plum breeding and production sector with annual export figures (2009) of close to 9 million cartons (5.25 kg equivalent cartons) comprising 35 different plum cultivars (PPECB Information portal: http://info.ppecb.com). Plums exported from South Africa to European markets are currently harvested relatively unripe and ripen slowly whilst in transit for up to 42 days in the cold chain to the market. To prevent physiological disorders such as chilling injury and gel breakdown most plum cultivars are stored at a dual temperature regime whereby the fruit are first stored at -0.5°C for 8 to10 days followed by an increase in temperature to 7.5°C for a further 5 to 7 days after which the temperature is dropped again to -0.5°C for up to 25 days. The protocol is flexible as some cultivars are more susceptible to the disorders than others. With plum consumer preference now shifting towards flavour and taste (SASPA/Richmond Towers, UK consumer research de-brief, 2006) it has become important to further analyse the aroma profiles of Japanese plums including the possible effects of cold storage to ensure that the flavour persists throughout the marketing operation.

Inter-specific hybrids between Japanese plums and apricots (*P. salicina x P. armeniaca*) are commonly known as plumcots and are bred for their sweet taste and complex but excellent flavour (Blažek, 2007). 'Flavor King' is such a plumcot that has become a valued cultivar in South Africa over the last decade.

The aim of this study was to investigate the aroma profiles of six Japanese plum cultivars (Pioneer, Sapphire, Laetitia, Songold, Larry Anne and Angeleno) and one plumcot (Flavor King) at three different functional stages: harvest (commercial harvest intended for export), fruit left to ripen on the tree and fruit exposed to the commercial cold storage regimes used for the cultivar in South Africa. Inter- and intra-

cultivar comparisons using discriminate analysis were used to describe any shifts in the aroma profiles caused by ripening and cold storage.

2. Material and Methods

2.1 Fruit selection, harvesting and storage conditions:

Fruit from six commercial plum (*Prunus salicina* Lindl.) cultivars and one plumcot grown near Stellenbosch (Western Cape, South Africa) were used in this study. The cultivars (in order of ripening and harvest) were Pioneer, Sapphire, Laetitia, Flavor King (plumcot), Songold, Larry Anne and Angeleno. Fruit were collected at two different picking dates from commercial orchards, trained to a flat trellis system. The first picking date coincided with the commercial harvest when fruit flesh firmness was close to the average parameters as stated by the South African governmental export regulations (Table 1). At this picking date we identified and tagged ten trees that were not harvested. These trees were left to bear their fruit until ripe and ready to eat at a flesh firmness of ± 9.81 - 29.43 N (±1 - 3 kg). Fruit of similar size and colour were selected from the middle of the canopy approximately 1.5 m from the orchard floor. Fruit were transported to our laboratory, stored at ambient temperature and processed within 12 hours of harvest. A minimum of six fruit for both picking dates were processed to determine quality parameters and aroma profiles as described below. A further 80 fruit from the first picking date were packed and cold-stored for up to 42 days according to commercial export protocols (Table 1). After storage the fruit were transferred to 15°C and left to ripen to a flesh firmness of ± 9.81 - 29.43 N (± 1 - 3 kg) after which a minimum of six fruit per cultivar were processed to determine quality parameters and aroma profiles (as described below). The study was conducted over two plum harvest seasons (2008 and 2009). The cultivars Pioneer, Laetitia and Angeleno contained more samples as they formed part of a bigger study investigating the aroma volatile dynamics throughout ripening (as described in Paper 2).

2.2 Determination of fruit quality parameters

Refer to Section 2.2 of Paper 2 for material and methods.

The range, mean and standard deviation values were determined for each quality parameter and are presented in Table 2.

2.3 Aroma volatile sample preparation

Refer to Section 2.3 of Paper 2 for material and methods.

2.4 HS-SPME conditions

Refer to Section 2.4 of Paper 2 for material and methods.

2.5 GC TOF-MS conditions and compound identification

Refer to Section 2.5 of Paper 2 for material and methods.

2.6 Data classifications and statistical analysis

The collection and storage of the fruit resulted in the creation of three distinct functional sample groups namely, 'Harvest', 'Tree-ripened' and 'Stored'. The 'Harvest' group was represented by fruit picked during the commercial harvest and processed immediately. These fruit are considered physoplogically mature but due to "early" South African plum harvest practices are not "ripe-and-ready-to-eat". The 'Tree-ripened' group contained fruit that were left on the tree to ripen and which were only picked and processed when considered "ripe-and-ready-to-eat" at a flesh firmness of ± 9.8 - 29.4 N (±1 - 3 kg). The 'Stored' group was limited to fruit that were picked during the commercial harvest and then stored according to commercial export protocols and ripened.

Aroma volatile data were subjected to a one-way analysis of variance (ANOVA) to evaluate the significance of possible differences between the functional groups. Comparison testing was done on the mean values of each compound using Fisher's LSD (Least Significant Difference) test ($p = 0.05$ level).

For this study Discriminant Analysis (DA) was performed on the three functional groups within each cultivar to establish if the groups are distinctly different in terms of their aroma profiles and to characterise the groups we used the stepwise method to identify the aroma volatiles that contribute significantly to the separation patterns. This will aid in the understanding the effects that commercial cold storage had on the aroma profiles of harvested fruit and how they differed from fruit ripened on the tree. Inter-cultivar DA was also performed for all the functional groups to identify possible differences and/or similarities amongst the seven cultivars and possibly identify cultivars that were more sensitive to a shift in aroma caused by commercial cold storage.

All calculations and modeling were performed using XLSTAT Version 2010.4.01. The results of the DA are described using the following statistical terms, figures and tables (Microsoft Windows Help for XLSTAT Version 5.1.2600.5512):

- **Discriminant factors**: When analysing the functional groups within each cultivar, there are only two discriminant factors (F1 and F2) that will describe 100% of the variance because there are only three groups. The inter-cultivar analysis, however, has up to six discriminant factors (F1 to F6) that will describe 100% of the variance because the seven cultivars are now considered as groups. (The maximum number of factors is equal to k-1, when n>p>k, where n is the number of observations, p the number of explanatory variables, and k the number of groups.)
- **Observations chart (figure):** This figure represents each of the observations (samples) on the factors axes. It allows confirming that the groups are well discriminated on the factor axes extracted from the original explanatory variables.

- **Variables chart (figure):** This figure shows how the initial variables (aroma compounds) are correlated with the two factors and aims to describe the factor axes.
  Note that observation and variable charts are usually discussed together by overlaying the four quadrants. (Quadrants are numbered clockwise starting with 1st quadrant in the upper right-hand corner).
- **Confusion matrix (table):** This table summarises the reclassification of the observations, and allows to quickly asses the percentage of well classified observations, which is the ratio of the number of observations that have been well classified over the total number of observations.
- **Variable selection table**: This table represents a summary of the variables that contribute most to the groups and are determined using a forwards stepwise analysis to build a model of discrimination. This table also shows the statistics used to evaluate the goodness of fit of the model (determination coefficient for the model (Partial R^2), F-ratio test (F statistic), and p-value at a significance level of 0.05 (Pr>F)).

3. Results and discussion

3.1 Quality parameters:

Fruit firmness was the maturity index used to create the three functional classes. The 'Harvest' and 'Stored' fruit were picked (and processed in the case of the 'Harvest' group) when the average fruit firmness was well within the export window and requirements (Table 1). Both the 'Tree ripened' and the 'Stored' groups were processed when the firmness was ± 9.8 - 29.4 N (± 1 - 3 kg) and assumed to be 'ripe-and-ready-to-eat'. All cultivars reflected this practice except for the 'Stored' group of 'Angeleno' fruit where the average firmness was 50.2 (±3.04) N (5.1 (±0.31) kg). 'Angeleno' fruit seem to soften at a much slower rate when ripened after storage compared to the other cultivars and appeared to have a 'rubbery' texture as opposed to the soft texture of the other cultivars. The samples were processed at a higher firmness as the onset of decay would have preceded a fruit firmness of ≤ 29.4 N (≤3 kg).

The other quality parameters, which were not controlled, showed obvious differences when comparing the functional groups (Table 2). These include slight weight loss during storage and increased TSS during tree ripening as would be expected (Table 2). Average fruit weight of the 'Stored' group was always found to be less than in the 'Harvest' group for all the cultivars. Similarly, the sugar (TSS) levels of the 'Stored' fruit were also lower than those of the 'Harvest' fruit due to the fruits' inability to replenish the carbohydrates metabolised during biological processes once separated from the tree. 'Tree-ripened' fruit were often bigger in size and weight and had higher sugar content when compared to 'Harvest' and 'Stored' fruit as they had a longer opportunity to develop these attributes while attached to the tree during ripening. The opposite pattern was observed for acid levels as a decrease in acids is recorded in the 'Tree-ripened' and 'Stored' samples. This is expected as it is well known that acid levels drop during the ripening process to produce a more palatable fruit. The cultivars with the highest sugar levels were

samples from 'Flavor King' and 'Larry Anne' fruit in the 'Tree-ripened' group with levels as high as 19.0% Brix ('Larry Anne') and 21.0% Brix ('Flavor King'). Although the lowest average acid level was present in the 'Tree-ripened' samples of 'Angeleno' with a value as low as 0.68 (±0.07) % malic acid it was followed by the 'Tree-ripened' samples of again 'Flavor King' (0.85 (±0.28) % malic acid) and 'Larry Anne' (1.00 (±0.23) % malic acid), resulting in relatively high sugar-to-acid ratios.

3.2 General trends in the aroma profiles of the seven plum cultivars:

A total of 62 aroma volatile compounds was identified for all seven cultivars. The mean value for each volatile compound in each functional group of each cultivar is presented in Table 3a and b. To simplify the discussion the data were regrouped according to the frequency in which the compounds occurred in each cultivar (Table 4).

Of the 62 compounds 31 (50%) were only present in one of the seven cultivars and for the purposes of this study classified to be 'unique' to that particular cultivar although we in no way suggest that it is not present in other plum cultivars not included in this study or at levels below our detection range (Table 4). Due to the low frequency of occurrence it is likely that these components, when present in ripe fruit, contribute to the cultivar specific aroma of plums rather than the general plum aroma. Of the remaining 31 compounds the following 11 compounds were measured in at least six of the seven cultivars and thus termed 'generic': hexanal, 2-hexenal, *trans* linalool oxide, *cis* linalool oxide, linalool, α-terpineol, p-menth-1-en-9-al, β-damascenone, hotreinol, 2-bornene, β-ionone. This high frequency of occurrence makes it more likely that these 11 compounds contribute to the general plum aroma. The remaining 20 compounds were shared amongst two to five of the cultivars and classified as 'frequent', implying that they are often found in plum cultivars. See Table 4 for the specific cultivars, compounds and frequency of occurrence.

3.3 'Pioneer' aroma profiles:

Of the total of 62 compounds identified in this study, only 20 were detected in 'Pioneer' (Table 3a and 4). All 20 compounds were measured in all three of the functional groups indicating that ripening and storage did not compromise the number of aroma compounds detected (Table 3a). Two 'unique' compounds were present, α-terpinene and unidentified 1(Table 4). The 'Harvest' samples were characterised by 12 of the 20 compounds measuring at their highest levels. The 'Tree-ripened' group showed most samples having significantly lower levels compared to the 'Harvest' group with only hexanal increasing to a level almost twice that of 'Harvest' group. When comparing the 'Stored' group to the 'Tree-ripened' group 50% of the aroma compounds have significantly different levels. Stored 'Pioneer' fruit seem to have increased levels of limonene, limonene oxide, β-cymene, α-terpinene, 2H-pyran,3,6.dihydro-4-methyl-2-(2-methyl-1-propenyl), 2-bornene, β-ionone and 4-(2,4,4-trimetyl-cyclohexa-1,5-dienyl)but-3-en-2-one. Only the levels of hexanal and 2-hexenal decreased significantly during storage (Table 3a).

The DA results indicated in both the confusion matrix (Table 5(b)) and the observation chart (Fig 1(a)) that the aroma profiles of the three functional groups were distinctly different from each other with an average of 92.31% of the samples correctly assigned. The variables plot (Fig 1(c)) confirmed that the 'Harvest' group contained the most samples at high levels with many compounds situated in quadrant 1 closest to the grouping of the 'Harvest' samples. A stepwise DA selected eight compounds as contributing to the separation of the groups with 2H-pyran,3,6.dihydro-4-methyl-2-(2-methyl-1-propenyl), 2-hexenal and β-ionone topping the list (Table 5(a)). When performing a DA using only the eight selected compounds the three functional groups remained distinct with 90.77% of the samples correctly assigned. This proves that these eight compounds are important during the ripening and storage of 'Pioneer' as they continued to separate the functional classes even when tested in isolation. Interestingly, six of the selected eight compounds were also 'generic' compounds possibly indicating that the general plum aroma of the three functional groups of 'Pioneer' is, indeed, different.

3.4 'Sapphire' aroma profiles:

For the 'Sapphire' samples from all three functional groups we measured a total of 26 different compounds (Table 4) which were present in all the groups except for n-hexyl acetate that was not detected in the 'Harvest' samples (Table 3a). Of the 26 compounds 18 showed no significant differences between any of the three functional groups implying that the means of the groups are relatively equal with little difference in up to 70% of the aroma profile. Interestingly, 'Sapphire' contained six unique compounds including nonanal, α-pinene, 1,3,cyclo-hexadiene1,3,5,5,tetra-methyl, eugenol methylether, 2-isopropylidene-5-methylhex-4-enal and *cis*-geranylacetone (Table 4). These compounds account for 23% of the total number of compounds detected for 'Sapphire', which was the cultivar with the second highest number of unique compounds, only surpassed by 'Flavor King' which is not a true plum, but a plum x apricot hybrid. Cold storage affected only six of the aroma compounds significantly in such a way that four of the six compounds (2-hexenal, 4-(2,6,6-trimethyl-cyclohexa-1,5,-diemethyl)but-3-en-2-one), γ-decalactone and 4-(2,4,4-trimetyl-cyclohexa-1,5-dienyl)but-3-en-2-one) had drastically increased their levels with the latter increasing by up to five fold when compared to fruit ripened on the tree (Table 3a). On the contrary, the levels of 2-isopropylidene-5-methylhex-4-enal and α-pinene (two unique compounds) failed to rise when ripened after cold storage as in the case of tree-ripened fruit, but remained at the same relatively low levels as seen in the harvest fruit.

The apparent similarities expressed by the significance testing mentioned above were not confirmed in the DA. In fact, the three functional groups of 'Sapphire' appeared to be more distinct than those of 'Pioneer' with 100% correctly assigned samples (Table 6(b)) and clearly separated groups with an increased distance between the groups compared to 'Pioneer' (Figure 2(a)). The only hint of similarity was seen in the variables chart where the compounds all seem to be equally spread throughout the 4 quadrants, most with similar distances from the origin (Figure 2(c)). The stepwise DA also illustrated a

divergence amongst the functional groups by selecting eight compounds as contributing most to the pattern (Table 6(a)). The two top compounds (2-hexenal and γ-decalactone) were also hugely affected by storage. Although the divergence amongst the groups tended to lessen and the percentage correctly assigned compounds dropped to 94.44% (Figure 2(b) and Table 6(c)) when the DA was repeated using only the eight selected compounds, the functional groups remained different from each other implying that the eight compounds were indeed contributing to the variance.

3.5 'Laetitia' aroma profiles:

A total of 22 compounds (Table 3a and 4) was measured for the 'Laetitia' samples with all present in the 'Harvest' samples, but only 20 present in the 'Tree-ripened' samples and 17 in the 'Stored' samples. Four of the compounds found in 'Laetitia' were unique, of which only one could be successfully identified (menthol). Similar to 'Pioneer', most of the compounds (17 of the 22) were at their highest levels in the 'Harvest' samples with a significant decrease in levels as the fruit ripened with phenol and unidentified 2 disappearing from the profile. The 'Tree-ripened' samples did, however, express higher levels of 2-hexanal, β-ionone, linalool, hotreinol and especially 1-hexanol, with levels more than 47 times those found in the 'Harvest' samples. The aroma profile of the cold stored samples differed from the 'Tree-ripened' samples by exhibiting even further decreasing levels for most of the compounds and a further disappearance of three compounds (2-ethylfuran, 2-hexen-4-olide and bisabalol oxide B) from the profile. The 'Stored' profile appeared to be weaker in every sense with no compounds showing any significant increases (Table 3a).

The DA confirmed the profiles mentioned above with a 100% separation of the functional groups (Table 7(b) and Fig. 3(a)) and most compounds associated with the 'Harvest' and 'Tree-ripened' groups (Fig. 3(c)). The stepwise analysis selected eight compounds as important contributors to the separation of the groups with 1-hexanol, β-damascenone and p-menth-1-en-9-al as the biggest role players (Table 7(a)). The importance of these compounds was verified when the confusion matrix and observation charts continued to show well separated groups (Fig. 3(b) and a 98.33% score on correctly assigned samples (Table 7(c)).

3.6 'Flavor King' aroma profiles:

This plum x apricot hybrid had the most aroma volatiles of all the cultivars, a total of 32 compounds was detected with 10 uniquely associated with 'Flavor King' (Table 4). Twenty-nine compounds made up the aroma profile of the 'Harvest' group with *cis*-3-hexenol, octyl acetate and pentamethylene acetate not present at harvest (Table 3a). Further characteristics of the 'Harvest' samples include significantly higher levels of hexanal, 2-hexenal, 2-hexen-4-olide, *trans* and *cis* linalool and hotreinol compared to the 'Tree-ripened' group and significantly lower levels of butyl acetate, n-hexylformate, heptyl acetate, p-menth-1-en-9-al, β-ionone and the two γ-lactones (decalactone and dodecalactone) that seemed to be associated

more with ripe fruit. Cold storage of 'Flavor King' fruit seemed to alter the aroma profile of the fruit considerably with a shift of almost 60% when compared to the profile of fruit ripened on the tree. No less than 19 components showed significant differences in their levels when exposed to extensive cold storage with either increases in esters such as butyl acetate, amylacetate, butyl butanoate, *cis*-3-hexenyl acetate as well as *cis*-3-hexenol and hotreinol or decreases in hexanal, linalool, ocimenol, heptyl acetate, octyl acetate, the two ionones (α and β) and the two γ-lactones (decalactone and dodecalactone) (Table 3a). These differences were spread amongst both 'generic' and 'unique' compounds suggesting that the general aroma and cultivar specific aroma changed during storage (Table 4).

The observation chart (Fig. 4(a) of the DAs illustrated the differences in the three functional groups with large distances between the groups. When comparing the scale of the two axes to those of the 'Pioneer', 'Sapphire' or 'Laetitia' charts it became evident that the divergence between the functional groups and their associated aroma profiles (Fig. 4(c)) was much greater. The cause of this separation pattern was explained in more detail by the nine components identified by the stepwise analysis as the major contributors (Table 8(a)). Hexenal, pentamethylene acetate and α-ionone were selected as the top most compounds responsible for the differences in the aroma profiles. When reworking the DA using only the nine compounds from the stepwise list the three functional groups remained distinct, although from the scale of the axes the groups were now closer to one another and more similar compared to the true plums such as 'Laetitia' and 'Sapphire'. Both confusion matrixes (Table 8(b) and (c)) showed 100% correctly assigned samples.

3.7 'Songold' aroma profiles:

Similar to 'Pioneer', we identified a total of 22 compounds in all the samples of 'Songold' (Table 3b and Table 4). Only two 'unique' compounds were detected but they could not be identified without a significant amount of uncertainty. All 22 compounds maintained their presence from the 'Harvest' to the 'Tree-ripened' phase, but during cold storage one component, p-cymen-8-ol, decreased to undetectable levels (Table 3b). Following the pattern of 'Laetitia' and 'Pioneer' the 'Songold' 'Harvest' samples also had the majority of the compounds at their highest levels, including five of the 'generic' compounds. The fruit left to ripen on the tree showed rising levels of 1-hexanol, 2-ethylfuran, 2-hexen-4-olide and β-ionone whilst maintaining relatively high levels of the generic compounds β-damascenone, hexanal and 2-hexenal already present at harvest. Cold storage conditions altered the ripening of the 'Songold' in such a way that nine of the 22 compounds in the 'Stored' functional group were significantly different from the 'Tree-ripened' fruit. Most of the affected compounds decreased drastically during storage indicating failure to increase their levels except for 1-hexanol that was found to accumulate levels of more than double compared to the 'Tree ripened' fruit and more than 52 times its harvest level. The compounds 4-(2,4,4-trimetyl-cyclohexa-1,5-dienyl)but-3-en-2-one and unidentified 6, however, managed to maintain

their harvest levels during storage suggesting a failure to metabolise these compounds as seen in the 'Tree-ripened' samples or possibly a loss followed by a resynthesisation.

As expected, the DA pattern of 'Songold' also indicated three distinct functional groups (Fig. 5(a)) with no confusion in the groups (Table 9(b)). The variables plot confirmed the aroma profiles described above (Fig. 5(c)) with most compounds found in quadrant 1 similar to the 'Harvest' samples and specific individual compounds scattered in the third and fourth quadrants in correlation with the 'Tree-ripened' and 'Stored' groups. Stepwise analysis selected five variables as the main compounds responsible for the separation of the groups (Table 9(a)) with relatively high partial R^2 values for the first four compounds. Interestingly, phenol that was only also measured in the 'Harvest' fruit of 'Laetitia' topped the list and was again mostly associated with the 'Harvest' samples although now present in all three groups. The effects of these compounds are real as they continued to separate the functional groups when tested in isolation although the distances between the groups seem to be closer than before (Fig. 5(b) and Table 9(c)).

3.8 'Larry Anne' aroma profiles:

'Larry Anne' contained the most (27) aroma compounds of the true plum cultivars tested (Table 3b and Table 4). It represented all the 'generic' and four 'unique' compounds of which two (2-[(2E)-2-butenyl]-1,3,5-trimethylbenzene and *cis*-p-menth-2,8-dienol) were positively identified. In general, all the compounds were present in all of the functional stages with the exception of n-hexyl acetate being absent from the 'Harvest' samples. Again, as seen in most of the plum cultivars tested, the 'Harvest' samples contained the highest levels of the majority of the compounds with decreasing trends during ripening. Significantly lower levels were only measured for hexanal and 2-hexen-4-olide and, similarly to 'Flavor King', low levels were also detected in the 'Harvest' samples for the two γ-lactones (deca- and dodeca-) and β-ionone. These five compounds, together with n-hexyl acetate, accounted for the high level compounds found in the 'Tree-ripened' fruit. As in the case of 'Flavor King', cold storage seemed to have a confounding effect on the aroma profile of the fruit with 56% of the total number of compounds altered when compared to fruit left to ripen on the tree. The general pattern caused by long term storage at low temperatures seemed to be the decreasing of compound levels to amounts significantly different to both the 'Harvest' and 'Tree-ripened' groups. 2-Hexen-4-olide, β-ionone, γ-dodecalactone and n-hexy lacetate also followed this trend but did not dip below the levels detected in the 'Harvest' fruit. Five compounds (p-menth-1-en-9-al, 2-hexenal benzaldehyde and the two linalool oxides (*cis* and *trans*)) showed levels much higher after storage when compared to tree-ripened fruit. Interestingly, hexanal and γ-decalactone seemed to be unaffected by cold storage and have accumulated to a similar level as found in the 'Tree-ripened' fruit.

In some aspects the DA results for 'Larry Anne' also seemed to be similar to those of 'Flavor King'. There was a large divide between the three functional groups, the values of the x-axis were also much larger

than those found in the other true plum cultivars (Figure 6(a)) and the variables chart was scattered with compounds a similar distance from the origin (Figure 6(c). Nine main compounds creating this divergence pattern were identified by the stepwise DA (Table 9(a)) with the top five compounds from the 'frequent' classification, no 'unique' compounds were on the list and only three 'generic' compounds were found at the bottom of the list. In the light of these results it is expected that the confusion matrixes (Table 10(b) and (c)) both show 100% correctly assigned samples. When only the nine compounds selected by the stepwise analysis were used in a DA the observations chart (Fig. 6(b) showed the functional groups now closer to one another and more similar to the patterns seen in the other cultivars although the aroma profiles (Fig. 6(d)) remained distinct.

3.9 'Angeleno' aroma profiles:

'Angeleno' samples had a total of 23 detectable compounds with three 'unique' (2H-pyran,2-ethenyltetrahydro-2,6,6 tri-methyl, unidentified 5 and benzyl acetate) compounds and only six of the seven 'generic' compounds (no β-ionone) present (Table 3b and Table 4). The 'Harvest' samples contained all 23 compounds and were characterised by high levels of most compounds except for 1-hexanol and hotreinol that were significantly higher in the 'Tree-ripened' fruit. All other compounds found in the aroma profile of 'Tree-ripened' samples were at much lower levels with 2-hexen-4-olide, p-cymen-8-ol and bisabalol oxide B decreasing to below detectable levels. The fruit exposed to cold storage had aroma profiles significantly different to the 'Tee-ripened' fruit with the levels of nine components altered. Most of the altered components had levels higher than that of the 'Tree-ripened' fruit but similar to that of the 'Harvest' fruit, suggesting that the lower temperature prevented them from being metabolised. In contrast to this, hexanal levels had decreased to below those of both the 'Harvest' and 'Tree ripened' samples and benzaldehyde was no longer detectable. The levels of unidentified 5, however, increased significantly under cold storage conditions. Similar to the 'Tree-ripened' group, the aroma profile of the 'Stored' group also lacked both 2-hexen-4-olide and p-cymen-8-ol.

The DA of 'Angeleno' appeared to be similar to that of 'Pioneer' with the three functional groups in a closer association than in the other cultivars (Fig. 7(a) and some overlap between the 'Tree-ripened' and 'Harvest' groups resulting in only 95.59% of the samples correctly assigned (Table 11(a)). The 'Stored' functional group did, however, remain distinct from the rest indicating that although the 'Tree-ripened' and 'Harvest' groups shared some characteristics the 'Stored' group had a completely different aroma profile. The variables chart (Fig. 7(c)) agreed with the aroma profiles described above with the compounds scattered amongst quadrants 2, 3 and 4 and none in quadrant 1 where the 'Tree-ripened' samples were situated, indicating the relatively low levels of the compounds once the fruit had ripened on the tree. The patterns observed in the DA are mainly created by the five compounds listed in Table 11(a) as selected by the stepwise analysis. The unidentified 5 component that had significantly increased levels under storage conditions, topped the list followed by 1-hexanol and hexanal that were associated with 'Tree-

ripened' and 'Harvest' fruit, respectively. When the DA was repeated using only the five selected compounds the overlap between the aroma profiles of the 'Tree-ripened' and 'Harvest' increased with more confusion between the assigned samples (Table 11(c)) but the 'Stored' group still remained divergent.

3.10 Comparing the different functional groups of the cultivars:

From the intra-cultivar results presented thus far it was evident that the functional groups within each cultivar each had a different aroma profile. To compare the functional groups of the cultivars with one another the DA was repeated using the cultivars as the dependant variables. This inter-cultivar analysis aimed to recognise and describe the similarities and/or differences amongst the cultivars within each functional group.

From the observations plot for the 'Harvest' groups (Fig. 8(a)) it was visually evident that three of the cultivars ('Flavor King', 'Sapphire' and 'Larry Anne') were well separated from a cluster representing the remaining cultivars ('Pioneer', 'Laetitia', 'Songold' and 'Angeleno'). This suggested that the aroma profiles of 'Flavor King', 'Sapphire' and especially 'Larry Anne' were already very different from the rest of the cultivars at harvest. The cluster formation of 'Angeleno', 'Laetitia', Songold' and, to a lesser degree, 'Pioneer' suggested that these cultivars had more similar aroma profiles at harvest. When compared with Fig. 8(b) the aroma profiles were illustrated relative to the seven cultivars with 81.13% of the variance described by Factors 1 and 2. One should, however, be cautious of the scale of these plots as it might appear that the cluster of cultivars was very similar and overlapping when they were in fact, well separated when analysed in isolation from 'Sapphire', 'Flavor King' and 'Larry Anne' (inserted graph in Fig. 8(a)) with all the cultivars having 100% correctly assigned samples (Table 12(b)). The stepwise analysis identified 38 of the 62 compounds as significant contributors to the pattern with limonene oxide and *cis*-3-hexenyl acetate, two 'frequent' compounds, at the top of the list. Nine of the 11 'generic' compounds were also on the list and could possibly indicate that the general plum aroma of the seven cultivars was different at harvest. It should also be kept in mind that 'Harvest' fruit were believed to be physiologically mature although not yet ripe and therefore it is expected that the aroma profiles might still be under developed. When the 38 selected compounds were retested in isolation the observation chart did not change much and the confusion matrix remained at 100% implying that these compounds were indeed responsible for the divergent aroma profiles (Fig. 8(c) and (d) and Table 12(c)).

Once the fruit had ripened on the tree the DA showed a divergence pattern similar to that of 'Harvest' with 'Angeleno', 'Laetitia', Songold' and 'Pioneer' again forming a cluster and 'Flavor King', 'Sapphire' and 'Larry Anne' again well separated (Fig. 9(a)). The biggest difference between the 'Harvest' and 'Tree-ripened' pattern was that 'Flavor King' and 'Larry Anne' had now swapped positions and that 'Flavor King' was distinctly different from the rest of the cultivars as pointed out by the increased scale on the x-axis.

This might be expected as ‘Flavor King’ is a plum x apricot hybrid and thus strongly influenced by an apricot aroma profile that separates it from the true plums. The variables chart (Fig. 9(b) also illustrated the difference in the ‘Flavor King’ profile by lumping all of the compounds unique to ‘Flavor King’ in the top part of the second quadrant similar to where the ‘Flavor King’ samples were nested. For the true plums this analysis indicated that the likeness of the aroma profiles did not appear to change much as the fruit ripened, except for ‘Larry Anne’ fruit that seem to be more similar to the other cultivars once it had ripened compared to the harvest stage. The pattern in the ‘Tree-ripened’ aroma profiles seemed to be governed by more compounds as for the ‘Harvest’ group with 46 different compounds selected by the stepwise analysis (Table 13(a)). Interestingly, the list was crowned by *cis*-p-menth-2,8-dienol and amylacetate that belonged to the ‘unique’ collection of compounds for ‘Larry Anne’ and ‘Flavor King’ respectively. Although nine of the ‘generic’ compounds were present, the list seemed to be heavily populated with the ‘unique’ compounds that aid in segregating each cultivar from the rest. Fig. 9(c) and (d) showed a virtually unchanged distribution of the seven cultivars although the scale was reduced indicating that the compounds selected could present the same separation pattern. The distinctness of the seven cultivars was again highlighted by the 100% correctly assigned samples for both Tables 13 (b) and (c).

When the DA of the ‘Stored’ samples (Fig. 10 and Table 14) was studied it appeared that the seven cultivars maintained the pattern of separation seen in the ‘Harvest’ and ‘Tree-ripened’ group with the cluster (‘Angeleno’, ‘Laetitia’, Songold’ and “Pioneer’) again present and ‘Flavor King’, ‘Sapphire’ and ‘Larry Anne’ again showing large divergence towards one another and towards the cluster. Interestingly, ‘Flavor King’, and ‘Larry Anne’ had again swapped positions and were now similar to what was seen in the observations chart of the ‘Harvest’ samples with ‘Larry Anne’ to the far right of the graph. The scales of both axes had noticeably increased indicating a further drift amongst the aroma profiles of the ‘Stored’ samples. ‘Sapphire’ had also changed position and had moved further away from ‘Flavor King’ when compared to the ‘Harvest’ pattern. Compared to the ‘Tree-ripened’ distribution pattern the biggest difference brought about by the low temperature exposure seemed to be in the positioning of ‘Larry Anne’ and ‘Flavor King’ relative to the rest of the cultivars. They had not just changed position, but also increased their distance from the other cultivars suggesting a large shift in their aroma profiles. This effect confirmed what was seen in the intra-cultivar analysis of these two cultivars where the apparent changes in the aroma profiles caused by cold storage were also most severe. The stepwise DA identified 34 of the 62 compounds as the main contributors to the pattern seen in the observations chart (Table 14(a)) with again the ‘unique’ compounds, unidentified 9 and unidentified 8, belonging to ‘Larry Anne’ and ‘Flavor King’ respectively, topping the list. Again it is important to bear in mind that although this discussion labeled some cultivars as ‘like’ or ‘similar’ with respect to their aroma profiles they all remained 100% distinct with no apparent overlap as indicated by Tables 14(b) and (c).

The seemingly larger difference in the aroma profiles of 'Sapphire', 'Flavor King' and 'Larry Anne' compared to the cluster cultivars in all three of the functional classes was highlighted by the 'frequent' compounds γ-decalactone, γ-dodecalactone and n-hexyl acetate that occurred exclusively in these three cultivars.

4. General discussion

Cold storage extends the postharvest life of fruit by lowering the respiration and ethylene biosynthesis rates, thereby delaying ripening and senescence. Counter productive to this, the lowering of respiration rates and ethylene production has a negative impact on the aroma quality of fruit by lowering the supply of precursors (especially fatty acid precursors) to the biochemical pathways leading to the formation of aroma compounds (Song and Bangerth, 2003). Interestingly, the enzymes (lipoxygenase and β-oxidative enzymes in the case of fatty acids) required to synthesize the aroma compounds from the precursors are not the limiting factor (Song and Bangerth, 2003). The results above showed that the ripening of plums and the effects of cold storage seemed to be cultivar specific with unique profiles for each of the functional groups indicating a shift in the aroma as the fruit ripened and/or were exposed to long term storage conditions. This individual response also makes it difficult to comment on the possible differences that dual temperature storage (-0.5 °C and 7.5 °C) might have, compared to the single temperature storage (only -0.5 °C) that was used in the case of 'Angeleno'. To asses this it is suggested that the same cultivar should be stored using both regimes and then compared.

When considering the different chemical groups measured within the seven cultivars there were some prominent compounds and general trends worth discussing even if there is no existing literature on plum storage studies from which to draw direct comparisons.

The alcohol group was mostly characterised by 1-hexanol that was present in three of the seven cultivars. In 'Angeleno' and 'Laetitia' it was strongly associated with 'Tree-ripened' fruit with significantly higher levels that were not maintained during storage. 1-Hexanol is commonly described as having a 'green' aroma and has been widely identified in ripe European (Ismail et al., 1981b) and Japanese plums (Gómez and Ledbetter, 1994) and plumcots (Gómez and Ledbetter, 1997). Interestingly it has been recorded to be present at a lower level in ripe plums with a strong plum-like odour and is said to have a negative, overpowering effect on compounds imparting plum-like odours (Ismail et al., 1981b).

The aldehydes, hexanal and 2-hexenal, were very prominent in all the cultivars measured in the present study and were usually present in relatively high values at harvest and throughout ripening. Ismail et al. (1981a) and Guichard et al. (1990) describe these compounds as having a strong 'green' aroma associated with immature fruit and diminishing in concentration as ripening proceeds (Gómez and Ledbetter, 1997) rather than remaining relatively constant as found in this study. Some plum aroma

studies also identified these compounds in relatively high amounts in ripe plums (Ismail et al., 1981a and b; Gómez and Ledbetter, 1994). Etievant et al. (1986) reported that the levels of both these aldehydes increased after deep-freezing and thawing of plum samples implying that their values can thus be inflated if samples have been subjected to such procedures prior to assessment. Raffo et al. (2008) also warned against massive formation of these C_6-aldehydes originating from increased lipoxygenase activity associated with the crushing of fruit and suggested an enzyme deactivation step with saturated $(NH_4)_2SO_4$ during sample homogenisation to prevent inflated aldehyde levels in the results. In spite of such efforts Raffo et al. (2008) still found that these compounds represented the main fraction of the whole volatile compounds isolated from peach samples. Similar to our storage results Raffo et al. (2008) reported that they did not observe univocal effects of cold storage on hexanal and 2-hexenal in two peach cultivars after two weeks at 1°C. In the light of the high presence and apparent importance of these compounds it is not surprising that they were both often identified via the stepwise DA as important contributors to the separation patterns of intra-cultivar functional groups. Another aldehyde worth mentioning is p-menth-1-en-9-al, which was measured in relatively high amounts in all seven plum cultivars in the present study but which has not been reported in any plums or stone fruit related studies. It is, however, commonly found in citrus honey (Soria et al., 2009). Cold storage caused p-menth-1-en-9-al levels to increase and decrease in some cultivars but four out of the seven showed no significant differences associated with low temperature exposure.

Esters (together with alcohols and aldehydes) are reported to form the most numerous chemical substances identified in plum extracts (Crouzet et al., 1990), contribute to the fruity aroma in plums (Gómez and Ledbetter, 1994) and some can act as a molecular tracer of apricot aromatic quality (Guillot et al., 2002). In contrast to this we found esters (ten in total) to be limited to mainly the plumcot 'Flavour King' with only n-hexal acetate and benzyl acetate measured in two other true plum species. Gómez and Ledbetter (1997) also reported the presence of numerous esters during the ripening of a plumcot accession, 'P251-002', with three being significant to the aroma profile although they did not appear in either parent cultivars (Gómez and Ledbetter, 1993). In a recent study comparing the aroma profiles of six Japanese plums Lozano et al. (2009) reported n-hexyl acetate as one of the esters present in the greatest proportions with similar levels detected in ripe 'Larry Anne' and 'Songold' and lower levels in 'Angeleno' ('Suplumsix'). We failed to detect n-hexyl acetate in any of our 'Songold' or 'Angeleno' samples, however the levels found in 'Larry Anne', Sapphire' and 'Flavor King' had strong links to ripe plum samples and seemed to develop during the ripening process as the mature but unripe fruit in the 'Harvest' group had significantly lower levels. Cold storage had an inhibiting effect on the accumulation of n-hexyl acetate and resulted in samples with levels lower than the 'Tree-'ripened' but higher than the 'Harvest' fruit. Butyl acetate, although only measured in 'Flavour King', reached a level almost three times that found in tree-ripened fruit and almost 19 times that found in harvest fruit. This significant increase suggested that low temperatures can favour the formation and accumulation of this specific

ester. Butyl acetate had been reported in other tree-ripened Japanese plum cultivars (Gómez and Ledbetter, 1994). However, Lozano et al. (2007), similar to the present findings, also failed to quantify any levels for ripe 'Songold', 'Larry Anne' and 'Angeleno' ('Suplumsix') samples.

For the furan chemical group we could not find univocal patterns amongst the compounds although 2-hexen-4-olide seemed to be associated mostly with harvest fruit as three of five cultivars had higher levels compared to ripe and stored samples. Even the 'generic' compounds *trans*- and *cis*- linalool oxides showed ambiguous trends during ripening and storage for the cultivars in the present study.

The hydrocarbon compounds appeared equally uneventful except for relatively high levels of hotreinol recorded in six of the seven cultivars. Only 'Flavour King' was sensitive to cold storage effects with hotreinol levels in the "Stored' functional group remaining at a level equal to that of the 'Harvest' group and higher than that of the 'Tree-ripened' group indicating that prolonged exposure to low temperatures interrupts its metabolism. Interestingly, the stepwise DA results of four of the seven cultivars identified hotreinol as a prominent compound in the separation patterns observed amongst the three functional groups. Hotreinol had not been identified in any other plum or stone fruit cultivars but is commonly found in other fruit types such as nectarines (Engel et al., 1988), grapes (Williams et al., 1982), passion fruit (Engel and Tressl, 1983) and papaya (Schreier et al., 1985) as well as in citrus honey (Soria et al., 2009).

The lactones (also termed cyclic esters) detected in 'Larry Anne', Sapphire' and 'Flavour King' are important as they have been identified in previous plum studies (Williams and Ismail, 1981; Gómez and Ledbetter, 1994) and are suggested to be indicative of ripeness as they increase during the ripening process (Gómez and Ledbetter, 1997). Williams and Ismail (1981) also suggested that γ-decalactone is responsible for creating a plum-like odour. In the present study the levels of the two lactones, γ-decalatone and γ-dodecalactone, both increased significantly as the fruit ripened on the tree but in the case of γ-dodecalactone fail to do the same during cold storage where similar or significantly lower levels were measured. The effect of cold-storage on the γ-decalactone levels seemed to be cultivar specific with increasing, decreasing and stable levels detected in different cultivars. In a cold storage study done on peaches (Raffo et al., 2008) lactone levels increased after one week of storage at 1°C but drastically decreased after two weeks of storage under similar conditions, implying that the ability of the fruit to perform lactone accumulation and, consequently, to develop its aroma is reduced during prolonged cold storage.

Norisoprenoids are volatile C_9-C_{13} fragments from the degradation of C_{40}-carotenoids, which have extremely low aroma thresholds (Mahattanatawee et al., 2005). Carotenoids are widely found in the plant kingdom and many fruit types have been documented to contain norisoprenoids, including stone fruit such as peaches (Raffo et al., 2008) apricots (Guillot et al., 2002) and plums (Gómez and Ledbetter,

1994; Williams and Ismail, 1981; Crouzet et al., 1990). The most prominent norisoprenoid measured in the present study was β-damascenone with high levels in both the 'Harvest' and 'Tree-ripened' fruit. This compound was also described as having a camphor-like fruity aroma associated with the flavour of cooked plums (Williams and Ismail, 1981). β-Damascenone levels seemed to be unaffected by cold storage and maintained its relatively high levels except for 'Songold' and 'Larry Anne' that showed a significant drop after storage. The other two norisoprenoids, α and β ionones, showed similar trends with diminished levels after cold storage. Although Raffo et al. (2008) did not identify any of the three norisoprenoids mentioned above in their study on cold storage effects on peaches, they did find a decrease in the total amount of norisoprenoids in white-fleshed peaches after exposure to low temperature storage conditions.

In the terpenes group linalool and α-terpineol are the most prominent. Both have been shown to have higher levels in the "Mature green" stage compared to the "Commercial ripe" and "Tree ripe" stages as identified for apricots and plumcots by Gómez and Ledbetter (1997). In the present study no univocal pattern could be established for the levels of either of the two terpenes suggesting a cultivar-specific reaction to ripening and cold storage. Linalool has a floral woody aroma and a sensory evaluation done by Williams and Ismail (1981) found it to be repeatedly associated with a plum-like odour region of the chromatogram.

5. Conclusion

In conclusion, it is difficult to establish a general pattern to describe the reaction of plum aroma to prolonged cold storage as each cultivar has a unique and complex aroma profile already established at the unripe harvest stage, that diverges even further during tree-ripening and storage. It is, however, clear that some cultivars such as 'Flavor King' and 'Larry Anne' are more sensitive to cold storage and develop aroma profiles that are even more different from their 'Tree-ripened' profiles when compared to the other cultivars. From the results it was also evident that the current commercial storage regimes, although favourable for quality parameters such as firmness, colour and sugar levels, altered the aroma profile of plums in such a way that it was neither 'Harvest'-like nor 'Tree-ripened'-like and delivered an end product of different aromatic quality. To overcome this it is suggested that the present study is expanded to include revised storage regimes and/or postharvest manipulations aimed at bridging the gap between the now diverged aroma profiles of stored and tree-ripened plums. This should also be linked to consumer acceptance studies to determine the preferred aroma profiles and eating quality of export plums.

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Table 1: Commercial harvest and storage conditions of the six plum and one plumcot cultivars.

Commercial harvest and storage conditions	'Pioneer'	'Sapphire'	'Laetitia'	'Flavor King' (plumcot)	'Songold'	'Larry Anne'	'Angeleno'
Required firmness range at harvest	9.50 – 5.50 kg 93.1 – 54.0 N	9.50 – 5.00 kg 93.1 – 49.1 N	8.50 – 4.00 kg 83.4 – 39.2 N	8.50 – 5.50 kg 83.40 – 54.0 N	9.00 – 5.00 kg (4.50 kg if 13% TSS) 88.3 – 49.1 N (44.2 N if 13% TSS)	9.00 – 5.50 kg 88.3 – 54.0 N	8.00 – 4.00 kg 78.5 – 39.2 N
Actual mean firmness at commercial harvest (±std dev)	6.81(±1.17) kg 66.8 (±11.51) N	6.81(±1.10) kg 66.8(±10.75) N	6.45 (±0.77) kg 63.3 (±7.57) N	7.35 (±0.70) kg 72.1(±6.90) N	7.06 (±0.80) kg 69.3 (±7.82) N	7.27 (±1.01) kg 71.3 (±9.91) N	6.37 (±0.78) kg 62.5 (±7.68) N
Total cold storage time (days)	28 d	42 d	42 d	42 d	42 d	42 d	42 d
Cold storage protocol	8d @ -0.5℃ 5d @ 7.5℃ 15d @ -0.5℃	10d @ -0.5℃ 7d @ 7.5℃ 25d @ -0.5℃	10d @ -0.5℃ 7d @ 7.5℃ 25d @ -0.5℃	10d @ -0.5℃ 7d @ 7.5℃ 25d @ -0.5℃	10d @ -0.5℃ 7d @ 7.5℃ 25d @ -0.5℃	10d @ -0.5℃ 7d @ 7.5℃ 25d @ -0.5℃	42d @ -0.5℃

Table 2: Mean (±std dev) of the quality parameters determined for each functional group for the six plum and one plumcot cultivars. (To convert Newton to kilogram, divide by 9.81)

	'Pioneer'			**'Sapphire'**			**'Laetitia'**			**'Flavor King' (plumcot)**		
Non-volatile parameters	**Harvest** *n* = 27	**Tree- Ripened** *n* = 20	**Stored** *n* = 18	**Harvest** *n* = 12	**Tree- Ripened** *n* = 12	**Stored** *n* = 12	**Harvest** *n* = 38	**Tree- Ripened** *n* = 10	**Stored** *n* = 12	**Harvest n = 12**	**Tree- Ripened n = 12**	**Stored n = 12**
Firmness (N)	*66.8 (±11.51)*	*21.3 (±6.99)*	*14.0 (±2.77)*	*66.8 (±10.75)*	*18.9 (±4.17)*	*15.5 (±3.47)*	*63.3 (±7.57)*	*16.6 (±3.30)*	*19.8 (±2.77)*	72.1 (±6.90)	22.5 (±15.8)	17.0 (±2.88)
Weight (g)	*60.31 (±10.53)*	*68.55 (±8.75)*	*58.92 (±7.58)*	*102.75 (±19.72)*	*82.58 (17.20)*	*88.17 (±16.55)*	*91.39 (±9.26)*	*118.92 (±18.74)*	*85.92 (±15.8)*	102.50 (±9.22)	90.67 (±6.88)	99.08 (±16.19)
Sugar (% Brix)	*10.55 (±1.20)*	*11.55 (±1.98)*	*9.83 (±0.66)*	*11.39 (±1.03)*	*12.97 (±1.85)*	*12.53 (±1.17)*	*11.20 (±1.69)*	*12.73 (±2.55)*	*11.38 (±1.71)*	15.36 (±0.81)	17.59 (±2.33)	14.38 (±1.45)
Acid (% malic acid)	*1.86 (±0.21)*	*1.37 (±0.26)*	*1.39 (±0.09)*	*1.50 (±0.17)*	*1.10 (±0.19)*	*1.24 (±0.18)*	*1.63 (±0.20)*	*1.21 (±0.48)*	*0.92 (±0.15)*	1.30 (±0.20)	0.85 (±0.28)	0.91 (±0.11)
Sugar:Acid	*5.73 (±0.84)*	*8.62 (±1.74)*	*7.08 (±0.54)*	*7.70 (±1.01)*	*11.93 (±1.77)*	*10.32 (±1.56)*	*6.82 (±1.66)*	*12.79 (±6.66)*	*12.64 (±2.49)*	12.23 (±2.95)	22.51 (±6.63)	15.96 (±2.64)

	'Songold'			**'Larry Anne'**			**'Angeleno'**		
Non-volatile parameters	**Harvest** *n* = 12	**Tree- Ripened** *n* = 12	**Stored** *n* = 12	**Harvest** *n* = 12	**Tree- Ripened** *n* = 12	**Stored** *n* = 12	**Harvest** *n* = 38	**Tree- Ripened** *n* = 18	**Stored** *n* = 12
Firmness (N)	*69.3 (±7.82)*	*28.7 (±3.82)*	*16.2 (±2.49)*	*72.0 (±9.91)*	*20.0 (±12.09)*	*14.9 (±2.89)*	*62.5 (±7.68)*	*29.7 (±6.37)*	*50.2 (±3.06)*
Weight (g)	*106.83 (±15.23)*	*129.00 (±13.11)*	*95.33 (±10.35)*	*111.08 (±30.79)*	*103.25 (±35.53)*	*80.00 (±7.75)*	*89.63 (±7.96)*	*79.64 (±11.99)*	*88.00 (±10.6)*
Sugar (% Brix)	*11.31 (±0.60)*	*14.94 (±2.53)*	*11.85 (±1.09)*	*16.14 (±1.83)*	*16.52 (±2.49)*	*13.97 (±1.04)*	*14.76 (±0.91)*	*15.32 (±0.92)*	*14.12 (±0.20)*
Acid (% malic acid)	*1.62 (±0.13)*	*1.22 (±0.09)*	*1.20 (±0.15)*	*1.82 (±0.18)*	*1.00 (±0.23)*	*1.28 (±0.38)*	*0.91 (±0.16)*	*0.68 (±0.07)*	*0.78 (±0.05)*
Sugar:Acid	*7.01 (±0.64)*	*12.18 (±1.59)*	*10.12 (±2.29)*	*8.89 (±0.59)*	*17.53 (5.86)*	*11.49 (±2.31)*	*16.74 (±3.56)*	*22.57 (±1.87)*	*18.29 (±1.44)*

Table 3a: Chemical groups and means of the aroma volatiles found in **'Pioneer', 'Sapphire', 'Laetitia'** and **'Flavor King'**. The retention time (RT) order represents the chronological order in which the compounds were separated, starting with hexanal at ±5.3 min. and ending with bisabalol oxide B at ±29.8 min. * indicates compounds identified via the NIST library and ** indicates identification via NIST library and confirmation using commercial standards. H = 'Harvest', TR = 'Tree-ripened', S = 'Stored' functional groups. ND = not detected. Abb = abbreviation. Different letters (a, b and c) indicate significant differences between values within each functional group according to the Fischer LSD test (p = 0.05).

Chemical group and RT order	Abb	Aroma compounds	'Pioneer'			'Sapphire'			'Laetitia'			'Flavor King'		
			H	TR	S	H	TR	S	H	TR	S	H	TR	S
Acids														
46	Nona	Nonanoic acid*	ND	ND	ND	0.094 a	0.082 a	0.052 a	ND	ND	ND	0.024 b	ND a	ND a
55	Eug	Eugenol methylether*	ND	ND	ND	0.002 a	0.091 a	0.033 a	ND	ND	ND	ND	ND	ND
Alcohols														
4	Hex	1-Hexanol**	ND	ND	ND	ND	ND	ND	0.051 a	2.417 b	0.219 a	ND	ND	ND
8	3Hexe	*cis*-3-Hexenol*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND a	0.102 a	1.081 b
37	cMenth	*cis*-p-Menth-2,8-dienol*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
39	Cymol	p-Cymen-8-ol*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Aldehydes														
1	Hexa	Hexanal**	0.293 b	0.580 c	0.040 a	0.660 a	1.095 a	0.588 a	0.148 b	0.028 a	0.003 a	0.762 b	0.240 a	0.086 a
3	Hexe	2-Hexenal**	3.353 b	2.757 b	1.886 a	1.910 a	1.777 a	4.603 b	0.906 b	1.350 c	0.455 a	0.771 b	ND a	ND a
5	Non	Nonanal*	ND	ND	ND	0.620 a	0.651 a	1.030 a	ND	ND	ND	ND	ND	ND
11	Benz	Benzaldehyde**	ND	ND	ND	ND	ND	ND	0.025 b	0.008 a	0.006 a	0.038 a	0.036 a	0.032 a
36	Iso	2-Isopropylidene-5-methylhex-4-enal*	ND	ND	ND	0.041 a	0.220 b	0.056 a	ND	ND	ND	ND	ND	ND
43	pMenth	p-Menth-1-en-9-al*	0.103 a	0.077 a	0.111a	0.098 a	0.093 a	0.251 a	0.056 b	0.028 a	0.036 ab	0.469 a	0.989 b	0.598 a
Esters														
2	But	Butyl acetate*	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.866 a	17.700 a	52.685 b
9	Hexfor	n-Hexylformate*	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.224 a	1.418 b	0.543 a
10	Amyl	Amylacetate*	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.183 a	0.284 a	0.631 b
12	Hexyl	n-Hexyl acetate*	ND	ND	ND	ND a	0.385 b	0.221 ab	ND	ND	ND	4.529 a	25.412 b	13.922 ab
19	Butbu	Butylbutanoate*	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.009 a	0.060 a	0.457 b
20	3Hexac	*cis*-3-Hexenyl acetate*	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.275 a	0.406 a	1.028 b
31	Benzyl	Benzyl acetate**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
33	Hep	Heptyl acetate*	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.019 a	0.123 b	0.012 a
41	Oct	Octyl acetate*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND a	0.197 b	0.017 a
50	Pent	Pentamethylene acetate*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND a	0.090 b	0.003 a
Furans														
7	Eth	2-Ethylfuran*	ND	ND	ND	ND	ND	ND	0.061 b	0.006 a	ND a	ND	ND	ND
13	Pyran2	2H-Pyran,2-ethenyltetrahydro - 2,6,6 tri-methyl*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
14	Hexen	2-Hexen-4-olide*	ND	ND	ND	ND	ND	ND	0.066 b	0.002 a	ND a	0.053 b	ND a	ND a
24	tLinox	*trans*-Linalool oxide**	0.093 a	0.094 a	0.135 a	0.079 a	0.048 a	0.084 a	0.108 b	0.052 a	0.068 ab	0.769 b	0.299 a	0.337 b
25	cLinox	*cis*-Linalool oxide**	0.034 a	0.021 a	0.030 a	0.057 a	0.034 a	0.032 a	0.038 b	0.013 a	0.018 a	0.256 b	0.109 a	0.111 a
34	Pyran3	2H-Pyran,3,6.dihydro-4-methyl-2-(2-methyl-1-propenyl)*	0.025 c	0.009 a	0.016 b	0.130 a	0.065 a	0.064 a	ND	ND	ND	0.108 b	0.042 a	0.057 ab
62	Bisa	Bisabalol oxide B*	ND	ND	ND	ND	ND	ND	0.010 b	0.001 a	ND a	ND	ND	ND

Chemical group and RT order	Abb	Aroma compounds	'Pioneer'			'Sapphire'			'Laetitia'			'Flavor King'		
			H	TR	S	H	TR	S	H	TR	S	H	TR	S
Hydrocarbons														
28	Hot	Hotrienol*	0.182 a	0.132 a	0.173 a	0.400 a	0.495 a	0.721 a	0.004 a	0.008 a	0.005 a	0.799 b	0.377 a	0.774 b
32	Cyclo	1,3, Cyclo-hexadiene 1,3,5,5,tetra-methyl*	ND	ND	ND	0.021 a	0.031 a	0.020 a	ND	ND	ND	ND	ND	ND
44	Bor	2-Bornene*	0.018 c	0.004 a	0.011 b	0.019 a	0.020 a	0.058 a	0.017 b	0.003 a	0.004 a	0.010 b	0.008 ab	0.001 a
53	2But	2-[(2E)-2-Butenyl]-1,3,5-trimethylbenzene*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
56	Trimeth	4-(2,4,4-Trimetyl-cyclohexa-1,5-dienyl) but-3-en-2-one*	0.013 a	0.028 a	0.072 b	0.021 a	0.034 a	0.176 b	0.021 b	0.003 a	0.004 a	0.008 a	0.007 a	0.002 a
59	Trimeth6	4-(2,6,6-Trimethyl-cyclohexa-1,5,-diemethyl)but-3-en-2-one*	ND	ND	ND	0.021 a	0.029 a	0.087 b	ND	ND	ND	0.008 ab	0.009 b	0.002 a
Lactones														
58	Deca	γ-Decalactone*	ND	ND	ND	0.011 a	0.173 a	0.537 b	ND	ND	ND	0.094 a	1.255 b	0.010 a
61	Dodeca	γ -Dodecalactone*	ND	ND	ND	0.004 a	0.055 a	0.054 a	ND	ND	ND	0.059 a	0.974 b	0.012 a
Norisoprenoids														
47	aIon	α-Ionone**	0.060 a	0.046 a	0.087 a	ND	ND	ND	ND	ND	ND	0.198 b	0.109 b	0.009 a
51	bDam	β- Damascenone**	0.099 b	0.068 ab	0.034 a	0.182 a	0.238 a	0.363 a	0.193 b	0.035 a	0.008 a	0.502 a	0.513 a	0.308 a
60	bIon	β- Ionone**	0.005 a	0.005 a	0.011 b	0.024 a	0.036 a	0.052 a	0.010 a	0.018 b	0.006 a	0.012 a	0.028 b	0.004 a
Phenols														
15	Phe	Phenol*	ND	ND	ND	ND	ND	ND	0.061 a	ND a	ND a	ND	ND	ND
Terpenes														
6	Pin	α-Pinene*	ND	ND	ND	0.039 a	0.079 b	0.024 a	ND	ND	ND	ND	ND	ND
17	Limox	Limonene oxide **	0.013 b	0.004 a	0.011 b	ND	ND	ND	ND	ND	ND	ND	ND	ND
18	Car	3-Carene **	0.004 b	0.001 a	0.001 a	0.157 a	0.046 a	0.014 a	ND	ND	ND	ND	ND	ND
21	Cym	β-Cymene**	0.009 c	0.001 a	0.004 b	ND	ND	ND	ND	ND	ND	ND	ND	ND
22	Lim	D-Limonene**	0.007 c	0.002 a	0.005 b	ND	ND	ND	ND	ND	ND	ND	ND	ND
23	aTerp	α-Terpinene*	0.003 c	0.001 a	0.002 b	ND	ND	ND	ND	ND	ND	ND	ND	ND
27	Lina	Linalool**	0.049 b	0.024 a	0.042 ab	0.286 a	0.576 a	0.628 a	0.011 a	0.049 a	0.004 a	0.190 b	0.152 b	0.041 a
29	Oci	Ocimenol*	ND	ND	ND	0.062 a	0.057 a	0.018 a	ND	ND	ND	0.041 b	0.044 b	0.004 a
30	Menth	Menthol*	ND	ND	ND	ND	ND	ND	0.013 b	0.006 ab	0.001 a	ND	ND	ND
40	aTerpol	α-Terpineol**	0.055 a	0.043 a	0.069 a	0.206 a	0.235 a	0.477 a	0.034 b	0.011 a	0.008 a	0.191 a	0.261 a	0.163 a
57	Gera	*cis*-Geranylacetone*	ND	ND	ND	0.007 a	0.013 a	0.016 a	ND	ND	ND	ND	ND	ND
Unidentified														
16	U2	Unidentified 2	ND	ND	ND	ND	ND	ND	0.035 a	ND a	ND a	ND	ND	ND
26	U1	Unidentified 1	0.013 b	0.003 a	0.004 a	ND	ND	ND	ND	ND	ND	ND	ND	ND
35	U5	Unidentified 5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
38	U6	Unidentified 6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
42	U9	Unidentified 9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
45	U3	Unidentified 3	ND	ND	ND	ND	ND	ND	0.097 b	0.047 ab	0.029 a	ND	ND	ND
48	U7	Unidentified 7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
49	U8	Unidentified 8	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.052 a	0.040 a	0.069 a
52	U4	Unidentified 4	ND	ND	ND	ND	ND	ND	0.066 b	0.054 ab	0.019 a	ND	ND	ND
54	U10	Unidentified 10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table 3b: Chemical groups and means of the aroma volatiles found in **'Songold', 'Larry Anne',** and **'Angeleno'**. The retention time (RT) order represents the chronological order in which the compounds were separated, starting with hexanal at ±5.3 min. and ending with bisabalol oxide B at ±29.8 min. * indicates compounds identified via the NIST library and ** indicates identification via NIST library and confirmation using commercial standards. H = 'Harvest', TR = 'Tree-ripened', S = 'Stored' functional groups. ND = not detected. Abb = abbreviation. Different letters (a, b and c) indicate significant differences between values within each functional group according to the Fischer LSD test (p = 0.05).

Chemical group and RT order	Abb	Aroma compounds	'Songold'			'Larry Anne'			'Angeleno'		
			H	TR	S	H	TR	S	H	TR	S
Acids											
46	Nona	Nonanoic acid*	ND	ND	ND	ND	ND	ND	ND	ND	ND
55	Eug	Eugenol methylether*	ND	ND	ND	ND	ND	ND	ND	ND	ND
Alcohols											
4	Hex	1-Hexanol**	0.008 a	0.184 b	0.422 c	ND	ND	ND	0.116 a	0.644 c	0.355 b
8	3Hexe	*cis*-3-Hexenol*	ND	ND	ND	ND	ND	ND	ND	ND	ND
37	cMenth	*cis*-p-Menth-2,8-dienol*	ND	ND	ND	0.218 b	0.135 a	0.122 a	ND	ND	ND
39	Cymol	p-Cymen-8-ol*	0.003 b	0.001 ab	ND a	ND	ND	ND	0.002 b	ND a	ND a
Aldehydes											
1	Hexa	Hexanal**	0.205 b	0.265 b	0.065 a	0.723 a	1.360 b	1.053 ab	0.284 b	0.232 b	0.096 a
3	Hexe	2-Hexenal**	1.253 a	1.286 a	0.808 a	2.030 a	2.574 a	3.669 b	1.072 a	0.831 a	1.367 a
5	Non	Nonanal*	ND	ND	ND	ND	ND	ND	ND	ND	ND
11	Benz	Benzaldehyde**	0.009 ab	0.012 b	0.002 a	0.018 a	0.015 a	0.055 b	0.011 b	0.004 a	ND a
36	Iso	2-Isopropylidene-5-methylhex-4-enal*	ND	ND	ND	ND	ND	ND	ND	ND	ND
43	pMenth	p-Menth-1-en-9-al*	0.146 b	0.052 a	0.094 ab	0.705 c	0.374 a	0.548 b	0.128 b	0.069 a	0.127 b
Esters											
2	But	Butyl acetate*	ND	ND	ND	ND	ND	ND	ND	ND	ND
9	Hexfor	n-Hexylformate*	ND	ND	ND	ND	ND	ND	ND	ND	ND
10	Amyl	Amylacetate*	ND	ND	ND	ND	ND	ND	ND	ND	ND
12	Hexyl	n-Hexyl acetate*	ND	ND	ND	ND a	0.471 b	0.120 a	ND	ND	ND
19	Butbu	Butylbutanoate*	ND	ND	ND	ND	ND	ND	ND	ND	ND
20	3Hexac	*cis*-3-Hexenyl acetate*	ND	ND	ND	ND	ND	ND	ND	ND	ND
31	Benzyl	Benzyl acetate**	ND	ND	ND	ND	ND	ND	0.003 b	0.001 a	0.002 ab
33	Hep	Heptyl acetate*	ND	ND	ND	ND	ND	ND	ND	ND	ND
41	Oct	Octyl acetate*	ND	ND	ND	ND	ND	ND	ND	ND	ND
50	Pent	Pentamethylene acetate*	ND	ND	ND	ND	ND	ND	ND	ND	ND
Furans											
7	Eth	2-Ethylfuran*	0.065 a	0.162 b	0.012 a	ND	ND	ND	0.044 b	0.003 a	0.014 a
13	Pyran2	2H-Pyran,2-ethenyltetrahydro -2,6,6 tri-methyl*	ND	ND	ND	ND	ND	ND	0.010 a	0.001 a	0.011 a
14	Hexen	2-Hexen-4-olide*	0.053 a	0.199 b	0.009 a	0.050 a	0.230 b	0.053 a	0.060 b	ND a	ND a
24	tLinox	*trans*-Linalool oxide**	0.185 b	0.065 a	0.081 a	0.780 c	0.300 a	0.507 b	0.044 b	0.011 a	0.038 b
25	cLinox	*cis*-Linalool oxide**	0.049 b	0.009 a	0.022 a	0.329 c	0.132 a	0.182 b	0.017 b	0.004 a	0.016 b
34	Pyran3	2H-Pyran,3,6.dihydro-4-methyl-2-(2-methyl-1-propenyl)*	0.036 b	0.009 a	0.004 a	0.202 b	0.037 a	0.054 a	ND	ND	ND
62	Bisa	Bisabalol oxide B*	ND	ND	ND	ND	ND	ND	0.002 b	ND a	0.001 ab

Chemical group and RT order	Abb	Aroma compounds	'Songold'			'Larry Anne'			'Angeleno'		
			H	TR	S	H	TR	S	H	TR	S
Hydrocarbons											
28	Hot	Hotreinol*	ND	ND	ND	0.861 b	0.535 a	0.619 a	0.009 a	0.039 b	0.037 b
32	Cyclo	1,3, Cyclo-hexadiene 1,3,5,5,tetra-methyl*	ND	ND	ND	ND	ND	ND	ND	ND	ND
44	Bor	2-Bornene*	ND	ND	ND	0.030 b	0.009 a	0.010 a	0.003 b	0.001 a	0.001 a
53	2But	2-[(2E)-2-Butenyl]-1,3,5-trimethylbenzene*	ND	ND	ND	0.093 b	0.083 b	0.024 a	ND	ND	ND
56	Trimeth	4-(2,4,4-Trimetyl-cyclohexa-1,5-dienyl) but-3-en-2-one*	0.019 b	0.002 a	0.016 b	ND	ND	ND	ND	ND	ND
59	Trimeth6	4-(2,6,6-Trimethyl-cyclohexa-1,5,-diemethyl)but-3-en-2-one*	0.016 a	0.009 a	0.012 a	ND	ND	ND	ND	ND	ND
Lactones											
58	Deca	γ -Decalactone*	ND	ND	ND	0.002 a	0.487 b	0.435 b	ND	ND	ND
61	Dodeca	γ -Dodecalactone*	ND	ND	ND	0.001 a	0.318 b	0.095 a	ND	ND	ND
Norisoprenoids											
47	aIon	α-Ionone**	0.393 b	0.169 a	0.080 a	0.019 c	0.011 b	0.003 a	0.087 b	0.030 a	0.081 b
51	bDam	β- Damascenone**	0.275 b	0.280b	0.069 a	0.184 c	0.116 b	0.066 a	0.150 b	0.062 a	0.086 a
60	bIon	β- Ionone**	0.006 a	0.015 b	0.008 ab	0.029 a	0.078 b	0.013 a	ND	ND	ND
Phenols											
15	Phe	Phenol*	0.077 b	0.001a	0.001 a	ND	ND	ND	ND	ND	ND
Terpenes											
6	Pin	α-Pinene*	ND	ND	ND	ND	ND	ND	ND	ND	ND
17	Limox	Limonene oxide **	ND	ND	ND	0.117 c	0.055 b	0.032 a	ND	ND	ND
18	Car	3-Carene **	ND	ND	ND	0.060 b	0.011 a	0.011 a	0.004 b	0.001 a	0.003 ab
21	Cym	β-Cymene**	0.040 a	0.008 b	0.006 b	0.019 b	0.003 b	0.005 b	ND	ND	ND
22	Lim	D-Limonene**	ND	ND	ND	0.053 b	0.020 a	0.024 ab	0.013 b	0.003 a	0.017 b
23	aTerp	α-Terpinene*	ND	ND	ND	ND	ND	ND	ND	ND	ND
27	Lina	Linalool**	0.059 b	0.013 a	0.025 a	0.688 b	0.622 b	0.337 c	0.129 b	0.083 a	0.108 ab
29	Oci	Ocimenol*	ND	ND	ND	0.169 b	0.035 a	0.040 a	0.008 b	0.002 a	0.003 a
30	Menth	Menthol*	ND	ND	ND	ND	ND	ND	ND	ND	ND
40	aTerpol	α-Terpineol**	0.018 b	0.006 a	0.007 b	0.815 b	0.277 a	0.273 a	0.077 b	0.026 a	0.079 b
57	Gera	*cis*-Geranylacetone*	ND	ND	ND	ND	ND	ND	ND	ND	ND
Unidentified											
16	U2	Unidentified 2	ND	ND	ND	ND	ND	ND	ND	ND	ND
26	U1	Unidentified 1	ND	ND	ND	ND	ND	ND	ND	ND	ND
35	U5	Unidentified 5	ND	ND	ND	ND	ND	ND	0.040 a	0.033 a	0.187 b
38	U6	Unidentified 6	0.055 ab	0.045 a	0.097 b	ND	ND	ND	ND	ND	ND
42	U9	Unidentified 9	ND	ND	ND	0.096 b	0.043 a	0.039 a	ND	ND	ND
45	U3	Unidentified 3	ND	ND	ND	ND	ND	ND	ND	ND	ND
48	U7	Unidentified 7	0.110 b	0.057 a	0.052 a	ND	ND	ND	ND	ND	ND
49	U8	Unidentified 8	ND	ND	ND	ND	ND	ND	ND	ND	ND
52	U4	Unidentified 4	ND	ND	ND	ND	ND	ND	ND	ND	ND
54	U10	Unidentified 10	ND	ND	ND	0.057 ab	0.078 b	0.027 a	ND	ND	ND

Table 4: Specific cultivars and their aroma compounds. Abb = abbreviation

	Abb	Aroma compound	Cultivars compound was detected in
'Generic'	Hexa	Hexanal	All
	Hexe	2-Hexenal	All
	tLinox	*trans*-Linalool oxide	All
	cLinox	*cis*-Linalool oxide	All
	Lina	Linalool	All
	aTerpol	α-Terpineol	All
	pMenth	p-Menth-1-en-9-al	All
	bDam	β- Damascenone	All
	Hot	Hotreinol	'Pioneer', 'Sapphire', 'Laetitia', 'Flavor King', 'Larry Anne', 'Angeleno'
	Bor	2-Bornene	'Pioneer', 'Sapphire', 'Laetitia', 'Flavor King', 'Larry Anne', 'Angeleno'
	bIon	β- Ionone	'Pioneer', 'Sapphire', 'Laetitia', 'Flavor King', 'Songold', 'Larry Anne'
'Unique'	aTerp	α-Terpinene	'Pioneer'
	U1	Unidentified 1	'Pioneer'
	Non	Nonanal	'Sapphire'
	Pin	α-Pinene	'Sapphire'
	Cyclo	1,3, Cyclo-hexadiene 1,3,5,5,tetra-methyl	'Sapphire'
	Iso	2-Isopropylidene-5-methylhex-4-enal	'Sapphire'
	Eug	Eugenol methylether	'Sapphire'
	Gera	*cis*-Geranylacetone	'Sapphire'
	U2	Unidentified 2	'Laetitia'
	Menth	Menthol	'Laetitia'
	U3	Unidentified 3	'Laetitia'
	U4	Unidentified 4	'Laetitia'
	U7	Unidentified 7	'Songold'
	U6	Unidentified 6	'Songold'
	cMenth	*cis*-p-Menth-2,8-dienol	'Larry Anne'
	U9	Unidentified 9	'Larry Anne'
	2But	2-[(2E)-2-Butenyl]-1,3,5-trimethylbenzene	'Larry Anne'
	U10	Unidentified 10	'Larry Anne'
	But	Butyl acetate	'Flavor King'
	U8	Unidentified 8	'Flavor King'
	Pent	Pentamethylene acetate	'Flavor King'
	3Hexe	*cis*-3-Hexenol	'Flavor King'
	Hexfor	n-Hexylformate	'Flavor King'
	Amyl	Amylacetate	'Flavor King'
	Oct	Octyl acetate	'Flavor King'
	Butbu	Butylbutanoate	'Flavor King'
	3Hexac	*cis*-3-Hexenyl acetate	'Flavor King'
	Hep	Heptyl acetate	'Flavor King'
	Benzyl	Benzyl acetate	'Angeleno'
	U5	Unidentified 5	'Angeleno'
	Pyran2	2H-Pyran,2-ethenyltetrahydro-2,6,6tri-methyl	'Angeleno'
'Frequentt'	Limox	Limonene oxide	'Pioneer', 'Larry Anne'
	Cymol	p-Cymen-8-ol	'Songold', 'Angeleno'
	Phe	Phenol	'Laetitia', 'Songold'
	Nona	Nonanoic acid	'Sapphire', 'Flavor King',
	Bisa	Bisabalol oxide B	'Laetitia', 'Angeleno'
	Hex	1-Hexanol	'Laetitia', 'Songold', 'Angeleno'
	Eth	2-Ethylfuran	'Laetitia', 'Songold', 'Angeleno'
	Cym	β-Cymene	'Pioneer', 'Songold', 'Larry Anne'
	Lim	D-Limonene	'Pioneer', 'Larry Anne', 'Angeleno'
	Hexyl	n-Hexyl acetate	'Sapphire', 'Flavor King', 'Larry Anne'
	Deca	γ -Decalactone	'Sapphire', 'Flavor King', 'Larry Anne'
	Dodeca	γ -Dodecalactone	'Sapphire', 'Flavor King', 'Larry Anne'
	Trimeth6	4-(2,6,6-Trimethyl-cyclohexa-1,5,-diemethyl)but-3-en-2-one	'Sapphire', 'Flavor King', 'Songold'
	Car	3-Carene	'Pioneer', 'Sapphire,', 'Larry Anne', 'Angeleno'
	Oci	Ocimenol	'Sapphire', 'Flavor King', 'Larry Anne', 'Angeleno'
	Trimeth	4-(2,4,4-Trimetyl-cyclohexa-1,5-dienyl)but-3-en-2-one	'Pioneer', 'Sapphire', 'Laetitia', 'Flavor King', 'Songold'
	aIon	α-Ionone	'Pioneer', 'Songold', 'Flavor King', 'Larry Anne', 'Angeleno'
	Benz	Benzaldehyde	'Laetitia', 'Flavor King' Songold', 'Larry Anne' 'Angeleno'
	Hexen	2-Hexen-4-olide	'Laetitia', 'Flavor King' Songold', 'Larry Anne' 'Angeleno'
	Pyran3	2H-Pyran,3,6.dihydro-4-methyl-2-(2-methyl-1-propenyl)	'Pioneer', 'Sapphire', 'Flavor King', 'Songold', 'Larry Anne'

Table 5: DA results for the three functional groups of **'Pioneer'**. (a) Variable selection table using the stepwise method' (see Table 3 for explanation of compound abbreviations). Confusion matrixes indicating the percentage correctly predicted samples for the three classes using all the compounds (b) and using only the eight compounds identified by the stepwise method (c).

(a)

Variable IN/OUT	Status	Partial R^2	F	Pr > F
Pyran3	IN	0.354	16.973	< 0.0001
Hexe	IN	0.255	10.459	0.0001
bIon	IN	0.342	15.563	< 0.0001
Hot	IN	0.189	6.855	0.002
Hexa	IN	0.216	8.013	0.001
pMenth	IN	0.201	7.153	0.002
tLinox	IN	0.148	4.876	0.011
aTerp	IN	0.150	4.864	0.011

(b)

from \ to	Stored	Harvest	Tree-ripened	Total	% correct
Stored	16	0	2	18	88.89%
Harvest	0	26	1	27	96.30%
Tree-ripened	0	2	18	20	90.00%
Total	16	28	21	65	92.31%

(c)

from \ to	Stored	Harvest	Tree-ripened	Total	% correct
Stored	16	0	2	18	88.89%
Harvest	0	27	0	27	100.00%
Tree-ripened	2	2	16	20	80.00%
Total	18	29	18	65	90.77%

Table 6: DA results for the three functional groups of **'Sapphire'**. (a) Variable selection table using the stepwise method' (see Table 3 for explanation of compound abbreviations). Confusion matrixes indicating the percentage correctly predicted samples for the three classes using all the compounds (b) and using only the eight compounds identified by the stepwise method (c).

(a)

Variable IN/OUT	Status	Partial R^2	F	Pr > F
Hexe	IN	0.433	12.600	<0.0001
Deca	IN	0.350	8.616	0.001
Hexa	IN	0.322	7.364	0.002
Lina	IN	0.349	8.047	0.002
Deca	OUT	0.127	2.190	0.130
Trimeth	IN	0.455	12.518	0.000
Hexyl	IN	0.224	4.185	0.025
Hot	IN	0.197	3.425	0.047
blon	IN	0.255	4.627	0.019

(b)

from \ to	Stored	Harvest	Tree-ripened	Total	% correct
Stored	12	0	0	12	100.00%
Harvest	0	12	0	12	100.00%
Tree-ripened	0	0	12	12	100.00%
Total	12	12	12	36	100.00%

(c)

from \ to	Stored	Harvest	Tree-ripened	Total	% correct
Stored	12	0	0	12	100.00%
Harvest	0	12	0	12	100.00%
Tree-ripened	0	2	10	12	83.33%
Total	12	14	10	36	94.44%

Table 7: DA results for the three functional groups of **'Laetitia'**. (a) Variable selection table using the stepwise method' (see Table 3 for explanation of compound abbreviations). Confusion matrixes indicating the percentage correctly predicted samples for the three classes using all the compounds (b) and using only the eight compounds identified by the stepwise method (c).

(a)

Variable IN/OUT	Status	Partial R^2	F	Pr > F
Hex	IN	0.741	81.576	< 0.0001
bDam	IN	0.368	16.274	< 0.0001
pMenth	IN	0.600	41.243	< 0.0001
U3	IN	0.225	7.818	0.001
Menth	IN	0.184	5.961	0.005
Phe	IN	0.159	4.918	0.011
Hexa	IN	0.116	3.360	0.043
Trimeth	IN	0.116	3.285	0.046

(b)

from \ to	Stored	Harvest	Tree-ripened	Total	% correct
Stored	12	0	0	12	100.00%
Harvest	0	38	0	38	100.00%
Tree-ripened	0	0	10	10	100.00%
Total	12	38	10	60	100.00%

(c)

from \ to	Stored	Harvest	Tree-ripened	Total	% correct
Stored	12	0	0	12	100.00%
Harvest	0	38	0	38	100.00%
Tree-ripened	0	1	9	10	90.00%
Total	12	39	9	60	98.33%

Table 8: DA results for the three functional groups of **'Flavor King'**. (a) Variable selection table using the stepwise method' (see Table 3 for explanation of compound abbreviations). Confusion matrixes indicating the percentage correctly predicted samples for the three classes using all the compounds (b) and using only the nine compounds identified by the stepwise method (c).

(a)

Variable IN/OUT	Status	Partial R^2	F	Pr > F
Hexe	IN	0.878	118.376	< 0.0001
Pent	IN	0.672	32.750	< 0.0001
alon	IN	0.491	14.973	< 0.0001
Hot	IN	0.367	8.699	0.001
Nona	IN	0.269	5.345	0.011
Trimeth	IN	0.445	11.247	0.000
3Hexe	IN	0.311	6.099	0.007
Butbu	IN	0.370	7.637	0.002
Hexfor	IN	0.333	6.237	0.006

(b)

from \ to	Stored	Harvest	Tree-ripened	Total	% correct
Stored	12	0	0	12	100.00%
Harvest	0	12	0	12	100.00%
Tree-ripened	0	0	12	12	100.00%
Total	12	12	12	36	100.00%

(c)

from \ to	Stored	Harvest	Tree-ripened	Total	% correct
Stored	12	0	0	12	100.00%
Harvest	0	12	0	12	100.00%
Tree-ripened	0	0	12	12	100.00%
Total	12	12	12	36	100.00%

Table 9: DA results for the three functional groups of **'Songold'**. (a) Variable selection table using the stepwise method' (see Table 3 for explanation of compound abbreviations). Confusion matrixes indicating the percentage correctly predicted samples for the three classes using all the compounds (b) and using only the five compounds identified by the stepwise method (c).

(a)

Variable IN/OUT	Status	Partial R^2	F	Pr > F
Phe	IN	0.544	19.665	< 0.0001
bDam	IN	0.589	22.966	< 0.0001
pMenth	IN	0.657	29.650	< 0.0001
aIon	IN	0.536	17.324	< 0.0001
Pyran3	IN	0.274	5.475	0.010

(b)

from \ to	Stored	Harvest	Tree- ripened	Total	% correct
Stored	12	0	0	12	100.00%
Harvest	0	12	0	12	100.00%
Tree-ripened	0	0	12	12	100.00%
Total	12	12	12	36	100.00%

(c)

from \ to	Stored	Harvest	Tree- ripened	Total	% correct
Stored	12	0	0	12	100.00%
Harvest	0	12	0	12	100.00%
Tree-ripened	0	0	12	12	100.00%
Total	12	12	12	36	100.00%

Table 10: DA results for the three functional groups of **'Larry Anne'**. (a) Variable selection table using the stepwise method' (see Table 3 for explanation of compound abbreviations). Confusion matrixes indicating the percentage correctly predicted samples for the three classes using all the compounds (b) and using only the nine compounds identified by the stepwise method (c).

(a)

Variable IN/OUT	Status	Partial R^2	F	Pr > F
Limox	IN	0.869	109.615	< 0.0001
Oci	IN	0.496	15.752	< 0.0001
U10	IN	0.504	15.771	< 0.0001
Pyran3	IN	0.443	11.919	0.000
Cym	IN	0.216	4.003	0.029
Deca	IN	0.581	19.448	< 0.0001
blon	IN	0.358	7.516	0.003
Lina	IN	0.311	5.875	0.008
Hexe	IN	0.283	4.927	0.016

(b)

from \ to	Stored	Harvest	Tree- ripened	Total	% correct
Stored	12	0	0	12	100.00%
Harvest	0	12	0	12	100.00%
Tree-ripened	0	0	12	12	100.00%
Total	12	12	12	36	100.00%

(c)

from \ to	Stored	Harvest	Tree- ripened	Total	% correct
Stored	12	0	0	12	100.00%
Harvest	0	12	0	12	100.00%
Tree-ripened	0	0	12	12	100.00%
Total	12	12	12	36	100.00%

Table 11: DA results for the three functional groups of '**Angeleno**'. (a) Variable selection table using the stepwise method' (see Table 3 for explanation of compound abbreviations). Confusion matrixes indicating the percentage correctly predicted samples for the three classes using all the compounds (b) and using only the six compounds identified by the stepwise method (c).

(a)

Variable IN/OUT	Status	Partial R^2	F	Pr > F
U5	IN	0.302	14.038	< 0.0001
Hex	IN	0.389	20.392	< 0.0001
Hexa	IN	0.329	15.427	< 0.0001
Benz	IN	0.355	17.077	< 0.0001
Hexe	IN	0.128	4.485	0.015
Hot	IN	0.150	5.308	0.008

(b)

from \ to	Stored	Harvest	Tree- ripened	Total	% correct
Stored	18	0	0	18	100.00%
Harvest	0	38	0	38	100.00%
Tree-ripened	0	3	9	12	75.00%
Total	18	41	9	68	95.59%

(c)

from \ to	Stored	Harvest	Tree- ripened	Total	% correct
Stored	18	0	0	18	100.00%
Harvest	0	37	1	38	97.37%
Tree-ripened	0	4	8	12	66.67%
Total	18	41	9	68	92.65%

Table 12: DA results for the **'Harvest'** functional group of the seven cultivars. (a) Variable selection table using the stepwise method' (see Table 3 for explanation of compound abbreviations). Confusion matrixes indicating the percentage correctly predicted samples for the three classes using all the compounds (b) and using only the 38 compounds identified by the stepwise method (c).

(a)

Variable IN/OUT	Status	Partial R^2	F	Pr > F
Limox	IN	0.974	899.020	< 0.0001
3Hexac	IN	0.899	212.620	< 0.0001
aTerp	IN	0.778	83.016	< 0.0001
aIon	IN	0.681	50.127	< 0.0001
Trimeth6	IN	0.663	45.953	< 0.0001
2But	IN	0.569	30.594	< 0.0001
Lina	IN	0.484	21.591	< 0.0001
Bisa	IN	0.403	15.404	< 0.0001
U5	IN	0.360	12.747	< 0.0001
Hexe	IN	0.263	8.035	< 0.0001
pMenth	IN	0.240	7.038	< 0.0001
Hexa	IN	0.262	7.854	< 0.0001
Lim	IN	0.212	5.924	< 0.0001
Hot	IN	0.203	5.575	< 0.0001
tLinox	IN	0.277	8.316	< 0.0001
Pin	IN	0.196	5.237	< 0.0001
Eug	IN	0.598	31.761	< 0.0001
Non	IN	0.241	6.731	< 0.0001
Bor	IN	0.220	5.936	< 0.0001
bDam	IN	0.198	5.131	< 0.0001
Hep	IN	0.298	8.774	< 0.0001
Lina	OUT	0.063	1.393	0.222
But	IN	0.419	14.917	< 0.0001
Hexfor	IN	0.443	16.309	< 0.0001
3Hexac	OUT	0.079	1.756	0.114
Deca	IN	0.249	6.792	< 0.0001
Cyclo	IN	0.251	6.828	< 0.0001
bIon	IN	0.237	6.277	< 0.0001
Bisa	OUT	0.074	1.615	0.149
U1	IN	0.194	4.839	0.000
Iso	IN	0.142	3.322	0.005
Car	IN	0.242	6.349	< 0.0001
Trimeth6	OUT	0.076	1.636	0.143
Lina	IN	0.193	4.739	0.000
Butbu	IN	0.145	3.328	0.005

Variable IN/OUT	Status	Partial R^2	F	Pr > F
Dodeca	IN	0.155	3.585	0.003
Pyran3	IN	0.158	3.636	0.002
U6	IN	0.158	3.592	0.003
Oci	IN	0.139	3.078	0.008
aTerpol	IN	0.131	2.843	0.013
Hex	IN	0.120	2.555	0.023
Trimeth6	IN	0.115	2.409	0.032
Hexen	IN	0.111	2.286	0.041
Pyran2	IN	0.131	2.731	0.016

(b)

from \ to	Angeleno	Flavor King	Laetitia	Larry Anne	Pioneer	Sapphire	Songold	Total	% correct
Angeleno	38	0	0	0	0	0	0	38	100.00%
Flavor King	0	12	0	0	0	0	0	12	100.00%
Laetitia	0	0	38	0	0	0	0	38	100.00%
Larry Anne	0	0	0	12	0	0	0	12	100.00%
Pioneer	0	0	0	0	27	0	0	27	100.00%
Sapphire	0	0	0	0	0	12	0	12	100.00%
Songold	0	0	0	0	0	0	12	12	100.00%
Total	38	12	38	12	27	12	12	151	100.00%

(c)

from \ to	Angeleno	Flavor King	Laetitia	Larry Anne	Pioneer	Sapphire	Songold	Total	% correct
Angeleno	38	0	0	0	0	0	0	38	100.00%
Flavor King	0	12	0	0	0	0	0	12	100.00%
Laetitia	0	0	38	0	0	0	0	38	100.00%
Larry Anne	0	0	0	12	0	0	0	12	100.00%
Pioneer	0	0	0	0	27	0	0	27	100.00%
Sapphire	0	0	0	0	0	12	0	12	100.00%
Songold	0	0	0	0	0	0	12	12	100.00%
Total	38	12	38	12	27	12	12	151	100.00%

Table 13: DA results for the **'Tree-ripened'** functional group of the seven cultivars. (a) Variable selection table using the stepwise method' (see Table 3 for explanation of compound abbreviations). Confusion matrixes indicating the percentage correctly predicted samples for the three classes using all the compounds (b) and using only the 46 compounds identified by the stepwise method (c).

(a)

Variable IN/OUT	Status	Partial R^2	F	Pr > F
cMenth	IN	0.914	146.790	< 0.0001
Amyl	IN	0.831	67.345	< 0.0001
Hex	IN	0.733	37.081	< 0.0001
Trimeth6	IN	0.699	30.987	< 0.0001
Hexfor	IN	0.627	22.102	< 0.0001
U7	IN	0.592	18.831	< 0.0001
Menth	IN	0.536	14.854	< 0.0001
Hexe	IN	0.535	14.554	< 0.0001
bIon	IN	0.362	7.093	< 0.0001
Benzyl	IN	0.353	6.722	< 0.0001
pMenth	IN	0.343	6.353	< 0.0001
Hep	IN	0.420	8.689	< 0.0001
Oci	IN	0.315	5.434	0.000
Eug	IN	0.286	4.668	0.000
aIon	IN	0.356	6.357	< 0.0001
Trimeth	IN	0.495	11.096	< 0.0001
Pyran3	IN	0.264	4.000	0.002
Cym	IN	0.235	3.377	0.006
U7	OUT	0.139	1.773	0.118
2But	IN	0.264	3.949	0.002
U3	IN	0.229	3.221	0.008
Lim	IN	0.232	3.221	0.008
Lina	IN	0.188	2.425	0.036
bDam	IN	0.220	2.919	0.014
tLinox	IN	0.238	3.183	0.009
Hot	IN	0.381	6.165	< 0.0001
3Hexe	IN	0.381	6.062	< 0.0001
Cyclo	IN	0.195	2.341	0.043
Lina	OUT	0.103	1.109	0.368
U7	IN	0.237	3.010	0.012
Oct	IN	0.220	2.684	0.023
Hexyl	IN	0.234	2.855	0.017
Lim	OUT	0.165	1.849	0.106
3Hexac	IN	0.385	5.835	< 0.0001
Hexfor	OUT	0.125	1.339	0.255

Variable IN/OUT	Status	Partial R^2	F	Pr > F
Butbu	IN	0.279	3.607	0.004
Oct	OUT	0.136	1.471	0.205
Benz	IN	0.284	3.706	0.004
U8	IN	0.469	8.110	< 0.0001
Lim	IN	0.241	2.860	0.017
Nona	IN	0.223	2.534	0.031
U1	IN	0.228	2.556	0.030
Eth	IN	0.224	2.448	0.037
Hexen	IN	0.237	2.585	0.029
Trimeth	OUT	0.182	1.859	0.107
But	IN	0.338	4.247	0.002
Limox	IN	0.315	3.750	0.004
cLinox	IN	0.246	2.612	0.028
U1	OUT	0.189	1.864	0.107
U4	IN	0.239	2.517	0.034
Bor	IN	0.253	2.653	0.027
U9	IN	0.315	3.526	0.006
Lim	OUT	0.186	1.752	0.130
Oct	IN	0.238	2.393	0.043
Dodeca	IN	0.294	3.120	0.012
cMenth	OUT	0.174	1.581	0.175
Gera	IN	0.314	3.434	0.007
Pin	IN	0.272	2.734	0.024

(b)

from \ to	Angeleno	Flavor King	Laetitia	Larry Anne	Pioneer	Sapphire	Songold	Total	% correct
Angeleno	12	0	0	0	0	0	0	12	100.00%
Flavor King	0	12	0	0	0	0	0	12	100.00%
Laetitia	0	0	10	0	0	0	0	10	100.00%
Larry Anne	0	0	0	12	0	0	0	12	100.00%
Pioneer	0	0	0	0	20	0	0	20	100.00%
Sapphire	0	0	0	0	0	12	0	12	100.00%
Songold	0	0	0	0	0	0	12	12	100.00%
Total	12	12	10	12	20	12	12	90	100.00%

(c)

from \ to	Angeleno	Flavor King	Laetitia	Larry Anne	Pioneer	Sapphire	Songold	Total	% correct
Angeleno	12	0	0	0	0	0	0	12	100.00%
Flavor King	0	12	0	0	0	0	0	12	100.00%
Laetitia	0	0	10	0	0	0	0	10	100.00%
Larry Anne	0	0	0	12	0	0	0	12	100.00%
Pioneer	0	0	0	0	20	0	0	20	100.00%
Sapphire	0	0	0	0	0	12	0	12	100.00%
Songold	0	0	0	0	0	0	12	12	100.00%
Total	12	12	10	12	20	12	12	90	100.00%

Table 14: DA results for the **'Stored'** functional group of the seven cultivars. (a) Variable selection table using the stepwise method' (see Table 3 for explanation of compound abbreviations). Confusion matrixes indicating the percentage correctly predicted samples for the three classes using all the compounds (b) and using only the 34 compounds identified by the stepwise method (c).

(a)

Variable IN/OUT	Status	Partial R^2	F	Pr > F
U9	IN	0.984	925.920	< 0.0001
U8	IN	0.826	69.447	< 0.0001
Phe	IN	0.797	56.801	< 0.0001
Pin	IN	0.677	30.038	< 0.0001
U3	IN	0.588	20.186	< 0.0001
Lim	IN	0.549	17.039	< 0.0001
Hexen	IN	0.519	14.913	< 0.0001
aTerp	IN	0.517	14.624	< 0.0001
U5	IN	0.442	10.673	< 0.0001
Hexa	IN	0.375	7.989	< 0.0001
U7	IN	0.332	6.544	< 0.0001
Eth	IN	0.487	12.321	< 0.0001
U10	IN	0.347	6.831	< 0.0001
Nona	IN	0.282	4.981	0.000
Oct	IN	0.288	5.067	0.000
Butbu	IN	0.365	7.088	< 0.0001
Amyl	IN	0.432	9.259	< 0.0001
Oct	OUT	0.013	0.156	0.987
Cyclo	IN	0.286	4.880	0.000
Gera	IN	0.333	6.004	< 0.0001
Non	IN	0.426	8.790	< 0.0001
bIon	IN	0.311	5.267	0.000
But	IN	0.284	4.571	0.001
cMenth	IN	0.251	3.806	0.002
2But	IN	0.827	53.401	< 0.0001
Cymol	IN	0.268	4.031	0.002
Hex	IN	0.300	4.635	0.001
Benzyl	IN	0.328	5.195	0.000
Hexe	IN	0.272	3.924	0.002
Hexyl	IN	0.239	3.242	0.008
Bisa	IN	0.200	2.536	0.029
U4	IN	0.206	2.589	0.027
Oci	IN	0.188	2.273	0.049

Deca	IN	0.456	8.119	< 0.0001
Lina	IN	0.384	5.915	< 0.0001
Hexe	OUT	0.071	0.729	0.628
Hep	IN	0.376	5.715	0.000
Car	IN	0.448	7.588	< 0.0001

(b)

from \ to	Angeleno	Flavor King	Laetitia	Larry Anne	Pioneer	Sapphire	Songold	Total	% correct
Angeleno	18	0	0	0	0	0	0	18	100.00%
Flavor King	0	12	0	0	0	0	0	12	100.00%
Laetitia	0	0	12	0	0	0	0	12	100.00%
Larry Anne	0	0	0	12	0	0	0	12	100.00%
Pioneer	0	0	0	0	18	0	0	18	100.00%
Sapphire	0	0	0	0	0	12	0	12	100.00%
Songold	0	0	0	0	0	0	12	12	100.00%
Total	18	12	12	12	18	12	12	96	100.00%

(c)

from \ to	Angeleno	Flavor King	Laetitia	Larry Anne	Pioneer	Sapphire	Songold	Total	% correct
Angeleno	18	0	0	0	0	0	0	18	100.00%
Flavor King	0	12	0	0	0	0	0	12	100.00%
Laetitia	0	0	12	0	0	0	0	12	100.00%
Larry Anne	0	0	0	12	0	0	0	12	100.00%
Pioneer	0	0	0	0	18	0	0	18	100.00%
Sapphire	0	0	0	0	0	12	0	12	100.00%
Songold	0	0	0	0	0	0	12	12	100.00%
Total	18	12	12	12	18	12	12	96	100.00%

Figure 1: Observations and Variables charts for **'Pioneer'** using all the compounds ((a) and (c)) and using only the compounds identified in the stepwise method of a DA ((b) and (d)). Percentages indicate the corresponding percentage of variance described by each factor. (See Table 3 for explanation of compound abbreviations).

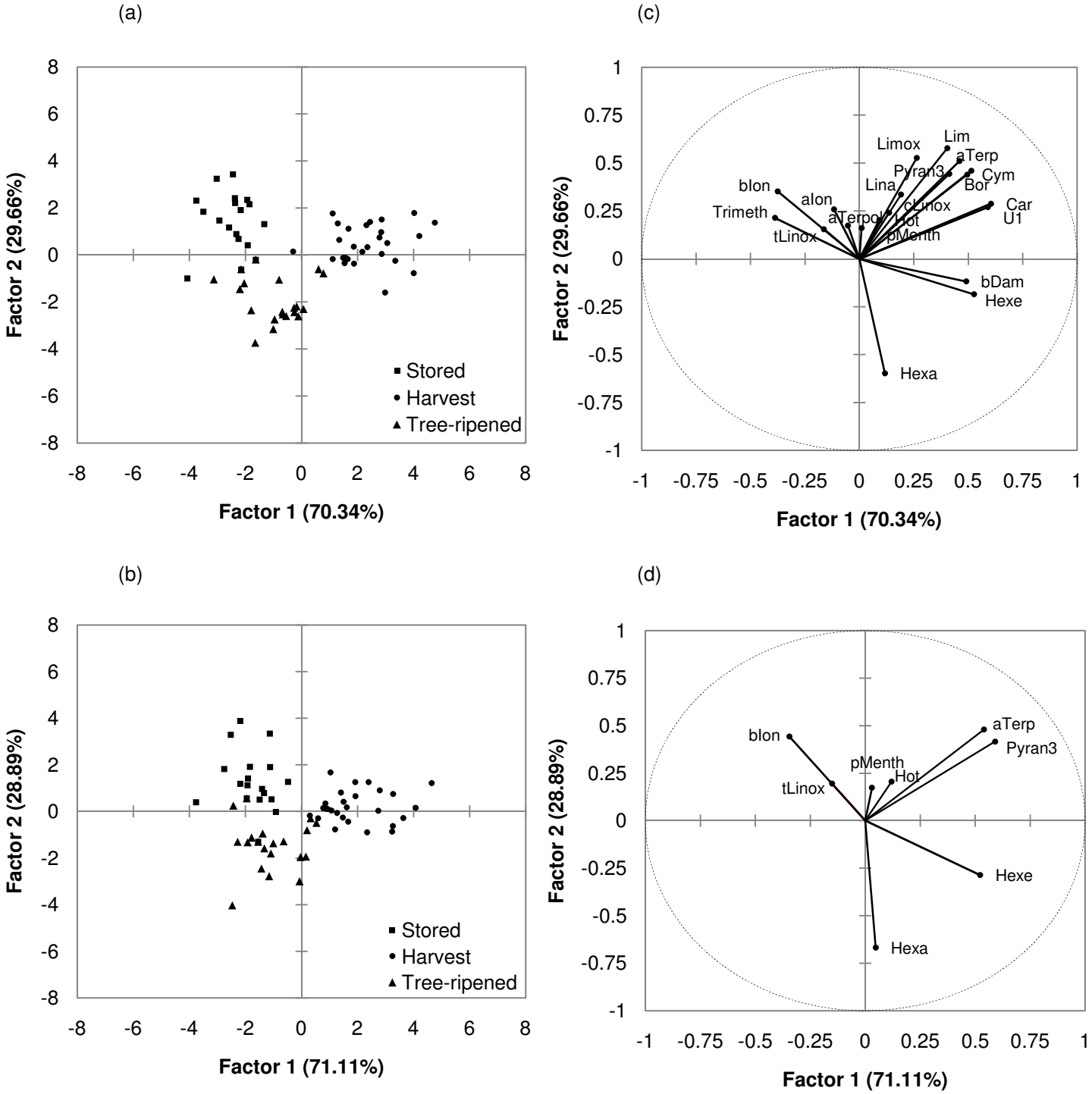


Figure 2: Observations and Variables charts for **'Sapphire'** using all the compounds ((a) and (c)) and using only the compounds identified in the stepwise method of a DA ((b) and (d)). Percentages indicate the corresponding percentage of variance described by each factor. (See Table 3 for explanation of compound abbreviations).

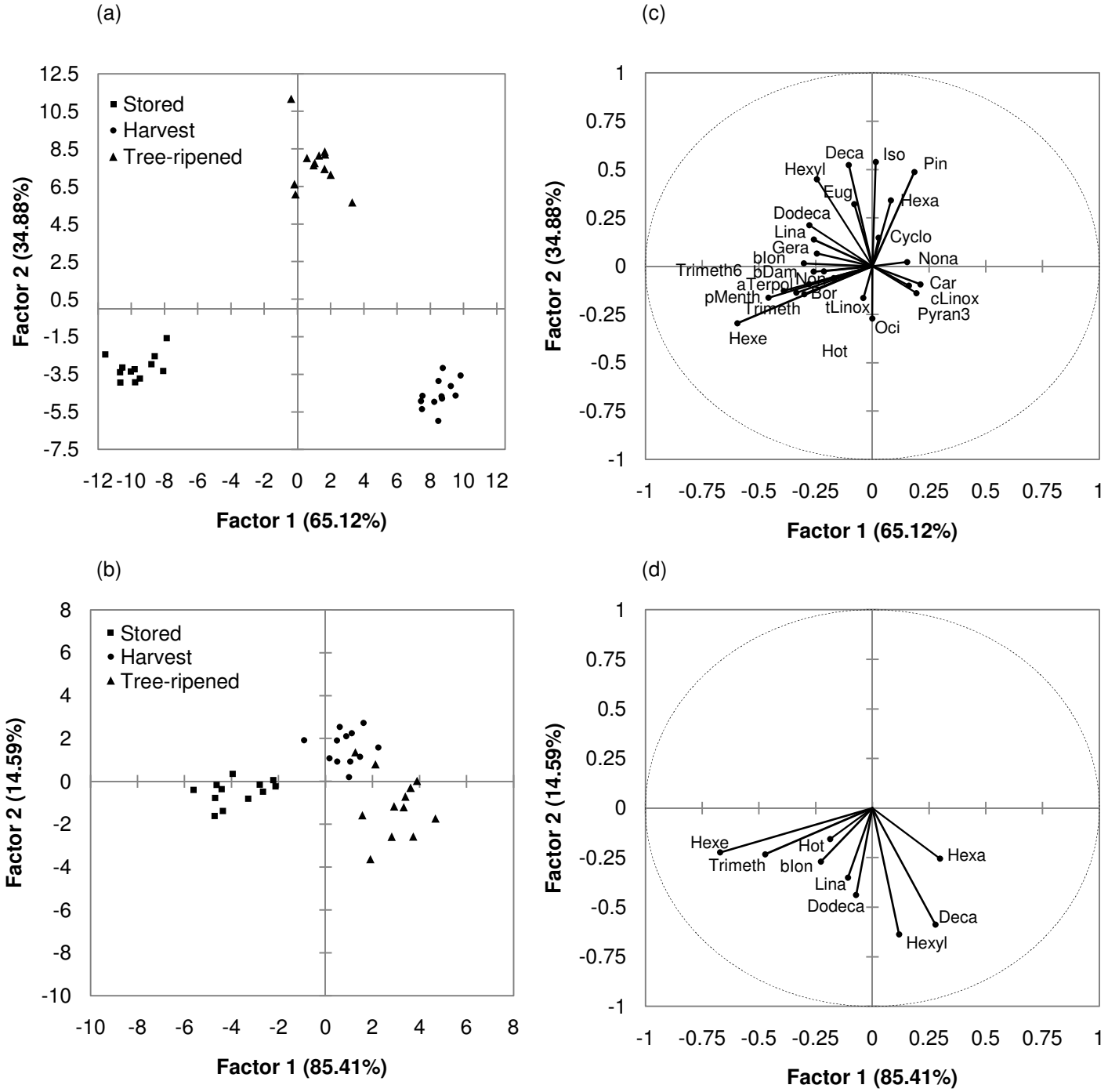


Figure 3: Observations and Variables charts for **'Laetitia'** using all the compounds ((a) and (c)) and using only the compounds identified in the stepwise method of a DA ((b) and (d)). Percentages indicate the corresponding percentage of variance described by each factor. (See Table 3 for explanation of compound abbreviations).

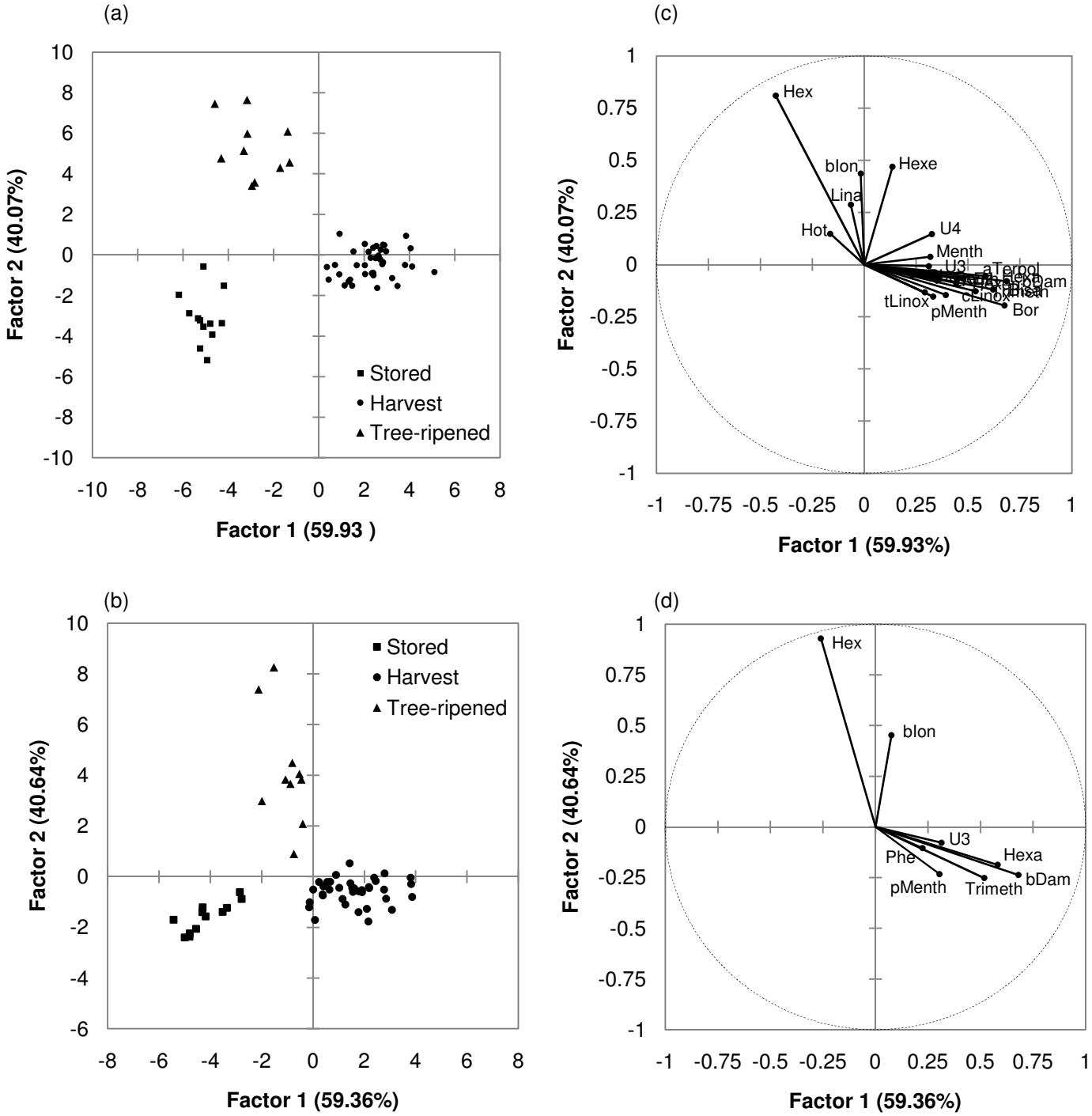


Figure 4: Observations and Variables charts for **'Flavor King'** using all the compounds ((a) and (c)) and using only the compounds identified in the stepwise method of a DA ((b) and (d)). Percentages indicate the corresponding percentage of variance described by each factor. (See Table 3 for explanation of compound abbreviations).

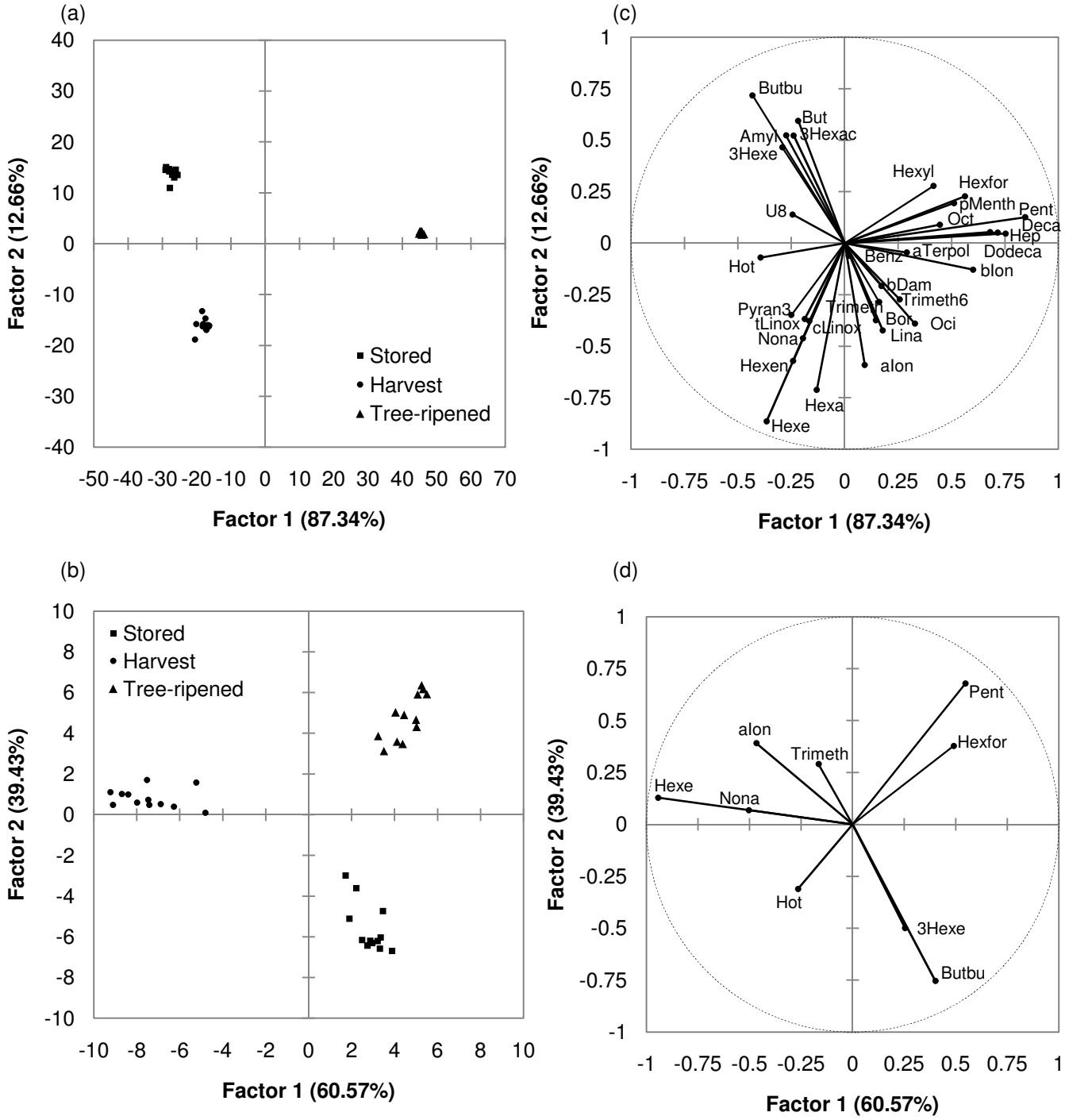


Figure 5: Observations and Variables charts for **'Songold'** using all the compounds ((a) and (c)) and using only the compounds identified in the stepwise method of a DA ((b) and (d)). Percentages indicate the corresponding percentage of variance described by each factor. (See Table 3 for explanation of compound abbreviations).

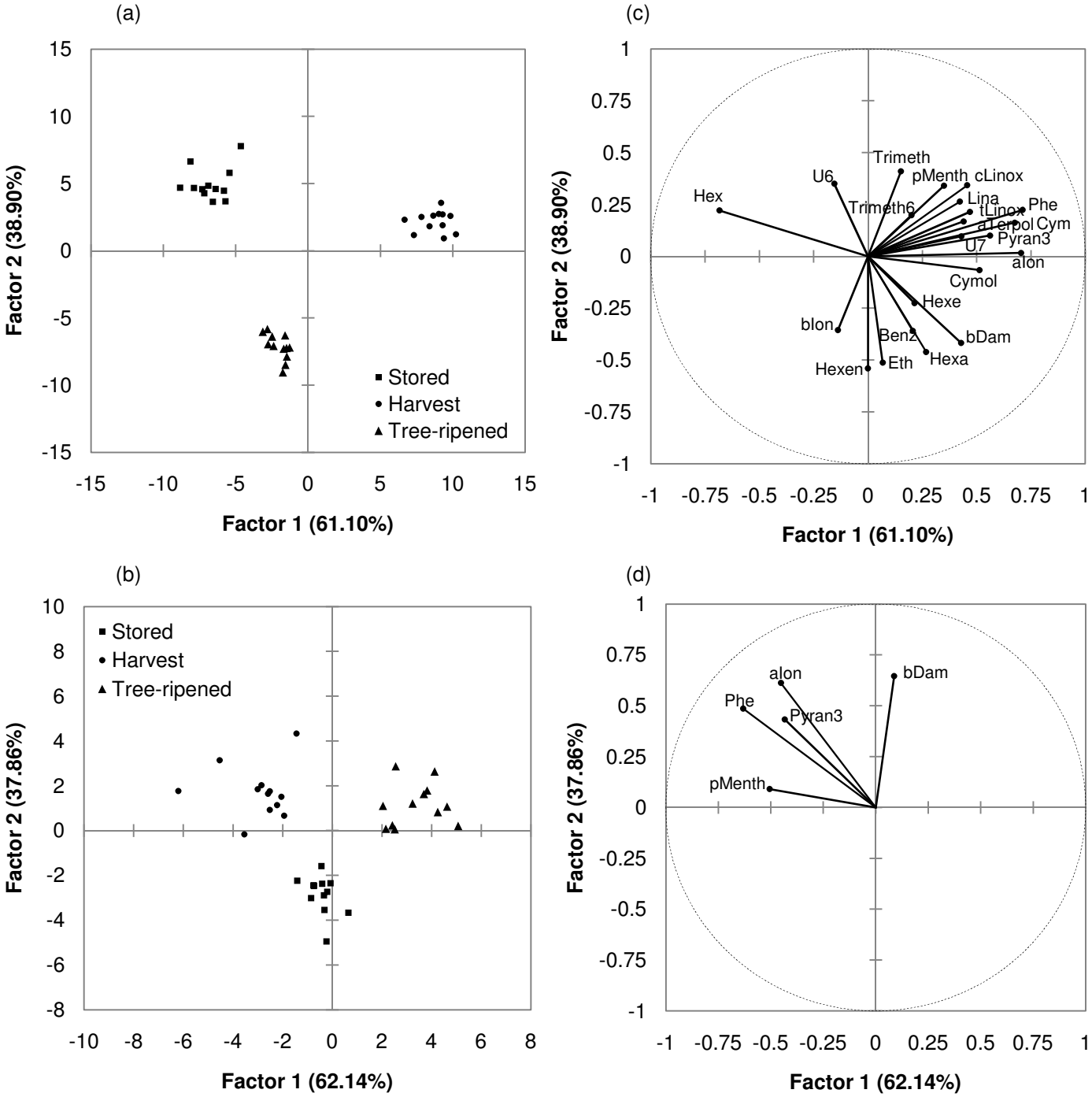


Figure 6: Observations and Variables charts for '**Larry Anne**' using all the compounds ((a) and (c)) and using only the compounds identified in the stepwise method of a DA ((b) and (d)). Percentages indicate the corresponding percentage of variance described by each factor. (See Table 3 for explanation of compound abbreviations).

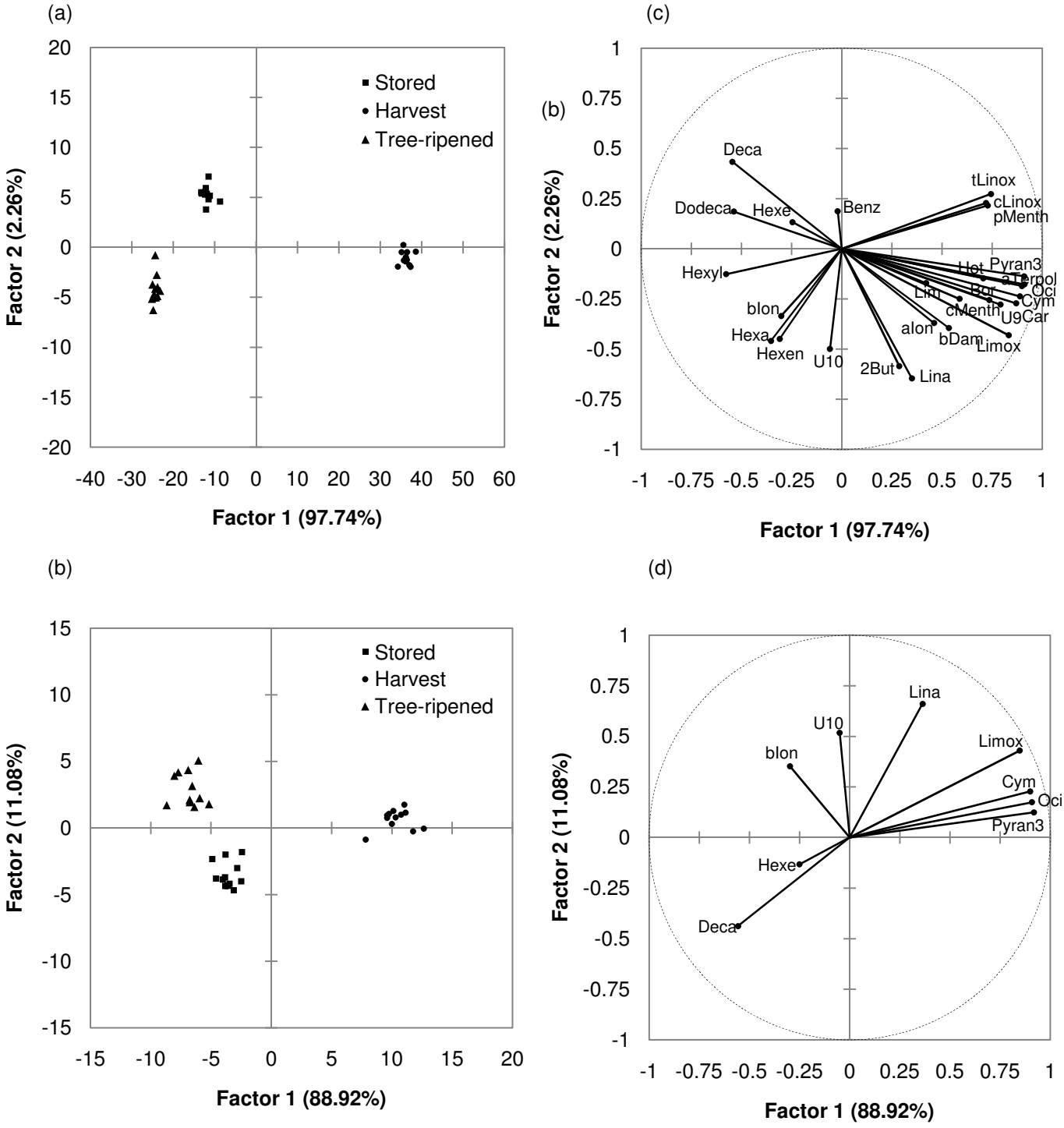


Figure 7: Observations and Variables charts for **'Angeleno'** using all the compounds ((a) and (c)) and using only the compounds identified in the stepwise method of a DA ((b) and (d)). Percentages indicate the corresponding percentage of variance described by each factor. (See Table 3 for explanation of compound abbreviations).

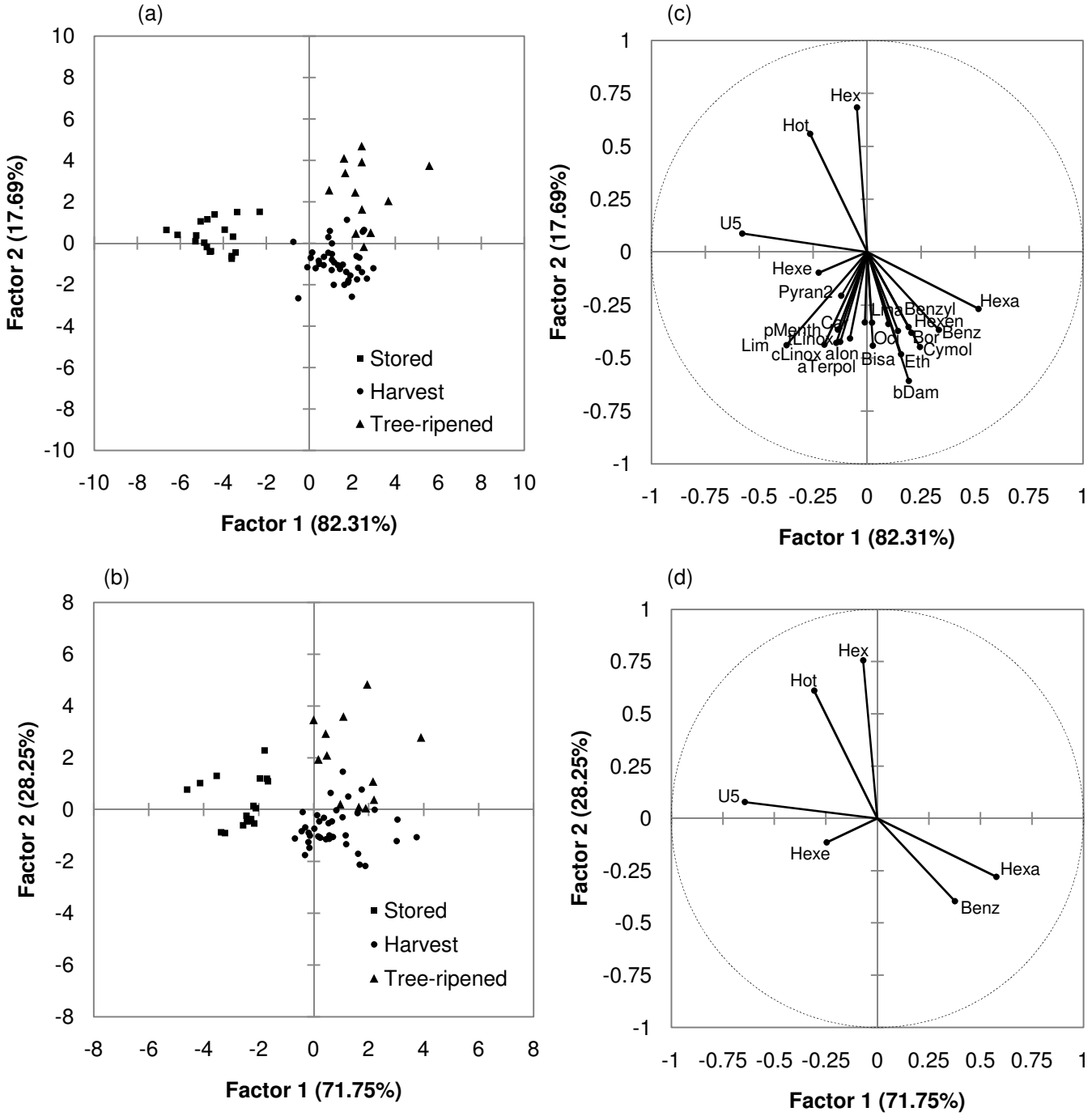
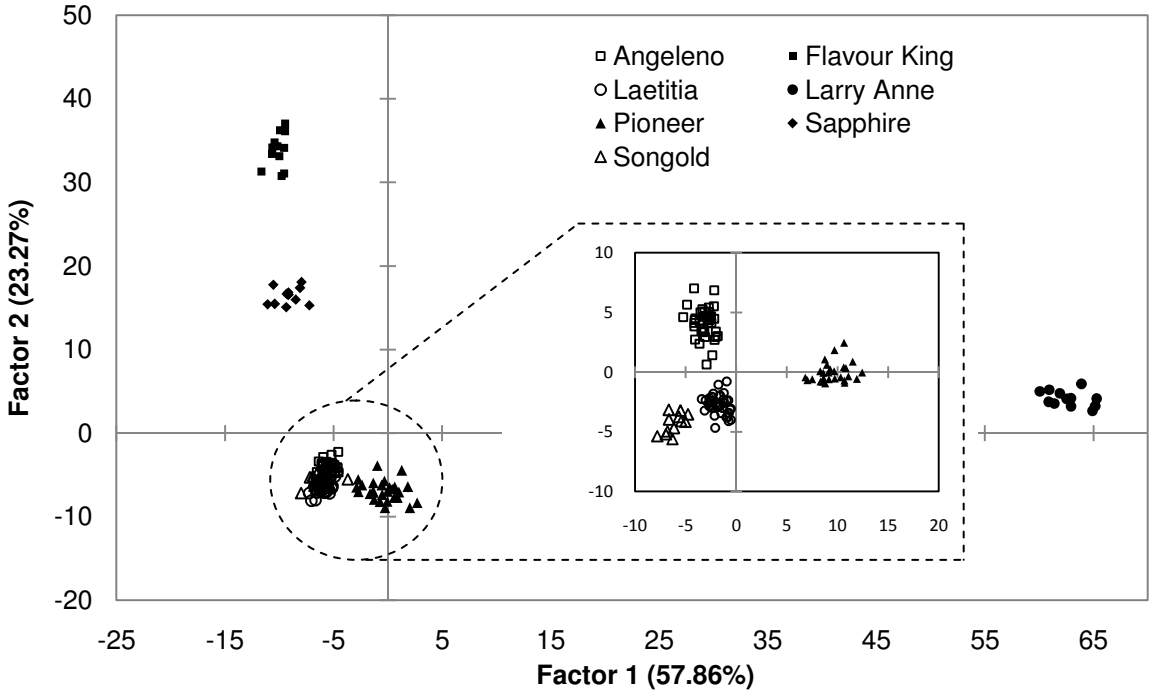
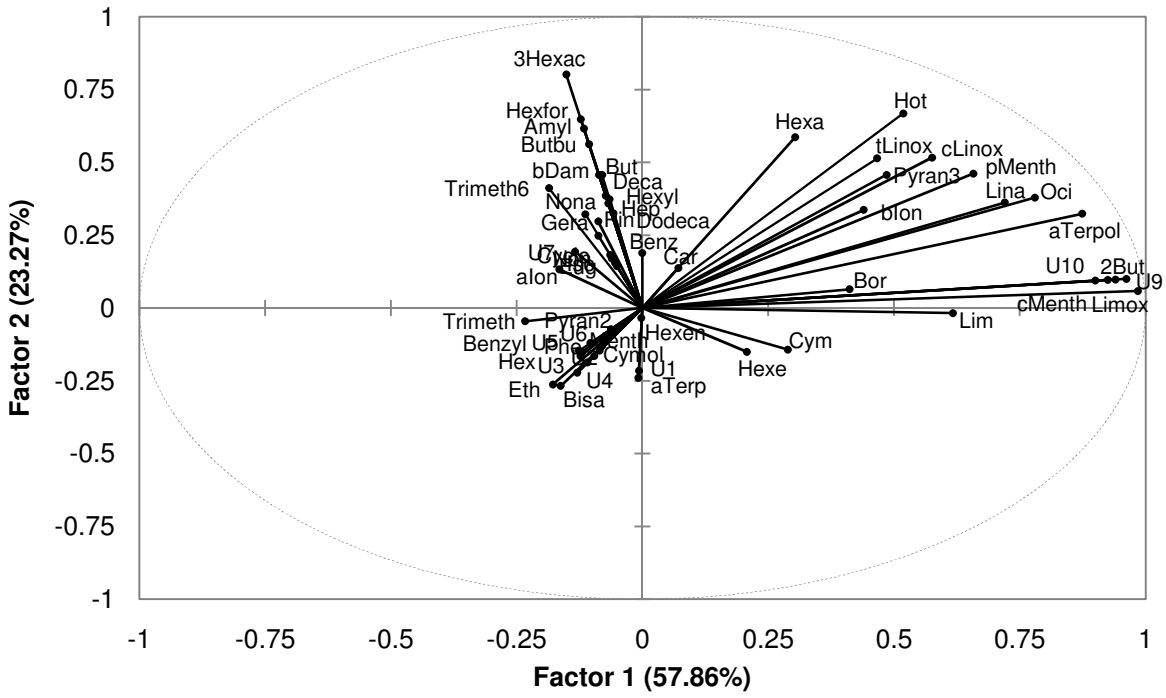


Figure 8: Observations and Variables charts for the **'Harvest'** functional group using all the compounds ((a) and (b)) and using only the compounds identified in the stepwise method of a DA ((c) and (d)). Percentages indicate the corresponding percentage of variance described by each factor. (See Table 3 for explanation of compound abbreviations).

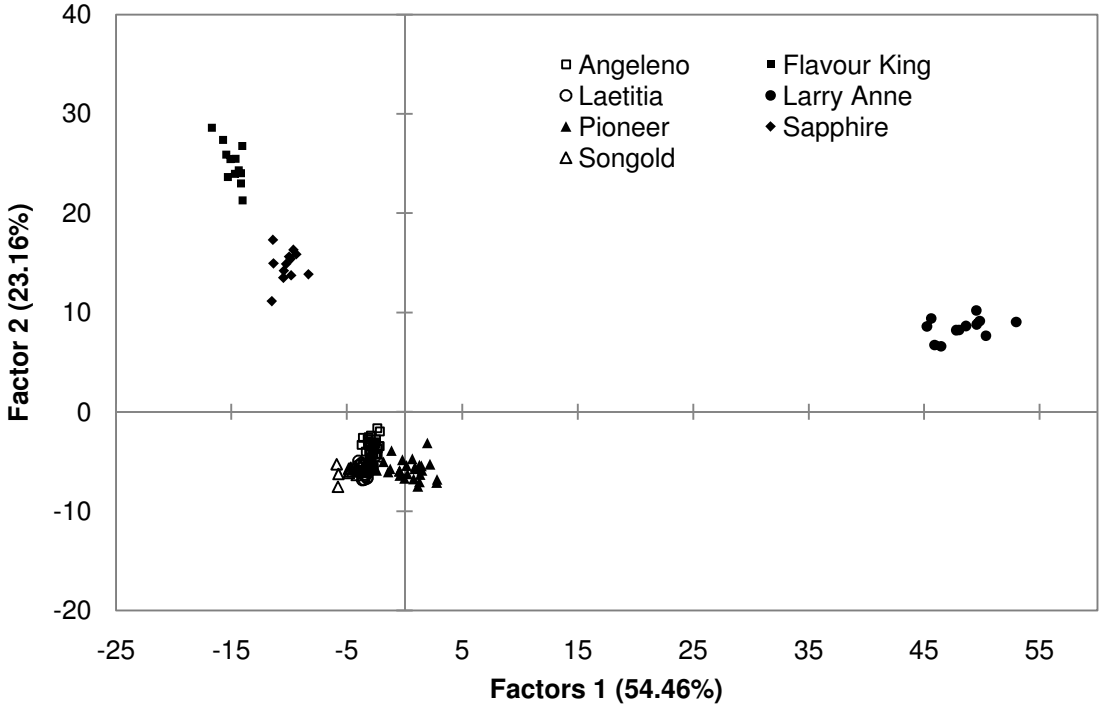
(a) Inserted graph: Observations plot from a DA using only the 'cluster' cultivars



(b)



(c)


Angeleno
Flavour King
Laetitia
Larry Anne
Pioneer
Sapphire
Songold
Factor 2 (23.16%)
Factors 1 (54.46%)
40
30
20
10
0
-10
-20
-25
-15
-5
5
15
25
35
45
55

(d)

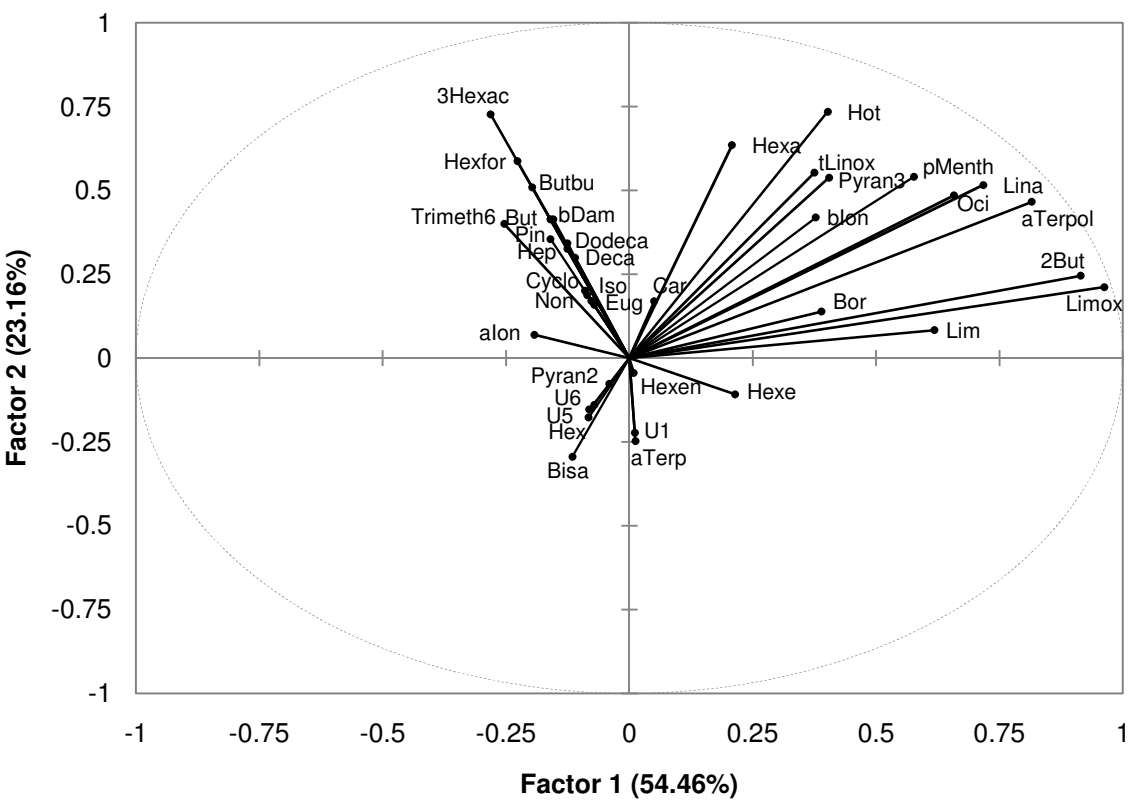
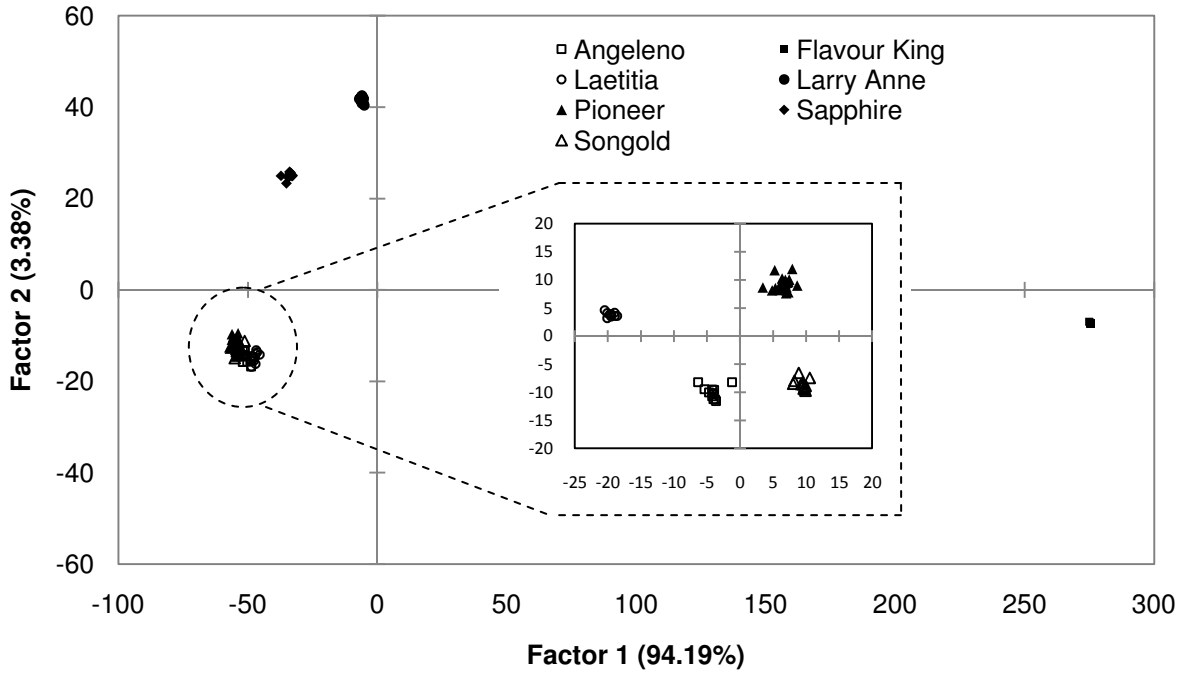
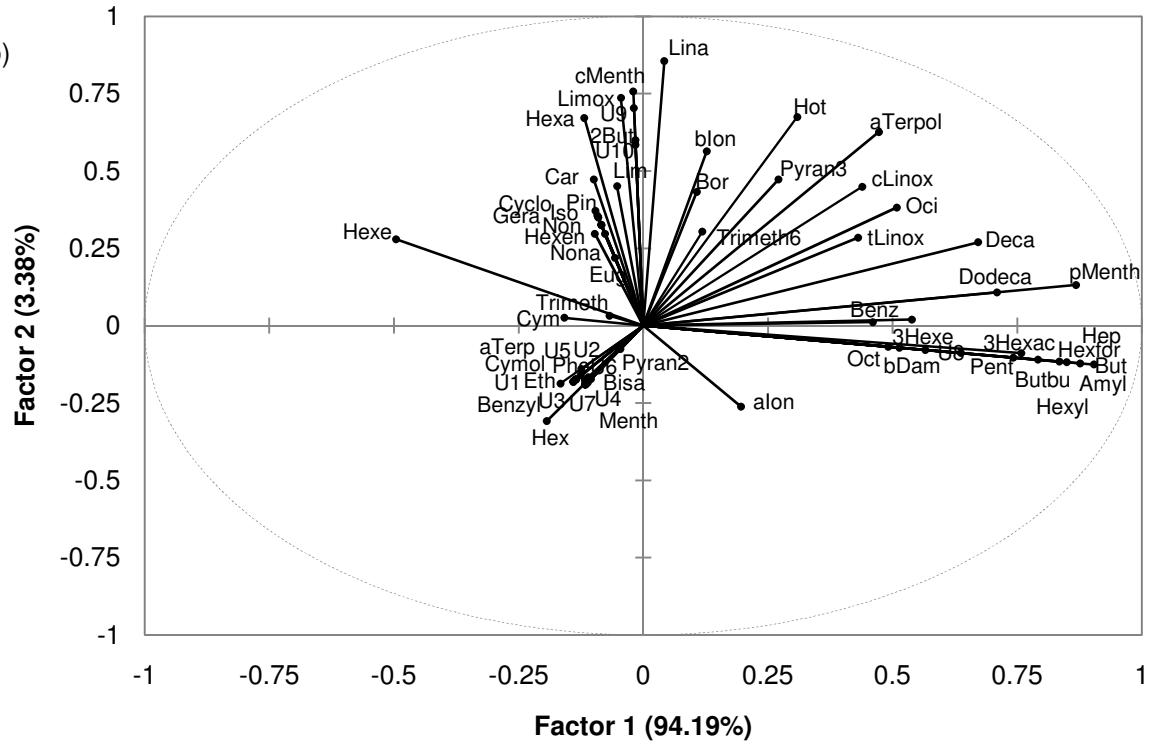

3Hexac
Hot
Hexa
Hexfor
tLinox
Pyran3
pMenth
Lina
Butbu
Oci
Trimeth6
But
bDam
bIon
aTerpol
Pin
Dodeca
Hep
Deca
2But
Cyclo
Iso
Car
Nor
Eug
Bor
Limox
aIon
Lim
Pyran2
Hexen
Hexe
U6
U5
Hex
U1
aTerp
Bisa
Factor 2 (23.16%)
Factor 1 (54.46%)
1
0.75
0.5
0.25
0
-0.25
-0.5
-0.75
-1
-1
-0.75
-0.5
-0.25
0
0.25
0.5
0.75
1

Figure 9: Observations and Variables charts for the **'Tree-ripened'** functional group using all the compounds ((a) and (b)) and using only the compounds identified in the stepwise method of a DA ((c) and (d)). Percentages indicate the corresponding percentage of variance described by each factor. (See Table 3 for explanation of compound abbreviations).

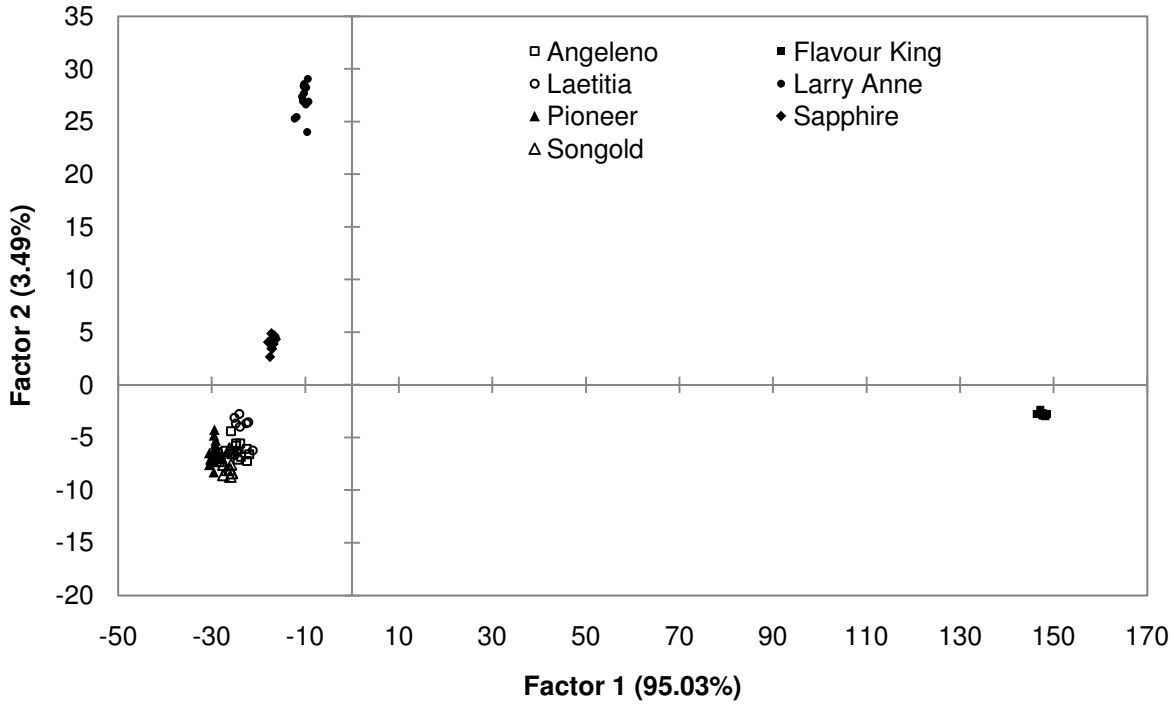
(a) Inserted graph: Observations plot from a DA using only the 'cluster' cultivars



(b)



(c)


Angeleno
Flavour King
Laetitia
Larry Anne
Pioneer
Sapphire
Songold
Factor 2 (3.49%)
Factor 1 (95.03%)
35
30
25
20
15
10
5
0
-5
-10
-15
-20
-50
-30
-10
10
30
50
70
90
110
130
150
170

(d)

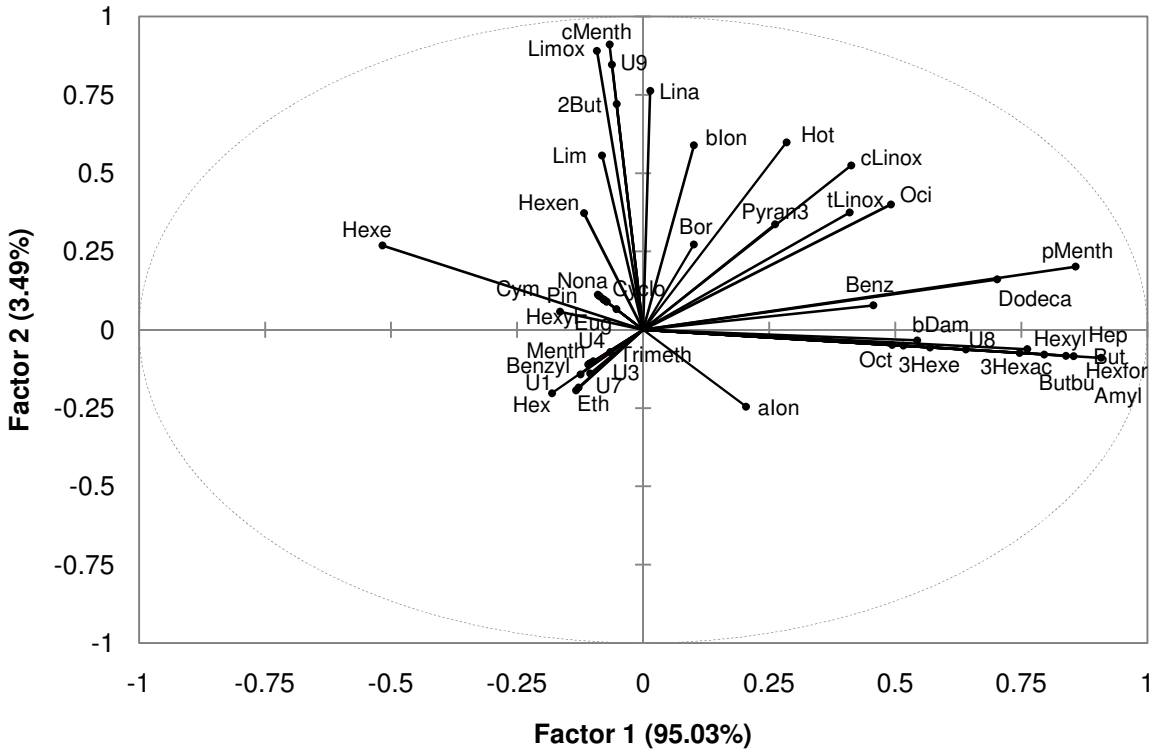
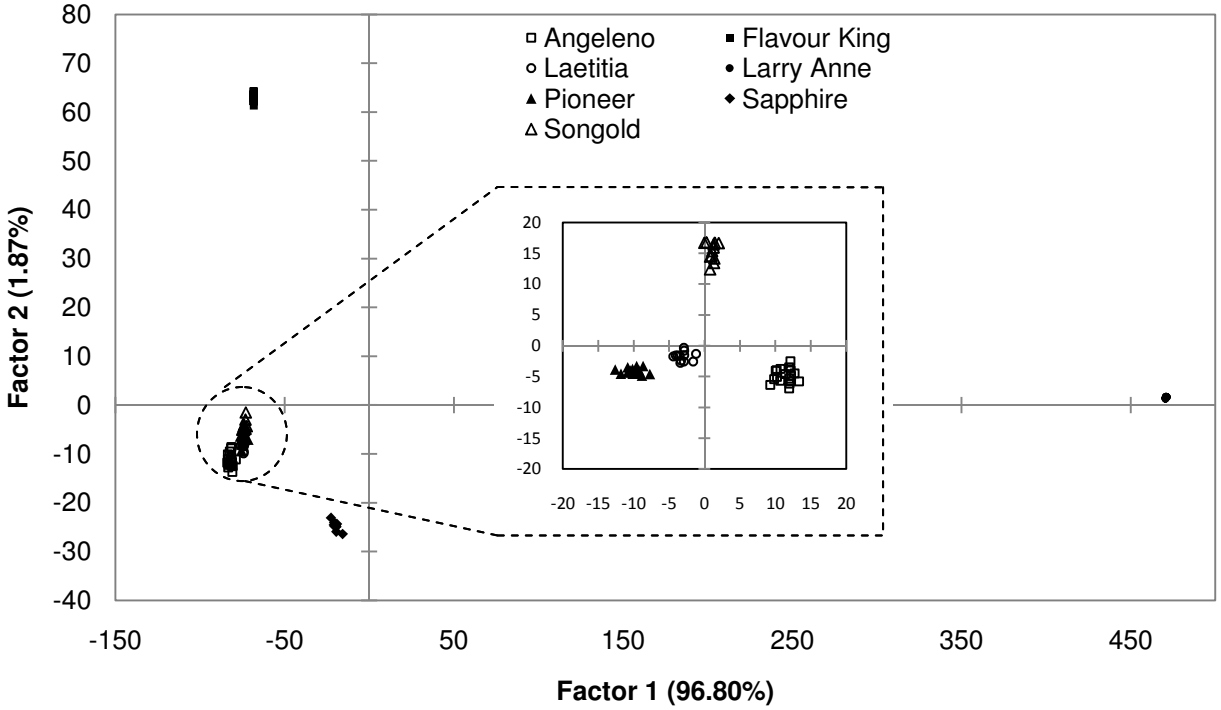
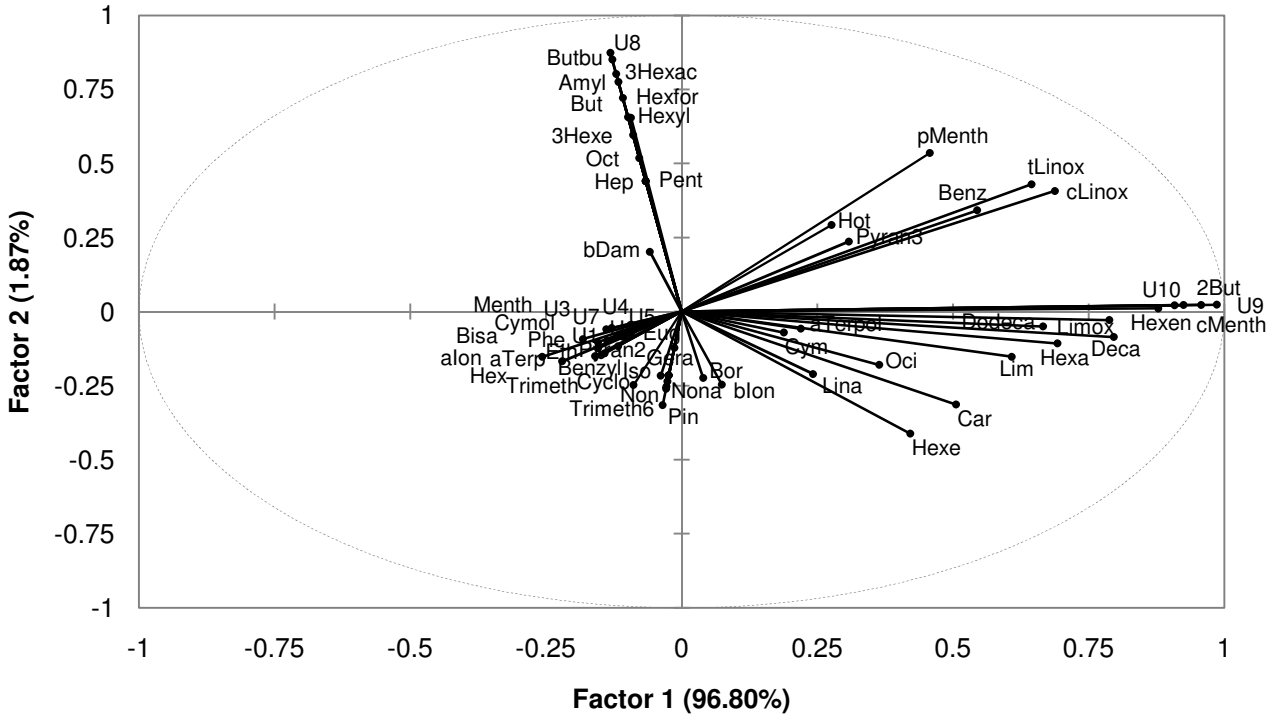

cMenth
Limox
U9
Lina
2But
bIon
Hot
Lim
cLinox
Hexen
tLinox
Oci
Pyran3
Hexe
Bor
pMenth
Benz
Cym
Pin
Nona
Cyclo
Dodeca
Hexyl
Eug
bDam
U8
Hexyl
Hep
U4
Menth
Trimeth
Oct
3Hexe
3Hexac
But
Hexfor
Benzyl
U3
Butbu
U1
U7
Amyl
Hex
Eth
aIon
Factor 2 (3.49%)
Factor 1 (95.03%)
1
0.75
0.5
0.25
0
-0.25
-0.5
-0.75
-1
-1
-0.75
-0.5
-0.25
0
0.25
0.5
0.75
1

Figure 10: Observations and Variables charts for the **'Stored'** functional group using all the compounds ((a) and (b)) and using only the compounds identified in the stepwise method of a DA ((c) and (d)). Percentages indicate the corresponding percentage of variance described by each factor. (See Table 3 for explanation of compound abbreviations).

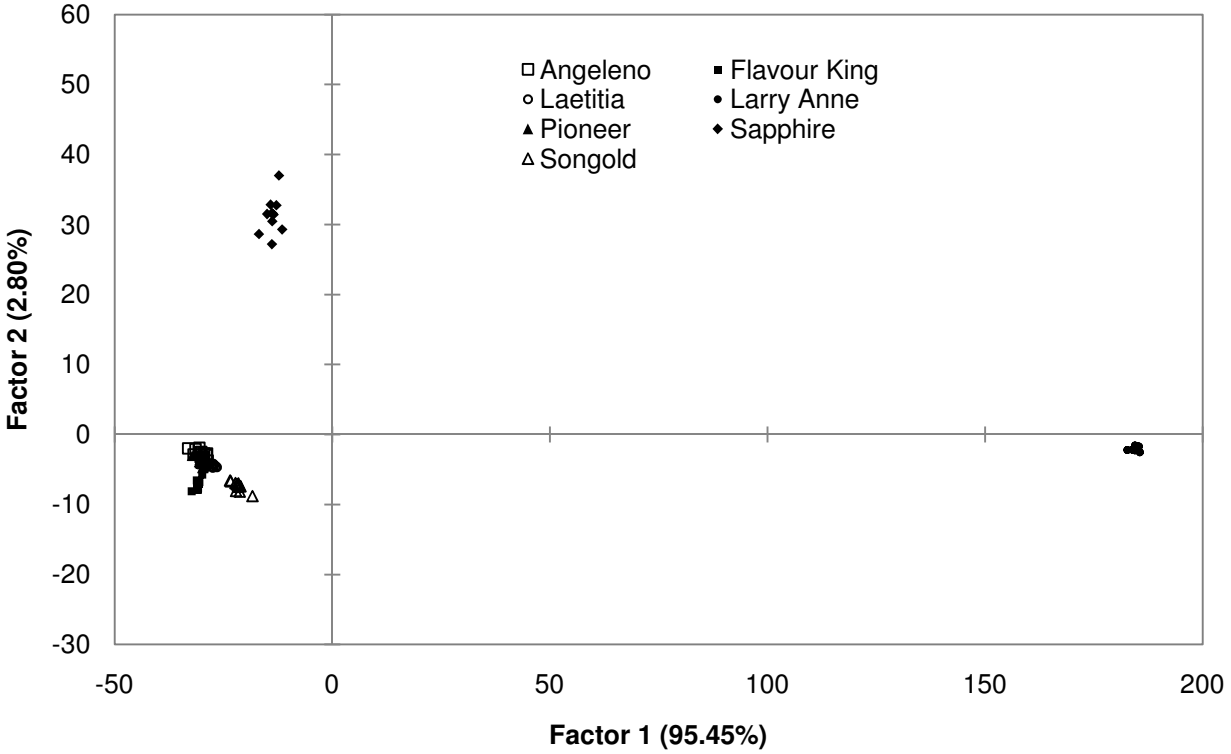
(a) Inserted graph: Observations plot from a DA using only the 'cluster' cultivars



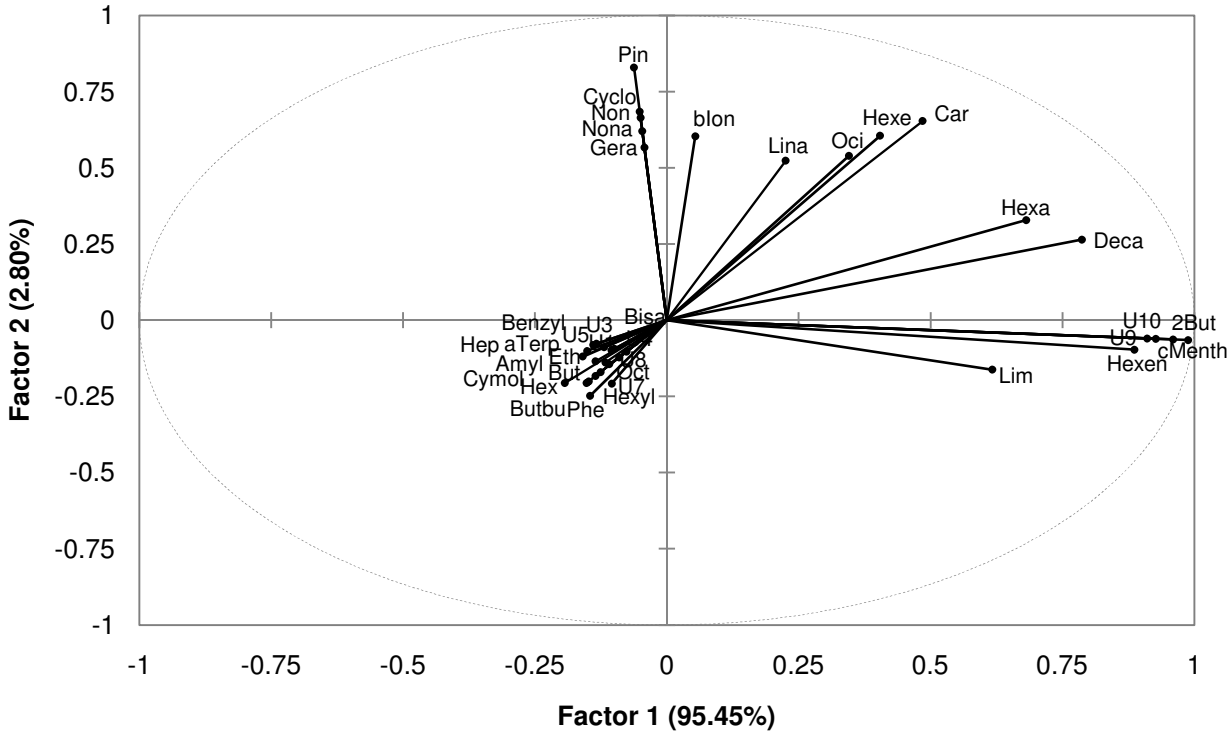
(b)



(c)


Angeleno
Flavour King
Laetitia
Larry Anne
Pioneer
Sapphire
Songold
Factor 2 (2.80%)
Factor 1 (95.45%)
60
50
40
30
20
10
0
-10
-20
-30
-50
0
50
100
150
200

(d)


Pin
Cyclo
Non
Nona
Gera
bIon
Lina
Oci
Hexe
Car
Hexa
Deca
Bisa
Benzyl
U3
Hep a
Terp
Amyl
Cymol
Hex
But
Hexyl
Lim
U10
2But
U9
cMenth
Hexen
Factor 2 (2.80%)
Factor 1 (95.45%)
1
0.75
0.5
0.25
0
-0.25
-0.5
-0.75
-1
-1
-0.75
-0.5
-0.25
0
0.25
0.5
0.75
1

GENERAL DISCUSSION AND CONCLUSION

The first paper of this dissertation, dealing with NIR spectroscopy as a non-destructive alternative to assessing quality parameters in plums, started off as a method development investigation to assist in minimising sample variation in the aroma volatile study. Pilot results were very promising and the decision was taken to explore this in tandem with the main topic. Ultimately we did not use NIR spectroscopy to assist us in the selection of samples as initially intended. The reason for this is two-fold, firstly, we opted to create maturity and functional groups and firmness provided us with a bigger range (compared to the sugar levels) to accommodate the groups. Firmness is also currently used in most commercial practices as a maturity index. Secondly, doing the NIR study simultaneously with the aroma study meant that we had to continue to do the traditional destructive testing in any case as part of the model building. Although using NIR spectroscopy to analyse and predict quality had been done before on plums this study included cultivar specific models of Japanese plums that have not been found in the literature. The models presented here also contain a comprehensive data set made up of plum samples stretching across a wider range of maturity and ripeness compared to similar studies described in the literature. The results indicated that prediction models derived from NIR spectroscopy can indeed accurately predict quality parameters in plums, especially sugar levels and sugar-to-acid ratios. Both cultivar specific and multi-cultivar models perform well provided that special care is given when choosing calibration and validation data sets in terms of cultivar, seasonality and the range of the variable. Failure to do so can lead to increased prediction errors. The results compared well to similar studies (see Table 3, Paper 1 for references) with R^2 values of TSS rating amongst the highest found in the literature. Future work should include investigating effects such as orchard and area on the predictability of the models as this will shed light on the possible influences of pre harvest practices, soil types and climate on predictability. From a research point of view it would be interesting to assess if NIR spectroscopy can be used to detect other cell micro structural effects such as changes in membrane and cell wall integrity and link this to maturity indexes and/or physiological disorders in plums.

The aroma volatile results discussed in Papers 2 and 3 form the major part of this dissertation and should be seen as a continuum with respect to aroma dynamics in plums starting with the immature, unripe, plum reaching maturity and then either being left to ripen on the tree or being harvested and cold stored followed by commercial ripening. A complete set of data describing all the different stages referred to above is presented for three cultivars (Pioneer, Laetitia and Angeleno). In Paper 2 the results indicated that the aroma profiles were more similar during the immature stage with increasing divergence towards maturity. This study confirmed that major compounds found in plums seem to be within the aldehydes and the terpene alcohols groups with surprisingly few ester compounds measured. In Paper 3 more cultivars were investigated together with the effects of post harvest cold storage. Although the results showed that all seven cultivars tested had significant differences in their aroma profiles after cold storage

no univocal pattern could be found within any of the chemical groups. This suggests that plum aroma volatiles have a complex and cultivar-specific reaction towards long term cold storage and that the compounds react individually rather than as a specified group as patterns of increase, decrease and no change have been observed during storage in most of the chemical groups. Interestingly, the inclusion of more cultivars in Paper 3 resulted in the detection of more esters although it should be noted that most of the esters were found in the plumcot cultivar (Flavor King). This indicated that the extraction method used throughout this study was not as insensitive to the detection of esters as once thought after the completion of Paper 2. 'Flavor King' also depicted a ripe aroma profile furthest diverged from the six true plum cultivars possibly pointing towards the effect of interspecific hybridisation on aroma profiles. It was also clear that some cultivars (Flavour King and Larry Anne) were more affected by cold storage than others (Pioneer). Comparing the results to other literature was not possible as no plum aroma studies directed at cold storage could be found. A possible reason for the lack of literature regarding prolonged cold storage could be that most northern hemisphere plum production takes place close to the market and does not necessitate long haul storage. This makes the current study novel and ground-breaking in its approach.

When comparing the results and discussions of Paper 2 and 3 they often seem ambiguous, e.g. in Paper 2 ten components were described as unique compared to only eight in Paper 3; 'Pioneer' is said to have five unique components in Paper 2 and in Paper 3 only two. Such seemingly contradictory statements are, however, easily explained when the scale of the two papers is taken into account. Paper 2 only dealt with three cultivars and in Paper 3 we discuss the results of seven cultivars. The more cultivars one includes in the investigation the more likely it is that similar compounds may be discovered which in turn decrease the chances of a compound being unique. Another example that illustrates the importance of scale is the separation patterns observed in Paper 2 compared to those of Paper 3. In Paper 2 'Pioneer', 'Laetitia' and 'Angeleno' seemed well separated in terms of all three ripening classes (Figures 5, 6 and 7). In Paper 3, this divergence seemed to have disappeared and the three cultivars are bundled into a cluster (Fig 8(a) and 9(a)). Again, this is a matter of scale; in Paper 3 we also presented data on cultivars that were aromatically very different to that of Pioneer, Laetitia and Angeleno and thus make the inter-cultivar differences amongst these three cultivars seem much smaller while they were in fact based on the same data as in Paper 2. Such obscurities can make the discussions seem incomprehensible if scale is not taken into account.

Future studies that will complement this dissertation include the identification of critical impacting compounds (possibly from the list of 'generic' compounds) that are responsible for the classic plum flavour by exposing the profiles to a sniffing panel and simultaneously assessing the preferred plum flavour by exposing intact fruit with different aroma profiles to a taste panel. Some of the detected compounds have very low odour threshold, e.g., norisoprenoids, incorporating this into the analysis may

make it more representable and realistic. Another spin-off from this research would be to investigate different pre- and postharvest practices currently in use or improve such practices with the aim to bridging the gap between the current aroma profile of export plums and that of the tree-ripened or a consumer-preferred profile. An attempt should also be made to incorporate aroma profiles into the breeding programs of plums in order to ensure that our next generation of plum cultivars will include flavour as an inherent characteristic and potentially maintain this during storage.

In conclusion, this study did not just contribute to our understanding of aroma volatiles in Japanese plums but also attempts to describe the dynamics thereof during maturation and ripening. This adds to the pool of fundamental knowledge essential to the applied studies that aim to improve pre-harvest manipulations and postharvest storage regimes in order to deliver a premium export product. The results further illustrated that the current harvest and storage practices do alter the aroma profile of plums compared to fruit ripened on the tree. Although some cultivars are more affected by this than others it is clear that we are delivering an end product that is possibly different to what we intended. The section on NIR spectroscopy as a tool to predict quality parameters provides the plum industry with a well researched basis when considering the improvement and modernisation of current practices.

APPENDIX 1: METHOD DEVELOPMENT

NOTE: The following appendix is intended to be only a brief description of some of the method development work preceding this study and meant for internal use only.

Introduction:

As discussed in the general introduction and literature review, the methodology of an aroma volatile investigation typically requires four steps starting with sample/substrate preparation (if whole fruit is used this step is obsolete/reduced), step two: extraction of the volatile compounds, step three: separation of the volatiles and finally step four: identification of the volatiles. The sample preparation step and especially the extraction step define the nature of the chemical groups that will be separated and identified, making it almost impossible to compare results from studies not using the same methodology (Crouzet et al., 1990). We used HS-SPME-GC-TOFMS comprising of Headspace Solid-Phase Microextraction (HS-SPME) with identical conditions in all our extractions coupled with gas chromatography (GC) to separate the compounds and time-of-flight-mass-spectroscopy (TOF-MS) for identification. HS-SPME-GC-TOFMS was preceded by a sample preparation step as described in Paper 2. This appendix will focus on the method development steps that gave rise to specific conditions during the sample preparation and HS-SPME steps. The following four aspects were investigated:

A: HS-SPME method development:

1. HS-SPME fibre selection
2. HS-SPME-GC-TOFMS repeatability

B: Sample preparation development:

1. Effect of sample oxidation (browning) on aroma profile
2. Effect of sample freezing on aroma profile

A1: HS-SPME fiber selection

Background

HS-SPME was developed by Pawliszyn and co-workers in 1990 (Arthur and Pawliszyn, 1990) as a new extraction technique using a fused-silica fiber that is coated on the outside with an appropriate film of several polymeric stationary phases. This acts as a 'sponge' to concentrate the aroma volatiles in the head-space above the sample onto the film (Kataoka et al., 2000). The volatiles are then desorbed in a GC injector port at a temperature equal to the boiling point of the least volatile analyte (Kataoka et al., 2000). The analytes are concentrated at the head of the GC column and is ready for separation. Several kinds of fibers are commercially available and differ in their affinity for certain analyte classes. Choosing the optimal fiber for your application is vital and will determine the chemical classes detected in the

results. The main advantages of HS-SPME over other extraction methods are simplicity speed, solvent-free, high-sensitivity, small sample volume, lower cost and simple automation (Kataoka et al., 2000).

Objectives

According to Crouzet et al. (1990), esters, aldehydes and alcohols are the chemical groups most important in plum aroma. After considering the affinity of the seven commercially available silica-coated fibers for these compounds we were left to choose between the poly(dimethylsiloxane) (PDMS, 100 µm) and the PDMS/Carboxen (75 µm) fibers. The objective of this experiment was to test the suitability of these two fibers using several commercially obtained aroma volatiles that have been associated with plum aroma in the literature. A qualitative and quantitative approach will be used to compare the performance of the fibers.

Materials and procedures

The two fibers, (PDMS (100 µm) and PDMS/CAR (75 µm), purchased from Supelco, USA, were exposed to the following 11 aroma compounds obtained from Sigma, St. Louis, MO, USA: 1-hexanol, benzaldehyde, 2-phenylethanol, ethyl nonanoate, methyl cinnamate, γ-decalactone, hexanal, linalool, nonanal, γ-octalactone and damascenone. The compound 2-octanol that does not naturally occur in plums was included as it was considered as an internal standard. All compounds were prepared as a 100 ml stock solution in ethanol at a concentration of 500 mg/L. This was diluted down in water to a final concentration of 50 µg/L (50 ppb). Duplicate solutions were run under similar conditions as in Paper 2.

Results

The table below contains the peak areas detected by the two fibers:

Standards	**Retention Times**	**Peak area**	
		PDMS/CAR (75µm)	**PDMS (100µm)**
Hexanal	5.48	6243	0
1-Hexanol	8.05	3304	0
Benzaldehyde	11.20	25225	0
2-Octanol	12.74	20256	3396
Linalool	16.00	156227	4954
Nonanal	16.17	103093	3413
2-Phenylethanol	16.50	1533	0
γ-octalactone	20.73	4764	0
Ethyl nonanoate	21.82	75459	65899
Methyl cinnamate	22.05	8194	0
	24.11	26592	10722
Damascenone	24.15	1192	153
γ-decalactone	26.26	15619	11455

Conclusion

From the results it is clear that the PDMS/CAR fiber did not just detect more of the compounds, but was also more sensitive by producing larger peak areas. PDMS/CAR (75 µm) is recommended as the optimum fiber to use when analysing plum aroma volatiles.

A3: HS-SPME-GC-TOFMS repeatability

Background

Now that the HS-SPME fibre choice has been made the method as a whole should be tested in terms of repeatability. High repeatability in a method means that the instrumentation is stable and that conditions do not change much from one sample to another. In our case, one sample took up to one hour to do, thus if six samples are run consecutively the lag time between sample 1 and sample 6 will be six hours. This means that sample 6 has had six hours of "waiting time" that could increase sample variability. Thus, if high repeatability can be proved for our experiment the results will have a two-fold implication: the instrumental conditions are stable and do not contribute to sample variability and the lag time between samples does not change the sample in such a way that it produces different results.

Objective

Experimental repeatability will be tested to determine the variability produced by the instrumentation or method (i.e. lag time) between two identical samples.

Materials and procedures

A pool of plum ('Laetitia') puree was prepared by blending three de-pitted plums. The puree was treated with ascorbic acid (0.1%) and salt (1 g / 5ml) as indicated in Paper 2. 2-Octanol (50 ppb) was added as an internal standard. Five millilitres of the bulk was then aliquoted into six different HS-SPME glass bottles and HS-SPME-GC-TOFMS was performed as described in Paper 2. The chromatograms were obtained and an average relative peak area for 13 compounds was calculated relative to that of the internal standard. The standard deviation of the 6 samples was determined for each average peak area and then expressed as a percentage of the average peak area. The smaller the percentage, the better the repeatability. The average percentage was then calculated across the 13 compounds to indicate the overall repeatability.

Results

Aroma volatile	Average relative	STDEV	STDEV as % of
trans-Linalool oxide	0.1674	0.0131	7.81
cis-Linalool oxide	0.0552	0.0045	8.10
Phenol	0.0034	0.0001	2.62
Linalool	0.0051	0.0005	9.74
Menthol	0.0107	0.0006	5.23

Aroma volatile	Average relative	STDEV	STDEV as % of
α-Terpineol	0.0415	0.0024	5.74
2-Bornene	0.0304	0.0013	4.38
Unidentified 1	0.0207	0.0019	9.01
Unidentified 2	0.2127	0.0140	6.58
β-Damascenone	0.2006	0.0107	5.36
4-(2,4,4-Trimetyl-cyclohexa-1,5-	0.0701	0.0060	8.52
β-Ionone	0.0391	0.0026	6.61
Bisabalol oxide B	0.0169	0.0015	9.04
		Average	6.83 %

Conclusion:

The repeatability varied for the different compounds, but was on on average between 6 and 7% of the mean. Compared to other literature this is acceptable and means that the HS-SPME-GC-TOF method was stable across samples and that a lag time of 6 hours beteen identical samples did not influence the results.

B1: Effect of sample oxidation (browning) on aroma profile

Background

As mentioned in the literature review and Paper 2, the plum aroma literature warns that flesh browning as result of enzymatic oxidation of phenolic compounds into quinones can alter the aroma profile of the fruit. During sample preparation when plums are cut, destoned and milled and the flesh is exposed to oxygen containing air the risk of deterioration is high. As Crouzet et al. (1990) explained flesh browning can be avoided by using various methods such as using ascorbic acid as an oxygen trap, adding sulphur dioxide to combine with the phenolic substrates and methanol mediated deactivation of the responsible proteins.

Objective

The aim of this investigation was to test if flesh browning changes the aroma profile of the plums used under our sample preparation conditions.

Materials and methods

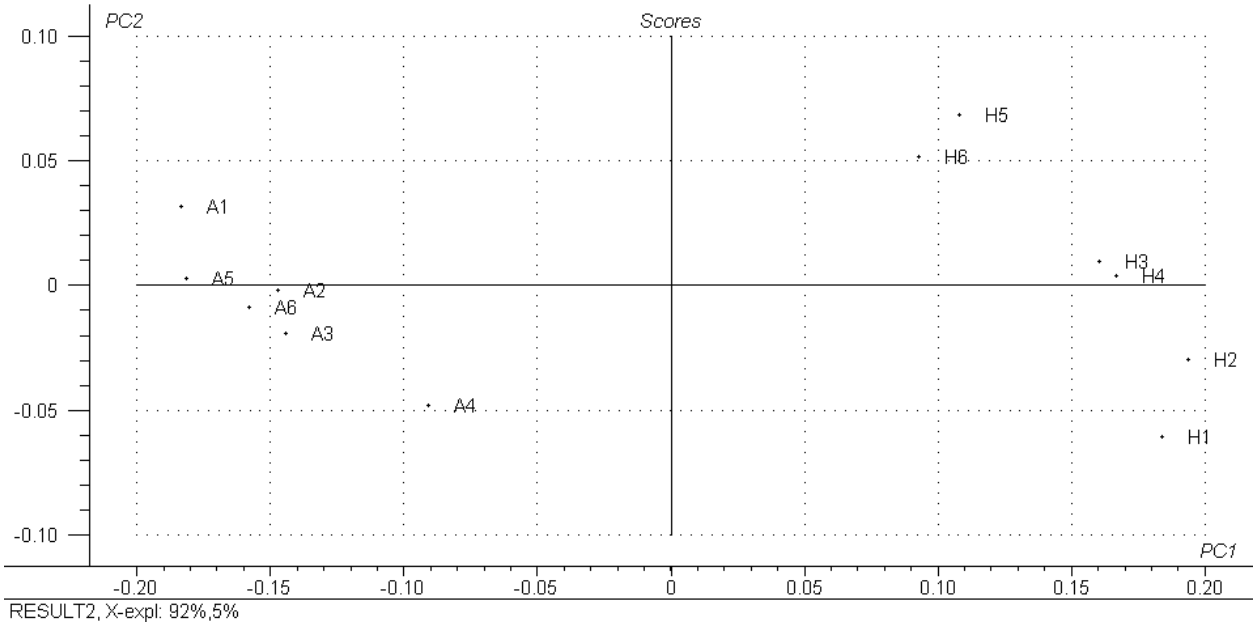
Imported plums were purchased at the local supermarket and a puree (>160 ml) was created by blending the depitted fruit pieces. Immediately after blending, 80 ml of the puree was decanted and treated with an ascorbic acid (0.1% final concentration) solution made up in water. The mixture was stirred thoroughly and 2-octanol (50 ppb final concentration) was added. Six aliquots of 5 ml were prepared in HS-SPME glass bottles and 1 g of NaCl was added to each bottle. The samples were exposed to HS-SPME-GC-TOFMS conditions as described in Paper 2. As a control, similar samples were prepared and analysed without the addition of the ascorbic acid. The chromatographs were obtained and the relative peak

heights of 14 components were determined for each sample. These results were used to run a PCA (Unscrambler version 4.0) to identify possible differences between the treated and untreated samples.

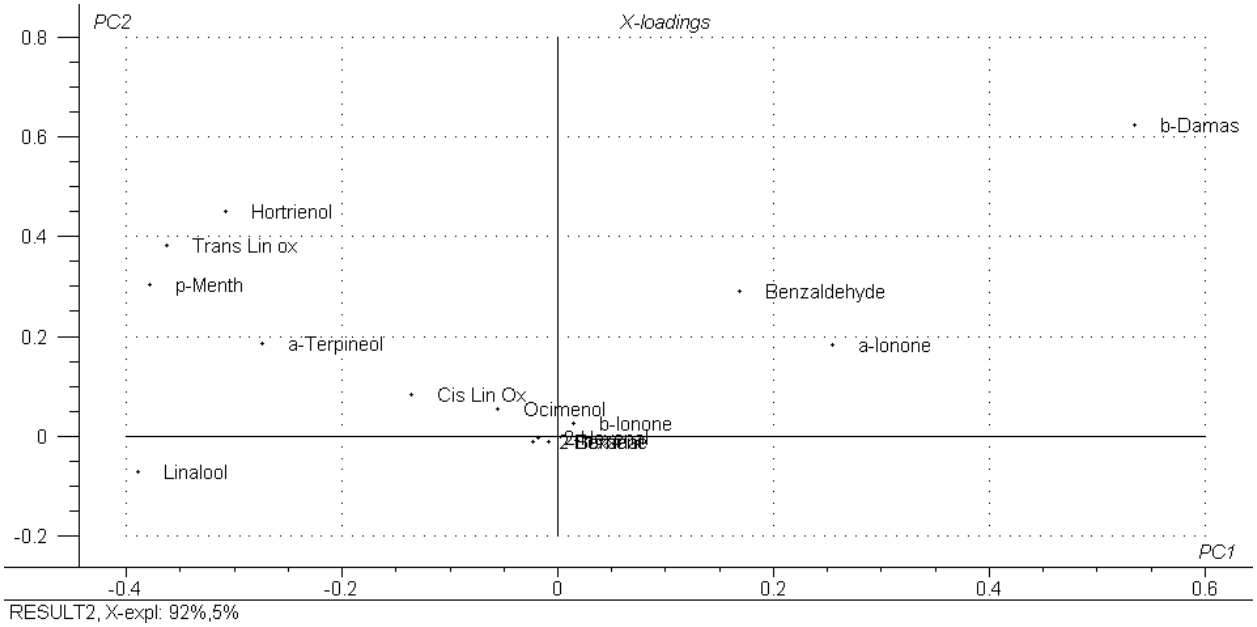
Results

The following figures represent the (a) scores and (b) loadings plots of the PCA. A1 to A6 represent the samples treated with ascorbic acid and sample H1 to H6 represent the untreated samples.

(a) Scores plot



(b) Loadings plot



Conclusion:

The PCA results show two that the samples (scores) treated with the ascorbic acid are clearly different from the untreated samples in the terms of aroma volatiles (loadings). This means that that if samples are not treated with ascorbic acid to prevent the oxidation and ultimate browning of the samples once they are exposed to oxygen during the sample preparation step, the aroma profiles change. If untreated the results will include the effects of oxidation and the aroma profile will not be representing that of the original plums. It is thus advised that all samples are treated with ascorbic acid immediately after blending.

B1: Effect of sample freezing on aroma profile

Background

To simplify the logistics of sample processing samples are often frozen at -80° after collection and then thawed and analysed at a later stage. In the case of plums the aroma literature warns against this procedure as it can alter the aroma profiles of the samples possibly due to cell structure disruption and consequent decompartmentation during freezing and thawing that leads to abnormal exposure of enzymes and substrates. See general introduction for references.

Objective

The objective of this investigation was to test if the aroma volatile profile of plum samples are altered once exposed to freezing (-80°C) and thawing prior to processing and analysis.

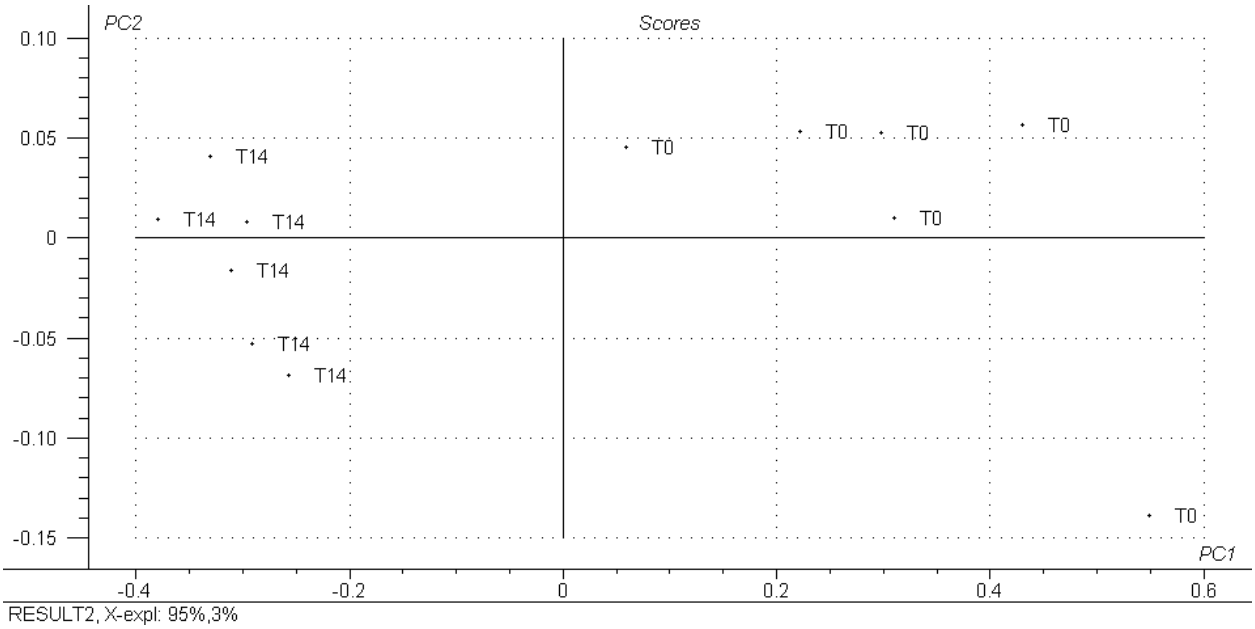
Materials and methods

Imported plums were purchased at the local supermarket and a puree (>160 ml) was created by blending the destoned fruit pieces. The puree was diluted (1:4) in water, stirred thoroughly and 2-octanol (50 ppb final concentration) was added. Half of the solution (80 ml) decanted into a PET bottle was left frozen at -80°C for 14 days. As a control the other half (80 ml) of the solution was not frozen, but rather used to prepare six aliquots of 5 ml in HS-SPME glass bottles after which 1 g of NaCl was added to each sample. The control (fresh) samples were immediately exposed to HS-SPME-GC-TOFMS conditions as described in Paper 2. Fourteen days later the frozen samples were thawed at room temperature and six aliquots were prepared and analysed similarly to the fresh samples. The chromatographs of all the samples were obtained and the relative peak heights of 14 components were determined for each sample. These results were used to run a PCA (Unscrambler version 4.0) to identify possible differences between the frozen and fresh samples.

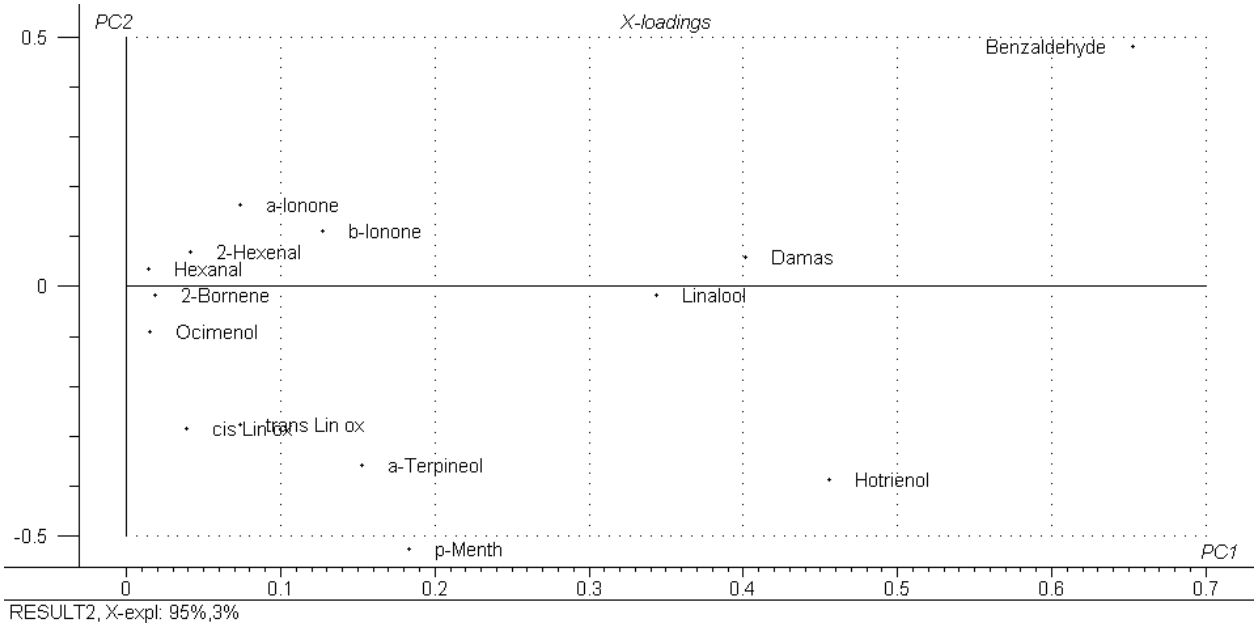
Results

The following figures represent the (a) scores and (b) loadings plots of the PCA. T0 represents the fresh (unfrozen) samples and T14 represents the frozen samples.

(a) Scores plot



(b) Loadings plot



Conclusion:

The PCA results shows two that the frozen samples (scores) are clearly different from the fresh samples in the terms of aroma volatiles (loadings). This means that that if samples frozen at -80℃ for 14 days and then thawed prior to analysis the aroma profiles changes. If frozen the results will include the effects of freezing and thawing and the aroma profile will not be representing that of the original plums. It is thus, advised that all samples are processed and analysed fresh.

REFERENCES:

Arthur, C.L., Pawliszyn, J., 1990. Solid phase microexreaction with termal desorption using fused silica optical fibers. Anal. Chem. 62, 2145-2148.

Crouzet, J., Etievant, P., Bayonove, C. 1990. Stoned fruit: apricot, plum, peach, cherry. In: Food Flavours Part C. The flavour of fruits. Morton, I.D., Macloud, A.J. (Eds). Elsievier Science Publishing Company INC, New York. pp 54-71.

Kataoka, H., Lord, H.L., Pawliszyn, J., 2000. Applications of solid-phase microextraction in food analysis. J. Chromatogr. A. 880, 35-62.

APPENDIX 2: PAPER 2

This appendix contains the results from the discriminant analysis (tables and figures) performed separately for the two plum harvest seasons (2008 and 2009). To simplify the presentation the results are divided into three sections (A, B and C) with two sub-sections each:

Section A: 'Pioneer'

A1: 'Pioneer' 2008 season

A2: 'Pioneer' 2009 season

Section B: 'Laetitia'

B1: 'Laetitia' 2008 season

B2: 'Laetitia' 2009 season

Section C: 'Angeleno'

C1: 'Angeleno' 2008 season

C2: 'Angeleno' 2009 season

SECTION A: 'PIONEER'

A1: 'Pioneer' 2008 season

Table 1:

(a) Variable selection table using a stepwise discriminant procedure

(See Table 2 of Paper 2 for explanation of compound abbreviations)

Variable IN/OUT	Status	Partial R^2	F statistic	Probability > F
Cym	IN	0.665	37.713	< 0.0001
bDam	IN	0.343	9.676	0.000
Bor	IN	0.221	5.095	0.011
Limox	IN	0.216	4.819	0.014
Lim	IN	0.265	6.124	0.005

(b) Discriminant Confusion matrix: All compounds

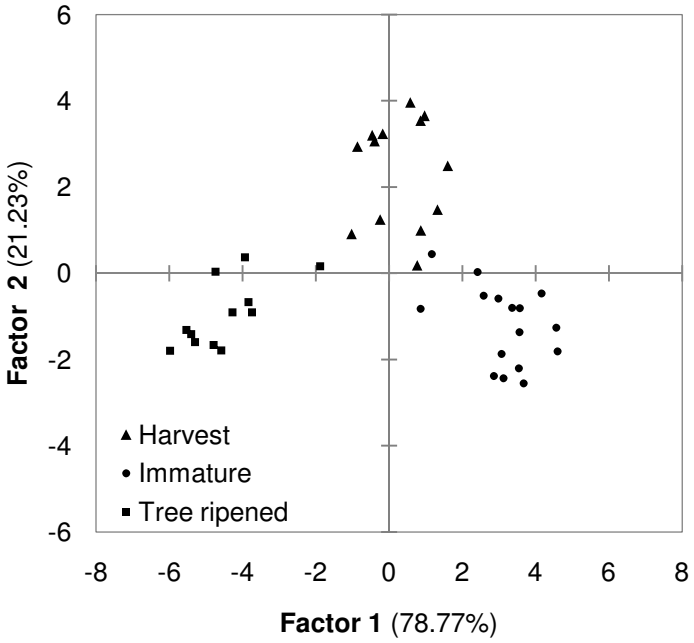
from \ to	Harvest	Immature	Tree ripened	Total	% correct
Harvest	13	0	0	13	100.00%
Immature	1	15	0	16	93.75%
Tree ripened	0	0	12	12	100.00%
Total	14	15	12	41	97.56%

(c) Discriminant Confusion matrix: 'Stepwise' compounds only

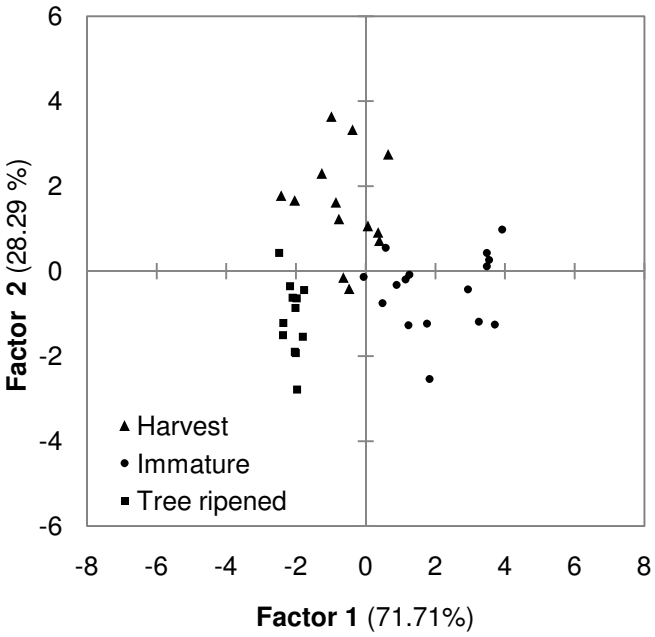
from \ to	Harvest	Immature	Tree ripened	Total	% correct
Harvest	12	0	1	13	92.31%
Immature	2	14	0	16	87.50%
Tree ripened	0	0	12	12	100.00%
Total	14	14	13	41	92.68%

Figure 1:

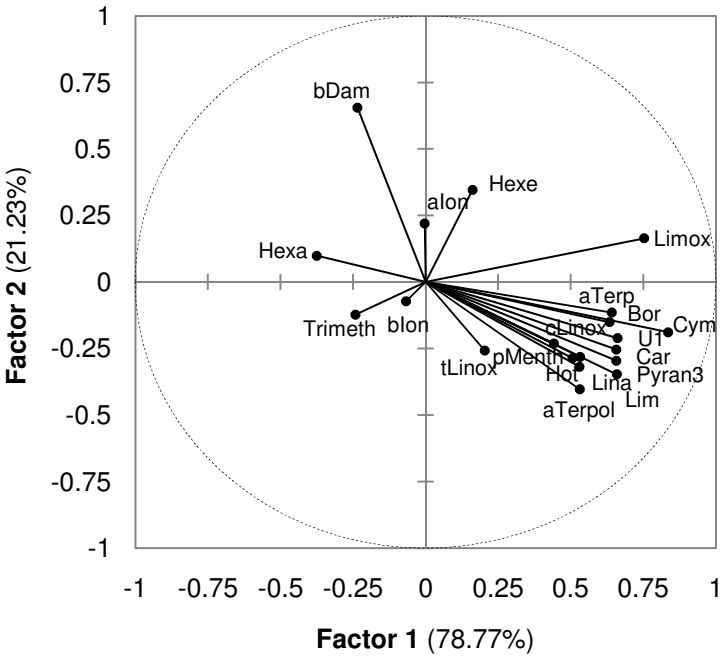
(a) Observations chart using all compounds



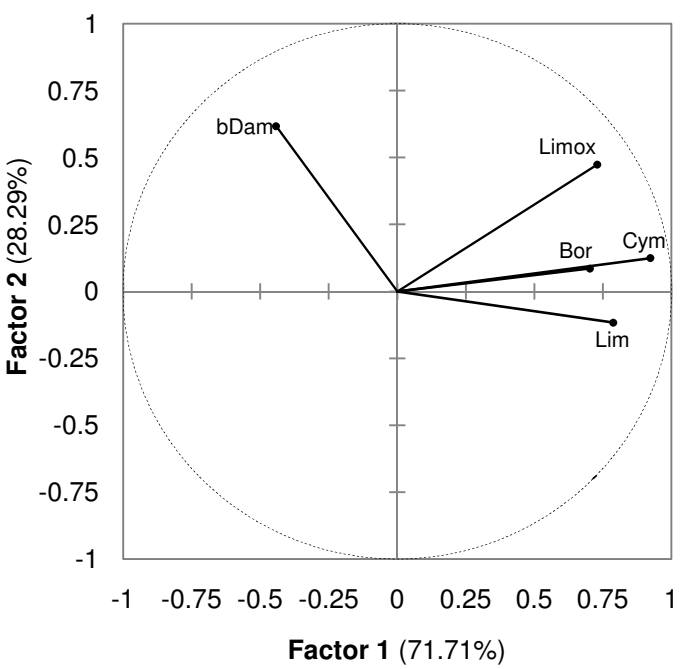
(c) Observations chart using 'stepwise' compounds only



(b) Variables chart using all compounds



(d) Variables chart using 'stepwise' compounds only



A2: 'Pioneer' 2009 season

Table 1:

(a) Variable selection table using a stepwise discriminant procedure

(See Table 2 of Paper 2 for explanation of compound abbreviations)

Variable IN/OUT	Status	Partial R^2	F statistic	Probability > F
Hot	IN	0.562	23.770	< 0.0001
Hexa	IN	0.305	7.897	0.001
blon	IN	0.236	5.396	0.009

(b) Discriminant Confusion matrix: All compounds

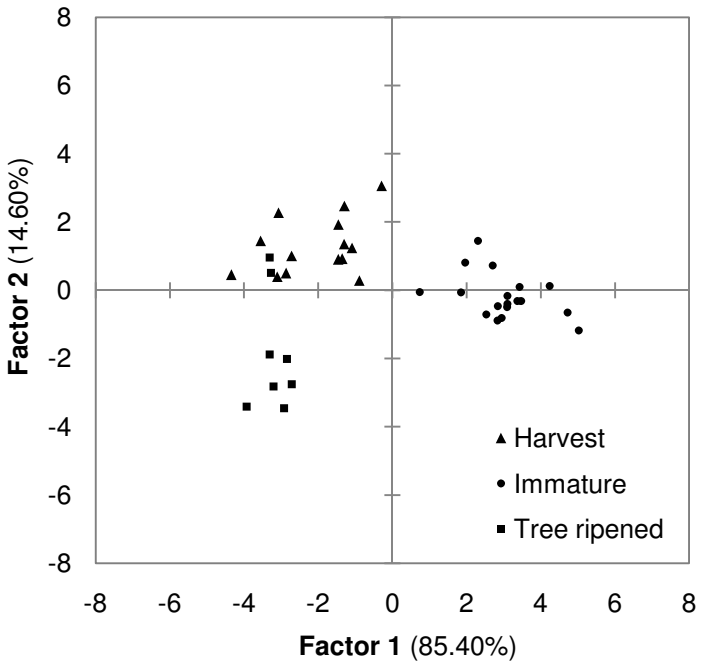
from \ to	Harvest	Immature	Tree ripened	Total	% correct
Harvest	14	0	0	14	100.00%
Immature	0	18	0	18	100.00%
Tree ripened	2	0	6	8	75.00%
Total	16	18	6	40	95.00%

(c) Discriminant Confusion matrix: 'Stepwise' compounds only

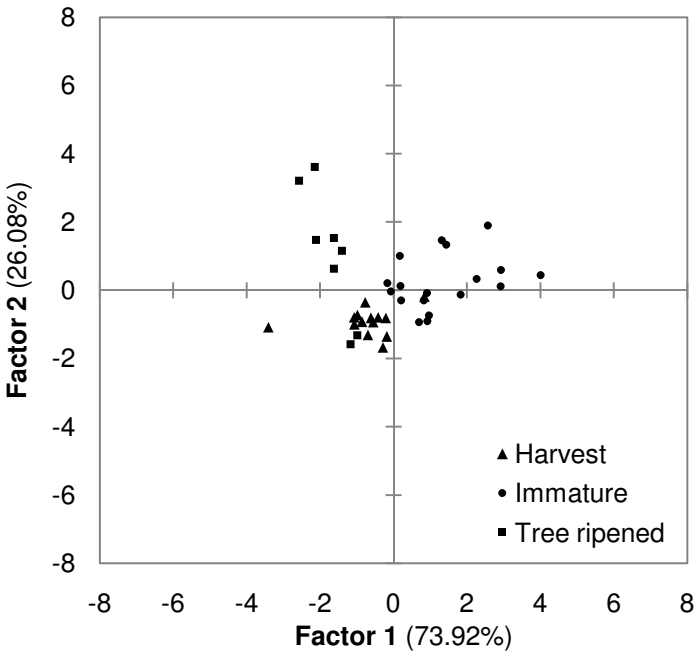
from \ to	Harvest	Immature	Tree ripened	Total	% correct
Harvest	13	1	0	14	92.86%
Immature	2	16	0	18	88.89%
Tree ripened	2	0	6	8	75.00%
Total	17	17	6	40	87.50%

Figure 1:

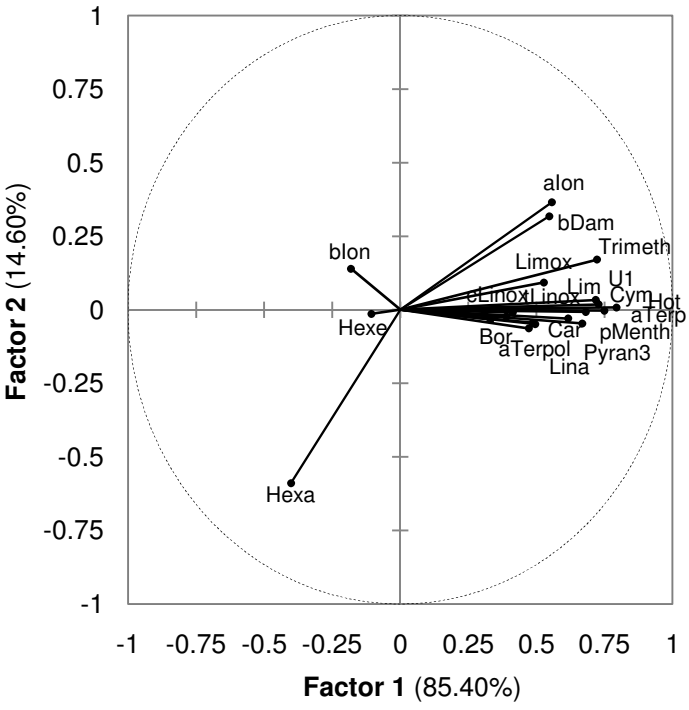
(a) Observations chart using all compounds



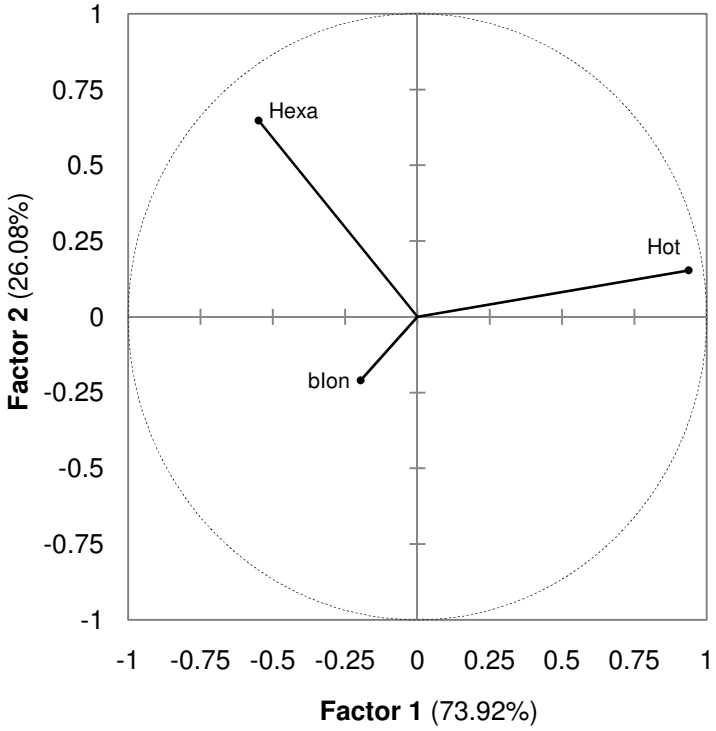
(c) Observations chart using 'stepwise' pounds only



(b) Variables chart using all compounds



(d) Variables chart using all 'stepwise' compounds only



SECTION B: 'LAETITIA'

B1: 'Laetitia' 2008 season

Table 1:

(a) Variable selection table using a stepwise discriminant procedure

(See Table 2 of Paper 2 for explanation of compound abbreviations)

Variable IN/OUT	Status	Partial R^2	F statistic	Probability >F
Hex	IN	0.475	19.430	< 0.0001
bDam	IN	0.290	8.570	0.001
Bisa	IN	0.363	11.675	< 0.0001
U4	IN	0.484	18.738	< 0.0001
Hex	OUT	0.043	0.901	0.414
bIon	IN	0.283	7.879	0.001
Phe	IN	0.226	5.679	0.007
Hot	IN	0.163	3.688	0.034
cLinox	IN	0.158	3.482	0.041

(b) Discriminant Confusion matrix: All compounds

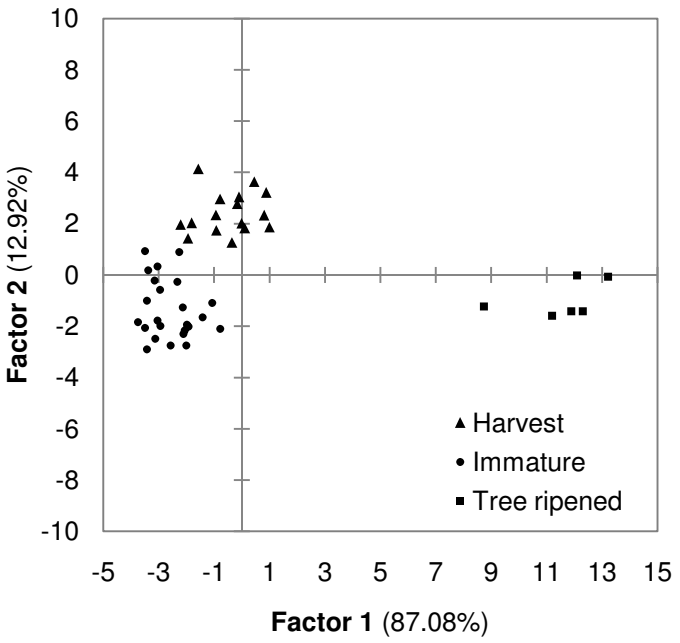
from \ to	Harvest	Immature	Tree ripened	Total	% correct
Harvest	16	0	0	16	100.00%
Immature	0	24	0	24	100.00%
Tree ripened	0	0	6	6	100.00%
Total	16	24	6	46	100.00%

(c) Discriminant Confusion matrix: 'Stepwise' compounds only

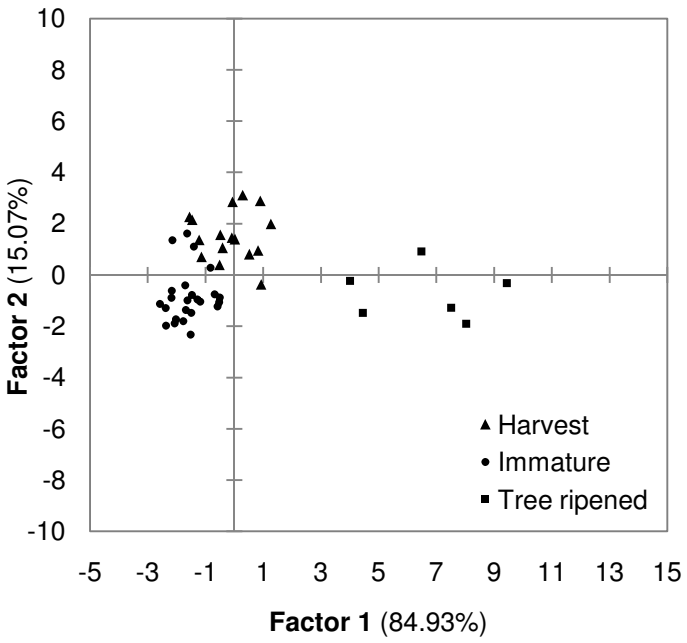
from \ to	Harvest	Immature	Tree ripened	Total	% correct
Harvest	16	0	0	16	100.00%
Immature	3	21	0	24	87.50%
Tree ripened	0	0	6	6	100.00%
Total	19	21	6	46	93.48%

Figure 1:

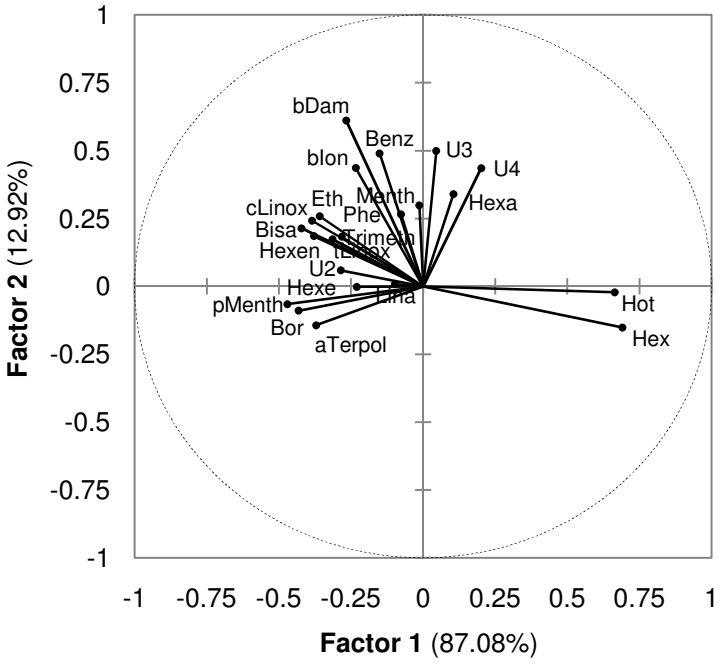
(a) Observations chart using all compounds



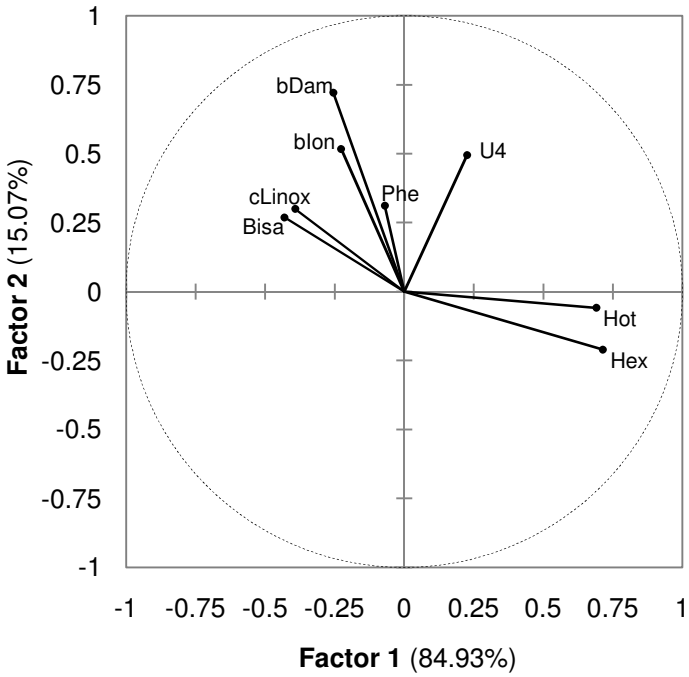
(c) Observations chart using 'stepwise' compounds only



(b) Variables chart using all compounds



(d) Variables chart using 'stepwise' compounds only



B2: ‘Laetitia’ 2009 season

Table 1:

(a) Variable selection table using a stepwise discriminant procedure

(See Table 2 of Paper 2 for explanation of compound abbreviations)

Variable IN/OUT	Status	Partial R^2	F statistic	Probability > F
Hex	IN	0.857	95.623	< 0.0001
cLinox	IN	0.406	10.604	0.0001
bDam	IN	0.424	11.036	0.0001
U3	IN	0.383	9.004	0.001
Trimeth	IN	0.246	4.579	0.019
Bor	IN	0.206	3.496	0.045

(b) Discriminant Confusion matrix: All compounds

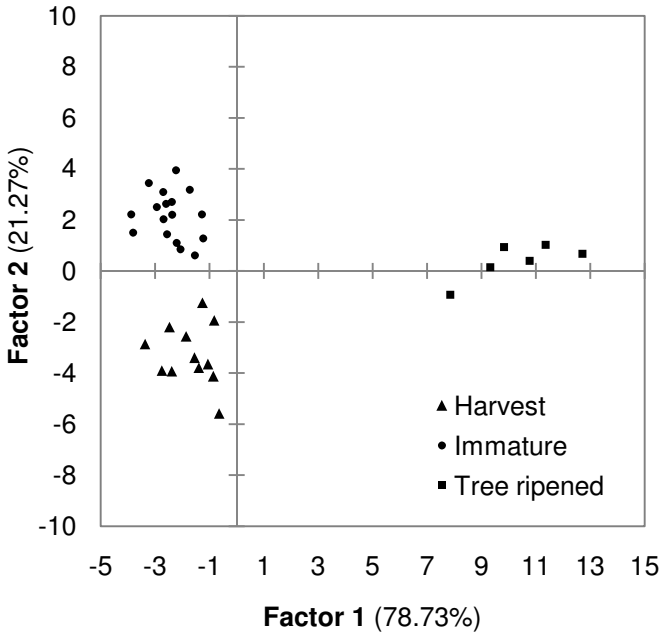
from \ to	Harvest	Immature	Tree ripened	Total	% correct
Harvest	12	0	0	12	100.00%
Immature	0	17	0	17	100.00%
Tree ripened	0	0	6	6	100.00%
Total	12	17	6	35	100.00%

(c) Discriminant Confusion matrix: ‘Stepwise’ compounds only

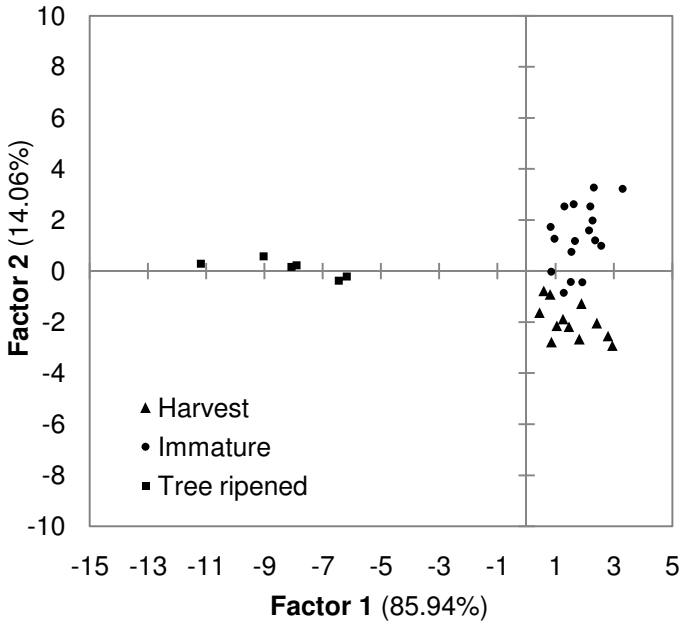
from \ to	Harvest	Immature	Tree ripened	Total	% correct
Harvest	12	0	0	12	100.00%
Immature	3	14	0	17	82.35%
Tree ripened	0	0	6	6	100.00%
Total	15	14	6	35	91.43%

Figure 1:

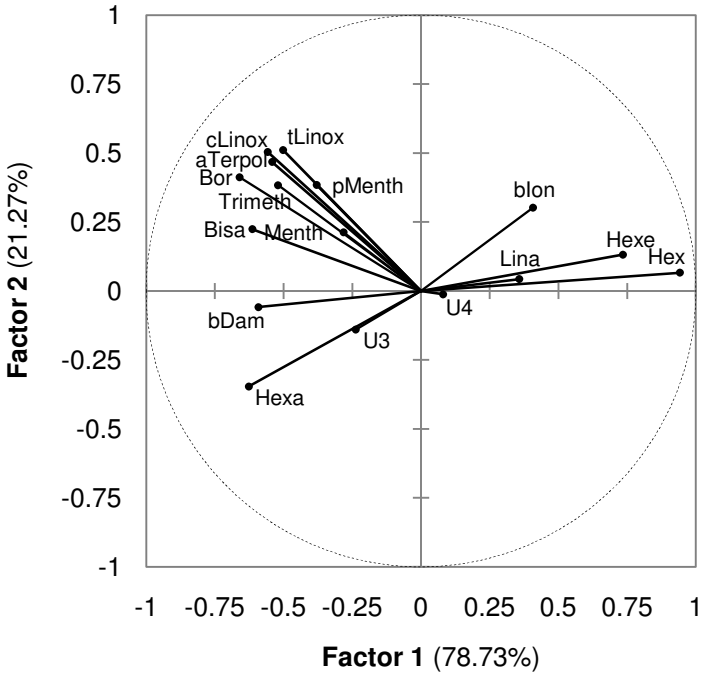
(a) Observations chart using all compounds



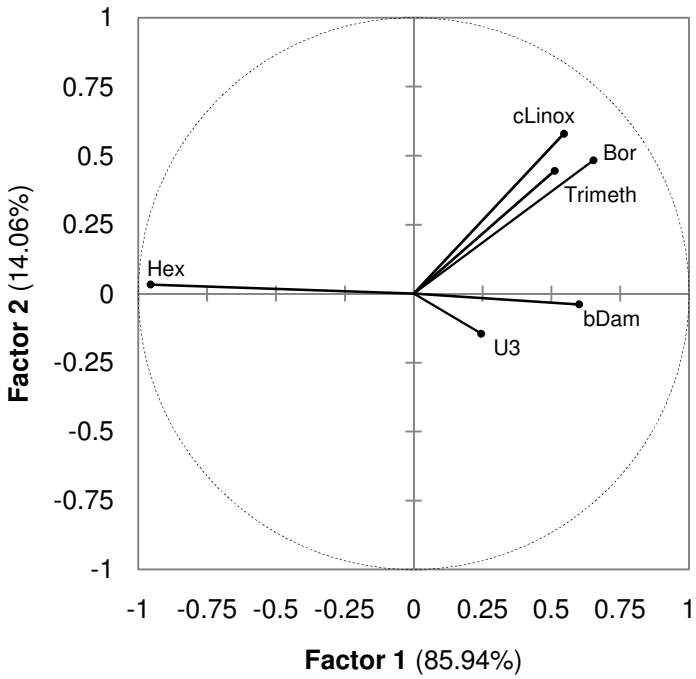
(c) Observations chart using 'stepwise' compounds only



(b) Variables chart using all compounds



(d) Variables chart using all 'stepwise' compounds only



SECTION C: 'ANGELENO'

C1: 'Angeleno' 2008 season

Table 1:

(a) Variable selection table using a stepwise discriminant procedure

(See Table 2 of Paper 2 for explanation of compound abbreviations)

Variable IN/OUT	Status	Partial R^2	F statistic	Probability > F
Hex	IN	0.892	157.206	< 0.0001
aTerpol	IN	0.312	8.374	0.001
Lina	IN	0.195	4.365	0.020
U5	IN	0.187	4.027	0.027

(b) Discriminant Confusion matrix: All compounds

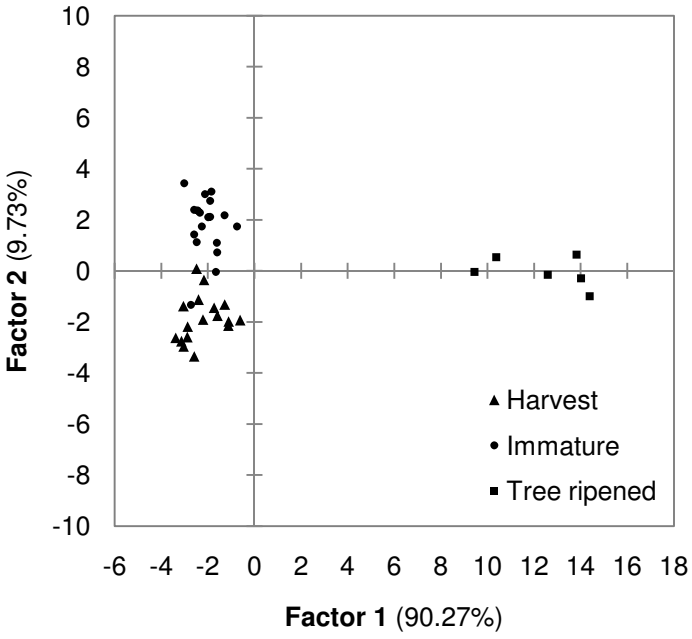
from \ to	Harvest	Immature	Tree ripened	Total	% correct
Harvest	16	1	0	17	94.12%
Immature	1	17	0	18	94.44%
Tree ripened	0	0	6	6	100.00%
Total	17	18	6	41	95.12%

(c) Discriminant Confusion matrix: 'Stepwise' compounds only

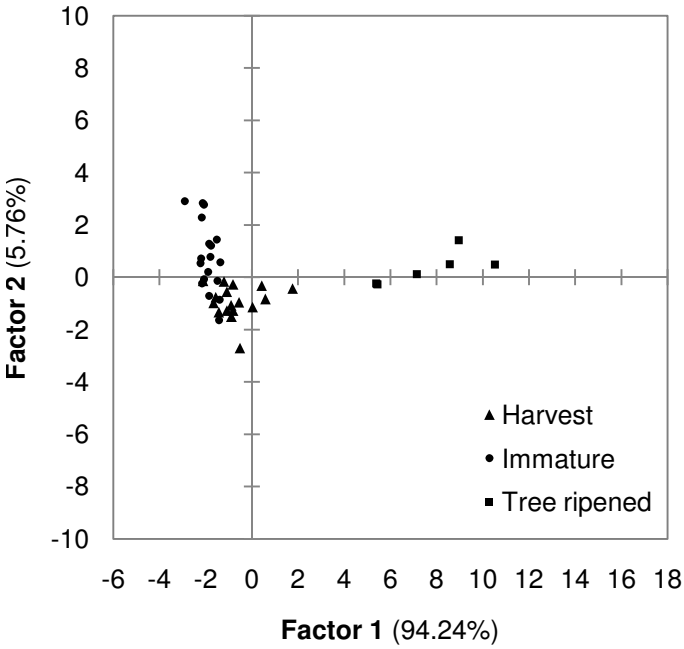
from \ to	Harvest	Immature	Tree ripened	Total	% correct
Harvest	16	1	0	17	94.12%
Immature	3	15	0	18	83.33%
Tree ripened	0	0	6	6	100.00%
Total	19	16	6	41	90.24%

Figure 1:

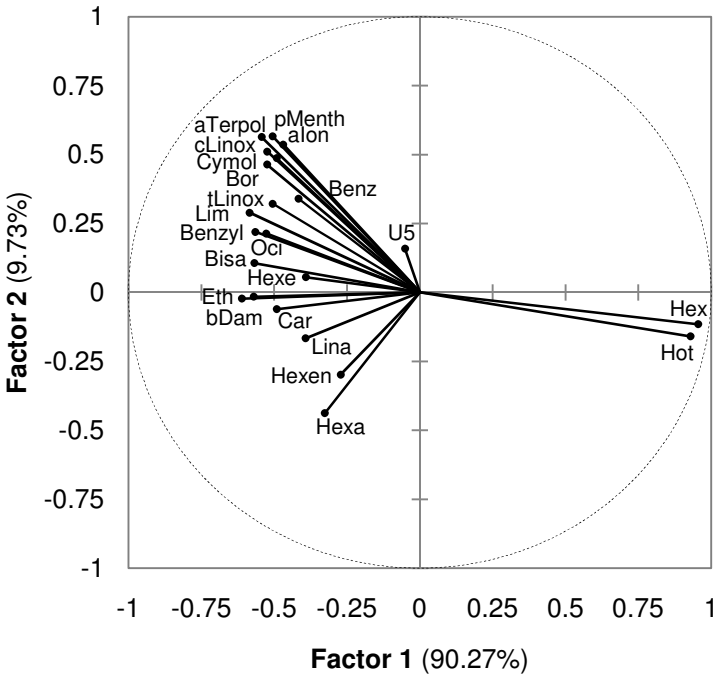
(a) Observations chart using all compounds



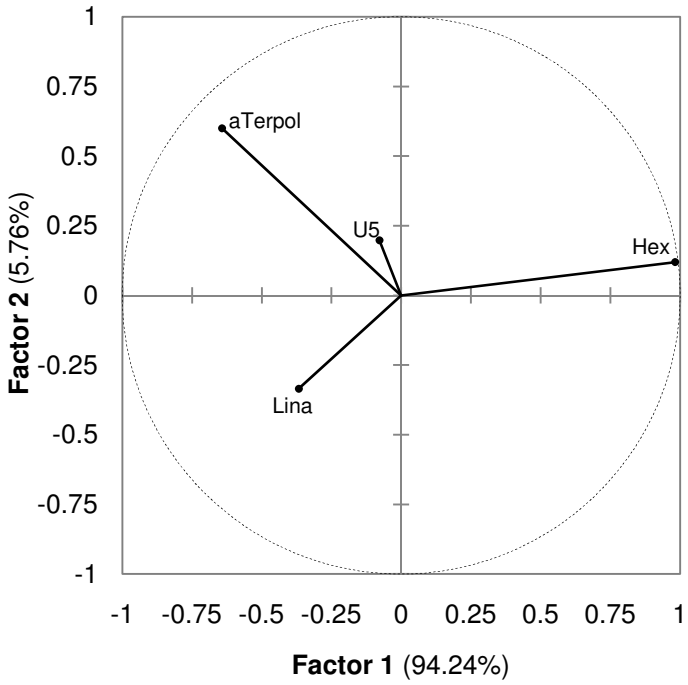
(c) Observations chart using 'stepwise' compounds only



(b) Variables chart using all compounds



(d) Variables chart using 'stepwise' compounds only



C2: ‘Angeleno’ 2009 season

Table 1:

(a) Variable selection table using a stepwise discriminant procedure

(See Table 2 of Paper 2 for explanation of compound abbreviations)

Variable IN/OUT	Status	Partial R^2	F statistic	Probability > F
Bisa	IN	0.563	21.900	< 0.0001
Hex	IN	0.374	9.866	0.000
Hexe	IN	0.243	5.129	0.012
Pyran2	IN	0.245	5.031	0.013
Lim	IN	0.214	4.091	0.027

(b) Discriminant Confusion matrix: All compounds

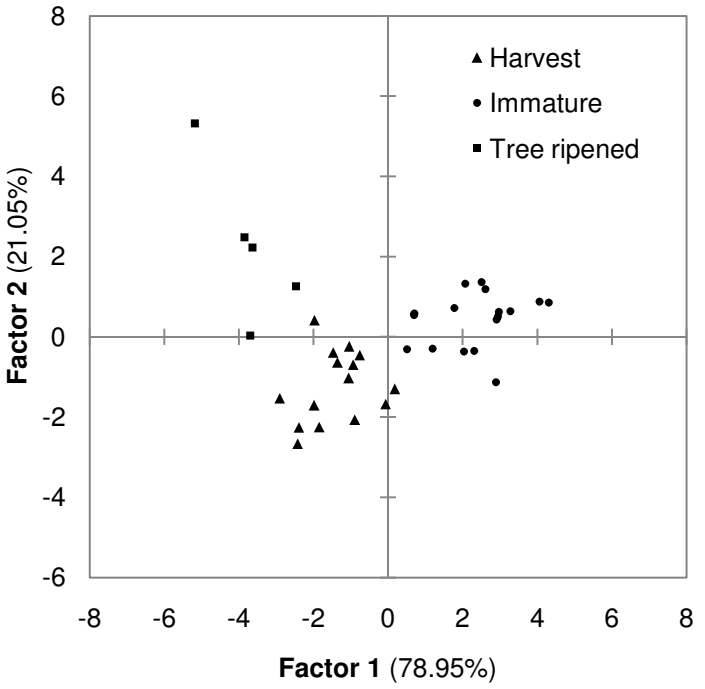
from \ to	Harvest	Immature	Tree ripened	Total	% correct
Harvest	15	0	0	15	100.00%
Immature	0	17	0	17	100.00%
Tree ripened	1	0	4	5	80.00%
Total	16	17	4	37	97.30%

(c) Discriminant Confusion matrix: ‘Stepwise’ compounds only

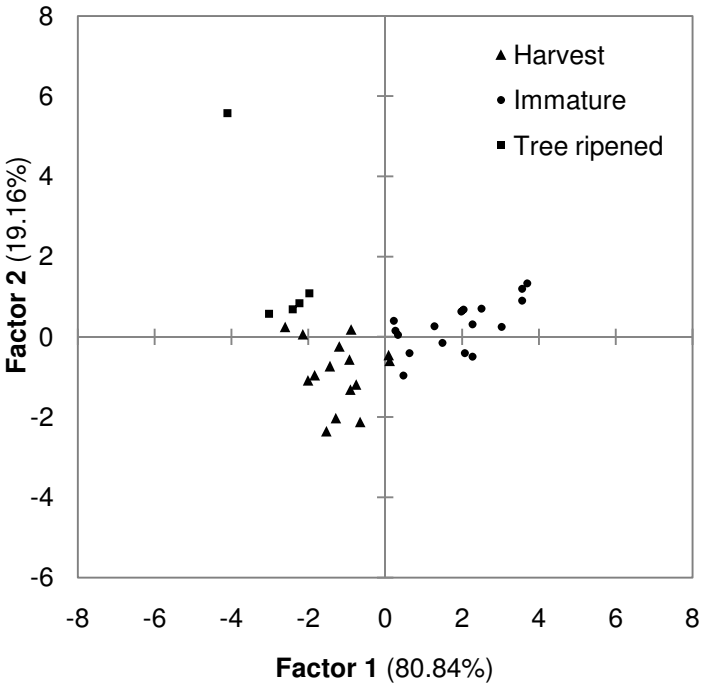
from \ to	Harvest	Immature	Tree ripened	Total	% correct
Harvest	15	0	0	15	100.00%
Immature	1	16	0	17	94.12%
Tree ripened	0	0	5	5	100.00%
Total	16	16	5	37	97.30%

Figure 1:

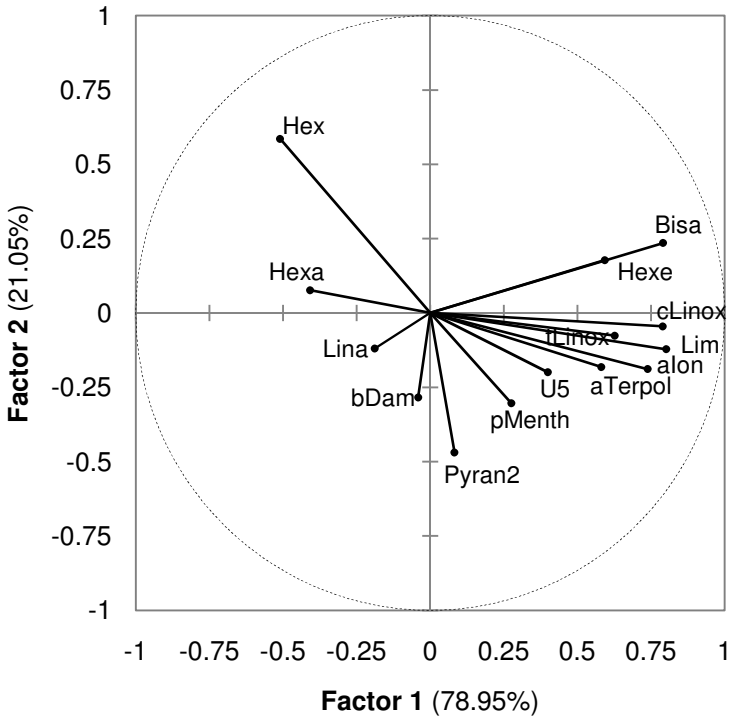
(a) Observations chart using all compounds



(c) Observations chart using 'stepwise' compounds only



(b) Variables chart using all compounds



(d) Variables chart using all 'stepwise' compounds only