INVESTIGATION OF ENVIRONMENTAL STAINING AND STORAGE ON DISCOLOURATION AND COOKING QUALITY IN FABA BEAN (*VICIA FABA* L.)

Syed Muhammad Nasar-Abbas

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Abstract

Faba bean (*Vicia Faba* L.) ranks third worldwide in overall production among cool-season legume crops and is used as a main source of protein both for food and feed purposes in many parts of the world. Australia is a major exporter of faba beans and the price received depends on the quality of the seed especially colour of the seed coat. Consumers don't like faba beans that are dark coloured or that have blemishes on the seed coat. Environmental staining and storage discolouration deteriorate seed colour causing substantial economic losses to growers and grain handlers.

To investigate the influence of environmental conditions, especially during pod/seed development and maturity, on the degree of environmental staining, field trials were conducted using four faba bean varieties (Fiord, Fiesta, Ascot and Cairo) in a range of environmental conditions under the Mediterranean-type climate of south western Australian grain belt. Although a majority of seeds had good colour but 3-25% were stained up to an unacceptable level across the trials and this varied with location and variety. Seeds formed later in plant development (located on the upper nodes of the plant) were more stained than seeds formed earlier (located on the lower nodes). This may be due to end of season environmental factors, such as high temperature and light intensity, and water and nutrient stress. Similarly seeds formed on small and weak plants, which may have developed under stressful conditions, had more staining than seeds formed on normal sized and healthy plants. Genotypic variation was also evident with Fiord showing greater staining than Ascot, Fiesta and Cairo. The cause of environmental staining appears to be complex but was associated with phenolic contents.

Storage discolouration was influenced by a number of factors including temperature, seed moisture content, light and storage period and these were critical in determining storage life. Changes in colour difference index (ΔE^*_{ab} values) calculated from L^*, a^* and b^* colour coordinates determined by a chroma meter, demonstrated that the higher the temperature and seed moisture content the faster the rate of change in colour. Seeds with ≤ 12% seed moisture content and stored at ≤ 25°C retained colour in an acceptable range for one year. Exposure to light (350 μ mol m^{-2} s^{-1}) caused testa darkening in one month that equaled testa darkening after 12 months storage in the dark at the same temperature (20 ± 2°C). Cotyledon colour also turned dark in samples stored at ≥ 37°C.

Losses in total free phenolics, total tannins and condensed tannins (proanthocyanidins) occurred with increased darkness of testa and cotyledons during storage.

Modified atmosphere packaging techniques were applied to study the effect of different gases and to manage seed coat colour darkening during storage. This included flushing with carbon dioxide, nitrogen, oxygen or ethylene, and vacuum packaging followed by storage at 30°C in the dark for one year. Changes in ΔE^*_{ab} values demonstrated that relative to control (flushing with air), nitrogen reduced colour darkening, whereas storage in oxygen accelerated colour darkening. Seeds flushed with oxygen also had higher losses of phenolic compounds demonstrating that colour darkening was likely to be due to oxidative transformation of phenolic contents.

Adverse storage conditions not only affected colour but also reduced cooking quality of faba bean. Seeds stored at ≤ 25°C demonstrated minor changes whereas seeds stored at ≥ 37°C had substantial changes in most of the physical and chemical characteristics examined including hydration and swelling coefficients, acid detergent fibre, lignin and tannin contents. Solutes and electrolytes leaching after 18 h soaking substantially increased with increased storage temperature. Faba bean hardness, examined by the hard-to-cook test, also increased with increased storage temperature. There was a high negative correlation ($r^2 = 0.98$) between storage temperature and cooking ability of faba bean. There was a three-fold increase in lignin content of faba bean stored at 50°C compared to those stored at 5°C and it was correlated with bean hardness ($r^2 = 0.98$). Reduction in free phenolics was negatively correlated ($r^2 = 0.75$) with bean hardness.

The environmental staining in faba bean can be minimized with correct choice of varieties, robust agronomic practices to establish and maintain healthy plants and the use of mechanical graders and colour sorters. For minimizing storage discolouration faba beans must be dehydrated to ≤12% seed moisture content and stored in insulated bins (silos) or at least bins painted white and constructed under trees shades. In addition occasional flushing with N_2 will further help reduce the colour darkening. The above approaches will improve quality, market opportunities, price and hence profitability of faba bean in the farming systems.

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I dedicate this thesis to the loving memories of my beloved parents and parents-in-law.

Thesis Structure

This thesis is composed of a General Introduction, Literature Review, four research chapters and a General Discussion. The research chapters have each been submitted for publication in refereed scientific journals. In addition the research was presented/ published at international and national conferences during the course of study and these are listed below

Publications arising from this thesis

Journals

1. Nasar-Abbas SM, Plummer JA, Siddique KHM, White P, D'Antuono M and Harris D (2007). Effect of site, harvesting stage and genotype on environmental staining in faba bean (*Vicia faba* L.). Submitted to *Australian Journal of Agriculture Research* (manuscript accepted with revision).

2. Nasar-Abbas SM, Plummer JA, Siddique KHM, White P, Harris D, Dods K and D'Antuono M (2007). Faba bean seeds darken rapidly and phenolic content falls when seeds are stored at higher temperature, moisture and light intensity. Submitted to *LWT- Food Science and Technology* (manuscript under review).

3. Nasar-Abbas SM, Plummer JA, Siddique KHM, White P, Harris D and Dods K (2007). Nitrogen retards and oxygen accelerates colour darkening in faba bean (V*icia faba* L.) during storage. Submitted to *Postharvest Biology and Technology* (in press, available online).

4. Nasar-Abbas SM, Plummer JA, Siddique KHM, White P, Harris D and Dods K (2007). Cooking quality of faba bean after storage at high temperature and the role of lignins and other phenolics in bean hardening. *LWT-Food Science and Technology* (in press, available online).

Conferences and workshops

1. Nasar-Abbas SM, Plummer JA, Siddique KHM, White P, Harris D, Dods K and D'Antuono M (2006). Environmental staining and storage discolouration in faba bean (*Vicia faba* L.). *Proceedings of International Workshop on Faba bean Breeding and Agronomy (25-27 October), Cordoba, Spain*, pp 27-28.

2. Nasar-Abbas SM, Plummer JA, Siddique KHM, White P, Harris D, Dods K and D'Antuono M (2006). Effect of harvesting stage, location and genotype on environmental staining in faba bean (*Vicia faba* L.). *Proceedings of 13th Australian Society of Agronomy Conference (10-14 September), Perth, Australia,* p112.

3. Nasar-Abbas SM, Plummer JA, Siddique KHM, White P, Harris D and Dods K (2004). Investigation of environmental staining and storage discolouration in faba bean (*Vicia faba* L.). *Abstracts ComBio 2004 combined conference (26-30 September), Perth, Australia,* p. 129.

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6. Nasar-Abbas SM, Plummer JA, Siddique KHM, White P and Harris, D (2004). Investigation of environmental staining and storage discolouration in faba bean (*Vicia faba* L.). *Pulse Genetic Enhancement Group Annual Workshop (April 1). Centre for Legumes in Mediterranean Agriculture, University of Western Australia, Perth.*

7. Nasar-Abbas SM, Plummer JA, Siddique KHM, White P and Harris, D (2004). Factors Affecting Storage Discolouration in Faba Bean (*Vicia faba* L.). *Faba*

bean Breeders Meeting 2005. Department of Agriculture and Food Western Australia.

8. Nasar-Abbas SM, Plummer JA, Siddique KHM, White P and Harris, D (2006). Factors affecting seed coat colour of faba bean (*Vicia faba* L.) during storage. *Proceedings of Rottnest Postgraduate Summer School (February 12-14), School of Plant Biology, University of Western Australia, Perth.*

9. Nasar-Abbas SM, Plummer JA, Siddique KHM, White P and Harris, D (2005). Factors affecting storage discolouration in faba bean (*Vicia faba* L.). *CLIMA Seminar Series (May 13)*, *Centre for Legumes in Mediterranean Agriculture, University of Western Australia, Perth*.

10. Nasar-Abbas SM, Plummer JA, Siddique KHM, White P and Harris, D (2004). Investigation of environmental staining and storage discolouration in faba bean (*Vicia faba* L.), *Proceedings of Rottnest Postgraduate Summer School (February 21-23), School of Plant Biology, University of Western Australia, Perth.*

Reports

1. Nasar-Abbas SM, Plummer JA, Siddique KHM, White P and Harris D (2006). Physicochemical changes in faba bean (*Vicia faba* L.) after long term storage at high temperature, *Biennial Research Report 2005-2006*, Centre for Legumes in Mediterranean Agriculture (submitted).

2. Nasar-Abbas SM, Plummer JA, Siddique KHM, White P, Harris D and Dods K (2006). Factors affecting seed coat colour of faba bean during storage, *Crop Updates 2006 - Pulses*. Department of Agriculture, Western Australia pp. 128-131.

3. Nasar-Abbas SM, Plummer JA, Siddique KHM, White P and Harris D (2004). Investigation of Environmental Staining and Storage Discolouration in Faba bean (*Vicia faba* L.). *Biennial Research Report 2003-2004*, Centre for Legumes in Mediterranean Agriculture, p. 71.

4. Nasar-Abbas SM, Plummer JA, Siddique KHM, White P and Harris D (2004). Environmental Staining and Storage Discolouration in Faba bean, *Crop Updates 2004 - Pulses*. Department of Agriculture, Western Australia, p.16.

5. Nasar-Abbas SM, Plummer JA, Siddique KHM, White P and Harris, D (2003). Environmental Staining and Storage Discolouration in Faba bean, *Crop Updates 2003 - Pulses*. Department of Agriculture, Western Australia, pp.15-17.

Media Releases

1. "Beans colour is in the bag", The Weekly Times, 23 Nov, 2005, page 95
2. "Curing coloured beans", Australian Grain, November – December 2005, page 24
3. "Research shed light on dark beans", Farm Weekly, Dec 1, 2005, page 12
4. "Storage colours faba bean fading", Countryman, Dec1 2005, page 15

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CHAPTER ONE

General Introduction

General Introduction

Faba bean (*Vicia faba* L.) is an important grain legume around the world and especially in Australia, which is a major exporter. Colour of seed testa is one of the important criteria in marketing of faba bean for human consumption. Faba bean has seed discolouration that leads to poor quality and reduced market price. Seed can be stained in the field before harvesting or discolour with storage. Both types of discoloration cause substantial economic losses to the grower and grain traders. Discoloured seeds are not acceptable in markets for human consumption and go to animal feed at a discounted price. Preharvest staining can be caused by certain pests and diseases but this accounts for a minor proportion of the problem compared to another type of staining commonly known as environmental staining. The cause of environmental staining is unknown, but environmental conditions especially during pod and seed formation, and at maturity are thought to have a large effect on the degree of staining. A better understanding of environmental factors that lead to the discolouration phenomenon is required to control/minimise the problem.

Faba bean colour darkening during storage at ambient temperature, especially under Australian conditions, is another issue that reduces its market value. Seed coat colour which is buff/beige at harvest (in most of the varieties), changes to dark brown or chocolate brown depending on the storage conditions. Darkened seeds are not sought after in the human consumption market and downgraded to feed market at low price. There has been no comprehensive analysis of the impact of different storage factors such as temperature, seed moisture content and light that could be used to find and standardise optimum storage conditions to minimise the problem. In addition, active packaging techniques using different gases and proper packaging materials can be useful in providing viable solution to further control or reduce the colour darkening during storage.

Besides colour, storage under unfavourable conditions can also affect other quality parameters of faba beans, especially cooking quality. A detailed study of the physicochemical changes affected by storage parameters that can lead to the hard-to-cook defect in faba bean would be useful in developing strategies to maintain quality during storage.

The main aims of the studies presented in this thesis were:

1. To investigate environmental factors associated with preharvest seed coat staining in faba bean (Chapter 3).

2. To study the factors affecting seed coat colour changes in faba bean during storage so that optimum storage conditions can be developed to minimise the problem (Chapter 4).

3. To assess technique/s and practice/s that can be helpful in minimising discoloration in faba bean Chapter 5).

4. To quantify physicochemical changes under different storage conditions that lead to hard-to-cook defect in faba bean during storage (Chapter 6).

CHAPTER TWO

Literature Review

Literature Review

2.1. Faba bean

Vicia faba L. is most commonly known as faba bean and this term will be used in this thesis. It is also known as horse bean, Windsor bean, English bean, tick bean, fava bean, field bean and pigeon bean. Faba beans are sometimes classified into subspecies according to varieties and their uses in various countries. Thus, small-seeded types (*V. faba* var. minor or var. equina) are called field beans in the United Kingdom and Europe or horse beans in Europe and Asia where they are mainly used as animal feed. The large seeded types (*V. faba* var. major) are widely known as broad beans, Windsor or straight beans and are eaten fresh as a vegetable and for dry seeds.

Faba beans have been found in some of the earliest known human settlements and are referred to in legends and lore throughout recorded history (Anonymous 2006b). Faba bean is one of the earliest domesticated food legumes in the world. Although its origin is still unclear (Ladizinsky *et al.* 1988) it is widely believed to have originated in the Mediterranean-West Asia region probably in the late Neolithic period (Bond 1995; Cubero 1974). Faba beans have been found in old Egyptians tombs (Stephens 2003). In fact they were "*the beans*", in rural cyclopedias prior to the 20th century: *bean* was the species known by botanists first of all as *Faba bona* and later as *Vicia faba* (Cubero 2006). Ancient Greeks and Romans used beans for voting in their assemblies; a white bean being used to cast a yes vote, and a black bean for no (Anonymous 2006a). Romans thought that the souls of the deceased were hiding in faba bean seeds and an important festival was the *Fabaria*, with offerings of cakes made from faba bean flour (Cubero 2006).

Faba bean (*V. faba*) ranks third worldwide in overall production among cool-season legume crops, following field pea and chickpea and it is used as a main source of protein both for food and feed purposes in many parts of the world (FAO 2003). Faba bean is a crop with increasing importance in Australia. It is attractive to farmers because of its potential to produce high grain yields as compared to other grain legumes in a range of dryland environments (Loss and Siddique 1997; Siddique *et al.* 1999; Siddique *et al.* 1993; Thomson *et al.* 1997), for the rotation benefits derived from breaking disease cycles of cereals (Felton *et al.* 1998) and for adding significant amounts of fixed

nitrogen to soils (Fan *et al.* 2006). Faba bean is now one of the major pulse crops of Australia with an area under cultivation of about 183,000 ha that produces 329,000 tonnes (Pulse Market Overview April 2006). Australia is the world's largest exporter of faba beans (Anonymous 2005).

2.2. Seed discolouration problem and its significance

Colour is a ratio relating a human response to physical energy, like its audio counterpart the decibel. Colour fills our lives and affects our choices and preferences of objects as diverse as cars, clothes and food (Anonymous 2007a). Colour is one of the most important sensory and quality attributes of almost all foods. Despite other quality characteristics such as size, soundness, firmness and the absence of foreign materials, legume seeds are also quality graded on the basis of their testa colour and gloss (Sathe *et al.* 1984). The degree of lightness, shade and uniformity of colour are important indicators of bean quality (Yoshida *et al.* 1991). Seed coat or testa colour is one of the most important quality characteristics in marketing of faba and other beans for human consumption (Aparicio-Fernandez *et al.* 2006; Park and Maga 1999; Quenzer *et al.* 1978; Yoshida *et al.* 1991). Faba beans have several genetically determined colours such as beige, black, brown, green, red, spotted, violet and white (Nozzolillo *et al.* 1989). The typical colour of seed coats in most cultivated varieties in Europe is pale buff to buff (Nilan 1967). Genetically, seed testa colour ranges from white to purple in different varieties of faba bean but the preferred colour has variously been described as beige, light tan or buff (AGWEST 1998). Other researchers describe light-brown as the most common seed coat colour in faba beans and 91% of accessions in the ICARDA faba bean collection have this colour at harvest (Robertson and El-Sherbeeny 1991). The controversy over the most common colour may be due to the difference in describing the name of colour as it is hard to distinguish between light-brown and buff or beige. Wild forms of this species generally have black seeds which, together with the fact that mutations from recessive to dominant are very uncommon, indicates that the black seed colour most likely is the original one (Nilan 1967).

Seed discolouration is a major concern in faba bean marketing for human consumption and it can be divided into two major classes; preharvest staining and postharvest colour darkening.

2.2.1. Preharvest staining

Preharvest staining is caused by different biotic and abiotic factors (Table 1). The most common biotic factor is the fungal disease, ascochyta blight caused by *Ascochyta fabae* (White *et al.* 2004). Ascochyta appears to be the main cause of disease-related seed discolouration in the higher rainfall region of the eastern part of Australia (Raynes and Bretag 2001). *Botrytis fabae* causes chocolate spot, which can infect pods and seeds but it is a far less common cause of discolouration. Other causal organisms of discolouration in faba bean are Pea Seed-borne Mosaic Virus (PSbMV) and Broad Bean Stain Virus (BBSV). Certain insects such as *Melanacanthas scutellaris* may damage the seed and leave stains. Disease-related discoloration caused by different organisms accounts for only 2.8% of discolouration (White *et al.* 2004) and is generally managed by selecting the resistant varieties or by the application of suitable pesticides. Seed discolouration caused by different organisms is beyond the scope of this thesis.

The most common abiotic cause of preharvest discolouration is known as environmental staining. It is the major cause of faba bean discoloration in Australia. A survey of about 30 crops produced in Western Australia between 1997 and 1999 (mainly cv. Fiord) showed that on average, 14.5% (1997), 7.5% (1998) and 18.5% (1999) of seeds were discoloured (White and Pope, unpublished data). The lowest proportion of discoloration in an individual crop was 0.2% while the highest was 52%. In 1999 the preharvest discolouration was divided distinctly between disease and environmental staining. Environmental staining accounted for 15.7% of the discolouration (White *et al.* 2004). Environmentally stained seeds have a dark brown to black stain of seed testa (Table 1).

Environmental staining is not well understood but it is thought to be caused by one or several environmental factors including temperature, rainfall/drought, light, frost or nutrients (Hughes and Sandsted 1975; Muzika 1993; White 2002). Some varieties are more susceptible to environmental staining than the others. Advanced lines within the Australian National Faba Bean Breeding Program are screened for environmental staining with the result that new releases (cvs. Fiesta, Manafest and Farah) show considerably less environmental staining than the older varieties (cvs. Fiord or Ascot) (White *et al.* 2004).

Table 1. Different types of discolouration in faba bean caused by biotic and abiotic factors (adapted from White *et al*. 2004)

Discolouration Type	**Main Factors**
Disease discolouration	    *Ascochyta blight* *Chocolate spot* *PSbMV*[1] *BBSV*[2]
Insect discolouration	 Melanacanthas scutellaris
Environmental staining	  *Unknown* [3] *Split pods*[4]
Storage darkening	 *Unknown*[5]

[1]PSbMV = Pea Seed-borne Mosaic Virus; [2]BBSV = Broad Bean Stain Virus; [3] May be due to some environmental factors; [4]Possibly due to exposure to light after pod splitting; [5]May be due to transformation of phenolic contents

2.2.2. Postharvest colour darkening

Postharvest colour darkening, also known as storage discolouration, is another major problem faced by the faba bean industry. Most of the faba bean varieties have beige/buff or light brown colour at harvest but this changes to medium brown, dark brown and even chocolate brown depending upon the storage conditions and duration.

Storage conditions contribute to the changes in seed colour of a range of grain legumes. Temperature, relative humidity (RH), seed moisture content (SMC), oxygen and light appear to be factors with temperature the most important. High temperature (≥ 24^{o}C) and high RH (≥ 80%) accelerated darkening in kidney beans (*Phaseolus vulgaris* L.) while beans stored at low temperature (1^{o}C) and RH (30%) retained their original colour for one year (Hughes and Sandsted 1975). Storage of chickpea (*Cicer arietinum* L.) at 33-35°C and 75% relative humidity for 160 days caused testa colour darkening which

was reflected by decrease in Hunter 'L' value and increase in total colour difference (ΔE) (Reyes-Moreno *et al.* 2000). Lentil (*Lens culinaris* Medic.) seeds exposed to moderately high temperature (20^{o} and 30^{o}C) at high RH (100%) turned brown in 3 weeks or less while at cool temperature (5^{o}C) with same RH (100%) browning did not occur before 5 weeks (Nordstorm and Sistrunk 1979; Nozzolillo and De Bezada 1984). Similarly little change in seed coat colour occurred in Rwandan dry beans (*Phaseolus vulgaris*) stored at 4°C for 24 months (Edmister *et al.* 1990). Light red kidney beans also retained their original colour for one year when stored at 1°C (Gunes and Lee 1997). Even at moderately low temperature (10°C) darkening was slow in adzuki beans (*Vigna angularis*) (Yousif *et al.* 2003b). At high temperatures (40^{o}C) and humidity (100% RH) there was a substantial (79%) increase in colour score (darker colour) of faba bean after just one week (Davies 1994).

In south west Australia faba bean crop is harvested in late spring to early summer (October-December) and grain is stored on farm in uninsulated bins for the next couple of months. The air temperature frequently reaches 40 °C over summer (Australian Bureau of Meteorology), which can quickly cause colour darkening (Davies 1994).

While RH seems to be less important than temperature, seed moisture contents (SMC) none the less, is likely contributor to postharvest colour darkening in faba beans and other beans during storage. In a detailed study the colour stability of 32 lines of pinto beans (*Phaseolus vulgaris*) and great northern beans (*Phaseolus vulgaris*) to which different levels of moisture were added was evaluated in an accelerated shelf-life testing chamber for up to 10 weeks using Hunter values and National Bureau of Standards (NBS) unit of colour difference (ΔE) techniques. Hunter L^{*} values decrease and a^{*} values increase as storage time increases, while b^{*} values do not change with storage. Beans with an extra 10% added moisture show much more colour change than samples with 5% added moisture (Park and Maga 1999). Similarly increases in colour darkening in Rwandan dry beans positively related to increases in water activity (a_w) across a range of storage temperatures (Edmister *et al.* 1990).

Light also enhances colour darkening in beans. Growers of light red kidney beans (*Phaseolus vulgaris*) often observed that beans darkened in the pods when harvest was delayed after pods and seeds were fully mature (Hughes and Sandsted 1975). It might

be due to the effect of light as growers also observed that bean seeds placed in the light near a window darkened rapidly. Scientific investigation indicated that ultraviolet and cool-white light darken kidney beans in storage (Hughes and Sandsted 1975).

The above mentioned factors i.e. storage temperature, seed moisture content and light may also be responsible for colour darkening in faba beans. A comprehensive study was required to investigate these factors so that recommendations could be developed for growers and grain traders for proper storage to control or at least minimise the seed discolouration in faba beans.

2.3. Involvement of phenolic compounds in colour development and discolouration in beans

Phenolic compounds are ubiquitous in the plant kingdom and many of them are involved in colour development in different parts of plants including seeds (Beninger and Hosfield 2003; Slattery *et al.* 1982). Chemical compounds found in seed coats of legumes include tannins, lignins and non-tannin polyphenolics compounds and concentration of these compounds may differ depending on the level of pigmentation in the seed coat (Asiedu *et al.* 2000). The seed colour of some beans is determined by the presence and concentration of flavonol glycosides, anthocyanins and condensed tannins (proanthocyanidins) (Beninger and Hosfield 2003).

Naturally, faba bean varieties have a range of colours that varies from beige, brown and red to violet. In faba bean seed coats, flavonoid aglycones, corresponding to eight genetically determined colours, reveal that myricetin predominates over quercetin in beige, black, brown, green, red, and violet seeds. Kaempferol is present in substantial amounts only in spotted seeds. White seeds have only trace amounts of quercetin and kaempferol and are the only ones without proanthocyanidins. Flavones of the epigenin type occur in all coloured but not white seed (Nozzolillo *et al.* 1989). Anthocyanins (malvidin, delphinidin, petunidin, and cyanidin glycoside) are only present in violet seeds and are obviously responsible for their colour. The dark colour of black, brown and red seeds possibly results from unidentified polymers (Nozzolillo *et al.* 1989). In different field pea (*Pisum sativum* L.) varieties, the sum of free phenolic acids, those liberated from soluble esters and glycosides, was higher for coloured seed coats (78 µg/g dry matter) than for the white seed coats (17µg/g dry matter). Phenolic

compounds, such as protocatechuic, gentisic and vanillic acids dominated in coloured seed coats, while ferulic and coumaric acids occurred in white seed coats (Troszynska and Ciska 2002). The phenolic composition of two varieties of lentils, Pardina and Castellana, revealed that the seed coat was very rich in catechins, procyanidins dimers and trimers and it contained minor concentrations of glycosides of quercetin, myricetin, letulin and apigenin (Duenas *et al.* 2002).

Phenolic compounds are substances that possess an aromatic ring bearing one or more hydroxyl substituents (Morton *et al.* 2000). There are around 5000 plant phenolic compounds identified (Bors *et al.* 1996). These compounds play a role in the structural stability of plant material as their chemical structure enables them to form a variety of esters and other linkages. Phenolic compounds are present in abundance in vascular bundles and structural features of plants. Thus, seeds, skins of fruits, stems and leaves are rich sources of phenolics (Morton *et al.* 2000). Tannins are among the most important phenolic compounds found in plants. The word tannin refers to polyphenols, while condensed tannins are known as proanthocyanidins. Proanthocyanidins comprise a group of polyhydroxyflavan-3-ol oligomers and polymers linked by carbon-carbon bonds between flavonol subunits (Hagerman *et al.* 1997). These are secondary products of plant metabolism and besides their role in certain defence mechanisms against pathogens and pests they are involved in colour determination of plant parts. The chemistry of tannins is complex and not well understood. Tannins are classified into two groups: condensed tannins (proanthocyanidins) which are polymers of flavonols and yield anthocyanidins when heated in acid solution, and hydrolysable tannins which produce gallic acid as a degradation product. The anthocyanins comprise the largest group of water soluble phenolic compounds in the plant kingdom occurring in at least 27 families, 73 genera and a multitude of species (Bridle and Timberlake 1997). The most widely distributed group of flavonoids in beans is the proanthocyanidins (Beninger and Hosfield 2003; De Mejia *et al.* 2003; Guzman-Maldonado *et al.* 1996). Anthocyanins have only been reported in black and blue-violet coloured beans (Beninger and Hosfield 2003; Romani *et al.* 2004).

Proanthocyanidins have been detected in different varieties of common beans. They usually range in concentration from 9 to 38 mg catechin equivalents per g, mainly in the seed coat (Aparicio-Fernandez *et al.* 2005; De Mejia *et al.* 2003). Flavan, the basic structural unit of condensed tannins is made up of two benzene rings linked together by

a three carbon atom fragment. This unit is also the basic structure of anthocyanidins and their glycosides (anthocyanins), the latter generally being responsible for the scarlet, red, mauve, purple and blue colours in plants organs. Methylation, hydroxylation or glycosidation are the possible modifications of the basic unit of polymers resulting in marked variability of the physiological and biochemical properties of these substances. These properties range from auxin transport regulators (Jacob and Rubery 1988) and protectors of inner tissues against visible and /or UV irradiation (Salatino *et al.* 1988) to activators of nodulation genes in *Rhizobium* (Firmin *et al.* 1986).

The white varieties of legumes usually contain tannins in lower concentrations than those with red, black or bronze seed coats. Tannin contents of black-seeded common beans were 2.48 tannic acid equivalent compared with 0.5 tannic acid equivalent in white cultivars (Carmona *et al.* 1991). Similarly, differences in tannin contents had been found between pigmented and unpigmented seed coats of faba beans (Cabrera and Martin 1989; Marquardt *et al.* 1978; Van Der Poel *et al.* 1991). Tannin contents were high in pigmented faba bean cultivars and low in unpigmented cultivars (Bos and Jetten 1989). Tannins share some precursors with anthocyanin pigments in their biosynthetic pathway. This is the reason for the well known relationship between white flowered plants and absence of tannins in their seeds (Dickinson *et al.* 1957). Flavones and flavonoles were in greater concentration in dark brown than beige or green seeds (Troszynska and Ciska 2002). There are some faba bean lines that have white flowers and white seed coats. Those lines contain very low quantities of tannin and are generally known as “tannin free lines”.

2.3.1. Involvement of phenolics in environmental staining

Environmental factors are known to alter the activity of some of the enzymes involved in flavonoid biosynthesis. The intensity and duration of light can have substantial effect on plant biochemistry especially on phenolic biosynthesis. In field peas, varietal differences in seed coat colour were minor but light intensity affected flavonol (a colouring compound in plants) content in seeds and leaves (Herrmann and Woldecke 1977). Shoots of *Zostera marina* L. plants grown for 4 weeks under high light intensity had higher concentrations of phenolic compounds than those grown under low light intensity (Vergeer *et al.* 1995). Total contents of phenols and anthocyanins were substantially higher in sun-exposed grape berries than in shaded berries (Crippen and Morrison 1986) and there were more bulk phenolics and condensed tannins produced

under full sun than shade in leaves of *Betula nana* L. (Graglia *et al.* 2001). Leaf phenolics increased in tulip poplar (*Liriodendron tulipifera*) and dogwood (*Cornus florida*) when sunlight was greater and tannins in dogwood saplings dropped substantially in deep shade (Dudt and Shure 1994). Tobacco (*Nicotiana tabacum* L.) plants exposed to 16 h photoperiods had three times higher concentrations of total phenolics in leaves than those exposed to 8 h photoperiods (Tso *et al.* 1970). Cell browning occurred in broad bean leaflets irradiated with red light (Rahman *et al.* 2002) . Browning in broad bean leaf tissues was also observed when inoculated with non-pathogenic *Botrytis cinerea*, and the browning was attributed to phenolic compounds (Mansfield and Hutson 1980). Hence light intensity, longer duration and warming at the later stage of plant development under Australian climatic conditions may similarly increase phenolic contents in faba bean seeds that are reflected in the formation of seed coat discolouration.

The mechanisms that account for the increase of phenolics with increased light intensity and longer photoperiods may be that the light stimulates most of the enzymes involved in phenolic biosynthesis of plants. In particular, the activity of phenylealanine ammonia-lyase, a key enzyme in the synthetic pathway of phenolics, increases under the influence of light (Hahlbrock and Scheel 1989) . The synthesis of phenylpropanoids and derived compounds (e.g. condensed tannins) competes directly with the synthesis of protein, and therefore with plant growth, because of a common precursor, phenylalanine (Haukioja *et al.* 1998). Another mechanism is explained by carbon/nutrient balance (Bryant *et al.* 1983; Waterman *et al.* 1984). The photosynthetic activity of a plant at high light intensity may be so high in relation to the quantity of nitrogen available that, once its limited supply has been used in the production of primary metabolites (amino acids, proteins), the remaining carbohydrates can only be used to produce nitrogen free molecules, such as phenolics (Waterman *et al.* 1984). In this way phenolic compounds are regarded as storage compounds of carbohydrates, which are produced at times when plants cannot convert carbohydrates into growth. Indeed nitrogen fertilization can result in a linear decrease in foliage concentrations of phenolic compounds. This inverse relation between nitrogen availability and phenolic contents is found in a range of species (Buchsbaum *et al.* 1990; Giertych *et al.* 1999; Muzika 1993) and it may occur in faba bean.

Faba bean is a nitrogen fixing plant and it would require a shortage of nitrogen to promote phenolic production as described above. Nodulation and nitrogen fixation is regulated by different factors including soil moisture, temperature and nutrient supply especially K. Low moisture and high temperature (30°C) reduce nitrogen fixation and suppress the growth rate and faba bean is even more sensitive than other beans (Sangakkara *et al.* 1996b). Under water stress conditions, due to high temperature and low rainfall under south west Australian conditions, nitrogen fixation and its supply to immature seeds at the top nodes of the plant may not keep pace with the high demand of nitrogen from the high rate of photosynthesis that is accelerated by high light intensity for long photoperiods and this may stimulate production of phenolics in faba bean plants.

There is some controversy over the carbon/nitrogen balance hypothesis (Hamilton *et al.* 2001; Koricheva 2002) but this lies mainly with the universality of the hypothesis. Production of compounds, such as terpenoids and alkaloids, may not follow the carbon/nitrogen balance hypothesis but production of phenolics, especially condensed tannins, generally follows the trend (Haukioja *et al.* 1998; Koricheva 2002; Muzika 1993).

Not only nitrogen but general supply of nutrients to plants affects phenolic metabolism. Plant phenols accumulates under conditions when plant growth is limited by mineral nutrients (Ilvessalo and Tuomi 1989). Vegetation growing on low-nutrient soils can contain relatively high concentrations of secondary products, such as tannins, compared with vegetation growing on nutrient-rich soils (McKey *et al.* 1978). In rain-forests, plants growing in dense stands on exceptionally poor soils contained higher concentrations of phenolics than the average for that site (McKey *et al.* 1978). The level of total phenolics usually decreased after fertilizer application as studied in many plants (Bryant *et al.* 1987; Larsson *et al.* 1986; Tuomi *et al.* 1984). The content of Ca is correlated with phenolic contents and liming caused an increase in phenolic contents in needles of Scots pine (Giertych *et al.* 1999). Accumulation of Zn was also associated with increases in phenolic contents (Giertych *et al.* 1999). High K supply had a positive effect on nitrogen fixation, shoot and root growth and on water potential in beans and the impact was more prominent in faba bean (Sangakkara *et al.* 1996a). Thus nutrient availability, especially at the end of season when water stress limits nutrient uptake, may influence phenolic contents and hence discolouration in faba bean.

Environmental staining in faba beans may have some similarities to that of black point in wheat and barley. Black point is a dark discolouration of the embryo end of wheat and barley grains. The discolouration is not limited to the area around the embryo but can also extend to the ventral surface of the kernels (Conner and Davidson 1988). Some studies associate it with the presence of certain fungi but most studies fail to establish any direct link between fungi and the development of black point symptoms in wheat and barley (Ellis *et al.* 1996; Jacobs and Rabie 1987; Williamson 1997). Environmental conditions (rain, humidity, temperature) affected the production of black point symptoms (Conner *et al.* 1992; Fernandez *et al.* 1994; Rees *et al.* 1984). Peroxidase activity found in the black point region of wheat and barley suggested that phenol peroxidases were involved in the dark discolouration (Cochrane 1994b; Williamson 1997). The phenols and peroxidases involved in producing black point symptoms in barley can increase under stressful environmental conditions (Cochrane 1994a). In wheat, hydrogen peroxide levels increased under stress as with drought (Smirnoff and Colombe 1988) and had been shown to triple within minutes when temperature dropped below 5°C (Okuda *et al.* 1991). A similar stress related phenomenon might be occurring in faba bean.

2.3.2. Involvement of phenolics in storage discolouration

Phenolic compounds are found in almost all legumes with faba bean being specially rich. Phenolic acid content of faba bean (155 mg kg^{-1}) is much higher than other legumes e.g. field pea (11 mg kg^{-1}), chickpea (11 mg kg^{-1}), navy bean (14mg kg^{-1}), lima bean (36 mg kg^{-1}), lupine (44 mg kg^{-1}), mung bean (86 mg kg^{-1}), lentil (113 mg kg^{-1}) and cowpea (121 mg kg^{-1}) (Sosulski and Dabrowski 1984). Changes in phenolic contents may be involved in colour darkening of seed coats in faba and other beans. However, among phenolic compounds, tannins appear to be the main group involved in the discolouration process in legumes. Proanthocyanidins are the predominant tannin group found in legume seeds. They are located mainly in seed coats and play an important role in the defence system of seeds that are exposed to oxidative damage caused by environmental factors such as light, oxygen, free radicals and metal ions (Troszynska and Ciska 2002). Tannin concentration was high in coloured seed coats (38-43 mg g^{-1}) and low in white coated beans (1.3 mg g^{-1}) while values ranged from 3.8 to 5.9 mg g^{-1} in cotyledons (Elias *et al.* 1979). The content of condensed tannins was

1560 mg of catechin equivalent per100 g of coloured seed coats while no condensed tannins occurred in white seed coats of field peas (Troszynska and Ciska 2002).

In faba bean, the amount of proanthocyanidins varies from 0 (white seeds) to 35 mg g^{-1} (brown seeds) of seed coat dry weight (Nozzolillo *et al.* 1989). White flowering and white seeded varieties are free from condensed tannins in the testa while coloured varieties are not. In addition, the darker the natural testa colour, the higher the polyphenols (tannin) content (Rawlands and Corner 1962). According to the observations of Paull (Faba bean breeder, Waite Agricultural Research Institute, University of Adelaide, South Australia; personal communication) faba bean varieties with white testa and white hila show very little colour changes during storage. Tannin-containing faba bean seed coats darkened with time so that after 6 months of storage at room temperature, seeds can easily be classified as tannin-containing or tannin-free, based on coat colour (Crofts *et al.* 1980). Tannin-free seeds had no seed coat darkening with time or when exposed to oxygen (Crofts *et al.* 1980; Marquardt *et al.* 1978). These preliminary studies suggest that tannins are the principal phenolic compounds involved in darkening/ discolouration in faba beans and this requires confirmation and quantification.

Some studies reveal that discolouration in beans is due to development of tannins from smaller molecular weight non-tannin material during storage. In black beans (*Phaseolus vulgaris*) the percentage changes in tannin contents suggest that the larger molecular weight tannins increase at the expense of the lower molecular weight fractions presumably due to their polymerisation (Sievwright and Shipe 1986). Under normal storage conditions, lentil seeds discoloured gradually over a 2-year period from the olive green of freshly harvested seeds to light yellow and finally deep brown (Nozzolillo and De Bezada 1984). Coats of both green and brown lentil seeds contained a high amount of proanthocyanidins (condensed tannins), both procyanidins and prodelphinidin, but brown seeds contained much lower amounts of low molecular weight proanthocyanidins (soluble tannins) than that of green seeds. Browning appeared to be a result of polymerisation of soluble tannins to brown-coloured high molecular weight polymers (condensed tannins). Testa tannin content of three chickpea (*Cicer arietinum* L.) varieties decreased substantially with colour darkening after storage at high temperature (33-35°C) and high relative humidity (75%) (Reyes-Moreno *et al.*

2000). The same reaction may be involved in faba bean seed coat darkening, and this needs to be investigated.

Alternatively, oxidation of phenolic compounds may be the main phenomenon that cause colour darkening in faba bean (Marquardt *et al.* 1978). The phenolic compounds vary widely in complexity but the common characteristic of all these compounds is that they are readily oxidised and undergo phenolic reactions (Bors *et al.* 1996). Pure flavonoid compounds such as anthocyanins, quercetin glycosides, and proanthocyanidins (condensed tannins) that are present in the seed coats of common beans, have substantial antioxidant activity relative to BHT (butylated hydroxytoluene), a commercial antioxidant used in foods (Beninger and Hosfield 2003). They play an important role in the defence system of seeds by exposing themselves to oxidative damage caused by environmental factors such as light, O_2, free radicals and metal ions (Troszynska and Ciska 2002). Stanley (1992a) also suggests that colour darkening in beans during storage is probably caused by air- and light-catalysed oxidation of leucoanthocyanidins, a group of phenolic compounds. Storage of faba beans with low oxygen concentration (2%) was effective in reducing darkening compared with storage in air (Black and Brouwer 1998a).

It appears that phenolic compounds, especially tannins, may be involved in darkening of faba beans during storage and it is possibly due to oxidation of phenolic compounds. Studies are required to investigate the affect of oxygen and other gases in colour darkening problems of faba bean during storage so that possible measures to control/minimise the problem can be determined.

2.4. Management strategies to minimise colour darkening during storage

Refrigeration or cold storage is a gentle method of food preservation. It acts by reducing the rate of changes in food products. It is likely it could also be used in faba bean to reduce physicochemical changes that result in colour darkening and other deteriorative affects on quality but economy of its usage is a matter of concern. Refrigeration is generally costly and mostly used for high value food products. Modified atmosphere packaging (MAP), on the other hand, can be a method of choice in case of grains. Modified atmosphere packaging is a food preservation method that maintains the natural quality of food products and extends storage life. It has been defined as ‘the enclosure of food products in a high gas barrier film in which the gaseous environment has been changed or modified to slow respiration rates, reduce microbial growth and

retard enzymatic spoilage with the intent of extending shelf life' (Young *et al.* 1988). Modified atmosphere packaging generally refers to a package in which air is removed, i.e. vacuum packaging, or air is replaced with desired gas/gases. With MAP, the gas composition surrounding the produce inside the package is different from the gas composition outside the package. Outside, the gas composition is always close to 78.1 kPa nitrogen, 20.95 kPa oxygen, 0.93 kPa argon, and 0.036 kPa carbon dioxide. Several different types of packages and packaging techniques have been developed to modify atmosphere. The modification of the atmosphere generally implies a reduction of oxygen content and/or an increase of the CO_2 or N_2 concentration and this may assist in reducing discolouration in faba beans. In some cases changing the level of carbon monoxide, ethylene, ethanol or other compounds in the atmosphere can contribute to shelf life extension. Modified atmosphere can be created passively by the respiration activity of produce inside the package (product modified MAP) or actively, by introducing the desired gas mixture (gas packing). Other active ways of obtaining modified atmosphere are the use of gas generators and scrubbers (controlled-atmosphere packaging), evacuation of air (hypobaric storage, vacuum packaging), or addition of chemical systems that absorb or generate gases or volatile compounds (active packaging) in packages (Gorris and Peppelenbos 1999).

Modified atmosphere packaging is usually used for shelf life extension in fresh products such as fruits and vegetables but it can also be used for dehydrated foods. Below water activity (a_w) 0.70 microbial growth is not a factor but deterioration can result from oxidation of lipids, vitamins and pigments such as chlorophyll and carotene, and certain phenolic compounds. Reduction of oxygen levels by nitrogen flushing or vacuum packaging has been used commercially for freeze-dried products, ground coffee, roasted nuts, powdered whole milk, and dehydrated potato flakes (Fierheller 1991) and it is an approach that may be useful in faba beans.

Flushing with N_2 and CO_2 are the most commonly applied modified atmosphere techniques. The usual gas for flushing dehydrated foods is N_2. It is inert with a low fat and moisture solubility (Fierheller 1991). Carbon dioxide has been proposed for packaging of nuts (Holaday *et al.* 1979) and may be suitable for dried legumes such as faba beans. Adsorption of the CO_2 by the nuts creates a vacuum. The adsorption phenomenon is similar to gas adsorption by charcoal and can be used on a variety of grains, oilseeds, legumes, rice and corn (Ooraikul 1991). Phenylalanine ammonia-lyase

activity, tanning ability and polyphenol levels were measured in cherimoya (*Annona cherimola* Mill.) fruit treated with 20% CO_2 + 20% O_2 + 60% N_2 during chilling temperature (6^oC) storage. Tanning ability and the early increase in tannin polyphenols induced by chilling temperature were reduced by CO_2 treatment (Maldonado *et al.* 2002). Carbon dioxide, solely or in combination with other gases, is helpful in reducing oxidation (Holaday *et al.* 1979) so it might be helpful in reducing oxidation related discoloration in faba beans.

As discussed earlier, colour darkening in faba bean is probably due to oxidative alteration of phenolic compounds (Marquardt *et al.* 1978) and this may be reduced by controlling the supply of oxygen. Storage of faba bean with low O_2 concentration results in reduced colour darkening and varieties with high tannin content darken more in air than low tannin varieties, suggesting that darkening of seed coats was possibly due to oxidation of polyphenolics, such as tannins (Black and Brouwer 1998a). Similarly, changes in total phenolic acids of minimally processed lettuce (*Lactuca sativa*) leaves were reduced when they were stored in MAP conditions of low O_2 (2-3%) and high CO_2 (12-14%) compared with storage in air and this controls browning (Gil *et al.* 1998). The availability of O_2 in air during storage is regarded as the main source of oxidation (measured as increase in colour darkening of beans), hence, traditional methods of storage of faba bean in the Middle East employ the use of underground pits which are filled completely with seeds to minimise air volume (El-Refai *et al.* 1988).

Involvement of oxidation processes in colour darkening of faba bean is also supported by the fact that when beans, grains or their products are stored under low O_2 atmosphere, their quality deterioration is substantially reduced. Low O_2 (5-10 kPa) and high CO_2 (5 kPa) in the packaging of snow pea pods was helpful in maintaining their quality by retarding changes in organic acids, free amino acids and sugar contents, and sensory attributes (Pariasca *et al.* 2001). There was a more stable nutritional quality (vitamins and minerals content) of green beans stored under 3% O_2 + 3% CO_2, than under atmospheric air storage (Sanchez-Mata *et al.* 2003). Black beans stored for one year at 30 ± 3^oC and 70-80% relative humidity under a modified atmosphere (containing N_2 and CO_2) in an impermeable container, loose quality in terms of hardening, at a slower rate than beans stored in air (in mesh bags) in the same environment (Aguilera and Rivera 1990). Oxidation due to the available oxygen in air is a factor that causes colour darkening of faba bean during storage. Hence any method of

packaging that can control availability of environmental O_2 could be helpful in reducing the darkening process. Vacuum packaging and/or use of other gases in faba bean packaging may be able to control or reduce the discolouration.

Flushing with N_2 demonstrates a marked effect in reducing colour darkening in different foods and food products. Drying of field pea seeds under N_2 and in a vacuum inhibited seed coat browning compared to seeds dried in air or O_2 (Marbach and Mayer 1974). Enzymatic browning in minimally processed apple pieces was successfully inhibited for long storage times by application of a modified atmosphere of 80% N_2 and 20% CO_2 (Nicoli *et al.* 1994) or 100% N_2 (Soliva-Fortuny *et al.* 2001). Similarly, nitrogen flushing was more effective than other gas treatments in preventing browning of cut potatoes (Gunes and Lee 1997). The mode of action of N_2 in reducing colour darkening may be associated with the reduction in oxidative transformation of phenolic compounds. Hence use of CO_2, N_2 or vacuum may be helpful in reducing the oxidation related changes in faba bean including oxidation of phenolic compounds that may help in reducing colour darkening. It is also very economical and an easy to use method compared with cold storage.

Another effective way of controlling or minimising discolouration problem can be the development of reduced-tannin or tannin-free varieties. Some tannin free lines already exist and these are almost resistant to environmental staining and storage colour darkening, under ambient conditions for a reasonable time period but there are concerns about their acceptability for human consumption. Tannins and other phenolic compounds are recognized as herbivore-deterrent secondary metabolites. They assist plants in the prevention of herbivore and insect attack (Estiarte *et al.* 1994). Tannin free lines are less resistant to some pathogens and pests specifically during germination and seedling establishment. Other concerns are low yield, smaller seed size and effect on sensory quality. White faba bean (tannin free lines have almost white seed coats) may not be very acceptable to consumers as they are used to using coloured varieties. Most (91%) of the faba bean accessions in the ICARDA collection have beige or light brown colour at harvest (Robertson and El-Sherbeeny 1991) and this is the colour consumers are familiar with. Reduction or elimination of tannins may also affect the flavour and taste of food products prepared from reduced tannin or tannin-free varieties as plant phenolic compounds are responsible for giving foods flavour, colour and taste (Daniel *et al.* 1999).

The development of reduced or tannin-free varieties with required characteristics is a lengthy process. In the short term, investigation on methods that can help reduce the discolouration in existing commercial varieties is required and MAP technique provides a potential solution.

2.5. Effect of storage conditions on different quality parameters other than colour

Grain legumes occupy an important place in human nutrition, especially in the dietary pattern of low-income groups of people. Legumes, considered as poor man's meat, are generally a good source of nutrients (Tharanathan and Mahadevamma 2003). They are an important and inexpensive source of protein, dietary fibre and starch for a large part of the world's population, mainly in developing countries and in vegetarian communities (Perla *et al.* 2003; Tharanathan and Mahadevamma 2003). Among the commonly consumed food legumes, faba beans occupy an important position in human nutrition and are consumed in large quantities in the Middle East, north Africa and China. In Egypt faba beans are mostly used to prepare four popular dishes, namely Medammis (stewed beans), Falafel (deep fried dough), Bissara (poured paste) and Nabet soup (boiled germinated beans) (Bakr 1996). Legume seeds are mostly preserved in dry storage at ambient temperature to maintain a year-round supply of this food source. High temperature during storage can cause deteriorative affects on legume seed quality (El-Refai *et al.* 1988; Noaman *et al.* 1988). The main type of deterioration is increased hardness of cotyledons or loss of cookability (ability to soften with cooking), followed by deterioration of texture, colour and flavour and loss of nutritive value (Cunha *et al.* 1993; Martin-Cabrejas *et al.* 1997; Yousif *et al.* 2003a).

2.5.1. Effect of storage conditions on cooking quality of beans

Cooking is the oldest method of processing legumes however, new methods such as microwave heating can also be used (Khatoon and Prakash 2006). Cooking makes the grain softer, destroys antinutritional factors and improves palatability and digestibility. Presoaking is almost a prerequisite for cooking whole legumes to soften their outer coat and reduce cooking time. Physicochemical properties of dry beans including colour, flavour and cooking properties influence consumer preference and processors standards (Reyes-Moreno and Paredes-Lopez 1993).

Hardness of cotyledons is commonly described as the "hard-to-cook" (HTC) phenomenon and is characterized by a requirement for extended cooking time. The HTC defect is one of the most important acceptability characteristics of beans because the cooking time required for beans to reach an acceptable texture also greatly influences consumer perception of bean quality (Affrifah and Chinnan 2005). The cooking quality of beans is defined as the cooking time required for beans to reach a cooked texture considered acceptable to consumers (Moscoso *et al.* 1984). Hard-to-cook beans need additional energy during preparation, have inferior nutritional qualities and therefore poor acceptance by consumers. Long cooking time is also one of the factors responsible for underutilization of legume seeds (Deshpande *et al.* 1984). Hence studies are required to find the affect of storage at ambient conditions of high temperature in the grain belt of south west Australia on the cooking quality of faba bean.

There are a number of factors during storage that affect cooking quality of beans. The most important are temperature, seed moisture content or relative humidity and duration of storage. Storage of beans at high temperature and high moisture content or relative humidity generally results in longer cooking times compared with those stored at low temperature and low moisture content. The quality of beans can be preserved by maintaining the moisture content or water activity at a level where the undesirable reactions, usually associated with the hard-to-cook defect, are inhibited (Reyes-Moreno and Paredes-Lopez 1993). According to Aguilera and Rivera (1990), the optimal moisture content for storage of black beans is 10-14%, which is adequate for delaying hardening and minimizing the mechanical damage during handling of beans. The cooking time of pinto and lima beans with 13% moisture contents increased threefold after 1 year storage at 25°C whereas, with 10% moisture contents, it was favourably comparable with beans stored at −23°C (Morris and Wood 1956).

High storage temperature, in addition to accelerating colour darkening, enhances many processes that deteriorate the cooking quality of legume seeds. Fresh beans (*Phaseolus vulgaris*) exhibited a mean cooking time of 60 min but when stored for six months at 37°C and 76% relative humidity the cooking time increased more than 300 min (Antunes and Sgarbieri 1979). Common beans (*P. vulgaris*) stored under adverse conditions of high temperature and relative humidity developed a hardening defect characterized by extending cooking time for cotyledon softening (Hincks and Stanley 1986; Jones and Boulter 1983). The cooking time of red and black beans (*P. vulgaris*)

stored at 29°C for 3.5 months was 2.45 and 2.41 times greater than those stored at 4°C (Maurer *et al.* 2004). Black beans stored at 4-5°C and 50-60% relative humidity exhibited quality characteristics of fresh beans, such as shorter cooking time and percentage of hard shell whereas beans stored at ambient conditions of 23-25°C and 30-50% relative humidity lost these characteristics (Berrios *et al.* 1999). Similarly cooking time of three chickpea varieties substantially increased when stored for 160 days under unfavourable conditions of 33-35°C and 75% relative humidity. It ranged from 112-142 min for fresh to 146-213 min for stored chickpeas (Reyes-Moreno *et al.* 2000). Storage of cowpeas at 30°C for 18 months increased seed hardness from 15.8 to 91.2 Newton (*N*) g^{-1}, whereas seeds stored at −18°C showed no change (Liu *et al.* 1992). Similarly, after 120 days storage at 33-35°C, hardness of four common bean varieties increased by 3 to 6 *N* $seed^{-1}$ (Reyes-Moreno *et al.* 1994) and hardness of cooked black beans stored for 200 days at 30°C increased by 5 *N* $seed^{-1}$ (Del Valle and Stanley 1995). Black beans stored for 2 years at ambient temperature (23-25°C) demonstrated longer cooking time than beans stored at refrigerated temperature (4-5°C) (Berrios *et al.* 1999). Similarly faba beans stored at ambient conditions of high temperature in Australia may acquire HTC characteristic. Investigation is required so that optimum storage conditions can be determined to minimise the defect.

The deterioration in texture quality of beans measured as the hard to cook phenomenon is caused by different structural and chemical changes in seeds that occur during long-term storage. These changes are accelerated under different storage conditions especially high storage temperature. Jones and Boulter (1983) describe HTC in common beans as due to reduced imbibition value and reduced pectin (middle lamella) solubility that cause a reduction in rate of cotyledon cell separation during cooking. Water absorption (18 h soak) was negatively correlated ($r = -0.69$ to -0.81) with cooking time of the common beans (Castellanos and Guzman-Maldonado 1995). Increased cooking time was associated with increased content of Ca, Mg and pectin in the seed (Muller 1967). Scanning electron microscopy of stored common beans (HTC) exhibited a dense packing of cotyledon cells with no separation between them as seen in fresh beans (Paredes-Lopez *et al.* 1989). The strong adhesion between cells observed for hard seeds might partially explain the reduced water uptake and consequently the lower rate of cooking (Hincks and Stanley 1987; Molina *et al.* 1975). Although cooking increases the digestibility of proteins, prolonged cooking will increase the percentage of leached solids and destroy the heat-labile vitamins (Giami and Okwechime 1993). Also,

prolonged cooking of legumes decreases the protein quality of the cooked product owing to an increase in Maillard browning causing lysine to be rendered unavailable (Walker and Kochhar 1982).

Several hypotheses have been proposed to explain the causes of bean hardening (Garcia *et al.* 1998; Maurer *et al.* 2004) however, most researchers agree that the defect develops mainly in cotyledons (Reyes-Moreno and Paredes-Lopez 1993). Hincks and Stanley (1986) proposed a multiple mechanism of bean hardening which includes phytate loss as a minor contributor and phenol metabolism as a major contributor during storage. Hard-to-cook beans (*P. vulgaris*) had more pectates and three times more phenolics in the soluble pectic fraction (Garcia *et al.* 1998). The presence of more ferulic acid bound to soluble pectin in HTC beans may cause changes in cell adherence, thereby inhibiting cell separation when beans are cooked. This is supported by work of Srisuma *et al*. (1989), which shows that hydroxycinnamic acids (especially ferulic acid), associated with hardening, increased in HTC beans (*P. vulgaris* var. Seafarer) compared to control beans. Analysis of black common beans using Fourier Transform Infrared Spectroscopy revealed that more phenolics were associated with the soluble pectin fraction of HTC beans than in control beans (Maurer *et al.* 2004). Studies on changes in phenolic contents of faba bean in relation to cooking quality will be useful in understanding the problem and any possible solution.

Water absorption is one of the important quality factors associated with the bean hardness defect (Berrios *et al.* 1999). A loss in water absorption during soaking for a stipulated time period is a good indicator of the loss in bean quality during storage. Red kidney beans stored at 32°C for 9 months exhibited a 10% decrease in bean hydration compared to those stored at 2°C (Moscoso *et al.* 1984). Storage of faba beans for 9 months in underground pits or in tin cans stored at room temperature in Egypt led to a gradual reduction in hydration and swelling coefficients (El-Refai *et al.* 1988). Adzuki beans (*Vigna angularis*) stored at 30°C for 6 months absorbed less water than those stored at 20°C or 10°C (Yousif *et al.* 2002). Seeds of 9 cultivars of dry beans (*Phaseolus vulgaris* L.) stored at 16°C for 21 days had imbibition values between 0.61 and 0.73 whereas values for beans stored at 45^{o}C ranged from 0.61 to 0.66 (Jimenez *et al.* 1989). Similarly substantial reduction in water absorption capacities of chickpea varieties occurred when they were stored under unfavourable conditions of high temperature (33-35°C) and high relative humidity (75%)(Reyes-Moreno *et al.* 2000)

Low hydration and swelling coefficients of beans stored at high temperature can be due to structural and chemical changes in the testa making it harder and less permeable to water so that it acts as a barrier, preventing water reaching the cotyledons. This is supported by the observation that water sorption was much faster in hulled dried green beans than those with intact testa (Liu *et al.* 1992). The dehulled beans are known as dhal in the Indo-Pak subcontinent and it cooks quicker due to its faster water sorption compared to whole seeds with intact testa (Kon *et al.* 1973). The water diffusivity in beans is a complex function of the microstructure and chemical composition of bean, and especially the testa (Deshpande and Cheryan 1986; Liu *et al.* 2005). Microstructural features, such as thin bean coat, coat pores and large and open micropyle and hilum, tend to influence sorption rates (Deshpande and Cheryan 1986).

Secondly, structural and chemical changes in cotyledons can render them resistant to water absorption. Scanning electron microscopy revealed large intercellular spaces and small adhesion areas between cotyledon cells in black beans (*P. vulgaris* L.) stored at 4-5^{o}C and small intercellular spaces and large adhesion areas between cotyledon cells in beans stored at 23-25°C for 2 years (Berrios *et al.* 1998). Examination of soft cooked beans by scanning electron microscopy indicated cell separation and extensive disruption of the cell wall whereas HTC beans (stored at high temperature and high RH conditions) exhibited remnant linkages between cotyledon cells, minimal changes in the appearance of the cell wall and the presence of ungelatinized starch granules (Aguilera and Rivera 1990). Lignin-like materials deposited around bean cotyledon cells promote hardening, both as a result of its own mechanical strength as well as its action in preventing water imbibition and swelling (Hincks and Stanley 1986; 1987). This was also observed in black beans stored at 34°C for 6 months (Jones and Boulter 1983; Plhak *et al.* 1989) and in common beans (*P. vulgaris*) (Paredes-Lopez *et al.* 1989). Like other beans, water absorption studies on faba beans stored at different temperatures will provides a simple means to determine the effect of storage temperature on cooking quality.

Loss of solids and electrolytes during soaking of beans is an indication of cooking quality deterioration (Ching and Schoolcraft 1968; Jackson and Varriano-Marston 1981; Parrish and Leopold 1978). After 12 h soaking, the loss of solids and electrolytes from black beans stored at refrigeration temperatures (4-5°C) for 2 years was 10.5 mg g^{-1} compared with 18.6 mg g^{-1} for beans stored at 23-25°C for the same time period

(Berrios *et al.* 1999). Hentges *et al.* (1991) also reported that cowpeas (*Vigna unguiculata*) and dry beans (*P. vulgaris*) stored at 29°C for 24 months lost more solids and electrolytes than seeds stored at 5°C. Fresh black beans exhibited relatively minor solid loss and low electrolyte losses compared to HTC beans stored at 41°C for 55 days (Jackson and Varriano-Marston 1981). Electric conductivity of the soaked water after 12 h soaking of HTC black beans (stored at 23-25°C) was nearly twice(818 µohm) that of beans (414 µohm) stored at 4-5°C for 2 years (Berrios *et al.* 1999). Similarly, after accelerated aging of cowpeas at 40°C and 100% relative humidity solute leakage and electric conductivity showed progressive increases as aging period increased from 1 to 6 days (Asiedu *et al.* 2000). Increase in solute leakage and electrolyte loss with storage under Australian conditions of high temperature may also occur in faba beans causing a loss of nutrients. Studies are required to determine the level of electrolyte losses in relation to storage temperature.

2.5.2. Effect of storage conditions on chemical properties of beans

Storage under unfavourable conditions also affects major and minor nutrients of beans. Storage of faba bean for nine months led to a moderate reduction in their ash contents (El-Refai *et al.* 1988). Berrios *et al*. (1999) observed no change in protein contents in black beans (*Phaseolus vulgaris* L.) stored for 2 years at refrigerated temperature (4-5^{o}C) and beans stored at ambient temperature (23-25°C). In contrast, appreciable decreases in crude protein contents have been reported in different beans by some researchers. Crude protein content of faba bean stored in tin cans at room temperature decreased from 29.2 to 19.8% in 9 months (El-Refai *et al.* 1988). Protein content decreased from 9.3% (good cooking lentils) to 8.8% in seed coat of poor cooking lentils (*Lens culinaris* Madik) (Bhatty 1995). The oxidation of fats and the rate of rancidity development are highly dependent on the temperature; in that way the higher the temperature the higher is the rate of rancidity (Harris *et al.* 1972; Nogala-Kalucka and Gogolewski 2000). Similar changes in nutrient composition are expected in faba beans that are stored at adverse storage conditions of high temperature in Australia.

Storage conditions cause changes in composition and thickness of testa and cotyledon cell walls in beans and this affects imbibition of water during soaking and cooking which are very important in determining cooking time. Adzuki beans stored at 30°C for 6 months had higher cell wall contents compared to beans stored at 10°C or 20°C (Yousif and Deeth 2003). Scanning electron microscopy of HTC common beans

showed a thickening of walls at the cell junction (Garcia *et al.* 1998). Rozo (1982) also observed an increase in cell wall thickness of red kidney beans stored at 40°C. The cell wall thickness may be attributed to lignification (Hincks and Stanley 1987) and/or due to tannins binding with protein components of cell wall and middle lamella (Sievwright and Shipe 1986). Lignin content of mung beans (*Vigna radiata* L. Wilczek) was 7 times higher in hard seeds than normal seeds (Rodriguez and Mendoza 1990). The lignification mechanism assumes that aromatic compounds, migrating from seed coats (Stanley 1992a) and biosynthesized using aromatic amino acids released from protein bodies (Hohlberg and Stanley 1987), accumulate at cell wall surfaces where they act as precursors in lignification-link reactions. Lignin serves as a matrix around the polysaccharide components of some plant cell walls, providing additional rigidity and compressive strength as well as rendering the walls hydrophobic and water impermeable (Whetten and Sederoff 1995). Lignins, when bound to the cell wall polysaccharide, confers rigidity to the cell wall (Lewis and Yamamoto 1990). Lignins deposited around bean cotyledon cells promote hardening, both as a result of their own mechanical strength as well as their action in preventing water imbibition and swelling (Hincks and Stanley 1986; 1987). During the development of seeds of two legumes, *Crotalaria spectabilis* Roth and *Sesbania exaltata,* soluble phenolics of seed coat converted to lignins and that resulted in onset of impermeability of seed coats (Egley *et al.* 1985). Several studies conclude that lignification of the middle lamella in stored legumes might partially explain their decreased cookability (Del Valle and Stanley 1995; Varriano-Marston and Jackson 1981). There is evidence that deposition of lignin can even occur in cells that are no longer alive (Pickett-Heaps 1968) and this may play a major role in the cotyledon cell wall hardening that renders faba and other beans hard to cook. Similar changes in cell wall contents, especially lignins, may happen in faba beans during storage under adverse conditions. Studies on cell wall contents, including lignin content changes, under a range of storage conditions will be helpful in understanding the HTC phenomenon in faba beans.

2.5.3. Involvement of Phenolic compounds in bean hardening

Phenolic contents of beans change with storage depending upon the time and conditions and this may affect the cooking quality. The content of polyphenols of dry beans (*P. vulgaris*) stored for 5 years at 30-40°C was lower than freshly harvested beans across a range of cultivars (Martin-Cabrejas *et al.* 1997). The total amount of phenolics per gram in common beans (*P. vulgaris*) stored at 35°C/75%RH for 6.5 months (making it HTC)

was one fifth of those stored at 4°C/40% RH for the same time period (Garcia *et al.* 1998). There was a negative correlation (r = −0.30) for cooking time and total polyphenols in four cultivars of Nigerian cowpeas (*Vigna unguiculata* L. Walp) (Giami and Okwechime 1993). Polyphenol content decreased as maturity of winged bean (*Psophocarpus tetragonolobus*) increased, with a concomitant increase in cooking time over similar maturity stages (Kadam *et al.* 1982). For seed coats of red kidney beans stored under adverse conditions (40^{o}C, 80% RH) the total soluble condensed tannin decreased as hardness developed and the correlation between these two variables was highly significant ($r = -0.85$, $P \leq 0.01$) (Rozo *et al.* 1990). Beans (*P. vulgaris*) stored at 30°C for one year demonstrated increase in hardness with decrease in tannin content compared with beans stored at 15°C (Stanley 1992b). Davies (1994) found a decrease in proanthocyanidins (condensed tannins) of faba beans after accelerated aging at 40°C and 100% RH. Phenolic, particularly tannin, contents of beans demonstrate a correlation with hardness which may also exist in faba bean. A study on the changes in phenolic contents in relation to faba bean hardness will be useful in understanding the hard-to-cook phenomenon.

In contrast to these studies some researchers conclude that hardness may be due to other changes beside tannins mainly present in bean testa (Deshpande *et al.* 1982). This is because significant hardening also occurs in white varieties of beans (*P. vulgaris*) containing low concentrations of tannins compared to black varieties (Stanley 1992b). Perhaps there are two (or more) types of bean hardness: (1) hardness related to seed coat (testa) impermeability and (2) hardness related to cotyledon impermeability (Morris *et al.* 1950). The former type may involve lignins as well as tannins whereas the latter type may mainly involve lignification as cotyledons are very low in tannin contents compared with testa. Hence cotyledon hardening may be similar in white and coloured varieties/cultivars because both are very low in tannins and it may primarily depend upon involvement of lignins in the hardening process. Coloured varieties may differ in testa hardening. Testa hardening may be greater in case of coloured varieties/cultivars as it may involve tannins, in addition to lignins, in the hardening process. As cotyledons constitute the major proportion (about 90%) (Deshpande *et al.* 1982) of beans, extra hardening of testa due to tannins in coloured varieties/cultivars may not cause an appreciable difference compared with seed hardening of white varieties/cultivars. Studies on the changes in phenolic contents of faba beans in relation to hard-to-cook

phenomenon are required to establish a relationship that can be exploited to manage the hardness problem during storage.

2.6. Conclusions and Research Objectives

Seed discolouration is a serious problem in faba bean that reduces its value and market opportunities for human consumption. Seed can be stained in the field before harvesting or discolour during storage. The cause of preharvest discolouration, most commonly known as environmental staining is unknown but a number of environmental factors especially during pod and seed formation are thought to have a large effect on the degree of staining. A study of a range of environmental factors under different field conditions is required to identify the factor/s responsible for or involved in environmental staining. The following hypotheses are addressed in Chapter 3.

Hypothesis 1: Faba bean plants grown under more stressful conditions produce seeds with more environmental staining

Hypothesis 2: Genotype influences environmental staining via a G x E interaction. New varieties stain less than old varieties.

Discolouration in faba beans also happens after harvest when seeds are stored under unfavourable conditions. Seed colour darkens from beige or buff (the original colour at harvest) to dark brown or even chocolate brown and this depends upon a number of storage factors including temperature, moisture (seed moisture content or relative humidity of the storage environment) and light. These factors need to be investigated thoroughly to understand the colour darkening phenomenon and to find practices and methods to control or minimise it.

Hypothesis 3: Higher storage temperature, seed moisture content and light intensity enhance storage discolouration in faba bean. Some varieties are worse than the others (addressed in Chapter 4).

Phenolics, which are mostly present in the seed testa of faba and other beans, are involved in colour development. Changes in phenolic contents especially tannins are evident in different legumes in relation to colour darkening of seed testa during storage.

The phenolic compounds vary widely in their structural and functional properties but the common characteristic of all these compounds is that they are readily oxidised.

Hypothesis 4: Phenolic compounds are involved in colour darkening of faba bean seeds during storage (addressed in Chapter 4).

Hypothesis 5: Modified atmosphere packaging can reduce colour darkening during storage (addressed in Chapter 5).

Storage under unfavourable conditions not only causes colour darkening but it also affects other quality parameters in beans especially the cookability. Storage under unfavourable conditions develops the hard-to-cook defect in many legumes and this reduces their consumer acceptability. Faba beans are stored under high temperature conditions in Australia that quite often exceeds 40°C (in summer months) and this may deteriorate cooking quality. Storage under cooler temperatures will be helpful in maintaining the cooking quality but it requires extra cost. A study is required to find out the maximum temperature at which faba bean can retain cooking quality for a whole year.

Hypothesis 6: Storage at high temperature deteriorates cooking quality of faba bean but there is an acceptable maximum temperature at which faba beans can maintain their cooking quality (the hypothesis is addressed in Chapter 6).

CHAPTER THREE

Effect of site, harvesting stage and genotype on environmental staining in faba bean (*Vicia faba* L.)

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Effect of site, harvesting stage and genotype on environmental staining in faba bean (*Vicia faba* L.)

Syed M. Nasar-Abbas[1,2*], Julie A. Plummer[1,3], Peter White[4,2], Kadambot H. M. Siddique[2,3], Mario D'Antuono[4], David Harris[5,2] and Ken Dods[5,2]

[1]*School of Plant Biology, Faculty of Natural and Agricultural Sciences, The University of Western Australia, 35 Stirling Hwy, Crawley WA 6009, Australia*
[2]*Centre for Legumes in Mediterranean Agriculture (CLIMA), The University of Western Australia, 35 Stirling Hwy, Crawley WA 6009, Australia*
[3]*Institute of Agriculture, The University of Western Australia, 35 Stirling Hwy, Crawley WA 6009, Australia*
[4]*Department of Agriculture and Food Western Australia, 3 Baron-Hay Court, South Perth WA 6151, Australia*
[5]*Chemistry Centre Western Australia, 125 Hay St, East Perth WA 6004, Australia*
**Corresponding author; e-mail: abbass01@cyllene.uwa.edu.au*

Abstract

Seed discolouration due to environmental staining in faba bean leads to poor quality and reduced market price. Environmental staining in faba bean is characterised by a dark brown, grey or black discolouration of the seed coat at harvest. Its cause is unknown, but it does not appear to be caused by a pathogen. Environmental conditions during pod and seed formation and at maturity are thought to have a large effect on the degree of environmental staining. To test the hypothesis that seeds formed under stressful conditions will have a higher degree of staining, faba bean seeds were harvested at two different stages of maturity from trials located in a range of environmental conditions under a Mediterranean-type climate of south west Australia over two seasons. Four faba bean varieties were studied (Fiord, Fiesta, Ascot and Cairo).

The majority of seeds had good colour but across the trials 3-25% were stained up to an unacceptable level and this varied with location and variety. Seeds formed later in plant development (located on the upper nodes of the plant) had more staining than seeds formed earlier (located on the lower nodes). Seeds formed on small and weak plants, had more staining than seeds formed on normal sized healthy plants. Fiord showed a greater amount of staining than Ascot, Fiesta and Cairo when grown in the mild, southern environments. Early harvesting (at physiological maturity) did not reduce environmental seed staining compared with harvesting at full maturity.

Chemical analysis of seed testa and cotyledons revealed that total phenolic contents of the testa and cotyledons increased with staining. An increase in Zn and Na and a decrease in K concentration in the testa were also associated with increased staining levels.

Keywords; Pulses, grain legumes, seed discoloration, phenolics, nutrients

Introduction

Australia is a major exporter of faba bean for human consumption. The price received for the grain depends mainly on the colour of the seed coat. Faba beans that are dark coloured or that have blemishes are not acceptable to consumers. In Australia up to 20% of the faba bean seeds harvested per annum are discoloured and potentially not suitable for human consumption. Preharvest discolouration of seeds is broadly divided into two types; disease discolouration caused by fungal and viral diseases and environmental staining. Environmental staining in faba beans is characterized by a dark brown, grey or black discolouration of the seed coat at harvest. The causes of environmental staining are largely unknown and it is usually the main form of discolouration in Western Australia (White *et al.* 2004). Casual observations suggest it is related to environmental stress and is induced when seeds mature under hot and dry conditions. Environmental factors, such as air temperature, water and nutrient availability, and light intensity and photoperiod affect plant metabolism in faba bean, lupin and other legumes (Chetia *et al.* 2005; Keeve *et al.* 1999) and affect various aspects of seed colour at harvest in a range of species (Buchsbaum *et al.* 1990; Herrmann and Woldecke 1977; Muzika 1993). These factors may also play a role in faba bean staining. The extent of staining appears to vary among different varieties. Advanced lines within the Australian National Faba Bean Improvement Program are screened for environmental staining with the result that recent varieties (e.g. Fiesta) show less environmental staining than older varieties (Fiord or Ascot) (White *et al.* 2004).

Phenolic compounds play an important role in many aspects of plant metabolism, including colour development in seeds and other plant parts (Beninger and Hosfield 2003). Phenolic compounds are also involved in the interaction of plants with the external environment (Zucker 1983) and may be involved in faba bean seed coat staining and discolouration. Phenolic compounds are carbon-based secondary compounds synthesised at least partially, through the phenylpropanoid pathway.

Environmental factors can have profound effects on phenotypic variation in quantitative patterns of secondary metabolism (Waterman and Mole 1989). Warm conditions, light intensity and duration, and lack of nutrient availability increase bulk phenolics and condensed tannins, whereas fertilisation and shading generally decrease concentrations (Graglia *et al.* 2001; Muzika 1993). Seeds developing towards the end of the growing season are normally born at the upper part of the plant canopy and are exposed to increased light intensity and higher temperatures and water stress that may affect discolouration intensity.

Seeds formed under more stressful environment will have more staining was the hypothesis which was tested by conducting field trials at different locations in the grain belt of south west Australia. The selected sites exposed faba bean plants to a range of environmental conditions which may affect the level and intensity of environmental staining.

Materials and Methods

Field Trials

To study factors in relation to seed staining in faba beans, the Australian National Faba Bean Improvement Programme's trials at five locations were used and these had a range of environmental conditions. The trials were located at Katanning, Borden (2003), York, Mingenew and Dongara (2004) within the grain belt of south west Australia. The details of each location are given in Table 1.

Table 1. Trial and soil characteristics at 5 sites in Western Australia

Location	Latitude	Longitude	pH at depths (cm)			Soil type
			10	40	65-90	
Borden	34.07 S	118.26 E	5.0	5.5	4.5	Red brown non-cracking clay
Katanning	33.69 S	117.58 E	5.5	5.5	7.0	Grey deep sandy duplex
York	31.96 S	116.85 E	5.0	7.5	9.0	Red deep loamy duplex
Dongara	29.24 S	115.07 E	7.5	7.5	8.5	Red brown cracking clay
Mingenew	29.26 S	115.61 E	5.5	5.5	6.0	Red loamy earth

Four commercial faba bean varieties in Australia (Fiord, Fiesta, Ascot and Cairo) were sampled from each experiment laid out in a randomised complete block design with 4 replicates. Standard management practices for the control of fungal diseases with prophylactic applications of mancozeb and chlorothanolin was used. As a result there

was very little disease on the foliage of plants and no sign that the disease occurred on the pods or seeds in this study. Insect damages were controlled by spraying insecticides as and when required. Plants were first harvested at physiological maturity (the plants started to turn brown but still had patches of green and were soft and pliable). The second harvest was 2-4 weeks later (depending upon the weather conditions especially air temperature of the site) at full maturity when plants were completely brown, dry and brittle.

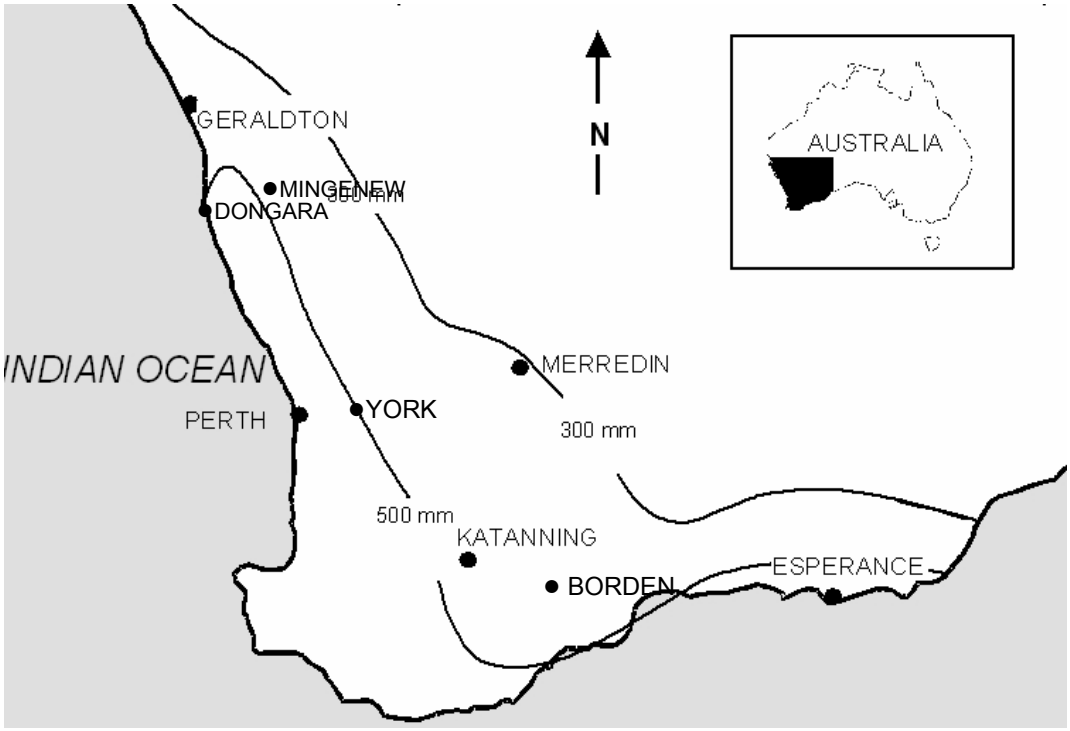

Figure 1. Map indicating the trial location in the grain-belt of south west Australia

Plant measurements

Five normal sized (similar to most of the plants in a trial) and 5 small (smaller and weaker than the normal plants in a trial) plants were randomly selected and individual plants were harvested at ground level from each plot (except Borden and Mingenew sites where only normal sized plants could be collected). The main stem of each plant was selected for detailed study. Seeds were separated by hand from pods at each node and were scored for staining using the following scale; non-stained = 1 (no sign of discoloration seen by the naked eye), slightly stained = 2 (small area generally around hilum lightly stained), moderately stained = 3 (brown grains on most of the seed surface), highly stained = 4 (dark brown to black patches on most of the seed surface),

badly stained = 5 (seed almost black due to severe staining all over the seed surface). The staining was distinct from seed coat darkening that is commonly seen in faba beans during storage (Nasar-Abbas *et al.* 2007b).

Seed bearing nodes on the main stem were divided into 3 groups; top 2 nodes (group 3), next 2 lower nodes (group 2) and rest of the nodes at the bottom of the plant (group 1). Seeds at each node were counted, scored for staining level and packed in separate paper bags. The bags were stored at 37°C for one week for dehydration and then seed weights were measured for each bag.

Statistical analysis

Within each site the interactions of the 3 groups of nodes with plant size, variety and harvest on the average staining score per node and average seed weight per node were examined by fitting a linear mixed model to these data. The model included fixed effects for variety, harvest, plant size and node groupings and interactions between these factors. Random effects in the model included terms representing the split plot structure of harvests within plots.

Genotype by environment interactions on staining score, average seed weight and percent unacceptably stained seeds (≥ "moderately stained" seeds) were examined by fitting a linear mixed model to data from normal plants at each site. Small plants, harvested at Dongara, Katanning and York, were excluded from this analysis as they were not representative of the crop. The model included fixed effects for site, variety, harvest and interactions between these factors. The node, from which seed was collected, was ignored in this analysis across sites. Random effects in the model included terms representing the split plot structure of harvests within plots at each site and a correlation model allowed for different experimental variance at each site.

The relationship between staining score and average seed weight was also examined using a linear mixed model with the same random model as described above. The fixed model incorporated intercepts and linear responses to average seed weight which were allowed to change with all combinations of variety, site and harvest. All analyses were carried out using the REML procedure in GenStat (GenStat for Windows, 9th Edition, VSN International Ltd, Rothamsted, England).

Determination of Phenolic Constituents

Total free phenolics, tannins and condensed tannins (proanthocyanidins) in faba bean (variety Fiord) seeds environmentally stained to different levels were determined in testa and cotyledons separately. Testa of seeds (3 x 10 seed) were manually removed and the hilum excised and discarded. Testa and cotyledons were ground separately with a grinder (IKA® A11 basic, IKA®-WERKE GmbH & Co. Germany). Ground testa (0.2 g) and cotyledons (2 g) were extracted with 20 ml of 70% v/v aq. acetone (analytical grade) by applying 20 min ultrasonic treatment at 4°C followed by overnight mechanical tumbling. Extracts were analysed for total phenolics using the Folin-Ciocalteu's Phenol Reagent (Merck) according to the method of Makkar *et al*. (1993). Total phenolic compounds were calculated from a prepared standard curve of tannic acid (Merck) in an identical matrix. Tannins were complexed with polyvinylpolypyrrolidone (Sigma) and unbound phenolics determined as above. Total tannins were calculated by subtracting non-tannin phenolics from total phenolics. Condensed tannins (proanthocyanidins) were determined according to the methods of Porter *et al*. (1986).

Mineral content analysis

Samples prepared for phenolic analysis (detailed above) were analysed for P, K, Na, Ca, Mg, S, B, Cu, Fe, Mn, Mo and Zn contents by using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) with Radial Mini Torch ARL 3580 B after digestion with a mixture of nitric and perchloric acids (McQuaker *et al.* 1979).

Results

Environmental conditions

Growing season at Katanning and Borden was during May to December; at York May to November and at Dongara and Mingenew it was May to October. The total rain fall received during the growing season was 354, 384, 334, 170, and 350mm at Katanning, Borden, Dongara, Mingenew and York, respectively. The monthly average minimum and maximum air temperature varied between 6-12°C and 15-29°C at Katanning, 6-12°C and 15-27°C at Borden, 8-11°C and 19-25°C at Dongara, 5-12°C and 18-29°C at Mingenew, and 3-11°C and 16-28°C at York, respectively (Figure 2). The minimum air temperature, at Katanning and York, sometimes dropped to −2°C and at Borden to 1°C.

Seed staining

Across all sites about 20% to 53% of seeds had some staining and this was mostly in the "slightly stained" category. Three to 25% were "moderately stained" or worse across the trials (Table 2 and 3).

Table 2. Proportion (%) of unstained seeds and stained seeds classified under 5 categories. Seeds were pooled for 4 varieties grown during 2003 and 2004 at 5 locations in south west Australia

Location	Level of staining				
	Non-stained	Slightly stained	Moderately stained	Highly stained	Badly stained
Borden	67.9 ± 5.0	27.7 ± 2.5	4.3 ± 2.6	0.2 ± 0.1	0.0 ± 0.0
Katanning	46.6 ± 4.7	30.3 ± 1.5	18.7 ± 2.6	4.2 ± 1.0	0.2 ± 0.1
York	73.8 ± 1.5	22.0 ± 1.0	4.0 ± 0.7	0.2 ± 0.1	0.0 ± 0.0
Dongara	80.0 ± 1.3	14.6 ± 0.7	4.5 ± 0.9	0.9 ± 0.3	0.1 ± 0.1
Mingenew	78.2 ± 2.7	18.3 ± 2.3	2.8 ± 1.2	0.0 ± 0.0	0.0 ± 0.0

Means ± s.e. of four varieties

Table 3. Proportion (%) of unstained seeds and stained seeds classified under 5 categories. Seeds were pooled variety wise from trials grown during 2003 and 2004 at 5 locations in south west Australia

Variety	Level of staining				
	Non-stained	Slightly stained	Moderately stained	Highly stained	Badly stained
Ascot	71.2 ± 7.8	21.3 ± 3.7	6.6 ± 3.5	0.8 ± 0.8	0.1 ± 0.1
Cairo	71.0 ± 6.5	22.0 ± 3.4	6.0 ± 3.2	1.0 ± 0.8	0.0 ± 0.0
Fiesta	72.2 ± 3.8	23.1 ± 2.4	4.1 ± 2.4	0.4 ± 0.4	0.1 ± 0.1
Fiord	64.4 ± 9.9	23.6 ± 4.7	10.2 ± 4.8	1.8 ± 1.4	0.0 ± 0.0

Means ± s.e. of five locations

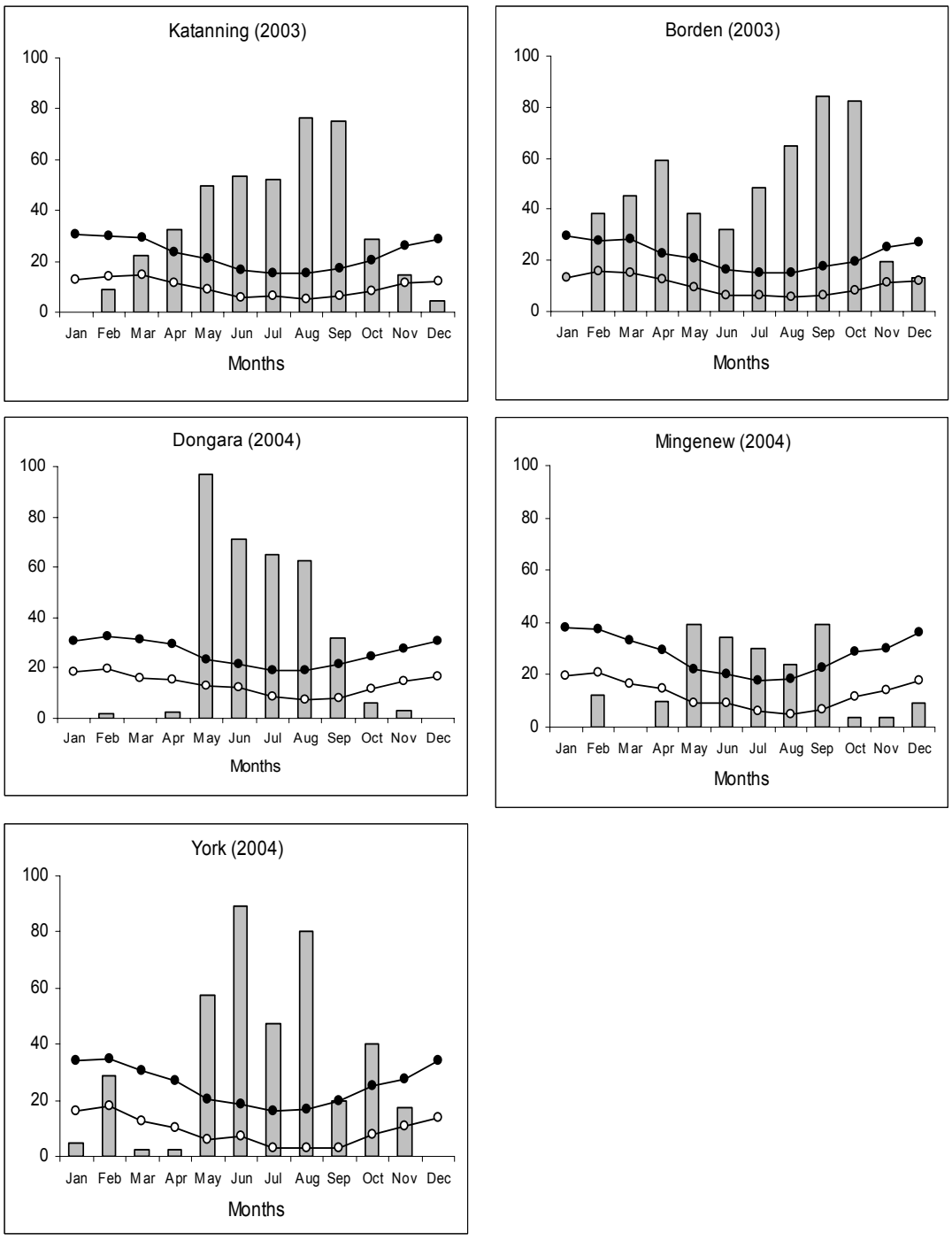

Figure 2. Weather data at trial sites in Western Australia during 2003 and 2004: —○—, Minimum air temperature (°C); —●—, Maximum air temperature (°C); Bars (histogram) represent monthly rainfall (mm).

Staining varied with location, variety and position of the seed on the plant and some trends were evident. Seeds from the top of the plant (group 3 seeds) had more staining ($P<0.001$) than those at the lower nodes of the plant Figure 3). Seeds from the middle of the plant (group 2) had staining which was generally similar to that in seeds at the

bottom of the plant (group 1) and this trend was consistent at all locations (Figure 3). Plant size also demonstrated a clear effect on staining. Seeds from smaller and weaker plants had higher staining ($P<0.001$) than those from normal sized plants (Figure 3).

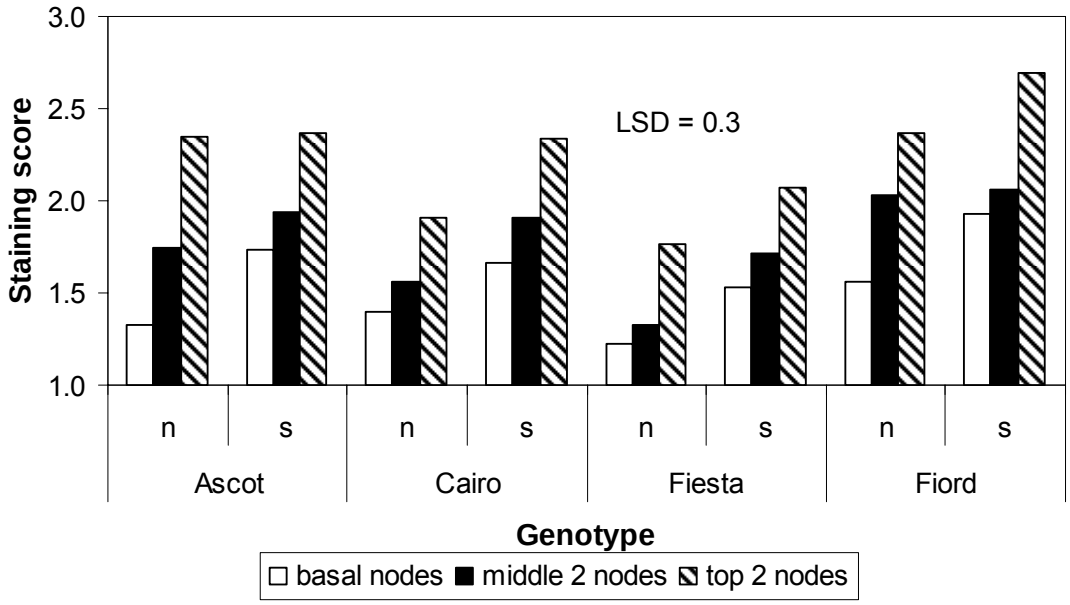

Figure 3. Effect of genotype, plant size and seed position in the plant canopy on environmental staining in faba beans (n = normal sized plants; s = smaller plants).

Varieties performed differently at Katanning and Borden. Ascot, Fiesta and Cairo had seeds of similar staining level and this was less than Fiord (Figure 4). All varieties had the same level of staining at the other sites. Katanning and Borden generally had higher staining level compared to other sites.

Staining of seeds harvested at physiological maturity was similar to that of seeds harvested at full maturity (Figure 5). The trend was similar at all sites and for all varieties.

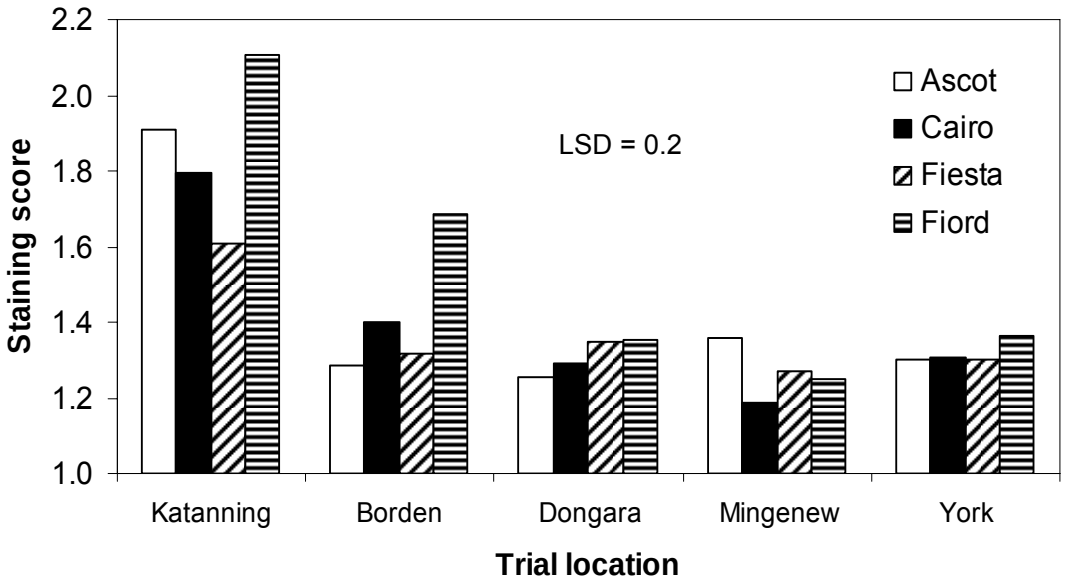

Figure 4. Effect of site and genotype on environmental staining in faba beans grown in grain belt at south west Australia

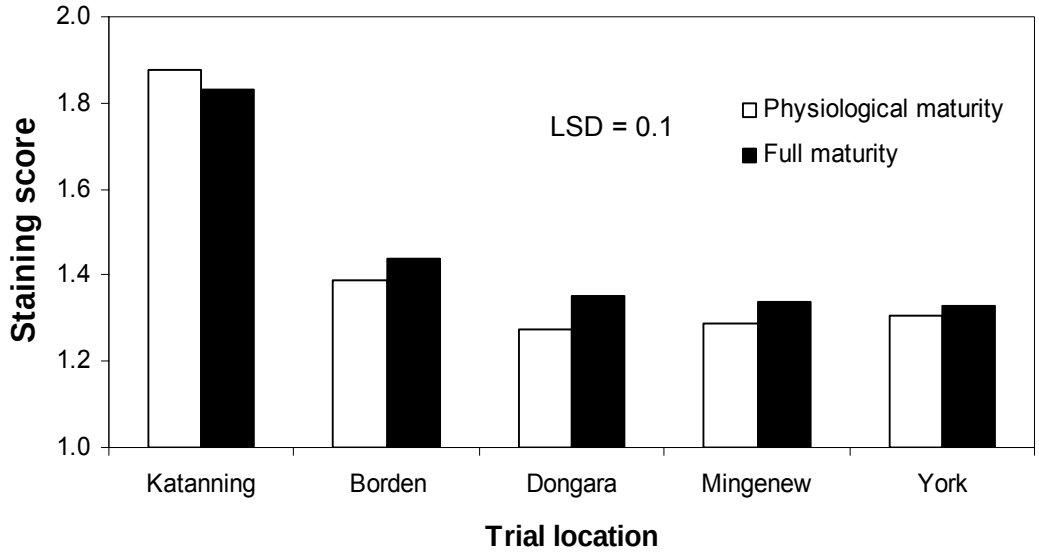

Figure 5. Effect of harvesting at physiological maturity and at full maturity on staining of faba beans grown at different locations in the grain belt at south west Australia.

Seed Weight

Seed weight varied (P<0.001) with node groups for each variety. The staining score response to average seed weight was the same for all sites, excluding Katanning. At these sites staining decreased by 0.075 ± 0.007 score for every increase of 0.1 g in average seed weight. Again seeds borne on smaller and weaker plants were lower in weight than those of normal plants and this was associated with staining. Seeds from smaller plants had higher (P<0.001) staining than those from normal plants. This was reflected in a linear decline in staining score as seed weight increased (Figure 6).

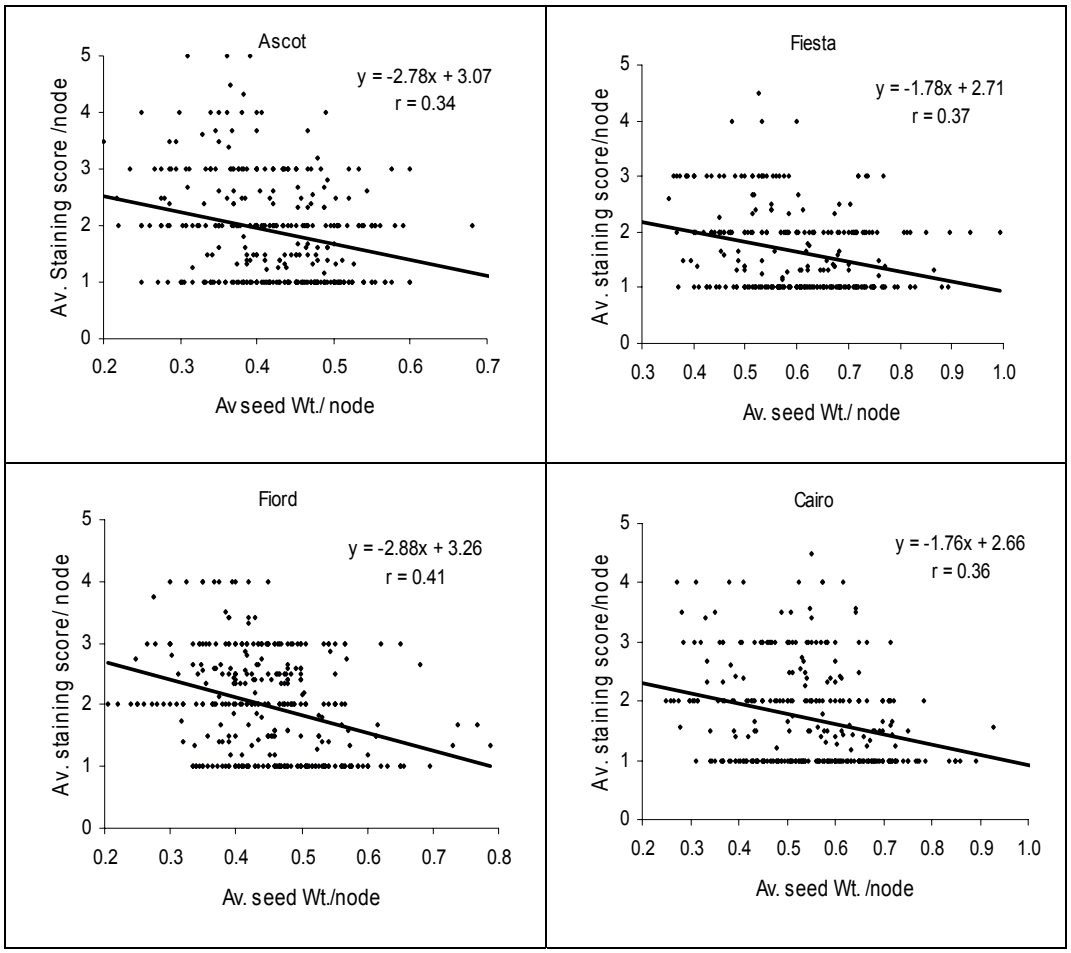

Figure 6. Correlation between seed size (average seed weight per node) and staining level in different varieties of faba bean.

Genotype by environment interactions for staining score and average seed weight revealed that only site had an effect (P<0.001) on the staining score of seeds from normal plants Katanning had significantly higher staining levels than all other sites followed by Borden which was also higher than Mingenew which was significantly higher than York and Dongara (Figure 7).

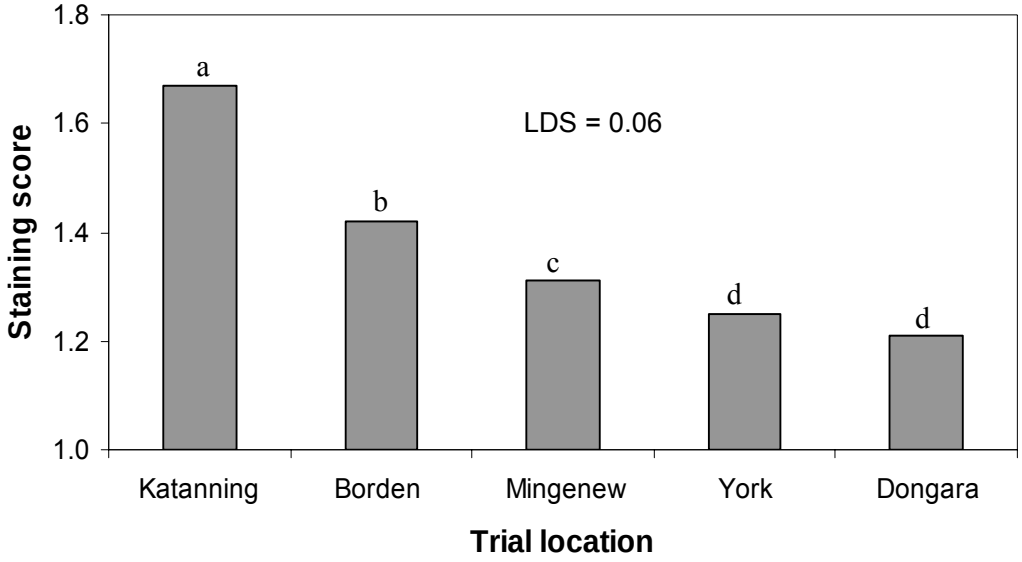

Figure 7. Effect of site on staining of faba beans grown in the grain belt at south west Australia (columns denoted by different letters are significantly different at P<0.001).

There was a site by variety by harvest interaction for both average seed weight and percentage of seeds with unacceptable staining (P<0.001). At most sites and at most times of harvest Ascot and Fiord had lower average seed weights than Cairo and Fiesta and there was very little difference in seed size between early and late harvest. At Borden and Katanning, Fiord had a higher percentage of unacceptable seed than other varieties but this was not the case at Dongara, Mingenew or York where there was no difference between varieties. At Katanning only Fiesta had a low percentage of unacceptable seed at both harvests.

Seed Phenolics

Faba bean testa was very rich in phenolic contents as compared to cotyledons and they were associated with staining. Total free phenolic contents increased with the increase in staining intensity. In testa, it increased from 62 mg g^{-1} in non-stained seeds through

67 mg g^{-1} in slightly stained seeds to 70 mg g^{-1}in highly stained seeds (Table 4). Total free phenolics also increased in cotyledons of stained seeds. They were1.9 through 2.2 to 2.4 mg g^{-1}in normal seeds, slightly stained seeds and highly stained seeds, respectively.

Table 4. Phenolic contents of faba bean (variety Fiord) samples with different levels of environmental staining

Staining level	Total phenolics (mg of tannic acid g^{-1})	Non-tannin phenolics (mg of tannic acid g^{-1})	Total tannins (mg of tannic acid g^{-1})	Proanthocyanidins (mg of leucocyanidin g^{-1})
Testa				
Non stained seeds	62.1 ± 0.4a	17.5 ± 0.4a	44.5 ± 0.7a	44.5 ± 0.3a
Slightly stained seeds	66.7 ± 0.4b	21.3 ± 0.7b	45.4 ± 0.9b	45.6 ± 0.2b
Highly stained seeds	70.3 ± 0.6c	24.1 ± 0.4c	47.0 ± 0.6b	46.5 ± 0.5b
Kernels				
Non-stained seeds	1.9 ± 0.1a	2.0 ± 0.2a	ND	ND
Slightly stained seeds	2.2 ± 0.1b	2.3 ± 0.2b	ND	ND
Highly stained seeds	2.4 ± 0.1c	2.4 ± 0.1c	ND	ND

Means, ± s.d of 3 observations, sharing the same letter in a column are not significantly different ($p \leq 0.05$) according to Tukey's HSD test. ND= not detected.

Seed nutrient contents

Analysis of seeds for macro and micro elements revealed that testa contain much higher concentrations of Na, Ca, Mg and B as compared to cotyledons. There was an increase in Na, Ca, Mn and Zn concentration and a decrease in K content in the testa of stained seeds as compared to the testa of not stained seeds. Other elements including P, Mg, S, B, Cu, Fe, and Mo did not have a definite trend (Table 5).

Table 5. Macro and micronutrient composition of faba bean (variety Fiord) samples with different levels of environmental staining

Staining level	Macronutrients (g kg^{-1})						Micronutrients (mg kg^{-1})					
	P	K	Na	Ca	Mg	S	B	Cu	Fe	Mn	Mo	Zn
Testa												
Non stained seeds	0.3	3.6	3.6	3.3	2.2	0.3	21	1.3	56	22	<2	5.4
Slightly stained seeds	0.3	3.5	4.5	3.3	2.2	0.3	23	1.5	150	27	<2	7.5
Highly stained seeds	0.2	3.1	4.4	3.6	2.1	0.3	21	1.1	24	24	<2	6.8
Kernels												
Non-stained seeds	3.3	8.7	0.7	0.4	0.9	1.6	6	8.8	59	11	<2	29
Slightly stained seeds	3.3	8.7	0.9	0.4	0.9	1.6	6	8.2	65	11	<2	28
Highly stained seeds	3.3	9.0	1.0	0.4	0.9	1.6	6	7.8	57	12	<2	30

Discussion

Most seeds were either un-stained or slightly stained and individually they would usually be suitable for marketing for human consumption depending on the price and demand. However, seeds are sold in bulk and a small proportion (about 5% at most of the locations) that had a staining level of "moderately stained" and above may cause substantial problems in marketing the produce for human consumption. According to Australian Pulse Trading Standards 2006-2007 (Anonymous 2007b) maximum 1%, 3% and 7% (by weight) discoloured seeds are the acceptable limits for Canning grade, No.1 grade and No. 2 grade faba beans, respectively, for domestic as well as international markets. Thus most of the faba bean produced in this study would come under No. 2 grade which may not be acceptable as such in certain markets for human consumption. The problem with staining may be further exacerbated by storage discolouration (Nasar-Abbas *et al.* 2007b). So that over time 5% discoloured seeds can quickly exceed the 7% level if stored incorrectly.

Seeds borne on the top of the plant had more staining and that was associated with higher phenolic contents. This might be due to their greater exposure to light and high temperatures than seeds lower in the canopy. High light intensity, high temperature and longer photoperiod can increase concentrations of phenolic compounds which are involved in colour development in different parts of plants (Beninger and Hosfield 2003; Graglia *et al.* 2001; Tso *et al.* 1970; Vergeer *et al.* 1995). Faba bean crops in the

grain belt of south west Australia mature during October-December (during late spring/early summer). Seeds developing at the end of growing season are at the top of the plant and are exposed to high light intensity, warmer temperatures and increasing day length (13-14 h). So higher light intensity, higher temperature coupled with longer duration at the later stage of plant development may similarly increase phenolic contents in faba bean seeds that may lead to increased seed discolouration.

Environmental staining in faba beans may have some similarities to that of black point in wheat and barley which is a dark discolouration of the embryo end and ventral surface of kernels (Conner and Davidson 1988). Environmental conditions (rain, humidity, temperature) are also reported to affect the production of black point symptoms (Conner *et al.* 1992; Fernandez *et al.* 1994). Peroxidase activity found in the black point region of wheat and barley suggests that phenol peroxidases are involved in the dark discolouration (Cochrane 1994b; Williamson 1997). The phenols and peroxidases involved in producing black point symptoms in wheat and barley can increase under stressful environmental conditions such as drought (Cochrane 1994a; Smirnoff and Colombe 1988). The same phenomenon might have occurred in faba bean.

The varietal difference was more prominent at southern sites (higher staining sites) with Fiord demonstrating clearly higher staining than the other varieties (Figure 4). At other sites the overall level of staining was probably too low for varietal differences to be evident. These observations suggest that the problem may be successfully managed in the future by selection and subsequently production of varieties with low staining susceptibility. Staining was associated with certain sites and a careful selection of site for varieties may also help in reducing the problem. Staining in the future may only be a problem in certain environments (such as Katanning) or in certain years and that if we could predict these conditions better management of the problem would be improved. Fiord and Ascot are older varieties whereas Fiesta and Cairo were selected from breeding populations that had been developed partly on the basis of reduced environmental staining. The benefits of this selection by breeders were borne out across these locations.

Small and weak plants had more staining than normal plants and this may have been due to lack of nutrient availability, which would affect phenolic metabolism. Small and weak plants may have been due to reduced access to water, due to restricted root growth

or poor soil structure. Similarly plants developing in dry seasons after late sowing appear to have more staining (White *et al.* 2004). It can also be explained by the carbon/nutrient balance mechanism (Bryant *et al.* 1983; Koricheva 2002) which is disturbed by reduction in nutrient mobility due to reduced or lack of water availability in the later stage of plant growth under south west Australian conditions and this could lead to increased phenolic production. The reduced growth of faba bean plants in a population may be due to competition among plants or due to unequal distribution of water or nutrients in a field. Plants that grow in a dense population may not get adequate nutrients leading to reduced growth and enhanced phenolic metabolism resulting in more stained seeds. Plant phenols accumulate under conditions when plant growth is limited by the supply of mineral nutrients. It is supported by the observation that in rain-forests, plants growing in dense stands on exceptionally poor soils contain higher concentration of phenolics than the average for that site (McKey *et al.* 1978). Certain agronomic practices including maintenance of proper plant to plant distance, proper fertiliser application, better site selection and adequate sowing time for that site can be helpful in controlling this factor and may help in reducing the staining.

The hyper and hypo availability of certain nutrients affect phenolic metabolism. Accumulation of Ca and Zn and a reduction in K content was associated with higher phenolic contents (Table 4) and this is supported by the results from other researchers. The content of Ca was positively correlated with phenolic contents and liming caused an increase in phenolic contents in needles of Scots pine (Giertych *et al.* 1999). Accumulation of Zn was also associated with increase in phenolic contents in needles of Scots pine (Giertych *et al.* 1999). The decrease in K may be associated with a greater loss of K cations through membranes because of an increase in their permeability caused by increased contents of phenolics (Glass and Dunlop 1974). The data for nutrient changes presented here are preliminary and provide insufficient information. This aspect requires specific experiments focused on the effect of different macro- and micro-nutrients on seed staining in faba bean.

Seeds formed in the later stage of plant growth (i.e. on the top nodes) had more staining than those at the lower nodes and these seeds were also, generally, smaller in size (Loss *et al.* 1997). Separating out smaller seeds by using a gravity table may also separate most of the discoloured seeds because they are smaller. This can provide an economical way of improving the quality of the produce. Further processing using electronic colour

sorters can ensure top quality product. These practices are especially useful for high value faba beans for canning markets.

Conclusions

Environmental staining adversely affects on the quality and marketing of faba bean for human consumption. The problem is complex and involves a number of factors. Southern sites of the grain belt had a greater level of staining than the northern sites but the reason for this is unclear. It could be a combination of temperature, rainfall, light intensity, soil fertility, water logging or other factors but a few clear trends have emerged from this study that lead to certain practices that can help reduce the problem. Correct choice of varieties and location with robust agronomic practices to establish healthy plants can minimise the staining at harvest. After harvest, the correlation between staining and small seeds may allow processing of seeds using mechanical grading and electronic colour sorters to ensure good quality product for human consumption. Further screening and evaluation of wide rage of germplasm for reduced level of environmental staining are important strategies for the future.

CHAPTER FOUR

Faba bean seeds darken rapidly and phenolic content falls when seeds are stored at higher temperature, moisture and light intensity

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Faba bean seeds darken rapidly and phenolic content falls when seeds are stored at higher temperature, moisture and light intensity

Syed M. Nasar-Abbas[1,2,*], Julie A. Plummer[1,3], Kadambot H.M. Siddique[2,3], Peter F. White[4,2], David Harris[5,2], Ken Dods[5] and Mario D'Antuono[4]

[1]*School of Plant Biology, Faculty of Natural and Agricultural Sciences, The University of Western Australia, 35 Stirling Hwy, Crawley WA 6009, Australia*
[2]*Centre for Legumes in Mediterranean Agriculture (CLIMA), The University of Western Australia, 35 Stirling Hwy, Crawley WA 6009, Australia*
[3] *Institute of Agriculture, The University of Western Australia, 35 Stirling Hwy, Crawley WA 6009, Australia*
[4]*Department of Agriculture and Food, Western Australia, 3 Baron-Hay Court, South Perth WA 6151, Australia*
[5]*Chemistry Centre Western Australia, 125 Hay St, East Perth WA 6004, Australia*
**Corresponding author; e-mail: abbass01@cyllene.uwa.edu.au*

Abstract

Faba beans (*Vicia faba* L.) cv. Fiesta with seed moisture content (SMC) modified to 8, 10, 12 and 14% were packed in polyethylene lined aluminium foil bags and stored at 5, 15, 20, 25, 30, 37, 45, 50 or 60°C (± 2°C) for one year. Samples were analysed for moisture content and seed coat (testa) colour at 0, 0.5, 1, 2, 3, 4, 6, 8, 10 and 12 months of storage using a chroma meter. Changes in ΔE^*_{ab} values calculated from L^*, a^* and b^* colour coordinates demonstrated that storing seeds at higher temperature and SMC darkened testa colour. The initial beige testa colour changed to light brown, dark reddish brown or almost black depending on storage conditions. The higher the temperature and SMC the faster the rate of change in colour (ΔE^*_{ab} values). A continuous increase in L^* and b^* values was found in all samples with the passage of time whereas a^* values first increased and then decreased in samples stored at relatively high temperatures (≥ 37°C). Seeds with 8% SMC had more stable testa colour compared to seeds with a SMC higher than 8%. Exposure to light (350 μ mol m^{-2} s^{-1}) caused testa darkening in one month that equalled to testa darkening after 12 months storage in the dark at the same temperature (20 ± 2°C). Cotyledon colour also turned dark in samples stored at ≥ 37°C. A loss in total free phenolics, total tannins and condensed tannins (proanthocyanidins) was found with increased darkness of testa and cotyledons during storage. A predictive model for testa colour changes has been developed that allows farmers and grain traders to calculate the storage life of faba beans based on the temperature and moisture conditions under which they are stored.

Keywords*: Vicia faba* L., legumes; seed coat; cotyledons; discolouration; phenolics

Introduction

Colour of seed testa is important for the marketing of faba bean for human consumption. Across different varieties, seed testa colour ranges from white to purple. The most common (91% of accessions) seed coat colour in faba bean at harvest is light brown or beige (Robertson and El-Sherbeeny 1991). Colour darkening of faba bean reduces its value and market opportunity. Consumers and processors are reluctant to purchase darkened seed because colour is an index of quality or freshness and consumers associate dark colour with old seed (Hughes and Sandsted 1975). Furthermore, during heat processing or canning the immersion liquid or broth changes to a dark muddy colour (Dickinson *et al.* 1957). Thus dark seeds are unacceptable to the unprocessed as well as the canning market. Storage conditions strongly influence the stability of seed colour in many types of beans. In other legumes there is some evidence that temperature, relative humidity, seed moisture content (SMC) and light are the main factors (Davies 1994; Hughes and Sandsted 1975; Nordstorm and Sistrunk 1977; Nozzolillo and De Bezada 1984; Park and Maga 1999).

Phenolic compounds are involved in colour development in many seeds (Merghem *et al.* 2004; Nozzolillo *et al.* 1989). Phenolic compounds in seeds affect sensory characteristics such as colour, flavour, astringency and hardness (Shahidi and Naczk 1989). Proanthocyanidins (condensed tannins) are the predominant and most widely distributed group of flavonoids (a class of phenolic compounds) found in legume seeds (Beninger and Hosfield 2003). Tannin concentrations were high in coloured seed coats and low in seed coats of white beans (*Phaseolus vulgaris*) (Elias *et al.* 1979) and peas (*Pisum sativum* L.) (Troszynska and Ciska 2002). In faba bean, the amount of proanthocyanidins varied from 0 (white seeds) to 3.5% (brown seeds) of seed coat dry weight (Nozzolillo *et al.* 1989). Again white-seeded varieties were free from condensed tannins and seed coats of tannin-free seeds did not darken with time or under oxidizing conditions (Crofts *et al.* 1980). These studies suggest that tannins are the principal phenolic compounds involved in darkening and discolouration of faba beans.

This study aimed to assess the rate and intensity of colour darkening of faba bean seed using a range of storage conditions and to correlate this with phenolic contents. Once

known, optimum storage condition could be used to minimise darkening and hence maintain seed colour for extended periods.

Materials and Methods

Plant material

Faba beans (*Vicia faba* L.), cvs. Fiesta, Fiord, Ascot and Manafest, were grown at Borden (longitude, 118.26 E; latitude, 34.07 S), Western Australia as part of the normal trial activities of the National Faba Bean Improvement Program. Beans were harvested in December 2003 and kept at 5°C in the dark until used for experiments in February 2004. Good colour (beige/buff) and healthy seeds (free from insect damage, visible viral or fungal attack or broken testa) were individually selected for the experiments.

Effect of seed moisture content and storage temperature

The moisture contents of seeds (cv. Fiesta) were modified to 8.4, 10.3, 11.8 and 13.6 % (hereafter referred to as 8, 10, 12 and 14% respectively) by dehydration over silica gel or rehydration in a 75% RH chamber (Wexler 1997). Initial and final seed moisture contents were determined by applying a standard air-oven method (AACC 2000). Seed samples (3 x 25 g) were placed in polyethylene lined aluminium foil bags (10 x 10 cm) and sealed using an impulse heat sealer. Bags were placed in plastic containers and stored at 5, 15, 20, 25, 30, 37, 45, 50 or 60°C (± 2°C) in controlled temperature storage rooms or hot air ovens. A minimum-maximum thermometer was placed in the storage box to monitor temperature changes during storage. Seeds were removed and left at room temperature (20 ± 2°C) for one hour and then analysed for moisture content (weight gain/loss of the bag) and seed coat (testa) colour at 0, 0.5, 1, 2, 3, 4, 6, 8, 10 and 12 months of storage. Colour was measured and then they were immediately re-sealed and returned to the respective storage temperatures.

Effect of genotype vs storage temperature

Four prominent and commercial faba bean varieties in Australia (Fiord, Fiesta, Ascot and Manafest) were selected. Seed moisture contents were adjusted to ~12% as described above. Seed samples (3 x 25 g) were placed in polyethylene lined aluminium foil bags (10 x 10 cm) and sealed using an impulse heat sealer. Bags were placed in plastic containers and stored at 37°C (± 2°C) in controlled temperature storage room.

Seed coat (testa) colour was measured at 0, 0.5, 1, 2, 3, 4, 6, 8, 10 and 12 months of storage as described above.

Effect of light vs genotype

Seed samples (3 x 25 g) of four varieties (Fiord, Fiesta, Ascot and Manafest) with 12% SMC were placed in a single layer in bags prepared from a transparent polyethylene material (10 x 10 cm). The bags were placed in a controlled environment room at 20 ± 2°C (to minimise the effect of storage temperature) under artificial light (GroLux, T8, SYLVANIA, Germany) with photosynthetic photon flux of 350 μ mol m^{-2} s^{-1} measured by a Quantum Meter (QMSW, Apogee Instruments, USA). To measure the light intensity received by seeds the meter detector was covered with the same transparent polyethylene used for packaging the samples. Samples were analysed for testa colour at 0, 0.5, 1, 2, 3, 4, 6, 8, 10 and 12 months storage interval. Both sides of seeds were exposed to the light source by weekly turning the bags upside down. Control samples were packed in polyethylene lined aluminium bags and stored in dark at the same temperature.

Storage temperature and the kernel (cotyledon) colour

Faba bean (cv. Fiesta) samples with 12% SMC were dehulled using a mechanical dehuller equipped with an aspirator (S. K. Engineering, India). The kernels (3 x 25 g) were placed in polyethylene lined aluminium foil bags and sealed as above. Samples were stored at 37 ± 2°C and analysed for moisture content and colour changes at 0, 0.5, 1, 2, 3, 4, 6, 8, 10 and 12 months storage interval.

Colour measurement

Seed coat colour was determined by a Minolta CR-310 chroma meter (Minolta, Japan) using the Granular-Materials Attachment CR-A50. Data were collected for L^*, a^* and b^* values. L^* value represents lightness, a^* value greenness and redness and b^* value blueness and yellowness. A white porcelain plate (L^* = 97.75, a^* = −0.08, and b^* = +1.77) supplied with the instrument was used for calibration.

In order to ascertain the practical significance of changes in objective measures of faba bean testa colour during storage, Colour Difference Index (ΔE^*_{ab}) was calculated from L^*, a^* and b^* colour coordinates by the equation (Anonymous 1991):

$$\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{½}$$

Where $\Delta L^* = L^*_1 - L^*_2$, $\Delta a^* = a^*_1 - a^*_2$ and $\Delta b^* = b^*_1 - b^*_2$

Initial L^*, a^* and b^* values (subscript by 1) and values at each storage interval (subscript by 2) were used to develop ΔE^*_{ab} values and this was used to compare colour changes in samples.

Discolouration acceptability level

Faba beans (cv. Fiesta) having a range of discolouration attained after storage for one year at different temperatures were photographed by a professional photographer using a digital camera (Nikon D100; 6Mp, Japan). The photograph (Figure 1) was sent to local and foreign grain handlers, exporters/importers and faba bean breeders/scientists and their comments were sought on the maximum acceptable level of discolouration for local and international marketing. According to their comments the samples with 12% SMC stored at ≤ 25°C for one year (Figure 1) were acceptable for marketing for human consumption. The maximum acceptable discolouration level was then back calculated in L^*, a^* and b^* values and used as reference for acceptance of a sample

Colour changes were also compared with the scale based on changes in Colour Difference Index (ΔE^*_{ab}) (Anonymous 1998). It describes that ΔE^*_{ab} between 0 to 0.5 is a trace difference and impossible to be detected by human eyesight, 0.5 to 1.5 is slightly discernible and hard to detect by eye, 1.5 to 3.0 is noticeable and able to be detected by a trained panel, 3.0 to 6.0 is appreciable and detectable by ordinary people, a difference of 6.0 to 12.0 is large and indicates a large detectable difference in the same colour group and larger than 12.0 is extreme and indicates a shift to another colour group.

Determination of phenolic constituents

Total free phenolics, tannins and condensed tannins (proanthocyanidins) were determined in testa and cotyledons separately. Testa of 20 seeds (cv. Fiesta) were manually removed and the hilum excised and discarded. The testa was then ground with a grinder (IKA® A11 basic, IKA®-WERKE GmbH & Co. Germany). Cotyledons were ground separately. Testa (0.2 g) and cotyledons (2 g) were extracted with 20 ml of 70% v/v aq. acetone (analytical grade) by applying 20 min ultrasonic treatment at 4°C followed by overnight mechanical tumbling. Extracts were analysed for total phenolics by using the Folin-Ciocalteu's Phenol Reagent (Merck) according to the method of Makkar *et al*. (1993). Total phenolic compounds were calculated from a prepared standard curve of tannic acid (Merck) in an identical matrix. Tannins were complexed with polyvinylpoly-pyrrolidone (Sigma) and unbound phenolics determined as above

(Makkar *et al.* 1993). Total tannins were calculated by subtracting non-tannin phenolics from total phenolics. Condensed tannins (proanthocyanidins) were determined according to the methods of Porter *et al*. (1986).

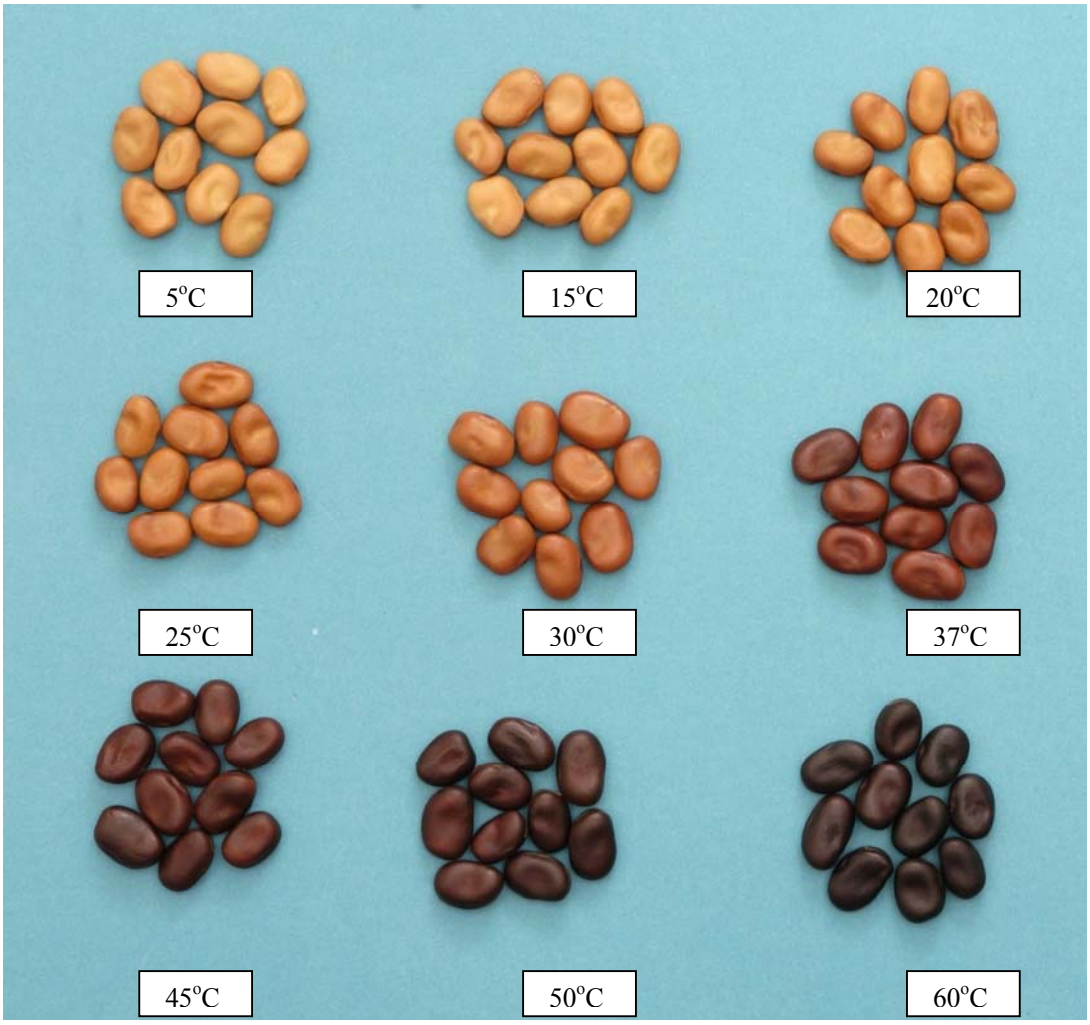

Figure 1. Effect of storage temperature on colour of faba bean (cv. Fiesta) seeds after12 months.

Statistical analysis

An analysis of variance was carried out using SPSS 10.0 for Windows and means were separated using Tukey's Honestly Significant Difference (Tukey's HSD) test at a significance level of 0.05. Changes in Colour Difference Index (ΔE^*_{ab}) of faba bean stored with different SMC at various temperatures were used to develop a predictive model in GenStat 2005 (GenStat for Windows, 8th Edition, VSN International Ltd, Rothamsted, England).

Results

Effect of storage temperature and duration on the stability of testa colour

Storage temperature and duration influenced faba bean colour. It changed from beige (initial colour) to medium brown in seeds stored at lower temperatures (≤ 25°C) but changed to dark reddish brown and almost black in seeds stored at higher temperatures (≥ 37°C) after 12 months (photograph 1). Both temperature and duration of storage influenced L^*, a^* and b^* values (Figure 2). The higher the temperature the faster the rate of change in L^*, a^* and b^* values. There was a continuous decrease in L^* and b^* values with the passage of time at all temperatures. Lightness and yellowness in the initial beige coloured seeds was masked as colour changed through brown to dark reddish-brown. On the other hand, a^* values increased and then decreased in samples stored at high temperatures (≥ 37°C). The a^* values increased sharply after two weeks to a maximum (a^*= 16.8) and then decreased in seeds stored at 60°C (Figure 2), whereas seeds stored at temperatures of 37, 45 and 50°C attained their maximum a^* values (a^*~ 16) after 1, 2 and 4 months respectively, followed by a continuous decrease indicating a similar path accelerated by temperature. Samples stored at temperatures ≤ 30°C did not achieve a similar high a^* value after one year in storage. This change in a^* values reflects a change in the red component of bean colour which increased due to an initial turning of bean colour to reddish-brown and then decreased due to a loss of the red component and an increase in darkness (L^*).

The Colour Difference Index (ΔE^*_{ab}) for faba bean seeds increased during storage at all temperatures. Substantial colour changes (ΔE^*_{ab} values) were found during storage in all seed samples particularly those stored at higher temperatures (≥ 37°C). Appreciable colour changes detectable by ordinary people (Anonymous 1998) occurred after 4 months at 5°C, after 2 months at 15 and 20°C, after 1 month at 25°C and after only two weeks in samples stored at or above 30°C (Figure 2).

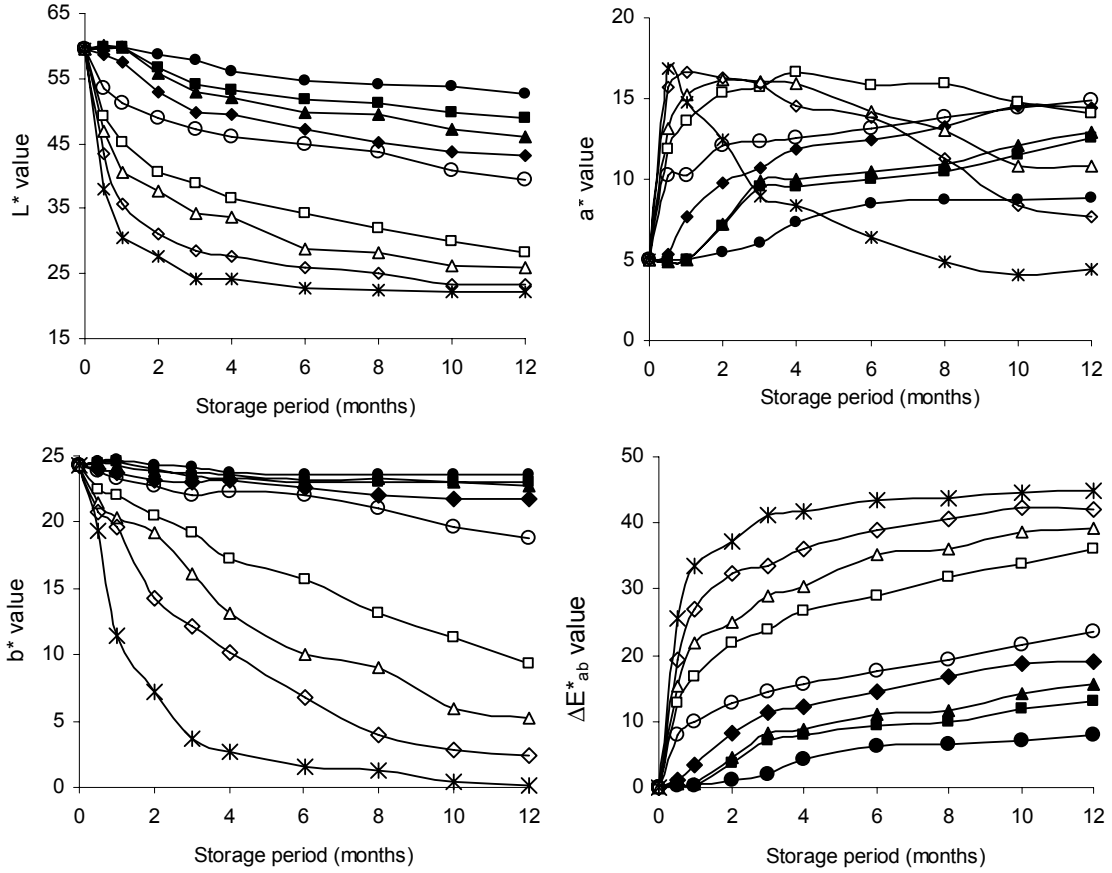

Figure 2. Effect of storage time and temperature on L^*, a^* and b^* colour coordinates and ΔE^*_{ab} values of faba bean (cv. Fiesta) seeds (5°C; —●—, 15°C; —■—, 20°C; —▲—, 25°C; —♦—, 30°C; —○—, 37°C; —□—, 45°C; —Δ—, 50°C; —◊—, 60°C; —x—).

Genotype response to storage temperature

The Colour Difference Index (ΔE^*_{ab}) increased over time with storage at 37°C for 12 months (Figure 3). All of the four varieties behaved similarly to this adverse storage temperature (37°C).

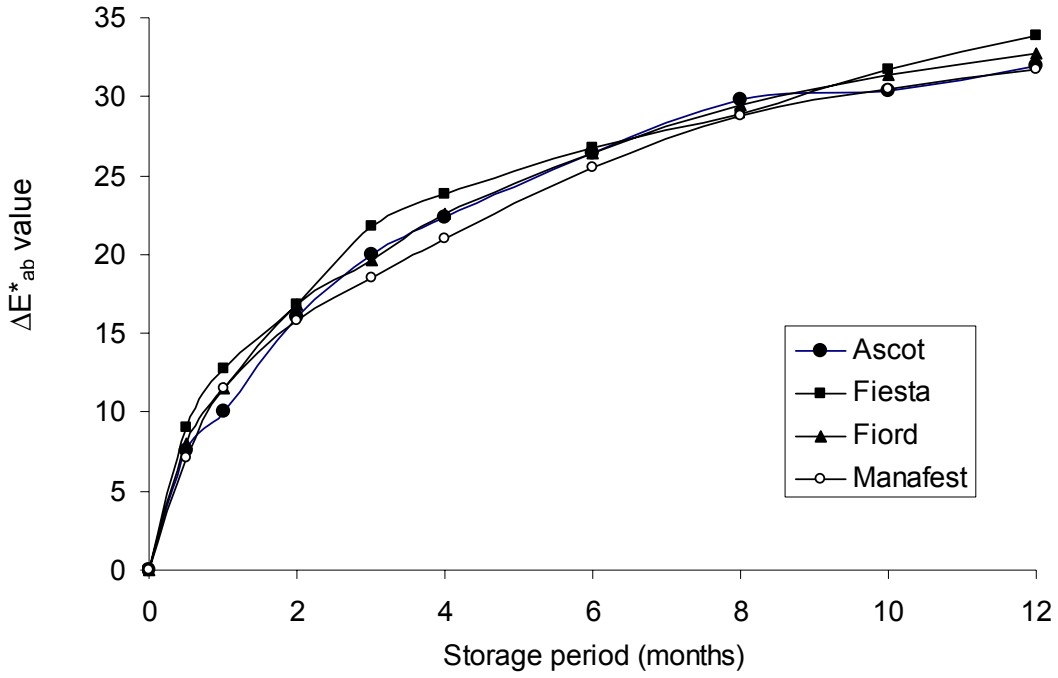

Figure 3. Effect of storage at 37°C on ΔE^*_{ab} of different faba bean varieties.

Effect of seed moisture content on the stability of testa colour

Seed moisture content was also an important factor affecting colour darkening. The higher the seed moisture content the faster was the darkening process at a set temperature (Figure 4). Samples with 8% SMC were less susceptible to discolouration compared to higher SMC. Seeds with 8% SMC had a change of 27 points in ΔE^*_{ab} values after 12 months storage at 37°C whereas seeds with 10, 12 and 14% SMC exhibited the same level of change in just 8, 6 and 3 months respectively (Figure 5).

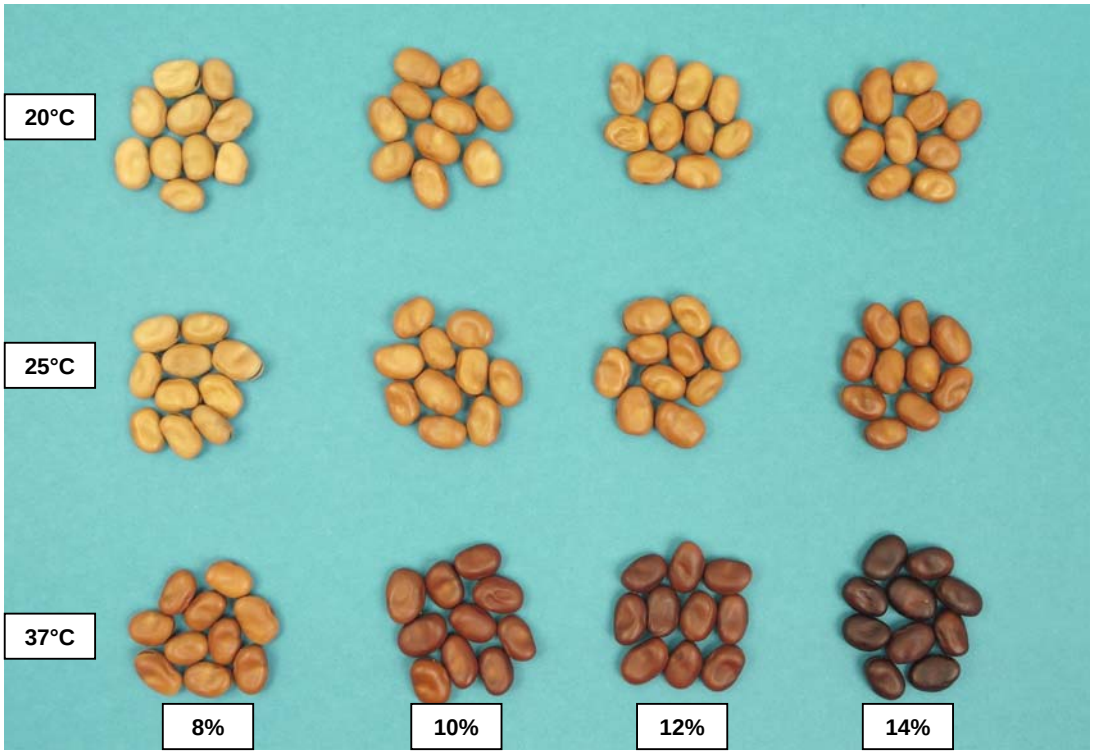

Figure 4. Effect of seed moisture content on the colour of faba bean (cv. Fiesta) stored at different temperatures for 12 months.

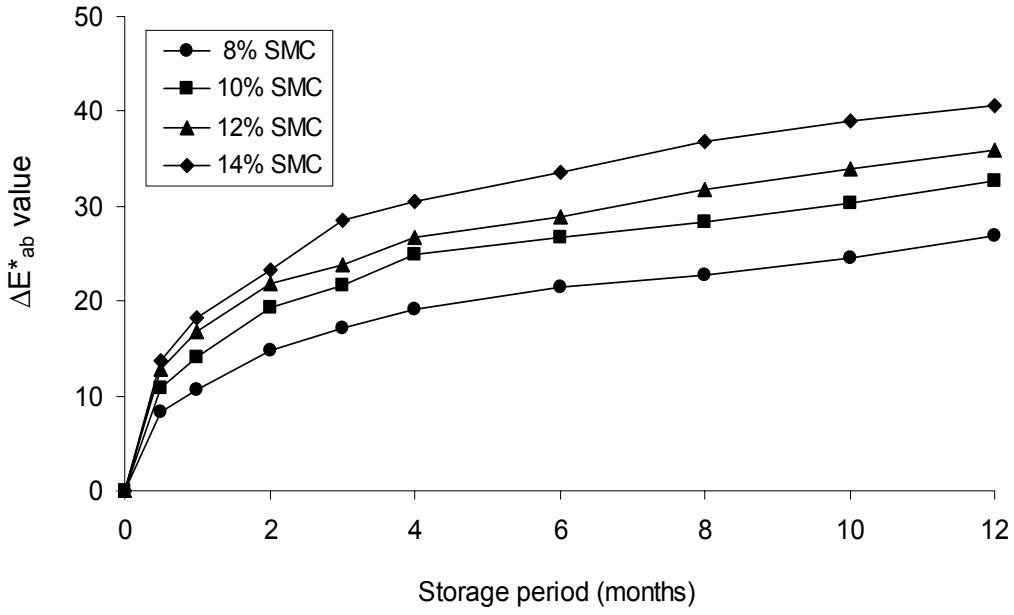

Figure 5. Effect of seed moisture content on ΔE^*_{ab} in faba bean (cv. Fiesta) stored at 37°C.

Effect of light on the stability of testa colour

Light caused a substantial increase in colour darkening (Figure 6). Seeds stored under light darkened much faster than those stored in dark. Appreciable colour changes detectable by ordinary people (Anonymous 1998) were measured after just 2 weeks storage at 20 ± 2°C. Varieties behaved differently to light. Manafest and Fiesta darkened more rapidly than Ascot and Fiord (Figure 7).

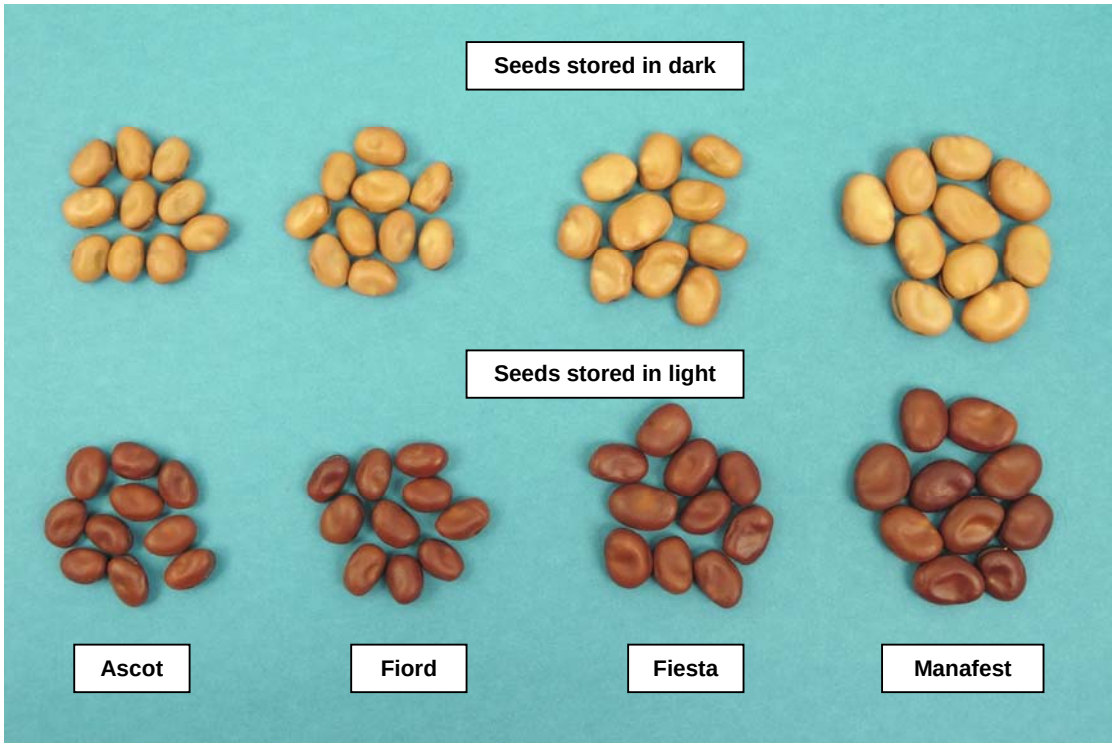

Figure 6. Effect of light on the colour darkening of different varieties of faba beans after one year storage at 20°C.

Effect of storage temperature on the kernel (cotyledon) colour

Not only testa colour, but also kernel colour of faba beans darkened also during storage at 37°C (Figure 8). Cotyledon colour darkened less than testa colour but differences were still large (Anonymous 1998). Cotyledons showed a change of 6 points in ΔE^*_{ab} values after 8 months storage at 37°C.

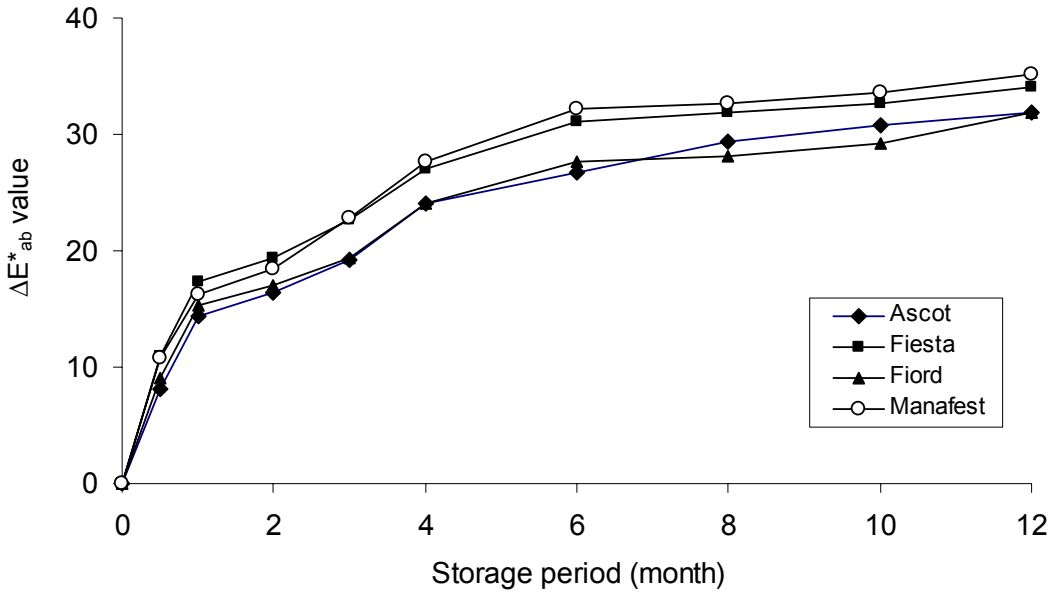

Figure 7. Effect of light on ΔE^*_{ab} in different faba bean varieties stored at 20°C for 12 months.

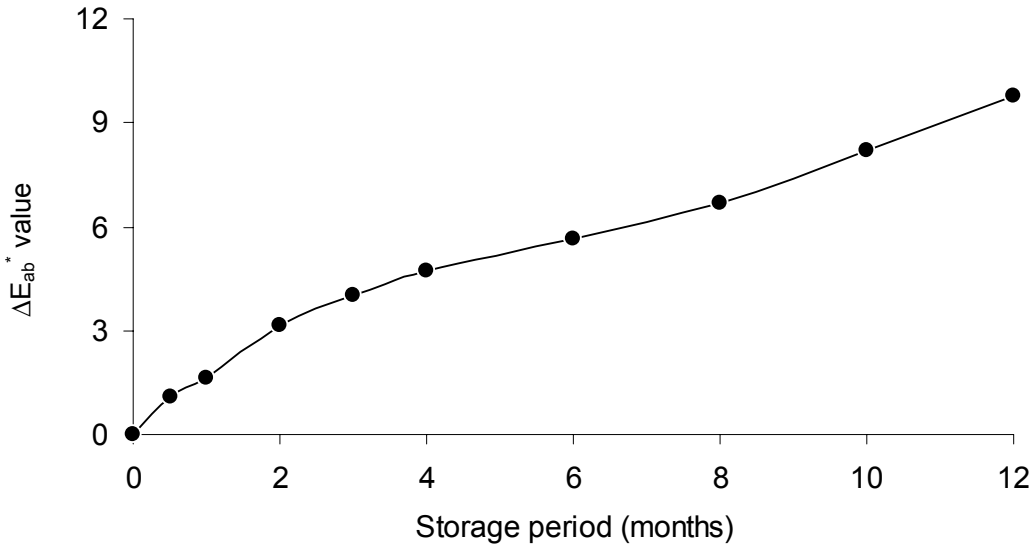

Figure 8. Effect of storage temperature (37°C) on ΔE^*_{ab} for cotyledons of faba bean (cv. Fiesta) stored for 12 months.

Predictive model for seed coat colour changes

Change in Colour Difference Index (ΔE^*_{ab}) of faba bean seeds with different SMC stored at a range of temperature for 12 months is expressed in Eq.I

$$Y = a^*(T + SMC + T \times SMC) + b^*(T + SMC + T \times SMC)\, k^P \qquad \text{Eq-I}$$

Where Y= change in ΔE^*_{ab} values, T is storage temperature in °C, SMC is % seed moisture content, P is storage period in months.

a, b and k are constants with the following values

$a = 0.063$, $b = -0.058$, and $k = 0.583$

The equation accounted for 94% of the ΔE^*_{ab} of Fiesta when stored under constant temperature and SMC in dark.

Changes in Phenolic constituents with change in colour

Storage at different temperatures for 12 months led to substantial reduction in total free phenolic constituents especially in the testa and there was a greater decrease with higher storage temperature resulting in more darkening (Table 1). The reduction in total free phenolics after 12 months storage ranged from 5% at 5°C to 76% at 50°C.

Tannins were the major proportion of total phenolics in the testa of faba bean. Tannin contents were negatively correlated with colour darkening in faba bean but the decrease was not significant for seeds stored under cooler temperatures up to 25°C (Table 1). Non-tannin phenolics also decreased, with an accompanying increase in darkening, with higher storage temperature. Testa of freshly harvested faba bean seeds contained 18.8 mg g^{-1} non-tannin phenolics (Table 1) which decreased by 12- 86% for seeds stored over the range of 5-50°C after 12 months. Proanthocyanidins (condensed tannins), which were the predominant group among tannins also substantially decreased (Table 1) with an increased storage temperature especially higher temperatures (≥ 37°C).

Storage at higher temperature (≥ 25°C) also affected total phenolic contents of the cotyledon. Total phenolics of cotyledons consistently decreased with increased storage temperature especially storage at higher temperatures (≥ 37°C) in darkness (Table 3).

Table 1. Phenolic constituents of testa of faba bean (cv. Fiesta) stored at different temperatures for 12 months

Storage treatment	Total free phenolics (mg of tannic acid g^{-1})	Non-tannin phenolics (mg of tannic acid g^{-1})	Total tannins (mg of tannic acid g^{-1})	Proanthocyanidins (mg of leucocyanidin g^{-1})
Control (fresh)	62.4 ± 0.4 a	18.8 ± 0.4 a	43.6 ± 0.6 a	40.7 ± 0.1 a
5°C in dark	59.5 ± 0.3 b	16.5 ± 0.3 b	43.0 ± 0.3 a	38.7 ± 1.4 a
15°C in dark	57.1 ± 0.9 bc	15.7 ± 0.1 bc	41.4 ± 0.8 a	35.7 ± 1.3 b
25°C in dark	55.9 ± 1.3 c	15.2 ± 0.5 cd	40.7 ± 0.9 a	34.8 ± 1.4 b
37°C in dark	50.6 ± 0.9 d	14.5 ± 0.4 d	36.1 ± 1.2 b	30.2 ± 1.0 c
45°C in dark	41.2 ± 0.7 e	11.3 ± 0.4 e	30.0 ± 1.1 c	24.2 ± 0.4 d
50°C in dark	15.0 ± 0.8 f	2.7 ± 0.1 f	12.2 ± 0.8 d	5.9 ± 0.1 e

Means (± s.e., n = 3) sharing the same letter in a column are not significantly different ($p \leq 0.05$) according to Tukey's HSD test.

Table 2. Phenolic constituents of testa of faba beans (cv. Fiesta) stored at 20°C under light and dark for different time periods

Storage duration (months)	Total free phenolics (mg of tannic acid g^{-1})	Non-tannin phenolics (mg of tannic acid g^{-1})	Total tannins (mg of tannic acid g^{-1})	Proanthocyanidins (mg of leucocyanidin g^{-1})
0 Control (fresh)	62.4 ± 0.4 a	18.8 ± 0.4 a	43.6 ± 0.6 a	40.7 ± 0.1 a
1 (under light)	52.4 ± 1.0 c	14.6 ± 0.4 c	37.8 ± 0.6 c	31.9 ± 1.4 c
3 (under light)	46.5 ± 1.2 d	12.1 ± 0.4 d	34.4 ± 1.4 d	28.8 ± 1.0 d
6 (under light)	42.3 ± 0.8 e	11.6 ± 0.2 d	30.8 ± 0.7 e	24.2 ± 1.0 e
9 (under light)	36.0 ± 1.3 f	9.5 ± 0.2 e	26.4 ± 1.5 f	20.3 ± 0.6 f
12 (under light)	33.5 ± 0.9 f	8.3 ± 0.1 f	25.2 ± 0.9 f	17.6 ± 1.2 f
12 (in dark)	56.6 ± 0.7 b	15.6 ± 0.2 b	41.1 ± 0.4 b	35.5 ± 0.3 b

Means (± s.e., n = 3) sharing the same letter in a column are not significantly different ($p \leq 0.05$) according to Tukey's HSD test.

Table 3. Total free phenolic contents of cotyledon of faba beans (cv. Fiesta) stored at different temperatures in dark and stored at 20°C under light for 12 months

Treatments	Total phenolics (mg of tannic acid g^{-1})
Freshly harvested	1.71 ± 0.01 a
Stored at 5°C in dark	1.62 ± 0.06 ab
Stored at 15°C in dark	1.61 ± 0.06 ab
Stored at 20°C in dark	1.58 ± 0.05 ab
Stored at 25°C in dark	1.52 ± 0.03 bc
Stored at 37°C in dark	1.46 ± 0.06 cd
Stored at 45°C in dark	1.34 ± 0.04 de
Stored at 50°C in dark	1.29 ± 0.04 e
Stored at 20°C in light	1.59 ± 0.01 ab

Means (± s.e., n = 3) sharing the same letter in the column are non-significant ($p \leq 0.05$) according to Tukey's HSD test

Discussion

It is possible to store faba beans without substantial darkening. Our results show that seed coat colour darkening in faba bean was slow at moderate to low temperatures (≤ 25°C) and it was slowest and therefore had best colour retention after 12 months at 5°C. Low temperature also slows seed coat discolouration in other legumes. Little change in seed coat colour occurred in Rwandan dry beans (*Phaseolus vulgaris*) stored at 4°C for 24 months (Edmister *et al.* 1990). Light red kidney beans (*Phaseolus vulgaris* L.) also retained their original colour for one year when stored at 1°C (Gunes and Lee 1997). In lentil seeds (*Lens culinaris* Medic.) there was no darkening at 5°C (Nordstorm and Sistrunk 1979) and it was slow in adzuki beans (*Vigna angularis*) at 10°C (Yousif *et al.* 2003b). So similar to other legumes, storage at 5°C best protected faba bean colour during long term storage.

Storage of faba bean at high temperatures (≥ 30°C) accelerated colour darkening especially at ≥ 37°C. This supports earlier evidence that high temperature storage is an important factor causing colour darkening in faba bean and other legume seeds (Amarowicz *et al.* 2004; Cunha *et al.* 1993; Quast and Silva 1977; Sorour and Uchino 2004). Davies(1994) also found that storage of faba beans at 40°C caused a substantial increase in colour darkening. Adzuki beans (Yousif *et al.* 2003b), Rawandan dry beans

(Edmister *et al.* 1990) and lentil seeds (Nozzolillo and De Bezada 1984) also darkened when stored at 30°C. Seeds stored at high temperatures (≥ 37°C) darkened to an unacceptable level of marketing for human consumption in less than 3 months.

In general, faba bean seed coat darkening increased with increased temperature but duration of storage must be taken into account. Long term storage caused colour darkening even at intermediate temperatures (15, 20 and 25°C) as in other legumes. Storage at 24°C for one year increased darkening in light-red kidney beans (Hughes and Sandsted 1975) and Rwandan dry beans colour darkened when stored at 23°C for 24 months (Edmister *et al.* 1990). Long term storage of faba bean at temperatures ≤ 25°C darkened seed coat colour but the darkening level was in the acceptable range for marketing for human consumption after 12 months. This contrasted with storage at ≥ 37°C which caused substantial darkening after just 2 weeks and the seeds became unacceptably dark for human consumption in less than 3 months. All of the four commercial varieties darkened at similar rate at high temperature giving no choice of suitability for storage. Other varieties may behave differently such as some tannin free lines (with white testa) are known to be resistant, to some extent, to storage discolouration (Paull, personal communication).

The accelerated colour darkening process in faba bean at high temperature (≥ 37°C) is a serious concern for on-farm storage in south west Australia. The faba bean crop is harvested in the beginning of summer (November-December) and grain is stored on farm for the next couple of months. The air temperature may rise above 40°C (Bureau of Meteorology, Western Australia), which can quickly cause colour darkening and lower the quality of produce. Conversely storage of faba bean at refrigeration temperatures (~5°C) would protect faba bean colour during long term storage but its practical use, especially considering the cost of storage, would be prohibitive commercially. A maximum storage temperature, which would keep faba bean colour darkening to an acceptable level for marketing for human consumption, was ≤ 25°C and this may be practical at commercial level.

Seed moisture content was also recognized as an important factor in discolouration of faba bean. Seeds with higher SMC darkened at faster rate than those having lower SMC. Seeds stored at 8% SMC were very resistant to colour darkening as compared to those with high SMC. High SMC and/or high relative humidity in the storage environment have been identified by other researchers as major factors responsible for the

deterioration of quality traits including colour of other species of bean. In pinto beans (*Phaseolus vulgaris*) seeds with 10% added moisture had greater colour change (decrease in Hunter L^* values and increase in a^* values) than control seeds or seeds with 5% added moisture (Park and Maga 1999). Increases in colour darkening in Rwandan dry beans positively related to increase in water activity (a_w) across a range of storage temperatures (Edmister *et al.* 1990).

Farmers need to harvest faba beans early and at high moisture contents (14-15%) to preserve seed quality and maximise yield. If seeds are harvested too dry then there is a risk of damage (splits and cracks). Harvesting early is important because the longer the crop remains in the field the more vulnerable it is to loss from lodging and pod shedding. Our results revealed that a 14-15% moisture content of faba bean accelerates discolouration considerably during storage. So, in order to maintain faba bean colour for human consumption during long term storage, faba bean could be dehydrated to 8-10% SMC after harvesting. The extra cost of dehydration and reduced yield (by weight) may be compensated for by the higher sale price and this requires a cost-benefit analysis.
Light also substantially affected faba bean colour during storage. Testa darkening under light storage for one month was equal to darkening in 12 months storage in dark at the same temperature (20 ± 2°C). The observed light acceleration of colour darkening in faba bean extends earlier research on the effect of light on other legumes. Ultraviolet and cool-white light darkened light-red kidney beans during storage (Hughes and Sandsted 1975). Similarly parts of faba bean seeds were observed to darken when they were exposed to light when pods split on the plant. Growers of light-red kidney beans also observed darkening of beans in pods when harvest was delayed after pods and seeds were fully mature (Hughes and Sandsted 1975).

Fiesta and Manafest varieties responded more to light compared to Fiord and Ascot. This might be due to their bigger seed size which provided more exposure to light when seeds were placed on their side so that the source of light was on a wider surface, as was the case in this experiment.

Colour darkening in faba beans due to light may be of less concern to producers because seeds get exposed to light for a very short period. There is generally little pod splitting in field. After harvesting faba beans are stored in metal bins/silos where no light can

penetrate. The only possibility of exposure to light is when they are packed in 50-100 kg white polypropylene weave bags at around 650 denier (most commonly used packing material), which is semi transparent, and subsequent storage where they are exposed to light/sunlight. Either this practice should be avoided or a different, non-transparent material should be used in storage bag manufacture.

Substantial colour changes in kernel (cotyledon) colour were also determined in seeds stored at higher temperature (37°C). Darkening of faba bean cotyledons is important for the dishes/products where cotyledon colour is visible e.g. Falafel (deep fried dough) and Bissara (poured paste) in Egypt and other Middle Eastern countries. This affects sensory quality of the products and hence their marketability.

The predictive model for seed colour changes will be helpful for farmers and exporters/importers to calculate and predict the storage life of faba beans. This will enable them to determine the limit of storage for colour changes to remain acceptable for marketing for human consumption and hence increase profitability.

A substantial reduction in phenolic compounds was associated with colour darkening in faba beans. Total free phenolic contents of testa demonstrated a 5% to 76% decrease whereas non-tannin phenolics demonstrated a 12% to 86% decrease in seeds stored across a temperature range of 5-50°C. Polyphenols in other legumes behave similarly (Hincks and Stanley 1986). A range of cultivars of dry beans (*Phaseolus vulgaris*) stored for 5 years under tropical conditions (30-40°C, 75% RH) exhibited an 11% to 38% decrease in total polyphenols and a substantial decrease in non-tannin polyphenols as compared with freshly harvested beans (Martin-Cabrejas *et al.* 1997). A reduction in polyphenol content was found at all stages of seed development in winged beans (*Psophocarpus tetragonolobus* L.) (Kadam *et al.* 1982). The reduction in total free phenolics and non-tannin phenolics is probably due to polymerization of existing polyphenolic compounds, resulting in insoluble, high molecular weight polymers. Browning in lentil seeds is also assumed to be the result of polymerisation of low molecular weight phenolic precursors to brown-coloured high molecular weight products (Nozzolillo and De Bezada 1984). The decrease in phenolic constituents with the increase in colour darkening may also be due to oxidative degradation of particular phenolic compounds (Marquardt *et al.* 1978). Phenolic compounds vary widely in complexity but their common characteristic is that they are readily oxidised and undergo

phenolic reactions (Bors *et al.* 1996). Indeed when faba beans are flushed with oxygen darkening accelerates, whereas flushing with nitrogen reduces it (Nasar-Abbas *et al.* 2007a). Further, storage of several varieties of faba beans under low oxygen concentration reduces colour darkening suggesting that darkening is due to oxidation of polyphenolics (Black and Brouwer 1998a). Oxidation of polyphenols, and especially non-tannin polyphenols, might also be due to peroxidase enzyme activity which continues during postharvest storage (Fry 1986). Others suggest that the darkening is probably due to a combination of Maillard (non-enzymatic) browning and chemical changes involving phenolic compounds (Edmister *et al.* 1990). It is possible that any or all of these processes are involved in the complex chemistry associated with seed discolouration of faba bean.

Total tannin and condensed tannin (proanthocyanidins) groups of phenolics also showed a continuous decrease with the increase in darkening of seed testa. This supports studies in different beans. A decrease in proanthocyanidins of faba beans with colour darkening is also caused by accelerated aging at 40°C and 100% RH (Davies 1994). In lentils there is a substantial reduction in proanthocyanidin contents as they change colour from green to dark brown during storage (Nozzolillo and De Bezada 1984). Tannins increase gradually in black beans (*Phaseolus vulgaris*) during storage at 5°C for 6 months whereas they increase, reach a plateau and then decline when stored at elevated temperatures of 30°C and 40°C (Sievwright and Shipe 1986). This suggests that tannins continue to develop from smaller molecular weight non-tannin material during storage but at higher temperatures there is a loss of tannins due to their binding with macro-molecules (proteins).

Our studies revealed that at lower temperatures (≤ 25°C) a non-significant reduction in tannin contents occurred whereas at higher temperatures (≥ 37°C) significant reductions were observed. This might have been due to a balance between development of tannins from smaller molecular weight, non-tannin material (Bors *et al.* 1996; Hughes and Sandsted 1975; Marquardt *et al.* 1978) and subsequent binding with proteins at lower temperatures. At high temperatures (≥ 37°C) this balance may have shifted towards binding with proteins due to increased biochemical activity (Sievwright and Shipe 1986). The loss in tannin content might also be due to their strong antioxidant activity (Shahidi *et al.* 2001). Proanthocyanidins (condensed tannins) are damaged by oxidative reactions, as they play an important role in the defence system of seeds exposed to

oxidative damage caused by environmental factors such as light, oxygen, free radicals and metal ions (Amarowicz *et al.* 2004; Troszynska and Ciska 2002). Proanthocyanidins are known to prevent lipid oxidation as reducing agents, free radical scavengers and chelators of pro-oxidant catalytic metals. Tannins are 15-30 times more effective in the quenching of peroxyl radicals than simple phenolics (Hagerman *et al.* 1998).

Light changed phenolic contents in the testa but it was not effective in changing cotyledon phenolic content. Light may only affect the testa of beans. The testa, which is the outermost portion of the seed, may filter or block light from reaching the cotyledons. The testa however, would not be able to insulate the cotyledons from a constant external temperature and the whole seed would quickly equilibrate with air temperature. Ultraviolet and cool-white light darken kidney beans in storage but seeds darkened by light decrease very little in cooking quality in contrast to seeds darkened by high storage temperature and relative humidity (Hughes and Sandsted 1975). Darkening caused by light probably involves only pigment changes in the seed coat whereas darkening caused by high temperature involves changes in constituents throughout the seed. Similar light induced changes in the seed testa, but not cotyledons, may also occur in faba bean.

CHAPTER FIVE

Nitrogen retards and oxygen accelerates colour darkening in faba bean (V*icia faba* L.) during storage

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Nitrogen retards and oxygen accelerates colour darkening in faba bean (V*icia faba* L.) during storage

S.M. Nasar-Abbas[a,b*], J.A. Plummer[a,c], K.H.M. Siddique[b,c], P.F. White[d], D. Harris[e] and K. Dods[e]

[a]*School of Plant Biology, Faculty of Natural and Agricultural Sciences, The University of Western Australia, 35 Stirling Hwy, Crawley WA 6009, Australia*
[b]*Centre for Legumes in Mediterranean Agriculture (CLIMA), Faculty of Natural and Agricultural Sciences, The University of Western Australia, 35 Stirling Hwy, Crawley WA 6009, Australia*
[c] *Institute of Agriculture, The University of Western Australia, 35 Stirling Hwy, Crawley WA 6009, Australia*
[d]*Department of Agriculture and Food, Western Australia, 3 Baron-Hay Court, South Perth WA 6151, Australia*
[e]*Chemistry Centre WA, 125 Hay St, East Perth WA 6004, Australia*
**Corresponding author; e-mail: abbass01@cyllene.uwa.edu.au*

Abstract

Modified atmosphere packaging (MAP) techniques were applied in order to control seed coat (testa) colour darkening in faba bean during long term storage. These techniques included flushing with carbon dioxide, nitrogen, oxygen or ethylene, and vacuum packaging. Seeds flushed with air were used as the control. After MAP treatments, samples were stored at 30°C in dark for one year. Seed coat colour was measured at 0, 0.5, 1, 2, 3, 4, 6, 8, 10 and 12 months of storage using a chroma meter. Changes in Chroma (C^*), hue angle ($h°$) and Colour Difference Index (ΔE^*_{ab} values) calculated from L^*, a^* and b^* colour coordinates demonstrated that relative to control, nitrogen was effective in reducing colour darkening by an appreciable level, whereas storage in oxygen accelerated the colour darkening process. Ethylene had some effect whereas the other MAP treatments were ineffective in reducing colour darkening in faba beans.

Analytical studies revealed that tannin compounds were the major constituents of total phenolics in faba bean of which proanthocyanidins were the predominant component group. Tannin concentration was negatively correlated with colour darkening in faba bean. Air, vacuum and ethylene treated samples showed similar changes in phenolic constituents after 12 months storage but samples flushed with CO_2 and especially those flushed with O_2 had much higher losses in phenolic constituents demonstrating that colour darkening is likely to be due to oxidative transformation of phenolic contents.

Flushing with N_2, which reduced colour darkening and tannin losses, would be useful in maintaining quality and improving market opportunities and acceptance during long term storage of faba beans. Nitrogen could be used to flush faba bean in airtight silos for bulk storage as well as in small individual packets that could go directly onto the supermarket shelf as a premium product.

Keywords: Modified Atmosphere Packaging; Carbon dioxide; Ethylene; Seed coat colour; Phenolics; Tannins

Introduction

Seed coat (testa) colour is one of the most important visual characteristics in marketing faba bean seed for human consumption. Genetically, seed testa colour ranges from white to purple in different varieties of faba bean but the preferred colour has variously been described as beige, light tan or buff (AGWEST 1998). Seed coat colour of most faba bean varieties is beige or buff at harvest but changes to light brown, dark brown or almost black depending upon the storage time and conditions. Seeds with dark brown testa colour are not accepted in overseas markets as consumer associate it with old seed and poor cooking and sensory qualities.

Storage conditions have a major influence on the stability of seed coat colour in beans. Environmental factors such as temperature, seed moisture content and light cause discolouration in faba and other beans ((Nasar-Abbas *et al.* 2007b). In addition, oxidation due to environmental O_2, has been reported as a major factor responsible for discolouration in various beans (Black and Brouwer 1998b; Stanley 1992a). Discolouration in dry beans may involve oxidation of phenolic compounds especially proanthocyanidins (condensed tannins) which are the predominant and most widely distributed group of flavonoids found in legume seeds (Beninger and Hosfield 2003). The phenolic compounds vary widely in complexity but the common characteristic of all these compounds is that they are readily oxidised and undergo phenolic reactions (Bors *et al.* 1996). The involvement of phenolic compounds (mostly proanthocyanidins) in faba bean discolouration is supported by the observations that high tannin faba bean varieties darken more in air than low tannin varieties (Black and Brouwer 1998b) and the white-seeded varieties, which are free from proanthocyanidins, do not darken with time or under oxidizing conditions (Crofts *et al.* 1980).

Storage discolouration related to atmospheric oxidation is often controlled by modified atmosphere packaging (MAP) techniques. With modified atmosphere packaging, the gas composition surrounding the produce, or seeds in this case, is different from the gas composition outside the package. Outside, the gas composition is always close to 78.1 kPa N_2, 20.95 kPa O_2, 0.93 kPa argon and 0.036 kPa CO_2. MAP, acts by altering metabolic processes including reducing the respiration rate (Jayas and Jeyamkondan 2002). Modified atmospheres can be obtained by gas generators and scrubbers (controlled-atmosphere packaging), evacuation of air (hypobaric storage, vacuum packaging), replacement of air with an alternative gas, or addition of chemical systems that absorb or generate gases or volatile compounds (active packaging) in packages (Gorris and Peppelenbos 1999). MAP may be applied to minimise discolouration in faba beans.

The modification of the atmosphere generally implies a reduction in O_2 content and/or an increase in the CO_2 or N_2 concentration and in some cases changes in the level of carbon monoxide, ethylene, ethanol or other compounds in the atmosphere. Flushing with N_2 and CO_2 are the most commonly applied modified atmosphere techniques but the usual gas for flushing dehydrated foods is N_2. Nitrogen is inert with a low fat and moisture solubility (Fierheller 1991). CO_2 has been proposed for packaging nuts (Holaday *et al.* 1979) and may be suitable for grain legume storage. Adsorption of the CO_2 by the nuts creates a vacuum. The adsorption phenomenon is similar to gas adsorption by charcoal and silica gel and can be used on a variety of grains including oilseeds, legumes, rice and corn (Fierheller 1991).

The objective of this study was to test the hypothesis that oxidation of phenolic contents may be the main cause of discolouration in faba bean and this might be controlled/minimised by the use of MAP techniques of flushing with different gases.

Materials and Methods

Plant Material

Faba bean (*Vicia faba* L.) cv. Fiesta, was grown in 2004 growing season (May to December) at Borden (longitude, 118.26 E; latitude 34.07 S), Western Australia as part of the National Faba Bean Improvement Program's field evaluation activity. Faba bean was harvested in December 2003 and kept at 5^{o}C in the dark until used for experiments in February 2004. Good colour (beige/buff) and healthy seeds (free from insect damage,

visible viral or fungal attack or broken testa) were individually selected. The average seed weight was 73.2 g per 100 seeds.

Modified Atmosphere Packaging and storage

Seed moisture content was maintained at 12% by dehydrating over silica gel. Initial and final seed moisture contents were determined by applying the air-oven method (AACC 2000). Seed samples (~25 g each) were packed in bags (~10 x 10 cm) prepared from polyvinyl chloride (PVC) sheet with a thickness of 300 μm and sealed with an impulse heat sealer. Air from the bags was removed with a syringe needle attached to a vacuum system and then the required gas, CO_2, N_2, O_2 or ethylene, (BOC Gases Australia Limited) was used to fill bags through the same needle. The procedure was repeated 3 times to ensure the removal of air traces from the bags and this was immediately followed by sealing of the access flush point. Samples packed in bags filled with air acted as controls. After MAP treatments, the packs were placed in plastic storage boxes and stored at 30^{o}C (an adverse storage temperature) in the dark (Nasar-Abbas *et al.* 2007b). The gas composition inside the bag could not be determined but according to the information provided by the manufacturer/supplier the PVC sheets were impermeable to the gases used in the experiment.

Colour measurement

Samples were analysed for changes in seed coat (testa) colour at 0, 0.5, 1, 2, 3, 4, 6, 8, 10 and 12 months storage interval. Seed coat colour was determined by a Minolta CR-310 chroma meter (Minolta, Japan) using the Granular-Materials Attachment CR-A50. Data were collected for L^*, a^* and b^* values. L^* = lightness, ranging from 0 (black) to 100 (white), a^*= bluish-green/red-purple hue component and b^*= yellow/blue hue component (McGuire 1992). A white porcelain reference plate (L^*= 97.75, a^*= −0.08, and b^*= +1.77) supplied with the instrument was used for calibration. For each storage period independent samples of seeds stored in separate packages were used which were discarded after taking the measurements.

In order to ascertain the practical significance of changes in objective measures of faba bean testa colour during storage, Chroma (C^*), hue angle (h°) and Colour Difference Index (ΔE^*_{ab}) was calculated from L^*, a^* and b^* colour coordinates. Chroma represents colour saturation which varies from dull (low value) to vivid colour (high value) and hue angle is defined as a colour wheel with red-purple at an angle of 0°, yellow at 90°,

bluish green at 180°, and blue at 270° (McGuire 1992). The values for the above were computed using the following equations (Anonymous 1991; McGuire 1992):

$C^* = (a^{*2} + b^{*2})^{½}$ Eq = I

$h° = [\tan^{-1}(b^*/a^*)/6.2832] \times 360$ Eq = II

where $a^* > 0$ and $b^* > 0$

$\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{½}$ Eq = III

Where $\Delta L^* = L^*_1 - L^*_2$, $\Delta a^* = a^*_1 - a^*_2$ and $\Delta b^* = b^*_1 - b^*_2$

Initial L^*, a^* and b^* values (subscript by 1) and values at each storage interval (subscript by 2) were used to develop ΔE^*_{ab} values and this was used to compare colour changes in samples.

Determination of Phenolic Constituents

Total free phenolics, tannins and condensed tannins (proanthocyanidins) were determined in testa and cotyledons separately. A sample (10 g) of each treatment was ground to powder with a grinder (IKA® A11 basic, IKA®-WERKE GmbH & Co. Germany). Ground sample (1 g) was extracted with 20 ml of 70% v/v aq. acetone (analytical grade) with 20 min ultrasonic treatment at 4°C followed by overnight mechanical tumbling in dark. Extracts were analysed for total phenolics using Folin-Ciocalteu's Phenol Reagent (Merck) according to the method of Makkar et al. (1993). Total phenolic compounds were calculated from a prepared standard curve of tannic acid (Merck) in an identical matrix. Tannins were complexed and precipitated with polyvinylpolypyrrolidone (Sigma) and unbound phenolics determined as above (Makkar *et al.* 1993). Total tannins were calculated by subtracting non-tannin phenolics from total phenolics. Condensed tannins (proanthocyanidins) were determined according to the methods of Porter et al. (1986).

Statistical analysis

An analysis of variance was carried out using SPSS 10.0 for Windows and means were separated using Tukey's Honestly Significant Difference (Tukey's HSD) test at $p \leq 0.05$.

Results

MAP and testa colour

Composition of the gaseous storage environment affected faba bean testa colour during storage. Compared with the control (air), vacuum packaging and CO_2 did not reduce colour darkening. The changes in L^*, C^* and $h°$ values showed similar decreasing trends

in both cases (Fig. 1a, b and c). Lightness and yellowness in the initial beige coloured seeds were masked as colour changed through light brown to dark brown. Ethylene had some effect in reducing colour darkening in faba beans. Whilst changes in L^*, a^* and b^* colour coordinates were small, they accumulated in ΔE^*_{ab} values (Fig. 1d.).

Oxygen accelerated colour darkening in faba bean and substantially changed L^*, C^* and $h°$ values even after 1-2 months. Compared to control, L^* and $h°$ values decreased faster with the passage of time. In contrast, C^* value remained almost constant for first 3 months and then decreased at a faster rate.

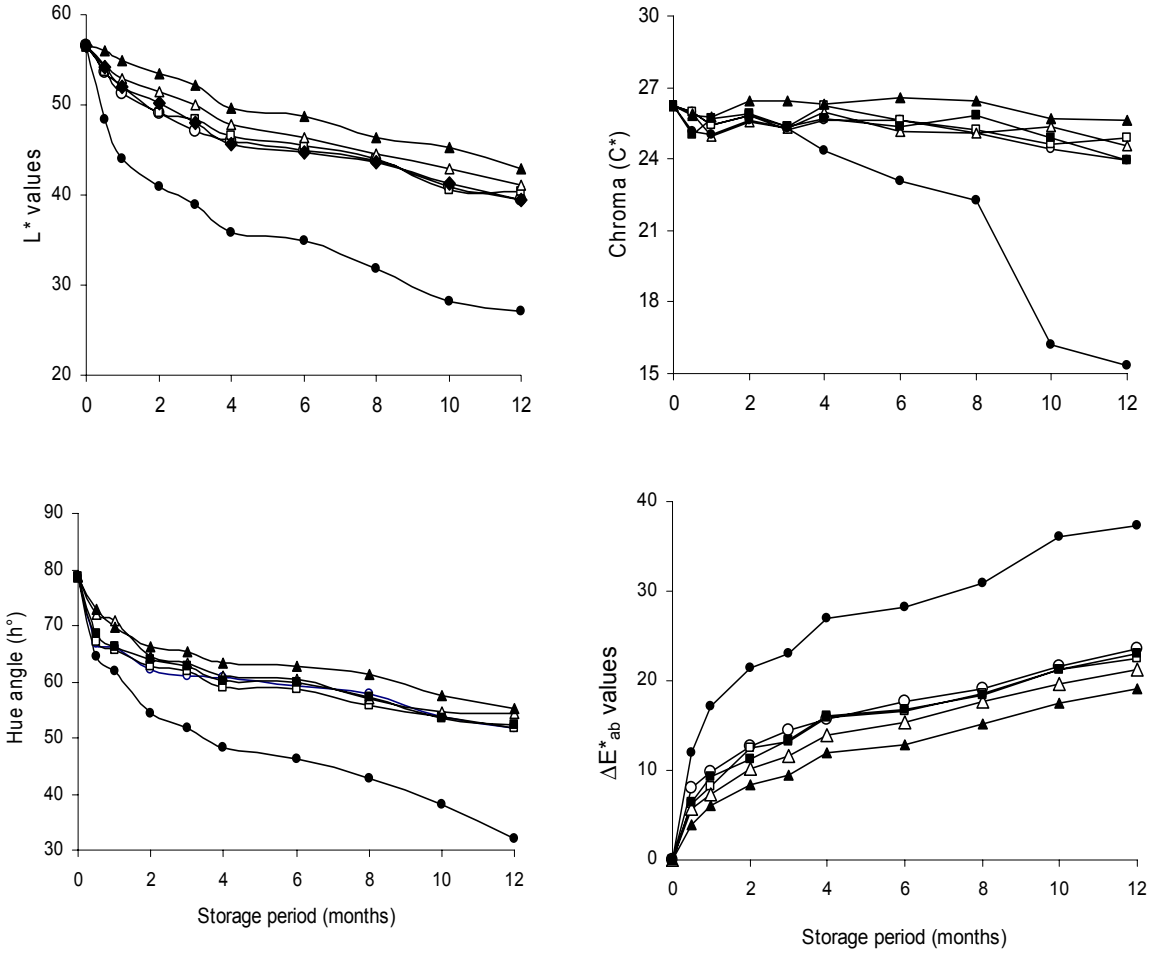

Figure 1. Effect of different modified atmosphere packaging on L^*, C^*, $h°$ and ΔE^*_{ab} values of faba bean seeds stored at 30°C for 12 months: air; —○—, vacuum; —□—, ethylene; —Δ—, O_2; —●—, CO_2; —■—, N_2; —▲—.

Compared to other MAP treatments, flushing with N_2 reduced colour darkening in faba beans. Nitrogen substantially reduced changes in L^* C^* and $h°$ values (Fig. 1a, b and c) and this was reflected in the stability of Colour Difference Index (ΔE^*_{ab} values) compared to other MAP treatments (Fig. 1d).

3.2. Phenolic Constituents

Phenolic constituents were correlated with colour darkening in faba bean (Table 1). Freshly harvested seeds contained 9.04 mg g^{-1} total phenolics of which a major part (4.25 mg g^{-1}) was tannins (Table 1). Among tannins, proanthocyanidins (condensed tannins) dominated (96%) phenolic compounds. Storage for 12 months led to substantial changes in phenolic constituents but this varied with MAP treatments. Air, vacuum and ethylene treated samples showed similar changes in phenolic constituents after 12 months storage but samples flushed with CO_2 and especially those flushed with O_2 demonstrated a much higher transformation change in extractable phenolic constituents. Seeds stored after flushing with CO_2 or O_2 had a demonstrated transformational loss of 33% and 50% respectively, in their total extractable phenolic contents. Samples flushed with N_2, on the other hand, lost only 13% of their total extractable phenolics after 12 months storage compared to freshly harvested seeds (Table 1).

Table 1. Phenolic constituents of faba beans stored under different modified atmosphere packaging for 12 months

Treatments	Total free phenolics (mg tannic acid g^{-1})	Non-tannin phenolics (mg tannic acid g^{-1})	Total tannins (mg of tannic acid g^{-1})	Proanthocyanidins (mg leucocyanidin g^{-1})
Control (Freshly harvested)	9.04 ± 0.05 a	4.79 ± 0.03 a	4.25 ± 0.02 a	4.07 ± 0.05 a
Air	7.14 ± 0.03 c	3.36 ± 0.11 c	3.78 ± 0.08 b	3.21 ± 0.03 c
Vacuum	7.29 ± 0.02 c	3.29 ± 0.05 c	4.04 ± 0.06 ab	3.31 ± 0.01 c
Ethylene	7.22 ± 0.04 c	3.32 ± 0.09 c	3.90 ± 0.06 b	3.30 ± 0.03 c
Carbon dioxide	6.07 ± 0.04 d	3.00 ± 0.05 c	3.07 ± 0.05 c	2.56 ± 0.02 d
Oxygen	4.48 ± 0.04 e	2.36 ± 0.05 d	2.12 ± 0.09 d	1.39 ± 0.03 e
Nitrogen	7.83 ± 0.04 b	3.89 ± 0.15 b	3.94 ± 0.13 ab	3.64 ± 0.04 b

Means (± s.e., n = 3) sharing the same letter in a column are not significantly different ($p \leq 0.05$) according to Tukey's HSD test.

Tannins were the major proportion of total phenolics in faba bean of which proanthocyanidins were the predominant group. Tannin contents were also negatively correlated with colour darkening in faba bean. Seeds stored after flushing with O_2 had more colour darkening than other MAP treatments and had much higher loss in tannin contents (Table 1). Seeds flushed with N_2 had less colour darkening and demonstrated a lower level of tannin transformation.

Discussion

Flushing with O_2 substantially accelerated the colour darkening process in faba bean accompanied by a demonstrated reduction in phenolic contents. Total phenolic contents had a high negative correlation ($r = -0.92$) with ΔE^*_{ab} values; the total colour difference from freshly harvested beans (Fig. 2). This is supported by earlier studies that colour darkening in faba bean is probably due to oxidative alteration of phenolic compounds (Marquardt *et al.* 1978). Phenolic compounds vary widely in complexity but the common characteristic of all these compounds is that they are readily oxidised (Bors *et al.* 1996). Tannins, which were a dominant group among phenolics of faba bean testa, are well known for their antioxidant activities (Beninger and Hosfield 2003). They play an important role in the defence system of seeds by exposing themselves to oxidative damage caused by environmental factors such as light, O_2, free radicals and metal ions (Troszynska and Ciska 2002). Storage of faba bean with low O_2 concentration results in reduced colour darkening and varieties with high tannin content darken more in air than low tannin varieties, suggesting that darkening of seed coats is possibly due to oxidation of polyphenolics such as tannins (Black and Brouwer 1998b). Stanley (1992a) also suggests that colour darkening in beans during storage is probably caused by air- and light-catalysed oxidation of leucoanthocyanidins, a group of phenolic compounds. Similarly, changes in total phenolic acids of minimally processed lettuce (*Lactuca sativa*) leaves are reduced when they are stored in MAP conditions of low O_2 (2-3%) and high CO_2 (12-14%) compared with storage in air and this controls browning (Gil *et al.* 1998). The availability of O_2 in air during storage is regarded as the main source of oxidation (measured as increase in colour darkening of beans), hence, traditional methods of storage of faba bean in the Middle East employ the use of underground pits which are filled completely with seeds to minimise air volume (El-Refai *et al.* 1988).

Involvement of oxidation processes in colour darkening of faba bean is also supported by the fact that when beans, grains or their products are stored under low O_2

atmosphere, their quality deterioration, including colour changes, is substantially reduced. Low O_2 (5-10 kPa) and high CO_2 (5 kPa) in the packaging of snow pea pods is helpful in maintaining their quality by retarding changes in organic acids, free amino acids and sugar contents, and sensory attributes (Pariasca *et al.* 2001). There is a more stable nutritional quality (vitamins and minerals content) of green beans stored under 3% O_2 + 3% CO_2, than under atmospheric air storage (Sanchez-Mata *et al.* 2003). Black beans stored for one year at 30 ± 3°C and 70-80% relative humidity under a modified atmosphere (containing N_2 and CO_2) in an impermeable container, lose quality in terms of hardening, at a slower rate than beans stored in air (in mesh bags) in the same environment (Aguilera and Rivera 1990).

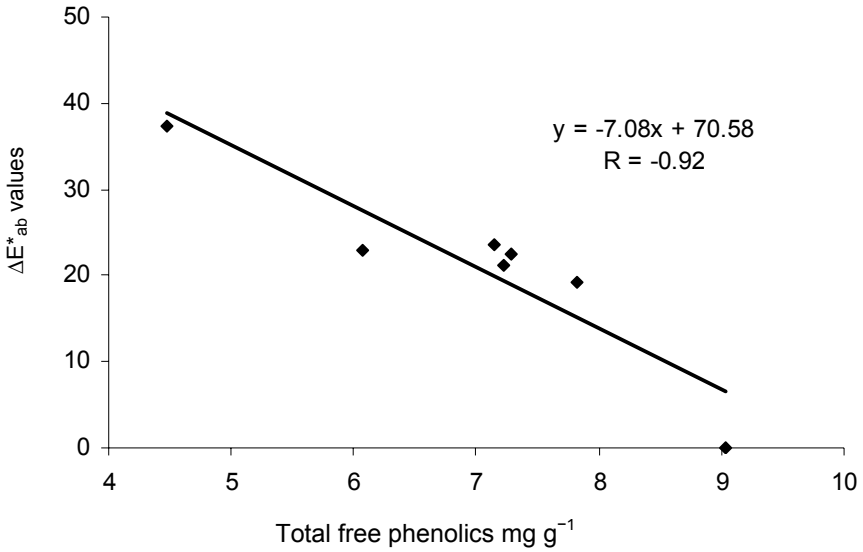

Figure 2. Correlation between total phenolic contents and colour darkening (ΔE^*_{ab}) in faba beans stored under different modified atmosphere packaging for 12 months.

Flushing with N_2 demonstrated a marked effect in reducing colour darkening in faba bean. The mode of action of N_2 in reducing colour darkening may be associated with the reduction in oxidative transformation of phenolic compounds. This is supported by studies in other foods and food products. Drying of field pea seeds under N_2 and in a vacuum inhibits seed coat browning compared to seeds dried in air or O_2 (Marbach and Mayer 1974). Enzymatic browning in minimally processed apple pieces can be successfully inhibited for long storage times by application of a modified atmosphere of 80% N_2 and 20% CO_2 (Nicoli *et al.* 1994) or 100% N_2 (Soliva-Fortuny *et al.* 2001). Similarly, nitrogen flushing is more effective than other gas treatments in preventing browning of cut potatoes (Gunes and Lee 1997). The results here indicate that some

oxidative reactions involved in colour darkening in faba bean can be minimised by flushing with N_2.

Faba bean flushed with CO_2 adsorbed almost all of the gas in the pouches which is similar to peanuts and oil seeds (Holaday *et al.* 1979), which also adsorb a large amount of CO_2 . Carbon dioxide, solely or in combination with other gases, is helpful in reducing oxidation (Holaday *et al.* 1979) and maintaining other quality parameters in foods (Nicoli *et al.* 1994; Pariasca *et al.* 2001) but it did not retard colour darkening and demonstratably not oxidative transformation of phenolic compounds in faba beans. This may be due to its interaction with phenolic compounds. Storage of strawberry with air enriched with up to 40% CO_2 increases colour darkness (increased L* value) and is accompanied with reduction of anthocyanin content (Gil *et al.* 1997). High CO_2 concentrations (> 73%) as a result of MAP destabilize anthocyanin derivatives in the skin of apples (Lin *et al.* 1991). Similar reactions with CO_2 might have occurred with faba bean testa that contains high quantities of proanthocyanidins (Nasar-Abbas *et al.* 2007b).

Conclusion

Oxidative transformation of phenolic contents caused by the presence of environmental oxygen was one of the major factors that caused seed coat darkening in faba beans during storage. Strong correlations between extractable phenolic transformation and colour degradative effects further emphasise the strong linkage between extractable phenolic compositional changes and faba bean colour changes.

One of the practical ways to avoid the availability of oxygen during storage of faba beans is by modifying the gaseous environment of the storage bins using N_2. The N_2 application may not require additional high cost as most of the farm storage bins in Australia are now constructed airtight (rolled steal) with pressure relief valves for CO_2 fumigation (Jayas and Jeyamkondan 2002). These farm storage bins can be successfully used for N_2 flushing for faba bean storage. This practice in addition to other measures, such as maintaining low seed moisture content and low temperature (Nasar-Abbas *et al.* 2007b), can minimise colour darkening in faba beans during long term storage. This would improve quality, market opportunities, price and hence profitability. Faba bean can also be packed in individual small packets flushed with nitrogen that could then go directly onto the supermarket shelf as a premium product.

CHAPTER SIX

Cooking quality of faba bean after storage at high temperature and the role of lignins and other phenolics in bean hardening

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Cooking quality of faba bean after storage at high temperature and the role of lignins and other phenolics in bean hardening

Syed M. Nasar-Abbas[1,2*], Julie A. Plummer[1,3], Kadambot H. M. Siddique[2,3], Peter White[4,2], David Harris[5,2] and Ken Dods[5,2]

[1]*School of Plant Biology, Faculty of Natural and Agricultural Sciences, The University of Western Australia, 35 Stirling Hwy, Crawley WA 6009, Australia*
[2]*Centre for Legumes in Mediterranean Agriculture (CLIMA), The University of Western Australia, 35 Stirling Hwy, Crawley WA 6009, Australia*
[3]*Institute of Agriculture, The University of Western Australia, 35 Stirling Hwy, Crawley WA 6009, Australia*
[4]*Department of Agriculture and Food Western Australia, 3 Baron-Hay Court, South Perth WA 6151, Australia*
[5]*Chemistry Centre Western Australia, 125 Hay St, East Perth WA 6004, Australia*
**Corresponding author; e-mail: abbass01@cyllene.uwa.edu.au*

Abstract

Selected physical and chemical characteristics of faba beans (*Vicia faba* L.) cv. Fiesta were studied after 12 months storage at 5, 15, 25, 37, 45 or 50 °C (± 2 °C) in relation to the hard-to-cook phenomenon. In comparison with control (seeds stored at 5°C), seeds stored at 15 and 25°C demonstrated non significant ($p \leq 0.05$) changes in most of the physical and chemical characteristics including hydration and swelling coefficients, acid detergent fibre, lignin and tannin contents, whereas seeds stored at ≥ 37°C demonstrated significant changes ($p \leq 0.05$). Solutes and electrolytes leaching after 18 h soaking substantially increased with increased temperature. Faba bean hardness tested by the hard-to-cook test also increased substantially with increased storage temperature. After 8 h soaking followed by 2 h cooking, the puncture force required for seeds stored at 5°C was 3.3 *N* seed^{-1} whereas seeds stored at 50°C required a much higher puncture force of 15.2 *N* seed^{-1}. There was a high negative correlation ($r^2 = 0.98$) between storage temperature and cooking ability of faba bean. Substantial increases in acid detergent fibre and lignin contents occurred with increased storage temperatures. Acid detergent fibre content was 9.6% for seeds stored at 5°C (control) and this increased to 11.2% for seeds stored at 50°C. There was a three-fold increase in lignin content of faba bean stored at 50°C compared to those stored at 5°C and it was correlated with bean hardness ($r^2 = 0.98$). Storage at high temperatures for 12 months led to a substantial reduction in total free phenolics especially in the testa and there was a greater reduction with increasing storage temperature. There was 70% reduction in total free phenolics in the testa of faba beans stored at 50°C compared to seeds stored at 5°C. Reduction in free

phenolics was negatively correlated (r^2 = 0.75) with bean hardness. These results are discussed in relation to possible mechanisms associated with the development of hard-to-cook defect in faba bean.

Keywords: Vicia faba L.; hard-to-cook; lignins; phenolics; physicochemical properties

Introduction

Legume seeds are mostly preserved in dry storage at ambient temperature to maintain year-round supply of this important protein food source. High temperature during storage, in countries such as Australia where air temperatures in summer may rise up to 40°C, can cause deteriorative affects on legume seed quality. The main form of deterioration is increased hardness of cotyledons or loss of cookability (ability to soften with cooking), followed by deterioration of colour, texture and loss of nutritive value (Martin-Cabrejas *et al.* 1997; Yousif *et al.* 2003a). Hardness of cotyledons is commonly described as the "hard-to-cook" (HTC) phenomenon and is characterized by a requirement for extended cooking time. Hard-to-cook beans need additional energy during preparation and may have inferior nutritional qualities in terms of protein, fats and mineral contents. Long cooking time is also one of the factors responsible for wider underutilization of legume seeds (Deshpande *et al.* 1984). Water absorption, soluble solids and electrolyte leaching are important quality factors associated with the bean hardness defect (Berrios *et al.* 1999) and are good indicators of the loss in bean quality during storage.

Several hypotheses have been proposed to explain the cause of bean hardening, including involvement of phenolic compounds (Garcia *et al.* 1998; Maurer *et al.* 2004). Hincks and Stanley (1986) proposed multiple mechanisms of bean hardening which include phenol metabolism as a major contributor during storage. Lignins are complex phenolic heteropolymers deposited in cell walls. Lignins reinforce and waterproof walls of specialized cells and play a fundamental role in mechanical support (Boudet *et al.* 1995). The deposition of lignin-like material around bean cotyledon cells promotes hardening (Hincks and Stanley 1987) and thus lignins may also be involved in HTC phenomenon in beans.

Faba bean, which is one of the major grain legume crops in Australia and many other parts of the World , also exhibit deteriorative changes when stored under unfavourable conditions (El-Refai *et al.* 1988) that adversely affect its value and market opportunities. However, there is little information regarding the effect of high temperature during

storage on quality parameters of faba beans. The objective of this study was to determine the effect of storage (12 months period) at different temperatures on the cooking quality of faba bean and to determine whether changes in lignin, phenolic and other contents relate with the development of HTC phenomenon in faba beans.

Materials and Methods

Plant Material

Faba beans (*Vicia faba* L.), cv. Fiesta, were grown at Borden (longitude, 118.26 E; latitude, 34.07 S), Western Australia as part of the field evaluation of the Australian National Faba Bean Improvement Program. Faba beans were harvested in December 2003 and kept at 5°C in the dark until used for experiments in February 2004. Good colour (beige/buff) and healthy seeds (free from insect damage, visible viral or fungal attack or broken testa) were individually selected. Average seed weight was 73.2 g per 100 seeds.

Conditions and duration of storage

Seed samples (3 x 25 g) were placed in polyethylene lined aluminium foil bags (10 x 10 cm) and sealed with an impulse heat sealer. Bags were placed in plastic storage boxes and stored at 5, 15, 25, 37, 45 and 50°C for one year to induce different levels of deterioration.

Physical properties

Hydration coefficient (Imbibition value)

Hydration coefficient was determined by soaking 10 g of bean seeds at room temperature (25°C) in 50 ml deionised water (ratio of 1:5). After 18 h the beans were removed from the soaking water, cut into two halves along the fissure and separated into the testa and cotyledon parts. Free water was removed by blotting paper and the sample was reweighed. Gain in weight was taken as the amount of water absorbed and expressed as the hydration coefficient (El-Refai *et al.* 1988).

$$\text{Hydration coefficient} = \frac{\text{Weight of bean seeds after soaking}}{\text{Weight of bean seeds before soaking}} \times 100 \qquad \text{Eq. I}$$

Swelling coefficient

The volume of raw bean seeds before and after soaking in deionised water for 18 h at 25°C was determined by water volume displaced in a graduated cylinder and expressed as the swelling coefficient (El-Refai *et al.* 1988).

$$\text{Swelling coefficient} = \frac{\text{Volume of bean seeds after soaking}}{\text{Volume of bean seeds before soaking}} \times 100 \qquad \text{Eq. II}$$

Electrolytes and solutes leaching

After 18 h soaking in deionised water the soak water was collected and leached electrolytes were quantified by assessing conductivity with a digital conductivity meter (PW 9527, Digital Conductivity Meter, Philips) in μ S cm^{-1} at 25°C (Hentges *et al.* 1991). To measure solutes leached from beans during soaking, soak water was evaporated, dried in a hot air oven (105°C), cooled in a desiccator, weighed and expressed as mg g^{-1} dry weight of beans.

Hard-to-cook test (puncture force)

Whole beans (3 x 10 g) were soaked at room temperature (25°C) in 50 ml distilled water (ratio of 1:5) in closed lid glass jars (100 ml capacity). After 8 hours jars were placed in a hot air oven (105°C) for 2 hours followed by cooling at room temperature (25°C) for 30 min. Hardness was measured using the method given by Reyes-Moreno et al. (1994). Ultra Test (Mecmesin Ltd., EU) equipped with a flat 2 mm diameter steel punch was used with a crosshead speed of 30 cm min^{-1}. A total of 3 x 10 beans were punched individually for each treatment and the mean peak force was calculated in Newtons per seed (N $seed^{-1}$).

Chemical analysis

Proximate composition

Proximate composition was determined using methods given by Association of Official Analytical Chemists (AOAC 2000) in triplicate (or otherwise mentioned) on raw material and expressed on a dry weight basis. Moisture content was determined by method 925.10, ash by method 923.03 and crude protein by method 968.06 using Truespec® CN, Carbon/Nitrogen determinator (LECO Corporation, MI, USA). Crude protein was calculated as N x 5.70. Crude fat was determined by using AOAC method 963.15 except that hexane was used as a solvent instead of petroleum ether.

Acid detergent fibre (ADF) and lignin (H_2SO_4) contents

Acid detergent fibre and lignin were determined by applying AOAC method 973.18. Cationic detergent in 0.5 M H_2SO_4 (20 g cetyle trimethylammonium bromide per 1 L 0.5 M H_2SO_4) was used to remove acid-labile carbohydrates, proteins and fats leaving a fibrous residue that is primarily cellulose and lignin. Sulphuric acid lignin is defined as the residue remaining after cellulose and other organic matter in acid detergent fibre is solubilised by 72% H_2SO_4.

Phenolic Constituents

Total free phenolics, tannins and condensed tannins (proanthocyanidins) were determined in testa and cotyledons separately. Testa of seeds (3 x 10) was manually removed and the hilum excised and discarded. Testa and cotyledons were ground separately with a grinder (IKA® A11 basic, IKA®-WERKE GmbH & Co. Germany). Ground testa (0.2 g) and cotyledons (2 g) were extracted with 20 ml of 70% v/v aq. acetone (analytical grade) by applying 20 min ultrasonic treatment at 4°C followed by overnight mechanical tumbling. Extracts were analysed for total phenolics using the Folin-Ciocalteu's Phenol Reagent (Merck) according to the method of Makkar *et al*. (1993). Total phenolic compounds were calculated from a prepared standard curve of tannic acid (Merck) in an identical matrix. Tannins were complexed with polyvinylpolypyrrolidone (Sigma) and unbound phenolics determined as above. Total tannins were calculated by subtracting non-tannin phenolics from total phenolics.

Statistical analysis

Analysis of variance was conducted using SPSS 11.0 for Windows and the means were separated according to Tukey's Honestly Significant Difference (Tukey's HSD) test at a significance level of 0.05.

Results

Effect of storage temperature on some physical properties

Substantial differences occurred in physical characteristics of faba beans stored at different temperatures (Table 1) for one year. Hydration and swelling coefficients that reflect the capacity to imbibe water in a reasonable length of soaking time was substantially affected by storage temperature. After 18 h soaking at 25°C, the hydration coefficient was significantly ($p \leq 0.05$) lower in samples stored at higher temperatures, especially those stored at ≥ 37°C, compared to samples stored at lower temperatures (≤

25°C). A layer of free water was present between the cotyledons and testa and in the fissure between cotyledons of hard beans which had been stored at ≥ 37°C.

The swelling coefficient behaved in the same way as hydration coefficient because swelling depends mainly upon the amount of water absorbed. The swelling capacity of beans decreased with increased storage temperature (Table 1). Compared with seeds stored at 5°C, there was only 3% decrease in hydration and swelling coefficients of seeds stored at 25°C whereas seeds stored at ≥ 37°C had a decrease of 10-18 % in hydration coefficient and 7-19% decrease in swelling coefficient.

Table 1. Changes in physical properties of faba beans stored at different temperatures for 12 months

Storage temperature (°C)	Hydration coefficient	Swelling coefficient	Electric conductivity (μ S cm^{-1})	Solutes leaching (mg g^{-1})	Hardness (N seed^{-1})
5 (Control)	193 ± 3 a	215 ± 4 a	827 ± 23 a	3.8 ± 0.4 a	3.3 ± 0.2 a
15	191 ± 2 a	210 ± 2 ab	905 ± 19 a	6.6 ± 0.5 ab	5.2 ± 0.3 b
25	188 ± 2 a	209 ± 2 ab	1115 ± 55 b	7.4 ± 0.2 b	7.1 ± 0.2 c
37	174 ± 2 b	201 ± 3 b	2523 ± 49 c	18.1 ± 0.7 c	10.7 ± 0.2 d
45	170 ± 3 b	189 ± 4 c	3216 ± 61 d	24.8 ± 1.8 d	13.7 ± 0.3 e
50	158 ± 2 c	175 ± 2 d	3467 ± 60 e	36.1 ± 1.8 e	15.2 ± 0.3 f

Means (± s.e., n = 3 except hardness where n = 30) sharing the same letter in the column are non significant (p=0.05) according to Tukey's HSD test.

Solute and electrolyte leakage increased with increased storage temperature. Faba bean stored at ≥37 °C exhibited 18-36 mg g^{-1} solute leakage whereas seeds stored at ≤25°C had only 4-7 mg g^{-1} solute leakage (Table 1). Solute leakage was directly proportional to electric conductivity of the soaked water demonstrated by a correlation coefficient of $r^2 = 0.93$ (Figure1) and negatively correlated ($r^2 = 0.99$) with hydration and swelling coefficients (Figure 2).

Bean hardness tested by the hard-to-cook test increased substantially with increased storage temperature. After 8 h soaking followed by 2 h cooking, the puncture force required for seeds stored at 5°C was 3.3 N seed^{-1}, whereas seeds stored at 50°C required a much higher puncture force of 15.2 N seed^{-1} (Table 1). There was a high level of negative correlation ($r^2 = 0.98$) between storage temperature and cooking ability of bean (Figure 3).

Effect of storage on some chemical properties

Chemical properties of faba bean changed after storage at different temperatures for 12 months (Table 2). A slight but continuous reduction in ash content occurred with increased storage temperature. Crude fat content remained almost constant for beans stored at ≤25°C but slightly increased in samples stored at ≥37°C. Protein content on the other hand did not change with storage temperature.

Substantial increases in ADF and lignin contents occurred with increased storage temperatures. Acid Detergent Fibre content was 9.6% for seeds stored at 5°C (control) and this increased to 11.2% for seeds stored at 50°C. There was a three-fold increase in lignin content of faba bean stored at 50°C compared to those stored at 5°C. Both ADF and lignin contents demonstrated a high positive correlation (r^2) of 0.97 (Figure 4) and 0.98 (Figure 5) respectively, with bean hardness.

Storage at high temperatures for 12 months led to a substantial reduction in total free phenolic constituents especially in the testa and there was a greater decrease with increasing storage temperature (Table 2). There was 70% reduction in total free phenolics in the testa of faba beans stored at 50°C compared to seeds stored at 5°C. Reduction in free phenolics was negatively correlated ($r^2 = 0.75$) with bean hardness (Figure 6). Tannins were the major proportion (72-82%) of total phenolics in the testa of faba beans and they decreased with increased storage temperature.

Total phenolic contents of cotyledons also consistently decreased with increased storage temperature especially storage at temperatures ≥ 37°C (Table 2).

Table 2. Changes in some chemical constituents (dry weight basis) of faba bean stored at different temperatures for 12 months.

Storage Temperature (°C)	Ash (%)	Crude protein (%N x 5.70)	Crude fat (%)	ADF (%)	Lignins (%)	Total phenolics in testa (mg of tannic acid g^{-1})	Total tannins in testa (mg of tannic acid g^{-1})	Total phenolics in cotyledons (mg of tannic acid g^{-1})
5 (Control)	2.81 ± 0.01 a	24.0	1.64	9.6 ± 0.1 c	0.31 ± 0.03 d	59.5 ± 0.3 a	43.0 ± 0.3 a	1.62 ± 0.06 a
15	2.80 ± 0.01 ab	23.5	1.57	9.7 ± 0.2 c	0.34 ± 0.05 d	57.1 ± 0.9 ab	41.4 ± 0.8 a	1.61 ± 0.06 a
25	2.82 ± 0.02 a	24.5	1.55	10.0 ± 0.1 bc	0.43 ± 0.01 d	55.9 ± 1.3 b	40.7 ± 0.9 a	1.52 ± 0.03 ab
37	2.79 ± 0.01 ab	24.2	1.63	10.4 ± 0.2 b	0.71 ± 0.08 c	50.6 ± 0.9 c	36.1 ± 1.2 b	1.46 ± 0.06 bc
45	2.77 ± 0.03 ab	23.5	1.70	10.7 ± 0.1 b	0.86 ± 0.03 b	41.2 ± 0.7 d	30.0 ± 1.1 c	1.34 ± 0.04 cd
50	2.76 ± 0.01 c	23.5	1.81	11.2 ± 0.2 a	1.03 ± 0.02 a	15.0 ± 0.8 e	12.2 ± 0.8 d	1.29 ± 0.04 d

Means (± s.e. of n = 3 except for crude protein and crude fat where n = 2) for whole seeds except for crude fat and crude protein.
Means sharing the same letter in the column are non significant (p=0.05) according to Tukey's HSD test.

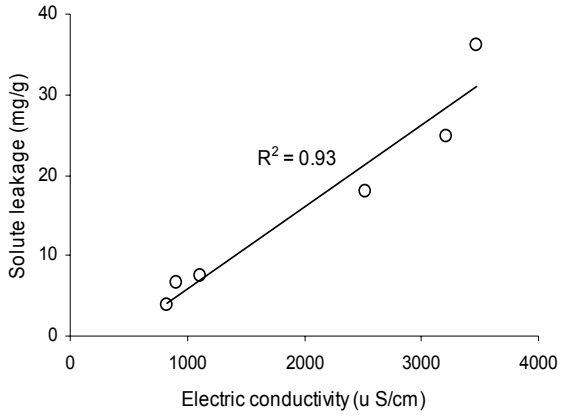


Figure 1. Correlation between solute leakage and electric conductivity of soaked water after 18 h soaking of faba bean at 25°C.

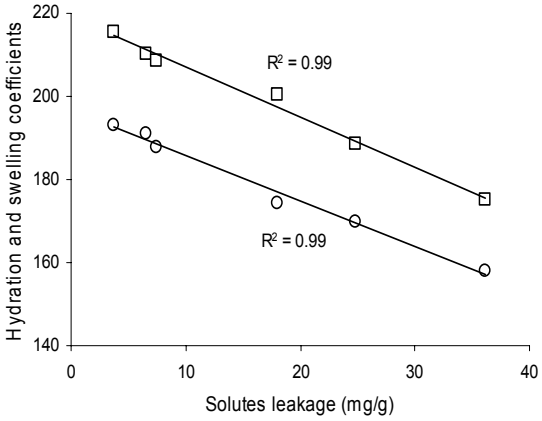


Figure 2. Correlation between solute leakage and hydration and swelling coefficients of faba bean after 18 h soaking at 25°C; hydration coefficient—○—, swelling coefficient —□—.

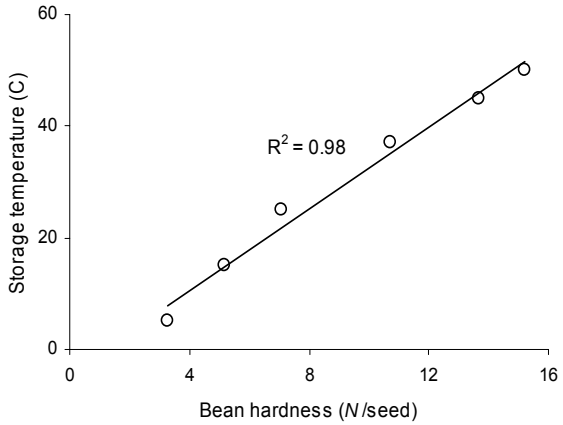


Figure 3. Correlation between storage temperature and cooking quality (bean hardness) of faba bean.

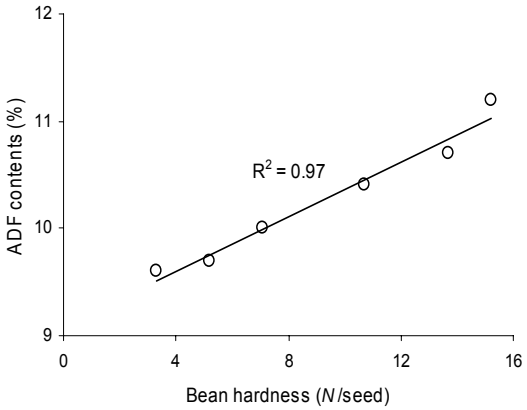


Figure 4. Correlation between changes in ADF contents and bean hardness in stored faba beans.

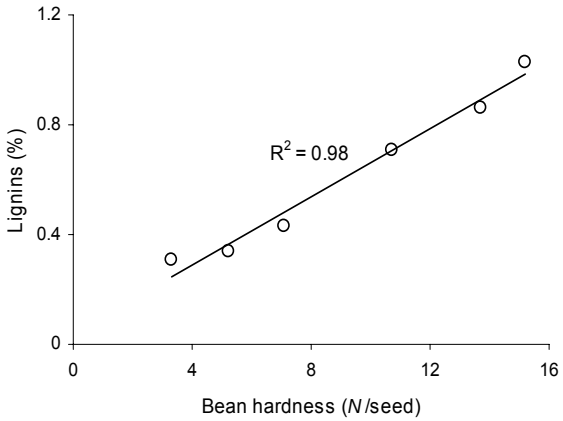


Figure 5. Correlation between changes in lignin contents and bean hardness in stored faba beans.

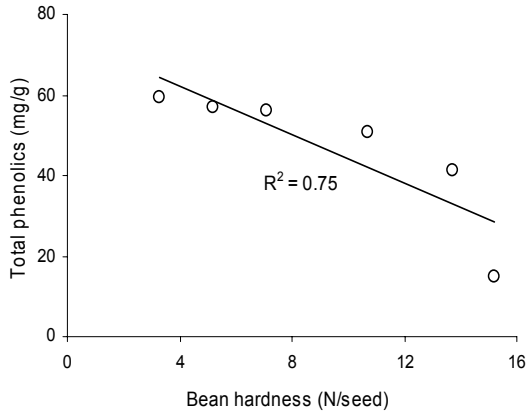


Figure 6. Correlation between changes in total phenolic content and bean hardness in stored faba beans.

Discussion

Effect of storage on some physical properties

Hardness is a textural problem where beans fail to soften sufficiently during the normal cooking process. The storage conditions played an important role in the hardening process of faba beans stored for a long period of time. A linear increase in the hard-to-cook (HTC) state was observed with storage temperature. Faba beans stored for an extended period under unfavourable conditions of high temperature (≥ 37°C) developed a harder texture after cooking compared with beans stored under favourable conditions (≤ 25°C) for the same time period. The cooking time of grain legumes varies with species and variety but hard to cook phenomenon as a function of extended storage time and high temperature is a common characteristic across various grain legumes. Extended storage of cow peas at 30°C increased seed hardness from 15.8 to 91.2 $N\ g^{-1}$, whereas seeds stored at −18°C showed no change (Liu et al. 1992). Even after medium term storage at 30-35°C, hardness of many common bean (*Phaseolus* spp) varieties increased by 3 to 6 $N\ seed^{-1}$ (Del Valle and Stanley 1995; Reyes-Moreno *et al.* 1994) .

Changes associated with hard-to-cook phenomenon are accelerated under high storage temperature and lead mainly to reduced hydration (imbibition) and swelling coefficients which in turn reduce cookability of seeds following long term storage. Storage of faba bean at lower temperatures 5-25°C had little effect but at temperatures ≥37°C, both hydration and swelling coefficients substantially decreased. Similar changes occur in a number of other grain legumes. Red kidney beans (*Phaseolus vulgaris* L.) stored at 32°C for 9 months exhibit a 10% decrease in bean hydration compared to those stored at 2°C (Moscoso *et al.* 1984). Storage of faba beans for nine months in underground pits or in tin cans stored at room temperature in Egypt leads to gradual reduction in hydration and swelling coefficients (El-Refai *et al.* 1988). Adzuki beans (*Vigna angularis*) stored at 30^{o}C for 6 months absorb substantially less water than those stored at 20°C and 10°C (Yousif *et al.* 2002).

Low hydration and swelling coefficients following storage at high temperature can be due to structural and chemical changes in the testa making it harder and less permeable to water so that it acts as a barrier, preventing water reaching the cotyledons. Water sorption is much faster in hulled dried beans than those with intact testa (Liu *et al.*

1992) and hence the preference for faster cooking dehulled beans, known as dhal, in the Indo-Pakistan subcontinent (Kon *et al.* 1973). Alternatively, structural and chemical changes in cotyledons can render them resistant to water absorption. Scanning electron microscopy reveals large intercellular spaces and small adhesion areas between cotyledon cells in black beans (*P. vulgaris* L.) stored at 4-5°C and small intercellular spaces and large adhesion areas between cotyledon cells in HTC beans stored at 23-25°C for 2 years (Berrios *et al.* 1998). Microscopic examination of soft cooked beans indicates cell separation and extensive disruption of the cell wall whereas HTC beans (stored at high temperature and high RH conditions) exhibit remnant linkages between cotyledon cells and minimal changes in the appearance of the cell wall (Aguilera and Rivera 1990). Scanning electron microscopy of stored HTC common beans (*P. vulgaris*) exhibits a dense packing of cotyledon cells with no separation between them as seen in fresh beans (Paredes-Lopez *et al.* 1989). The strong adhesion between cells observed for hard seeds might partially explain the reduced water uptake and consequently the slower rate of cooking (Hincks and Stanley 1987).

The reduced hydration coefficient was partially due to leaked solutes (including electrolytes) from cotyledons during imbibition which was much greater in harder beans, especially those stored at ≥37°C. A high negative correlation (r^2 = 0.99) was found between solute leakage and hydration coefficient (Figure 2). This also occurs in HTC black beans that are stored at higher temperatures. After 12 h soaking, the loss of solids and electrolytes from black beans stored at refrigeration temperatures (4-5°C) for 2 years is 10.5 mg g^{-1} compared with 18.6 mg g^{-1} for black beans stored at 23-25°C (Berrios *et al.* 1999). Leached solids may affect the hydration rate of beans in two ways. Solids leached into the soaking water increase the concentration of the solution, which in turn adversely affects the rate of water absorption by beans. Black beans soaked for 18 h in 1.75M sucrose solution have a substantial reduction (from 2.3 to 1.8) in imbibition value compared with imbibition in water (Jones and Boulter 1983). The soaking solution from beans also contains sugars as soaking of lentil, faba bean, common bean and cowpea in water for 12 h causes a loss of 20-27% in sucrose and 27-35% in raffinose family oligosaccharides (Abdel-Gawad 1993). Secondly, solute (including electrolyte) removal from bean cells may reduce their water affinity and water holding capacity in accordance with the osmotic principles.

Effect of storage on some chemical properties

There was a small but continuous reduction in ash content (sum of minerals content) with increased storage temperature (Table 2). The reason remains unknown but our results are in agreement to those of El-Refai et al. (1988) who demonstrated that storage of faba bean for nine months led to a moderate reduction in ash content with no loss of phosphorus, iron, calcium and magnesium contents. There were no appreciable changes in crude protein content of the samples stored at different temperatures as found in black beans (Berrios *et al.* 1999). In contrast, appreciable decreases in crude protein contents have been reported. In faba bean stored in tin cans at room temperature crude protein content decreased from 29.2 to 19.8% in 9 months (El-Refai *et al.* 1988).

In our study there was a slight increase in crude fat content with increased storage temperature especially at temperatures ≥ 45°C. This might be due to increased rancidity especially at higher temperature. Dry beans (*P. vulgaris*) with high moisture content stored at high temperature for a long period become rancid (Morris and Wood 1956). The oxidation of fats and the rate of rancidity development are highly dependent on temperature, and the higher the temperature the higher the rate of rancidity (Nogala-Kalucka and Gogolewski 2000). Slight changes in proximate composition suggest a negligible loss in nutritive value of aged or HTC seeds but there might be a loss of heat sensitive vitamins due to long cooking time in case of HTC beans (Garcia et al. 1998).
Reduced water absorption that leads to poor cookability of faba bean may be due to substantial increases in acid detergent fibre and lignin contents that reflect increases in faba bean testa and cotyledon cell wall fraction (Yousif and Deeth 2003) stored at higher temperatures (≥ 37°C). Cell wall contents of adzuki bean stored at 30°C for 6 months increase compared to beans stored at 10°C or 20°C (Yousif and Deeth 2003). High temperature (≥37°C) might have caused a thickness of cell walls as occurs in common beans stored at 35°C for 6.5 months (Garcia *et al.* 1998). This cell wall thickening in beans may be attributed to lignification (Hincks and Stanley 1987). Lignin serves as a matrix around the polysaccharide components of some plant cell walls, providing additional rigidity and compressive strength as well as rendering the walls hydrophobic and water impermeable (Whetten and Sederoff 1995). The lignin content increased during storage and this would enhance the HTC character. In legumes, during seed development, soluble phenolics of the seed coat can convert to lignins and this results in onset of impermeability of seed coats (Egley *et al.* 1985). Lignin-like

materials deposited around bean cotyledon cells promote hardening, both as a result of mechanical strength from the lignin as well as its action in preventing water imbibition and swelling (Hincks and Stanley 1986; 1987). Deposition of lignin can also occur in dead cells (Pickett-Heaps 1968) and this probably plays a major role in the cotyledon cell wall hardening that renders faba and other beans hard to cook. Lignification of the middle lamella can also occur during storage of legumes resulting in a further decrease in cookability (Del Valle and Stanley 1995).

The decrease in cooking quality of faba bean was also associated with phenolic constituents. There was a continuous and substantial decrease in total phenolics with the increase in storage temperature especially in the seeds stored at ≥ 37°C. A similar decrease in phenolic compounds is observed in common beans stored at high temperature (35°C) compared with beans stored at 4°C and it is associated with HTC phenomenon (Garcia *et al.* 1998). Similarly in four cultivars of cowpeas (*Vigna unguiculata* L Walp) there is a negative correlation between cooking time and total polyphenols (Giami and Okwechime 1993). There is also evidence of a causal relationship between decreased polyphenolic contents and cooking time during seed development of winged beans (*Psophocarpus tetragonolobus* L. DC) (Kadam *et al.* 1982).

Tannins, which were the major proportion of total phenolic content of faba beans testa, also decreased with increased storage temperature and this had an adverse effect on bean hardening. Similarly in red kidney beans stored under high temperature there is a strong correlation between decreasing condensed tannins (proanthocyanidins) and increasing hardness (Rozo *et al.* 1990). A decrease in tannin content and increase in hardness occurs in beans stored at 30°C for one year (Stanley 1992b).

The decrease in phenolic constituents with increase in storage temperature may be due to oxidative degradation, which can be accelerated at higher temperature. Phenolic compounds vary widely in complexity but the common characteristic of all these compounds is that they are readily oxidised and undergo phenolic reactions (Bors *et al.* 1996) . Storage of faba bean after flushing with N_2 retards, whereas flushing with O_2 accelerates reduction in phenolic contents, including tannins and proanthocyanidins (Nasar-Abbas *et al.* 2007a). Colour darkening in beans during storage accompanied by

seed hardening is probably caused by air- and light-catalysed oxidation of leucoanthocyanidins, a group of phenolic compounds (Stanley 1992a). Oxidation of phenolic compounds may directly affect permeability and cookability of seeds. The permeability to water of seed coats of field pea (*Pisum sativum* L.) is related to phenolic contents in the seed coat and to their level of oxidation as oxidation processes may cause structural changes which affect permeability to water (Marbach and Mayer 1974).

Some researchers dispute the role of phenolic compounds in bean hardness (Deshpande *et al.* 1982) because hardening occurs in white varieties of beans containing low concentrations of tannins (Stanley 1992b). However, hardness is related to seed coat (testa) impermeability and also to cotyledon impermeability. In the testa it may involve lignins as well as tannins, whereas in cotyledons it may be primarily lignification as cotyledons have low concentrations of phenolic compounds. The ratio of testa to cotyledon tannin contents is about 9:1 in dry beans (Deshpande *et al.* 1982). Here the tannin concentrations of cotyledon were too low to be detected using a standard method given by Makkar *et al*. (1993). Hence hardness of cotyledons which have very low tannin concentrations in both white and coloured faba bean varieties probably depends on lignins. Testa hardening however may be greater in coloured varieties as both tannins are probably involved in the hardening process. Cotyledons constitute the major proportion (about 90%) of beans and so extra hardening of testa due to tannins in coloured varieties may not cause an appreciable difference with seed hardening.

Conclusions

Twelve months storage of faba beans caused substantial deteriorative changes in physicochemical properties and those changes were temperature dependent. The higher the temperature the faster was the deteriorative effect. Main changes were increased ADF and lignin content (cell wall component) and reduced phenolic contents, which were correlated with hydration and swelling coefficients and cooking quality of faba beans. Beans stored at ≤25°C demonstrated appreciable stability in most of the physicochemical properties. Proximate analysis revealed that there was little or no effect on nutritive value of beans stored at different temperatures. Bean hardening (hard-to-cook) after storage under unfavourable temperature conditions is a complex phenomenon but it was mainly attributed to lignification of cotyledon cell wall and oxidative damage to phenolic contents.

CHAPTER SEVEN

Isolation and identification of phenolic compounds involved in testa colour darkening in faba bean during storage

Isolation and identification of phenolic compounds involved in testa colour darkening in faba bean during storage

Introduction

It has been known in the previous chapters that phenolic compounds especially tannins are associated with testa colour darkening in faba beans during storage. Tannins are a heterogenous mix of polyphenols of vegetable origin with high molecular weight. They are divided into two main groups: hydrolysed and condensed tannins. The latter presents complex structures such as dimerics, trimerics, oligomerics and polymerics (Ferreira and Nogueira 2000). Seed coats of the majority of the common varieties of legumes are known to contain appreciable amounts of proanthocyanidins (Merghem *et al.* 2004).

Several studies have demonstrated the presence of many groups and classes of phenolic compounds in faba beans. Faba beans contain proanthocyanidins, the highest level being in the seed coats (Cansfield *et al.* 1980; Griffiths 1981; Merghem *et al.* 2004; Nozzolillo *et al.* 1989). Three classes of flavonoids have been identified by HPLC fitted with a photodiode array detector: flavones, flavonols and anthocyanins. The largest amount of flavonols is extracted from the coats of green and red seeds, and the least from brown seeds (Nozzolillo *et al.* 1989). Myricetin is present in the immature seeds of two cultivars of broad bean (Herrmann and Woldecke 1977). Faba bean seed coat extract contains (+)-gallocatechin, (−)-epigallocatechin, (+)-catechin and (−)-epicatechin units. The major condensed tannins of faba bean comprise six compounds identified as two A-type procyanidin dimers, the procyanidin dimers B_1, B_2 and B_3, and a procyanidin trimer (Merghem *et al.* 2004).

The presence or absence of different types of phenolics is responsible for the natural testa colour in faba bean. Naturally, faba bean varieties have a range of colours that varies from beige, brown and red to violet. In faba bean seed coats, myricetin predominates over quercetin in beige, black, brown, green, red, and violet seeds. Kaempferol is present in substantial amounts only in spotted seeds. White seeds have only trace amounts of quercetin and kaempferol and are the only ones without

proanthocyanidins. Flavones of the apigenin type occur in all coloured but not white seed (Nozzolillo *et al.* 1989).

These studies demonstrate presence and involvement of different phenolic compounds in colour development of seed coat but there are no studies on the identification of compounds involved in seed coat colour darkening during storage and this requires further investigation. The aim of this study was to isolate and identify those compound/s using various analytical techniques.

Material and methods

Reagents

Methanol, HPLC grade, (Univar, USA), Acetone, analytical grade (Univar, USA), Acetonitrile, HPLC solvent (Baker chemicals, USA), ethyl acetate, dichloromethane and chloroform, liquid chromatography and UV spectrophotometry reagent (Mallinckrodt Chemicals, USA).

Plant Materials

Stored samples

Faba bean (cv. Fiesta) samples packed in polyethylene lined aluminium foil bags and stored at 5, 15, 25, 37, 45 and 50°C for 12 months (details in Chapter 4) were used. They had testa colour from beige through light brown, dark brown to chocolate brown depending upon the storage temperature. Higher the storage temperature; the darker was the colour.

Bulk sample

A bulk sample (20 kg) of freshly harvested faba bean (cv. Fiesta) was collected and stored at 5°C until used after about 3 months.

Sample preparation and extraction

Stored samples

Testa of 20 seeds from each of the samples stored at different temperatures was manually removed, hilum excised and discarded and testa ground with a grinder (IKA® A11 basic, IKA®-WERKE, Germany). The ground testa (0.2 g) material was extracted with 20 ml each of methanol, 70% aq. acetone (v/v) or 2% HCl-methanol (v/v) (Carmona *et al.* 1991; Hong and Wrolstad 1990; Makkar *et al.* 1993; Nozzolillo *et al.* 1989). Extraction was facilitated by applying 20 min ultrasonic treatment at 4°C

followed by overnight mechanical tumbling at room temperature. The extracts were stored at refrigeration temperature in dark for further analysis.

Bulk sample

Seeds were dehulled using a mechanical dehuller. Hulls (1 kg) were extracted with methanol. Hulls were divided into two portions of 500 g each. Each portion was placed in a 2 l glass bottle and 1.5 l methanol was added and tumbled overnight. After 18 h the extract was vacuum filtered through nylon filter (0.45 µm) and the procedure was repeated 3 times. Extracts were pooled and concentrated *in vacuo* in a rotary evaporator at 40°C. Near the final stage of concentration process, the extract was separated by itself into two parts, greenish and dark brown. Both extracts were collected separately and stored at refrigeration temperature in dark for further analysis.

HPLC analysis

Extracts of stored samples were analysed for phenolic contents by using a HPLC (Waters 2695) equipped with Waters 2996 Photodiode Array Detector. A Phenomenex Luna® C18 (4.0 x 150 mm, 5 µm) protected with a guard column packed with the same material was used for all analyses. The temperature of the column oven was 35°C. Gradient elution was performed using Acetonitrile (anhydrous for HPLC) as solution A and 10 mM ammonium formate buffer (pH 3) as solution B. The linear gradient was from 10% A/90% B to 90% A/10% B over 45 min and the flow rate was 0.5 ml/min. The injection volume was 20µl for all samples. The wavelength used was 200-600 nm. The chromatograms presented here were recorded at 280 nm.

Similarly extracts from the bulk sample were also analysed. A small quantity (2 mg) of each of dark brown and greenish samples was redissolved in 1ml methanol and analysed by HPLC procedure.

Partitioning

The dark brown extract (25 g) obtained from the bulk sample was redissolved in 150 ml methanol by adding 50 ml portions (3 x) successively. The reconstituted extract was mixed with 300 ml distilled water in a 1 l separating funnel. Then 400 ml dichloromethane (CH_2Cl_2) was added, mixed and left to separate for 24 h. Four layers were visible but only the lowest layer was completely separated from others. The lowest layer (dichloromethane extract) was removed and dried *in vacuo* using a rotary

evaporator (Buchi Rotavapour R-200, Switzerland) at 40°C. The extract weight was 0.49 g.

The rest of the material was left for further 24 h but it failed to separate clearly. A further 200 ml distilled water was added, mixed and left to stand for 24 h but this also failed to separate. Then 200 ml chloroform ($CHCl_3$) was added, mixed and left to stand. After one hour the lowest layer separated. It was collected in a flask and dried in a rotary evaporator at 40°C. The chloroform extract weighed 0.31 g.

Ethyl acetate (400 ml) was added to the remaining extract, mixed and kept for separation. After one hour it was separated and the lower layer was removed. The process was repeated with a further 400ml ethyl acetate and the extracts were pooled and dried as above. This ethyl acetate extract weighed 0.69 g.

The remaining extract was mixed with 400 ml *n*-butanol (butan-1-ol), separated and dried as above. The *n*-butanol extract weighed 8.05 g. The remaining water based extract could not be concentrated and was kept as such at 4°C in dark.

Individual extracts were analysed for phenolic compounds by HPLC. Each extract (2 mg) was dissolved in 1ml methanol and then 100 µg flavone (Sigma) was added as an internal standard.

Isolation and purification of peaks identified by HPLC analysis in n-butanol extract of the bulk sample

Separation by Sephadex LH-20 column

Isolation and purification of the peak at retention time (R_t) 1.0 min was attempted as it was the most prominent peak that was consistent with colour darkening in stored samples. The *n*-butanol extract (3 g) was dissolved in 2-3 ml methanol and 1ml deionized water. The extract was separated on a Sephadex LH-20 column (25 x 500 mm) eluted sequentially with methanol (1 l), dichloromethane: methanol, 1:1 (v/v) (150 ml), 70% aq. Acetone (v/v) (150 ml) and acetone (150 ml). Eluants were collected in 25 ml-bottles. In total 36 bottles were collected for methanol and 6 bottles for each of the other solvents. The eluants in individual bottles were analysed by HPLC and pooled accordingly. The pooled fractions were dried by rotary evaporator followed by freeze drying and stored in a freezer in dark. Each dried fraction (2 mg) was reconstituted in 1 ml methanol and analysed by HPLC.

Separation by reverse phase C-18 column

Further purification of the dried fraction was carried out by dissolving in 1ml methanol and separating on a C-18 column (Silica gel 100 C18-Reverse Phase, Fluka) by eluting sequentially with 100 ml each of 5%, 10%, 20%, 50% v/v aq. methanol and 100% methanol. The fractions were separately collected as above in 25 ml glass bottles and pooled according to the presence of peaks after HPLC analysis. The pooled fractions were concentrated by rotary evaporator to remove methanol and then freeze dried to remove water. The dried samples were reconstituted with methanol and again analysed by HPLC as above. Attempts were made to identify the compounds on the basis of their NMR spectra.

^{1}HNMR analysis

^{1}H NMR spectrum was obtained for the isolated peak using CD_3OD solvent on a Brucker AM-200 instrument. Mass spectrum was measured using a VG Autospec mass spectrometer (70 eV) (Wang *et al.* 2000) .

Identification of compounds by comparing with standards

To identify the compound/s demonstrating changes with the change in faba bean testa colour during storage, solutions (2 mg/ml methanol) of epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate (Sigma, St. Louis, MO, USA) were used following the compounds identified by Merghem *et al*. (2004). The prepared solutions were run on HPLC using the same method (given above) and peaks were compared for their retention times.

Results

Comparison of extraction solvents

Methanol gave better results as it produced clear and separate peaks (Figure 1) whereas 70% acetone produced its own peaks which masked some of the sample peaks (Figure 2).

In case of extraction with 2% HCl-methanol, concentrations may be higher (Carmona *et al.* 1991) but there were many more peaks, most probably due to acid-hydrolysis of the compounds (Figure 3). The aim was not to determine total concentration of phenolic compounds but to isolate and identify them in their original form so methanol was selected for bulk extraction.

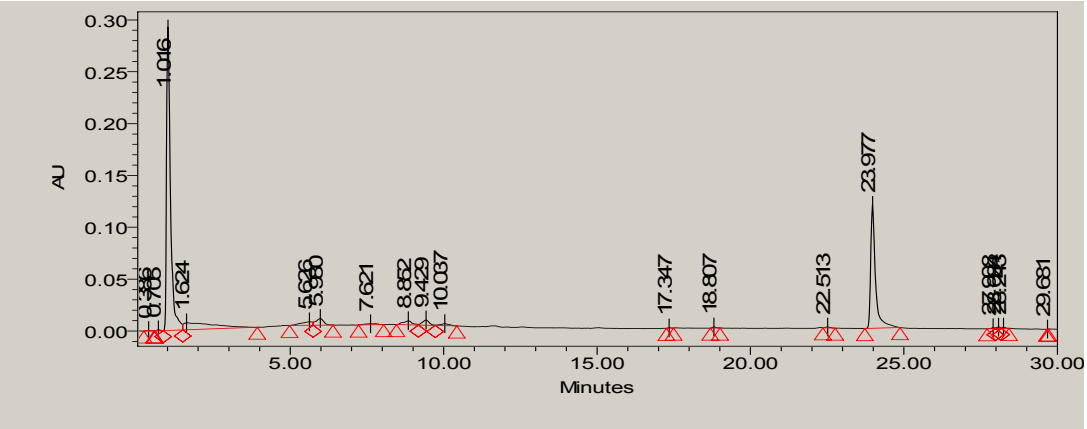


Figure 1. HPLC chromatogram of methanol extract of faba bean testa (sample stored at 5°C for 12 months).

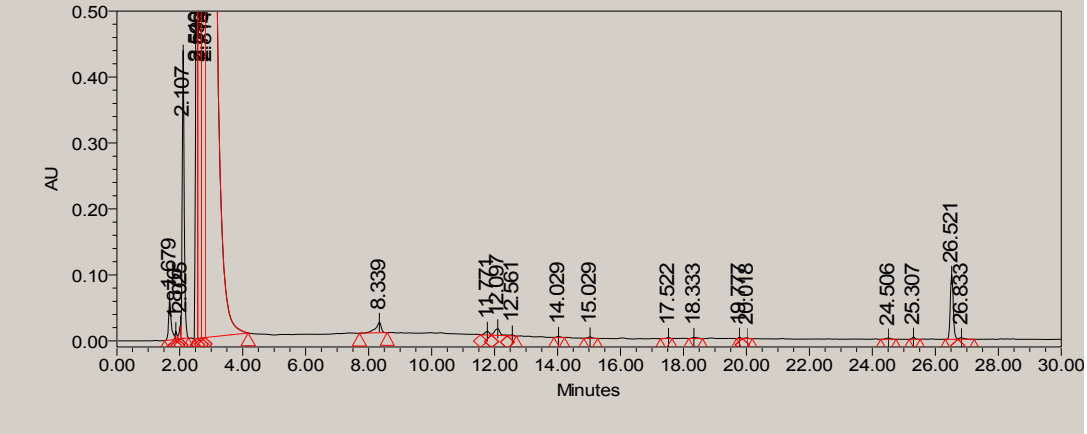


Figure 2. HPLC chromatogram of 70% acetone extract of faba bean testa (sample stored at 5°C for 12 months).

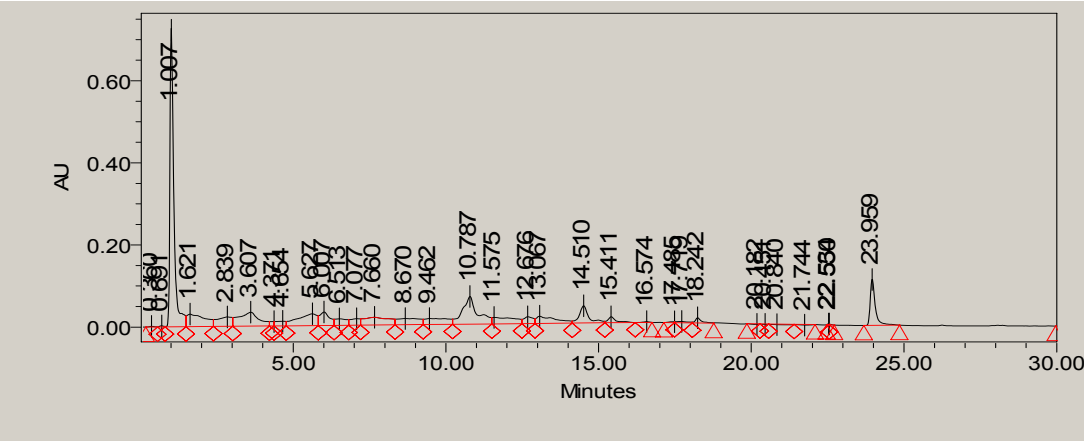
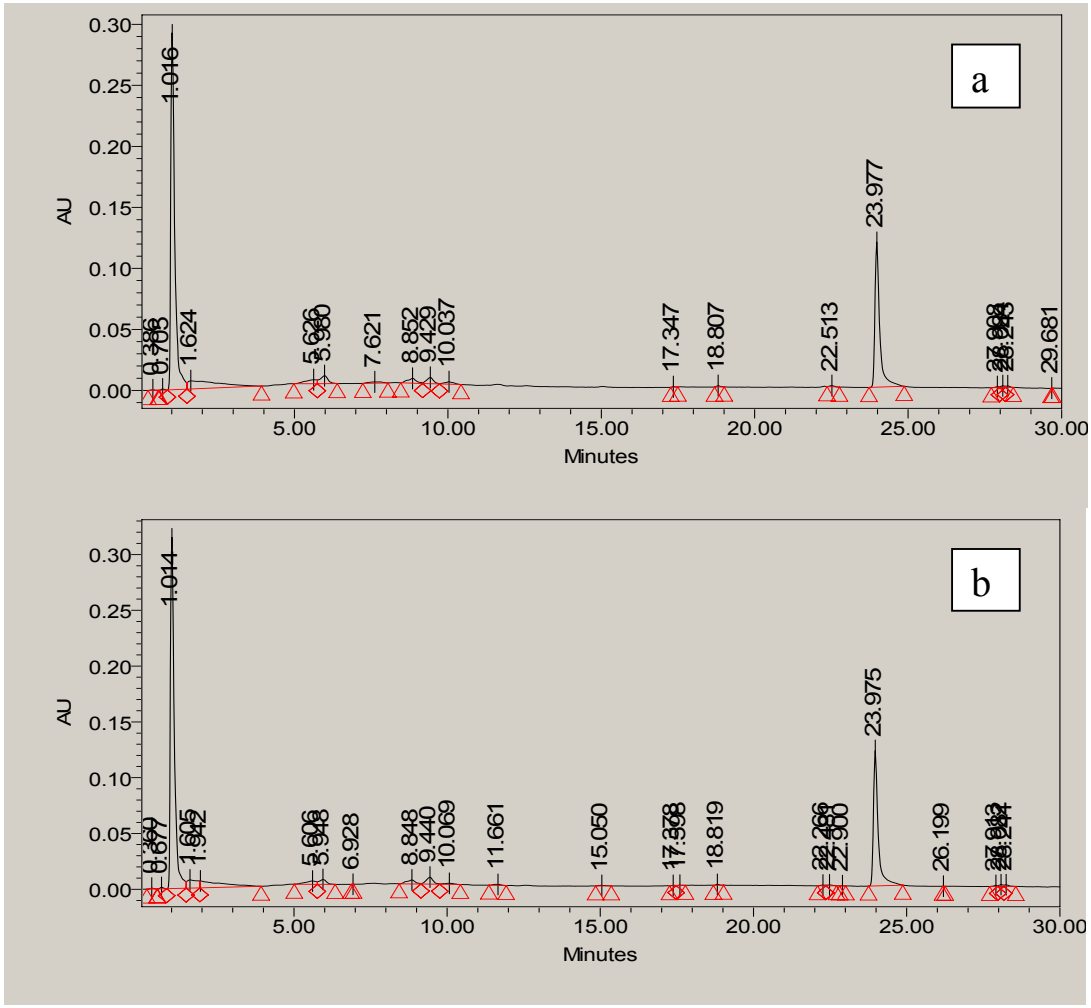


Figure 3. HPLC chromatogram of 2% HCL-methanol extract of faba bean testa (sample stored at 5°C for 12 months).

Analysis of stored samples

HPLC analyses of methanol extracts of samples with varying degree of colour darkening due to storage at 5, 15, 25, 37, 45 and 50°C for 12 months revealed a few peaks consistent with changes in colour darkening of faba bean testa (Figure 4a-f).



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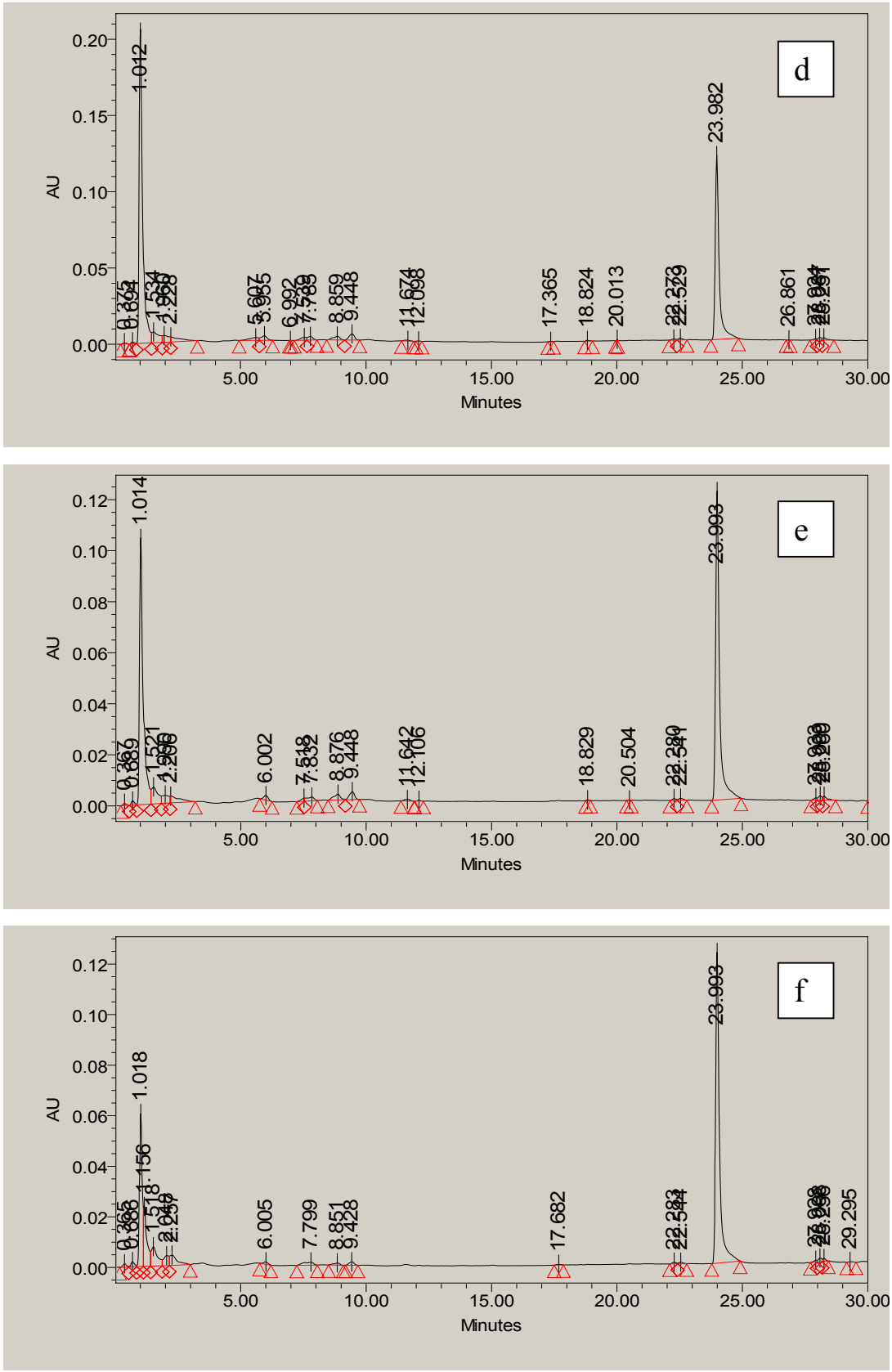

Figure 4. HPLC chromatogram of methanol extracts of faba bean testa stored at 5 (a), 15 (b), 25 (c), 37 (d), 45 (e) and 50°C (f) for 12 months.

The peaks at R_t 1.0, 5.5 and 6.0 min demonstrated a consistent decrease in their peak area divided by peak area of internal standard (Figure 5). Comparing with peak representing internal standard (R_t 24.0), the compound represented by the peak at R_t 1.0 is clearly decreasing with increase in colour darkening of faba bean.

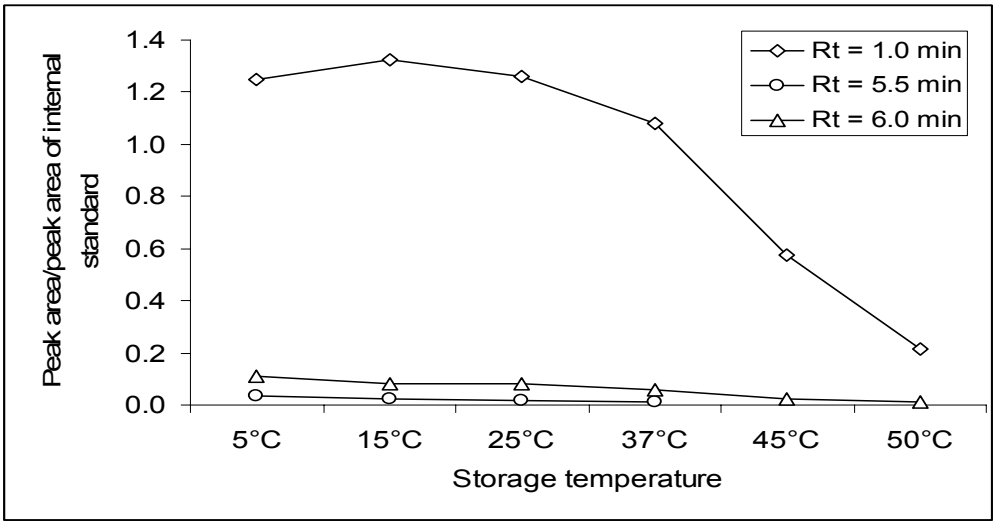

Figure 5. HPLC peaks that generally consistently decreased with increased colour darkening in faba beans stored at different temperatures.

Analysis of the bulk sample

HPLC chromatograms of dark brown and light green extracts obtained by bulk extraction (Figures 6a and b) show that one of the prominent peaks (R_t 1.0 min) was present in the dark brown fraction.

Separation of the compound by fractionation of brown extract using different solvents followed by HPLC analysis, demonstrated that the compound was present in *n*-butanol extract (Figure 7).

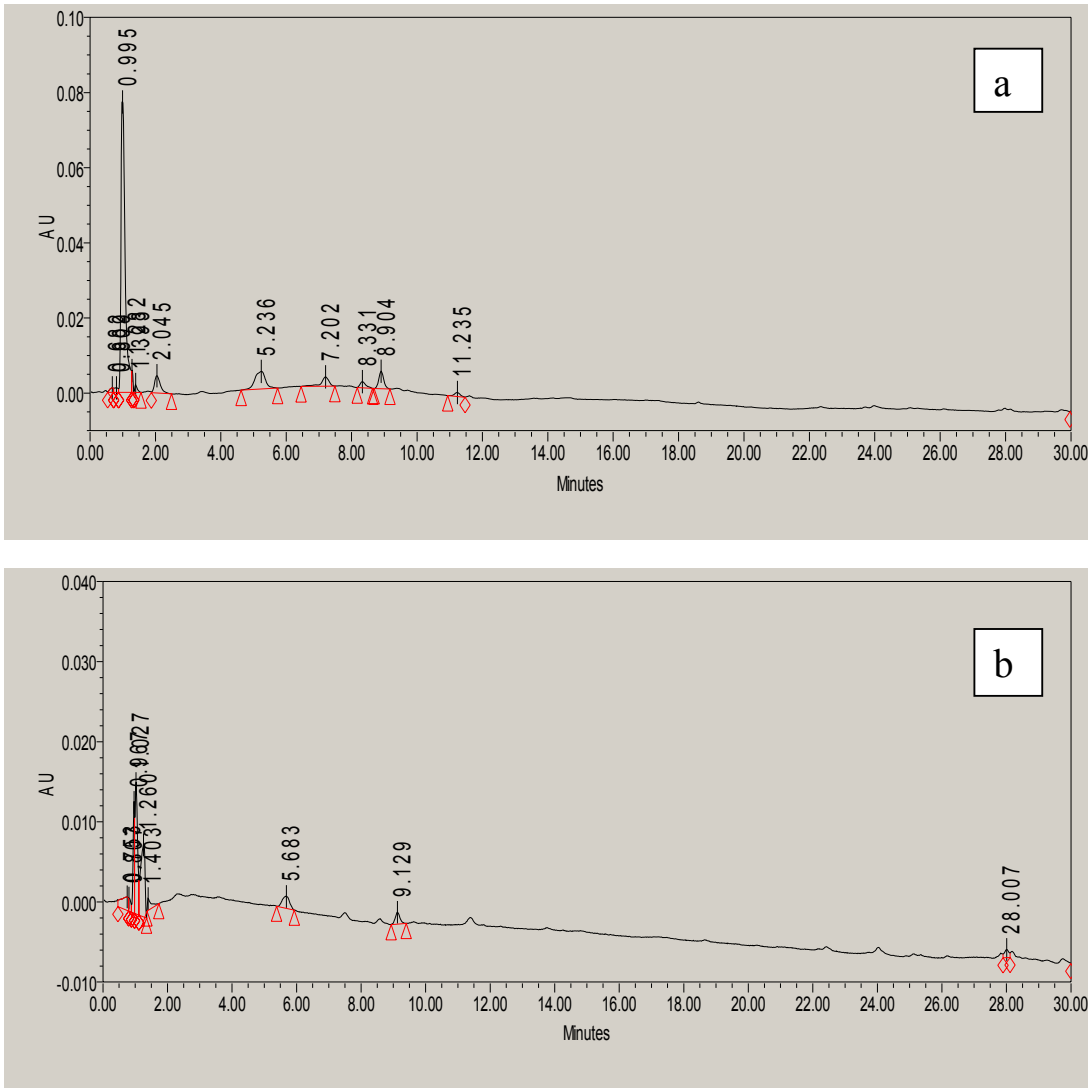

Figure 6a and b. HPLC chromatogram of dark brown (a) and greenish (b) parts of methanol bulk extract: dark brown (a) showing the presence of most of the required peaks.

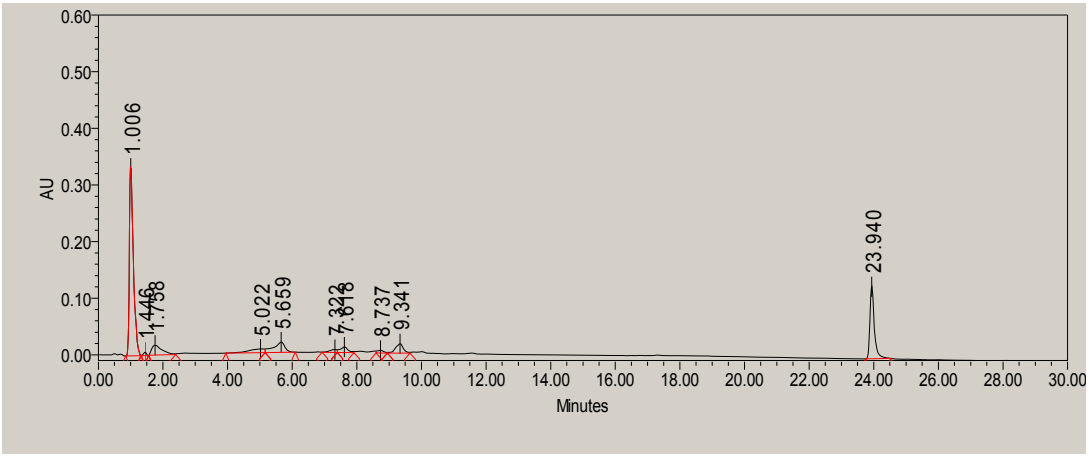

Figure 7. HPLC chromatograms for *n*-butanol extract showing the major peak at R_t 1.0 min. and flavone (internal standard) peak at R_t 23.9 min.

Other extracts either did not have the selected peak or there were many other peaks that may cause a hindrance in further isolation and purification processes. So the *n*-butanol extract was further processed using a Sephadex LH 20 column to isolate and purify the selected peak. Separation of *n*-butanol extract by using a Sephadex LH-20 column showed that the fraction 11-17 had an almost isolated peak (Figure 8).

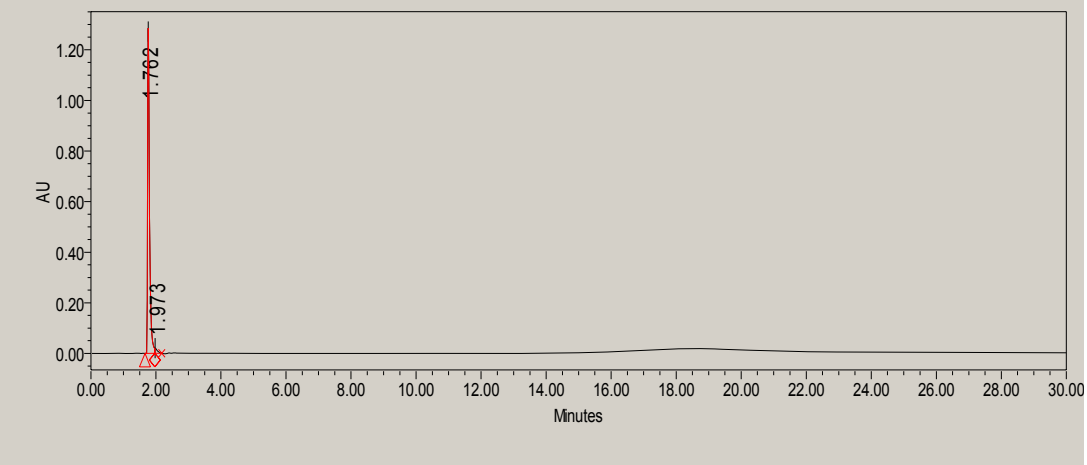

Figure 8. HPLC chromatogram of the isolated peak from *n*-butanol extract fractionated by LH-20 column.

The chromatogram shows a different R_t (1.76 min) for the peak but the UV spectrum demonstrated that it was the same peak which had a R_t of 1.0 min in previous chromatograms. The peak had maximum absorption at 230.9 nm with a small peak at 278.1 nm. The R_t for the peak might have changed due to a variation in the method.

^{1}H NMR analysis of isolated peak

^{1}H NMR spectrum (Figure 9) revealed that the peak identified by HPLC was a mixture of more than one compound. So, further purification was required for its identification which was carried out using a C-18 column as described above. The eluants obtained after using C-18 column were analysed by HPLC analysis but none of the fractions displayed the particular peak. The compound in the illusive peak may have been transformed into another compound during the concentration and drying procedure. Several repeated attempts failed to isolate the compound.

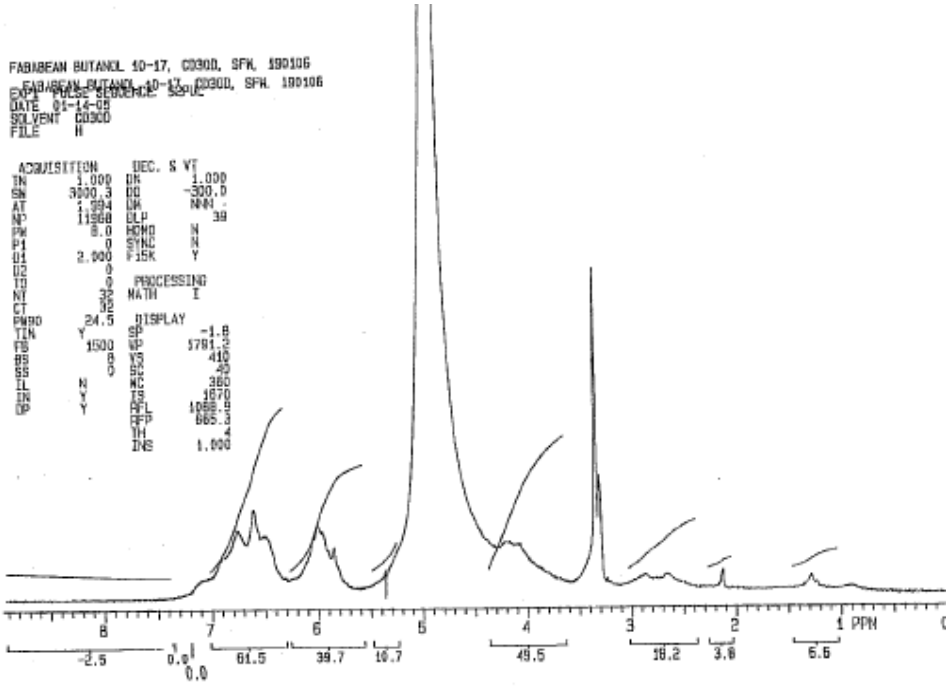

Figure 9. ^{1}H NMR spectrum of the isolated peak shown in the Figure 8.

Identification by comparing with standards

Attempts were made to identify the peaks using samples of compounds known to occur in faba bean (Merghem *et al.* 2004) including epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate. None of them developed a peak comparable with the retention time of the isolated peak. This means the peak was not among compounds we used as standards.

Discussion

A few phenolic compounds associated with colour darkening in faba bean were detected and separated. One of them at R_t of 1.0 min was very prominent but attempts to isolate and identify were not successful. The analysis of phenolic compounds, especially tannins has been difficult because of the structural complexity of these compounds (Schofield *et al.* 2001). It is also very difficult to isolate individual phenolic compounds as they are very unstable due to their sensitivity to heat, light and other factors such as oxidation (Bors *et al.* 1996; Okuda *et al.* 1989). Some more sophisticated procedures are required to isolate and purify the individual compounds especially for concentration and drying of the extracts. The most commonly used method is by rotary evaporators. Concentration using rotary evaporators, even *in vacuo*, is generally carried out at 30-40°C. The temperature may be high enough to introduce structural and chemical

changes in some phenolic compounds. The evaporation process may take hours especially when concentrating a water extract or water + organic solvent extract. Large and complex tannins are easily degraded into smaller tannins by water or dilute acids especially at elevated temperatures in just 30 min (Beasley *et al.* 1977; Okuda *et al.* 1989; Okuda *et al.* 1993). Freeze drying is an alternative way to dry extracts. It is suitable for water extracts but in case of water + organic solvent extracts, organic solvents should be removed before freeze drying otherwise it may damage the instrument. However, removal of organic solvents is commonly with a rotary evaporator.

Light during rotary evaporation may also adversely affect many phenolic compounds which are sensitive to light (Okuda *et al.* 1989). It is hard to run a rotary evaporator in dark because it requires a constant surveillance during running.

The use of rotary evaporators to concentrate and dry different types of extracts is quite common practice in analytical procedures. It may be suitable for concentration and drying of stable compounds present in a sample but not helpful when chasing a less stable compound. An alternative method of concentrating and drying of a phenolic extract could be the use of a nitrogen stream. The process can be run at low temperatures and can be managed in dark. Time did not permit further separation, isolation and drying of the compound/s using this technique.

The compounds responsible for colour darkening in faba bean remain illusive and their extraction, purification and identification will require extensive and delicate manipulations and analyses.

CHAPTER EIGHT

General Discussion

General Discussion

This study has contributed significantly to the understanding of seed discolouration in faba beans (*Vicia faba* L.). It provides greater understanding about different types of discolouration in faba beans and strategies to minimise their occurrence. Seed discolouration is a problem in many legume seeds (Edmister *et al.* 1990; Gunes and Lee 1997; Nozzolillo and De Bezada 1984; Yousif *et al.* 2003b) but it is not as critical as in faba bean especially when sold for human consumption. Other legume seeds may naturally be dark coloured and colour changes may not be distinguishable by eye or colour changes may be so minor and hence do not cause problems with consumer acceptance. In case of faba bean most commercial varieties are light in seed coat colour (beige/buff or light brown colour) (AGWEST 1998; Nilan 1967; Robertson and El-Sherbeeny 1991) and even a small change is easily visible. They also change colour very fast (Davies 1994) from beige to dark brown and even chocolate brown under unfavourable conditions.

The aim of this study was to investigate seed discolouration problems in faba bean which reduce its quality, value and market opportunity. Two types of discolouration were investigated; preharvest staining most commonly known as environmental staining and postharvest colour darkening or storage discolouration. To investigate different factors affecting environmental staining, data were collected from field trials at five locations in the southwestern Australian grain belt (Chapter 3). While a substantial proportion (20-53%) of seeds had some staining, 3-25% had a staining level that is generally not acceptable for human consumption in local and international markets. The problem was very complex and in field trials a few clear trends were evident. Seeds borne on the top of the plant had more staining and this was associated with higher phenolic contents. In southwestern Australia, faba beans are sown at the start of winter and harvested at the beginning of summer. Seeds borne in later stages of plant development are on the top nodes in the canopy and get greater exposure to light and high temperatures than those in the lower parts of the plant. A variety of mechanisms can account for the increase of phenolics with increased temperature, light intensity and longer photoperiods. This may be associated with the stimulation of enzymes involved in phenolic biosynthesis, in particular, the activity of phenylalanine ammonia-lyase, a

key enzyme in the synthetic pathway of phenolics (Barnes and Jones 1984; Flores *et al.* 2005; Sacher *et al.* 1972). It can also be explained by the carbon/nutrient balance mechanism (Bryant *et al.* 1983; Koricheva 2002; Waterman *et al.* 1984) which is disturbed by reduction in nutrient mobility due to reduced or lack of water availability in the later stage of plant growth under terminal soil moisture stress conditions in southwestern Australia and this could lead to increased phenolic production. This was supported by the increased prevalence of stained seeds in smaller and weaker plants than normal plants. Increase in Zn and Ca concentrations and a decrease in K contents were also associated with higher staining/phenolic contents and this is supported by the results from other researchers (Giertych *et al.* 1999; Glass and Dunlop 1974). Further detailed experiments that impose hyper and hypo availability of different nutrients are required to study their effect on discolouration phenomena.

Genetic variation in the susceptibility to discolouration was also evident. Fiord, an old variety, was more stained in Katanning and Borden sites than the other three varieties. This provides a base for detailed varietal suitability trials at different locations in the grain belt so that recommendations about suitable varieties for a certain area can be provided to farmers and growers. Targeted breeding and selection efforts to develop varieties less susceptible to environmental staining can be the best long term solution as some advanced lines within the Australian National Faba Bean Breeding Program were screened for environmental staining with the result that new varieties (Fiesta, Manafest and Farah) show considerably less environmental staining than the older varieties (Fiord or Ascot) (White *et al.* 2004). Some tannin free lines are resistant to environmental staining (Paull, personal communication) but there are concerns about their yield, pest and disease susceptibility and especially consumer acceptability for seed size, colour and taste. Development of reduced tannin varieties has the potential to reduce discolouration without affecting consumer acceptability and plant resistance to pests and diseases but sensory evaluation studies will be required for successful marketing of the product for human consumption.

Environmental staining is very complex and involves a number of factors that are hard to manage but short term practical solution lies in the choice of location and varieties and postharvest processing. Faba bean is more sensitive to environmental factors than the other legumes (Sangakkara *et al.* 1996a; 1996b). Plants that had good and vigorous growth had less staining of their seeds than weak plants. Hence, good agronomic

practices that assist in maintaining adequate nutrient, water supply and healthy plants throughout the growing season may reduce the problem substantially. In addition, postharvest processing practices, such as mechanical size grading, can be used to remove most of the discoloured seeds, which are generally smaller in size than the normal seeds within a seed lot of a specific variety. Further grading using electronic colour sorters can give top quality beans. It will add to costs but the produce could gain access to high value human consumption especially canning markets. The costs/ benefits of such systems would need to be investigated.

Seed colour darkening during storage at ambient temperature is another issue that reduces market value of faba beans. Darkened seeds are not relished by humans and go to animal feed and get lower prices in the market. A number of factors such as temperature, seed moisture content (SMC), light and storage period were studied (Chapter 4) in order to find a possible solution. Changes in colour difference index (ΔE^*_{ab} values) calculated from L^*, a^* and b^* colour coordinates, determined by a chroma meter, demonstrated that the higher the temperature and SMC the faster the rate of change in colour. The seeds with ≤ 12% SMC and stored at ≤ 25°C retained colour in acceptable range of marketing for human consumption for one year, whereas seeds stored at high temperatures (≥ 37°C) darkened to an unacceptable level in less than 3 months. Farmers need to harvest faba beans early and at high moisture contents (14-15%) to reduce risk of damage and maximise yield. Our results revealed that a 14-15% moisture content of faba bean accelerates discolouration considerably during storage. So, in order to maintain faba bean colour for human consumption during long term storage, seed must be dehydrated to < 12% SMC after harvesting using an appropriate dehydration technique using aerated silos. The extra cost of dehydration and reduced yield (by weight) may be compensated by the higher sale price and this requires a simple cost-benefit analysis.

A predictive model for seed colour changes was developed that will be helpful for farmers and traders to calculate and predict the storage life of faba beans and to assist in the cost- benefit analysis. This will enable them to determine the limit of storage at determined temperatures for colour changes to remain within an acceptable range of marketing for human consumption. This would provide farmers and grain traders with greater knowledge about their current and future faba bean supplies and potentially increase overall profitability.

Light also substantially accelerated colour darkening in faba beans. Exposure to light (350 μ mol m^{-2} s^{-1}) caused testa darkening in one month that equalled testa darkening after 12 months storage in the dark at the same temperature (20 ± 2°C). Colour darkening in faba beans due to light may be of less concern to producers because seeds get exposed to light for a very short period of time. After harvesting faba beans are stored in metal bins/silos where no light can penetrate. The only possibility of exposure to light is when they are packed in 50-100 kg bags made of white polypropylene weave which is around 650 denier (the most commonly used packing material). This material is semi transparent and beans can be exposed to light/sunlight during subsequent open storage and transport. Further studies are required to find out the effect of light/sunlight on faba bean colour packed in this and other alternate, light blocking materials which may reduce seed darkening.

In contrast to environmental staining, colour darkening during storage was associated with loss in total free phenolics, total tannins and condensed tannins (proanthocyanidins) in testa as well as cotyledons. In the case of environmental staining, phenolics may be developing with plant/seed growth under certain stressful conditions but in storage discolouration colour changes appears to be due to degradation or conversion of phenolics by oxidative transformation processes (Amarowicz *et al.* 2004; Troszynska and Ciska 2002). In general, phenolics contents appeared to be involved in testa colour darkening but identification of actual compounds among phenolics responsible for the colour changes would be a key to understand the problem. In this project a few compounds were located in the HPLC chromatograms that were associated with colour darkening. Efforts were made to isolate and identify those compounds but could not succeed mainly due to their instability (Chapter 7). Further research work is required using more sensitive and delicate procedures to identify the compound/s so that future strategies can be developed to control the problem.

To control storage colour darkening, cold storage might be the most effective method but it is not an economical way to store dry beans. Modified atmosphere packaging techniques on the other hand might be an economically viable method. To study the effect of different gases on colour darkening, flushing with carbon dioxide, nitrogen, oxygen or ethylene, and vacuum packaging were applied for storage at 30^{o}C (mean maximum air temperature of southwestern Australia in summer months). Changes in

ΔE^*_{ab} values demonstrated that relative to control (flushing with air), nitrogen was effective in reducing colour darkening by an appreciable level, whereas storage in oxygen accelerated the colour darkening process. Colour darkening was associated with losses in phenolic constituents. Highest losses were found in seeds stored after flushing with O_2 suggesting that colour darkening is likely to be due to oxidative transformation of phenolic contents.

Application of N_2 to control colour darkening is practicable and economical in Australia and other countries. Flushing with N_2 may not require additional high costs as most farm storage bins in Australia are now constructed airtight (rolled steal) with pressure relief valves and bins are generally used for CO_2 fumigation (Jayas and Jeyamkondan 2002). These farm storage bins can be used for N_2 flushing for faba bean storage and experimentally this would be easy to test and confirm. An on farm study is required to confirm the results and to calculate the cost-benefit of the method before promotion to the industry.

Adverse storage conditions of high temperature and high SMC not only affect colour but also deteriorate cooking quality of faba bean. Seeds stored at ≤ 25°C demonstrated minor changes, whereas seeds stored at ≥ 37°C demonstrated substantial changes in most physical and chemical characteristics including hydration and swelling coefficients, acid detergent fibre, lignin and tannin contents. Solute and electrolyte leaching after 18 h soaking substantially increased with increased temperature. Faba bean hardness, tested by the hard-to-cook test, also increased substantially with increased storage temperature. There was a high negative correlation (r^2 = 0.98) between storage temperature and cooking ability of faba bean. The same relationship between storage temperature and cookability prevails in other legumes such as cowpeas, common beans, red kidney beans and adzuki beans (Del Valle and Stanley 1995; Liu *et al.* 1992; Moscoso *et al.* 1984; Reyes-Moreno *et al.* 1994; Yousif *et al.* 2002). Hardness was associated with reduction in free phenolics and increase in lignin content. There was a three-fold increase in lignin content of faba beans stored at 50°C compared to those stored at 5°C and it was correlated with bean hardness (r^2 = 0.98). Reduction in free phenolics was negatively correlated (r^2 = 0.75) with bean hardness. To maintain cooking quality avoid storage at high temperature with high seed moisture contents. This may be achieved by dehydrating the beans after harvest and storing them in insulated/shaded bins.

The results presented in this thesis confirm that phenolics are mostly responsible for colour darkening in faba bean. Different groups of phenolics, mostly tannins and specifically proanthocyanidins, have been reported to be involved in colour formation and colour darkening in beans (Amarowicz *et al.* 2004; Martin-Cabrejas *et al.* 1997; Nozzolillo and De Bezada 1984; Troszynska and Ciska 2002), but actual compound/s could not be identified even after extensive investigation. The complex, unstable and illusive nature of these compounds makes them difficult to extract, purify and identify. For this reason the focus in this thesis was to manage the discolouration problem.

Research presented in this thesis indicated that storage temperature, seed moisture content, light and oxidation due to availability of O_2 from air are the major factors affecting colour darkening and cooking quality of faba bean during storage. On the basis of results presented, the following practices are recommended to maintain quality of faba beans for human consumption by minimising colour darkening and bean hardening. Faba beans must be dehydrated to $\leq$ 12% seed moisture content and stored in insulated bins (silos) or at least bins painted white and constructed in shaded areas protected from direct sunlight. In addition, occasional flushing with N_2 will further help reduce the colour darkening. This will improve quality, market opportunities, price and hence profitability. The recommendations contained here will greatly assist farmers and grain traders in improving the quality of faba bean, thus expanding the potential of profitable legume crop options in soutwestern Australia.

In conclusion, the hypotheses formed in Chapter 2 are assessed and accepted/rejected as given below.

Hypothesis 1.
Faba bean plants grown under more stressful condition produce seeds with more environmental staining

This hypothesis was supported as small and weak plants in a population that might have grown under water or nutrient stress had more staining than normal plants. Also seeds that developed on top nodes in the later stage of plant growth and were under stress due to high temperature, high light intensity and reduced nutrient mobility due to lack of water had more staining than the seeds borne in the early stage of seed bearing.

Hypothesis 2: Genotype influence environmental staining via a G x E interaction. New varieties stain less than the old varieties.

The hypothesis was supported and old variety Fiord had more staining at high staining sites.

Hypothesis 3: Higher storage temperature, seed moisture content and light intensity enhance storage discolouration in faba bean. Some varieties are worse than the others.

The first part of the hypothesis was supported. For the second part the varieties used in the experiments behaved almost similar to storage temperature but behaved differently to light. Varieties other than those used in our experiments may behave differently to storage temperature also.

Hypothesis 4: Phenolic compounds are involved in colour darkening of faba bean seeds during storage.

The hypothesis was supported

Hypothesis 5: Modified atmosphere packaging can reduce colour darkening during storage.

This hypothesis was supported and flushing with nitrogen was helpful in reducing colour darkening during storage.

Hypothesis 6: Storage at high temperature deteriorates cooking quality of faba bean but there is an acceptable maximum temperature at which faba beans can maintain their cooking quality.

This hypothesis was supported and faba beans stored at ≤ 25°C retained the cooking quality almost near to those stored at 5°C.

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Appendices I & II

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Cooking quality of faba bean after storage at high temperature and the role of lignins and other phenolics in bean hardening

Syed M. Nasar-Abbas[a,b,*], Julie A. Plummer[a,c], Kadambot H.M. Siddique[b,c], Peter White[b,d], David Harris[b,e], Ken Dods[b,e]

[a]*School of Plant Biology, Faculty of Natural and Agricultural Sciences, The University of Western Australia, 35 Stirling Hwy, Crawley, WA 6009, Australia*
[b]*Centre for Legumes in Mediterranean Agriculture, The University of Western Australia, 35 Stirling Hwy, Crawley, WA 6009, Australia*
[c]*Institute of Agriculture, The University of Western Australia, 35 Stirling Hwy, Crawley, WA 6009, Australia*
[d]*Department of Agriculture and Food Western Australia, 3 Baron-Hay Court, South Perth, WA 6151, Australia*
[e]*Chemistry Centre Western Australia, 125 Hay Street, East Perth, WA 6004, Australia*

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Abstract

Selected physical and chemical characteristics of faba beans (*Vicia faba* L.) cv. Fiesta were studied after 12 months storage at 5, 15, 25, 37, 45 or 50 °C (±2 °C) in relation to the hard-to-cook phenomenon. In comparison with control (seeds stored at 5 °C), seeds stored at 15 and 25 °C demonstrated non-significant ($p \leqslant 0.05$) changes in most of the physical and chemical characteristics including hydration and swelling coefficients, acid detergent fibre, lignin and tannin contents, whereas seeds stored at ⩾37 °C demonstrated significant changes ($p \leqslant 0.05$). Solutes and electrolytes leaching after 18 h soaking substantially increased with increased temperature. Faba bean hardness tested by the hard-to-cook test also increased substantially with increased storage temperature. After 8 h soaking followed by 2 h cooking, the puncture force required for seeds stored at 5 °C was 3.3 N seed^{-1} whereas seeds stored at 50 °C required a much higher puncture force of 15.2 N seed^{-1}. There was a high negative correlation ($r^2 = 0.98$) between storage temperature and cooking ability of faba bean. Substantial increases in acid detergent fibre and lignin contents occurred with increased storage temperatures. There was a three-fold increase in lignin content of faba bean stored at 50 °C compared to those stored at 5 °C and it was correlated with bean hardness ($r^2 = 0.98$). Storage at high temperatures for 12 months led to a substantial reduction in total free phenolics especially in the testa and there was a greater reduction with increasing storage temperature. Reduction in free phenolics was negatively correlated ($r^2 = 0.75$) with bean hardness.

Keywords: *Vicia faba* L.; Hard-to-cook; Lignins; Phenolics; Physicochemical properties

1. Introduction

Legume seeds are mostly preserved in dry storage at ambient temperature to maintain year-round supply of this important protein food source. High temperature during storage, in countries such as Australia where air temperatures in summer may rise up to 40 °C, can cause deteriorative affects on legume seed quality. The main form of deterioration is increased hardness of cotyledons or loss of cookability (ability to soften with cooking), followed by deterioration of colour, texture and loss of nutritive value (Martin-Cabrejas, Esteban, Perez, Maina, & Waldron, 1997; Yousif et al., 2003). Hardness of cotyledons is commonly described as the ''hard-to-cook'' (HTC) phenomenon and is characterised by a requirement for extended cooking time. Hard-to-cook beans need additional energy during preparation and may have inferior nutritional qualities in terms of protein, fats and mineral contents. Long cooking time is also one of the factors responsible for wider under-utilisation of legume seeds

*Corresponding author. School of Plant Biology, Faculty of Natural and Agricultural Sciences, The University of Western Australia, 35 Stirling Hwy, Crawley, WA 6009, Australia. Tel.: +61 8 6488 2418; fax: +61 8 6488 1108.
E-mail address: abbass01@cyllene.uwa.edu.au (S.M. Nasar-Abbas).

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(Deshpande, Sathe, & Salunkhe, 1984). Water absorption, soluble solids and electrolyte leaching are important quality factors associated with the bean hardness defect (Berrios, Swanson, & Cheong, 1999) and are good indicators of the loss in bean quality during storage.

Several hypotheses have been proposed to explain the cause of bean hardening, including involvement of phenolic compounds (Garcia, Filisetti, Udaeta, & Lajolo, 1998; Liu, 1995; Maurer, Ozen, Mauer, & Nielsen, 2004). Hincks and Stanley (1986) proposed multiple mechanisms of bean hardening which include phenol metabolism as a major contributor during storage. Lignins are complex phenolic heteropolymers deposited in cell walls. Lignins reinforce and waterproof walls of specialized cells and play a fundamental role in mechanical support (Boudet, Lapierre, & Grima-Pettenati, 1995). The deposition of lignin-like material around bean cotyledon cells promotes hardening (Hincks & Stanley, 1987) and thus lignins may also be involved in HTC phenomenon in beans.

Faba bean is one of the major grain legume crops in Australia and many other parts of the world. Australia produces 330 thousand tonnes (Anonymous, 2007) of faba bean whereas world production is around 4 million tonnes (FAO, 2003). Faba bean, like many other legumes, also exhibit deteriorative changes when stored under unfavourable conditions (El-Refai, Harras, El-Nemr, & Noaman, 1988) that adversely affect its value and market opportunities. However, there is little information regarding the effect of high temperature during storage on quality parameters of faba beans. The objective of this study was to determine the effect of storage (12 months period) at different temperatures on the cooking quality of faba bean and to determine whether changes in lignin, phenolic and other contents relate with the development of HTC phenomenon in faba beans.

2. Materials and methods

2.1. Plant material

Faba beans (*Vicia faba* L.), cv. Fiesta, were grown at Borden (Universal Transverse Mercator zone 50; 617,400 mE, 6,220,707 mN), Western Australia as part of the field evaluation of the Australian National Faba Bean Improvement Program. Faba beans were harvested in December 2003 and kept at 5 °C in the dark until used for experiments in February 2004. Good colour (beige/buff) and healthy seeds (free from insect damage, visible viral or fungal attack or broken testa) were individually selected. Average seed weight was 73.2 g per 100 seeds.

2.2. Conditions and duration of storage

Seed samples (3 × 25 g) were placed in polyethylene lined aluminium foil bags (10 × 10 cm) and sealed with an impulse heat sealer. Bags were placed in plastic storage boxes and stored at 5, 15, 25, 37, 45 and 50 °C for 1 year to induce different levels of deterioration.

2.3. Physical properties

2.3.1. Hydration coefficient (imbibition value)

Hydration coefficient was determined by soaking 10 g of bean seeds at room temperature (25 °C) in 50 ml deionised water (ratio of 1:5). After 18 h the beans were removed from the soaking water, cut into two halves along the fissure and separated the testa and cotyledon parts followed by free water removal by using a blotting paper and reweighing. Gain in weight was taken as the amount of water absorbed and expressed as the hydration coefficient (El-Refai et al., 1988):

$$\text{Hydration coefficient} = \frac{\text{Weight of bean seeds after soaking}}{\text{Weight of bean seeds before soaking}} \times 100. \quad (1)$$

2.3.2. Swelling coefficient

The volume of raw bean seeds before and after soaking in deionised water for 18 h at 25 °C was determined by water volume displaced in a graduated cylinder and expressed as the swelling coefficient (El-Refai et al., 1988):

$$\text{Swelling coefficient} = \frac{\text{Volume of bean seeds after soaking}}{\text{Volume of bean seeds before soaking}} \times 100. \quad (2)$$

2.3.3. Electrolytes and solutes leaching

After 18 h soaking in deionised water the soak water was collected and leached electrolytes were quantified by assessing conductivity with a digital conductivity meter (PW 9527, Digital Conductivity Meter, Philips) in $\mu S\,cm^{-1}$ at 25 °C (Hentges, Weaver, & Nielsen, 1991). To measure solutes leached from beans during soaking, soak water was evaporated, dried in a hot air oven (105 °C), cooled in a desiccator, weighed and expressed as $mg\,g^{-1}$ dry weight of beans.

2.3.4. Hard-to-cook test (puncture force)

Whole beans (3 × 10 g) were soaked at room temperature (25 °C) in 50 ml distilled water (ratio of 1:5) in closed lid glass jars (100 ml capacity). After 8 h jars were placed in a hot air oven (105 °C) for 2 h followed by cooling at room temperature (25 °C) for 30 min. Hardness was measured using the method given by Reyes-Moreno, Paredes-Lopez, and Barradas (1994). Ultra Test (Mecmesin Ltd., EU) equipped with a flat 2 mm diameter steel punch was used with a crosshead speed of $30\,cm\,min^{-1}$. A total of 3 × 10 beans were punched individually for each treatment and the mean peak force was calculated in Newtons per seed ($N\,seed^{-1}$).

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2.4. Chemical analysis

2.4.1. Proximate composition

Proximate composition was determined using methods given by Association of Official Analytical Chemists (AOAC, 2000) in triplicate (or otherwise mentioned) on raw material and expressed on a dry weight basis. Moisture by method 925.10, ash by method 923.03 and crude protein by nitrogen determination (method 968.06) using Truespec® CN, Carbon/Nitrogen determinator (LECO Corporation, MI, USA). Crude protein was calculated as $N \times 5.70$. Crude fat was determined by using AOAC method 963.15 except hexane was used as a solvent instead of petroleum ether.

2.4.2. Acid detergent fibre (ADF) and lignin (H_2SO_4) contents

Acid detergent fibre and lignin were determined by applying AOAC method 973.18. Cationic detergent in 0.5 M H_2SO_4 (20 g cetyle trimethylammonium bromide per 1 l 0.5 M H_2SO_4) was used to remove acid-labile carbohydrates, proteins and fats leaving a fibrous residue that is primarily cellulose and lignin. Sulphuric acid lignin is defined as the residue remaining after cellulose and other organic matter in acid detergent fibre is solubilised by 72% H_2SO_4.

2.4.3. Phenolic constituents

Total free phenolics, tannins and condensed tannins (proanthocyanidins) were determined in testa and cotyledons separately. Testa of seeds (3×10) were manually removed and the hilum excised and discarded. Testa and cotyledons were ground separately with a grinder (IKA® A11 basic, IKA®-WERKE GmbH & Co., Germany). Ground testa (0.2 g) and cotyledons (2 g) were extracted with 20 ml of 70% (v/v) aq. acetone (analytical grade) by applying 20 min ultrasonic treatment at 4 °C followed by overnight mechanical tumbling. Extracts were analysed for total phenolics using the Folin–Ciocalteu's Phenol Reagent (Merck) according to the method of Makkar, Bluemmel, Borowy, and Becker (1993). Total phenolic compounds were calculated from a prepared standard curve of tannic acid (Merck) in an identical matrix. Tannins were complexed with polyvinylpolypyrrolidone (Sigma) and unbound phenolics determined as above. Total tannins were calculated by subtracting non-tannin phenolics from total phenolics.

2.5. Statistical analysis

Analysis of variance was conducted using SPSS 11.0 for Windows and the means were separated according to Tukey's Honestly Significant Difference (Tukey's HSD) test at a significance level of 0.05.

3. Results

3.1. Effect of storage temperature on some physical properties

Substantial differences occurred in physical characteristics of faba beans stored at different temperatures (Table 1) for 1 year. Hydration and swelling coefficients that reflect the capacity to imbibe water in a reasonable length of soaking time was substantially affected by storage temperature. After 18 h soaking at 25 °C, the hydration coefficient was significantly ($p \leqslant 0.05$) lower in samples stored at higher temperatures, especially those stored $\geqslant 37$ °C, compared to samples stored at lower temperatures ($\leqslant 25$ °C). A layer of free water was present between the cotyledons and testa and in the fissure between cotyledons of hard beans which had been stored at $\geqslant 37$ °C.

The swelling coefficient behaved in the same way as hydration coefficient because swelling depends mainly upon the amount of water absorbed. The swelling capacity of beans decreased with increased storage temperature (Table 1). Compared with seeds stored at 5 °C, there was only 3% decrease in hydration and swelling coefficients of seeds stored at 25 °C whereas seeds stored at $\geqslant 37$ °C had a decrease of 10–18% in hydration coefficient and 7–19% decrease in swelling coefficient.

Solute and electrolyte leakage increased with increased storage temperature. Faba bean stored at $\geqslant 37$ °C exhibited 18–36 mg g^{-1} solute leakage whereas seeds stored at $\leqslant 25$ °C had only 4–7 mg g^{-1} solute leakage (Table 1). Solute leakage was directly proportional to electric

Table 1
Changes in physical properties of faba beans stored at different temperatures for 12 months

Storage temperature (°C)	Hydration coefficient	Swelling coefficient	Electric conductivity (μS cm^{-1})	Solutes leaching (mg g^{-1})	Hardness (N $seed^{-1}$)
5 (Control)	193±3a	215±4a	827±23a	3.8±0.4a	3.3±0.2a
15	191±2a	210±2ab	905±19a	6.6±0.5ab	5.2±0.3b
25	188±2a	209±2ab	1115±55b	7.4±0.2b	7.1±0.2c
37	174±2b	201±3b	2523±49c	18.1±0.7c	10.7±0.2d
45	170±3b	189±4c	3216±61d	24.8±1.8d	13.7±0.3e
50	158±2c	175±2d	3467±60e	36.1±1.8e	15.2±0.3f

Note: Means (±S.E., $n = 3$ except hardness where $n = 30$) sharing the same letter in the column are non-significant ($p = 0.05$) according to Tukey's HSD test.

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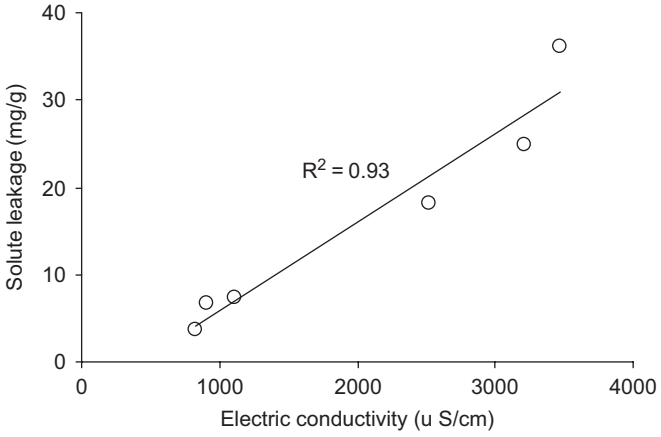

Fig. 1. Correlation between solute leakage and electric conductivity of soaked water after 18 h soaking of faba bean at 25 °C.

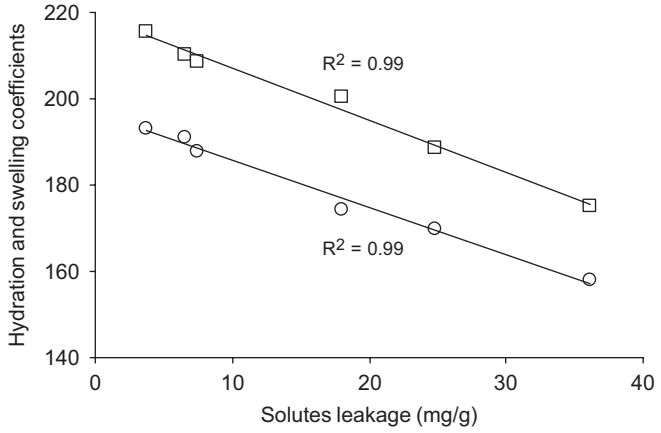

Fig. 2. Correlation between solute leakage and hydration and swelling coefficients of faba bean after 18 h soaking at 25 °C; hydration coefficient (–○–), swelling coefficient (–□–).

conductivity of the soaked water demonstrated by a correlation coefficient of $r^2 = 0.93$ (Fig. 1) and negatively correlated ($r^2 = 0.99$) with hydration and swelling coefficients (Fig. 2).

Bean hardness tested by the hard-to-cook test increased substantially with increased storage temperature. After 8 h soaking followed by 2 h cooking, the puncture force required for seeds stored at 5 °C was 3.3 N seed^{-1}, whereas seeds stored at 50 °C required a much higher puncture force of 15.2 N seed^{-1} (Table 1). There was a high level of negative correlation ($r^2 = 0.98$) between storage temperature and faba bean cooking quality (Fig. 3).

3.2. Effect of storage on some chemical properties

Chemical properties of faba bean changed after storage at different temperatures for 12 months (Table 2). A slight but continuous reduction in ash content occurred with increased storage temperature. Crude fat content showed a slight increase for the samples stored at ⩾37 °C but the results could not be statistically proved due to lack of

Fig. 3. Correlation between storage temperature and cooking quality (bean hardness) of faba bean.

observations. Protein content on the other hand did not change with storage temperature.

Substantial increases in ADF and lignin contents occurred with increased storage temperatures. Acid detergent fibre content was 9.6% for seeds stored at 5 °C (control) and this increased to 11.2% for seeds stored at 50°C. There was a three-fold increase in lignin content of faba bean stored at 50 °C compared to those stored at 5 °C. Both ADF and lignin contents demonstrated a high positive correlation (r^2) of 0.97 (Fig. 4) and 0.98 (Fig. 5), respectively, with bean hardness.

Storage at high temperatures for 12 months led to a substantial reduction in total free phenolic constituents especially in the testa and there was a greater decrease with increasing storage temperature (Table 2). There was 70% reduction in total free phenolics in the testa of faba beans stored at 50 °C compared to seeds stored at 5 °C. Reduction in free phenolics was negatively correlated ($r^2 = 0.75$) with bean hardness (Fig. 6). Tannins were the major proportion (72–82%) of total phenolics in the testa of faba beans and they decreased with increased storage temperature. Total phenolic contents of cotyledons also consistently decreased with increased storage temperature especially storage at temperatures ⩾37 °C (Table 2).

4. Discussion

4.1. Effect of storage on some physical properties

Hardness is a textural problem where beans fail to soften sufficiently during the normal cooking process. The storage conditions of faba beans played an important role in the hardening process of beans stored for a long period of time. A linear increase in the hard-to-cook (HTC) state was observed with storage temperature. Faba beans stored for an extended period under unfavourable conditions of high temperature (⩾37 °C) developed a harder texture after cooking compared with beans stored under favourable conditions (⩽25 °C) for the same time period. The cooking

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Table 2
Changes in some chemical constituents (dry weight basis) of faba bean stored at different temperatures for 12 months

Storage temperature (°C)	Ash (%)	Crude protein (%N × 5.70)	Crude fat (%)	ADF (%)	Lignins (%)	Total phenolics in testa (mg of tannic acid g^{-1})	Total tannins in testa (mg of tannic acid g^{-1})	Total phenolics in cotyledons (mg of tannic acid g^{-1})
5 (Control)	2.81±0.01a	24.0	1.64	9.6±0.1c	0.31±0.03d	59.5±0.3a	43.0±0.3a	1.62±0.06a
15	2.80±0.01ab	23.5	1.57	9.7±0.2c	0.34±0.05d	57.1±0.9ab	41.4±0.8a	1.61±0.06a
25	2.82±0.02a	24.5	1.55	10.0±0.1bc	0.43±0.01d	55.9±1.3b	40.7±0.9a	1.52±0.03ab
37	2.79±0.01ab	24.2	1.63	10.4±0.2b	0.71±0.08c	50.6±0.9c	36.1±1.2b	1.46±0.06bc
45	2.77±0.03ab	23.5	1.70	10.7±0.1b	0.86±0.03b	41.2±0.7d	30.0±1.1c	1.34±0.04cd
50	2.76±0.01c	23.5	1.81	11.2±0.2a	1.03±0.02a	15.0±0.8e	12.2±0.8d	1.29±0.04d

Means (±S.E. of $n = 3$ except for crude protein and crude fat where $n = 2$) for whole seeds except for crude fat and crude protein.
Means sharing the same letter in the column are non-significant ($p = 0.05$) according to Tukey's HSD test.

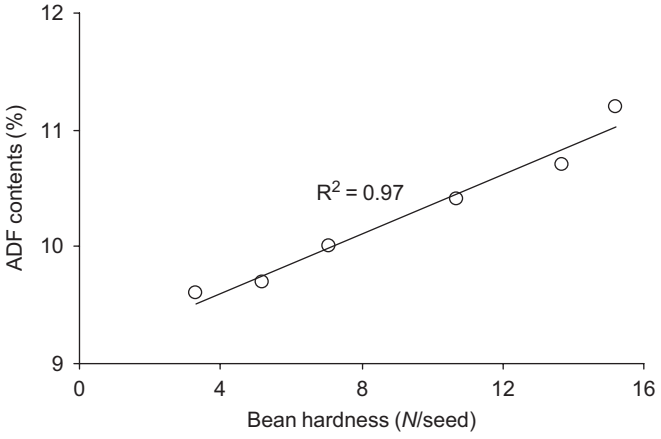

Fig. 4. Correlation between changes in ADF contents and bean hardness in stored faba beans.

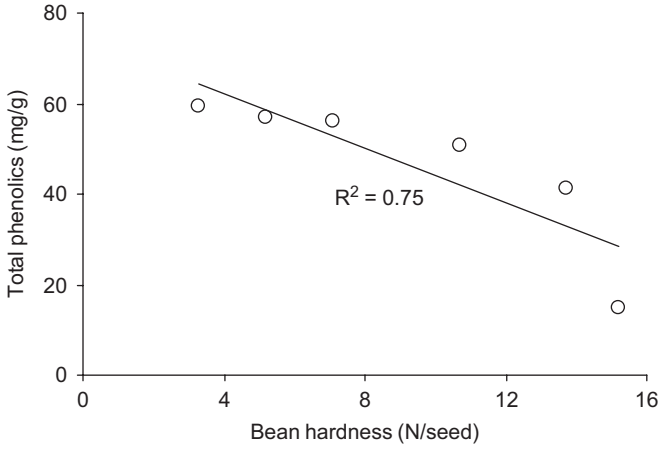

Fig. 6. Correlation between changes in total phenolic content and bean hardness in stored faba beans.

Fig. 5. Correlation between changes in lignin contents and bean hardness in stored faba beans.

time of grain legumes varies with species and variety but hard-to-cook phenomenon as a function of extended storage time and high temperature is a common characteristic across various grain legumes. Extended storage of cow peas at 30 °C increases seed hardness from 15.8 to 91.2 N g^{-1}, whereas seeds stored at −18 °C show no change (Liu, McWatters, & Phillips, 1992). Even after medium term storage at 30–35 °C, hardness of many common bean (*Phaseolus* spp.) varieties increases by 3–6 N $seed^{-1}$ (Del Valle & Stanley, 1995; Reyes-Moreno et al., 1994).

Changes associated with hard-to-cook phenomenon are accelerated under high storage temperature and lead mainly to reduced hydration (imbibition) and swelling coefficients which in turn reduce cookability of seeds following long term storage. Storage of faba bean at lower temperatures 5–25 °C had little effect but at temperatures ⩾37 °C, both hydration and swelling coefficients substantially decreased. Similar changes occur in a number of other grain legumes. Red kidney beans (*Phaseolus vulgaris* L.) stored at 32 °C for 9 months exhibit a 10% decrease in bean hydration compared to those stored at 2 °C (Mosco-so, Bourne, & Hood, 1984). Storage of faba beans for 9 months in underground pits or in tin cans stored at room temperature in Egypt leads to gradual reduction in hydration and swelling coefficients (El-Refai et al., 1988). Adzuki beans (*Vigna angularis*) stored at 30 °C for 6 months absorb substantially less water than those stored at 20 and 10 °C (Yousif, Deeth, Caffin, & Lisle, 2002).

Low hydration and swelling coefficients following storage at high temperature can be due to structural and

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chemical changes in the testa making it harder and less permeable to water so that it acts as a barrier, preventing water reaching the cotyledons (Liu et al., 1992). Alternatively, structural and chemical changes in cotyledons can render them resistant to water absorption (Aguilera & Rivera, 1990; Berrios, Swanson, & Cheong, 1998). The strong adhesion between cells observed for hard seeds might partially explain the reduced water uptake and consequently the slower rate of cooking (Hincks & Stanley, 1987).

The reduced hydration coefficient was partially due to leaked solutes (including electrolytes) from cotyledons during imbibition which was much greater in harder beans, especially those stored at $\geqslant 37\,^{\circ}C$. A high negative correlation ($r^2 = 0.99$) was found between solute leakage and hydration coefficient (Fig. 2). This also occurs in HTC black beans that are stored at higher temperatures. After 12 h soaking, the loss of solids and electrolytes from black beans stored at refrigeration temperatures (4–5 °C) for 2 years is $10.5\,mg\,g^{-1}$ compared with $18.6\,mg\,g^{-1}$ for black beans stored at 23–25 °C (Berrios et al., 1999). Leached solids may affect the hydration rate of beans in two ways. Solids leached into the soaking water increase the concentration of the solution, which in turn adversely affects the rate of water absorption by beans (Jones & Boulter, 1983). Secondly, solute (including electrolyte) removal from bean cells may reduce their water affinity and water holding capacity in accordance with the osmotic principles.

4.2. Effect of storage on some chemical properties

There was a small but continuous reduction in ash content (sum of minerals content) with increased storage temperature (Table 2). The reason remains unknown but our results are in agreement to those of El-Refai et al. (1988) who demonstrated that storage of faba bean for 9 months led to a moderate reduction in ash content with no loss of phosphorus, iron, calcium and magnesium contents. There were no appreciable changes in crude protein content of the samples stored at different temperatures as found in black beans (Berrios et al., 1999). In contrast, appreciable decreases in crude protein contents have been reported. In faba bean stored in tin cans at room temperature crude protein content decreased from 29.2% to 19.8% in 9 months (El-Refai et al., 1988).

Reduced water absorption that lead to poor cookability of faba bean may be due to substantial increases in acid detergent fibre and lignin contents that reflect increases in faba bean testa and cotyledon cell wall fraction (Yousif & Deeth, 2003) stored at higher temperatures ($\geqslant 37\,^{\circ}C$). Cell wall contents of adzuki bean stored at 30 °C for 6 months increase compared to beans stored at 10 or 20 °C (Yousif & Deeth, 2003). High temperature ($\geqslant 37\,^{\circ}C$) might have caused a thickening of cell walls as occurs in common beans stored at 35 °C for 6.5 months (Garcia et al., 1998). This cell wall thickening in beans may be attributed to lignification (Hincks & Stanley, 1987). Lignin serves as a matrix around the polysaccharide components of some plant cell walls, providing additional rigidity and compressive strength as well as rendering the walls hydrophobic and water impermeable (Whetten & Sederoff, 1995). Increased lignification occurred during storage would enhance the HTC character. In legumes, during seed development, soluble phenolics of the seed coat can convert to lignins and this results in onset of impermeability of seed coats (Egley, Paul, Duke, & Vaughn, 1985). Lignin-like materials deposited around bean cotyledon cells promote hardening, both as a result of mechanical strength from the lignin as well as its action in preventing water imbibition and swelling (Hincks & Stanley, 1986, 1987). Deposition of lignin can also occur in dead cells (Pickett-Heaps, 1968) and this probably plays a major role in the cotyledon cell wall hardening that renders faba and other beans hard to cook. Lignification of the middle lamella can also occur during storage of legumes resulting in their further decrease in cookability (Del Valle & Stanley, 1995).

The decrease in cooking quality of faba bean was also associated with phenolic constituents. There was a continuous and substantial decrease in total phenolics with the increase in storage temperature especially in the seeds stored at $\geqslant 37\,^{\circ}C$. A similar decrease in phenolic compounds is observed in common beans stored at high temperature (35 °C) compared with beans stored at 4 °C and it is associated with HTC phenomenon (Garcia et al., 1998). Similarly in four cultivars of cowpeas (*Vigna unguiculata* L. Walp) there was a negative correlation between cooking time and total polyphenols (Giami & Okwechime, 1993). There is also evidence of a causal relationship between decreased polyphenolic contents and cooking time during seed development of winged beans (*Psophocarpus tetragonolobus* L. DC) (Kadam, Kute, Lawande, & Salunkhe, 1982).

Tannins, which were the major proportion of total phenolic content of faba beans testa also decreased with increased storage temperature and this had an adverse effect on bean hardening. Similarly in red kidney beans stored under high temperature there is a strong correlation between decreasing condensed tannins (proanthocyanidins) and increasing hardness (Rozo, Bourne, Hood, & Van Soest, 1990). A decrease in tannin content and increase in hardness occurs in beans stored at 30 °C for 1 year (Stanley, 1992b).

The decrease in phenolic constituents with increases storage temperature may be due to oxidative degradation, which can be accelerated at higher temperature. Phenolic compounds vary widely in complexity but the common characteristic of all these compounds is that they are readily oxidised and undergo phenolic reactions (Bors, Heller, Michel, & Stettmaier, 1996). Storage of faba bean after flushing with N_2 retards, whereas flushing with O_2 accelerates reduction in phenolic contents, including tannins and proanthocyanidins (Nasar-Abbas et al., 2007). Colour darkening in beans during storage accompanied by

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seed hardening is probably caused by air- and light-catalysed oxidation of leucoanthocyanidins, a group of phenolic compounds (Stanley, 1992a). Oxidation of phenolic compounds may directly affect permeability and cookability of seeds. The permeability to water of seed coats of field pea (*Pisum sativum* L.) is related to phenolic contents in the seed coat and to their level of oxidation as oxidation processes may cause structural changes which affect permeability to water (Marbach & Mayer, 1974).

Some researches dispute the role of phenolic compounds in bean hardness (Deshpande, Sathe, Salunkhe, & Cornforth, 1982) because hardening occurs in white varieties of beans containing low concentrations of tannins (Stanley, 1992b). However, hardness is related to seed coat (testa) impermeability and also to cotyledon impermeability. In the testa it may involve lignins as well as tannins, whereas in cotyledons it may be primarily lignification as cotyledons have low concentrations of phenolic compounds. The ratio of testa to cotyledon tannin contents is about 9:1 in dry beans (Deshpande et al., 1982) and most researchers agree that the defect develops mainly in cotyledons (Reyes-Moreno & Paredes-Lopez, 1993). Here the tannin concentrations of cotyledon were too low to be detected using a standard method given by Makkar et al. (1993). Hence, hardness of cotyledons which have very low tannin concentrations in both white and coloured faba bean varieties, probably depends on lignins. Testa hardening however may be greater in coloured varieties as both tannins and lignins are probably involved in the hardening process. Cotyledons constitute the major proportion (about 90%) of beans and so extra hardening of testa due to tannins in coloured varieties may not cause an appreciable difference with seed hardening.

5. Conclusions

Twelve months storage of faba beans caused substantial deteriorative changes in physicochemical properties and those changes were temperature dependent. The higher the temperature the faster was the deteriorative effect. Main changes were increased ADF and lignin content (cell wall component) and reduced phenolic contents, which were correlated with hydration and swelling coefficients and cooking quality of faba beans. Beans stored at $\leqslant 25\,^{\circ}C$ demonstrated appreciable stability in most of the physicochemical properties. Proximate analysis revealed that there was little or no effect on nutritive value of beans stored at different temperatures. Bean hardening (hard-to-cook) after storage under unfavourable temperature conditions is a complex phenomenon but it was mainly attributed to lignification of cotyledon cell wall and changes in phenolic contents.

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Nitrogen retards and oxygen accelerates colour darkening in faba bean (*Vicia faba* L.) during storage

S.M. Nasar-Abbas [a,b,*], J.A. Plummer [a,c], K.H.M. Siddique [b,c], P.F. White [d], D. Harris [e], K. Dods [e]

[a] *School of Plant Biology, Faculty of Natural and Agricultural Sciences, The University of Western Australia, 35 Stirling Hwy, Crawley, WA 6009, Australia*
[b] *Centre for Legumes in Mediterranean Agriculture (CLIMA), Faculty of Natural and Agricultural Sciences, The University of Western Australia, 35 Stirling Hwy, Crawley, WA 6009, Australia*
[c] *Institute of Agriculture, The University of Western Australia, 35 Stirling Hwy, Crawley, WA 6009, Australia*
[d] *Department of Agriculture and Food, Western Australia, 3 Baron-Hay Court, South Perth, WA 6151, Australia*
[e] *Chemistry Centre WA, 125 Hay St, East Perth WA 6004, Australia*

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Abstract

Modified atmosphere packaging (MAP) techniques were applied in order to control seed coat (testa) colour darkening in faba bean during long-term storage. These techniques included flushing with carbon dioxide, nitrogen, oxygen or ethylene, and vacuum packaging. Seeds flushed with air were used as the control. After MAP treatments, samples were stored at 30 °C in dark for 1 year. Seed coat colour was measured at 0, 0.5, 1, 2, 3, 4, 6, 8, 10 and 12 months of storage using a chroma meter. Changes in chroma (C^*), hue angle (h°) and colour difference index (ΔE^*_{ab} values) calculated from L^*, a^* and b^* colour coordinates demonstrated that relative to controls, nitrogen was effective in reducing colour darkening by an appreciable level, whereas storage in oxygen accelerated the colour darkening process. Ethylene had some effect whereas the other MAP treatments were ineffective in reducing colour darkening in faba beans.

Analytical studies revealed that tannin compounds were the major constituents of total phenolics in faba bean of which proanthocyanidins were the predominant component group. Tannin concentration was negatively correlated with colour darkening in faba bean. Air, vacuum and ethylene treated samples showed similar changes in phenolic constituents after 12 months storage but samples flushed with CO_2 and especially those flushed with O_2 had much higher losses in phenolic constituents demonstrating that colour darkening is likely to be due to oxidative transformation of phenolic contents. Flushing with N_2, which reduced colour darkening and tannin losses, would be useful in maintaining quality and improving market opportunities and acceptance during long-term storage of faba beans. Nitrogen could be used to flush faba beans in airtight silos for bulk storage as well as in small individual packets that could go directly onto the supermarket shelf as a premium product.

Keywords: Modified atmosphere packaging; Carbon dioxide; Ethylene; Seed coat colour; Phenolics; Tannins

1. Introduction

Seed coat (testa) colour is one of the most important visual characteristics in marketing faba bean seed for human consumption. Genetically, seed testa colour ranges from white to purple in different varieties of faba bean but the preferred colour has variously been described as beige, light tan or buff (AGWEST, 1998). Seed coat colour of most faba bean varieties is beige or buff at harvest but changes to light brown, dark brown or almost black depending upon the storage time and conditions. Seeds with dark brown testa colour are not accepted in overseas markets as consumers associate it with seeds and poor cooking and sensory qualities.

Storage conditions have a major influence on the stability of seed coat colour in beans. Environmental factors such as temperature, seed moisture content and light that cause discolouration in faba and other beans (Nasar-Abbas et al., 2008). In addition, oxidation due to environmental O_2, has been reported as a major factor responsible for discolouration in various beans

* Corresponding author at: School of Plant Biology, Faculty of Natural and Agricultural Sciences, The University of Western Australia, 35 Stirling Hwy, Crawley, WA 6009, Australia. Tel.: +61 8 6488 2418; fax: +61 8 6488 1108.
E-mail address: abbass01@cyllene.uwa.edu.au (S.M. Nasar-Abbas).

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(Stanley, 1992; Black and Brouwer, 1998). Discolouration in dry beans may involve oxidation of phenolic compounds especially proanthocyanidins (condensed tannins) which are the predominant and most widely distributed group of flavonoids found in legume seeds (Beninger and Hosfield, 2003). The phenolic compounds vary widely in complexity but the common characteristic of all these compounds is that they are readily oxidised and undergo phenolic reactions (Bors et al., 1996). The involvement of phenolic compounds (mostly proanthocyanidins) in faba bean discolouration is supported by the observations that high tannin faba bean varieties darken more in air than low tannin varieties (Black and Brouwer, 1998) and the white-seeded varieties, which are free from proanthocyanidins, do not darken with time or under oxidizing conditions (Crofts et al., 1980).

Storage discolouration related to atmospheric oxidation is often controlled by modified atmosphere packaging (MAP) techniques. With modified atmosphere packaging, the gas composition surrounding the produce, or seeds in this case, is different from the gas composition outside the package. Outside, the gas composition is always close to 78.1 kPa N_2, 20.95 kPa O_2, 0.93 kPa argon, and 0.036 kPa CO_2. MAP, acts by altering metabolic processes including reducing the respiration rate (Jayas and Jeyamkondan, 2002). Modified atmospheres can be obtained by gas generators and scrubbers (controlled-atmosphere packaging), evacuation of air (hypobaric storage, vacuum packaging), replacement of air with an alternative gas, or addition of chemical systems that absorb or generate gases or volatile compounds (active packaging) in packages (Gorris and Peppelenbos, 1999). MAP may be applied to minimise discolouration in faba beans.

The modification of the atmosphere generally implies a reduction in O_2 content and/or an increase in the CO_2 or N_2 concentration and in some cases changes in the level of carbon monoxide, ethylene, ethanol or other compounds in the atmosphere. Flushing with N_2 and CO_2 are the most commonly applied modified atmosphere techniques but the usual gas for flushing dehydrated foods is N_2. Nitrogen is inert with a low fat and moisture solubility (Fierheller, 1991). CO_2 has been proposed for packaging nuts (Holaday et al., 1979) and may be suitable for grain legume storage. Adsorption of the CO_2 by the nuts creates a vacuum. The adsorption phenomenon is similar to gas adsorption by charcoal and silica gel and can be used on a variety of grains including oilseeds, legumes, rice and corn (Fierheller, 1991).

The objective of this study was to test the hypothesis that oxidation of phenolic contents may be the main cause of discolouration in faba beans and this might be controlled/minimised by the use of MAP techniques of flushing with different gases.

2. Materials and methods

2.1. Plant material

Faba bean (*Vicia faba* L.) cv. Fiesta, was grown in 2004 growing season (May–December) at Borden (118.26E longitude, 34.07S latitude), Western Australia as part of the National Faba Bean Improvement Program's field evaluation activity. Beans were harvested in December 2003 and kept at 5 °C in the dark until used for experiments in February 2004. Good colour (beige/buff) and healthy seeds (free from insect damage, visible viral or fungal attack or broken testa) were individually selected. The average seed weight was 73.2 g per 100 seeds.

2.2. Modified atmosphere packaging and storage

Seed moisture content was maintained at 12% by dehydrating over silica gel. Initial and final seed moisture contents were determined by applying the air-oven method (AACC, 2000). Seed samples (~25 g each) were packed in bags (~10 cm × 10 cm) prepared from polyvinyl chloride (PVC) sheet with a thickness of 300 μm and sealed with an impulse heat sealer. Air from the bags was removed with a syringe needle attached to a vacuum system and then the required gas, CO_2, N_2, O_2 or ethylene, (BOC Gases Australia Ltd.) was used to fill bags through the same needle. The procedure was repeated three times to ensure the removal of air traces from the bags and this was immediately followed by sealing of the access flush point. Samples packed in bags filled with air acted as controls. After MAP treatments, the packs were placed in plastic storage boxes and stored at 30 °C (an adverse storage temperature) in the dark (Nasar-Abbas et al., 2008). The gas composition inside the bag could not be determined but according to the information provided by the manufacturer/supplier the PVC sheets were impermeable to the gases used in the experiment.

2.3. Colour measurement

Samples were analysed for changes in seed coat (testa) colour at 0, 0.5, 1, 2, 3, 4, 6, 8, 10 and 12 months storage interval. Seed coat colour was determined by a Minolta CR-310 chroma meter (Minolta, Japan) using the Granular-Materials Attachment CR-A50. Data were collected for L^*, a^* and b^* values. L^* = lightness, ranging from 0 (black) to 100 (white), a^* = bluish-green/red-purple hue component and b^* = yellow/blue hue component (McGuire, 1992). A white porcelain reference plate ($L^* = 97.75$, $a^* = -0.08$, and $b^* = +1.77$) supplied with the instrument was used for calibration. For each storage period independent samples of seeds stored in separate packages were used which were discarded after taking the measurements.

In order to ascertain the practical significance of changes in objective measures of faba bean testa colour during storage, chroma (C^*), hue angle (h°) and colour difference index (ΔE^*_{ab}) was calculated from L^*, a^* and b^* colour coordinates. Chroma represents colour saturation which varies from dull (low value) to vivid colour (high value) and hue angle is defined as a colour wheel with red-purple at an angle of 0°, yellow at 90°, bluish-green at 180°, and blue at 270° (McGuire, 1992). The values for the above were computed using the following equations (Anonymous, 1991; McGuire, 1992):

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad \text{(I)}$$

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$$h^\circ = \left[\tan^{-1}\frac{b^*/a^*}{6.2832}\right] \times 360 \quad \text{(II)}$$

where $a^* > 0$ and $b^* > 0$.

$$\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad \text{(III)}$$

Where $\Delta L^* = L^*_1 - L^*_2$, $\Delta a^* = a^*_1 - a^*_2$ and $\Delta b^* = b^*_1 - b^*_2$

Initial L^*, a^* and b^* values (subscript by 1) and values at each storage interval (subscript by 2) were used to develop ΔE^*_{ab} values and this was used to compare colour changes in samples.

2.4. Determination of phenolic constituents

Total free phenolics, tannins and condensed tannins (proanthocyanidins) were determined in testa and cotyledons separately. A sample (10 g) of each treatment was ground to powder with a grinder (IKA® A11 basic, IKA®-WERKE GmbH & Co., Germany). Ground sample (1 g) was extracted with 20 mL of 70% (v/v) aq. acetone (analytical grade) with 20 min ultrasonic treatment at 4 °C followed by overnight mechanical tumbling in the dark. Extracts were analysed for total phenolics using Folin–Ciocalteu's Phenol Reagent (Merck) according to the method of Makkar et al. (1993). Total phenolic compounds were calculated from a prepared standard curve of tannic acid (Merck) in an identical matrix. Tannins were complexed and precipitated with polyvinylpolypyrrolidone (Sigma) and unbound phenolics determined as above (Makkar et al., 1993). Total tannins were calculated by subtracting non-tannin phenolics from total phenolics. Condensed tannins (proanthocyanidins) were determined according to the methods of Porter et al. (1986).

2.5. Statistical analysis

An analysis of variance was carried out using SPSS 10.0 for Windows and means were separated using Tukey's honestly significant difference (Tukey's HSD) test at $p \leq 0.05$.

3. Results

3.1. MAP and testa colour

Composition of the gaseous storage environment affected faba bean testa colour during storage. Compared with the control (air), vacuum packaging and CO_2 did not reduce colour darkening. The changes in L^*, C^* and h° values showed similar decreasing trends in both cases (Fig. 1a–c). Lightness and yellowness in the initial beige coloured seeds were masked as colour changed through light brown to dark brown. Ethylene had some effect in reducing colour darkening in faba beans. Whilst changes in L^*, a^* and b^* colour coordinates were small, they accumulated in ΔE^*_{ab} values (Fig. 1d).

Oxygen accelerated colour darkening in faba bean and substantially changed L^*, C^* and h° values even after 1–2 months. Compared to controls, L^* and h° values decreased faster with the passage of time. In contrast, C^* values remained almost constant for first 3 months and then decreased at a faster rate.

Compared to other MAP treatments, flushing with N_2 reduced colour darkening in faba beans. Nitrogen substantially reduced changes in L^* C^* and h° values (Fig. 1a–c) and this was reflected in the stability of the colour difference index (ΔE^*_{ab} values) compared to other MAP treatments (Fig. 1d).

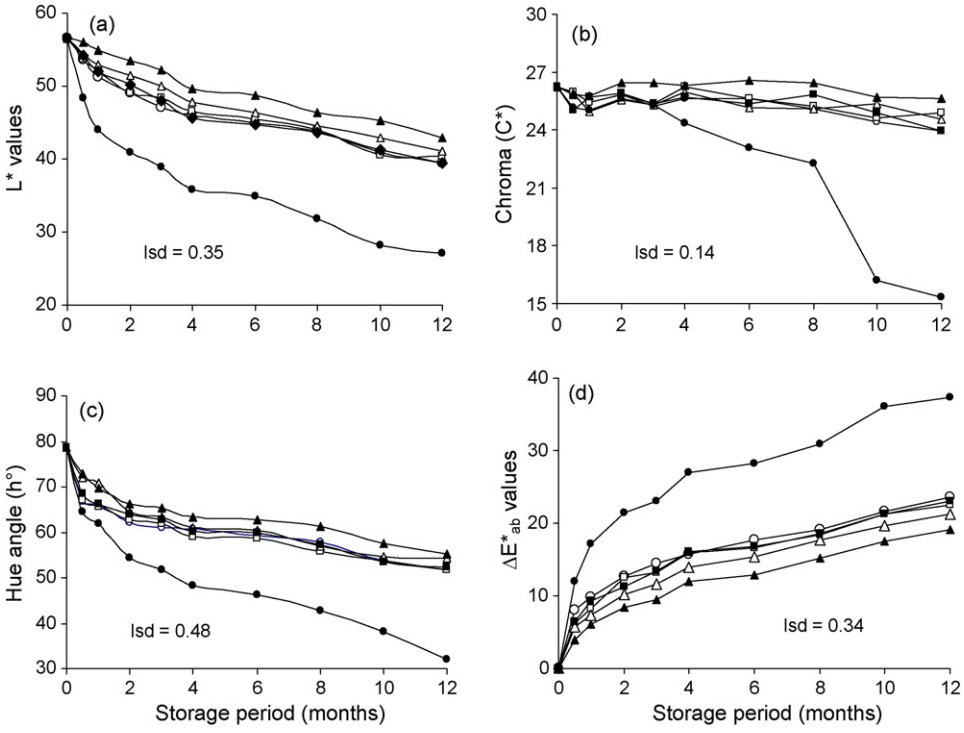

Fig. 1. Effect of different modified atmosphere packaging on: (a) L^*, (b) C^*, (c) h° and (d) ΔE^*_{ab} values of faba bean seeds stored at 30 °C for 12 months: air (◯), vacuum (□), ethylene (△), O_2 (●), CO_2 (■), and N_2 (▲).

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Table 1
Phenolic constituents of faba beans stored under different modified atmosphere packaging for 12 months

Treatments	Total free phenolics (mg tannic acid g^{-1})	Non-tannin phenolics (mg tannic acid g^{-1})	Total tannins (mg tannic acid g^{-1})	Proanthocyanidins (mg leucocyanidin g^{-1})
Freshly harvested	9.04 ± 0.05 a	4.79 ± 0.03 a	4.25 ± 0.02 a	4.07 ± 0.05 a
Air	7.14 ± 0.03 c	3.36 ± 0.11 c	3.78 ± 0.08 b	3.21 ± 0.03 c
Vacuum	7.29 ± 0.02 c	3.29 ± 0.05 c	4.04 ± 0.06 ab	3.31 ± 0.01 c
Ethylene	7.22 ± 0.04 c	3.32 ± 0.09 c	3.90 ± 0.06 b	3.30 ± 0.03 c
Carbon dioxide	6.07 ± 0.04 d	3.00 ± 0.05 c	3.07 ± 0.05 c	2.56 ± 0.02 d
Oxygen	4.48 ± 0.04 e	2.36 ± 0.05 d	2.12 ± 0.09 d	1.39 ± 0.03 e
Nitrogen	7.83 ± 0.04 b	3.89 ± 0.15 b	3.94 ± 0.13 ab	3.64 ± 0.04 b

Means (±S.E., $n = 3$) sharing the same letter in a column are not significantly different ($p \leq 0.05$) according to Tukey's HSD test.

3.2. Phenolic constituents

Phenolic constituents were correlated with colour darkening in faba bean (Table 1). Freshly harvested seeds contained 9.04 mg g^{-1} total phenolics of which a major part (4.25 mg g^{-1}) was tannins (Table 1). Among tannins, proanthocyanidins (condensed tannins) dominated (96%) phenolic compounds. Storage for 12 months led to substantial changes in phenolic constituents but this varied with MAP treatments. Air, vacuum and ethylene treated samples showed similar changes in phenolic constituents after 12 months storage but samples flushed with CO_2 and especially those flushed with O_2 demonstrated a much higher transformation change in extractable phenolic constituents. Seeds stored after flushing with CO_2 or O_2 had a demonstrated transformational loss of 33% and 50%, respectively, in their total extractable phenolic contents. Samples flushed with N_2, on the other hand, lost only 13% of their total extractable phenolics after 12 months storage compared to freshly harvested seeds (Table 1).

Tannins were the major proportion of total phenolics in faba bean of which proanthocyanidins were the predominant group. Tannin contents were also negatively correlated with colour darkening in faba bean. Seeds stored after flushing with O_2 had more colour darkening than other MAP treatments and had much higher loss in tannin contents (Table 1). Seeds flushed with N_2 had less colour darkening and demonstrated a lower level of tannin transformation.

4. Discussion

Flushing with O_2 substantially accelerated the colour darkening process in faba bean accompanied by a demonstrated reduction in phenolic contents. Total phenolic contents had a high negative correlation ($r = -0.92$) with ΔE^*_{ab} values; the total colour difference from freshly harvested beans (Fig. 2). This is supported by earlier studies where colour darkening in faba bean is probably due to oxidative alteration of phenolic compounds (Marquardt et al., 1978). Phenolic compounds vary widely in complexity but the common characteristic of all these compounds is that they are readily oxidised (Bors et al., 1996). Tannins which are a dominant group among phenolics of faba bean testa are well known for their antioxidant activities (Beninger and Hosfield, 2003). They play an important role in the defence system of seeds exposed to oxidative damage caused by environmental factors such as light, O_2, free radicals and metal ions (Troszynska and Ciska, 2002). Storage of faba bean with low O_2 concentration results in reduced colour darkening and varieties with high tannin content darken more in air than low tannin varieties, suggesting that darkening of seed coats is possibly due to oxidation of polyphenolics such as tannins (Black and Brouwer, 1998). Stanley (1992) also suggests that colour darkening in beans during storage is probably caused by air- and light-catalysed oxidation of leucoanthocyanidins, a group of phenolic compounds. Similarly, changes in total phenolic acids of minimally processed lettuce (*Lactuca sativa*) leaves are reduced when they are stored in MAP conditions of low O_2 (2–3%) and high CO_2 (12–14%) compared with storage in air and this controls browning (Gil et al., 1998). The availability of O_2 in air during storage is regarded as the main source of oxidation (measured as increase in colour darkening of beans), hence, traditional methods of storage of faba bean in the Middle East employ the use of underground pits which are filled completely with seeds to minimise air volume (El-Refai et al., 1988).

Involvement of oxidation processes in colour darkening of faba bean is also supported by the fact that when beans, grains or their products are stored under low O_2 atmosphere, their quality deterioration, including colour changes, is substantially reduced. Low O_2 (5–10 kPa) and high CO_2 (5 kPa) in the packaging of snow pea pods is helpful in maintaining their quality by retarding

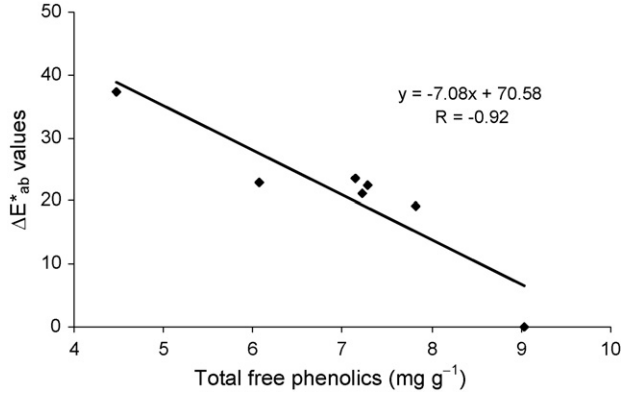

Fig. 2. Correlation between total phenolic contents and colour darkening (ΔE^*_{ab}) in faba beans stored under different modified atmosphere packaging for 12 months.

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changes in organic acids, free amino acids and sugar contents, and sensory attributes (Pariasca et al., 2001). There is a more stable nutritional quality (vitamins and minerals content) of green beans stored under 3% O_2 + 3% CO_2, than under atmospheric air storage (Sanchez-Mata et al., 2003). Black beans stored for 1 year at 30 ± 3 °C and 70–80% relative humidity under a modified atmosphere (containing N_2 and CO_2) in an impermeable container, lose quality in terms of hardening, at a slower rate than beans stored in air (in mesh bags) in the same environment (Aguilera and Rivera, 1990).

Flushing with N_2 demonstrated a marked effect in reducing colour darkening in faba bean. The mode of action of N_2 in reducing colour darkening may be associated with the reduction in oxidative transformation of phenolic compounds. This is supported by studies in other foods and food products. Drying of field pea seeds under N_2 and in a vacuum inhibits seed coat browning compared to seeds dried in air or O_2 (Marbach and Mayer, 1974). Enzymatic browning in minimally processed apple pieces can be successfully inhibited for long storage times by application of a modified atmosphere of 80% N_2 and 20% CO_2 (Nicoli et al., 1994) or 100% N_2 (Soliva-Fortuny et al., 2001). Similarly, nitrogen flushing is more effective than other gas treatments in preventing browning of cut potatoes (Gunes and Lee, 1997). The results here indicate that some oxidative reactions involved in colour darkening in faba bean can be minimised by flushing with N_2.

Faba beans flushed with CO_2 adsorbed almost all of the gas in the pouches which is similar to peanuts and oil seeds (Holaday et al., 1979), which also adsorb a large amount of CO_2. Carbon dioxide, solely or in combination with other gases, is helpful in reducing oxidation (Holaday et al., 1979) and maintaining other quality parameters in foods (Nicoli et al., 1994; Pariasca et al., 2001) but it did not retard colour darkening and not oxidative transformations of phenolic compounds in faba beans. This may be due to its interaction with phenolic compounds. Storage of strawberries with air enriched with up to 40% CO_2 increases colour darkness (increased L^* value) and is accompanied by a reduction of anthocyanin content (Gil et al., 1997). High CO_2 concentrations (>73%) as a result of MAP destabilize anthocyanin derivatives in the skin of apples (Lin et al., 1991). Similar reactions with CO_2 might have occurred with faba bean testa that contains high quantities of proanthocyanidins (Nasar-Abbas et al., 2008).

5. Conclusion

Oxidative transformation of phenolic contents caused by the presence of environmental oxygen was one of the major factors that caused seed coat darkening in faba beans during storage. Strong correlations between extractable phenolic transformation and colour degradative effects further emphasise the strong linkage between extractable phenolic compositional changes and faba bean colour changes.

One of the practical ways to avoid the availability of oxygen during storage of faba beans is by modifying the gaseous environment of the storage bins using N_2. The N_2 application may not require additional high cost as most of the farm storage bins in Australia are now constructed airtight (rolled steal) with pressure relief valves for CO_2 fumigation (Jayas and Jeyamkondan, 2002). These farm storage bins can be successfully used for N_2 flushing for faba bean storage. This practice in addition to other measures, such as maintaining low seed moisture content and low temperature (Nasar-Abbas et al., 2008), can minimise colour darkening in faba beans during long-term storage. This would improve quality, market opportunities, price and hence profitability. Faba bean can also be packed in individual small packets flushed with nitrogen that could then go directly onto the supermarket shelf as a premium product.

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