Faba bean (*Vicia faba* L.) seeds darken rapidly and phenolic content falls when stored at higher temperature, moisture and light intensity

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Abstract

Faba beans 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 over the storage period using a chroma meter. 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). 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). Seeds with 8% SMC had more stable testa colour compared to seeds with higher SMC. Exposure to artificial light (350 μ mol m^{-2} s^{-1}) substantially accelerated the colour darkening. Cotyledon stored at 37 ± 2 °C also darkened with the storage time. A loss in total free phenolics, total tannins and proanthocyanidins was found with increased darkness of testa and cotyledons during storage.

Keywords: Pulse; Seed coat; Cotyledons; Colour darkening; Phenolics

1. Introduction

Colour of seed testa is important for the marketing of faba bean for human consumption. Across different faba bean varieties, seed testa colour ranges from white to purple but the preferred colour has variously been described as beige, light tan or buff (AGWEST, 1998). Light brown or beige is also the most common (91% of accessions at ICARDA) seed coat colour in faba bean at harvest (Robertson & El-Sherbeeny, 1991), however it is not stable and darkens during storage. Seed coat colour may change to medium brown, dark brown and even chocolate brown depending upon the storage conditions and duration. Postharvest colour darkening of faba bean reduces its value and market opportunity. Consumers and processors are reluctant to purchase darkened seed because colour is considered as an index of quality or freshness and consumers associate dark colour with old seed (Hughes & Sandsted, 1975). Furthermore, during heat processing or canning the immersion liquid or broth changes to a dark muddy colour (Dickinson, Knight & Rees, 1957). Thus dark seeds are unacceptable to the unprocessed as well as the canning market.

Storage conditions strongly influence the stability of postharvest seed colour in many types of beans. In other legumes there is some evidence that temperature, relative humidity (RH), seed moisture content (SMC) and light are the main factors that affect the stability of seed colour during storage (Hughes et al., 1975; Nordstorm & Sistrunk, 1977; Nozzolillo & De Bezada, 1984; Park & Maga, 1999). High temperature ($\geq$ 24 °C) and high RH ($\geq$ 80%) accelerated darkening in kidney beans (*Phaseolus vulgaris* L.) while beans stored at low temperature (1 °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 postharvest testa colour darkening which was reflected by decrease in Hunter 'L' value and increase in total colour difference (ΔE) (Reyes-Moreno, Okamura-Esparza, Armienta-

Rodelo, Gomez-Garza & Milan-Carrillo, 2000). Lentil (*Lens culinaris* Medic.) seeds exposed to moderately high temperature (20 and 30 °C) at high RH (100%) turned brown in 3 weeks or less while at cool temperature (5 °C) with same RH (100%) browning did not occur before 5 weeks (Nordstorm & Sistrunk, 1979; Nozzolillo et al., 1984). Similarly little change in postharvest seed coat colour occurred in Rwandan dry beans (*Phaseolus vulgaris*) stored at 4 °C for 24 months (Edmister, Breene & Serugendo, 1990). Light red kidney beans also retained their original colour for one year when stored at 1°C (Gunes & Lee, 1997). Even at moderately low temperature (10 °C) darkening was slow in adzuki beans (*Vigna angularis*) (Yousif, Kato & Deeth, 2003).

This study aimed to assess the rate and intensity of postharvest colour darkening of faba bean using a range of storage conditions and to find the correlation of phenolic contents with postharvest colour darkening. Once known, optimum storage condition could be used to minimise darkening and hence maintain seed colour for extended periods.

2. Materials and methods

2.1. Plant Material

Faba beans (*Vicia faba* L.), cv. Fiesta, were grown at Borden (11.26 E longitude, 34.07 S latitude), 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. The average seed weight was 73.2 g per 100 seeds.

2.2. Effect of storage temperatures, seed moisture content and light on postharvest testa colour

The moisture contents of seeds were modified to 8.4, 10.3, 11.8 and 13.6 g/100g (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. Minimum-maximum thermometers were placed in the storage boxes to monitor temperature changes during storage. A part of the seeds with 12% SMC were placed in bags (10 x 10 cm) prepared using a transparent polyvinyl chloride (PVC) sheet and sealed as above. The bags were placed in a cool room at 20 ± 2 °C under artificial light (GroLux, T8, SYLVANIA, Germany) with photosynthetic photon flux of 350 μ mol $m^{-2}s^{-1}$ (Quantum Meter, QMSW, Apogee Instruments, USA). To measure the light intensity received by seeds the meter detector was covered with the same transparent PVC sheet used for the packaging samples.

Seeds were removed and left at room temperature (25 ± 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 conditions.

2.3. Effect of storage temperature on the kernel (cotyledon) colour

Faba bean 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.

2.4. Colour measurement

Seed coat colour was determined using 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 Eq. I (Anonymous, 1991):

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

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 postharvest colour changes in the samples.

2.5. Postharvest colour darkening acceptability level

Faba beans having a range of colour darkening 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 (Fig. 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 postharvest colour darkening for local and international marketing. According to their comments the samples with 12% SMC stored at ≤ 25 °C (Fig. 1) for one year were acceptable for marketing for human consumption. The maximum acceptable postharvest colour darkening was then back calculated in L^*, a^* and b^* values and used as reference for acceptance of a sample.

Postharvest colour changes were also compared with the scale based on changes in Colour Difference Index (ΔE^*_{ab}) (Anonymous, 1989). 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.

2.7. Determination of Phenolic Constituents

Total free phenolics, tannins and proanthocyanidins (PA) were determined in testa and cotyledons separately (Anonymous, 2000). Testa of 20 seeds were manually removed and the hilum excised and discarded (hilum consists of a small part of testa (~5%) and has blackish colour that does not obviously change during storage). 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 spectrophotometrical methods using the Folin-Ciocalteu’s Phenol Reagent (Merck). Total phenolic compounds were calculated from a prepared standard curve of tannic acid (Merck) under same set of conditions. Tannins were complexed with polyvinylpolypyrrolidone (Sigma) and unbound non-tannin phenolics were determined as above (Anonymous, 2000). Total tannins were calculated by subtracting non-tannin phenolics from total phenolics. Proanthocyanidins were determined according to Butanol-HCl method of Porter, Hrstich, & Chan (1986) given in (Anonymous, 2000).

2.8. Statistical analysis

Correlations and analyses of variance were carried out using SPSS 14.0 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).

3. Results

3.1. Effect of storage temperature and duration on the stability of postharvest testa colour

Storage temperature and duration influenced faba bean testa 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 (Fig. 1). Both temperature and duration of storage influenced L^*, a^* and b^* values (Fig. 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 (Fig. 2), whereas seeds stored at temperatures of 37, 45 and 50 °C attained their maximum a^* values (a^*~ 16) after 4, 2 and 1 month 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). The higher the storage temperature the higher the change in colour after a given time period. The data demonstrated a positive correlation (r = 0.85) between storage temperature and the ΔE^*_{ab} values. Appreciable postharvest colour changes detectable by ordinary people (Anonymous, 1989) 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 (Fig. 2).

3.2. Effect of seed moisture content and light on the stability of postharvest testa colour

Seed moisture content (SMC) was also an important factor affecting postharvest colour darkening expressed by changes in L^*, a^* and b^* values. The higher the seed moisture content the faster was the darkening process at a given temperature. There was a positive correlation (r = 0.88) between SMC and ΔE^*_{ab} values. Samples with 8% SMC were less susceptible to darkening compared to higher SMC. There was a continuous decrease in L^* and b^* values with the passage of time (Fig. 3). 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 (Fig. 3).

Light also caused a substantial increase in postharvest colour darkening. Seeds stored under light darkened much faster than those stored in dark. Storage under light caused a faster decrease in L^* and b^* values and a faster increase in a^* values (Fig. 4). Appreciable colour changes detectable by ordinary people (Anonymous, 1989) were measured just after 2 weeks storage under light at 20±2 °C.

3.3. Effect of storage temperature on the kernel (cotyledon) colour

Not only testa colour but also kernel colour of faba beans darkened during storage at 37 °C. Similar to testa colour cotyledon colour demonstrated a decrease in L^* and b^* values and an increase in a^* values (Fig. 5). Cotyledon colour darkened less than testa colour but differences were still large (Anonymous, 1989). Cotyledons showed a change of 6 points in ΔE^*_{ab} values after 8 months storage at 37 °C (Fig. 5).

3.4. Predictive model for postharvest seed coat colour changes

Using the data collected for changes in colour difference index (ΔE^*_{ab}) of faba bean seeds stored for 12 months under a range of storage conditions a predictive model was developed which is expressed in Eq. II:

$$Y = a\,(T + \mathrm{SMC} + T \times \mathrm{SMC}) + b(T + \mathrm{SMC} + T \times \mathrm{SMC})\,k^{P} \qquad \text{Eq. II}$$

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% variance provided that Fiesta variety is stored under constant temperature and SMC in dark.

3.5. Changes in Phenolic constituents with change in postharvest testa and cotyledon 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 postharvest 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, which were the predominant group among tannins also substantially decreased (Table 1) with an increased storage temperature especially higher temperatures (≥ 37 °C).

Storage under light at 20 °C caused substantial changes in phenolic contents compared with the samples stored in dark at the same temperature. Samples stored under light for 12 months showed a 46% decrease in total phenolics and 57% decrease in PA whereas samples stored in dark showed only 9% decrease in total phenolics and 13% decrease in PA (Table 2). 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 dark (Table 3).

4. Discussion

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

It is possible to store faba beans without substantial darkening. Our results show that postharvest 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 postharvest seed coat colour darkening in other legumes. Little change in seed coat colour occurs 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 retain their original colour for one year when stored at 1 °C (Gunes et al., 1997). In lentil seeds (*Lens culinaris* Medic.) there is no darkening at 5 °C (Nordstorm et al., 1979) and it is slow in adzuki beans (*Vigna angularis*) at 10 °C (Yousif et al., 2003). So similar to other legumes, storage at 5 °C best protected faba bean postharvest 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 postharvest colour darkening in faba bean and other legume seeds (Amarowicz, Troszynska, Barylko-Pikielna & Shahidi, 2004; Cunha, Sgarbieri & Damasio, 1993; Quast &

Silva, 1977; Sorour & Uchino, 2004). Davies (1994) also found that storage of faba beans at 40 °C causes a substantial increase in postharvest colour darkening. Adzuki beans (Yousif et al., 2003), Rawandan dry beans (Edmister et al., 1990) and lentil seeds (Nozzolillo et al., 1984) also darken when stored at 30 ^{o}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, postharvest 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 ^{o}C for one year increases darkening in light-red kidney beans (Hughes et al., 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 of marketing for human consumption after 12 months. This contrasted with storage at ≥ 37 °C which caused substantial darkening just after 2 weeks and the seeds became unacceptably dark (brown) for human consumption in less than 3 months.

The accelerated colour darkening process in faba bean at high temperature (≥ 37 °C) is a serious concern for on-farm storage in Western 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 the 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.

4.2. Effect of seed moisture content and light on the stability of postharvest testa colour

Seed moisture content was also recognized as an important factor in colour darkening of faba bean. Seeds with higher SMC darkened at faster rate than those having lower SMC. Seeds with 8% SMC were very resistant to colour darkening as compared to those with higher 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 have greater colour change (decrease in Hunter L^* values and increase in a^* values) than control seeds or seeds with 5% added moisture (Park et al., 1999). Increases in postharvest colour darkening in Rwandan dry beans positively relate 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. 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 postharvest colour darkening 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 for one month was equal to darkening in 12 months 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 darkens light-red kidney beans during storage (Hughes et al., 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 observe darkening of beans in pods when harvest is delayed after pods and seeds are fully mature (Hughes et al., 1975).

Postharvest 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 bags made of 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 non-transparent material should be used for packaging faba beans for retailing.

4.3. Effect of storage temperature on the kernel (cotyledon) colour

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.

4.5. Predictive model

The predictive model for postharvest seed coat 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.

4.6. Changes in phenolic constituents with change in postharvest testa and cotyledon colour

A substantial reduction in phenolic compounds was associated with postharvest 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 & Stanley, 1986). A range of cultivars of dry beans (*Phaseolus vulgaris*) stored for 5 years under tropical conditions (30-40 °C, 75% RH) exhibit an 11% to 38% decrease in total polyphenols and a substantial decrease in non-tannin polyphenols as compared with freshly harvested beans (Martin-Cabrejas, Esteban, Perez, Maina & Waldron, 1997). A reduction in polyphenol content is found at all stages of seed development in winged beans (*Psophocarpus tetragonolobus* L.) (Kadam, Kute, Lawande & Salunkhe, 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 et al., 1984). The decrease in phenolic constituents with the increase in colour darkening may also be due to oxidative degradation of particular phenolic compounds (Marquardt, Ward & Evans, 1978). Phenolic compounds vary widely in complexity but their common characteristic is that they are readily oxidised and undergo phenolic reactions (Bors, Heller, Michel & Stettmaier, 1996). Indeed when faba beans are flushed with oxygen darkening accelerates, whereas flushing with nitrogen reduces it (Nasar-Abbas, Plummer, Siddique, White, Harris & Dods, 2008). 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 & Brouwer, 1998). 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 postharvest seed coat colour darkening of faba bean.

Tannin especially PA (condensed tannin) may be involved in colour darkening of faba bean. Pinto bean variety with higher initial PA contents darkened faster than the one with lower PA contents (Beninger, Gu, Prior, Junk, Vandenberg & Bett, 2005). The continuous decrease in tannin contents including PA with the increase in darkening of seed testa supports studies in different beans. A decrease in PA 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 et al., 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 & 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 determined. This might have been due to a balance between development of tannins from smaller molecular weight, non-tannin material (Bors et al., 1996; Hughes et al., 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 et al., 1986). The loss in tannin content might also be due to their strong antioxidant activity (Shahidi, Chavan, Naczk & Amarowicz, 2001). Proanthocyanidins 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 & 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. 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.

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Table 1. Phenolic constituents of testa of faba beans stored at different temperatures for 12 months

Storage 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})
Control (Freshly harvested)	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 stored at 20 °C under artificial light and dark for different time periods

Storage period (months)	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})
0 (Freshly harvested; control)	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 stored at different temperatures in dark and stored at 20 °C under artificial light for 12 months

Treatments	Total free phenolics (mg tannic acid g^{-1})
Freshly harvested (control)	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 not significantly different ($p \leq 0.05$) according to Tukey's HSD test

Fig. 1. Effect of temperature on the colour of faba bean seeds after12 month storage in dark.

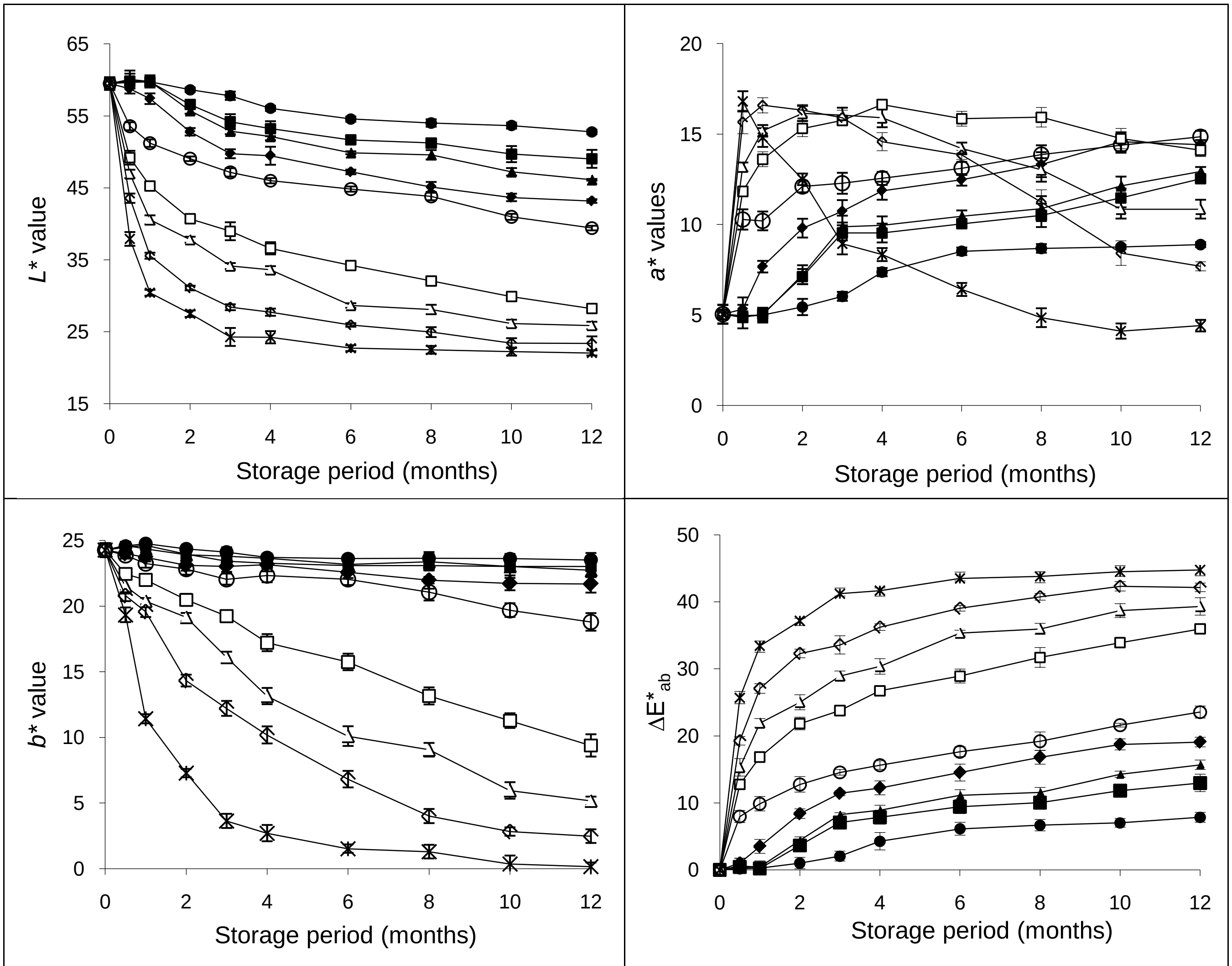

Fig. 2. Effect of storage time and temperature on L^*, a^* and b^* colour coordinates and ΔE^*_{ab} values of faba bean seeds stored in dark: 5 °C (●), 15 °C (■), 20 °C (▲), 25 °C (♦), 30 °C (○), 37 °C (□), 45 °C (Δ), 50 °C (◊), 60 °C (ж). Error bars = ±s.d., n = 9.

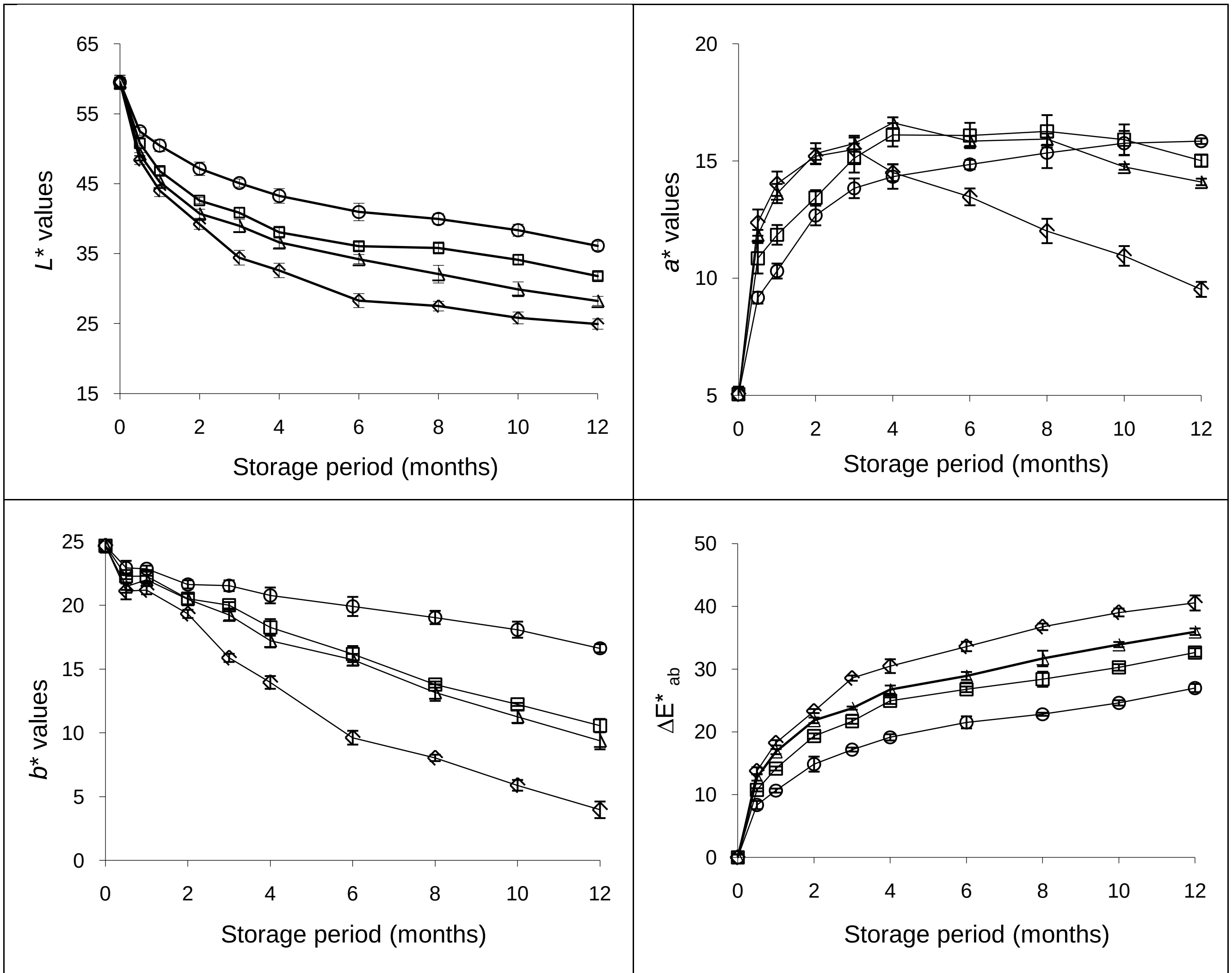

Fig. 3. Effect of seed moisture content on L^*, a^* and b^* colour coordinates and total colour change (ΔE^*_{ab} values) of faba bean seeds stored at 37 °C for 12 months in dark: 8% SMC (○), 10% SMC (□), 12% SMC (Δ), 14% SMC (◊). Error bars = ±s.d., n = 9.

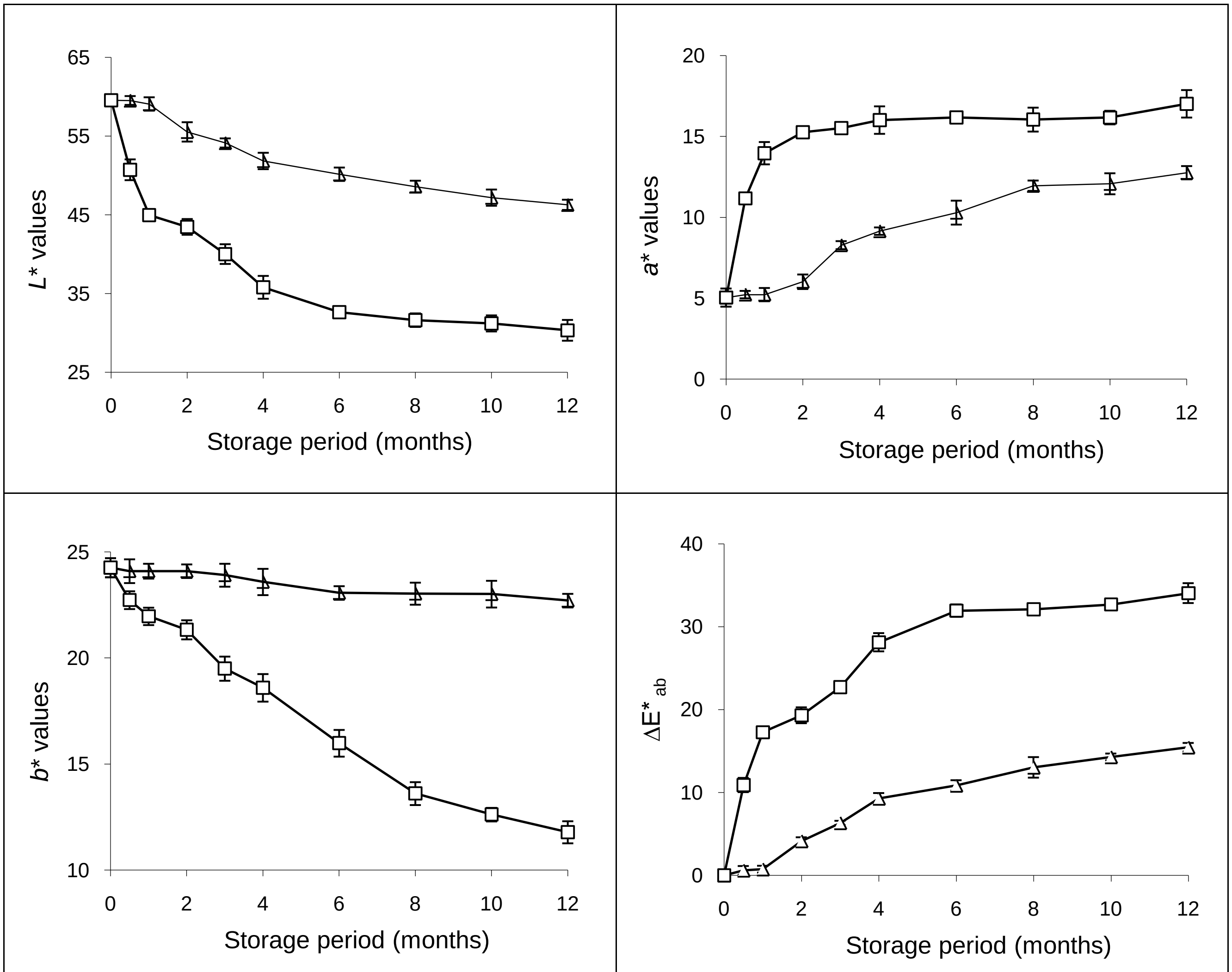

Fig. 4. Effect of light on L^*, a^* and b^* colour coordinates and total colour change (ΔE^*_{ab} values) of faba bean seeds stored at 20 °C for 12 months: in dark (Δ), under artificial light (□). Error bars = ±s.d., n = 9.

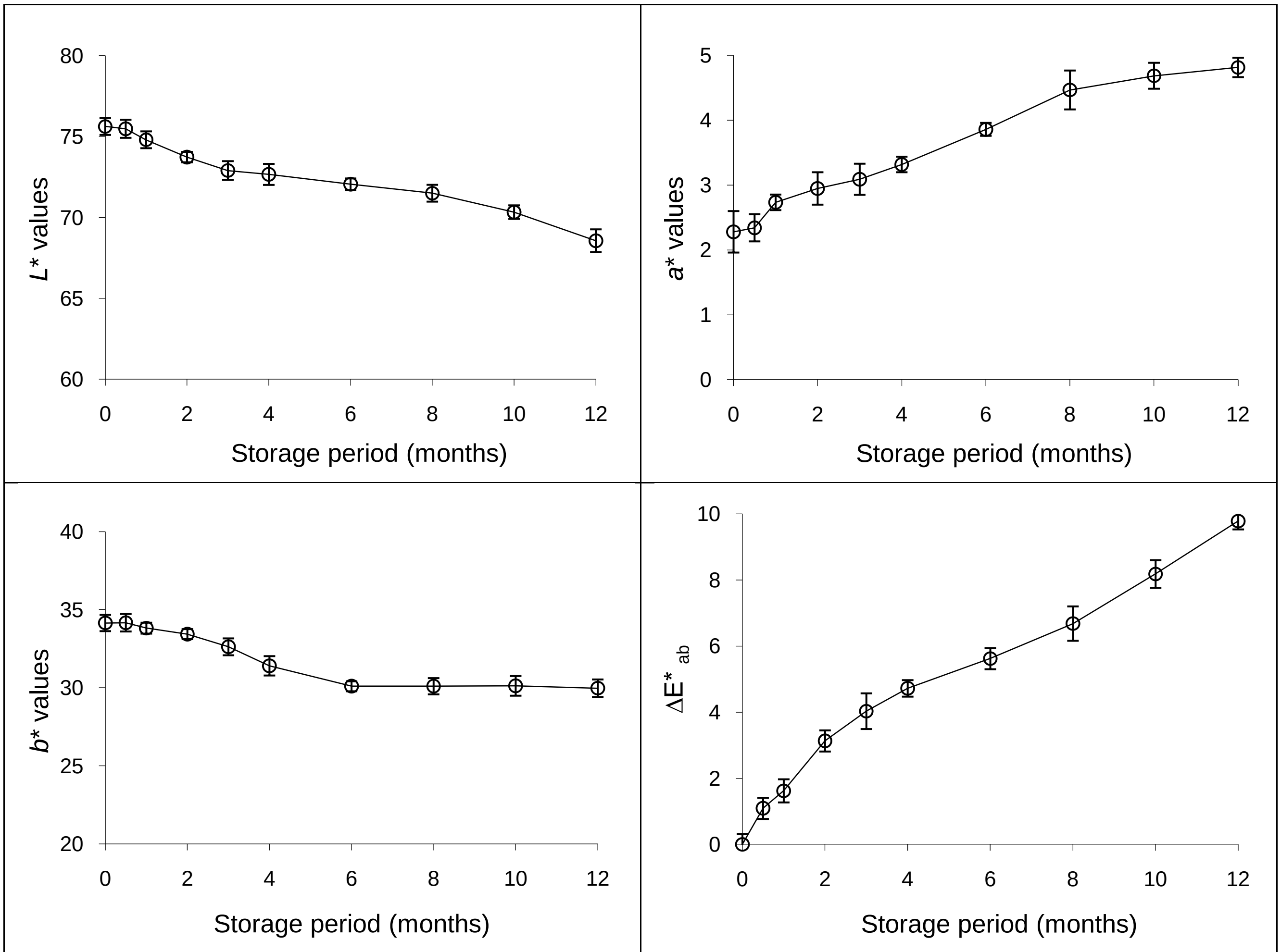

Fig. 5. Effect on L^*, a^* and b^* colour coordinates and total colour change (ΔE^*_{ab} values) of faba bean cotyledons stored at 37 °C for 12 months in dark (Error bars = ±s.d., n = 9).