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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 ($\triangle E^*_{ab}$ 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. Cotyledons 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.

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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 & Sandsted, 1975; Nordstorm & Sistrunk, 1977; Nozzolillo & De Bezada, 1984; Park & Maga, 1999). High temperature (≥24 °C) and high RH (≥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 & 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 ($\triangle E$) (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

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(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 & De Bezada, 1984). Similarly little change in postharvest seed coat colour occurred in Rwandan dry beans (*P. 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/100 g (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 × 25 g) were placed in polyethylene lined aluminium foil bags (10 × 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 × 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 × 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 ($\triangle E^*_{ab}$) was calculated from L^*, a^* and b^* colour coordinates by the Eq. 1 (Anonymous, 1991):

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

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

Initial L^*, a^* and b^* values (subscript by 1) and values at each storage interval (subscript by 2) were used to develop $\triangle E^*_{ab}$ 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 ($\triangle E^*_{ab}$) (Anonymous, 1989). It describes that $\triangle E^*_{ab}$ between 0 and 0.5 is a trace difference and impossible to be detected by human eyesight, 0.5–1.5 is slightly discernible and hard to detect by eye, 1.5–3.0 is noticeable and able to be detected by a trained panel, 3.0–6.0 is appreciable and detectable by ordinary people, a difference of 6.0–12.0 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.

2.6. 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

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