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# Increased stored soybean dietary fibre concentration is positively correlated to testa darkening measured chromaticity

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ABSTRACT

To assess efficacy of seed testa chromaticity indicator of dietary fibre changes in soybean (*Glycine max* L.), soybean samples were stored for zero (0 M), three months (3 M), six months (6 M) or nine months (9 M) at three moisture contents (MC) (9, 11 and 13%) and three temperatures (10, 20 and 30 °C). Stored soybean samples were analysed for seed testa colour and dietary fibre content. Extended storage (9 M) at high MC (13%) and temperature (30 °C) resulted in reduced chromaticity ($C^*ab$) and $L^*$ (darkening) of the testa colour value by 6.7% and 4.7% respectively. Furthermore, storage time and elevated MC (13%) had the biggest effect on dietary fibre levels with insoluble and soluble dietary fibre increasing significantly up to 9 M of storage, by 4.90% and 15.09% respectively with a total dietary fibre increase of 4.62%. This effect was enhanced with increased storage time. Use of testa colour as an indicator of the effect of storage time and conditions on soybean dietary fibre levels contributed to understanding the related impact on the nutritional quality of soybean. This study elucidated the role of post-harvest storage time and conditions on soybean dietary fibre levels as related to chromaticity.



## 1. Introduction

Soybeans are important grain legumes that have been grown in the Orient for many centuries and provide many functional components that promote human health and wellbeing such as linoleic and linolenic acids, vitamin E, oligosaccharides, isoflavons, phytosterols and phytates (Tripathi and Misra, 2005). Furthermore, soybeans are a rich source of oil, protein and dietary fibre (Liu, 1997).

Today, soybeans are an important broad acre crop that are produced and utilised in Australia and around the world. Projected global production for 2013/2014 is 281.7 million tonnes (United States Department of Agriculture, 2013) with Australian soybean production for 2012/13 estimated to be 87,250 tonnes (Australian Oilseeds Federation, 2013).

As a consequence of the grain production agronomic cycle, grain legumes need to be stored for extended periods of time (six to twelve months or longer). Storage time and conditions are of paramount importance to soybean physical and biochemical stability as well as nutritional and functional quality. This is due to the fact that the stored grain is a living biological entity which slowly deteriorates during storage due to post-harvest physiological activity (Yousif et al., 2007).

It has been noted that the stored legume grain testa colour undergoes a darkening process as a consequence of storage under improper storage conditions of high MC and high temperature for extended periods of time (Yousif et al., 2003).

Furthermore, it has also been reported that grain testa colour is related to the level of dietary fibre within the grain. This grain testa darkening has been found to strongly correlate with grain legume components such as dietary fibre content (Yousif and Deeth, 2003); with a positive relationship existing between testa colour and grain dietary fibre content in that darker grains are reported to exhibit higher levels of dietary fibre (Mustafa et al., 2011).

Previous works have also reported that one of the grain legume components that is greatly affected by storage time and conditions (MC and temperature) is dietary fibre which exhibited a large increase in amount with increased storage time. A number of authors reported storage related dietary fibre increase of 23% in "adzuki" bean (*Vigna angularis*) stored for 6 months at 30 °C/40% relative humidity (RH) (Yousif and Deeth, 2003); 11.2% in faba bean (*Vicia Faba* L.) stored for 12 months at 50 °C ambient RH (Nasar-Abbas et al., 2008) and 80% in red kidney beans (*Phaseolus vulgaris*)

*Abbreviations:* 0 M, Zero months; 3 M, Three months; 6 M, Six months; 9 M, Nine months; $a^*$, Red/green value; ANOVA, Analysis of Variance; AOAC, Association of Official Analytical Chemists International; $b^*$, Yellow/blue value; $C^*ab$, Chromaticity; CSIRO, Commonwealth Scientific and Industrial Research Organisation; $L^*$, Light/dark colour value; MC, Moisture content; RH, Relative humidity; SEM, Standard error of the mean.

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stored for 8 months at 40 °C/80% RH (Rozo, 1990). The variability in the amount of dietary fibre increase could be attributed to variation in grain legume species/varieties, varying storage treatments and different methods of analysis used.

The aim of this paper is to report on the effect of storage time and conditions (MC and temperature) on change of chromaticity of the soybeans testa and related change in the dietary fibre levels. The novel aspect of this study is the use of colour measurement "Chromaticity" of the soybean testa as an indicator of the level of dietary fibre in stored soybeans.

## 2. Materials and methods

The soybeans used were a culinary line, 98050-46, obtained from CSIRO Plant Industry Canberra Black Mountain laboratories. Post-harvest, soybeans were conditioned to higher MC of 11% (as a % of total weight) and 13%. Soybeans were placed in large trays in a cold room (4 °C, 80% RH), and periodically mixed to give an even uptake of moisture from the air. Soybeans were conditioned to a lower MC of 9% under ambient laboratory conditions of 21 °C and 45% RH.

Soybean MC was determined by the modified solids (total) and moisture in flour-air oven method AOAC, 925.10 (Association of Official Analytical Chemists International) (AOAC, 2005). Upon reaching the required MC (9, 11 and 13%), all soybean treatments were place separately in sealed food grade Nylon/Polyethylene bags, and allowed to equilibrate for a week before checking the final moisture content.

### 2.1. Storage conditions

Following the moisture conditioning to the correct MC, separate 4.2 kg lots of soybeans were stored at three temperatures 10, 20 and 30 °C in resealable food grade Nylon/Polyethylene bags (350 × 270 mm), placed in 5 L polypropylene, food grade plastic buckets with sealable lids for the following storage times; zero months (0 M), three months (3 M), six months (6 M) and nine months (9 M). The trial was replicated twice.

### 2.2. Analytical methods

#### 2.2.1. Chromaticity (C*ab) and L* colour values of soybeans

The $L^*$, $a^*$ and $b^*$ colour values were measured on the stored soybean treatments using a Konica Minolta Colorimeter CR series Chroma Meter CR-400 (Konica Minolta Sensing Americas Inc., Ramsey, USA). Soybeans (average weight 62.9 g) were placed in a glass light projection tube CR-A33e. Each soybean sample was measured 60 times and reported as an average. Between each measurement, soybeans were removed from the glass light projection tube, placed in a bowl and mixed. Data for each soybean treatment were represented via the use of a two dimensional plot of $C^*ab$ ($C^* = [a^2 + b^2]^{1/2}$) and $L^*$ colour values following the method of Kato and Meguro (1998) to provide a clearer illustration of differences in colour of the stored soybeans.

#### 2.2.2. Chemical analysis sample preparation

Soybeans (40 g) were placed in liquid nitrogen, and then milled in a Polymix hammer mill (Kinematic Dispersing and Mixing Technology-Model KM 80 - 60; Littau-Luzern Switzerland) with a 1 mm die. Following the milling stage, the sample was mixed thoroughly and placed in an airtight container to full capacity; all samples were stored at −18 °C until analyzed.

#### 2.2.3. Chemical composition analysis

Soybean chemical composition analysis was carried out in triplicate and averages reported. Moisture content was determined by the modified solids (total) and moisture in flour-air oven method AOAC, 925.10 (AOAC, 2005). Ash content was determined by the ash of flour direct method AOAC, 923.03 (AOAC, 2005). Total crude protein was determined by the protein in grains method AOAC, 979.09 (AOAC, 2005), and the resulting % N was converted into % total crude protein by multiplying by a factor of 6.25 (Alvarez et al., 2012). Lipid content was determined by the Soxhlet extraction method AOAC, 963.15 (AOAC, 2005). Available carbohydrates were calculated by difference (Chillo et al., 2011).

Insoluble and soluble dietary fibre content was determined by total dietary fibre in foods-enzymatic gravimetric method AOAC, 985.29 (AOAC, 2005), using Sigma–Aldrich total dietary fibre assay kit (TDF100A 1 KT) (Sigma–Aldrich Pty. Ltd Castle Hill, Australia). Total dietary fibre is the sum of the insoluble and soluble dietary fibre. All dietary fibre results (insoluble and soluble) have been corrected for lipid content (204.4 g $kg^{-1}$) via the following equation;

$$\text{Lipid corrected dietary fibre content} = \text{dietary fibre} \times \text{lipid coefficient}^*).$$

$$^*\text{lipid coefficient} = (1000 - 204.4)/1000 = 0.7956.$$

All dietary fibre results are reported on a dry basis prior to statistical analysis.

#### 2.2.4. Statistical analysis

Descriptive analyses were calculated and presented as mean ± the standard error of the mean (SEM). A three way mixed analysis of variance (ANOVA) with moisture content (9, 11 and 13%) and storage temperature (10, 20 and 30 °C) as among cohort factors plus storage time (0, 3, 6, 9 and 12 M) as a repeat measure. If the ANOVA revealed a significant interaction, then multiple comparisons (with Bonferroni adjustments where appropriate) were used to locate specific differences between storage conditions and/or time. The level of significance in this work is $P < 0.05$ unless otherwise stated. Statistical analysis was carried out via Statistical Package for Social Sciences (SPSS) version 17.0 for Windows (patch 14.0.2); IBM Corporation, New York, USA.

## 3. Results and discussion

### 3.1. Chromaticity (C*ab) and L* colour measurement

Colour measurement using the $L^*$, $a^*$, $b^*$ system is a standardised and approved method which has been used extensively by researchers to measure pulse grain colour quality (Kato et al., 2000). However the $L^*$, $a^*$, $b^*$ system is a relatively complicated three dimensional method for the expression of colour difference among different colour samples.

Another validated and simpler method for colour measurement is the chromaticity ($C^*ab$) and $L^*$ which is a two dimensional colour difference method. This method has been utilised successfully by Kato et al. (2000) to elucidate storage related changes to "adzuki" bean (*V. angularis*) seed testa. In comparison to other studies that measured soybean testa colour (Huang et al., 2012), the use of testa chromaticity measurements has the capacity to show difference among soybeans storage samples in a simple and direct way.

Soybean storage results indicated a significant reduction of the $C^*ab$ colour value with increased soybean MC (9, 11 and 13%) and storage temperature (20 and 30 °C), thereby significantly increasing soybean testa darkening. This effect is significantly enhanced with increased storage time (0, 3, 6 and 9 M) (Fig. 1). It is also evident that compared to 0 M; storage MC and temperature interact significantly with the higher soybean MC (13%) and higher storage