



Pigment loss from semolina to dough: Rapid measurement and relationship with pasta colour[☆]

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ABSTRACT

The yellow colour of pasta is due largely to the presence of carotenoid pigments in semolina. Some of the carotenoids can be degraded during pasta processing through oxidation induced by lipoxygenase (LOX), resulting in pasta colour loss. Measurement of LOX activity is complex and not practical as a screening tool, so there is a need to develop a rapid, cost effective, high throughput method for routine measurement of colour loss in breeding programs and the durum milling and pasta processing industries. After establishing a rapid micro-scale pigment extraction procedure, this study developed a method to quantify pigment loss due to enzymatic degradation in semolina. It can be conducted in parallel with a simple and reliable micro-scale semolina pigment content assay utilizing water saturated 1-butanol extraction. Water (0.15 ml) was added to semolina (200 mg) in a micro centrifuge tube. The mixture was then homogenized to simulate the mixing and kneading processes in pasta-making, and allowed to rest for 1.0 or 2.0 h. Pigments in the resulting dough were extracted by adding 0.85 ml of 1-butanol, followed by homogenization, resting, and centrifugation. Absorption at 435 nm was recorded and converted to yellow pigment concentration. The pigment loss from semolina to dough was found to be genotype dependent. Durum genotypes with low loss (0–3.8%) were characterized by the absence of a LOX gene duplication at the *Lpx-B1* locus, which was shown previously to be associated with a strong reduction in LOX activity in semolina. The loss was higher (5.5–21.8%) for genotypes carrying the LOX gene duplication. While the relationship between pigment content in semolina and pasta colour was significant ($r^2 = 0.55–0.61$), the relationship between pigment content in dough after oxidative loss and pasta colour was stronger ($r^2 = 0.59–0.90$). These results suggest that the method developed in this study could be used as a tool to select for reduced colour loss due to LOX activity.

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1. Introduction

Pasta yellowness is an important criterion for assessment of commercial quality of durum wheat and its end products. It depends on the semolina carotenoid content, carotenoid degradation by lipoxygenase (LOX), and pasta processing conditions. The extended conjugated double bonds typical of carotenoid molecules is an important chemical feature responsible for their attractive yellow colour because it forms the light absorbing chromophore (Britton, 1995; Rodriguez-Amaya and Kimura, 2004). The existence of visible colour in carotenoids requires at least seven conjugated

double bonds. The greater the number of conjugated double bonds, the higher the wavelength value for maximum absorption (Melendez-Martinez et al., 2007; Rodriguez-Amaya, 2001).

LOXs are a family of non-heme iron containing dioxygenases (Andreou and Feussner, 2009) that catalyse the insertion of molecular oxygen into polyunsaturated fatty acids (PUFAs) containing a (*cis,cis*)-1,4-pentadiene structure to yield unsaturated fatty acid hydroperoxides. Oxygen can be added to either end of the penta-diene system with high stereo specificity, and in the case of linoleic and α -linolenic acids, this leads to either the 9(S)- or 13(S)-hydroperoxy derivatives or both, depending on the specific iso-form of the enzyme (Liavonchanka and Feussner, 2006). Carotenoids are very reactive to hydroperoxides and colour loss is due to interruption of the polyene chain, in which the conjugated double bonds may either be cleaved or added (Krinsky and Johnson, 2005). In the case of pasta processing, some of the carotenoids in semolina are degraded resulting in loss of yellow colour through the introduction

Abbreviations: CWAD, Canada Western Amber Durum; LOX, lipoxygenase; TYP, total yellow pigment; WSB, water saturated n-butanol.

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of oxygen during the mixing process and activity of LOX (Borrelli et al., 1999). This might explain the poor relationship between pasta yellowness and semolina yellow pigment content observed in a recent study (Fu et al., 2011) which found that elevated yellow pigment concentration does not guarantee a desirable high yellowness of pasta. A protocol developed in that study effectively predicted pasta colour by evaluating semolina dough sheet colour over time. Dough sheet b^* values and spaghetti b^* values were significantly correlated. Semolina dough sheets can be easily prepared with only 30 g of material. Shortly after sheeting (30 min), dough sheet b^* values can be used to predict pasta yellowness without making the end product which involves mixing, extrusion and drying. However, colour of semolina dough sheets does not directly indicate the pigment loss in dough, and preparation of dough sheets can be time restrictive for screening thousands of lines in large breeding programs.

LOX is the major contributor of oxidative degradation of carotenoids in durum wheat (Borrelli et al., 2003; Borrelli et al., 2008). Significant progress has been made in the genetics of LOX to facilitate durum wheat improvement by developing new cultivars with low LOX activity. Carrera et al. (2007) reported the existence of duplication at the *Lpx-B1* locus and allelic variation for a deletion of the *Lpx-B1.1* copy. The deletion was associated with a 4.5-fold reduction in LOX activity and improved pasta colour but not semolina colour, suggesting reduced pigment degradation during pasta processing. De Simona et al. (2010) found that different mechanisms contribute to the final activity of LOX in semolina, a trait that, in association with pigment content, determines the yellow colour of the durum wheat end products. Differences in LOX activity were associated with: (i) variable gene expression; (ii) different combinations of LOXs isoforms; and (iii) different allelic forms. A recent study (Verlotta et al., 2010) revealed that the distribution of *Lpx-B1* genes and alleles among 85 genotypes defined three discrete haplotypes with high, intermediate and low LOX activity in mature grains, respectively.

While the importance of LOX in pasta colour is well recognized, the difficulty and cost associated with the measurement of LOX prevent it from being a selection tool in most durum breeding programs (Clarke, 2001). Several methodologies used for the measurement of lipoxygenase activity have been developed, including a spectrophotometric assay based on measurement of the absorbance at 234 nm produced by hydroperoxy lipid products, an oxygraphic assay based on the evaluation of oxygen consumption using an oxygen electrode, and by assessment of beta-carotene bleaching activity determined photometrically by monitoring the absorbance decrease at 460 nm due to beta-carotene disappearance (Borrelli et al., 1999; Gokmen et al., 2007; McDonald, 1979; Pastore et al., 2000). All three methods are tedious and require enzyme extraction and linoleic acid oxidation to form hydroperoxide, and are not practical as a screening tool for LOX activity in large breeding populations or as a method of quality assurance in the durum wheat milling and pasta processing industries. Furthermore, other enzymes such as peroxidases and polyphenoloxidases can contribute to bleaching and browning of pasta (Kobrehel et al., 1974; Taha and Sagi, 1987), which are not assessed by measuring LOX activity alone. Thus, there is a need to develop a method that is rapid, reproducible, easy, and suitable for adaptation to a high throughput assay format.

The objective of this study was to develop a rapid method to quantify pigment loss from semolina to dough in small samples, which in turn necessitated development of a simple and reliable micro method for the determination of yellow pigment in durum wheat. The assay reported here estimates pigment degradation during pasta processing and is a practical and useful assay to characterize the intrinsic quality of durum wheat.

2. Materials and methods

2.1. Durum wheat samples, milling, and DNA analysis

Six registered Canada Western Amber Durum (CWAD) wheat varieties (AC Avonlea, Brigade, Commander, Eurostar, AC Navigator, and Strongfield) grown in 2009 in Saskatchewan, Canada were used for developing the micro method for the determination of yellow pigment in durum wheat. All wheat samples were graded as No. 1 CWAD. Whole meal was prepared from each of the varieties by grinding 300 g of grain with a Perten 3100 laboratory mill equipped with a 0.8 mm sieve. Semolina was also produced based on the process described below.

Advanced durum wheat lines evaluated in the 2009 and 2011 Canadian Durum Wheat Variety Registration Trials were used for pasta processing and pigment loss measurement to develop and validate the protocols. Samples from the 2010 trial were collected, but were not included in the analysis because of adverse weather conditions that resulted in samples with poor intrinsic quality. Each trial included 25 experimental lines and five check varieties; a total of 23 lines were milled from the 2009 trial, and all 30 from the 2011 trial were milled. The trials were grown at nine (2009) and 11 (2011) locations in Saskatchewan, Alberta, and Manitoba. Preparation of the wheat composites for the durum variety registration trials was carried out at Semiarid Prairie Agricultural Research Centre, Agriculture and Agri-Food Canada, Swift Current, SK, Canada. Grain harvested at stations with high levels of disease, insect or weather damage was excluded from composites. The amount of wheat from each remaining station was chosen based on protein content and grade to prepare 6 kg composites for quality testing at a grain protein concentration of about 13%. Most samples were graded as No.1 or No. 2 CWAD, with a few graded as No. 3 CWAD.

Durum wheat was milled on a four stand Allis-Chalmers laboratory mill in conjunction with a laboratory purifier according to the mill flow described by Dexter et al. (1990). All lines were milled in duplicate or triplicate 2-kg batches. The mill room was controlled for temperature (21 °C) and humidity (60% R.H.). Samples were conditioned prior to milling to 16.5% moisture overnight. Semolina granulars were prepared by combining selected streams (from most refined to less refined) derived from the break, sizing and purifier systems until 65% extraction is reached. Semolina samples with this constant extraction rate were used for comparability in terms of pigment loss among the genotypes examined.

Each line in the 2009 and 2011 trials was evaluated for the duplication of LOX genes at the *Lpx-B1.1* locus. The DNA from each line was extracted from 3-day old leaves as described previously (Pozniak et al., 2007). Polymerase chain reaction (PCR) analysis was performed on 100 ng of DNA using the primers LOXB-L/R and PCR conditions described previously (Carrera et al., 2007). PCR amplicons were digested with *HaeIII* prior to gel electrophoresis on a 1.5% (w/v) agarose gel.

2.2. Micro-procedure for measuring pigment content in semolina and wholemeal

Total yellow pigment in semolina was assessed using AACC approved method 14-50. Briefly, water saturated n-butanol (WSB, 40 ml) was added to 8.0 g of semolina (14% m.b.), shaken and extracted overnight. Extracts were then filtered through Whatman No. 1 filter paper, and absorbance measured at 436 nm using a UV/Visible Ultrospec 3000 (Pharmacia Biotech, Cambridge, England). Absorbance was measured individually for two aliquots from each sample extract, and values were averaged and converted to yellow pigment using the coefficient for β -carotene prior to data analysis.

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