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Wheat flour granulometry determines colour perception

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ABSTRACT

Einkorn, durum wheat and Kamut® are rich of carotenoids, antioxidants with beneficial effects on human health. In the present study the effect of flour granule size on carotenoid content and colour was assessed; furthermore, the suitability of two colorimeters (Minolta Chroma meter CR-210 and Minolta Chroma meter II Reflectance), a spectrophotometer (Jasco V-650 with integrating sphere) and image analysis to define colour and determine carotenoid concentration in wheat flours of different granulometry was tested. Carotenoid content did not vary across flours of diverse size, except in the finest einkorn fraction (<80 μm), which had lower concentration. For all instruments colour coordinate L^* decreased and b^* increased with flour size growth, while a^* varied with the different devices. A Principal Components Analysis (PCA), performed considering carotenoid content and all colorimetric indices, distinguished einkorn from durum and Kamut®, and divided samples of different granulometry; a similar result was achieved by a PCA performed on the absorbance spectra from the integrating sphere. The PCA on image texture data classified the samples following flour size. In conclusion, flour colour is determined not only by carotenoid content but also by flour particle size: therefore, direct colour measurement seems not suitable to predict flour carotenoid content.

1. Introduction

Carotenoids are lipid-soluble pigments that play a relevant role in many essential functions of plants and animals. In humans they are the precursors of vitamin A and exert a positive function on health, because their antioxidant activity protects cells and tissues from free radicals and oxygen ion damages (Adom & Liu, 2002). Carotenoids are scarce in bread wheat (*Triticum aestivum* L. subsp. *aestivum*), but are more abundant in durum wheat (*Triticum turgidum* L. subsp. *durum* Desf.) and Kamut® (*T. turgidum* L. ssp. *turanicum* Jakub.), ranging from 1.5 to 4.8 mg/kg (Hidalgo, Brandolini, Pompei, & Piscozzi, 2006; Panfili, Fratianni, & Irano, 2004), and in einkorn (*Triticum monococcum* L. subsp. *monococcum*), where they vary between 5.3 and 13.6 mg/kg dm (Abdel-Aal et al., 2002; Brandolini, Hidalgo, & Moscaritolo, 2008; Hidalgo et al., 2006). The yellow colour imparted by carotenoids (mainly lutein and its fatty acid esters) has become a very important quality trait for pasta and other food products (Blanco et al., 2011).

In wheat flour, carotenoid evaluation is analytically performed by HPLC (Fratianni, Irano, Panfili, & Acquistucci, 2005; Leenhardt et al., 2006), a procedure that is very precise but also time-consuming and expensive. Easier, quicker and cheaper alternatives commonly adopted are the use of visible/near-infrared reflectance spectrophotometry on water-saturated butanol extracts (AACC International, 2012) or the direct measurement of flour colour by reflectance spectrophotometry (Oliver, Blakeney, & Allen, 1992). The direct flour analysis is based on the CIE colorimetric space (McCaig, 2002; Posner, 2009); the colour is classified in three dimensions: L^*, which measures brightness (0 = black and 100 = bright), a^*, where positive a^* indicates redness and negative a^* indicates greenness, and b^*, where positive b^* indicates yellowness and negative b^* indicates blueness (CIE Commission Internationale de l'Eclairage, 2004). CIE flour colour is determined mainly by a combination of brightness and yellowness: brightness is influenced by bran content, while yellowness is affected by carotenoid content of the endosperm (Hidalgo & Brandolini, 2008a; Oliver et al., 1992). Variation in L^* affects the measurement of b^*, and can lead to errors in estimating carotenoid content (Mares & Campbell, 2001).

Wheat flour carotenoid content and colour are influenced by inherent genotypic characteristics (Brandolini et al., 2008), environmental conditions (Hidalgo, Brandolini, & Ratti, 2009), stresses during grain production (Fratianni, Giuzio, Di Criscio, Zina, & Panfili, 2013; Hidalgo et al., 2009; Lachman, Hejtmánková, & Kotíková, 2013), milling (Posner, 2009) and storage conditions (Hidalgo & Brandolini, 2008b). In fact, milling procedures and extraction rates have a strong bearing on several components, such as ash, protein, pigments and damaged

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starch content (Posner, 2009) which, in turn, influence colour. Additionally, flour particle size, related to differences in wheat species, grain hardness and moisture content of the kernels at milling (Posner, 2009), is another possible source of variation (Symons & Dexter, 1991).

Several authors have tested different reflectance spectrophotometers and colorimeters for colour determination in wheat flour (e.g. Black & Panozzo, 2004; Dowell et al., 2006; Mares & Campbell, 2001; McCaig, 2002; Oliver et al., 1992), concluding that the instruments with visible wavelength sensors had good potential for predicting flour colour values with accuracy.

The aims of this research were a) to assess the influence of wheat flour granule size on yellow pigment content and colour and b) to evaluate the suitability of different instruments (two Minolta colorimeters, Chroma meter CR-210 and Chroma meter II Reflectance, and one Jasco V-650 spectrophotometer with integrating sphere) and of image analysis to define colour and to measure the carotenoid concentration in different wheat flours.

2. Materials and methods

2.1. Materials

The evaluation of protein, ash, pigment content and colour as a function of flour granulometry was performed on seven samples: two *T. monococcum* ssp. *monococcum*, the free-threshing line SAL98-8-3-2 (2011 harvest) and the hulled cultivar Monlis (three samples from plots fertilized with different nitrogen levels: 0, 40, and 80 kg/ha of N; 2012 harvest), one *T. turgidum* ssp. *turanicum* (Kamut®; 2012 harvest) and one *T. turgidum* ssp. *durum* (durum wheat) cv Saragolla (2011 and 2012 harvests). The accessions chosen are characterized by medium-to-high carotenoid content (Hidalgo et al., 2006). All accessions were cropped in Sant'Angelo Lodigiano (Italy), in the centre of the Po plain agricultural belt, following standard cultural practices (Castagna, Borghi, Di Fonzo, Heun, & Salamini, 1995). After harvesting, the kernels were stored at 5 °C. Just before milling, the seeds of einkorn cv Monlis were de-hulled with an Otake FC4S thresher (Satake, Japan); dehulling was not required for the other accessions, all free-threshing.

Refined flours were obtained using a Bona-GBR lab mill (Bona, Monza, Italy), that separates refined flour from bran and shorts. Flours of different granulometry were graded with an automatic lab sifter (Buhler Plain sifter, Buhler, Switzerland): about 800 g of flour was screened for 5 min through a set of different sifters with increasingly smaller mesh sizes (method 66–20, AACC American Association of Cereal Chemists, 1995). The flour samples were split into five different fractions: ≥200 μm, <200 and ≥160 μm (160–200), <160 and ≥120 μm (120–160), <120 and ≥80 μm (80–120), <80 μm and stored under vacuum at −20 °C until analysis.

2.2. Experimental

The dry matter of the different samples was measured as described in method 44-15A (AACC American Association of Cereal Chemists, 1995), ash content was assessed with AACC method 08-03 (AACC American Association of Cereal Chemists, 1995), and protein content (N × 5.7) was determined following method 46-10 (AACC American Association of Cereal Chemists, 1995). The extraction of total carotenoids was performed with saturated butanol, following AACC method 14-60.01 (AACC International, 2012); the extracted pigments were measured at 450 nm with a double-beam V650 spectrophotometre (Jasco, Japan). Total carotenoid content was then computed considering a calibration curve prepared with six different concentrations (from 0.25 to 5.00 mg/L) of lutein standard stock solution (Sigma-Aldrich, St. Louis, MO, USA). All measurements were performed twice; the results are presented as means, expressed as mg/kg on a dry matter basis (DM).

The flour colour was measured in triplicate using two Tristimulus colorimeters, Chroma meter CR-210 (Minolta Italia SpA, Italy) and Chroma meter II Reflectance (Minolta Camera Co., LTD, Japan). The analysis led to the determination of the values of coordinates L^* (luminosity), a^* (red–green) and b^* (yellow–blue). Additionally, the UV–VIS reflectance spectrum was recorded by a V-650 spectrophotometre (Jasco, Japan) with integrating sphere (PIN 757, Jasco, Japan). The flour samples were fitted into a container (diametre: 20 mm; height: 2 mm); the spectra were measured at wavelengths between 220 and 800 nm, with a 400 nm min^{-1} scansion speed, a 5 nm UV–VIS band broadness, and D2/WI lamp change at 340 nm. The spectra of each sample were recorded three times; the colour coordinates L^*, a^*, and b^* were determined with the spectrophotometre Spectra Manager software, using the parameters: light source C, standard observer 2°, data interval 5 nm and colour matching JISZ871-1999.

To perform image analysis, for each accession the images of three flour-filled Petri dishes were acquired with an Epson Perfection 3170 Photo flatbed scanner (Seiko Epson Corporation, Nagano, Japan), previously calibrated with IT8 Colour Targets system and SylverFast software v.6.1 (LaserSoft Imaging Inc., Kiel, D). During the acquisition process the samples were covered with a black box to prevent loss of light; the images, acquired at a resolution of 600 dpi (dots per inch) and a colour depth of 24 bits, were saved in uncompressed TIFF format. To create the final data set, two different ROIs were tested: 400 × 400 pixels and 250 × 250 pixels. The final results were similar, so a region of interest (ROI) of 250 × 250 pixels, representative of the whole sample surface, was extracted from each single Petri dish image using the Image-Pro Plus 7.0.1 software (Media Cybernetics, Inc., USA). This ROI was chosen because of the inferior analysis time required by the algorithm to process all the image data sets at the same time. On the whole data set the following evaluations were performed:

- Colour and homogeneity: the colour evaluation was performed in the RGB space colour, considering the red (R), green (G), blue (B) and intensity mean (I) parameters. The homogeneity of each surface was evaluated from the heterogeneity (HTG) parameter provided by the software Image-Pro Plus. This parameter, ranging from 0 (homogeneous surface) to 1 (heterogeneous surface), is used to characterize the surface of different types of substrates (Fongaro & Kvaal, 2013; Marti, Fongaro, Rossi, Lucisano, & Pagani, 2011). For each parameter, the values are reported as mean of the three images analysed.
- Image texture: the image texture of each region of interest was evaluated by means of the Gray Level Co-occurrence Matrix (GLCM) algorithm (Haralick, 1979). Fourteen different features (angular second moment, contrast, sum of squares, correlation, inverse different moment, entropy, sum average, sum variance, sum entropy, difference variance, different entropy, two information measures of correlation and maximum correlation coefficient) can be obtained by this method to describe the surface characteristic of the image analysed although generally, as reported by Zheng, Sun, and Zheng (2006), in the food field six of them (angular second moment, contrast, sum of squares, correlation, inverse different moment, entropy) are enough to find a good relation between image texture and food properties. In this work the following five Haralic Descriptors were calculated: Angular Second Moment (ASM), showing the uniformity of an image; Contrast (CON), showing the amount of local variation present in a image; Correlation (COR), showing the pixel linear dependencies; Entropy (ENT), a measure of statistical randomness related to image disorder and the Inverse Different Moment (IDM), illustrating the homogeneity of an image (Fongaro & Kvaal, 2013; Zheng et al., 2006). The images were processed with a revised plug in to run on image stacks, named GLCM_Texture (Cabrera, 2005), for the ImageJ v. 1.44c software (Schneider, Rasband, & Eliceiri, 2012). The GLCM algorithm was