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Distribution of carotenoids in endosperm, germ, and aleurone fractions of cereal grain kernels

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ABSTRACT

To compare the distribution of carotenoids across the grain, non-corn and corn cereals were hand dissected into endosperm, germ and aleurone fractions. Total carotenoid content (TCC) and carotenoid composition were analysed using spectrophotometry and HPLC. Cereal carotenoid composition was similar; however, concentrations varied significantly ($p < 0.05$). Endosperm fractions had TCC ranging from 0.88 to 2.27 and 14.17 to 31.35 mg/kg in non-corn cereals and corn, respectively. TCC, lutein and zeaxanthin in germ fractions were higher in non-corn cereals than in corn. Lutein and zeaxanthin contents were lower in non-corn cereal endosperms. The aleurone layer had zeaxanthin levels 2- to 5-fold higher than lutein among the cereals. Positive significant correlations ($p < 0.05$) were found between TCC, carotenoids analysed by HPLC and DPPH results. This study is the first to report on carotenoid composition of the aleurone layer. Our findings suggest that the aleurone of wheat, oat, corn and germ of barley have significantly enhanced carotenoid levels.

1. Introduction

Increased interest in functional foods requires more information on the phytochemicals including carotenoids in grain cereals that have health enhancing properties. Carotenoids are among the abundant families of pigments in nature that are responsible for the yellow, orange and red colours of fruits, vegetables and grains. They form part of the antioxidant system in seeds (Howitt & Pogson, 2006). Xanthophyll carotenoids, which include lutein and zeaxanthin are recognised for their antioxidant properties (Gentili & Caretti, 2011; Leenhardt et al., 2006; Miller, Sampson, Candeias, Bramley, & RiceEvans, 1996). Carotenoids act as radical scavengers and singlet oxygen quenchers (Leenhardt et al., 2006). Epidemiological studies have shown that carotenoid-rich foods reduce the risk of degenerative diseases, such as cancer, cardiovascular diseases, and age-related macular degeneration and also maintain skin health (Burkhardt & Boehm, 2007; Rice-Evans, Sampson, Bramley, & Holloway, 1997; Roberts, Green, & Lewis, 2009).

Although a minor component in cereals (Irakli, Samanidou, & Papadoyannis, 2011), some grains contain higher and others lower content of carotenoids compared to fruits and vegetables (Abdel-Aal et al., 2002; Humphries & Khachik, 2003). However, carotenoid content is an important characteristic in the utilisation of cereals such as durum wheat for pasta production (Hentschel et al., 2002). Several authors studied carotenoid content and composition in whole grain wheat (Abdel-Aal, Young, Rabalski, Hucl, & Fregeau-Reid, 2007; Adom, Sorrells, & Liu, 2003; Hentschel et al., 2002; Panfili, Fratianni, & Irano, 2004) maize or yellow corn (Kimura, Kobori, Rodriguez-Amaya, & Nestel, 2007; Luterotti & Kljak, 2010, and barley (Goupy, Hugues, Boivin, & Amiot, 1999). The main carotenoids in cereal grains are lutein and zeaxanthin (Hentschel et al., 2002; Panfili et al., 2004). Lutein was the most abundant carotenoid in 11 wheat varieties studied by Adom et al. (2003). Okarter, Liu, Sorrells, and Liu (2010) found higher levels of lutein and zeaxanthin in eight diverse whole wheat varieties than reported by Adom and others. Zeaxanthin was the dominant carotenoid in maize whereas lutein was the main component in oat, barley, spelt and durum wheat (Panfili et al., 2004). Studies on carotenoid content and composition mainly used durum wheat, bread wheat, specialty wheat (Einkorn, Khorasan) (Abdel-Aal et al., 2002, 2007; Hidalgo, Brandolini, Pompei, & Piscozzi, 2006) yellow corn (Hulshof, Kosmeijer-Schuil, West, & Hollman, 2007; Kurilich & Juvik, 1999a) while barley and oat were rarely used (Goupy et al., 1999; Panfili et al., 2004). Only a few studied distribution of carotenoids in grain kernels and their fractions (Borrelli, De Leonardis, Platani, & Troccoli, 2008; Hentschel et al., 2002; Panfili et al., 2004). There is limited or no information on carotenoid composition of the aleurone layer.

Although the study used hand dissected fractions, these fractions can be obtained mechanically at large scale during milling

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and dry fractionation processes (Antoine, Peyron, Lullien-Pellerin, Abecassis, & Rouau, 2004). The main objective of this study was therefore to investigate the distribution of total and individual carotenoids in endosperm, germ and aleurone fractions obtained by hand dissection of diverse cereals grains. A secondary objective was to determine the antioxidant activity of carotenoid extracts.

2. Materials and methods

2.1. Chemicals

Acetonitrile, methyl-t-butyl ether (MtBE) 1-butanol and methanol were purchased from Fisher Scientific (Whitby, ON, Canada). Carotenoid standards, lutein, zeaxanthin and β-cryptoxanthin were purchased from ChromaDex Inc. (Santa Ana, CA) and trolox (S)-(−)-6-hydroxy-2,5,7,8-tramethylchroman-2-carboxylic acid and 2,2-diphenyl- 1-picrylhydrazyl (95%) was obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA).

2.2. Samples

A study was conducted on 7 non-corn (2 barley varieties (purple and regular), 4 soft wheat varieties (Ambassador, Caledonia, Purple wheat and MSUD8006), 1 oat) and 3 yellow corn (USP1395XR, P1508HR and Dasca-flint corn) cereals. Samples analysed included 10 whole grains, 10 endosperm fractions, 10 germ fractions and 10 aleurone layer (only for HPLC).

2.3. Sample preparation

2.3.1. Hand dissection/grain fractionating

The grains were hand dissected to separate the outer pericarp (bran), inner layer (aleurone layer), germ and endosperm based on the procedure described by Stewart, Nield, and Lott (1988) with further modifications. Briefly, grain brush ends and germs were removed by a sharp scalpel under a magnifying glass and the seeds cut lengthwise. The grains were soaked in 0.1% sodium hypochlorite for 15–20 min to sterilize the surfaces and rinsed using sterile, distilled, deionised water. The seeds were placed in 10 cm petri dishes lined with two ashless filters, moistened with 10 mL of sterile, distilled, deionised water. The petri dishes were wrapped in aluminium foil and kept at room temperature (20 °C) for 2 days. The pericarp, aleurone layer and endosperm were separated using a scalpel and stored at −20 °C. Sample were freeze dried, ground using a multi-use blade grinder, model PCC770 (Loblaws Inc. MO, Canada) to pass through a 0.5 mm sieve screen and no residue >0.5 mm were discarded. The ground samples were stored at −20 °C before extraction and analysis.

2.3.2. Estimation of proportions of seed fractions

To determine the ratio of the germ, endosperm and bran to whole grain seed, 25 seeds of each grain sample were randomly selected and manually dissected under a magnifying glass. The weight of germ was calculated by subtracting weight of the endosperm with germ from weight of endosperm without germ. Isolated germs were randomly weighed to confirm the weight found by subtraction. The average weight of whole grain and percentage proportions of each fraction are shown in Table 1. The percentage proportions were later used to calculate the distribution of the TCC in the grain fractions.

2.4. Extraction of carotenoids

Carotenoids were extracted according to the method of Abdel-Aal et al. (2007) with some modifications. Briefly, 200 mg of ground samples (whole grain, endosperm and germ) were mixed with 2 mL of water–saturated butanol in tubes covered with black cap and aluminium foil in a fume hood. The mixtures were vortex for 30 s and carotenoids extracted by shaking for 15 min at speed of 40 using a horizontal rotary shaker (RKVSD, ART Inc. Laurel, MD). After shaking, the samples were left to stand for 60 min at room temperature in the dark and homogenised again before shaking for another 15 min. Lastly, the samples were allowed to stand for another 60 min. About 1.8 mL of extract were transferred into 2 ml brown micro-centrifuge tubes and centrifuged at 4000*g* and 20 °C using IEC Micromax Microcentrifuge (Thermo Electron Corporations, USA) for 5 min. All the procedures were carried out in the dark.

Table 1
Whole grain weight and percentage proportion of each fraction.

Cereal type	Weight (mg)	% proportion of whole grain		
	Whole	Bran	Germ	Endopserm
Purple barley	45.5	15.7	2.6	81.7
Non-pigmented barley	47.5	14.4	2	83.6
Mean-barley	**46.5**	**15.1**	**2.3**	**82.7**
Purple wheat	45.7	11.7	1.6	86.8
Ambassador wheat	47.6	12	2	86
Caledonia wheat	50.8	12.2	2.1	85.8
MSU D8006 wheat	48.8	12	1.9	86.1
Mean-wheat	**48.2**	**12.0**	**1.9**	**86.2**
Oat	38.4	8.7	1.8	89.5
DASCA corn	378.7	5.7	12.1	82
USP1395XR corn	360.6	5.8	11.2	83.0
P1508HR corn	394.6	5.2	10.7	84.1
Mean-yellow corn	**377.9**	**5.6**	**11.3**	**83.0**

Average weight of the various grain cereals ($n = 25$) and % proportions of the grain fractions (bran, germ and endosperm).

2.5. Spectrophotometric determination of TCC

Supernatants were transferred from micro-centrifuge tubes into a semi-micro quartz cuvet and absorbance measured at 450 nm (average absorbance for carotenoids in wheat and corn) using a Ultraspec 1100 pro, UV/Visible spectrophotometer (Biomicron Ltd., Cambridge, CB4 QFJ, England). All analysis was done in triplicate. Total carotenoid content (TCC) was calculated using the following equation and expressed as μg lutein equivalent/g sample.

$$C = (2 \times A)/S \times W[\mu g/g]$$

where C = lutein content, μg/g; A = absorbance reading, S = regression coefficient (the number that express the relationship which is created based on concentration of lutein working standard solutions in μg/mL and the absorbance); 2 = dilution factor (the dilution factor 2 is based on the total extracted volume of 2 ml) and W = sample weight, g (Abdel-Aal & Young, 2007, 2009).

2.6. Determination of carotenoid composition by HPLC

Fresh extractions were done to determine carotenoid composition using the same procedure described above up to centrifugation of the extracts. After centrifugation, the supernatant obtained was filtered through a 0.45 μm nylon disc filter into brown HPLC vials and stored at −20 °C overnight before analysis. HPLC analysis of carotenoid composition included the aleurone layer fraction and analysis was done in duplicate. The determination of carotenoid composition was done according to the method described by Abdel-Aal et al. (2007) with some modifications. Briefly, the chromatographic separation and quantification of carotenoids was carried out on an HPLC (Waters 2695) equipped with a photodiode array detector (PAD) (Waters 996) and