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Chemical characterization and biological effects of immature durum wheat in rats

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Abstract

A durum wheat cultivar, Simeto, was grown in experimental fields and samples were collected at various stages of development (from 9 to 45 days after flowering) to assess changes in chemical composition during maturation. Fructans, in particular low molecular weight fructo-oligosaccharides, accumulated in the first 2–3 weeks after anthesis. The cultivar was then grown in an open field and collected at 15 days after anthesis and at maturity 45 days after anthesis. Experimental diets containing 53% wholemeal from immature or mature wheat, were fed for 6 and 12 weeks to two groups of growing rats. Glutathione, vitamins C and E and total hydrophilic or lipophilic antioxidant concentrations were determined in mature and immature wholemeal. The effects of feeding immature and mature wheat diets for the two experimental periods on the immune system, antioxidant status and plasma lipids were studied. Feeding immature wheat increased the proliferation rates of lymphocytes, indicating a stimulating effect on the immune response, decreased the plasma triglycerides and cholesterol levels, indicating a positive effect on lipid profiles. Antioxidant concentrations in blood and lymphocytes did not change significantly.

Keywords: Immature wheat; Fructans; Antioxidants; Immune response; Plasma lipids

1. Introduction

Cereals have always been considered chiefly as dietary energy sources because of their high content of hydrolysable polysaccharides, but recently they have received attention as sources of compounds with added health benefits, these include fructose polymers (fructans and fructo-oligosaccharides) and antioxidant molecules such as glutathione, ascorbic acid, tocopherols, carotenoids and flavonoids (Adom et al., 2003; D'Egidio et al., 1997). Fructans are polymers consisting of chain of (2→6)-β-fructofuranosyl units sometimes branched through (2→1)-linkages, with a degree of polymerization (DP) from 3 to 60 substituting a sucrose molecule linked to the fructose residue of the disaccharide sucrose. Fructo-oligosaccharides (FOS), with an average DP 4–5 can be also produced from fructans by controlled chemical or enzymatic hydrolysis (Hendry and Wallace, 1993; Roberfroid et al., 1998). The concentration of fructans and antioxidant compounds in wheat grains depends on their phase of maturity, being significantly higher in the first period of ripening, and rapidly decreasing 2–3 weeks after anthesis (Paradiso et al., 2003). In a green-harvested wheat (cv. Freekeh), Humphries and Khachik (2003) found a notably higher concentration of antioxidant carotenoids in comparison with two bread wheat varieties of (cvs. Pioneer, Catoctin).

Previous studies have show that significant quantities of fructans are present in wheat grain during grain filling and that vitamin C and glutathione concentrations in immature wheat

Abbreviations: BHT, butylated hydroxytoluene; ConA, concanavalin A; DP, degree of polymerization; FOS, fructo-oligosaccharides; GSH, reduced glutathione; GSSG, oxidized glutathione; HDL, high density lipoprotein; HPLC, high performance liquid chromatography; LDL, low density lipoprotein; PBS, phosphate buffer saline; WSC, water soluble carbohydrates.

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kernels are much higher than in mature wheat (De Gara et al., 2003). The various physiological effects of fructans and antioxidants may prove beneficial for human health promotion and protection (Nardi et al., 2003). In particular, fructans appear to exert favourable effects on mineral absorption and on pre-biotic activity in experimental animals and in humans (Gibson, 1999; Niness, 1999), on reducing the levels of circulating lipids and glucose (Delzenne and Kok, 1999) and on modulating the immune response (Schley and Field, 2002). The antioxidant activity of whole wheat has been also associated with reduced risk of chronic diseases such as cardiovascular diseases and cancer, even though the preventative mechanisms by which antioxidants prevent the various diseases are still debated (Polidori, 2003). Most studies on fructans have involved inulin, a (2→1)-β-fructan extracted from chicory roots, which are rich in these fructans, as a supplement to normal diets of experimental animals or human volunteers.

Mature wheat, contains low concentration of fructans (1–4%), but is the most important dietary source of fructans, due to the high content of wheat products in the human diet (Van Loo et al., 1995). Thus, wheat with an increased level of fructans would be an interesting natural resource for the preparation of functional foods (Pagani et al., 2003).

The effects of feeding young rats with synthetic balanced diets containing immature and mature durum wheat grain on the immune system, antioxidant status and plasma lipids for two experimental periods were studied. The durum wholemeals were chemically characterized, with particular reference to their carbohydrate and antioxidant contents.

2. Materials and methods

2.1. Plant growth

A common Italian durum cultivar (Simeto) was grown in an experimental field at the Istituto Sperimentale per la Cerealicoltura in Rome on 10 m^2 plots in a randomised block design and with a sowing density of 450 seeds/m^2. The development stages of plants were followed to define the earing and flowering time, the latest considered as reference for the harvesting time. The samples were collected after 9, 13, 17, 21, and 28 days from flowering and at physiological maturation (45 days). Kernel fresh weight and moisture content were recorded.

The same cultivar was grown in a 1000 m^2 field and half the crop harvested at the milky stage (2 weeks after flowering) and the remainder at physiological maturity. The grain from field production was used for chemical analysis and nutritional experiments.

2.2. Carbohydrate and protein determination

Carbohydrates (fructans) and mono- and di-saccharides were analysed as described by D'Egidio et al. (1999). The soluble carbohydrates were extracted with 96% ethanol at 80 °C for 1 h to remove mainly mono- and di-saccharides and, after centrifugation (24,800 *g*, 15 min, 4 °C), fructans were extracted from the pellet with water at extraction at 100 °C for 2 h. The glucose and fructose contents of both fractions were determined enzymatically (glucose oxidase/peroxidase, Megazyme diagnostic kit, Megazyme International Ireland Ltd) and chemically (resorcinol/HCl) (Westhafer et al., 1982), respectively. The fructan content was determined from the sum of the fructose and glucose content of the water extract.

Protein (N×5.7) was determined from nitrogen assay by the Dumas combustion method (FP-428 Leco) and the starch content by enzymatic procedure (Megazyme diagnostic kit, Megazyme International Ireland Ltd).

Total dietary fibre was determined by enzymatic–gravimetric method (AOAC 16th Ed. Method 985.29, 1995).

2.3. Fructans characterization

Fructo-oligosaccharide and fructans in durum wheat samples were analyzed by high performance anion exchange chromatography (HPAEC) with pulsed amperometric detection (Corradini et al., 2004).

2.4. Antioxidant determinations

Vitamin C contents of durum kernels were determined according to Paradiso et al. (in press).

The glutathione pool (GSH plus GSSG) was assayed according to de Pinto et al. (1999).

Vitamin E was extracted and separated chromatographically using a System Gold HPLC (Beckman Coulter Inc., Fullerton, CA, USA). A C18 ODS-1 column (length 15 cm, i.d. 4.6 mm) with 3% dichloromethane in 97% methanol (v/v) as mobile phase was delivered at a flow rate of 1 ml/min with a 20 µl injection loop. Tocopherols in eluates were determined spectophotometrically at 290 nm. Vitamin E content was calculated from calibration curves obtained by using known concentration of α, β, δ and γ tocopherols.

Total antioxidant capability was measured by vigorously mixing 1 g of wholemeal with 2 ml 50 mM Na-phosphate buffer (pH 7.5) and 5 ml ethyl acetate. The homogenate was centrifuged at 4000 *g* for 10 min to separate the aqueous and organic phases. The two phases were collected separately and the hydrophilic and lipophilic antioxidant capabilities were measured (Arnao et al., 2001) using the 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABS)/horseradish peroxidase (HRP) decolouration method. The capability of the aqueous and organic phases to scavenge the ABTS radical cations was compared using a standard dose–response curve obtained by using 6-hydroxy-2,5,7,8-tetramethylchromano-2-carboxylic acid (trolox). Results were expressed as trolox equivalents.

2.5. Feeding experiments

Two groups of young male Sprague–Dawley rats, weighing 100–110 g were fed for 6 and 12 weeks with experimental diets containing mature and immature wheat. The composition of