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# Characterisation of oat (*Avena sativa* L.) oil bodies and intrinsically associated E-vitamers

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**Abstract**

Transmission electron microscopy of sections of oat (*Avena sativa* L.) grain suggested that the highest concentrations oil bodies were in the aleurone and germ rather than the starchy endosperm. Oil bodies recovered from homogenized tissues by centrifugation and washed in (9 M) urea were significantly ($P<0.05$) enriched in lipid (93.3 ± 1.4% dry wt) and low in protein (1.4 ± 0.2%) compared with unwashed (40.2 ± 1.9% lipid; 23.0 ± 1.8% protein), water-washed (78.6 ± 1.2% lipid; 7.8 ± 0.5% protein), and salt-washed (1 M NaCl) oil bodies (89.9 ± 0.4% lipid; 5.1 ± 0.4% protein). Washing significantly reduced ($P<0.05$) the total phenolic content of the oil bodies but significantly increased concentrations of E-vitamers, on a dry weight basis, suggesting an intrinsic association between the E-vitamers and oat oil bodies. The profile of E-vitamers in the oil bodies reflected that in oat grain with α-tocotrienol accounting for ca. 66% of the total E-vitamers. These E-vitamers may provide oxidative stability to the membrane and/or oil of oat oil bodies


*Keywords:* Tocopherol; Tocotrienol; E-Vitamers; Oats; Oil bodies

## 1. Introduction

Plant seeds store cellular triacylglycerols (TAGs) in distinct spherical droplets called oil bodies that act as energy stores for use in germination and post-germinative growth. Oil bodies (typically 0.5–2.5 μm in diameter) are composed of a TAG core surrounded by a monolayer of phospholipids in which is embedded small (15–26 kDa), basic proteins called oleosins, which are specific to oil bodies (Tzen et al., 1993). Classically plant oil bodies contain 94–98% (w/w) neutral lipids, 0.5–2% phospholipids and 0.5–3.5% proteins (Tzen et al., 1993). Oil bodies do not coalaesce, but remain as discrete organelles, even during long periods of storage within seeds (Slack et al., 1980), and this has been suggested to arise from 'steric hindrance' of oleosins and to the mutual repulsion of the electronegativity of oil body surfaces (Tzen and Huang, 1992; Tzen et al., 1992). Moreover, when oil bodies are isolated in aqueous media this stability is maintained.

Oil bodies have been extracted and characterised from a number of cereal species including maize (Ting et al., 1996; Tzen and Huang, 1992; Tzen et al., 1992); wheat (Jelsema et al., 1977) and rice (Chuang et al., 1996). However, there has been no similar characterisation for oil bodies from the common oat (*Avena sativa*). Oats contain comparatively high amounts of oil compared with other cereal species, with values between 6 and 18% reported (Peterson and Wood, 1997; Price and Parsons, 1975). Moreover, histological examination of the oat grain has revealed the presence of lipid bodies in situ (Peterson and Wood, 1997) and the oil body protein oleosin has been found in ground oat samples (Banas et al., 2000). In addition to their comparatively high oil levels, oats also contain significant levels of E-vitamers (compounds chemically and metabolically related to vitamin E and with the same biological activity). Total tocol (tocopherol) concentrations in oat varieties range from approximately 20–30 mg $kg^{-1}$ with α-tocotrienol the most abundant homologue (Peterson, 1995; Peterson and Qureshi, 1993). The tocol group of lipophilic phytochemicals is associated with high oil crops where they have a proposed role in maintaining the stability of cellular membranes and preventing the oxidation of intracellular lipids

*Abbreviations used:* HPLC, high performance liquid chromatography; SDS, sodium dodecyl sulfate; TAG, triacylglycerol; TEM, transmission electron microscopy; TPC, total phenolic content.

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(Fryer, 1992). Interestingly, a positive correlation has been found between tocotrienol and oil concentrations in a range of oat varieties (Peterson and Wood, 1997). Despite this implicit link between tocotrienol and oat oil, a physical link between oat oil bodies and E-vitamers has not been demonstrated.

In the current investigation, an attempt was made to characterise oat oil bodies and to determine if there is an intrinsic association between oat oil bodies and E-vitamers.

## 2. Experimental

### 2.1. Plant material

Oat grains (*Avena sativa*, sub-sp *nuda*) used were a high fat, naked variety called Fatso (seed bred by IGER, Aberystwyth, UK). The oats, harvested in August 2004 from farms in Kent, UK were supplied by Oat Services Ltd (Southampton, UK) and had a grower's reported oil content of 14.6%.

### 2.2. Electron microscopy of oil bodies in situ

Whole oat grains were initially fixed in 2.5% glutaraldehyde in cacodylate buffer (0.05 M, pH 7.4) for 48 h at room temperature before 1 mm slices of selected areas of the grains (aleurone, starchy endosperm and germ) were made using a scalpel blade. These slices were fixed for a further 1 h in fresh 5% buffered glutaraldehyde under vacuum at room temperature. The samples were then washed in buffer for 16 h before postfixing in 2% $OsO_4$ for 3 h at room temperature. The washed samples were dehydrated in a graded ethanol series followed by three 1h acetone steps and finally several changes of Spurr resin over a 38 h period at 36 °C under vacuum. The sections were finally embedded in fresh Spurr resin and polymerised at 70 °C under vacuum for 16 h. Using an ultramicrotome (Leica, UK) with a glass knife, 0.5 μm sections were cut and stained with toluidine blue for light microscopy. Ultra-thin (80 nm) sections were cut using a diamond knife, mounted on copper grids and stained using uranyl acetate and lead citrate before being observed in an electron microscope (JEOL 1010 TEM; JEOL Ltd, Herts UK).

### 2.3. Recovery and washing of oil bodies

Ground oat grains (ca. 100 g) were soaked in extraction media (1:5, w/v; 10 mM sodium phosphate buffer pH 7.5, 0.6 M sucrose) for 12 h before homogenisation (Kenwood BL315 blender; 1 min). The slurry was filtered through three layers of cheesecloth and the filtrate centrifuged at 10,000 *g* (Beckman J2-21 centrifuge; fixed rotor JA-10) for 20 min at 5 °C. The oil bodies, which floated to form a creamy pad on the top of the homogenate, were carefully removed and stored at 5 °C. For further analysis, this crude oil body fraction was used directly or after washing with distilled water, urea (9 M) or NaCl (1 M). Washing was used to remove extraneous material and passively associated compounds from the oil body preparation. For washing, crude oil body material was dispersed in the washing media (1:5, w/v), shaken vigorously for 10 min (700 oscillations $min^{-1}$; SF1 flask shaker, Stuart Scientific, UK) and then centrifuged (3000 *g*) before recovery of the cleaned oil body pad. In each case, the oil bodies were washed twice.

### 2.4. Proximate composition

The lipid content of freeze-dried oil body preparation (ca. 0.5–1 g) was determined gravimetrically by repeated extraction with diethyl ether (Murphy and Cummins, 1989). The protein content of defatted, freeze-dried oil bodies was determined using the bicinchoninic acid assay (Smith et al., 1985) after solubilisation of proteins in 2% sodium dodecyl sulfate (SDS) solution at 90 °C. Bovine serum albumin was used as a protein standard.

### 2.5. Protein analysis by SDS–PAGE

SDS solubilised proteins from crude and washed oil body samples were resolved by SDS–PAGE using a 15% polyacrylamide gel and then stained with Coomassie Blue R-250.

### 2.6. Light microscopy and particle size analysis

The integrity and behavior of urea washed oil bodies following suspension in different media was observed using light microscopy (Chuang et al., 1996). Washed oil bodies were resuspended in 1 ml of 10 mM phosphate buffer at pH 7.5, pH 6.5 and pH 7.5 buffer containing 5 μg of trypsin. The samples were left for 1 h at room temperature and then examined under a light microscope. Oil body size distributions (% frequency) in the different media were determined using a laser granulometer (Malvern Mastersizer S; Malvern Instruments, Worcs., England) fitted with a 320 mm lens and using a small sample dispersion unit with presentation code 3NAD with sample obscuration maintained at 13–25%.

### 2.7. Analysis of methanolic extracts

Phytochemicals were extracted from freeze-dried oil bodies (ca. 1 g; $n=3$) with 100% methanol (1:8, w/v) by continuous mixing for 24 h. Samples were centrifuged (3000 *g*) and the clear yellow supernatant (ca. 8 ml) was aliquoted into amber vials. Extracts were stored at −20 °C prior to analysis. Normal-phase high performance liquid chromatography (HPLC) with fluorescence detection (Jasco FP-920 fluorescence detector) was used to identify tocopherols/tocotrienols. Methanolic extracts (3 ml) were evaporated and then resuspended in hexane before separations were performed on an Inertsil 5 silica column (5 μm, 250 mm × 4.6 mm; Chrompack) using a mobile phase of hexane:1,4-dioxane (95:5, v/v) at a flow rate of 1.5 ml $min^{-1}$. Detection was performed at an excitation wavelength of 294 nm and emission wavelength of 326 nm. A Waters Alliance Separation Module, Waters Millenium Software and commercial standards were utilised. Concentrations are expressed on an oil body dry weight basis (mg $kg^{-1}$; $n=3, \pm$SD).