

Title Evaluation of ergothioneine and other antioxidants in cultivated mushrooms and factors affecting ergothioneine in *Agaricus bisporus*

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Abstract

Antioxidants, such as L-ergothioneine (ERGO) and polyphenols protect against oxidative stress. ERGO is present in fungi, however there is a lack of an assay to identify and quantify this antioxidant in fungi. Thus, utilizing High Performance Liquid Chromatography and Liquid Chromatography-Mass Spectroscopy, ERGO was quantified in *Agaricus bisporus* (white and brown strains) and specialty mushrooms, *Lentinula edodes*, *Pleurotus ostreatus*, *Pleurotus eryngii* and *Grifola frondosa*. Freeze dried mushroom powder was analyzed with two C18 columns in tandem utilizing an isocratic mobile phase consisting of aqueous buffer with 3% acetonitrile and 0.1% triethylamine. ERGO was identified by matching retention time and mass spectra of the authentic compound with mushroom samples while quantification was completed via absorbance at 254 nm. ERGO content of the mushrooms ranged from 0.4-2.0 mg/g dry weight (DW). Within *A. bisporus*, white button contained the least and portabellas contained the highest ERGO. No significant difference was found within the specialty mushrooms; however all contained significantly greater amounts than *A. bisporus*.

Total antioxidant capacity in *A. bisporus* and the specialty mushrooms was measured by oxygen radical absorbance capacity (ORAC_{total}), hydroxyl radical averting capacity (HORAC), peroxynitrite radical averting capacity (NORAC), superoxide radical averting capacity (SORAC) assays, and Folin-Ciocalteu reagent. Polyphenols ranged from 4.2-10.6 mg gallic acid equivalent/g dw. ORAC_{total} values ranged from 39-138 μmol of Trolox equivalents (TE)/g dw. HORAC values ranged from 3.0-13.6 μmol of caffeic acid equivalents/g dw. NORAC values ranged from 2.0-9.0 μmol of TE/g dw. SORAC values ranged from 0.37-2.6 kunit superoxide dismutase equivalents/g dw. *A. bisporus* mushrooms had a higher antioxidant capacity relative to the specialty mushrooms. A relationship was found between ORAC_{total} and polyphenols ($R^2 = 0.87$).

Multiple crop studies were completed to determine the influence of selected cultural and postharvest practices on the ERGO content in *A. bisporus*. Addition of histidine, a precursor of ERGO, to compost significantly increased the amount of ERGO produced. ERGO increased when extent of

mycelium growth increased in the compost. Results indicated ERGO concentration was increased by several stress factors, including dry compost and later flushes of the crop cycle. A shelf-life study indicated ERGO levels significantly decrease during postharvest storage.