

03/01/2018

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Certificate of Analysis

Customer: EnergyBalance Sample Identification: Batch #: B-15654 BL ID #: 18-0112 Description: Reumartis, powder, 8855 Date Received: 02/14/2018

Results:

Analysis	Result	Units
ORAC against peroxyl radicals	611	µmole TE/gram
ORAC against hydroxyl radicals	1,986	µmole TE/gram
ORAC against peroxynitrite	146	µmole TE/gram
ORAC against super oxide anion	3,139	µmole TE/gram
ORAC against singlet oxygen	4,228	µmole TE/gram
ORAC against hypochlorite	Not Detected	µmole TE/gram

There are six predominant reactive species found in the body: peroxyl radicals, hydroxyl radicals, peroxynitrite, super oxide anion, singlet oxygen and hypochlorite. ORAC 6.0 provides a measure of the total antioxidant power of a food/nutrition product against the six predominant reactive species.

The ORAC result is expressed as micromole Trolox equivalency (µmole TE) per gram.

References:

- [1] Ou, Boxin, Maureen Hampsch-Woodill, and Ronald L. Prior. "Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe." *Journal of agricultural and food chemistry* 49.10 (2001): 4619-4626.
- [2] Huang, Dejian, et al. "Development and validation of oxygen radical absorbance capacity assay for lipophilic antioxidants using randomly methylated β-cyclodextrin as the solubility enhancer." *Journal of Agricultural and Food Chemistry* 50.7 (2002): 1815-1821.
- [3] Ou, Boxin, et al. "Novel fluorometric assay for hydroxyl radical prevention capacity using fluorescein as the probe." *Journal of Agricultural and Food Chemistry* 50.10 (2002): 2772-2777.
- [4] Dubost, N. Joy, Boxin Ou, and Robert B. Beelman. "Quantification of polyphenols and ergothioneine in cultivated mushrooms and correlation to total antioxidant capacity." *Food Chemistry* 105.2 (2007): 727-735.
- [5] Zhang, Liliang, et al. "Novel high-throughput assay for antioxidant capacity against superoxide anion." *Journal of agricultural and food chemistry* 57.7 (2009): 2661-2667.
- [6] Ou, Boxin, Dejian Huang, and Maureen H. Woodill. "Method for assaying the antioxidant capacity of a sample." U.S. Patent No. 7,132,296. 7 Nov. 2006.

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ORAC 6.0 Method Description:

Oxygen Radical Absorbance Capacity (ORAC) tests are among the most acknowledged methods that measure antioxidant scavenging activity against oxygen radicals that are known to be involved in the pathogenesis of aging and many common diseases. ORAC 6.0 consists of six types of ORAC assays that evaluate the antioxidant capacity of a material against six primary reactive oxygen species (ROSs, commonly called "oxygen radicals") found in humans: peroxyl radical, hydroxyl radical, superoxide anion, singlet oxygen, peroxynitrite, and hypochlorite. This is a comprehensive panel that evaluates the antioxidant capacity of a material against oxygen radicals.

The ORAC 6.0 tests are based on evaluating the capacity of an interested material to protect a probe (a fluorescent probe or chromagen) from its damage by ROSs. In all ORAC assays, an ROS inducer is introduced to the assay system. The ROS inducer triggers the release of a specific ROS, which would degrade the probe and cause its emission wavelength or intensity change. When an antioxidant material presents in the environment, the antioxidant absorbs the ROS and preserves the probe from degradation. The degree of probe preservation indicates the antioxidant capacity of the material. Trolox is used as the reference standard, and the results are expressed as μ mole Trolox equivalency per gram (or milliliter) of a tested material.

The ORAC 6.0 values obtained from various natural and synthetic materials range widely due to the significantly different natures of these materials including fresh produce, liquid, extract, pure compound, cream, or oil. <u>Select ORAC 6.0 values of benchmark materials</u> are listed in our website to provide limited scale. Comparisons between materials from similar categories are more informative and valuable.

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