

06/27/2011

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## Certificate of Analysis

**Customer: Health Forever Product Limited****Sample Identification:**

Batch #: B-11452

BL ID #: 11-1161

Description: Jobelyn, Powder

Date Received: 06/15/2011

**Results:**

Test	Result	Units
Antioxidant power against peroxy radicals	3,549	μmole TE/gram
Antioxidant power against hydroxyl radicals	18,387	μmole TE/gram
Antioxidant power against peroxynitrite	269	μmole TE/gram
Antioxidant power against super oxide anion	11,417	μmole TE/gram
Antioxidant power against singlet oxygen	4,000	μmole TE/gram
Total ORAC <sub>FN</sub> (sum of above)	37,622	μmole TE/gram

\* The acceptable precision of the ORAC assay is < 15% relative standard deviation

There are five predominant reactive species found in the body: peroxy radicals, hydroxyl radicals, peroxynitrite, super oxide anion, and singlet oxygen. Total ORAC<sub>FN</sub> provides a measure of the total antioxidant power of a food/nutrition product against the five predominant reactive species.

Signed for and on behalf of Brunswick Laboratories

**Authorized Signature****Boxin Ou, Ph.D.****REFERENCES:**

- [1] Ou, B. *et al.*, *J Agric and Food Chem*, **2001**, 49 (10): 4619-4626.
- [2] Huang, D. *et al.*, *J Agric and Food Chem*, **2002**, 50 (7): 1815-1821.
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- [5] Dubost, N.J. *et al.*, *Food Chem*, **2007**, 105 (2): 727-735
- [6] Zhang, L. *et al.*, *J Agric and Food Chem*, **2009**, 57(7): 2661-2667.
- [7] Ou, B. *et al.*, Method for Assaying the Antioxidant Capacity of A Sample. US Patent 7,132,296 B2.

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**IMPROVING HEALTH THROUGH SCIENCE**

Figure 1: Radical Source and Antioxidant Capacity of Fruits

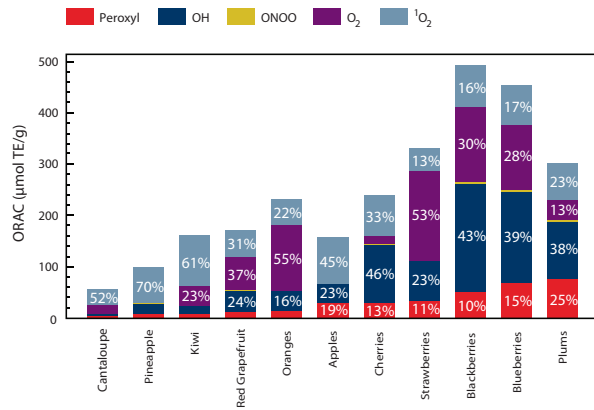
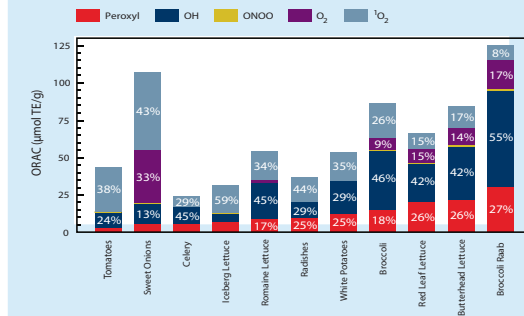
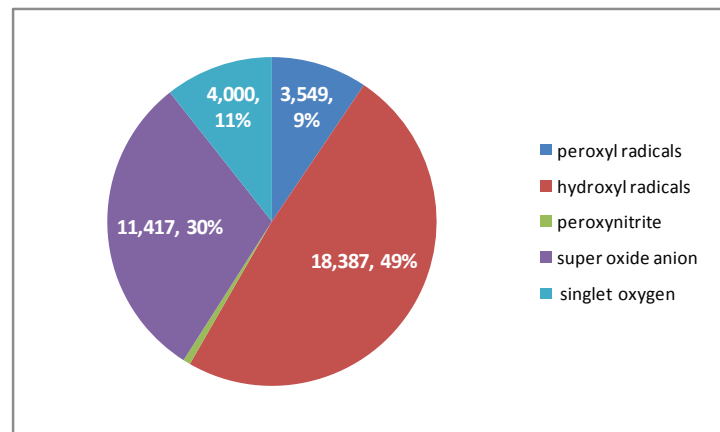
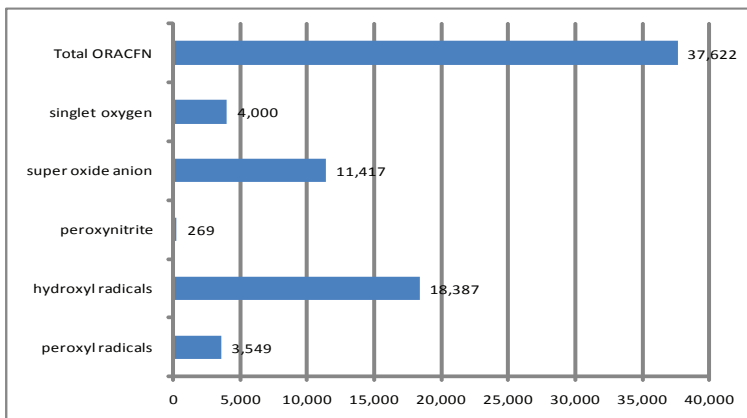


Figure 2: Radical Source and Antioxidant Capacity of Vegetables



## 5.0 fn Antioxidant Capacity of Jobelyn ORAC (μmol TE/g)

## 5.0 fn Antioxidant Capacity of Jobelyn ORAC (μmol TE/g)

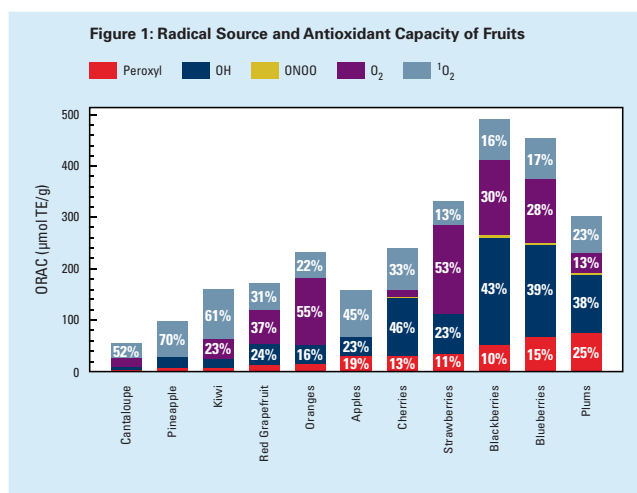


## ORAC5.0™ — Antioxidant Capacity Measurements of Botanicals and the Use of Multiple Radical Sources Using the Oxygen Radical Absorbance Capacity Assay (ORAC)

Several thousand polyphenolic compounds exist in plants and many of these have antioxidant capacity (AOC). Because of the difficulty of quantitating the individual antioxidant compounds, a method which provides a “sum” of the antioxidant components in plants and biological samples is useful. These types of assays are often referred to as “total” antioxidant capacity assays. However, there is in actuality no single assay which provides a “total” measurement of antioxidant capacity. In thinking about antioxidant capacity methods, one has to consider the oxidant source and the mechanism of reaction with potential antioxidants. Experimental evidence has suggested that there are six major reactive oxygen species (ROS) causing oxidative damage in the human body. These species include: superoxide anion ( $O_2^{\cdot-}$ ); hydrogen peroxide ( $H_2O_2$ ); peroxy radicals ( $ROO^{\cdot}$ ); hydroxyl radicals ( $HO^{\cdot}$ ); singlet oxygen ( $^1O_2$ ); and peroxynitrite ( $ONOO^-$ ). The peroxy radical is the most abundant free radical in the human body. Another one of the more relevant radicals in biological regulation is superoxide anion radical. The superoxide anion is formed by the reduction of molecular oxygen in the process of energy metabolism.

Accumulated evidence indicates that reactive oxygen species, such as peroxy radicals ( $ROO^{\cdot}$ ), hydroxyl radicals ( $HO^{\cdot}$ ), the superoxide anion ( $O_2^{\cdot-}$ ), and singlet oxygen ( $^1O_2$ ), are involved in the pathophysiology of aging and a multitude of diseases. To counteract the damage of the reactive oxygen species on living cells, a defense system is designed biologically to neutralize the reactive oxygen species or to prevent the reactive oxygen species from being generated in the first place. Depending on the reaction mechanisms, antioxidants are often classified into

two major categories: radical chainbreaking antioxidants and preventive antioxidants. Chainbreaking antioxidants convert reactive free radicals (e.g.,  $HO^{\cdot}$ ) to stable and thus nonaggressive molecules through hydrogen atom transfer reactions between  $HO^{\cdot}$  and the antioxidants. As a result, the autoxidation chain reactions between the free radicals and the cellular molecules are terminated. Preventive antioxidants inhibit the oxidation reaction from occurring by either converting the precursors of the reactive oxygen species to unreactive species or inhibiting the oxidation reaction. To counteract the assault of the superoxide anion reactive species, living cells have a biological defense system



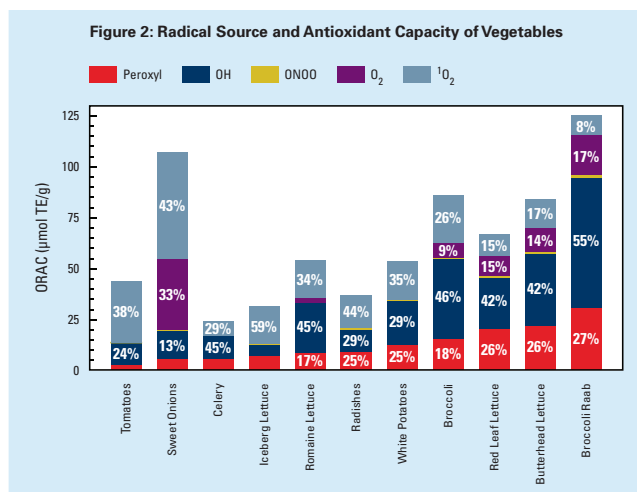
composed of enzymatic antioxidants to convert reactive oxygen species or reactive nitrogen species to harmless species. In contrast, no enzymatic action is known to scavenge  $\text{ROO}\cdot$ ,  $\text{HO}\cdot$ ,  $^1\text{O}_2$ , and  $\text{ONOO}\cdot$ , so the burden of defense relies on a variety of non-enzymatic antioxidants such as vitamin C, and vitamin E, and many phytochemicals that have the property of scavenging oxidants and free radicals. To comprehensively evaluate the oxidant-scavenging capacity of a food sample, assays have to be designed to include these reactive oxygen species. However, so far the majority of assays are designed to measure a sample's capacity to react with one oxidant (either organic radical or redox active metal complex). The peroxy radical has been the most frequently used reactive oxygen species in antioxidant capacity assays because it is the most relevant radical in lipid autoxidation and can be generated conveniently from azo compounds. The peroxy radical has been used as a radical source in the Oxygen Radical Absorbance Capacity (ORAC).

It is evident that the antioxidant defense "team" in living cells contains individual antioxidants that function in very different tasks in the battles against oxidative stress and reactive oxygen species. Therefore, it is imperative that to comprehensively evaluate the antioxidant capacity of food nutrients in vitro, we need a broad range of assays that can cover all aspects of antioxidant capacity. It is impossible to have a one-fits-for-all assay. Although there is a validated assay for peroxy radical absorbance capacity (ORAC) (1-5), no such assay had been developed for

any other of the reactive oxygen species, until Brunswick Laboratories first published methods for the analysis of antioxidant capacity using the hydroxyl, and superoxide anion radicals (6, 7). Brunswick Laboratories has subsequently addressed the other oxygen radical species [singlet oxygen ( $^1\text{O}_2$ ); and peroxynitrite ( $\text{ONOO}\cdot$ )] by developing assays that assess their contribution to total antioxidant capacity.

A sample of available data is presented in Figure 1 and 2 on fruits and vegetables. From this data, it is evident that there is little or no correlation among the different radical sources used to assess antioxidant capacity. One example that stands out is with tomatoes, which has a very low antioxidant capacity as measured with the peroxy radical, but much higher antioxidant capacity using the singlet oxygen radical (Figure 2). Furthermore, the original peroxy ORAC represents no more than 27% of the antioxidant potential of selected fruits and vegetables. The other radical assays added to the ORAC suite represent the preponderance of antioxidant potential.

By measuring all primary reactive oxygen species, ORAC5.0™ provides new opportunities which can be used in the formulation of nutritional products that deliver quantifiable, maximal protection against multiple radical sources.



## References

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