

Antioxidants and free radicals

Antioxidants are molecules that are capable of protecting the human body against so-called 'free radicals' and other harmful compounds that are released within the body as a result of oxidative reactions. Free radicals are very reactive oxygen metabolites which, when produced in excess amounts, can cause damage to tissues and organs. Common free radicals are for example superoxide anion (O_2^-), hydroxyl radical ($\cdot OH$) and lipid peroxy radical ($ROO\cdot$).

Antioxidants are able to neutralize free radicals by accepting the unpaired electron that makes free radicals so highly reactive. Scientific research has shown that antioxidants can play a protective role in a number of diseases in which oxidative stress and free radicals play a role such as cardiovascular and neurodegenerative diseases.

In humans, antioxidants can originate from external (food) sources as well as being produced endogenously. Well-known examples of antioxidants originating from food are vitamins such as ascorbic acid (vitamin C) and α -tocopherol (vitamin E), but also a number of plant-derived compounds possess strong antioxidative capacities. An important group of bioactive molecules showing potent antioxidative capacities are plant constituents such as anthocyanins (plant-pigments), carotenoids and polyphenols (especially flavonoids) from vegetables and fruits and fat-soluble antioxidants from vegetable oils.

Plants are capable of producing numerous amounts of very diverse molecules. Besides molecules originating from the so-called primary metabolism which mainly serve processes such as CO_2 assimilation, energy exchange and growth, plants contain many molecules which are products of the secondary metabolism. These secondary metabolites are all more or less produced to protect and maintain the plant itself. Some molecules fend off predators like insects and herbivores, while other molecules attract insects that are important for pollination. The amounts of secondary metabolites within a certain plant are very variable and are highly depending on all sorts of (environmental) factors. Thus, plants that are under attack by insects or fungi will contain higher concentrations of protective molecules than plants that are free of attacks. Also external factors such as soil conditions, soil hydrology and sunlight conditions can cause significant differences in (secondary) metabolite concentrations. Furthermore, in commercially important plants such as vegetables and fruits, specific varieties and cultivars also play an important role.

Plant-derived antioxidants

Plants can produce a huge variety of antioxidative molecules. Anthocyanins and pigments, responsible for many of the bright colors of fruits and flowers, offer the plant protection from free radicals originating from oxidative stress or exposure to UV radiation.

Flavonoids form another important group of molecules that possess strong antioxidant capacity and thereby are able to protect the plant from all kind of potential harmful oxidation processes. Because many antioxidants also belong to the secondary plant metabolites, the amounts of these molecules in plants can also significantly fluctuate depending on plant species and environmental conditions. Concentrations of plant antioxidants also highly depend on the developmental stage of the plant.

The fact that plants can react to outside threats by producing protective molecules, may very well explain some of the recent observations by several scientific study-groups, investigating the quality of organic vegetables and fruits. In press releases by leaders of this European research, it was revealed that organic crops show higher amounts of antioxidants than conventionally grown crops. It was shown that organic tomatoes, wheat, potatoes, cabbages and onions contain 20-40% more antioxidants. They also showed that organic milk contained on average about 60% more antioxidants, among which 50% more vitamin E and 75% more β -carotene. These findings seem to confirm the idea that plants in fact do not just 'randomly' produce all kind of constituents, but that these metabolites play a vital role in the survival strategy of the plant. After all, in organic farming no or far less pesticides are used which may give insects and fungi a bigger change to attack these plants. This will lead to enhanced stress levels in these plants. Therefore, these plants have to produce higher concentrations of protective molecules to maintain themselves.

So, by exposing plants to increased stress levels, these plants may be stimulated to produce higher amounts of secondary metabolites, thereby also producing higher concentrations of antioxidants.

Effect of *Immutines* on antioxidant capacity of cucumbers

In a total of three studies, effects of *Immutines*-addition to the water during the growing process of cucumbers were investigated. Cucumbers were rooted on rockwool and grown in greenhouses and during distinct periods, several concentrations of *Immutines* were added to the water which was administrated to the growing plants (0.5, 1, 1.5, 3 en 4 ml/m²/week, respectively).

From data delivered by the growing company, it appeared that after harvesting the average weight of the *Immutines*-treated cucumbers was slightly higher than that of the control cucumbers (non-treated cucumbers).

Additionally, a mineral analysis showed that the *Immutines*-treated cucumbers absorbed 9-20% more of the administrated mineral mixture compared to the control cucumbers.

By using the standardized and validated ORAC (Oxygen Radical Absorbance Capacity) method, the antioxidant capacity of the *Immutines*-treated cucumbers was determined and compared to that of the control cucumbers (non-treated cucumbers).

(Also see ORAC Europe reports of September 2008, May 2009 and July 2009).

From the results obtained by the antioxidant capacity determinations performed by ORAC Europe BV, the following conclusions can be drawn:

Antioxidant capacity:

- The antioxidant capacity of *Immutines*-treated cucumbers (expressed as hydrophilic ORAC value) was 29-37% higher than the antioxidant capacity of control cucumbers.

It appears that addition of *Immutines* to the water during the growth process may lead to a higher concentration of antioxidants or molecules with an antioxidative effect in these cucumbers, which ultimately results in a higher ORAC value (per unit of weight).

However, no concentration-dependent effects of *Immutines* administration were detected. The measured increases in antioxidant capacity after administration of *Immutines* in concentrations of 0.5, 1, 1.5, 3 en 4 ml/m²/week, were 8.7%, 36.6%, 33.3%, 37% en 29.7%, respectively.

The increase of antioxidant capacity already seems to reach a maximum level after administration of an *Immutines* concentration of 1 ml/m²/week (and higher). Probably, further increasing the *Immutines* concentration will not lead to a further increase in antioxidant capacity.

Determination of dry weight:

- The determined dry weights (by way of freeze-drying) of the *Immutines*-treated cucumbers (concentrations of 3 ml/m²/week and 4 ml/m²/week) were respectively 4.2% en 7.1% higher than the dry weight of control cucumbers. (No dry weights were determined of the 1 ml/m²/week and 1.5 ml/m²/week *Immutines*-treated cucumbers).

The experimental data of the by ORAC Europe BV performed analyses, are described in ORAC Europe test-reports 20080923, 20090604 en 20090703.

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