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Berlín, 16-02-2020

Determination of the Antioxidative Power (AP) of natural products/final products.

|                      |                                                       |
|----------------------|-------------------------------------------------------|
| TEST REPORT          | granavidaAP-02-2020                                   |
| Customer:            | contact person,                                       |
| Date of assignement: | customer -                                            |
| Samples:             | final products, pomegranate juice Granavida           |
| Samples receipt:     | 05-02-2020                                            |
| Storing.             | 2-8 °C                                                |
| Performed análisis:  | determination of the Antioxidative Power(AP) at t = 0 |
| Date of análisis:    | 11-02-2020                                            |

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Amtsgericht Berlin Charlottenburg  
HRB 105664B  
Geschäftsführer:  
Dr. T. Herrling, Dr. K. Jung

### **The Antioxidative Power (AP)**

The new antioxidative power (AP) method offers the determination of the all over antioxidative power of active ingredients, i.e. plant extracts, vitamins etc., by following the reducing activity against a stable test radical – diphenyl-picryl-hydrazyl (DPPH) with ESR spectroscopy. The AP method basically utilizes the well known DPPH method with a major difference that both the antioxidative capacity and the antioxidative activity are used to characterize the antioxidant under discussion. For this purpose different concentrations of the active ingredients are investigated by ESR spectroscopy and the decrease of the test radical spins is tracked accordingly for each set. With this innovative technique important kinetic information is additionally obtained which is completely neglected by most of the other test systems. Therefore, both the reaction time and the reduction potential of the antioxidants contribute to the calculation of the AP.

$$AP = n^{\circ} \text{ free radicals} / \text{mg} \cdot \text{min}$$

The resulting AP is expressed in antioxidative units (AU), where 1 AU corresponds to the activity of a 1 ppm solution of pure vitamin C (ascorbic acid) as a benchmark. This method allows a rapid and general applicable technique for the measurement of the AP of very different classes of substances.

The AP method allows:

- (1) to perform a standardized analytical method with benchmarked results,
- (2) to obtain a meaningful comparison of active ingredients regarding their capability to scavenge free radicals and/or to terminate radical chain reactions,
- (3) to control the variation within or between products, and to provide quality standards that are benchmarked for quality control and efficacy claims.

The AP method allows a functional Quality Control during the whole production process. It helps to choose the raw materials with the highest antioxidant activity, it is essential for the quality control during long term stabilities and stockage conditions and important for the creation of new products with high antioxidant activity.

Literature:

Jung K, Richter J, Kabrodt K, Lucke IM, Schellenberg I, Herrling T. The antioxidative power AP--A new quantitative time dependent (2D) parameter for the determination of the antioxidant capacity and reactivity of different plants. Spectrochim Acta A Mol Biomol Spectrosc. 63(2006):846-50.

Jung K, Sacher M, Blume G, Janßen F, Herrling T. How Active are Biocosmetic Ingredients? SÖFW-Journal 133 1/2 – 2007.

**results:**

|                             | Antioxidative Power |                      |
|-----------------------------|---------------------|----------------------|
|                             | AP (AU)             | t <sub>r</sub> (min) |
| Pomegranate juice granavida | 2425                | 0,37                 |

All data are means affected by a confidential range of 5%.

To better range the test product, the following table lists the AP values and reaction times of some antioxidants, fresh fruits/vegetables, and juices:

|                           | AP (AU)   | Reaction time t <sub>r</sub><br>(min) |
|---------------------------|-----------|---------------------------------------|
| <b>examples</b>           |           |                                       |
| Ascorbic acid (vitamin C) | 1.000.000 | 0,24                                  |
| Tocopherol (vitamin E)    | 404.000   | 0,33                                  |
| Tocopherol acetate        | 0         | -                                     |
| Fresh fruits:             |           |                                       |
| Black currant             | 430       | 1,05                                  |
| Grapefruit                | 450       | 0,33                                  |
| Orange                    | 230-850   | 0,24                                  |
| Cherry                    | 220       | 0,80                                  |
| Tomato                    | 59        | 0,80                                  |
| Apple                     | 40        | 2,85                                  |
| Pomegranate               | 670       | 0,93                                  |

### Discussion

The Antioxidant Power determines the *activity* of antioxidants and radical scavengers. The higher the capacity of a test substance to neutralize free radicals is and the higher the reaction velocity is, the higher is the AP. The AP values are benchmarked against ascorbic acid (vitamin C) and expressed in Antioxidative Units (AU).

In comparison to fresh pomegranate fruit the granaVida juice showed higher antioxidative activity, both in terms of capacity and reactivity.

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In this study only one sample of granaVida juice has been analysed. Thus, differences between different batches, different storing conditions or different proceedings can not be evaluated.

Dr. Katinka Jung