ORAC and DPPH free radical scavenging values of Ester-C® and PureWay-C™

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SUMMARY

Background: In this study we have investigated the antioxidant and free radical scavenging activity of Ester-C® and PureWay-C™. The antioxidant and free radical scavenging capability were measured using the (ORAC) and (DPPH) methods.

Materials and Methods: The free radical scavenging activity of Ester-C® and PureWay-C™ were measured by the reduction of 1,1-diphenyl-2-picryl hydrazyl (DPPH) (purple) to 1,1-diphenyl-2-picryl hydrazine (clear). Ester-C® and PureWay-C™ scavenging of peroxyl radical oxygen reactive species (Oxygen Radical Absorbance Capacity or ORAC) were determined by fluorescence spectrophotometry.

Results: When compared to calcium ascorbate-calcium threonate-dehydroascorbate (Ester-C®), vitamin C-lipid metabolites (PureWay-C™) exhibited higher free radical scavenging activity by the DPPH method and also showed stronger antioxidant activity by the ORAC method. Here, we found that calcium ascorbate-calcium threonate-dehydroascorbate (Ester-C®) its IC50 value for DPPH radicals was (8.08 μg/ml), but that vitamin C-lipid metabolites (PureWay-C™) exhibited stronger activities, and its IC50 value for DPPH radicals was (5.58 μg/ml): The IC50 value represents the concentration of the test samples where the inhibition of the test activity reached 50%, or where 50% of the radicals were scavenged by the test samples. The vitamin C-lipid metabolites (PureWay-C™) demonstrated free radical scavenging activity of nearly 75% reduction of DPPH at 10 μg/ml; 10.47% more than Ester-C® (65.13%) reduction of DPPH at 10 μg/ml and oxygen radical scavenging of over 3782 μM (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalents per gram of PureWay-C™; 11.9% more than Ester-C® (3380 μM (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalents per gram of Ester-C®.

Conclusion: These data demonstrate that the vitamin C-lipid metabolites (PureWay-C™) are more potent antioxidant and have higher significant free radical scavenging capabilities than Ester-C®.

Keywords: Vitamin C, DPPH, ORAC

BACKGROUND

Vitamin C is an important dietary component which is required for physiological and metabolic activities including healthy neuronal development [1, 2], prevention of neurodegenerative diseases [3, 4], wound healing [1, 5, 6], and a healthy immune system [1, 7, 10]. More recently a formulation of vitamin C-lipid metabolites has been shown to more rapidly stimulate neurite outgrowth and fibroblast adhesion and to protect cells of the immune system when compared to all other vitamin C formulations [2]. This increased rate of bioactivity suggests an increase in the bioavailability and rate of cellular uptake of vitamin C-lipid metabolites when compared to other vitamin C formulations.

Here, we measure the antioxidant capability and free radical scavenging properties of vitamin C-lipid metabolites (PureWay-C™) when compared to calcium ascorbate-calcium threonate-dehydroascorbate (Ester-C®). We also confirm that the vitamin C-lipid metabolites (PureWay-C™) maintain and deliver both high and effective antioxidant and free radical scavenging activity.

MATERIALS AND METHODS

Materials

Formulations of Ester-C® (Lot No. 101-0604-016): (E2) and PureWay-C™ (792-060607-A1): (P2) were provided to ChromaDex Analytics Research & Development Boulder, CO.

DPPH and ORAC Assays

The DPPH assay was carried out as described by Vani *et al*., 1997 [9] with some modifications. Briefly, 200 µl of various concentration of ascorbic acid solution (1000 mg PureWay-C™ or Ester-C® in 50 ml ultra pure water) were mixed with 50µl of 0.659mM 2,2-diphenyl-1-picryl hydrazyl (DPPH) solution and incubated at 25°C for 20 minutes. The absorbance was then read at 510 nm. A control reaction was carried out without the test sample.

The ORAC assay was performed as described by Cao et al., 1993 [10]. Briefly, 2,2'-Asobix (2-amidinopropane) dihydrochloride (AAPH) (0.414 g) was dissolved in 10 ml of 75 mM phosphate buffer to final concentration of 153 mM and kept on ice. The fluorescein stock solution was prepared at 4.19×10^{-3} mM in 75 mM phosphate buffer and kept at 4 °C in the dark. For the (+/-)-6-Hydroxy-2,5,7,8- tetramethylchromane-2-carboxylic acid, (Trolox®) standard preparation, 0.25 g of Trolox® dissolved was in 50 ml of phosphate buffer to yield 0.02 M stock. Next, PureWay-C™ or Ester-C® were dissolved in acetone/water mixture (50-50) and subsequently diluted with 75 mM phosphate buffer (pH 7.4) to a varying extent to yield 200, 100, 50, 25, 12.5, and 6.25 µM for the test reactions. The ORAC assay detects free radical damage to the fluorescein

and a loss of fluorescence. Antioxidants inhibit the free radical range damage to the fluorescent compound and prevent the reduction in fluorescence. Reactions containing the PureWay-C™, Ester-C® and blanks (solvent) were run in parallel using equivalent amounts of a generator of a radical oxygen species and the fluoroscein and the area under the curve from the experimental sample was calculated. After subtracting the area under the curve for the blank, the resultant difference is expressed as antioxidant activity of the PureWay-C™ or Ester-C®. Results from different concentrations are compared with Trolox® and the ORAC results are expressed as Trolox® equivalents (TE) per gram of sample.

RESULTS

In order to confirm that the vitamin C-lipid metabolites (PureWay-C™) formulation has more potent antioxidant activities and has stronger free radical scavenging capabilities than Ester-C®. ORAC and PDDH evaluations were conducted. Figure1 shows that PureWay-C™ was able to scavenge 75.60% of the PDDH free radicals at 10μg/ml and Ester-C® was able to scavenge 65.13% of the DPPH at 10μg/ml (Table 1). Gallic acid, a known scavenger, was used as a positive control. PureWay-C™ shows a classic dose dependency scavenging of DPPH free radicals (Table 1). By reaching 75.60% scavenging capability, a 10.47% more than Ester-C®, is also indicative of PureWay-C™ as an excellent free radical scavenger [9].

In addition to free radical scavenging, the antioxidant capabilities of PureWay-C™ and Ester-C® were measured by the ORAC method and the values obtained were compared (Table 2). PureWay-C™ showed stronger antioxidant activity on a gram basis than Ester-C® (Figure 2. For example, PureWay-C™ has over 3782 units of antioxidant activity per gram, 11.9% more than Ester-C® (Table 2). While these comparisons are limited in value, it can be concluded that PureWay-C™ is an excellent dietary supplement due to its antioxidant capabilities.

DISCUSSION

Vitamin C is an important dietary component to ensure healthy physiological and metabolic activities such as the development of a healthy nervous system [1, 2]; prevention of neurodegenerative diseases [3, 4]; wound healing in vitro [5], and in vivo [6]; and protection of the immune system from xenobiotics [1, 7, 8].

Vitamin C is a chemical reducing agent (antioxidant) in many intracellular and extracellular reactions such as oxidative DNA and protein damage, low-density lipoprotein oxidation, lipid peroxidation, oxidants and nitrosamines in gastric juice, extracellular oxidants from neutrophils and endothelium-dependent vasodilation. PureWay-C™, which exhibited potent antioxidant and free radical scavenging effect in vitro, as well as stronger antioxidant activity and higher free radical scavenging activity than Ester-C®, can serve as a good dietary candidate for further evaluation of its bio-efficacies and molecular and biological mechanism in vitro, as well as in vivo, on anti-oxidation effects, and may provide efficient antioxidant protection to humans and animals

from oxidation products and processes that contribute to the pathogenesis of cancer, cardiovascular diseases, and other age-related diseases by cytotoxic, genotoxic and proinflammatory mechanism and atherosclerosis.

CONCLUSION

PureWay-C™ is an excellent antioxidant and free radical scavenger, and delivers more effective antioxidant and free radical scavenging activities than Ester-C®.

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Table1. DPPH values comparing the free radical scavenging activity of PureWay-C™ with Ester-C®.

Percent Inhibition on DPPH radicals

Concentration tested (µg/ml)	Ester-C®	PureWay-C™ .
1	5.86	5.86
2.5	20.20	17.69
5	28.79	41.15
7.5	42.09	63.14
10	65.15	75.60

Percent Inhibition on 50% of the radicals

	Ester-C®	PureWay-C™	Gallic acid
IC 50 µg/ml (95% confidence)	8.08	5.58	1.86

Free radical scavenging activity of calcium ascorbate threonate-dehydroascorbate (Ester-C®), and vitamin C-lipid metabolites (PureWay-C™) were quantitatively determinated using a DPPH assay. The inhibitory effects of the two test sample on DPPH radicals followed dose-dependent manner. While both vitamin C preparations stimulated a significant reduction in the number of DPPH free radicals, PureWay-C™ showed a greater reduction than Ester-C®.

The IC50 value represents the concentration of the test samples where the inhibition of the test activity reached 50%, or where 50% of the radicals were scavenged by the test samples.

Table2. ORAC values comparing the antioxidant activity of PureWay-C™ with Ester-C®.

Nutrient source	ORAC (μM TE/g)	Reference
Ester-C®	3380	Present study
PureWay-C™	3782	Present study

The ORAC values of PureWay-C™ and Ester-C® were measured three times and the average is presented. Here the ORAC values are presented in μM Trolox® Equivalents/gram of substance tested. ORAC values can often be expressed as per serving, or per volume, etc.

FIGURE LEGENDS

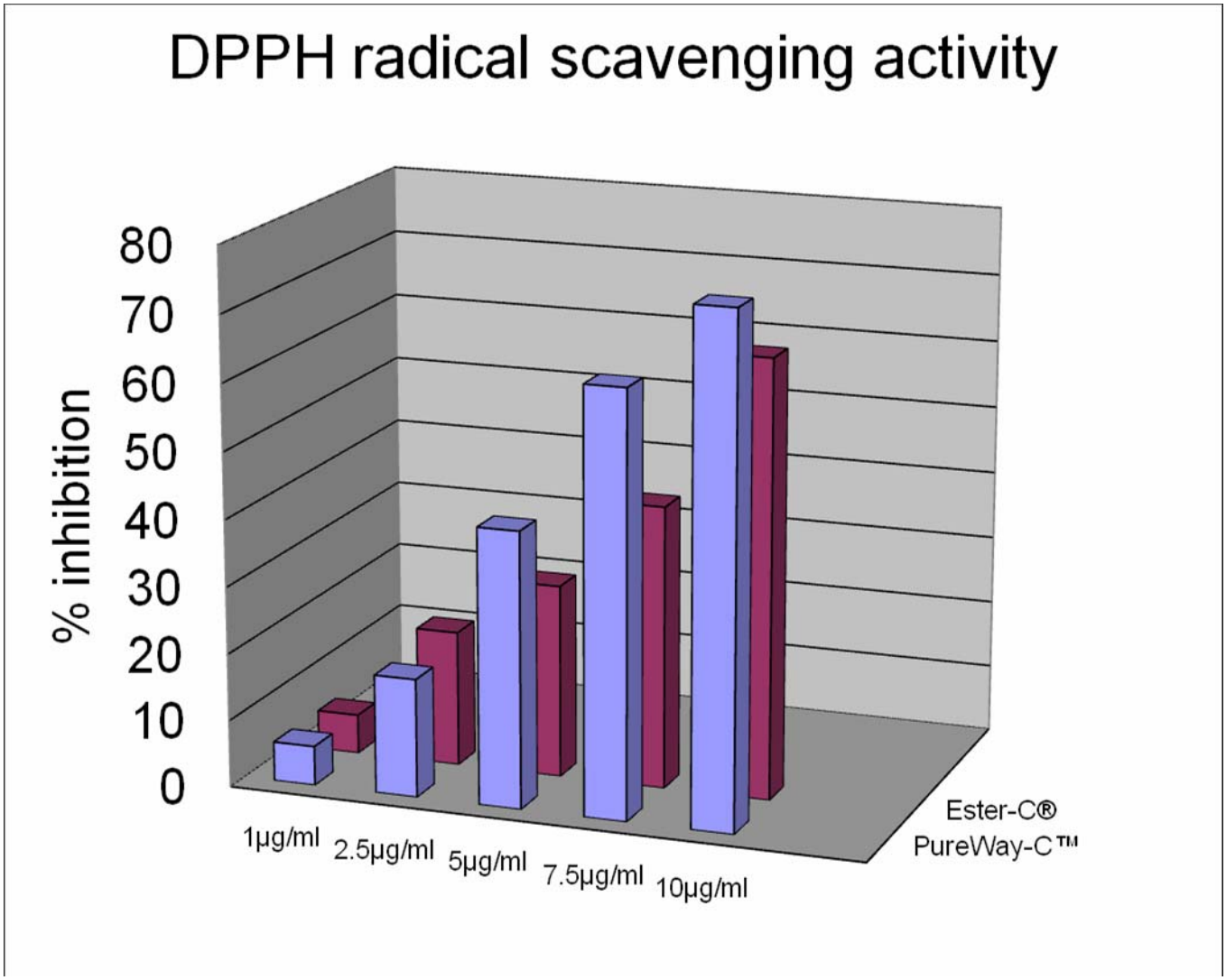

Figure1. Free radical scavenging activities of PureWay-C™ and Ester-C® measured using the DPPH assay. PureWay-C™ or Ester-C®, at concentrations ranging from 1 - 10 µg/ml, were mixed with 2,2-diphenyl-1-picryl hydrazyl (PDDH) solution and incubated at 25°C for 20 minutes. The absorbance was then read at 510 nm as described in the Materials and Methods section. A control reaction was carried out without the test samples. Each assay was performed in triplicate.

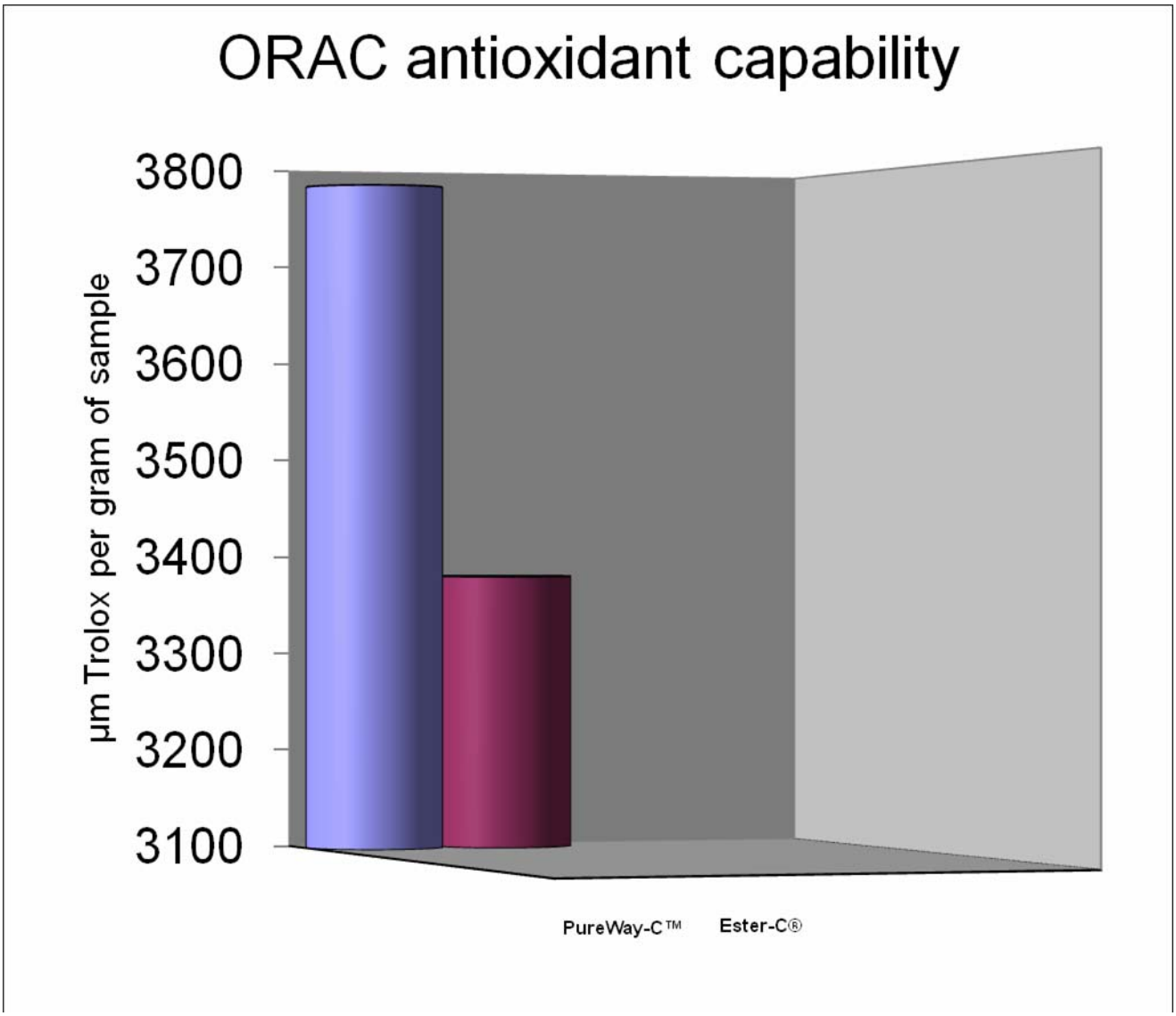

Figure2. The antioxidant capabilities of PureWay-C™ and Ester-C® measured using the ORAC assay. The PureWay-C™ and Ester-C® ORAC values were calculated using the regression equation between Trolox concentration and the net area under the curve (AUC) and are expressed as micromole Trolox equivalents per gram of sample.