Measurement Service

Analysis Report

BOC

REDOX TECHNOLOGIES

CUSTOMER DETAILS

Company: ChemCream, S.A.P.I. de C.V. Plaza
Marina Local D3 - D3, Marina Vallarta
Puerto Vallarta, Jalisco C.P. 48335, Mexico

SERVICE DETAILS

No. Report: 20220623001
Analysis request:
Total Antioxidant Capacity (TAC).
Hydrogen peroxide.
Redox potential.

SAMPLE DATA

No. Samples: 1

Sample ID : 140222

AETHEION ZCM65 Synergistic Skin Lotion 30mL

RECEPTION DATE: 2022/05/13

Sample preparation

Different media were used to solubilize the sample including Milli-Q water, 0.1 M PBS (phosphate buffer solution) pH 7.4, EtOH, MeOH and DMSO (dimethyl sulfoxide). The sample was insoluble in EtOH and MeOH but soluble in DMSO. Slurries were observed for Milli-Q water and PBS. The liquid fractions of the slurries, obtained after filtering with a glass microfiber filter 1.2 µm, were used for TAC assays. Clear filtrates were only obtained for sample slurries with concentrations ≤ 0.05 g/mL (**Figure 1**). Samples in Milli-Q water and PBS were prepared using two incubation (stirring) times at RT (30 min and overnight).

DMSO samples were also used for antioxidant analysis. These samples were prepared by dissolving 0.25 g of lotion in 1 mL of DMSO. After 30 min of stirring at RT, the samples were centrifuged at 5000 g for 5 min at RT.

The cosmetic sample was also highly soluble in the Non WaterBased Solution (NWBS) specially designed for measuring non water based samples with the eBQC-NI device.

ZCM65 lotion was shaking before sampling.

Metal instruments were nor used for sample preparation.

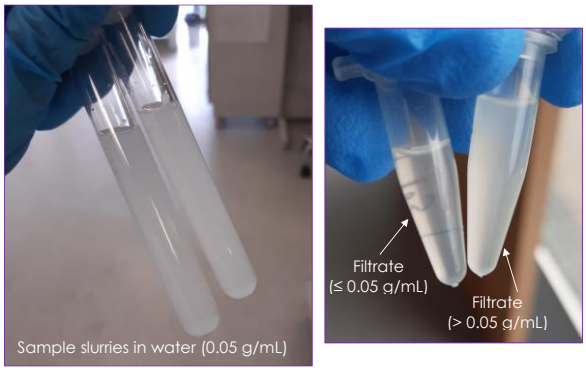

Figure 1. Sample slurries and filtrates in water.

ANALYSIS RESULTS: TAC/eBQC-NI

Assay: Total Antioxidant Capacity / eBQC-Natural Ingredients

Date: 2022/05/26

Analyzed by: María Díaz (PhD Project Leader)

Assay info:

eBQC-Natural Ingredients (e-BQC-NI) is a portable electrochemical device designed to determine the total antioxidant capacity of food samples, beverages, plant-based extracts and cosmetic samples. During the measurement, the sample is oxidized by applying a variable potential and the total antioxidant capacity is calculated using the voltammetric charge.

e-BQC-NI shows the total antioxidant capacity of the sample as "eBQC value".

e-BQC-NI results can be expressed as TEAC (Trolox Equivalents Antioxidant Capacity) by performing a standard curve with Trolox. Trolox is a water-soluble analogous of Vitamin E with very strong antioxidant activity. It is commonly used as standard or positive control in TAC assays.

Water Based and Non Water Based Solutions have been developed for measuring water based and non water based samples with the e-BQC- NI device. These solutions have been formulated to guarantee sample solubility without comprising the conductivity required for the electrochemical measurements.

RESULTS

Water Based (WBS) and Non Water Based (NWBS) NI solutions were tested to prepare the cosmetic sample. Due to low solubility of the sample in the WBS, the NWBS was used for the assay. The sample was dissolved in NWBS (0.25 g/mL) and centrifuged at 5000 g for 5 min at RT. The supernatant was then 1/10 diluted with NWBS for the analysis. The cosmetic sample was highly soluble in NWBS.

Table 1 shows the antioxidant capacity (eBQC value) obtained for control (NWBS) and cosmetic sample. eBQC-NI measurements were

ANALYSIS RESULTS: TAC/eBQC-NI

performed in triplicate. The eBQC measurements were registered after dropping 50 µL of the sample on the strip. The eBQC value recorded for orange juice is ten times the value registered for the diluted cosmetic sample.

Table 1. eBQC NI results for control and sample prepared in NWBS.

Sample		eBQC value	eBQC value (mean ± SD)
Sample	1	182	**176 ± 8**
	2	180	
	3	167	
NWBS	1	0	**0**
	2	0	
	3	0	

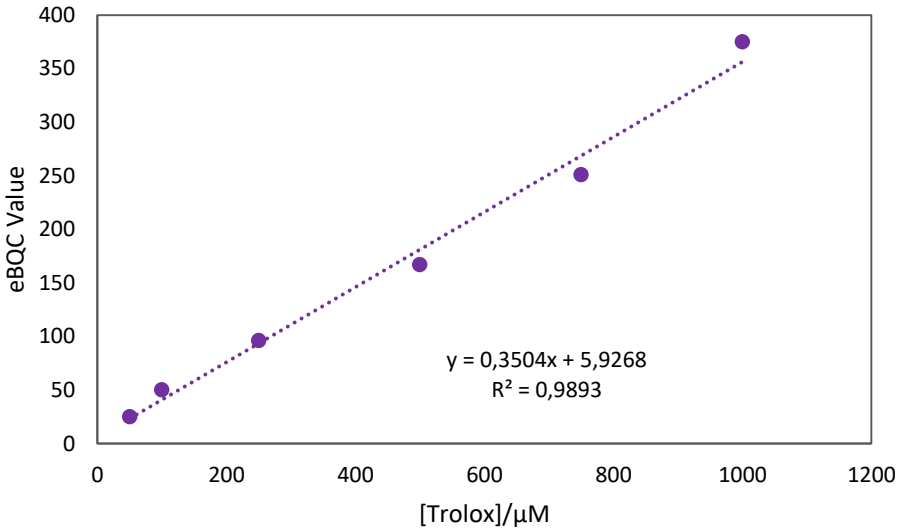

Figure 2. eBQC NI Trolox standard curve in NWBS.

A calibration curve using Trolox as standard (**Figure 2**) was performed in order to express eBQC-NI results as Trolox Equivalents Antioxidant Capacity (TEAC). The linear range for Trolox in NWBS was from 50 to 1000 µM.

TEAC value for the sample was calculated using the equation obtained from the linear regression of the standard curve. When working with diluted samples, TEAC values were multiplied by dilution factor to calculate the antioxidant capacity in the original sample.

ANALYSIS RESULTS: TAC/eBQC-NI

The TEAC value calculated for the cosmetic sample was **4863 ± 230 µM Trolox** (**19 ± 1 µM Trolox/g cream**).

Cosmetic samples prepared in Milli-Q water, 0.1 M PBS pH 7.4 and DMSO were also tested with the e-BQC-NI device. The liquid fractions of the slurries (water and PBS) obtained after filtering with glass microfiber filters (1.2 µm) were used for the assay. Water samples must be 1:2 diluted in WBS before performing the electrochemical measurement due to their low conductivity. The sample prepared in DMSO became turbid when dissolved in WBS and it was discarded for the assay.

Table 2 shows the antioxidant capacity (eBQC value) obtained for control and cosmetic samples prepared in water and PBS (0.05 g/mL) after 30 min of stirring. eBQC-NI measurements were performed in triplicate. The eBQC measurements were registered after dropping 50 µL of the sample on the strip. TEAC values calculated for the samples were < 50 µM Trolox and did not fall in the linear range of the standard curve (**Figure 3**, 50-1000 µM Trolox). Therefore, the antioxidant activity of these filtrates was very low. An increase in the antioxidant activity was not observed in the filtrates obtained after overnight stirring the samples in Milli-Q water or PBS.

Table 2. eBQC NI results for control and cosmetic samples prepared in Milli-Q water and PBS (30 min stirring).

Sample		eBQC value	eBQC value (mean ± SD)
Sample/Water	1	29	**30 ± 2**
	2	32	
	3	30	
Sample/PBS	1	23	**27 ± 3**
	2	25	
	3	30	
WBS	1	0	**0**
	2	0	
	3	0	

ANALYSIS RESULTS: TAC/eBQC-NI

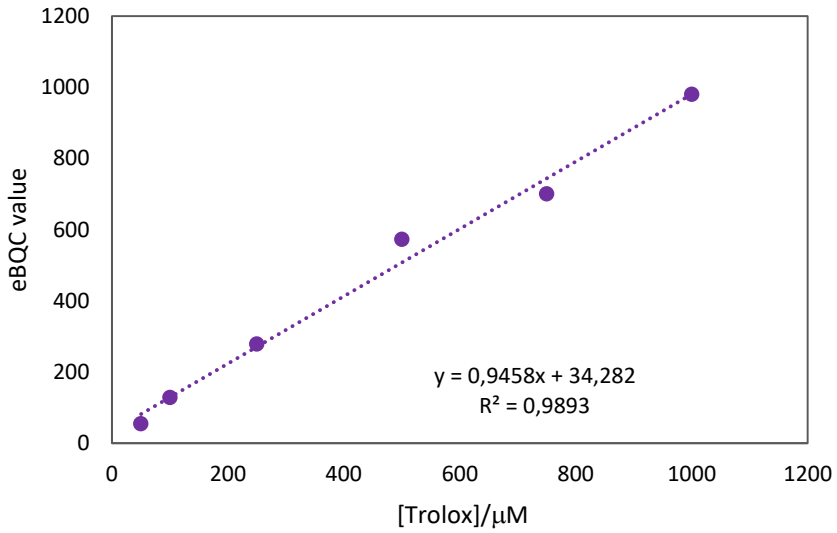

Figure 3. eBQC NI Trolox standard curve in WBS.

ANALYSIS RESULTS: TAC/Sectrophotometric assays

Assay: Total Antioxidant Capacity

KF01007 DPPH Assay Kit

KF01002 ABTS Assay Kit

KF01003 FRAP Assay Kit

KF01001 DMPD Assay Kit

Date: 2022/06/09

Analyzed by: Daniel Bayón (R&D Technician) and María Díaz (PhD Project Leader)

Assay info:

KF01007 DPPH Assay Kit

This TAC Assay Kit is based on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. In this method, the DPPH free radical (DPPH$^{\bullet}$), which is a deep purple colored (λ_{max} = 517 nm) stable organic nitrogen radical, is reduced by antioxidants to the colorless DPPH reduced form. Therefore, the absorbance decrease at 517 nm depends linearly on the antioxidant concentration. The synthetic antioxidant Trolox is used to standardize the sample TAC relative to Trolox (Trolox Equivalents Antioxidant Capacity, TEAC).

KF01002 ABTS Assay Kit

This TAC Assay Kit is based on the interaction between antioxidants and the pre-formed green–blue stable radical cationic chromophore, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate, ABTS$^{\bullet+}$). In the presence of antioxidants, the oxidized ABTS$^{\bullet+}$ radical is reduced to ABTS resulting in a discoloration of the solution, measured by the decrease in absorbance at 734 nm. Antioxidants scavenge ABTS$^{\bullet+}$ radical cation in a concentration dependent manner which correlates with a proportional decrease in color intensity. Trolox can be used to standardize the sample TAC relative to Trolox (Trolox Equivalents Antioxidant Capacity, TEAC).

ANALYSIS RESULTS: TAC/Spectrophotometric assays

KF01003 FRAP Assay Kit

The FRAP (Ferric Reducing Antioxidant Power) TAC Assay Kit is based on the reduction of Fe^{3+} to Fe^{2+} by antioxidants. This kit measures the antioxidant activity of compounds that are able to act as reductants. In the assay, at an acidic pH, the antioxidants present in the sample reduce a colorless Fe^{3+} complex to a blue colored Fe^{2+} complex which shows a maximum of absorbance at 593 nm. Absorbance recorded at 593 is, therefore, directly proportional to the total antioxidant capacity. The antioxidant potential of samples is determined based on an iron standard curve (iron standard provided with the kit) and results are expressed as Fe^{2+} equivalents (µM) or FRAP value.

KF01001 DMPD Assay Kit

The DMPD (N,N-dimethyl-p-phenylenediamine) TAC assay kit is based on the interaction between antioxidants and the pre-formed purple stable radical cation $DMPD^{\bullet+}$. At acidic pH and in the presence of a suitable oxidant solution $DMPD^{\bullet+}$ can be easily generated from DMPD. In the presence of hydrogen-donating antioxidants, the $DMPD^{\bullet+}$ radical is reduced to DMPD resulting in a discoloration of the solution, measured by the decrease in absorbance at 553 nm. Antioxidants scavenge $DMPD^{\bullet+}$ radical cation in a concentration dependent manner which correlates with a proportional decrease in color intensity. The synthetic antioxidant Trolox (included in the kit) is used to standardize the sample TAC relative to Trolox (Trolox Equivalents Antioxidant Capacity, TEAC).

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