Validation Test Report

Total Antioxidants Potential & Energy Capacity (TAPEC) measurement for Beverage's Antioxidants

Dr. Chua Chee Yong

Principle consultant
Eliddell BioTechnology Consultancy

Aug 2021

Total Antioxidants Potential & Energy Capacity (TAPEC) measurement for Beverage's Antioxidants

Author: Dr Chua Chee Yong

ABSTRACT:

The effect of Antioxidants in inhibiting free radicals generated endogenously or exogenously and their presence in some beverages are well known. There are some chemical methods such as Oxygen Radical Absorbance Capacity (ORAC), developed to define the antioxidant capacity of solid foods and aqueous solution. ORAC method uses a complex chemical array and custom designed optical instruments to arrive at their ORAC figure. Currently, there is an absence of comprehensive and easy measurement method to quantify a beverage's antioxidant capacity and its neutralizing strength. Hence, a Total Antioxidants Potential & Energy Capacity (TAPEC) method is developed to provide a unique and easy to use method to determine the strength and capacity of the antioxidants commonly present in beverages. TAPEC uses three basic references namely, Oxidation Reduction Potential (ORP) of pure water, standard oxidants using one liter of hypochlorous acid of TRO 0.2 mg/l, and standard antioxidant using Ascorbic acid (Vitamin C) as standard with concentration of 800mg in 100ml of pure water to establish the basic TAPEC scalar reference value. The various antioxidants content commonly present in the beverages are then tested, measured, and quantified by their respective TAPEC values with reference to the basic scalar value of standard antioxidant. TAPEC zero value represents nil strength of antioxidant with reference to the analogy of pure water with the pH value 7 as neutral solution. Using this measurement method, the TAPEC value per 100 ml of common beverage's antioxidant neutralization strength such as tea, coffee, orange juice, whisky, red wine, and entropy treated water were determined as 267, 148, 30, 11, 206 and 160, respectively. When tea, coffee, orange juice, whisky and red wine were treated by the entropy energy method, their TAPEC value per 100ml were increased to 400, 167, 91, 23 and 234, respectively.

KEY WORDS: TAPEC, Antioxidizing Strength Measurement, Beverage, Antioxidant, ORP Shift, TRO, Vitamin C

INTRODUCTION

There are many types of everyday beverages like drinking water, milk, coffee, tea, juice etc. Alcoholic beverages include low alcohol content wine, beer and high alcohol content whisky, brandy, and other liquors. Nowadays, consumption of beverages is not just to quench the thirst. Higher standards of living and wellness awareness has driven it beyond thirst-quenching to promoting better health and enjoyment, and many beverage producers are now promoting the health benefits of the antioxidant content in their drinks.

Due to the wide variations in the types and concentration of antioxidants content in different types of drinks, it becomes very difficult to align the claims on the actual antioxidant effects. More complex is the individual antioxidizing strength of different types of antioxidants together with their influence or combined interaction effects with other constituents in the drinks.

Primarily, the main health beneficial function of antioxidant is to neutralize the Reactive Oxygen Species in human body which can be produced by human body internally, or from external sources through ingestion or in contact with the environment.

Ideally, the preferred antioxidant measurement method or procedures must quantify the overall antioxidizing strength of the beverages by a scalar value to represent the strength of all the antioxidant contents in neutralizing a common Reactive Oxygen Species ROS. This value must encompass factors such as representative to the overall strength in neutralizing ROS, antioxidizing strength per 100 ml drink including all the antioxidant effects of constituents and interaction with other contents, and simple scalar value easy for users to understand the limits and control their own antioxidant consumption.

Considering all the factors mentioned above, Total Antioxidant Potential & Energy Capacity (TAPEC) as a beverage's antioxidants strength measurement method is developed to meet all the above requirements. This TAPEC measurement method and its scalar value provide a common platform for the industries and individual to align antioxidant strength capacity standard in a consistent way.

Antioxidant is well known to inhibit free radical or reactive oxygen species (ROS) generated endogenously (derived internally in human body) or exogenously (from external sources). Myke-Mbata et al (2018) defined the antioxidant effect as inhibiting oxidation of biomolecules by free radicals / reactive oxygen species (ROS). Free radicals are highly reactive to nucleic acids, lipids, and protein, leading to the abnormality in cellular metabolism, or cell death detrimental to health. Rafael Radi (2018) had also detailed the Redox pathways of hazardous excessive oxidation in cell and tissue components. These aspects of human redox molecular biochemistry, and its relationships to diseases and aging, lead to the development of preventive and therapeutic strategies of using antioxidant and its applications.

Since the neutralization of oxidative stress caused by antioxidant involved the transfer of electron and changes of redox state, the Oxidation-Reduction Potential (ORP) is therefore an important measurement to determine the oxidizing or reducing nature of the solution (APHA, 2002). A negative shift of the ORP mV value of a solution indicates the increased antioxidizing or reduced oxidizing tendency of a solution with reference to a standard reference which is marked as 0 mV ORP (ORP value reference to the Ag/AgCl reference cell is mostly used in ORP meters available in the market). In application to define an antioxidant state, for simplicity to consumers, common conservative practice in the market is to ensure ORP value read by OPR meter using Ag/AgCl reference cell to be negative (ORP < 0).

Se-Yeong Lee et al (2004) had developed the ORP methods for the measurement of antioxidant tendency as a simple and accurate method without the use of complex procedures and pretreatment. It can be reliably measured in nearly all aqueous solutions and generally not subject to interference from solution color, turbidity, colloidal matter, and suspended matter (ASTM, 2000).

In this development of Beverage antioxidants capacity strength measurement testing, Oxidation Reduction Potential (ORP) is a precursor parameter directly tied with the antioxidizing effect of an aqueous solution to neutralize free radicals, therefore ORP measurement is adopted in this development testing.

The ORP is the electromotive force, E_m, developed between a noble metal electrode and a standard reference electrode. This ORP is related to the solution composition by

$E_m = E^0 + 2.3\ RT/nF\ (\log A_{ox}/A_{red})$

Where E_m = ORP, E^0 = constant that depends on the choice of reference electrodes, F= Faraday constant 96485 C/ gmol, R = 8.314 Nm/gmol K, T = absolute temperature K, n = number of electrons involved in process reaction, A_{ox} and A_{red} = activities of the reactants in the process.

The ORP meter reference electrode used in TAPEC test is based on Ag/AgCl half-cell, the 0 mV reference of ORP meter refers to the same oxidation and reduction potential status as Ag/AgCl reference cell. However, this 0 mV reference point is not the true neutral REDOX (oxidation and reduction) state of the beverage. Beverage's neutral state shall be based on pure water which is used as a solvent in all REDOX lab tests. Pure water is neither oxidative nor reductive in the human body biological redox reactions. ORP mV reading of Pure water as measured by an ORP meter is therefore used as the true zero reference point for neutral water. The commonly acceptable water quality parameters, namely, pH, conductivity, and Total Residual Oxidants (TRO) measurement can also be applied to further define and specify the pure water quality that established as the zero point of the TAPEC scale. In addition, selection of appropriate compound as standard oxidant for the evaluation of neutralization strength of various beverages' antioxidant is equally important as establishing the TAPEC zero value. It needs to be representative and comparable to the level of reactive oxygen species redox activity in human bodies and representative to the beverage intake. Hydrogen peroxide as an oxidant is highly unstable under the ambient environment, and stabilizer is usually present in low concentration Hydrogen peroxide available in the market. Thus, its redox state has been altered and not suitable to use as standard oxidant. Another plausible way is the use of low concentration hypochlorous acid (HOCl) as a standard oxidant for TAPEC redox test. They are easily available and widely present in water supply sources which is used in beverages making process and for household consumption.

When utilities water is supplied from water treatment plant to the household tap, a small amount of disinfectant is usually added (eg chlorine gas, active oxidative substances) to ensure the transported water is free from disease causing bacteria. Chlorine gas (Cl_2) and water react to form hypochlorous acid (HOCl) and hydrochloric acid (HCl). The HOCl dissociates into hypochlorite ion (OCl^-) and hydrogen ion. This dissociation of HOCl is incomplete at pH 6.5 to 8.5. Both HOCl and OCl^- species are present to some extent. As the germicidal effect of HOCl is much higher than the of OCl^-, chlorination at lower pH is preferred. The HOCl and OCl^- species which are extremely reactive with the cell components of numerous microorganisms. Linders J, et al (2019) had included transient oxidants from oxidation process as active substance, and their derivatives as Total Residual Oxidants (TRO). Hence, TRO value is used to measure the residual concentration of this active substance in the water supply transmission network for domestic consumption.

Hypochlorite is also one of the most common external reactive oxygen species (ROS) ingested by human body. Sam C.H. and Lu HK (2009) stated in their review article that Hypochlorous acid (HOCl), as one of the reactive oxygen species (ROS)

released by neutrophils in periodontal pockets and have a role in regulating inflammation and healing in destroyed gingival and periodontal tissues.

Ahsan UK and Michael K (1994) had revealed the evolution of single molecular oxygen (singlet spin state dioxygen) in the study of the pH profile of the decomposition of aqueous hypochlorite. This observation had also suggested for the mechanisms of redox reactions involving H_2O_2 and singlet oxygen species in both chemical and biological systems.

The $^1O_2$* singlet oxygen which is the strong reactive oxygen species produced when hypo reacts with H_2O_2.

$$HOOH + OCl^- \dashrightarrow {}^1O_2{}^* + HOH + Cl^-$$

In hypochlorite hydrolysis, H_2O_2 is produced with H^+ and Cl^-. When under acidic condition, singlet oxygen $^1O_2$* (ROS) is produced. It is a free radical and sometimes it is denoted as [O].

It is therefore appropriate to use hypochlorite as standard oxidant yet representative for the beverages we consume daily also representative to the type of ROS relevant to our body.

In beverages' TAPEC antioxidant neutralization strength tests, it is also important to select the standard oxidant's concentration at a reasonable level which is representative of the reactive oxygen species concentration in human. Hence, the level of the chosen standard oxidant - hypochlorous acid (HOCl) is fixed at TRO 0.2 ppm (mg/L). A One-liter amount of this level of hypochlorous acid solution is used for the redox tests to increase the sensitivity of this test.

For the development of Total Antioxidant Potential & Energy Capacity (TAPEC) beverage antioxidants strength measurement method, the selection of the representative type of antioxidant associated to human health is critical for defining the measurement. This chosen antioxidant should be commonly acknowledged for its antioxidant effect and widely used in the community for health benefits. Ascorbic acid (Vitamin C) with a chemical formula of $C_6H_8O_6$ is commonly found in fresh fruits and vegetables and widely consumed by humans in the human history. Robert BG et al (2001) has given a detailed account for the human historical discovery of this ascorbic acid functions way back to 1628. In 1925, researchers in London have isolated antiscorbutic activity from a crude fraction of lemon and using animal assays demonstrated that the activity was destroyed by oxidation and protected by reducing agents. Ascorbic acid (Vitamin C) is an essential nutrient involved in the repair of tissue and the enzymatic production of certain neurotransmitters. It also functions as an antioxidant. Humans, unlike most animals, are unable to synthesize ascorbic acid (vitamin C) endogenously, so it is an essential dietary component. Hence, selecting the ascorbic acid as the representative antioxidant for TAPEC measurement scale is ideal. The upper vitamin C intake dosage for adult (19 years old and above) as stated in the US National Institutes of Health (2016) is 2000 mg per day. Typically, ascorbic acid manufacturer recommends consumption using a concentration of 800mg ascorbic acid in 100ml of water.

MATERIAL AND METHODS:

Reagents Used

The high purity water was obtained from a Milli-Q system (Milli-pore Corporation, Australia) with a resistivity of 18.2 MΩ-cm and less than 50 μg/L of organic carbon content. The chemicals/reagents used in this test include sodium hypochlorite (The Clorox Company, USA), 100% pharmaceutical grade Ascorbic Acid L-ascorbic acid (NutriBiotic, USA). The reference Oxidant solution is prepared by addition of 8 μL sodium hypochlorite in 1000mL of high purity water.

Liquid Solution Analysis

The various water quality parameters were determined using the standard methods (APHA, 1998). The ORP measurement was carried out by using Lutron YK-23RP ORP meter and the ORP-14 electrode. A Thermo Scientific EUTEH pH 150 meter with a pH probe was used for water pH value measurements. The Digital Themo sensor probe (for *in-situ* temperature measurement) of the Themo Scientific meter was immersed in the various test solution. The Hach DR 900 colorimeter (HACH USA) was used in the determination of the various water sample TRO value in ppm. The TRO water quality parameter was determined using the methods outlined in ISO 7393-2 (2017). For the electrical conductivity measurement for the micro volume sample, the HORIBA (Horiba, Japan) Compact conductivity meter EC 22 was used.

Entropy Treatment Process

The Entropy unit (Ecospec NovelTech, 480 W, water chamber platinum emitter with 1 liter capacity, as shown in Figure 1) was turned on and water was filled in the water tank. Water chamber at the touch screen panel was selected and activated the Entropy treatment by pressing the start button. Treated water was collected in the beaker by pressing the dispense button. This treated water was then used for testing purposes.

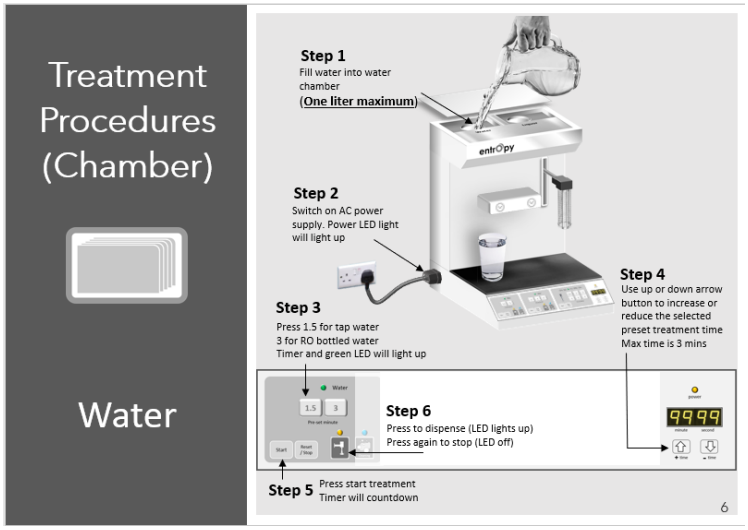

Figure 1 Entropy Treatment Unit

Entropy treatment is done for other beverages using probe to treat for one minute and used for testing purposes.

Beverage's Antioxidants Neutralization Strength Tests

The neutralization strength tests were conducted to determine the volume of the various beverages needed to neutralize one liter of standard oxidant (0.2ppm TRO hypochlorous acid) by shifting the solution ORP to pure water ORP value. The volume of the standard antioxidant (800mg/100ml concentration ascorbic acid) required to neutralize one liter of standard oxidant (TRO 0.2 HOCl solution) is first determined. To determine the TAPEC value of other beverages, the test beverage is titrated to neutralize the same one liter of standard oxidant. The ratio of the titrated volume of the test beverage to the volume needed by the standard antioxidant (ascorbic acid) is then multiplied by 1000 - the assigned TAPEC value per 100 ml of ascorbic acid with concentration of 800mg/100ml water. The resulting value is the TAPEC value per 100 ml of the respective test beverage's antioxidants value. The process of neutralizing the beverage's antioxidant against the standard oxidant solution is a redox titration process except that the end point is the ORP value of the pure water.

100ml of the standard antioxidant (ascorbic acid), tea, coffee, orange juice, entropy treated water, entropy treated tea, entropy treated coffee and entropy treated whisky were prepared and filled in the various glass beakers. Their respective water quality parameters (pH, Temperature, conductivity and ORP) were measured. One liter of pure water was prepared and its water quality parameters including TRO were obtained. A few One-liter standard oxidant was prepared by adding the known ml of sodium hypochlorite to obtain the 0.2ppm TRO concentration. Its water quality parameters were also measured. The respective redox process ORP values were then obtained when the gradual volume of that beverage was added. The volume of the various beverage's antioxidant required to arrive at the neutralization end point of pure water ORP shall be determined through the redox ORP titration curve against volume plot. The time response for each beverage's antioxidant gradual addition points including the redox end point will vary due to differences in the mechanism of antioxidant effect. This time response for the chemical-based mechanism will take a few minutes. For energy-based mechanism, it will take hours to a day.

RESULTS AND DISCUSSION:

The Liquid Quality Parameters for Various Standard and Test Solutions

The respective parameters measured for various standard and test solutions used in this beverage's antioxidants neutralization strength tests are tabulated in the two tables below:

Parameter	Pure water 1000 ml	Standard oxidant solution (HOCl) 1000ml	Reference antioxidant (Ascorbic acid) 100 ml
pH / temp.	7.45 / 23.0°C	6.86 / 22.9°C	2.29 / 22.8°C
Conductivity, µS/cm	3	5	533
ORP, mV	+375	+550	+175
TRO, ppm	0.0	0.2	-

Parameter	Tea 100 ml	Coffee (black) 100ml	Orange Juice 100ml	Whisky 100ml	Red wine 100ml	Entropy treated water 100ml
pH / temp.	4.93 / 34.2°C	5.01 / 35.5°C	3.71 / 22.5°C	3.86 / 20.6°C	3.25 / 25.2°C	8.00 / 25.5°C
Conductivity, µS/cm	593	1430	3040	34	2280	131
ORP, mV	+194	+71	+135	+270	+51	-302
TRO,ppm	-	-	-	-	-	-

Parameter	Entropy treated Tea 100 ml	Entropy treated Coffee (black) 100ml	Entropy treated Orange Juice 100ml	Entropy treated whisky 100ml	Entropy treated red wine 100ml
pH / temp.	4.98/ 27.6°C	4.94 / 38.0°C	3.72 / 26.1°C	3.97/ 23.5°C	3.27/ 27.7°C
Conductivity, µS/cm	469	1448	3470	32	2260
ORP, mV	-158	-115	-220	-134	-248
TRO,ppm	-	-	-	-	-

The solution quality parameters of pure water demonstrated that pure water contains no oxidant as TRO value is zero, and 3 µS/cm conductivity shows no other dissolved solids. Its ORP value is +375mV vs Ag/AgCl reference cell and this value should be taken as neutral status relevant to the human metabolic redox process. The standard oxidant (HOCl) solution ORP value was +550 mV which is +175 mV more positive than pure

water. Readings more positive than the +375 mV value also indicate that it is an oxidant as compared to pure water. For ascorbic acid, it is 200mV **more negative than pure water** indicating that it acts as antioxidant compared to pure water. The more negative ORP mV readings also observed for **untreated** tea, coffee, orange juice, whisky, red wine, and entropy treated water. Comparing with pure water of +375mV ORP, these untreated beverages are more negative than pure water with ORP magnitude of **-181mV, -304mV, -240mV, -105mV, -324mV and -680mV**, respectively. For the entropy treated beverages, their trend of the ORP shift with respect to the neutral state of pure water are similar. For example, Entropy treated coffee has an ORP of -115mV, which is -490mV more negative than pure water of +375mV (-115mV – (+375mV) = -490mV). This -490mV negative shift of Entropy treated coffee is more than the untreated coffee negative shift of -304mV (+71mV – (+375mV) = -304mV). For other Entropy treated beverages, the similar negative ORP shift magnitude with reference to pure water have the same trend. This is due to the vibrational energy is being imparted to the beverage and stored as the usable energy for the antioxidant effect.

The ORP changes with the gradual volume addition of Ascorbic Acid

The measured ORP changes with the gradual addition of Ascorbic Acid to One-liter standard oxidant solution are tabulated as shown below:

Volume of Ascorbic acid added, ml	Solution pH/Temperature	Solution ORP, mV	Solution Conductivity. µS/cm
0.0	6.86/ 22.9°C	+550	5
0.7	4.71 / 22.7°C	+200	12
0.9	4.38 / 22.5°C	+176	12
1.1	4.29 / 22.5°C	+167	14
1.3	4.28 / 22.5°C	+168	13
1.5	4.29 / 22.4°C	+167	13

The test results showed that with the addition of the ascorbic acid, the solution shifts toward more reducing mV and reached the ORP value of +167mV with the addition of 1.5ml ascorbic acid (standard oxidant total ORP shift is - 383mV). It only required 0.4 ml of the ascorbic acid solution to neutralize One-liter standard oxidant of 0.2ppm TRO HOCl solution.

The ORP changes with the gradual volume addition for Tea

The measured ORP changes with the gradual addition of Tea to One-liter standard oxidant solution are tabulated as shown below:

Volume of Tea added, ml	Solution pH/Temperature	Solution ORP, mV	Solution Conductivity. µS/cm
0.0	6.86 / 22.9°C	+550	5
0.5	5.94 / 23.0°C	+536	6
1.0	5.73 / 23.0°C	+478	7
1.5	5.71 / 23.0°C	+368	7

The test results showed that with the addition of tea, the solution shifts toward reducing potential and reached the ORP value of +368mV with the addition of 1.5ml (standard oxidant ORP shift of -182 mV). It required 1.5 ml of the tea to neutralize One-liter standard oxidant of 0.2ppm TRO HOCl solution.

The ORP changes with the gradual volume addition of Coffee

The measured ORP changes with the gradual addition of coffee to One-liter standard oxidant solution are tabulated as shown below:

Volume of coffee added, ml	Solution pH/Temperature	Solution ORP, mV	Solution Conductivity. µS/cm
0.0	6.86 / 22.9°C	+550	5
0.5	5.90 / 23.0°C	+548	6
1.0	5.85 / 23.0°C	+535	7
1.5	5.79 / 23.0°C	+480	9
2.0	5.67 / 23.0°C	+441	9
2.5	5.57 / 23.0°C	+378	9
3.0	5.57 / 23.0°C	+351	11

The test results showed that with the addition of coffee, the solution shifts toward reducing potential and reached ORP value of +351mV with the addition of 3.0ml (standard oxidant ORP shift of -199 mV). It required 2.7ml of the coffee solution to neutralize One-liter standard oxidant of 0.2ppm TRO HOCl solution.

The ORP changes with the gradual volume addition of orange juice

The measured ORP changes with the gradual addition of orange juice to One-liter standard oxidant solution are tabulated as shown below:

Volume of orange juice added, ml	Solution pH/Temperature	Solution ORP, mV	Solution Conductivity. µS/cm
0.0	6.86 / 22.9°C	+550	5
1.0	4.64 / 23.0°C	+545	43
2.0	4.37 / 22.9°C	+519	61
3.0	4.18 / 22.9°C	+497	64
4.0	4.15 / 22.9°C	+476	68
5.0	4.08 / 22.9°C	+459	72
6.0	4.01 / 22.9°C	+444	76
7.0	4.05 / 22.9°C	+432	81
8.0	4.02 / 22.9°C	+421	85
9.0	3.99 / 22.9°C	+411	90
10.0	3.97 / 22.9°C	+398	93
11.0	3.97 / 22.9°C	+397	96
12.0	3.97 / 22,9°C	+387	101
13.0	3.96 / 22.9°C	+383	103
14.0	3.93 / 22.9°C	+370	109

The test results showed that with the addition of the orange juice, the solution shifts toward reducing potential and reached the ORP value of +370mV with the addition of 14.0ml (standard oxidant ORP shift of -180 mV). It required 13.5 ml of the orange juice to neutralize One-liter standard oxidant of 0.2ppm TRO HOCl solution

The ORP changes with the gradual volume addition of Whisky

The measured ORP changes with the gradual addition of whisky to One-liter standard oxidant solution are tabulated as shown below:

Volume of Whisky added, ml	Solution pH/Temperature	Solution ORP, mV	Solution Conductivity. µS/cm
0.0	6.86 / 25.0°C	+550	5
2.5	6.63 / 25.1°C	+552	6
5.0	6.81 / 25.1°C	+531	7
7.5	6.67 / 25.1°C	+531	8
10.0	6.35 / 25.1°C	+520	8
12.5	6.26 / 25.1°C	+517	9
15.0	5.96 / 25.1°C	+520	10
17.5	5.52 / 25.1°C	+506	11
20.0	5.32 / 25.1°C	+491	13
22.5	5.18 / 25.1°C	+479	14

25.0	5.06 / 25.1°C	+462	15
27.5	4.83 / 25.1°C	+445	15
30.0	4.67 / 25.1°C	+426	15
32.5	4.61 / 25.1°C	+405	15
35.0	4.43 / 25.1°C	+391	16
37.5	4.43 / 25.1°C	+379	16
40.0	4.32/ 25.1°C	+369	16

The test results showed that with the addition of the whisky, the solution shifts toward reducing potential and reached the ORP value of +369mV with the addition of 40.0ml (standard oxidant ORP shift of -181 mV). It required 37.6 ml of the orange juice to neutralize One-liter standard oxidant of 0.2ppm TRO HOCl solution

The ORP changes with the gradual volume addition of red wine

The measured ORP changes with the gradual addition of red wine to One-liter standard oxidant solution are tabulated as shown below:

Volume of red wine added, ml	Solution pH/Temperature	Solution ORP, mV	Solution Conductivity. µS/cm
0.0	6.86 / 25.0°C	+550	5
0.5	4.75 / 25.3°C	+490	28
1.0	4.40 / 25.3°C	+448	30
1.5	4.03 / 25.3°C	+404	32
2.0	3.99 / 25.3°C	+371	33

The test results showed that with the addition of red wine, the solution shifts toward reducing potential and reached ORP value of +371mV with the addition of 2.0ml (standard oxidant ORP shift of -179 mV). It required 1.94 ml of the red wine solution to neutralize One-liter standard oxidant of 0.2ppm TRO HOCl solution.

The ORP changes with the gradual volume addition of Entropy treated water

The measured ORP changes with the gradual addition of Entropy treated water to ~~the~~ One-liter standard oxidant solution are tabulated as shown below:

Volume of Entropy treated water	Solution pH/Temperature	Solution ORP, mV	Solution Conductivity. µS/cm

added, ml			
0.0	6.86 / 22.9°C	+550	5
0.5	6.99 / 23.3°C	+558	7
1.0	6.99 / 23.3°C	+565	9
1.5	6.86 / 23.3°C	+520	11
2.0	6.92 / 23.7°C	+432	12
2.5	6.78 / 23.6°C	+376	15
3.0	6.62 / 23.0°C	+320	16

The test results showed that with the addition of the entropy treated water, the solution shifts toward slight oxidating then followed by rapid negative shift and reached the ORP value of +320mV with the addition of 3.0ml (standard oxidant ORP shift of -230 mV). It required 2.5 ml of the entropy treated water solution to neutralize One-liter standard oxidant of 0.2ppm TRO HOCl solution.

The ORP changes with the gradual volume addition of Entropy treated tea

The measured ORP changes with the gradual addition of Entropy treated tea to One-liter standard oxidant solution are tabulated as shown below:

Volume of entropy treated tea added, ml	Solution pH/Temperature	Solution ORP, mV	Solution Conductivity. µS/cm
0.0	6.86 / 22.9°C	+550	5
0.5	5.73 / 22.5°C	+451	5
1.0	5.46 / 22.5°C	+376	5
1.5	5.49 / 22.4°C	+342	5

The test results showed that with the addition of the entropy treated tea, the solution shifts toward reducing potential and reached the ORP value of +342mV with the addition of 1.5ml (standard oxidant ORP shift of -208 mV). It required 1.0 ml of the entropy treated tea solution to neutralize One-liter standard oxidant of 0.2ppm TRO HOCl solution.

The ORP changes with the gradual volume addition of Entropy treated coffee

The measured ORP changes with the gradual addition of Entropy treated coffee to One-liter standard oxidant solution are tabulated as shown below:

Volume of Entropy	Solution pH/Temperature	Solution ORP, mV	Solution Conductivity.

treated coffee added, ml			µS/cm
0.0	6.86 / 22.9°C	+550	5
0.5	5.75 / 22.6°C	+528	7
1.0	5.56 / 22.6°C	+512	9
1.5	5.34 / 22.5°C	+458	11
2.0	5.40 / 22.4°C	+412	12
2.5	5.39 / 22.5°C	+368	12

The test results showed that with the addition of the entropy treated coffee, the solution shifts toward reducing potential and reach the ORP value of +368mV with the addition of 2.5ml (standard oxidant ORP shift of -182 mV). It required 2.4 ml of the entropy treated coffee solution to neutralize One-liter standard oxidant of 0.2ppm TRO HOCl solution.

The ORP changes with the gradual volume addition of Entropy treated Orange Juice

The measured ORP changes with the gradual addition of Entropy treated orange juice to One-liter standard oxidant solution are tabulated as shown below:

Volume of Entropy treated Orange Juice added, ml	Solution pH/Temperature	Solution ORP, mV	Solution Conductivity. µS/cm
0.0	6.86 / 25.0°C	+550	5
0.5	4.93 / 24.0°C	+542	9
1.0	4.78 / 24.0°C	+510	12
1.5	4.56 / 24.0°C	+485	13
2.0	4.48 / 24.0°C	+476	14
2.5	4.39 / 24.0°C	+467	15
3.0	4.20 / 24.0°C	+455	17
3.5	4.16 / 24.0°C	+438	18
4.0	4.16 / 24.0°C	+398	23
4.5	4.16 / 24.0°C	+370	25

The test results showed that with the addition of the entropy treated orange juice, the solution shifts toward reducing potential and reached the ORP value of +370mV with the addition of 4.5ml (standard oxidant ORP shift of -180 mV). It required 4.4 ml of the entropy treated coffee solution to neutralize ~~the~~ One-liter standard oxidant of 0.2ppm TRO HOCl solution.

The ORP changes with the gradual volume addition of Entropy treated whisky

The measured ORP changes with the gradual addition of Entropy treated whisky to One-liter standard oxidant solution are tabulated as shown below:

Volume of entropy treated whisky added, ml	Solution pH/Temperature	Solution ORP, mV	Solution Conductivity. µS/cm
0.0	6.86 / 22.9°C	+550	5
2.5	6.38 / 25.3°C	+567	6
5.0	6.13 / 25.3°C	+555	7
7.5	5.63 / 25.3°C	+522	7
10.0	4.91 / 25.3°C	+500	8
12.5	4.48 / 25.4°C	+463	8
15.0	4.44 / 25.4°C	+411	9
17.5	4.38 / 25.4°C	+371	10

The test results showed that with the addition of the entropy treated whisk, the solution shifts toward reducing and reached the ORP value of +371mV with the addition of 17.5ml (standard oxidant ORP shift of -179 mV). It required 17.4 ml of the entropy treated whisky solution to neutralize One-liter standard oxidant of 0.2ppm TRO HOCl solution.

The ORP changes with the gradual volume addition of Entropy treated red wine

The measured ORP changes with the gradual addition of Entropy treated red wine to One-liter standard oxidant solution are tabulated as shown below:

Volume of Entropy treated red wine added, ml	Solution pH/Temperature	Solution ORP, mV	Solution Conductivity. µS/cm
0.0	6.86 / 25.0°C	+550	5
0.5	4.73 / 25.3°C	+484	21
1.0	4.39 / 25.3°C	+435	26
1.5	4.01 / 25.3°C	+399	29
1.8	4.02 / 25.3°C	+365	32

The test results showed that with the addition of entropy treated red wine, the solution shifts toward reducing potential and reach ORP value of +365mV with the addition of 1.8ml (standard oxidant ORP shift of -185 mV). It required 1.71 ml of the entropy treated red wine solution to neutralize One-liter standard oxidant of 0.2ppm TRO HOCl solution.

The Proposed TAPEC Scale

The volume of the various beverage's antioxidants required to neutralize the One-liter standard oxidant of 0.2ppm TRO HOCl solution and their volume ratio of ascorbic acid needed vs beverage's antioxidant needed are tabulated below:

Type of Beverages	Volume of the Beverages need to neutralize the Standard Oxidant, ml	Volume ratio of Ascorbic acid needed vs Beverages needed
Ascorbic acid 800mg/100ml	0.4	1.00
Tea	1.5	0.267
Coffee (Black)	2.7	0.148
Orange juice	13.5	0.030
Whisky	37.6	0.011
Red wine	1.94	0.206
Entropy treated water	2.5	0.160
Entropy treated Tea	1.0	0.400
Entropy treated Coffee	2.4	0.167
Entropy treated Orange juice	4.4	0.091
Entropy treated Whisky	17.4	0.023
Entropy treated red wine	1.71	0.234

Type of Beverage's antioxidant	Volume ratio of Ascorbic acid needed	TAPEC value per 100ml of the

	vs Beverage's antioxidant needed	Beverage's antioxidant
Ascorbic acid 800mg/100ml	1.00	1000
Tea	0.267	267
Coffee (Black)	0.148	148
Orange juice	0.030	30
Whisky	0.011	11
Red wine	0.206	206
Entropy treated water	0.160	160
Entropy treated Tea	0.400	400
Entropy treated Coffee	0.167	167
Entropy treated Orange Juice	0.091	91
Entropy treated Whisky	0.023	23
Entropy treated red wine	0.234	234
Pure Water	-	0

From the above test results, it is a feasible and practical method for measuring the Beverage's antioxidant neutralization strength by a redox titration process. The TAPEC value per 100 ml of untreated ascorbic acid, tea, coffee, orange juice, whisky, red wine and entropy treated water were determined as 1000, 267, 148, 30,11, 206 and 160, respectively. When tea, coffee, orange juice, whisky and red wine were treated by the entropy energy method, their TAPEC value per 100ml increased to 400,167, 91, 23 and 234, respectively.

The Total Antioxidants Potential & Energy Capacity (TAPEC) values so determined by this method provide a comprehensive guide to the consumption of these beverages in particular their antioxidants content or capacity. For example, the upper limit of daily ascorbic acid intake of 2000 mg as recommended by the medical practitioners and nutritionists is equivalent to 2500 TAPEC antioxidant scalar value.

To neutralize the oxidative stress in the human body, using the TAPEC value computation, and based on the ascorbic acid antioxidant daily dosage of 2500 TAPEC value, we can arrive at the equivalent of drinking of 1563ml of Entropy treated water {160 TAPEC in 100ml (2500/160 x 100ml)}

CONCLUSIONS:

TAPEC is a method developed for measuring the beverage's antioxidant neutralization strength using redox titration process. It uses pure water ORP as neutralization end point. The Total Antioxidants Potential & Energy Capacity (TAPEC) values were determined for various beverages based on this method and it provides a comprehensive guide to the consumption of these antioxidizing beverages. Three basic references namely, Oxidation Reduction Potential (ORP) of pure water, Standard Oxidants – hypochlorous acid TRO 0.2 mg/l and Standard Antioxidant using 800 mg Ascorbic acid (Vitamin C) in 100ml of pure water are adopted to establish the TAPEC scale reference value. TAPEC zero value represents nil strength of antioxidant with reference to the analogy of pure water with the pH value 7 as neutral solution. The TAPEC value per 100 ml of common beverage's antioxidant neutralization strength such as tea, coffee, orange juice, whisky, red wine and entropy treated water were determined as 267, 148, 30, 11, 206 and 160, respectively. When tea, coffee, orange juice, whisky and red wine were treated by the entropy energy method, their TAPEC value per 100ml increased to 400,167,91, 23 and 234, respectively.

NOTATIONS:

The following symbols are used in this paper:

CS = specific conductivity (µS /cm);
ORP = oxidation/reduction potential (mV
TAPEC = Total Antioxidants potential & energy capacity
TRO = Total residual oxidants (ppm)

REFERENCES:

American Public Health Association (APHA), American Water Works Association (AWWA), and Water Pollution Control Federation (WPCF). (2002). Standard Methods for the Examination of Water and Wastewater, Method 2580 – Oxidation-Reduction Potential (ORP), 22th Ed., American Public Health Association, Washington, D.C.USA

American Society of Testing and Material (ASTM) (2000), D1498-00 Standard Practice for Oxidation-Reduction Potential of Water, USA

Ahsan UK and Michael K (1994),Singlet molecular oxygen evolution upon simple acidification of aqueous hypochlorite: Application to studies on the deleterious health effects of chlorinated drinking water, Proceeding Nation Academic Science Vol 91: 12362-12364, USA

International Standard ISO 7393-2 (2017), Water quality – Deamination of free chlorine and total chlorine – Part 2: Colorimetric method using N, N-dialky-1,4-phenylenediamine, for routine control purposes, ISO, Geneva, Switzerland

Linders J, et al (2019) Methodology for the evaluation of ballast water management systems using active substances, GESAMP working group 34 report, 25-29, IMO. London, United Kingdom

Myke-Mbata B.K., et al. (2018), Antioxidant supplementation and Free Radicals Quelling: The Pros and Cons, Journal of Advances in Medicine and Medical Research 25(6): 1-13, United Kingdom

Rafael Radi. (2018), Oxygen radicals, nitric oxide, and peroxynitrite: Redox pathways in molecular medicine, PNAS Proceeding of the National Academy of sciences of United State of American, 115(23), 5839-5848, USA

Sam C.H. and Lu HK (2009), The role of hypochlorous acid as one of the reactive oxygen species in periodontal disease, Journal of Dental Science, Vol 4(2) : 45-54, Association for Dental Sciences of the Republic of China, ROC

Se-Yeong Lee., et al. (2004), Development of new method for antioxidant capacity with ORP-pH system, Biotechnology and Bioprocess Engineering, Vol 9:514-518, South Korea

Robert BG, et al. (2001), Handbook of Vitamins, 3rd edition, 529-568, Marcel Dekker, Inc, USA

US National Institutes of Health (2016), Fact Sheet for Health Professionals – Vitamin C, Office of Dietary Supplements, US National Institutes of Health, USA