Validation Test Report

The Existence and Sustainability of Antioxidizing Property of Entropy Treated Water Before Absorption into the Blood Stream

Dr. Chua Chee Yong

Principle consultant
Ellidell BioTechology Consultancy

February 2021

The Existence and Sustainability of Antioxidizing Property of Entropy Treated Water Before Absorption into the Blood Stream

Author: Dr Chua Chee Yong

ABSTRACT:

The health benefit of antioxidant is well documented, and the antioxidant state of water or beverages can be easily measured and identified by its Oxidation Reduction Potential (ORP) measured by ORP meter. But such beneficial antioxidizing water property must remain as antioxidizing after passing through the digestive tract and absorbed into blood stream at the intestine wall to provide the beneficial antioxidizing effect. This investigation is to confirm that the antioxidizing state of Entropy treated water is sustainable after passing through the stomach and duodenum before entering the blood stream at the intestine wall. It is also compared with untreated drinking water as control test. Stomach acid (HCl) and the duodenum ($NaHCO_3$) secretion from pancreas simulation tests were carried out to simulate the digestive tract conditions. The sustainable antioxidant effect of Entropy treated water has demonstrated to remain antioxidizing till it reaches the intestine before entering the blood stream. In comparison, untreated water remains as oxidizing when it reaches the intestine. In this simulated test, the final ORP of the Entropy treated water was –149 mV (minus 149 mV) after passing through the simulated acid and alkaline addition process and proven to be anti-oxidizing. Another identical simulation test was also carried out for alkaline water produced by Direct Current electrolysis. Alkaline water became very acidic and antioxidizing property is completely diminished once it is mixed with stomach HCl acid. Only after the addition of $NaHCO_3$ simulating the duodenum condition, the pH and ORP were brought back by $NaHCO_3$ to alkaline level and slightly reductive at -29mV (minus 29 mV) before entering the intestine.

KEY WORDS: Entropy Treatment Process, Oxidative Stress Neutralization, Antioxidant Effect, ORP Shift, Human Digestive Route

INTRODUCTION:

Free radicals can be generated in the human body endogenously (proceeding from within; derived internally) or exogenously (originating from outside: derived externally). They are highly active, can react with various important classes of biological molecules such as nucleic acids, lipids, and proteins, altering their normal redox state leading to an aberration in their role in cellular metabolism/ regulation and tissue structural architecture. This ultimately leading to cellular injury or cell death which is known to be deleterious to health. Myke-Mbata et al (2018) defined the antioxidant effect as inhibiting oxidation of biomolecules by free radicals. Its primary role will be decreasing or inhibiting synthesis, scavenge and neutralizing the effects of free radicals.

The tendency of a system, especially the physiological system of higher animals, to maintain internal stability, owing to the coordinated response of its parts to situation or stimulus that would tend to disturb its normal condition or function is known as homeostasis. It is this mechanism that maintains the activity of prooxidants, oxidants and antioxidants in a physiological balance in our body. Any tilt in this balance leads to oxidative stress. When the oxidative stress occurs, the free radicals attack the nearest stable molecule, abstracting its electron and the antioxidants will neutralize this oxidative stress. Rafael Radi (2018) had detailed the Redox pathways the hazard of excess oxidation in cell and tissue components that may compromise cell function and viability due to human aerobic life. These aspects of human redox biochemistry of the molecular, basis of diseases and aging lead to the development of preventive and therapeutic strategies including the role of the antioxidant effect and its application.

Since the neutralization of oxidative stress 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 value of a solution indicating the increased antioxidizing or reduced oxidizing capacity of a solution with reference to a neutral solution of 0 mV ORP. At its application state as antioxidant, it is essential to ensure ORP value to be negative (ORP < 0).** Se-Yeong Lee et al (2004) had developed the ORP methods for the measurement of antioxidant capacity 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).

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.

Human digestive tract has two key pH change zones, one in the stomach and one at duodenum. The stomach secrets HCl which performs important physiological functions including activating enzymes, hydrolysis of food components and inactivation of microorganisms. The stomach goes through contractive motions to mix the bolus with digestive juices, breakdown any large food fragments and transport the resulting materials to duodenum then to the small intestine. The ingested food component may remain inside the stomach for period up to a few hours. Typically, the amount of food remaining within the stomach after ingestion decreases by about 50% in 30 to 90 min. The partially digested food (chyme) will then leave the stomach and enters the small intestine. This is the region in the gastrointestinal (GI) tract that majority of food digested and absorbed. The duodenum is the upper part of the small intestine where $NaHCO_3$ is added from pancreas causing pH to increase from highly acidic (pH 1 to 3) in the stomach to around neutral (pH 7 to 8) in the duodenum, where the pancreatic enzymes work most efficient.

L Kalantzi , et al(2006) had shown that there may be large variation in both stomach and duodenum pH for human. Evans DF et al (1988) had conducted the gastrointestinal (GI) pH measurement of active human subjects using a pH sensitive radiotelemetry capsule passing freely through the gastrointestinal tract for up to 48 hours during normal GI transit. He reported the Gastric pH as highly acidic (range 1.0- 2.5) and the mean pH in the terminal ileum as 7.5. MeClemnets and Li (2010, reviewed several in vitro digestion models to test the efficacy of different approaches of controlling lipid digestion under the conditions that simulate the human gastrointestinal tract. This simulation approach can be adopted for the evaluation of Entropy treated water antioxidant effect. Chan, et al (2012) had also used the simulated gastrointestinal pH conditions for their antioxidant property evaluation. In view of this approach, it is rational to use simulated digestive tract conditions to determine the antioxidizing effect to our body for untreated and Entropy treated water.

This is a laboratory-based test, for the sole purpose as explained in the abovementioned. It is not a clinical test in any capacity.

MATERIAL AND METHODS:

Reagents Used

The Singapore Public Utilities Board supply water was used in this study. The high purity water for dilution 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 study include stabilized extra pure 9 weight % Hydrochloric Acid HCl (International Scientific, Singapore), $NaHCO_3$ Sodium Bicarbonate (RCI Labscan, Thailand). The saturated solution is prepared by dissolving 8 g of the $NaHCO_3$ in 100mL of high purity water and added to the untreated or treated water until the final solution reaches the pH value of 8.

Water Analysis

The various water quality parameters were determined using the standard methods (APHA, 1998). The ORP measurement was carried 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. Another Themo Scientific EUPEH CON 150 meter equipped with conductivity electrode (K = 1.0) was used to measure the conductivity. The Digital Themo sensor probe (for *in-situ* temperature measurement) of the Themo Scientific meter was immersed in the untreated and treated water during the Entropy treatment process.

Entropy Treatment Process

The Entropy unit (Ecospec NovelTech, 480 W, water chamber platinum emitter with 1 liter capacity, as shown in Figure 2) was turned on and tap water was filled in the inlet of the unit tank. Select the water treatment mode at the touch screen panel and activate the Entropy treatment. The treatment LED indicator light will light up when pressing the start button. The treated water was then collected in the beaker/glass by pressing the dispense button. This treated water was then used in the various tests.

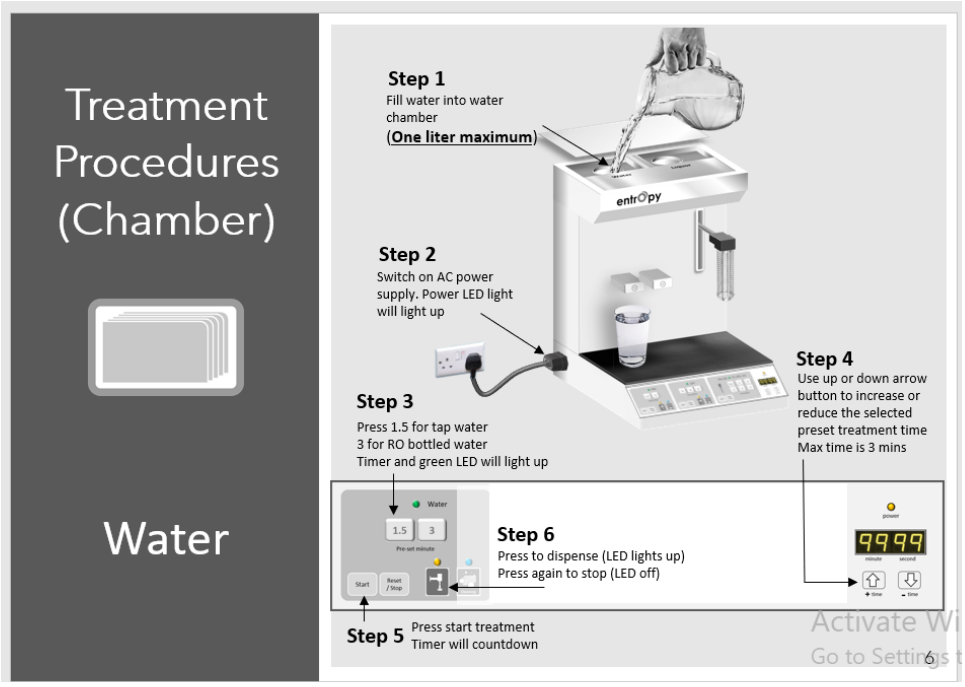

Figure 2 Entropy Treatment Unit

The Simulation Tests

The simulation tests were conducted to determine the final pH and ORP value of the Entropy treated and untreated water after the addition of the HCl and $NaHCO_3$. 200mL of the untreated and Entropy treated water were prepared and filled in the glass beaker. These two solutions were mixed with 9% w/w HCl and with a 1:1 ratio. Their respective water quality parameters (pH, Temperature, conductivity and ORP) were measured. And followed by addition of $NaHCO_3$ (saturated with pH around 8.2) into the resulting solution until the final solution reached pH of 8. Water quality parameters (pH, Temperature, conductivity and ORP) of the two final solutions were then measured.

RESULTS AND DISCUSSION:

The ORP for the Entropy Treated Water Under the Simulated Gastrointestinal pH Conditions

The respective parameter measured at various stages of the untreated and Entropy treated water tests were tabulated in the two tables below:

Parameter	Untreated water 200 ml	Entropy treated 200 ml	Add + 200 ml HCl	Further add + 55 ml $NaHCO_3$
pH / temp.	7.31 / 24.5°C	6.93 / 27.1°C	2.54 / 25.7°C	8.01 / 25.1°C
Conductivity, µS/cm	163.7	157.1	1083	6420
ORP, mV	+282	-254	-72	-149

Parameter	Untreated water (Control) 200 ml	Add 200 ml HCl	Further Add 55 ml $NaHCO_3$
pH / temp.	7.31 / 24.5°C	2.65 / 23.7°C	7.97 / 23.6°C
Conductivity, µS/cm	163.7	1067	6390
ORP, mV	+282	+345	+207

The above simulation tests showed the various water parameter changes between the Entropy treated water and untreated water when passing through the two pH change zones in the human digestive tract. When foods or drinks enter the mouth, the pH 7 condition in the mouth is unlikely to affect much the water pH or ORP values.

However, as the water or foods enter the stomach, the stomach acid HCl pH is anywhere between 1.0 to 2.5 which is very acidic and oxidizing. If water enters the stomach, we shall expect the water pH to be acidified by the stomach acid and swing to positive ORP or oxidizing state. The stomach secretes about 1500 ml of gastric acid per day which is about the same amount of our water intake per day. The holding size of our stomach is about 1000 ml, it is therefore reasonable to use the same amount of water and HCl for mixing to simulate stomach condition. After leaving stomach and before entering the small intestine, pancreas will secrete $NaHCO_3$ at duodenum to neutralize the acid and bring up the pH to about 8 in the small intestine before water is absorbed into the blood stream through the intestine wall. If antioxidant property is maintained in the water, it will also be absorbed through the intestine walls and goes into the blood stream bringing it to the cell to neutralize the free radicals produced by the cells if any.

In this simulation test, two sets of tests were conducted, one is without treatment and one with Entropy treatment. The water ORP and pH values were measured before and after pH 2.5 HCl addition, simulating stomach conditions. This is to observe how ORP and pH values are changed in the stomach. After HCl addition test, the solution is then added with $NaHCO_3$ to bring the solution up to pH 8 to simulate the water condition at duodenum before entering the intestines. At this point, if the treated water mixture solution ORP measured is anti-oxidizing compared with the oxidizing untreated water mixture, it then proves that Entropy treated water when entering the blood stream possesses the anti-oxidizing property.

From the test results, the final solution ORP of the Entropy treated water after the simulated acid and alkaline addition process was determined as –149 mV (in intestine) which is an anti-oxidizing water. This test result further confirmed Entropy treatment process can produce anti-oxidizing water and maintain its antioxidizing effect until it reaches the intestines for absorption into the blood stream.

The pH and ORP for the Direct Current Electrolysis Generated Alkaline Water Under the Simulated Gastrointestinal pH Conditions

Using Direct Current electrolysis together with a permeable barrier/membrane, the DC electrolysis can generate alkaline water at the cathode side. To evaluate the antioxidant capacity of the alkaline water at the cathode area, the same procedure as outlined in the previous section was carried for alkaline water. The respective parameters measured at various pH change stages at gastrointestinal (GI) tract for this alkaline water and untreated water tests were tabulated as shown below:

Parameter	Untreated water	DC electrolysis alkaline water 200 ml	Add 200 ml HCl	Further add 55 ml $NaHCO_3$
pH	7.31 / 24.5°C	8.67 / 23.90°C	2.49/ 22.0°C	8.02 / 21.8°C
Conductivity, µS/cm	163	138	1098	7320
ORP, mV	+282	-120	+67	-29

The above test simulated the conditions when the DC electrolysis alkaline water passes through the two pH changes zones in the human digestive tract.

The test results showed that the alkaline water changed to very acidic (pH 2.49) and oxidizing (ORP +67 mV), both the alkaline and antioxidizing properties diminished after it mixed with the stomach HCl.

After the addition of $NaHCO_3$ simulating pancreas secretion condition, the water pH was brought to pH 8 and ORP -29 mV, but this is because of $NaHCO_3$ addition, instead of the resilience of the alkaline water.

CONCLUSIONS:

The antioxidant effect of Entropy (uses pulsating capacitive field to excite and elevate the selective molecular bond vibration energy of water) treated water or beverage is sustainable after passing through the digestive tract to reach the intestine as demonstrated and proven in a series of tests conducted. In comparison, untreated water remains oxidizing throughout the various stages in the digestive tract. Entropy treated water negative ORP antioxidizing property is therefore utilizable for neutralizing the oxidative stress present internally in body cell process or derived from external factors.

NOTATIONS:

The following symbols are used in this paper:

CS = specific conductivity (µS /cm);
ORP = oxidation/reduction potential (mV
GI = Gastrointestinal

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), 22^{th} 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

Anastas, P.T. and Warner, J. C. (1998). Green Chemistry, Theory and Practice. Oxford University Press, Oxford, United Kingdom.

Chan.K. W., et al. (2012), Antioxidant Property Enhancement of Sweet Potato Flour under Simulated Gastrointestinal pH, International Journal of Molecular Sciences 13, 8987-8997, Switzerland

Mc Clements D.J. and Li Yan, et al. (2010), Review of in vitro digestion models for rapid screening of emulsion-based systems, Food & Function 1, 32-59, United Kingdom

Evans D.F., et al. (1988), Measurement of gastrointestinal pH profiles in normal ambulant human subjects, Gut, 29, 1035-1041, United Kingdom

Ecospec NoveTech (2020) Entropy User Manual, Singapore

L Kalantzi , et al(2006) Characterisation of the human upper gastrointestinal contents under conditions simulating bioavailability/bioequivalence studies, Pharm. Res. 23(1) 165-176 USA

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

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