

# IN-VITRO STUDY ON THE ANTIOXIDANT CAPACITY OF ALKALINE IONIZED WATER

Ngo Quoc Luan<sup>1\*</sup> and Ngo Khac Khong Minh<sup>2</sup>

<sup>1</sup>School of Education, Can Tho University

<sup>2</sup>Can Tho University of Technology

Email: ngoquocluan@ctu.edu.vn

## ARTICLE INFO

Received:

28/5/2025

Accepted:

11/9/2025

Published:

30/01/2026

**Keywords:** Antioxidant, ionized water, kangen water

## ABSTRACT

*This study assessed the antioxidant capacity of alkaline ionized water produced at laboratory scale using a Kangen TRIM ION H-2 device and a re-filtered domestic water source from Can Tho city. Two approaches were used. First, ORP measurements showed the alkaline ionized water had a negative ORP, which theory links with strong reducing and anti-oxidation potential. Yet this effect did not last when the water stayed in contact with air. The ORP rose quickly, mainly because dissolved hydrogen escaped from the water over time, reducing the water's reducing power. Second, the DPPH assay provided a practical estimate of antioxidant strength. Results indicated only weak antioxidant activity. Specifically, 19 liters of T3 water showed an antioxidant capacity equivalent to 46.2 mg of ascorbic acid for scavenging 1 mmol of DPPH. Overall, ORP suggested promising reducing conditions at first, but the DPPH test showed the real antioxidant effect was limited and decreased further with air exposure.*

## 1. INTRODUCTION

To mitigate health concerns associated with various diseases, particularly those linked to oxidative processes, pharmacological interventions have been widely employed. Nevertheless, in addition to their therapeutic benefits, many medications are associated with adverse side effects. Consequently, increasing attention has been directed toward the development of alternative and safer supportive approaches. One emerging research direction involves the use of alkaline ionized water (commonly known as Kangen water), which has attracted global scientific interest for its potential supportive roles in disease prevention and treatment.

Alkaline ionized water has been reported to enhance the decomposition activity of superoxide anion radicals, a phenomenon attributed to changes in its ionic product [1]. This property has prompted investigations into its potential effects on lowering blood pressure and reducing blood glucose and lipid levels [2]. As a result, alkaline

ionized water is considered a promising adjunct in the prevention of oxidation-related diseases, including diabetes, cancer, arteriosclerosis, and neurodegenerative disorders, as well as in alleviating certain side effects associated with hemodialysis [3]. Moreover, alkaline water with a pH of approximately 8.8 has been shown to inactivate pepsin, a key enzyme implicated in the pathogenesis of gastroesophageal reflux disease [4].

In Vietnam, a review of the existing literature indicates a lack of official publications specifically examining the free radical scavenging capacity of alkaline ionized water produced from local water sources. Therefore, the present study aims to address this gap by reporting laboratory-scale results on the antioxidant capacity of alkaline ionized water generated using the Kangen TRIM ION H-2 device (Japan) with urban tap water collected in Can Tho City.

## 2. RESEARCH METHODS

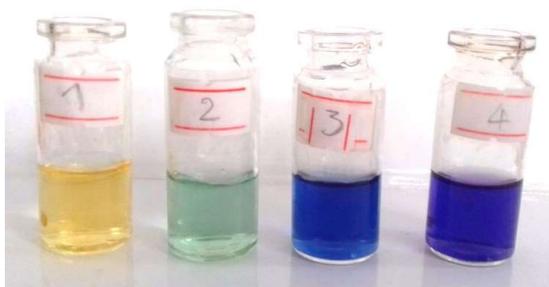
### 2.1. Chemicals, equipment

3-Stage water filtration system (Vietnam),

Kangen device (TRIM ION H-2, Japan), ORP meter (Orion star A329-Thermo Scientific, USA), UV-Vis meter (Prove-300, Merck, Germany), pH meter (Hanna, Romania), DPPH (>95%, Sigma-Aldrich, Germany), Ascorbic acid (99.7%, Xilong, China), Ethanol (96%, Vietnam), CH<sub>3</sub>COOH (China), CH<sub>3</sub>COONa (China), NH<sub>4</sub>OH (China), NH<sub>4</sub>Cl (China), Double distilled water (Vietnam), Micropipette, volumetric flask...

## 2.2. Research methods

The antioxidant capacity of alkaline ionized water at the laboratory scale was determined through two methods: measuring ORP values and testing for DPPH free radical scavenging.



*T1: Acidic electrolyzed water pH 5.82; T2: Neutral water pH 6.98; T3: Alkaline electrolyzed water pH 8.51; and T4: Alkaline electrolyzed water pH 9.96.*

**Figure 2. Electrolyzed water at different pH levels with synthetic pH indicator**

### 2.2.1. ORP measurement method

Acidic electrolyzed water (T1), filtered water (T2), alkaline electrolyzed water level 3 (T3), and alkaline electrolyzed water level 4 (T4) samples of approximately 200 mL were placed into corresponding numbered cups 1, 2, 3, and 4, respectively. The electrodes of the ORP meter were immersed in each cup, and the results were recorded immediately after the water was produced. The cups were left at room temperature, uncovered, and ORP values were measured at 15, 30, 60, and 120 minutes after production.

### 2.2.2. DPPH free radical scavenging method

The DPPH free radical scavenging method is a recognized method for determining antioxidant activity based on the ability to trap

free radicals created by DPPH (1,1-diphenyl-2-picrylhydrazyl) [5-7].

### Preparation of reagent (DPPH solution)

Weigh 39.4 mg of DPPH into a 100 mL volumetric flask, add 50 mL of EtOH, shake vigorously until completely dissolved, then add EtOH to make up to 100 mL, the concentration of the stock DPPH solution is 1 mM. This DPPH solution is allowed to stabilize in the dark for 30 minutes at 4°C, and is used to dilute to lower concentrations of DPPH solutions.

### Preparation of positive control solution

Weigh 20.1 mg of ascorbic acid into a 200 mL volumetric flask, add distilled water, shake vigorously until completely dissolved, then add distilled water to make up to 200 mL, the concentration of ascorbic acid is 0.57 mM (100.5 µg/mL). The standard solution is diluted to lower concentrations.

### Preparation of buffer solutions

*Buffer 4.76:* Measure 25 mL of 1M CH<sub>3</sub>COOH solution into a 100 mL beaker, then add 25 mL of 1M CH<sub>3</sub>COONa solution. Stir the mixture thoroughly with a magnetic stirrer and check the pH with a pH meter. If pH > 4.76, correct with 1M CH<sub>3</sub>COOH solution. If pH < 4.76, correct with 1M CH<sub>3</sub>COONa solution. The corrected buffer solution should be stored in a stoppered bottle.

*Buffer 10:* Weigh 2.67 g of NH<sub>4</sub>Cl into a 200 mL beaker and dissolve it in 80 mL of distilled water. Stir the solution thoroughly on a magnetic stirrer and check the pH with a pH meter. Slowly add concentrated NH<sub>3</sub> solution until the pH reaches 10. The corrected buffer solution is stored in a stoppered bottle.

*Buffer 8.5:* Measure 50 mL of ammonium buffer solution pH=10 into a 100 mL beaker. Stir the solution thoroughly on a magnetic stirrer and measure the pH with a pH meter. Slowly add NH<sub>4</sub>Cl (crystals) to the buffer solution until the pH reaches 8.5. The corrected buffer solution is stored in a stoppered bottle.

*Principles for measuring antioxidant capacity in each experimental treatment*

The test sample (including Kangen water and positive control) was dissolved in ethanol (the solvent was also the negative control) and DPPH was dissolved in 96% ethanol. Both the test sample and DPPH were investigated at appropriate concentrations to obtain a linear range. The absorbance of DPPH at a wavelength of  $\lambda = 517$  nm was determined by a UV-Vis meter after adding DPPH to the test sample solution and incubating for 15 minutes. The results of the tests (calculated using the formula in document [7]) are shown as the average value of at least 3 repeated tests  $\pm$

standard deviation  $s$  calculated using the formula:

$$s = \sqrt{\frac{1}{n-1} \sum_{i=1}^n (x_i - \bar{x})^2}$$

### 3. RESULTS AND DISCUSSION

#### 3.1. ORP measurement method

The ORP values of the electrolyzed water samples and the comparison sample at the time the electrolyzed water was first produced at the outlet of the electrolyzer and after several time points are presented in Table 1.

**Table 1. ORP measurement results of electrolyzed water samples and comparison sample (unit mV)**

Time after production (minutes)	Recommended level not to be used	Recommended usage level	T1	T2	T3	T4
0	>0	-400 - 0	446 $\pm$ 2	223 $\pm$ 2	-761 $\pm$ 3	-780 $\pm$ 3
15	>0	-400 - 0	388 $\pm$ 3	221 $\pm$ 2	-462 $\pm$ 3	-467 $\pm$ 2
30	>0	-400 - 0	349 $\pm$ 4	222 $\pm$ 2	-259 $\pm$ 3	-271 $\pm$ 3
60	>0	-400 - 0	299 $\pm$ 2	223 $\pm$ 1	-180 $\pm$ 2	-185 $\pm$ 2
120	>0	-400 - 0	268 $\pm$ 1	223 $\pm$ 1	-61 $\pm$ 1	-77 $\pm$ 1

Note: T1 = acidic electrolyte water; T2 = comparison sample; T3, T4 = alkaline electrolyte water.

The results indicated that at the time of production (0 min), both the acidic electrolyzed water sample (T1) and the filtered water sample (T2) exhibited positive oxidation–reduction potential (ORP) values that fell within the recommended range for drinking water. In contrast, the two alkaline electrolyzed water samples (T3 and T4) showed negative ORP values at this time point. During the period from 30 to 120 minutes after production, the ORP values of all samples were within the recommended range; however, at 0 and 15 minutes after production, the ORP values exceeded the recommended limits.

Under open storage conditions (15, 30, 60, and 120 minutes), the ORP values of the alkaline electrolyzed water samples (T3 and T4) increased markedly, whereas the ORP of the acidic electrolyzed water sample (T1) showed a slight decrease and that of the filtered

water sample (T2) remained nearly unchanged. This trend can be primarily attributed to the rapid loss of dissolved hydrogen in alkaline electrolyzed water over time, which directly affects its reducing capacity.

#### 3.2. DPPH free radical scavenging method

##### 3.2.1. Investigating the effect of pH on reaction systems

Based on the measured pH values of the electrolyzed water samples, which included acidic (pH 5.82), neutral (pH 6.98), alkaline (pH 8.51), and strongly alkaline conditions (pH 9.96), three commonly used laboratory buffer systems with corresponding pH values of 4.76, 8.50, and 10.00 were selected to adjust the pH of the reaction system in order to evaluate the effect of pH on the DPPH reaction.

The experimental results demonstrated that an increase in pH led to a corresponding increase in DPPH absorbance. Consequently, to accurately assess the DPPH free radical

scavenging activity of alkaline ionized water, it was necessary to employ a buffer system to maintain comparable pH conditions across all test samples. Additionally, the results indicated that increasing antioxidant concentration resulted in a significant decrease in absorbance of the reaction system under both acidic and neutral conditions, exhibiting a strong linear correlation. Based on these findings, all subsequent experiments were conducted at pH 4.76 using an acetate buffer solution to ensure consistency and reliability of the measurements.

3.2.2. Comparison of the antioxidant capacity of alkaline ionized water at level 3 (sample T3) and level 4 (sample T4) taken from

the TRIM ION H-2 electrolysis machine

The acidic (T1) and neutral (T2) electrolyzed water samples exhibited relatively high positive oxidation–reduction potential (ORP) values; therefore, in theory, they are considered to have negligible free radical scavenging capacity. Consistent with this assumption, when T1 and T2 were individually reacted with DPPH, the absorbance values showed substantial fluctuations and no discernible trend was observed. In contrast, the alkaline electrolyzed water samples (T3 and T4) produced more stable and reproducible absorbance results, indicating a more consistent interaction with DPPH.

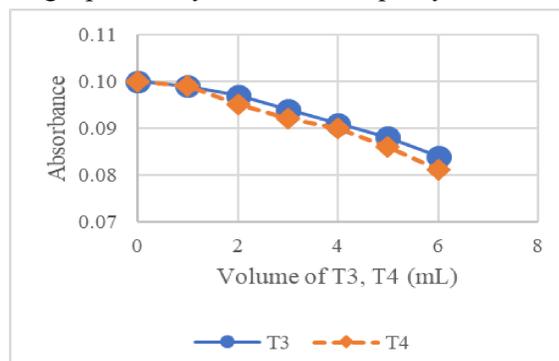
**Table 2. Results of the survey on the DPPH free radical scavenging ability of samples T3 and T4 under the same conditions**

No.	0	1	2	3	4	5	6
$V_{\text{DPPH}}$ (mL)	1	1	1	1	1	1	1
$V_{\text{đệm}}$ (mL)	0.3	0.3	0.3	0.3	0.3	0.3	0.3
$V_{\text{đm}}$ (mL)	6	5	4	3	2	1	0
$V_{\text{mãu}}$ (mL)	0	1	2	3	4	5	6
$V_{\text{tổng}}$ (mL)	7.3	7.3	7.3	7.3	7.3	7.3	7.3
Absorbance							
	<b>T3</b>	<b>0.100</b>	<b>0.099</b>	<b>0.097</b>	<b>0.094</b>	<b>0.091</b>	<b>0.088</b>
	<b>T4</b>	<b>0.100</b>	<b>0.099</b>	<b>0.095</b>	<b>0.092</b>	<b>0.090</b>	<b>0.086</b>

Samples labeled 0, 1, 2, 3, 4, 5, and 6 corresponded to increasing volumes (mL) of sample T3 (or T4). Each sample was first adjusted to pH 4.76 using 0.3 mL of buffer solution and subsequently diluted with solvent (distilled water) to achieve an equal total volume. Thereafter, 1 mL of 0.1 mM DPPH solution was added to each system. The mixtures were thoroughly shaken and incubated in the dark for 15 minutes. Absorbance (Abs) was then measured at 517 nm, and the obtained results are summarized in Table 2.

As illustrated in Figure 3, the reactivity of water samples T3 and T4 toward DPPH was essentially equivalent, indicating that increasing electrolysis intensity did not result in a proportional enhancement of radical scavenging activity. However, pH analysis revealed a critical distinction between the two samples. The moderately alkaline ionized water (T3) exhibited a pH value within the acceptable limits for domestic

and drinking water, whereas the strongly alkaline ionized water (T4) exceeded the permissible pH range specified by relevant water quality standards.



**Figure 3. Absorbance of T3 and T4 systems under the same investigation conditions**

Given that no significant difference in DPPH reactivity was observed between T3 and T4, and considering the safety and practical applicability of the water for human consumption, sample T3 was selected for subsequent experiments.

### 3.2.3. Determine the DPPH concentration corresponding to the sample's reactivity

The appropriate concentration of DPPH was defined as the concentration at which the radical was completely (100%) reduced by the antioxidant. The determination criteria included: (i) a gradual color change of the solution from purple to yellow, and/or (ii) a decrease in

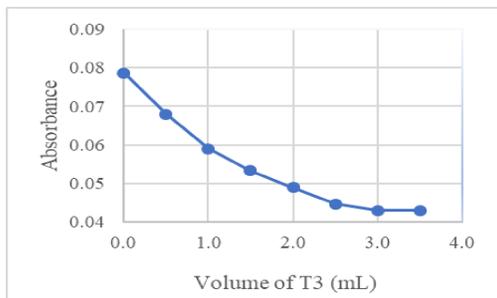
absorbance at 517 nm that reached a plateau in samples with high antioxidant concentrations, as indicated by at least two final samples exhibiting identical absorbance values.

Based on the dilution study conducted using water sample T3, the optimal DPPH concentration for subsequent experiments was determined to be 0.05 mM.

**Table 3. Results of the linearity survey with DPPH concentration of 0.05 mM**

No.	0	1	2	3	4	5	6	7	
$V_{DPPH}$ (mL)	1	1	1	1	1	1	1	1	
$V_{buffer}$ (mL)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	
$V_{solvent}$ (mL)	3.5	3.0	2.5	2.0	1.5	1.0	0.5	0	
$V_{sample}$ (mL)	<b>0</b>	<b>0.5</b>	<b>1.0</b>	<b>1.5</b>	<b>2.0</b>	<b>2.5</b>	<b>3.0</b>	<b>3.5</b>	
$V_{total}$ (mL)	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	
Absorbance at 517 nm	First time	0.079	0.069	0.061	0.053	0.05	0.045	0.043	0.043
	Second time	0.078	0.068	0.058	0.053	0.048	0.044	0.043	0.043
	Third time	0.079	0.067	0.058	0.054	0.049	0.045	0.043	0.043
	<b>Average</b>	<b>0.079</b>	<b>0.068</b>	<b>0.059</b>	<b>0.053</b>	<b>0.049</b>	<b>0.045</b>	<b>0.043</b>	<b>0.043</b>
s (%)	0.0005	0.0010	0.0015	0.0005	0.0010	0.0005	0	0	

### 3.2.4. Determine the linear range



**Figure 4. Absorbance of the systems as a function of sample volume. T3 increases by 0.5 mL**

Samples labeled 0, 1, 2, 3, 4, 5, 6, and 7 corresponded to increasing volumes of sample T3 (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 mL, respectively). To each system, 0.3 mL of buffer solution at pH 4.76 was added, followed by the addition of distilled water to adjust the total volume to 3.8 mL. Subsequently, 1 mL of 0.05 mM DPPH solution was added. The mixtures were thoroughly shaken and incubated in the dark for 15 minutes. Absorbance (Abs) was then measured at 517 nm, and the corresponding

results are summarized in Table 3.

From the graph in Figure 4, a linear range from sample 0 to sample 6 (0-3 mL sample T3) can be selected. This range will be applied to the SC% calculation formula.

### 3.2.5 Determining the volume of T3 that inhibits 50% of DPPH free radicals (determining $SC_{50}$ )

From the average absorbance of the systems from 0-6, calculate SC% using the formula:

$$SC\% = \left[ \frac{A_0 - A_x}{A_0 - A_{100}} \times 100 \right] \pm s$$

Where:

$A_0$  = absorbance of sample 0 (negative control, where 0% of DPPH is neutralized).

$A_x$  = absorbance of any sample.

$A_{100}$  = absorbance of the sample where 100% of DPPH is neutralized.

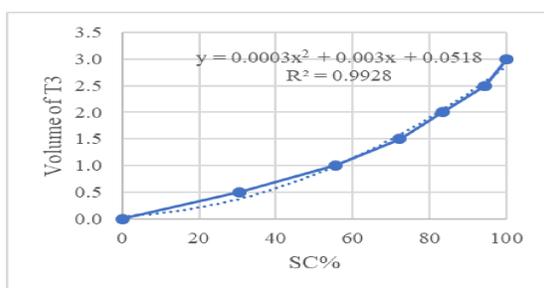
s = standard deviation.

The SC% calculation results are presented in Table 4.

From the equation representing the volume of alkaline ionized water as a function of SC%, with  $x = 50\%$ , we get  $y = 0.95$ . Therefore, the  $SC_{50}$  of alkaline ionized water is  $0.95 \text{ mL} / 0.05 \times 10^{-3} \text{ mmol DPPH}$ .

**Table 4. Results of calculating SC% of the systems with increasing T3 volume by every 0.5 mL**

No.	0	1	2	3	4	5	6
<b>T3 water volume (mL)</b>	<b>0.0</b>	<b>0.5</b>	<b>1.0</b>	<b>1.5</b>	<b>2.0</b>	<b>2.5</b>	<b>3.0</b>
Absorbance	0.079	0.068	0.059	0.053	0.049	0.045	0.043
<b>SC (%)</b>	<b>0.00</b>	<b>30.56</b>	<b>55.56</b>	<b>72.22</b>	<b>83.33</b>	<b>94.44</b>	<b>100.00</b>
Standard deviation (s, %)	±1.39	±2.78	±4.17	±1.39	±2.78	±1.39	±0.00

**Figure 5. Volume of alkaline ionized water according to % DPPH neutralized**

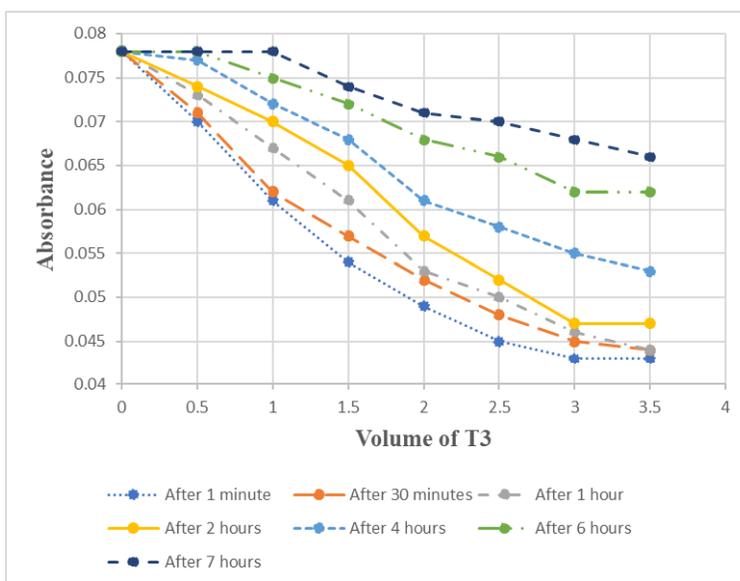
### 3.2.6 Investigation of the free radical scavenging ability of alkaline electrolyzed water over time after production

After collection, water sample T3 was exposed to ambient air at room temperature. The DPPH free radical scavenging activity was measured at multiple time points after production, specifically at 1 and 15 minutes, 30 minutes, and

1, 2, 3, 4, 5, 6, and 7 hours. Each experiment was conducted in triplicate, and the mean values were calculated. The corresponding results are presented in Table 5.

Analysis of the absorbance data presented in Table 5, together with the trend shown in Figure 6, indicates that the antioxidant activity of alkaline ionized water decreases progressively over time. Within the first 2 hours after production, the decline in activity is minimal; however, a more pronounced reduction is observed beyond this period. Notably, from 6 hours onward, the reaction solution exhibits a pale purple coloration, suggesting that the alkaline ionized water has nearly completely lost its antioxidant capacity.

### 3.2.7 Determination of $SC_{50}$ for the ascorbic acid positive control using the same DPPH concentration

**Figure 6. Absorbance of systems as a function of volume T3 and time**

**Table 5. Results of absorbance measurements of DPPH and T3 systems at different concentrations over time after production**

STT	0	1	2	3	4	5	6	7
V <sub>DPPH</sub> (mL)	1	1	1	1	1	1	1	1
V <sub>buffer</sub> (mL)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
V <sub>solvent</sub> (mL)	3.5	3.0	2.5	2.0	1.5	1.0	0.5	0
V <sub>sample</sub> (mL)	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
V <sub>total</sub> (mL)	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8
After 1 minute	0.078	0.070	0.061	0.054	0.049	0.045	0.043	0.043
After 15 minutes	0.078	0.071	0.062	0.055	0.051	0.047	0.044	0.044
After 30 minutes	0.078	0.071	0.062	0.057	0.052	0.048	0.045	0.044
After 1 hour	0.078	0.073	0.067	0.061	0.053	0.050	0.046	0.044
After 2 hours	0.078	0.074	0.070	0.065	0.057	0.052	0.047	0.047
After 3 hours	0.078	0.077	0.071	0.066	0.061	0.058	0.052	0.048
After 4 hours	0.078	0.077	0.072	0.068	0.061	0.058	0.055	0.053
After 5 hours	0.078	0.077	0.073	0.068	0.063	0.057	0.057	0.055
After 6 hours	0.078	0.078	0.075	0.072	0.068	0.066	0.062	0.062
After 7 hours	0.078	0.078	0.078	0.074	0.071	0.070	0.068	0.066

**Table 6. Survey results determining the range of positive control concentrations appropriate for DPPH levels**

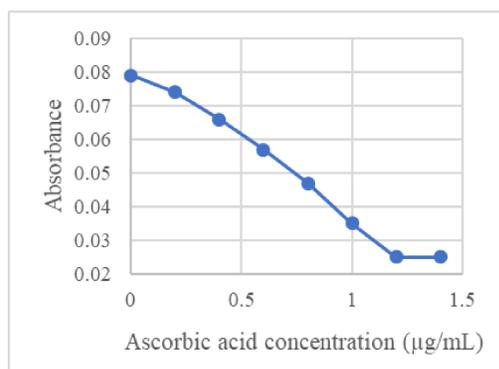
No.	0	1	2	3	4	5	6	7	
V <sub>DPPH</sub> (mL)	1	1	1	1	1	1	1	1	
<b>Ascorbic acid concentration (µg/mL)</b>	<b>0</b>	<b>0.2</b>	<b>0.4</b>	<b>0.6</b>	<b>0.8</b>	<b>1.0</b>	<b>1.2</b>	<b>1.4</b>	
V <sub>ascorbic acid</sub> (mL)	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	
V <sub>total</sub> (mL)	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	
Absorbance at 517 nm	First time	0.08	0.075	0.062	0.056	0.042	0.036	0.025	0.025
	Second time	0.078	0.073	0.068	0.058	0.051	0.035	0.025	0.025
	Third time	0.079	0.073	0.069	0.058	0.048	0.033	0.026	0.025
	<b>Average</b>	<b>0.079</b>	<b>0.074</b>	<b>0.066</b>	<b>0.057</b>	<b>0.047</b>	<b>0.035</b>	<b>0.025</b>	<b>0.025</b>
	s (%)	0.001	0.0015	0.0035	0.001	0.0045	0.0015	0.0005	0

**Table 7. Results of SC% calculation of ascorbic acid positive control**

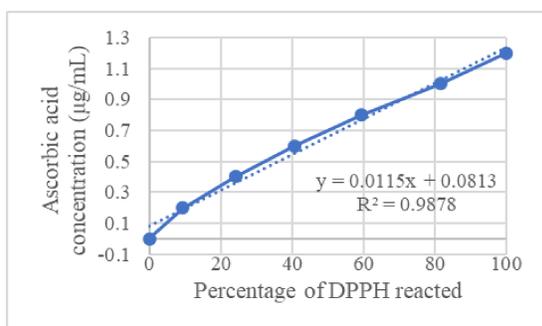
No.	0	1	2	3	4	5	6
<b>Ascorbic acid concentration (µg/mL)</b>	<b>0</b>	<b>0.2</b>	<b>0.4</b>	<b>0.6</b>	<b>0.8</b>	<b>1.0</b>	<b>1.2</b>
Absorbance	0.079	0.074	0.066	0.057	0.047	0.035	0.025
<b>SC%</b>	<b>0</b>	<b>9.26</b>	<b>24.07</b>	<b>40.74</b>	<b>59.26</b>	<b>81.48</b>	<b>100</b>
Standard deviation (s. %)	±1.85	±2.78	±6.48	±1.85	±8.33	±2.78	±0.93

The SC<sub>50</sub> value of ascorbic acid (positive control) was determined to enable comparison with sample T3 under identical assay conditions, using a DPPH concentration of  $0.05 \times 10^{-3}$  mmol. Following preliminary trials and serial dilutions, ascorbic acid solutions were prepared at concentrations of 1.4, 1.2, 1.0, 0.8, 0.6, 0.4, and 0.2 µg/mL, corresponding to  $7.95 \times 10^{-3}$ ,  $6.82 \times 10^{-3}$ ,  $5.68 \times 10^{-3}$ ,  $4.55 \times 10^{-3}$ ,  $3.41 \times 10^{-3}$ ,  $2.27 \times 10^{-3}$ , and  $1.14 \times 10^{-3}$  mM, respectively. Each concentration was reacted with 1 mL of 0.05 mM DPPH solution for subsequent analysis.

Based on Figure 7 and the absorbance data presented in Table 6, the linear response range of the ascorbic acid positive control was determined to be 0–1.2  $\mu\text{g/mL}$ . Within this linear range, the radical scavenging percentage (SC%) of the system was calculated for each tested concentration (Table 7). Linear regression analysis of ascorbic acid concentration versus SC% indicated that an SC value of 50% corresponded to an ascorbic acid concentration of 0.66  $\mu\text{g/mL}$ . Accordingly, the  $SC_{50}$  of ascorbic acid for 1 mL of 0.05 mM DPPH was calculated to be 2.31  $\mu\text{g}$  per  $0.05 \times 10^{-3}$  mmol DPPH.



**Figure 7. Absorbance of DPPH and ascorbic acid systems at different concentrations**



**Figure 8. Positive control concentrations as a percentage of neutralized DPPH**

### 3.2.8 Comparison of $SC_{50}$ values between water sample T3 and the ascorbic acid positive control

Comparison of the  $SC_{50}$  values of alkaline electrolyzed water sample T3 and the ascorbic acid positive control revealed that, under

identical experimental conditions with a DPPH concentration of  $0.05 \times 10^{-3}$  mmol, the antioxidant capacity of 0.95 mL of T3 alkaline water was equivalent to that of 2.31  $\mu\text{g}$  of ascorbic acid. When normalized to 1 mmol of DPPH, approximately 19 L of T3 alkaline water exhibited an antioxidant capacity equivalent to 46.2 mg of ascorbic acid per mmol of DPPH.

These results clearly demonstrate that the antioxidant capacity of alkaline electrolyzed water (T3) is substantially lower than that of ascorbic acid. From a practical consumption perspective, assuming an average adult intake of 2 L of T3 alkaline water per day, more than 9.5 days would be required to achieve an antioxidant capacity equivalent to 46.2 mg of ascorbic acid. This finding preliminarily suggests that alkaline ionized water is considerably less effective as an antioxidant source compared with direct vitamin C supplementation.

Nevertheless, the relatively low measured antioxidant activity of alkaline ionized water may be attributable to its intrinsic physicochemical properties, particularly the rapid loss of dissolved hydrogen upon exposure to air. Furthermore, the preparation, dilution, and measurement steps involved in the experimental procedure require a non-negligible amount of time, which may have further reduced the residual hydrogen concentration in the samples, thereby diminishing the observed antioxidant capacity.

## 4. CONCLUSION

The antioxidant capacity of alkaline ionized water produced using the Kangen TRIM ION H-2 device with filtered tap water from Can Tho City was evaluated at the laboratory scale using two complementary approaches. Oxidation–reduction potential (ORP) measurements indicated that freshly produced alkaline ionized water exhibited negative ORP values, suggesting a theoretical suitability for antioxidant applications. However, upon exposure to ambient air, the ORP increased rapidly, which can be attributed to the loss of dissolved hydrogen.

Assessment using the DPPH radical scavenging assay further confirmed that alkaline

ionized water possesses antioxidant activity; however, this activity was relatively weak. Specifically, approximately 19 L of T3 alkaline ionized water exhibited an antioxidant capacity equivalent to 46.2 mg of ascorbic acid per 1 mmol of DPPH. These findings indicate that, although measurable, the antioxidant capacity of alkaline ionized water is substantially lower than that of conventional antioxidants such as vitamin C.

Based on these results, the application of alkaline ionized water should be considered primarily in relation to other functional properties, including its acid–base characteristics or potential hygienic effects, rather than its antioxidant capacity. Any antioxidant effect appears to be transient and is most pronounced when the water is consumed within a limited time frame, approximately 30 to 120 minutes after production.

**Acknowledgement:** This study was partially funded by Can Tho University (project code TSV2019-110).

#### References

- [1] Hanaoka K., Sun D., Lawrence R., Kamitani Y., Fernandes G. The mechanism of the enhanced antioxidant effects against superoxide anion radicals of reduced water produced by electrolysis. *Biophysical chemistry*. 2004; 17:71-82.
- [2] Chan Y.M., Shariff Z.M., Chin Y.S., Ghazali S.S., Lee P.Y. and Chan K.S.

Associations of alkaline water with metabolic risks, sleep quality, muscle strength: A cross-sectional study among postmenopausal women. *PLOS One*. 2022; 17(10): 1-16.

[3] Shirahata S., Hamasaki T., Teruya K. Advanced research on the health benefit of reduced water. *Trends in Food Science & Technology*. 2012; 23(2): 124-131.

[4] Koufman J.A., Johnston N. Potential Benefits of pH 8.8 alkaline drinking water as an adjunct in the treatment of reflux disease. *Annals of Otolaryngology, Rhinology, and Laryngology*. 2012; 121(7): 431-434.

[5] Brand-Williams W., Cuvelier M.E. and Berset C. Use of a free radical method to evaluate antioxidant activity. *Lebensm.-Wiss. u.-Technol*. 1995; 28:25-30.

[6] Shela G., Olga M.B., Elena K., Antonin L., Milan C., Nuria G.M., Ratiporn H., Yong-Seo P., Soon-Teck J., and Simon T. Bioactive compounds and antioxidant potential in fresh and dried Jaffa® sweeties, a new kind of citrus fruit. *Journal of the Science of Food and Agriculture*. 2004; 84:1459-1463.

[7] Kumar G.P., Navyaa K., Ramya E.M., Venkataramana M., Anand T., Anilakumar K.R. DNA damage protecting and free radical scavenging properties of *Terminalia arjuna* bark in PC-12 cells and plasmid DNA. *Free Radicals and Antioxidants*. 2013; 3:35-39.

### NGHIÊN CỨU KHẢ NĂNG KHÁNG OXI HÓA *IN-VITRO* CỦA NƯỚC ĐIỆN GIẢI ION KIỀM

#### TÓM TẮT

*Khả năng kháng oxy hóa ở quy mô phòng thí nghiệm của nước điện giải ion kiềm với thiết bị Kangen TRIM ION H-2 và nguồn nước sinh hoạt tại Cần Thơ qua lọc lại đã được nghiên cứu bằng hai phương pháp. Phương pháp đo trị số ORP cho kết quả nước điện giải kiềm có trị số âm, về lý thuyết rất thích hợp dùng cho mục đích chống oxy hóa. Tuy nhiên, nếu để hở, chỉ số này tăng lại khá nhanh do mất hydrogen hòa tan. Phương pháp DPPH cho kết quả chứng tỏ nước điện giải ion kiềm cũng có khả năng kháng oxy hóa yếu: 19 L nước T3 có khả năng kháng oxy hóa tương đương với 46,2 mg ascorbic acid đối với 1 mmol DPPH.*

**Từ khóa:** Kháng oxy hóa (Chống oxy hóa), nước điện giải, nước kangen