The Removal of Colors in Non-carbonated Soft Drinks using Granular Activated Carbon for Coliform Bacteria Detection by Sanitary Indicator Medium

Naovarat Srisantisang* Pisit Vatanasomboon**
Piangchan Rojanavipart* Pipat Luksamijarulkul******

ABSTRACT

This research was designed to study the efficiency of non-carbonated soft drink color removal by using granular activated carbon (GAC) for coliform bacteria detection by SI medium. The conditions established in the study were to use 6 color tones of non-carbonated soft drinks (red, green, purple, blue, orange and yellow) which were removed by using 3 GAC amounts (100, 200, 300 mg per soft drink 1 mL) at two contact times of color removal, 30 and 60 minutes. The results showed that a higher amount of GAC and the increasing of contact time significantly increased color removal efficiency at *p-value* < 0.05, and that, also, the different color tones of soft drinks significantly affected different color removal efficiencies at *p-value* < 0.05. The highest color removal efficiency derived from using 300 mg/mL of GAC amount at 60 minutes, but was not the best suitable for every color tone. The best suitable condition of each color tone was selected from the lowest GAC amount and the shortest contact time for the color removal as well as affected the color of SI medium, which was changed to yellow color for a positive result, but was still purple color for a negative result within 24 hours. Using 200 mg/mL of GAC amount at 30 minutes was the best suitable condition for red-green-purple-orange color tones, using 300 mg/mL of GAC amount at 30 minutes was the best suitable condition for yellow color-tone, and using 300 mg/mL of GAC amount at 60 minutes was the best suitable condition for blue color-tone. The validity of coliform bacteria detection by SI medium for these removed-color soft drinks when compared with the standard MPN method were sensitivity value, specificity value and efficiency of test of more than 80%. Thus, GAC can be used to efficiently remove the colors of soft drinks for coliform bacteria detection by SI medium.

Key words: non-carbonated soft drink, color removal, granular activated carbon, SI medium, coliform bacteria detection

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Correspondence: Associate Professor Pisit Vatanasomboon, Department of Environmental Health Science, Faculty of Public Health, Mahidol University, Bangkok 10400

* Graduate Student in Master of Science Programme in Environmental Sanitation, Faculty of Public Health, Mahidol University

** Department of Environmental Health Science, Faculty of Public Health, Mahidol University

*** Department of Biostatistics, Faculty of Public Health, Mahidol University

**** Department of Microbiology, Faculty of Public Health, Mahidol University

Introduction

In the past, Department of Health, Ministry of Public Health did the research on the simple method to check total coliform bacteria - which is normal flora in the food pathway of human and mammals. This method was called SI-2 which was the name of culture medium for coliform bacteria detection.[1] Later, SI-2 was developed into Sanitary Indicator medium (SI medium). The weakness of SI medium is interference from intense color of food, because the intense color of food will interfere with the observation of color change in SI medium (If the result is positive, the clear purple color of SI medium will be changed into unclear yellow color or unclear yellow-purple color). Since a lot of non-carbonated soft drinks widely sold by venders usually had intense colors as well as many color tones, color removal from these non-carbonated soft drinks for coliform bacteria detection by SI medium is very necessary. If the colors are not removed, error from color vision is likely to happen and so researchers will misinterpret the result of coliform bacteria detection by SI medium.

Activated carbon is an adsorbent which is widely applied for the color removal in industries. For examples, it was used for water and wastewater color treatment, decolorization and purification of sugar in food industry, also decolorization of oils and fats.[2] Thus, activated carbon was expected to be applied for the removal of non-carbonated soft drink colors by adsorption process. There are two reports about the removal of Methylene Blue from aqueous solution by using activated carbon. It has been shown that the decrease of activated carbon particle size affects the increase of adsorption capacity.[3-4] So the smallest size of granular activated carbon should be selected, which could settle well without the filtration step of activated carbon, and the carbon could be easily used.

Therefore the granular activated carbon was used in the color removal of non-carbonated soft drinks for coliform bacteria detection by SI medium. The suitable conditions regarding the amount of granular activated carbon and the contact time of color removal for each color tone of non-carbonated soft drinks were studied. The study results could be applied correctly and conveniently by governmental officials or related personnel with the SI medium to detect coliform bacteria in non-carbonated soft drinks with intense colors.

Materials and Methods

This study was designed as an experimental research to determine the color removal efficiency by using granular activated carbon in removing non-carbonated soft drink colors for coliform bacteria detection by SI medium. Granular activated carbon (GAC) was made of coconut shells and activated by superheated steam. Also, it has an iodine number of 1,100 mg/g and particle size of 0.42-1.70 mm. Regarding non-carbonated soft drinks, they were prepared by mixing the finished syrup, i.e., sala syrup (red), cream soda syrup (green), grape syrup (purple), blueberry syrup (blue), orange syrup (orange) and pineapple syrup (yellow) at the ratio of finished syrup: distilled water of 1 : 4, which is the normal

ratio according to the label of the finished syrup. The experiment was divided into two parts including:

Part 1: The efficiency of non-carbonated soft drink color removal by using granular activated carbon (GAC) at different conditions

The 6 color tones of non-carbonated soft drinks [red (Carmoisine), green (Tartrazine and Brilliant Blue FCF), purple (Carmoisine and Brilliant Blue FCF), blue (Brilliant Blue FCF), orange (Tartrazine and Sunset Yellow FCF) and yellow (Tartrazine)] were removed by using 3 GAC amounts (100, 200, 300 mg per soft drink 1 mL) at 2 contact times of color removal (30 and 60 minutes) for study of color removal efficiency. The color removal efficiency was measured by UV-visible spectrometer to find different color intensities between before and after color removal. The value of color intensity was indicated by absorbance value from the spectrometer. It could be calculated by the following equation.

$$\text{Color removal efficiency (\%)} = \frac{(I_i - I_f) \times 100}{I_i}$$

Where; I_i means the initial color intensity of non-carbonated soft drinks

I_f means the final color intensity of non-carbonated soft drinks

The color intensity by spectrometer at the wavelength corresponding to the maximum absorbance (λ_{max}) for each food colorant type in color tones of non-carbonated soft drinks included Carmoisine at wavelength 516 nm[5], Brilliant Blue FCF at wavelength 630 nm, Tartrazine at wavelength 428 nm and Sunset Yellow FCF at wavelength 484 nm.[6-7] Moreover, pH value of soft drinks had been measured by pH meter after the color intensity of soft drink was measured by spectrometer.

Part 2: The validity of coliform bacteria detection by SI medium for removed-color non-carbonated soft drinks compared with the standard MPN method

(1) Preparing of non-carbonated soft drinks that were contaminated with coliform bacteria

Coliform bacteria had been cultured in Eosin methylene blue agar (EMB) before being mixed in sterile non-carbonated soft drinks. Mixed non-carbonated soft drinks with coliform bacteria had 6 diluted samples or characteristics (each color tone in one set).

(2) Coliform bacteria detection by MPN method

Non-carbonated soft drinks that were contaminated (6 samples) and uncontaminated (1 sample) with coliform bacteria as one set were

detected by MPN method for each color tone. The soft drinks were detected with the coliform bacteria amount for 3 sets by MPN method.[8-9] In addition, the standard of coliform bacteria in the street-side soft drink detected by the MPN method is less than 20 MPN/ 100 mL[10], so it was used for confirming the validity.

(3) Coliform bacteria detection by SI medium

(3.1) The suitable GAC amounts and contact times for removing non-carbonated soft drink colors are shown from the results of coliform bacteria detection by SI medium and comparison with the standard MPN method for seven samples of each color tone in the first set. Each sample of the seven samples from each color tone was also divided into 6 portions for coliform bacteria detection by SI medium. The divided samples had the color removed by using sterile GAC in 3 amounts (100, 200 and 300 mg per samples 1 mL) and 2 contact times (30 and 60 minutes). When sterile GAC in each amount was soaked in samples (1 mL) for 30 and 60 minutes, SI medium (6 mL) was added in the glassware which contained the non-carbonated soft drinks mixed with GAC. After that, these non-carbonated soft drinks in which was detected coliform bacteria by SI medium were preserved at room temperature for 17 and 24 hours. The test results were determined by comparing with the color indicator sheet of SI medium which was referenced from the Research and Laboratory Development Center, Department of Health, Ministry of Public Health 2006. The suitable GAC amount and contact time of each color tone was selected from GAC amount and contact time, removed color, and caused the color of SI medium to change to yellow color for a positive result but was still purple color for a negative result within 24 hours. The best suitable GAC amount and contact times were also the least values of the suitable GAC amount and contact times.

(3.2) The additive data for finding validity of coliform bacteria detection by SI medium occurred from the coliform bacteria detection of the second and third sets which used the best suitable GAC amount and contact times (from the results of the first set) for color removal of soft drinks.

Analyzing the data

Descriptive statistics was used to calculate the percent color removal efficiency from Part 1 experimental data, and then analyzed as the mean percentages and standard deviations for their combination of color tones of non-carbonated soft drinks, amounts of GAC and contact times. Part 2, experimental data, were analyzed to find the validity (percentage of sensitivity value, specificity value, positive predictive value, negative predictive value and test efficiency value) of coliform bacteria detection by SI medium in removed-color non-carbonated soft drinks when compared with the standard MPN method.

Inferential statistics, as the analysis of variance (ANOVA), was performed in the factorial experiment of 3 variables: color tones of non-carbonated soft drinks, amounts of GAC and contact times. The results of ANOVA table were assigned at $\propto$ level of 0.05.

Results

Part 1: The efficiency of non-carbonated soft drink color removal by using granular activated carbon (GAC) at different conditions

Regarding the comparisons of color removal efficiencies in non-carbonated soft drinks which had 6 color tones (red, green, purple, blue, orange and yellow) by using different GAC amounts (100, 200 and 300 mg/mL), the results showed that the higher amount of GAC gave more color removal efficiency than the lower amount of GAC follow as Figure 1 (Here below paragraph 1 of Part 1 in Results item). GAC amounts of 100, 200 and 300 mg/mL affected different color removal efficiencies at the level of significance 0.05 (*p-value* < 0.05). Furthermore, the increase of GAC amount affects the increase of pH value of non-carbonated soft drinks according to the alkalinity property of GAC. The study results showed that using GAC amount up to 300 mg/mL in the soft drinks gave pH values less than 9.

The study concerns the color removal efficiencies by using different contact times as 30 and 60 minutes in color removal of non-carbonated soft drinks by GAC, showed that the longer contact time of color removal gave more color removal efficiency than the shorter contact time of color removal follow as Figure 1 (Here below paragraph 1 of Part 1 in Results item). 30 and 60 minutes of contact times for color removal of the soft drinks affected different color removal efficiencies at level of significance 0.05 (*p-value* < 0.05).

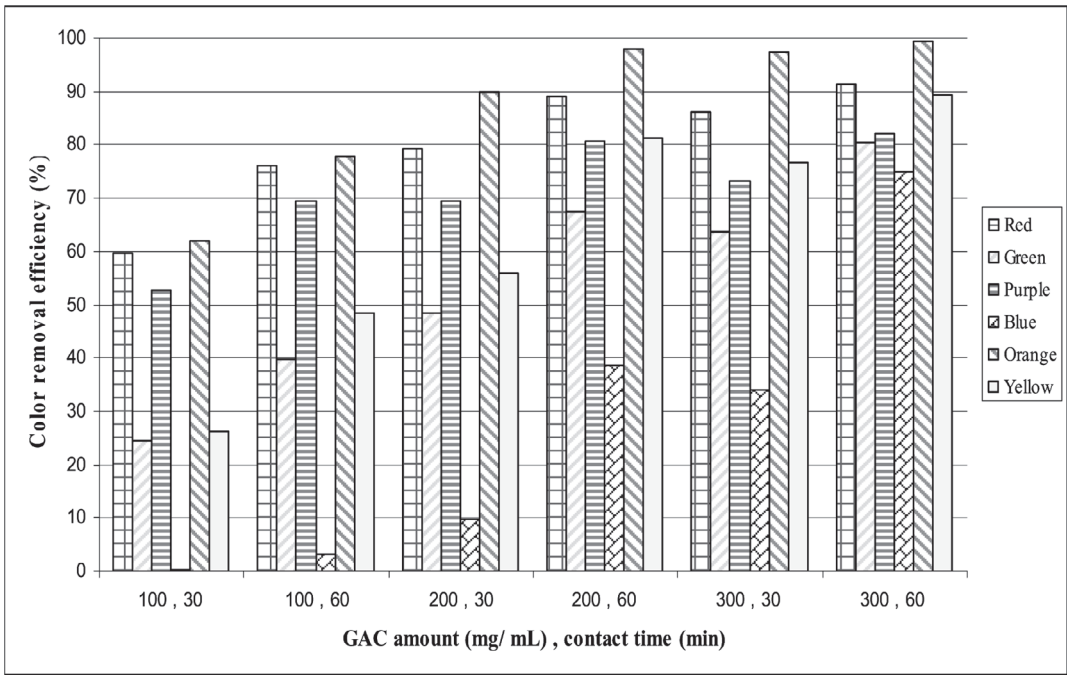

Figure 1 Color removal efficiency (%) of non-carbonated soft drinks at different conditions

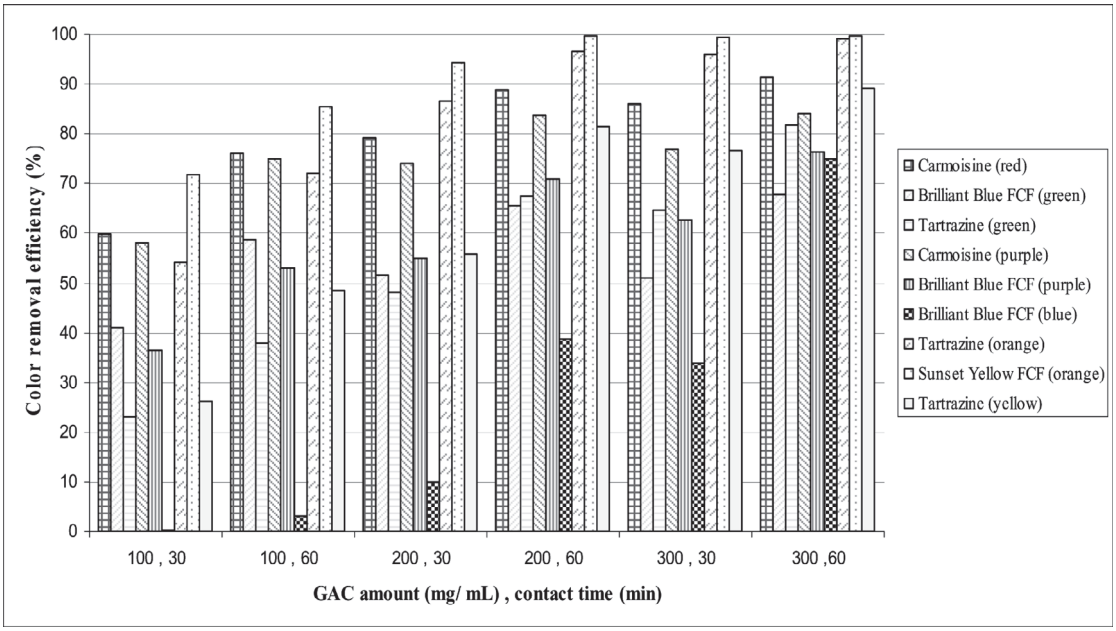

Figure 2 Color removal efficiency (%) of food colorants in non-carbonated soft drinks at different conditions

The efficiency of non-carbonated soft drink color removal when using different color tones at the same GAC amount and contact time showed that the different soft-drink color tones gave different color removal efficiencies follow as Figure 1 (Here below paragraph 1 of Part 1 in Results item) at level of significance 0.05 (*p-value* < 0.05). The orange color-tone soft drink gave the highest color removal efficiency, but the blue color-tone soft drink gave the lowest color removal efficiency. Moreover, each color tone of the soft drinks was prepared from one or two food colorants which were presented in Table 1 (Here below paragraph 3 of Part 1 in Results item). Food colorants of the soft drinks have 4 types, i.e., Carmoisine or Azorubine, Tartrazine, Brilliant Blue FCF and Sunset Yellow FCF. When color removal efficiencies in the soft drinks were considered according to food-colorant types, they decreased in a sequence as Sunset Yellow FCF (first rank), Carmoisine (second rank), Tartrazine (third rank) and Brilliant Blue FCF (fourth rank) which were shown in Figure 2 (Here below Table 1).

Table 1 The components of food colorants in non-carbonated soft drinks

Color tones	The components of food colorants (mg/ kg)	Amount in soft drinks	Percentages in soft drinks (%)
1. Red	Carmoisine or Azorubine	40.3	100.00
2. Green	Tartrazine	30.3	91.54
	Brilliant Blue FCF	2.8	8.46
3. Purple	Carmoisine or Azorubine	15.8	75.24
	Brilliant Blue FCF	5.2	24.76
4. Blue	Brilliant Blue FCF	30.7	100.00
5. Orange	Tartrazine	30.4	56.82
	Sunset Yellow FCF	23.1	43.18
6. Yellow	Tartrazine	54.6	100.00

Part 2: The validity of coliform bacteria detection by SI medium for removed-color non-carbonated soft drinks compared with the standard MPN method

Total studied non-carbonated soft drinks of 126 samples had color removed by the best suitable GAC amount and contact time for each color tone. The best suitable GAC amount and contact time of red color-tone soft drinks were 200 mg/mL of GAC amount at 30 minutes of contact time, green color-tone soft drinks were 200 mg/mL of GAC amount at 30 minutes of contact time, purple color-tone soft drinks were 200 mg/mL of GAC amount at 30 minutes of contact time, blue color-tone soft drinks were 300 mg/mL of GAC amount at 60 minutes of contact time, orange color-tone soft drinks were 200 mg/mL of GAC amount at 30 minutes of contact time and yellow color-tone soft drinks were 300 mg/mL of GAC amount at 30 minutes of contact time, which gave color removal efficiencies as 79.30%, 48.50%, 69.45, 74.92%, 89.87% and 76.63%, respectively. These results showed that each color tone of the soft drinks had different best suitable GAC amounts, with contact times and color removal efficiencies for coliform bacteria detection by SI medium.

After each color tone of non-carbonated soft drinks had been removed, these soft drinks were taken to detect the coliform bacteria by SI medium compared with the standard MPN method. The coliform-bacteria detection results within 24 hours by SI medium being compared with the standard MPN method are shown in Table 2 (Here below paragraph 2 of Part 2 in Results item). Data in the Table 2 were calculated the validity as follows : sensitivity value as 86.11% [93/ (93+15) x 100], specificity value as 100% [18/ (18+0) x

100], positive predictive value as 100% [93/(93+0) x 100], negative predictive value as 54.55% [18/(18+15) x 100] and efficiency of test value as 88.10% [(93+18)/ 126 x 100].

Table 2 Test results of coliform bacteria detection in removed-color non-carbonated soft drinks preserved in room temperature within 24 hours

Results from MPN method (Standard)	Results from SI medium		Total
	+ (Positive)	- (Negative)	
+ (Positive)	93 (TP)	15 (FN)	**108**
- (Negative)	0 (FP)	18 (TN)	**18**
Total	**93**	**33**	**126**

Note : TP = True Positive, FP = False Positive, FN = False Negative, TN = True Negative

Discussion

Part 1 : The efficiency of non-carbonated soft drink color removal by using granular activated carbon (GAC) at different conditions

The study results showed that the higher amount of GAC gave more color removal efficiency than the lower amount of the carbon, which is in agreement with the related researches of Sumarin and Wongraweekul, and Prayurnprohm.[11-12] The theory about the factors affecting adsorption mentioned that the increase of adsorbent surface area can affect the increase of the adsorption capacity.[13] Because the increase of GAC amount causes the increase of surface area in GAC, the increase of GAC amount can also affect the increase of removal efficiency of non-carbonated soft drink colors. The GAC amounts within 300 mg/mL used in this study gave pH values of less than 9 as the accepted values, because these pH values result in normal growth of bacteria.[14] Thus, GAC amounts up to 300 mg/mL do not disturb the growth of bacteria in the soft drinks, and can be used to remove the soft drink color for coliform bacteria detection by SI medium.

The results of this study showed that the longer contact time of color removal gave more color removal efficiency than the shorter contact time of the removal and this is in agreement with the related researches of Sumarin and Wongraweekul, and Purkait et al.[11,15] The adsorption process for soft-drink color removal has the dynamic for transport which involves three consecutive steps : 1) adsorbate (color) is transported through a surface film onto the exterior of adsorbent (GAC), 2) adsorbate (color) is diffused inside of the pore and/or the pore wall surface of adsorbent (GAC), and 3) adsorbate

(color) is attached onto an available interior site of adsorbent (GAC). In most cases of activated carbon (including GAC), the rate of organics (colors) adsorption is controlled by step one and two.[16]

The study results concerning color tones of soft drinks showed that the different soft-drink color tones affected the different color removal efficiencies. Adsorption theory was mentioned that the solubility factor of adsorbate (food colorant causes color tone in soft drink) and can affect adsorption capacity. The high solubility that presents a strong solute-solvent interaction can indicate a decrease of adsorption capacity, because the solute-solvent interaction can break its bond before the occurrence of the adsorption process.[13] Moreover, food colorants could be classified by their chemical structure in terms of sulfonic acid group (-SO3H). Their degrees of water solubility are determined by the number of sulfonic acid groups present and their positions in the molecule.[6,17] The color removal efficiency will decrease when this food colorant has a higher number of sulfonic acid groups and the suitable position in the molecule (more solubility). Brilliant Blue FCF (food colorant of blue color-tone soft drink) is classified as Triphenylmethane dye which has 3 sulfonic acid groups, and provides excellent solubility.[6,17] This characteristic of Brilliant Blue FCF caused the lowest color removal efficiency. Sunset Yellow FCF (food colorant of orange color-tone soft drink) which had the highest color removal efficiency and Carmoisine are classified as Sulfonated dye of Azo color that have the character of two sulfonic acid groups.[6,17] This characteristic of Sunset Yellow FCF and Carmoisine affected color removal efficiency more than Brilliant Blue FCF. Sunset Yellow FCF had more color removal efficiency than Carmoisine, and may occur because Sunset Yellow FCF has a suitable position in the molecule, lower than Carmoisine has. The Tartrazine that is in Pyrazolone dyes and has two sulfonic acid groups may be classified as azo color, which is similar to Sunset Yellow FCF and Carmoisine.[6,17] Tartrazine may have a suitable position in the molecule higher than Carmoisine. Thus, Tartrazine provides less color removal efficiency than Carmoisine.

Part 2 : The validity of coliform bacteria detection by SI medium for removed-color non-carbonated soft drinks compared with the standard MPN method

Regarding the validity of coliform bacteria detection by SI medium for removed-color non-carbonated soft drinks this study showed that sensitivity value, specificity value and efficiency of test more than 80% are the same as the study of Research and Laboratory Development Center, Department of Health, Ministry of Public Health 18. Thus, the overall study results concerning using of GAC in the studied conditions for removal of soft drink colors showed that the use of GAC does not affect the original validity of coliform bacteria detection by SI medium.

The best suitable GAC amount and contact time for color removal of 6 color-tone soft drinks in this study can be applied with intense-

color non-carbonated soft drinks which are prepared from finished syrup with water at a ratio of 1 : 4 and at a ratio of water to finished syrup of more than 4 times. However, the used GAC amount for soft-drink color removal of each color tone should not be more than the studied amount because the greater GAC amount may cause more adsorption of SI medium color and then the resultant interpretation is not clear.

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การกำจัดสีในเครื่องดื่มไร้คาร์บอเนตโดยใช้ถ่านกัมมันต์ชนิดเกล็ด เพื่อการตรวจโคลิฟอร์มแบคทีเรียด้วยอาหารตรวจเชื้อที่เป็นตัวชี้วัดด้านสุขาภิบาล

เนาวรัตน์ ศรีสันติแสง* พิศิษฐ์ วัฒนสมบูรณ์**
เพียงจันทร์ โรจนวิภาต*** พิพัฒน์ ลักษมีจรัลกุล****

บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาประสิทธิภาพการกำจัดสีในเครื่องดื่มไร้คาร์บอเนตโดยใช้ถ่านกัมมันต์ชนิดเกล็ดเพื่อการตรวจโคลิฟอร์มแบคทีเรียด้วยอาหารตรวจเชื้อที่เป็นตัวชี้วัดด้านสุขาภิบาล (SI medium) สีของเครื่องดื่มไร้คาร์บอเนต คือ แดง, เขียว, ม่วง, ฟ้า, ส้ม และเหลือง ถูกกำจัดสีออกด้วยถ่านกัมมันต์ชนิดเกล็ด ปริมาณ 100, 200 และ 300 มิลลิกรัมต่อเครื่องดื่มไร้คาร์บอเนต 1 มิลลิลิตร ที่ระยะเวลากำจัดสีเครื่องดื่ม 30 และ 60 นาที ผลการศึกษาพบว่า ปริมาณของถ่านกัมมันต์ที่สูงกว่า และการเพิ่มระยะเวลากำจัดสี ส่งผลให้ประสิทธิภาพการกำจัดสีมากกว่าอย่างมีนัยสำคัญทางสถิติ (p-value < 0.05) รวมทั้งโทนสีของเครื่องดื่มที่แตกต่างกัน ส่งผลให้ประสิทธิภาพการกำจัดสีที่แตกต่างกันอย่างมีนัยสำคัญทางสถิติ (p-value < 0.05) ประสิทธิภาพการกำจัดสีมากที่สุดที่เกิดจากการใช้ถ่านกัมมันต์ปริมาณ 300 มิลลิกรัมต่อเครื่องดื่ม 1 มิลลิลิตร ที่ระยะเวลากำจัดสี 60 นาที ไม่ใช่สภาวะที่เหมาะสมที่สุดในทุกโทนสีของเครื่องดื่ม แต่สภาวะที่เหมาะสมที่สุดในแต่ละโทนสีของเครื่องดื่มถูกเลือกจากปริมาณถ่านกัมมันต์และระยะเวลาที่ใช้น้อยที่สุดที่กำจัดสีเครื่องดื่มและส่งผลให้สีของ SI medium เปลี่ยนเป็นสีเหลือง (กรณีที่ตรวจพบโคลิฟอร์ม) และยังคงเป็นสีม่วง (กรณีตรวจไม่พบโคลิฟอร์ม) ภายใน 24 ชั่วโมง คือ การใช้ถ่านกัมมันต์ ปริมาณ 200 มิลลิกรัมต่อเครื่องดื่ม 1 มิลลิลิตร ที่ระยะเวลา 30 นาที เหมาะสมที่สุดสำหรับเครื่องดื่มสีแดง-เขียว-ม่วง-ส้ม, การใช้ถ่านกัมมันต์ ปริมาณ 300 มิลลิกรัมต่อเครื่องดื่ม 1 มิลลิลิตร ที่ระยะเวลา 30 นาที เหมาะสมที่สุดสำหรับเครื่องดื่มสีเหลือง และการใช้ถ่านกัมมันต์ปริมาณ 300 มิลลิกรัมต่อเครื่องดื่ม 1 มิลลิลิตร ที่ระยะเวลา 60 นาที เหมาะสมที่สุดสำหรับเครื่องดื่มสีฟ้า เมื่อหาค่าความถูกต้อง(validity) ของการตรวจโคลิฟอร์มแบคทีเรียด้วย SI medium ในเครื่องดื่มที่ถูกกำจัดสีออกด้วยสภาวะที่เหมาะสมที่สุด เปรียบเทียบกับวิธีมาตรฐาน MPN ได้ค่า sensitivity, specificity และ efficiency of test มากกว่า 80% แสดงให้เห็นว่าถ่านกัมมันต์ชนิดเกล็ดสามารถนำไปใช้กำจัดสีของเครื่องดื่ม เพื่อการตรวจโคลิฟอร์มแบคทีเรียด้วย SI medium ได้อย่างมีประสิทธิภาพ

คำสำคัญ: เครื่องดื่มไร้คาร์บอเนต, กำจัดสี, ถ่านกัมมันต์ชนิดเกล็ด, อาหารตรวจเชื้อ, ตรวจโคลิฟอร์มแบคทีเรีย

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* นักศึกษาหลักสูตร วทม. (สุขาภิบาลสิ่งแวดล้อม) คณะสาธารณสุขศาสตร์ มหาวิทยาลัยมหิดล
** ภาควิชาวิทยาศาสตร์อนามัยสิ่งแวดล้อม คณะสาธารณสุขศาสตร์ มหาวิทยาลัยมหิดล
*** ภาควิชาชีวสถิติ คณะสาธารณสุขศาสตร์ มหาวิทยาลัยมหิดล
**** ภาควิชาจุลชีววิทยา คณะสาธารณสุขศาสตร์ มหาวิทยาลัยมหิดล