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Metabolic Effect of Dietary Erythritol Intake on *Escherichia coli*

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Abstract

Artificial sweeteners are man-made substitutes for diet that needs low sugars or caloric intake. Recent studies have shown that AS consumption is possibly associated with metabolic disturbances and intestinal flora disturbances. Erythritol is a kind of 4-carbon sugar substitute in the form of sugar alcohol, which may contribute to the prevention of gingivitis by inhibit the biofilm formation of oral bacteria. Despite these studies, the data on how erythritol affects commensal flora causing pathogenicity to remain limited. In this study, microbiota (*Escherichia coli*) models were used to investigate the effects of different concentration of erythritol on the metabolism of bacteria, especially on *Escherichia coli*'s growth, transformation efficiency and hemolytic activity. The results showed that sweeteners decreased the bacteria's ability to normally grow in higher concentration, and form biofilms to varying degrees. And the addition of erythritol in low concentration may have an effect of promote the growth of *Escherichia coli*. Habitual consumption of artificial sweeteners in the diet continues to increase was associated with the maintenance of micro-ecological equilibrium in gut.

Keywords

Artificial Sweetener, Erythritol, Hemolytic Activity, *Escherichia coli*

1. Introduction

Erythritol is a kind of 4-carbon sugar substitute in the form of sugar alcohol, most of them function as low-calorie nonnutritive sweetener (NNS) in sugar-free or low-sugar products. It has 60~80% of sweetness that sucrose has, and it has characteristics such as high stability in multiple power of hydrogen condition and heat, safe due to no cariogenic potential and low glycemic index. Because of the way these molecules are structured, they can stimulate the sweet

taste receptors on your tongue. Additionally, erythritol is considered safe for consumption as it does not contribute to tooth decay (cariogenic potential) and has a low glycemic index, making it suitable for individuals concerned about blood sugar levels.

Thus, erythritol has been recently considered as a natural sugar substitute much healthier than other artificial sweeteners, and added to lots of food, since small amount of erythritol is present in nature. Erythritol is present in a range of fruits, including melon, watermelon, pears, and grapes, as well as in fermented foods like cheese and soy sauce [1]. Mammals, including humans, can also produce endogenous erythritol by pentose-phosphate pathway [2]. It is produced by enzymatic hydrolysis of corn or wheat starch to produce glucose, fermented by safe and suitable food-grade osmotic yeast. Once separated from the fermentation broth, erythritol is purified to a crystalline product of greater than 99% purity. Its main uses include confectionery, chewing gum, beverages, and baking products as non-nutritive sweetener, flavor enhancer, stabilizer, and thickener [1] [3]. According to Kannan and Baseman [4], we can determine that the intake of 0%, 2%, 5%, and 10% (1.0, 2.6, and 5.4 gm/kg/day to females and 0.9, 2.2, and 4.6 gm/kg/day to males) erythritol solution does not affect the basic metabolism of rats, and they examine no relation in nephrotoxicity, tumor-inducing or tumor-promoting changes. Its uses include The United States Food and Drug Administration (FDA) approved erythritol as trusted source for use as a food additive in the U.S. in 2001.

But lots of study have shown the side effects of artificial sweeteners (including aspartame, sucralose, and even xylitol which is also sugar alcohols) [5] [6], the previous work on these artificial sweetener shows that in high concentration, some artificial sweeteners, may cause bacteriostatic effects on *Escherichia coli* (*E. coli*) and affects its biofilm formation, overall, increasing the pathogenicity of *E. coli*. According to the research of Ruiz-Ojeda *et al*. [7], saccharin, sucralose (NNSs), and stevia (natural sweetener) have demonstrated alterations in gut microbiota, while more research is needed to fully understand the impact of sweeteners on the human gut microbiome.

However, it is important to note that concerns about the potential harmful effects of erythritol persist. For instance, certain studies have suggested that erythritol might have pesticidal properties due to its toxicity to *drosophila*, which raises questions about its impact on other organisms and the environment [8]. Additionally, a study on adolescents in 2017 proposed a potential association between erythritol derived from the internal metabolism of glucose and an increased risk of being overweight [2].

A recent study, published in the journal Nature Medicine in 2023, found that the common artificial sweetener erythritol was associated with an increased risk of heart attack and stroke. Researchers studied more than 4,000 Americans and Europeans and found that those with higher blood levels of erythritol had an increased risk of heart attack, stroke, or death. Laboratory and animal studies have

further found that erythritol seems to make it easier for platelets to activate and form blood clots. These clots can break off and travel to the heart, causing a heart attack, or to the brain, causing a stroke. This research has drawn attention to erythritol, and some experts recommend moderate dietary restriction of erythritol until more research is done.

However, there is also a study from 2023 that indicates that erythritol may have beneficial effects on the body. This study reviewed the safety, production, metabolism and health effects of erythritol. Studies have found that dietary erythritol has no effect on blood sugar and insulin, and induces the secretion of intestinal hormones, regulates satiety, and promotes weight loss. Long-term rodent studies have shown that erythritol intake can reduce body weight or fat content. However, observational studies have shown that plasma erythritol is positively associated with obesity and cardiometabolic disease. It is currently thought that these associations may be related to the impairment of glucose metabolism in the body or the glycolysis pathway induced by high-sugar diet. However, long-term clinical trials are needed to investigate the effects of long-term erythritol intake on body weight and metabolic disease risk [1].

To gain a comprehensive understanding of the metabolic effects of erythritol, further research is warranted. This ongoing investigation will enable a clearer and more informed assessment of the compound's overall impact on human health. By uncovering the potential benefits and risks associated with erythritol consumption, this research will empower dietitians and consumers to make well-informed decisions about incorporating erythritol into their diets. Ultimately, such knowledge will contribute to the development of healthier dietary practices and ensure the responsible and safe use of erythritol as a sugar substitute. Further studies should aim to explore erythritol's effects on various physiological processes, its interactions with gut bacteria, and its potential implications for metabolic health to provide a more comprehensive understanding of this chemical compound's effects on human well-being.

Escherichia coli is a type of bacteria normally lives in people's intestines, also present in human's digestive tract and oral cavity that creates multiple effects on human's metabolism. From a technical standpoint, this species can be easily isolated, cultured, and maintained in the laboratory, and is often used as a potential human fecal indicator. Therefore, *Escherichia coli* was used as a model to test our hypothesis whether artificial sweeteners at levels physiologically attainable in the small intestine would negatively affect bacteria in the gut microbiome. Its close relationship with human has made experiments done on *E. coli* can be an indication of potential pathogenic mechanism that undergoes in microbiota and may indirectly linked to the side effects erythritol may have on human's intestinal health. Janus *et al.* [9] found that erythritol reduces the biofilm formation of gingivitis bacteria such as *P. gingivalis* and *S. gordonii*. Thus, this work can be an initial exploration of whether erythritol is healthy for human's digestive function because its effect on human's intestinal flora, providing new insights into

erythritol and make more rational addition and use in food. To predict that over-consuming erythritol has the possibility to present side effects.

This study aims to provide specific approach to the metabolism of *E. coli* such as growth, hemolytic activity, and transformation efficiency, to see if different concentrations would affect these sides of metabolic activity, providing more understanding to the effect of erythritol on intestinal bacteria.

2. Materials and Methods

2.1. Growth Curve Assay

Bacterial Glycogen samples were retrieved from a −80˚C refrigerator and allowed to naturally dissolve at room temperature. The samples were thoroughly shaken to ensure uniformity. Next, 10 mL of culture medium with varying concentrations of erythritol (0%, 5%, 10%, and 20%) was inoculated with the Glycogen bacteria at a 1:1000 ratio. The cultures were incubated at 37˚C and 200 rpm for 2 days. Every 12 hours (0, 12, 24, 36, and 48 hours), 1000 μL samples were collected and transferred to a cuvette for spectrophotometric measurement of absorbance at 600 nm (OD600). The absorbance of the solution was measured three times using a spectrophotometer, with LB medium as a control to set the zero absorbance.

2.2. Hemolytic Activity Assay

Monoclonal bacterial groups from the Glycogen stock were retrieved from the −80˚C refrigerator, naturally dissolved, and inoculated at a 1:1000 ratio into medium containing various concentrations of erythritol (0%, 2.5%, 5%, 10%, and 20%) with LB medium. After 24 hours of incubation, a certain volume of the culture was used to streak blood agar plates and incubated overnight. Images of the blood agar plates were captured using a camera, and semi-quantitative analysis was performed using ImageJ software.

2.3. Transformation Efficiency Assay

Dh5*α* competent cells were retrieved from the −80˚C freezer and rapidly placed on ice. After thawing, the cells were added to a mixture and left on ice for 25 minutes. A heat shock was performed in a water bath at 42˚C for 45 seconds, followed by immediate cooling on ice for more than 3 minutes to minimize the reduction of transformation efficiency due to shaking. The cells were then recovered in 700 μL of LB medium at 37˚C and 200 rpm for 60 minutes. After centrifugation at 5000 rpm for 1 minute, the bacterial cells were collected, and approximately 200 μL of the resuspended bacteria was plated on LB agar containing antibiotics (ampicillin 50 μg/mL) and erythritol at various concentrations (0%, 2.5%, 5%, 10%, and 20%). The plates were placed upside down and incubated at 37˚C for 12 - 16 hours overnight. Subsequently, ImageJ software was used to calculate and analyze the gray area on the plates. After the transformation process, individual bacterial colonies were picked from each plate and

inoculated into 10 mL of LB medium. The optical density of the bacterial growth was measured at 0 h, 4 h, 8 h, 12 h, and 24 h.

3. Results

In the experiment, different concentrations of erythritol were used to investigate its effects on bacterial growth and physiological characteristics. By employing varying concentrations of erythritol, researchers aimed to assess its impact on bacterial growth rate, metabolic activity, as well as its potential for toxicity or beneficial effects. There is no current limited content for erythritol, however current recommendation of erythritol content are no more than 20%. The concentration range studied included 0%, 2.5%, 5%, 10%, and 20% to cover different physiological conditions, enabling researchers to understand how erythritol functions and its potential effects on bacteria. Conducting experiments with these concentrations helps gain a better understanding of erythritol's role in bacterial growth and metabolism, laying the foundation for further research.

3.1. Growth Curve

This experiment used the measurement of optical density of each sample to plot the growth curve, as an indication of the growing ability of bacteria. After exposure to different concentrations of artificial sweeteners erythritolfor an extended period of 48 hours, the effects of these artificial sweeteners on the growth of Escherichia coli in floating cultures were measured at regular 12-hour intervals.

A series of experiments conducted with *E. coli* revealed that exposure to erythritol at any measured point in time or any tested concentration had significant impact on the normal growth of these bacteria over the course of the experiment (**Figure 1**).

The mean optical density was lower with higher concentrations of erythritol, but interestingly, when theerythritol concentration was low (2.5%), the optical density was higher than the control group. Due to the increasing value of 2.5% to 5%, in contrast, exposure to a high concentration at 10% and 20% display a decreasing tide of the optical density (**Figure 1**). A one-way ANOVA test for differences in means across independent variables was made. Here, the independent variable is concentration of erythritol (with 4 levels and one control). And the dependent variable is the absorbance of the *E. coli* sample. The analysis found a significant interaction effect between time, but a not significant interaction between the concentration of erythritol.

We tested the sample's absorbance on a fixed period. The one-way ANOVA results about the erythritol's effect on *E. coli*'s growth curve (**Figure 1**) suggest that erythritol concentration does not have a significant impact on *E. coli*'s growth optical density. The findings have practical implications for food safety and microbiology research. Further studies are needed to explore the mechanisms underlying the effect of erythritol concentration on *E. coli* growth. As shown in **Figure 1**, the association was observed between erythritol concentration and

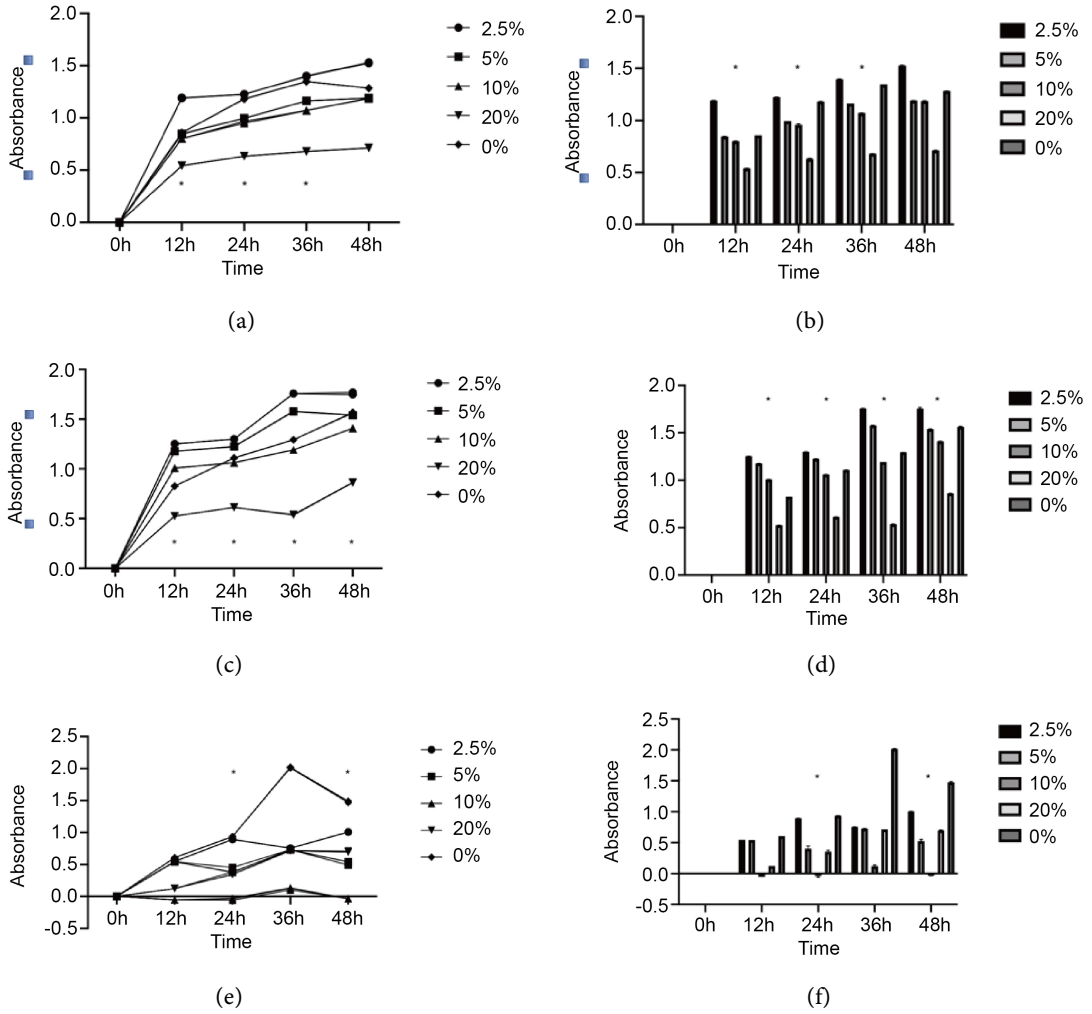


Figure 1. The growth curve of each experiment trial. (a) and (b), (c) and (d), (e) and (f) represent three repetition groups. The horizontal axis represents the time of the measurement and the vertical axis represents the absorbance of the *E. coli* sample), There is a significant difference overall in group 3's (e) and (f) 10% sample and 0% sample ($p = 0.0119 < 0.05$). And in group 1 ((a) and (b)), all the samples have significant difference in 12 h, 24 h, 36 h ($p < 0.0001$); in group 2 ((c) and (d)), all the samples have significant difference in 12 h, 24 h, 36 h, 48 h ($p < 0.0001$); and in group 3 ((e) and (f)), all the samples have significant difference in 24 h, 48 h ($p < 0.0001$).

E. coli ($p = 0.5062 > 0.05$ in **Figure 1(a)** and **Figure 1(b)**, $p = 0.3967 > 0.05$ in **Figure 1(c)** and **Figure 1(d)**, but $p = 0.0228 < 0.05$ in **Figure 1(b)**).

The control group represents the bacterial growth in the absence of erythritol. It serves as a baseline to compare the effects of different erythritol concentrations. As expected, the optical density of the control group increased steadily over time, representing the normal growth of *E. coli* in the absence of any external factors. At a low concentration of erythritol (2.5%), the optical density was higher than the control group, indicating that *E. coli* exhibited enhanced growth compared to the control. This observation suggests that at this concentration, erythritol may have a beneficial effect on *E. coli* growth, possibly acting as a growth enhancer or a nutrient source. At intermediate concentrations of eryt-

hritol (5% and 10%), the optical density exhibited a decreasing trend compared to the control. This suggests that higher concentrations of erythritol start to negatively affect *E. coli* growth, leading to reduced optical density over time.At the highest concentration of erythritol (20%), the optical density showed a sharp decrease, indicating that *E. coli* growth was severely inhibited at this concentration. High concentrations of erythritol might have toxic effects on the bacteria, leading to decreased growth or even cell death. The comparison with the control group highlights the differences in bacterial growth under different erythritol concentrations. The control group's growth curve serves as a reference to evaluate the effects of erythritol on *E. coli*. By comparing the optical density at different time points with the control group, researchers can identify how erythritol influences bacterial growth over time.

Upon analyzing the various samples at specific time intervals, it was observed that a notable difference existed between the samples in group 2 and group 3. This observation was substantiated by numerous p values recorded within a defined time frame, where the p values were found to be less than 0.05, indicating significant differences between the groups. However, when considering all the five examples collectively over a period of an hour, a slight similarity in trends was observed. Thus, the application of the multiple comparison of two-way ANOVA could prove beneficial in this scenario, whereas the use of the multiple comparison of one-way ANOVA would not be adequate, as it does not permit a comparison of sample differences at specific time intervals. Upon conducting one-way ANOVA, it was evident that there was no significant difference between the absorbance values in each group. This finding was reinforced by the p-value, which was greater than 0.05. Conversely, two-way ANOVA was found to have a higher likelihood of obtaining a p-value less than 0.05.

3.2. Hemolytic Activity

The hemolytic activity experiment measured the normal ability of bacteria to release hemolysin, which is a protein that can cause the red blood cell (RBS) to swell and burst in the Hypotonic solution and release the hemoglobin into the environment. Measuring this normal activity will provide another measurement to the bacteria stability and normal metabolism. In this experiment, we tested *E. coli*'s hemolytic activity after growing in solutions containing different concentration of Erythritol.

The results from the semiquantitative analysis were shown in **Figure 2**.

The analysis of the data obtained from the experiment involved plotting a concave curve using the grey values. Furthermore, a one-way ANOVA test was conducted using the same grey values, which revealed a statistically significant difference between the mean of each group. The test yielded a P value less than 0.0001, indicating that the concentration of erythritol has a substantial and meaningful impact on *E. coli*'s hemolytic activity.

The significant difference observed between the groups suggests that erythritol

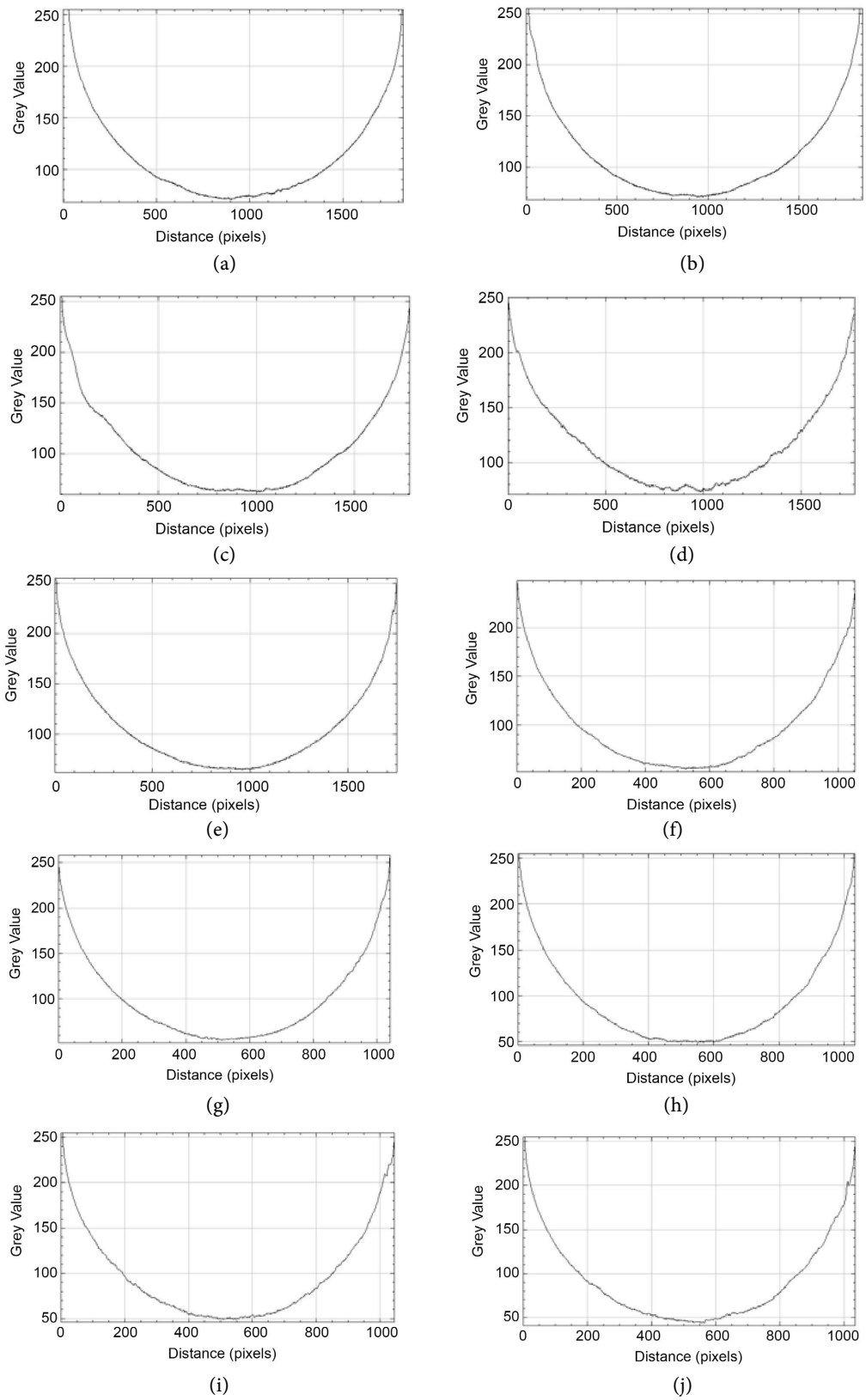


Figure 2. The gray value calculated using ImageJ Software (the concentration of erythritol is represented as the following: (a) and (f): 2.5% erythritol; (b) and (g) 5% erythritol; (c) and (h) 10% erythritol; (d) and (i) 20% erythritol; (e) and (j) 0% erythritol).

may act as a potential inhibitor of *E. coli*'s hemolytic activity. This finding is particularly important because hemolytic activity is a critical virulence factor in many pathogenic strains of *E. coli*. By inhibiting the release of hemolysin, erythritol may play a role in reducing the bacteria's pathogenicity and virulence.

The potential inhibitory effects of erythritol on *E. coli*'s hemolytic activity could be attributed to various mechanisms. Erythritol may interfere with the expression of genes involved in hemolysin production or affect the synthesis of the hemolysin protein itself. Additionally, erythritol might interfere with the interaction between the hemolysin and host cells, thereby preventing hemolysis and subsequent damage to RBCs.

The current experiment demonstrated that erythritol at various concentrations inhibited *E. coli*'s hemolytic activity. These contrasting findings may be attributed to differences in bacterial species, experimental conditions, or the specific virulence factors involved.

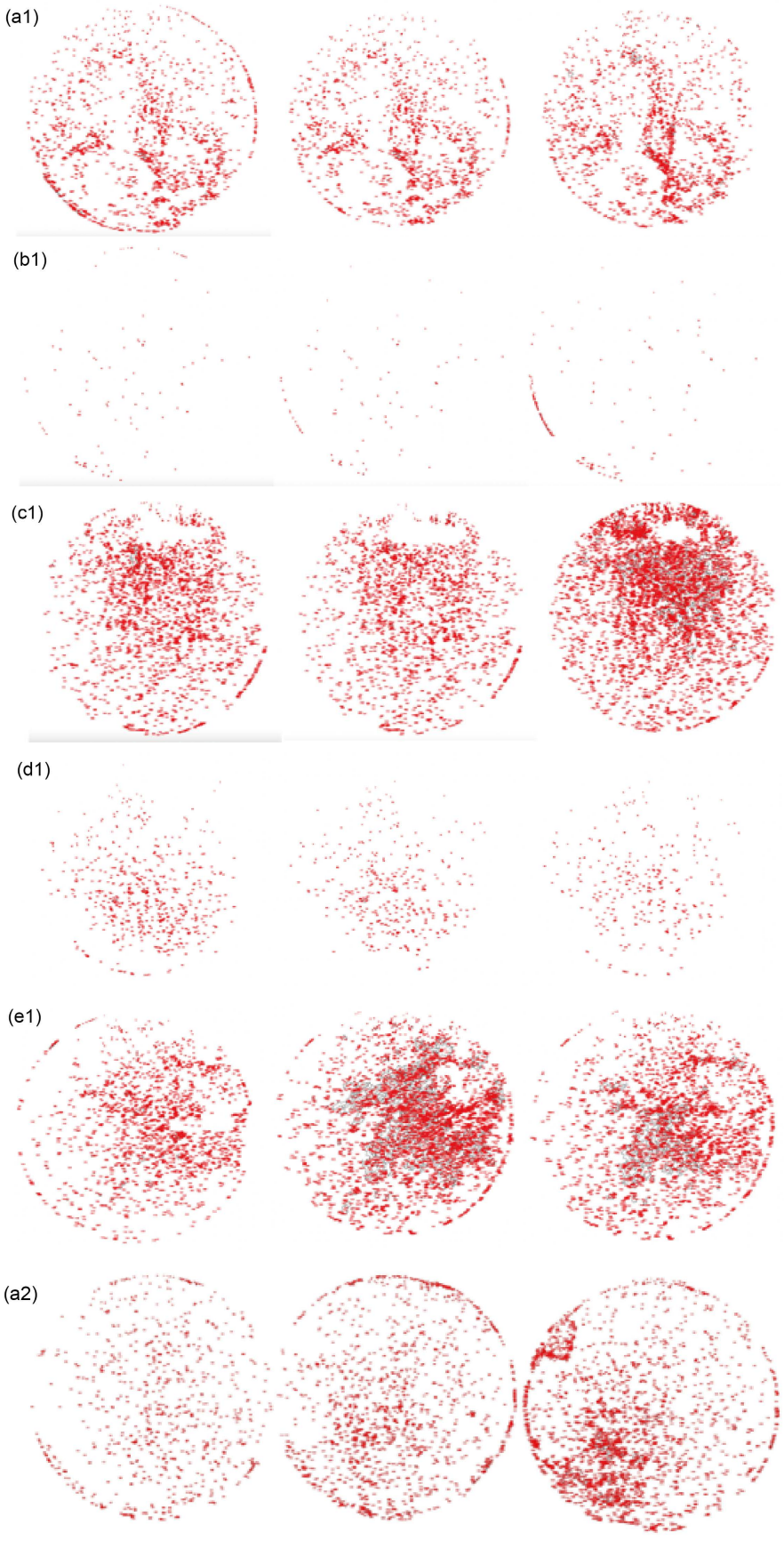
Regarding *E. coli*'s metabolism, the results indicate that erythritol's presence affects the expression or activity of factors related to hemolysin production. Hemolysin is a crucial component of *E. coli*'s pathogenicity, enabling the bacteria to damage host cells and contribute to the progression of infections. Erythritol's inhibitory effects on hemolytic activity may be linked to its impact on the regulation of genes or proteins involved in hemolysinsynthesis and release.

Overall, these findings have significant implications for the development of novel antimicrobial strategies targeting pathogenic *E. coli* strains. Understanding erythritol's inhibitory effects on hemolytic activity can aid in the design of therapeutic approaches that specifically disrupt the bacteria's virulence factors without promoting resistance development. Future research could delve deeper into the underlying mechanisms of erythritol's inhibitory effects and explore its potential applications in combating *E. coli* infections with hemolytic activity.

3.3. Transformation Efficiency

Bacteria transformation is the efficiency of transforming extracellular DNA and expressing it in the bacteria. It is an essential technique for prepare it to cloning and make protein expression and application using the DH5acompetent cells to import the DNA into the bacteria. The results from the semiquantitative analysis were shown in **Figure 3**.

The observed inhibition of bacterial growth by erythritol appeared to be concentration-dependent, as evidenced by the varying colony sizes and densities on the different petri dishes. Larger colony sizes are indicative of higher transformation efficiency, implying that erythritol's inhibitory effects may also extend to *E. coli*'s transformation efficiency. At lower erythritol concentrations, there seemed to be a disruption of membrane homeostasis and an effect on the membrane permeability towards the plasmid DNA, leading to decreased transformation efficiency (**Figure 3**). The results of this study align with previous research findings that suggest erythritol has the potential to inhibit bacterial growth.


(a1)
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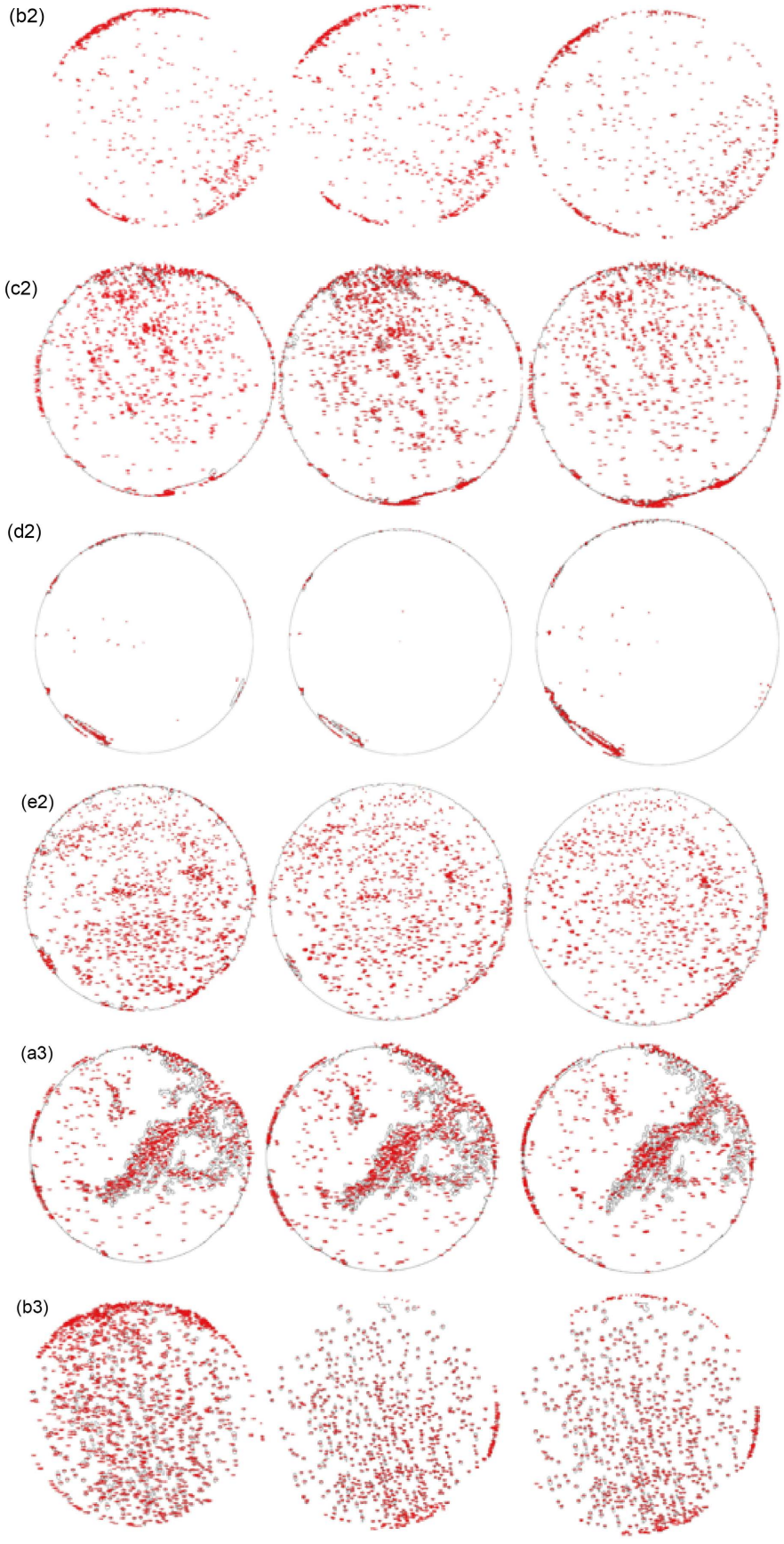
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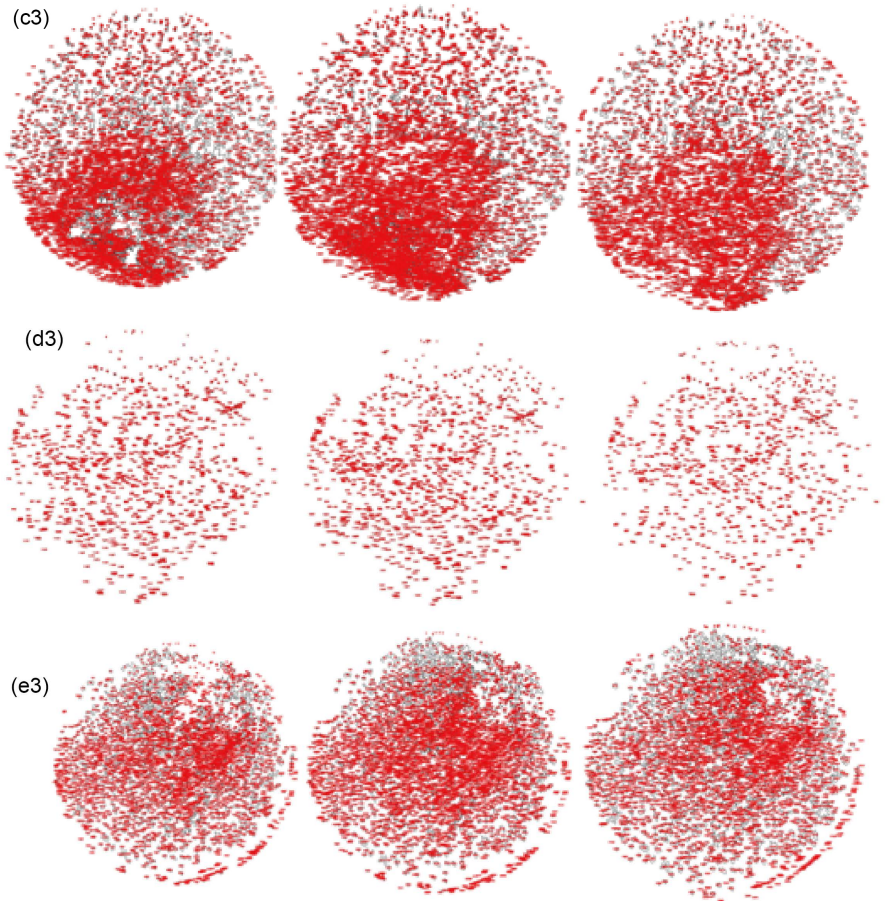


Figure 3. The results of cell counting analysis using Image J, a,b,c,d,e represents a group of samples with erythritol concentration of 2.5%, 5%, 10%, 20%,and 0%. And 1, 2, 3 represents the 3 repeated groups that was conducted.

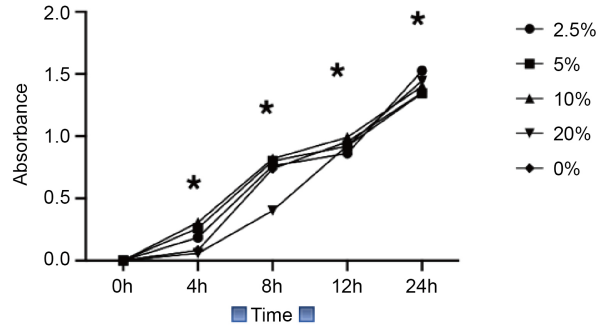
However, it is worth noting that the extent of this inhibition appears to be concentration-dependent, as evidenced by the varying colony sizes and densities observed in the different petri dishes (**Figure 3**). These findings may have significant implications in the development of effective antimicrobial agents that target *E. coli*, particularly in cases where the concentration of erythritol can be modulated to optimize its inhibitory effects. It is important to note, however, that further studies are needed to fully elucidate the mechanisms underlying erythritol's inhibitory effects on *E. coli* transformation. Future research efforts may also explore the potential applications of erythritol as a natural alternative to conventional antimicrobial agents, considering growing concerns about antibiotic resistance and the need for sustainable and eco-friendly solutions.

It is worth noting that the size of the *E. coli* colony is closely linked to its transformation efficiency, as a larger colony size implies a higher likelihood of successful transformation. Thus, the negative correlation between erythritol concentration and *E. coli* colony size growth strongly suggests that erythritol may inhibit *E. coli*'s transformation efficiency. At low concentration These results suggest there is a disruption of the membrane homeostasis and affects the

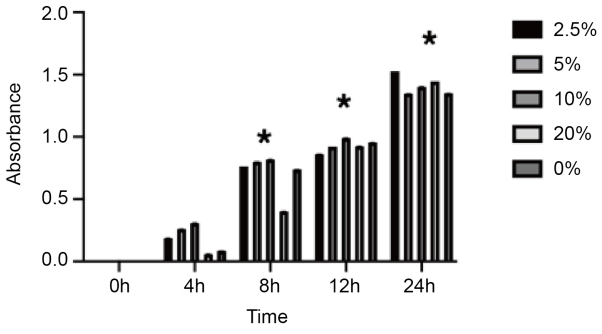
membrane permeability towards the plasmid.

We picked the unicolonial group of bacteria in each plate after the transformation is done, and add it into 10 mL of LB. We measure the optical density of each sample's growth in 0 h, 4 h, 8 h, 12 h, 24 h. Based on the results of the one-way ANOVA, there is no significant interaction between concentration of erythritol and absorbance over a 24 h period (Figure 4), which means that the effect of time on transformation efficiency may not be differ depending on the concentration of erythritol. Therefore, it is difficult to interpret the p-values for the row and column effects. However, the multiple comparison test on 4 h, 8 h, 12 h, and 24 h, all have a p-value < 0.0001. This suggests that the different concentrations of erythritol have a significant impact on transformation efficiency in a specific time.

Linking these results to *E. coli*'s metabolism, it is known that the efficiency of bacterial transformation depends on various factors, including membrane permeability, protein expression, and DNA repair mechanisms. Erythritol's inhibitory effects on transformation efficiency may disrupt these cellular processes, affecting the bacteria's ability to take up and express foreign DNA.



(a)



(b)

Figure 4. The growth curve of each experiment trial measured by optical density using an ultraviolet spectrophotometer. In multiple comparison of one-way ANOVA, $p = 0.9965 > 0.05$, suggesting that there are no significant differences of 5 samples with different erythritol concentration's optical density within 24 h period. However, there is a significant difference in the 5 samples in 4 h ($p < 0.0001$), 8 h ($p < 0.0001$), 12 h ($p < 0.0001$), and 24 h ($p < 0.0001$).

Overall, these experimental results highlight erythritol's potential as an antimicrobial agent, particularly in modulating bacterial transformation efficiency. Understanding erythritol's effects on *E. coli*'s metabolism and cellular processes can open avenues for the development of eco-friendly and sustainable antimicrobial strategies, considering the growing concerns about antibiotic resistance. However, further research is required to fully elucidate the underlying mechanisms and explore the practical applications of erythritol in antimicrobial therapy.

4. Discussion

Bacterial growth is one of the most well-studied features of metabolism. Since *E. coli* is not a bacterium that normally causes hemolysis, it generally does not exhibit strong hemolysis. However, *E. coli* can secrete toxins, such as enterotoxins and invasives, which can cause damage to host cells and tissues. [10] suggests that the addition of erythritol may not affect *E. coli*'s cytotoxicity in blood.

It may be inferred that the presence and concentration of erythritol will affect the metabolism and growth of *Escherichia coli*, thus affecting the conversion efficiency of the plasmid. The quantitative analysis of the data obtained from the experiment has yielded valuable insights into the relationship between erythritol concentration and *E. coli* transformation efficiency. There is a positive correlation between erythritol concentration and average cell count, which suggests that erythritol may have a stimulatory effect on *E. coli* growth. However, the analysis also reveals a negative correlation between erythritol concentration and average cell size, total area, and percentage area of the *E. coli* colonies, as evidenced by the decreasing trend observed in **Figure 5**.

The hemolytic activity of *Escherichia coli* can cause the rupture of red blood cells on the plate, releasing components such as hemoglobin, which can change the color of the plate and thus affect its grayscale value. The more obvious the

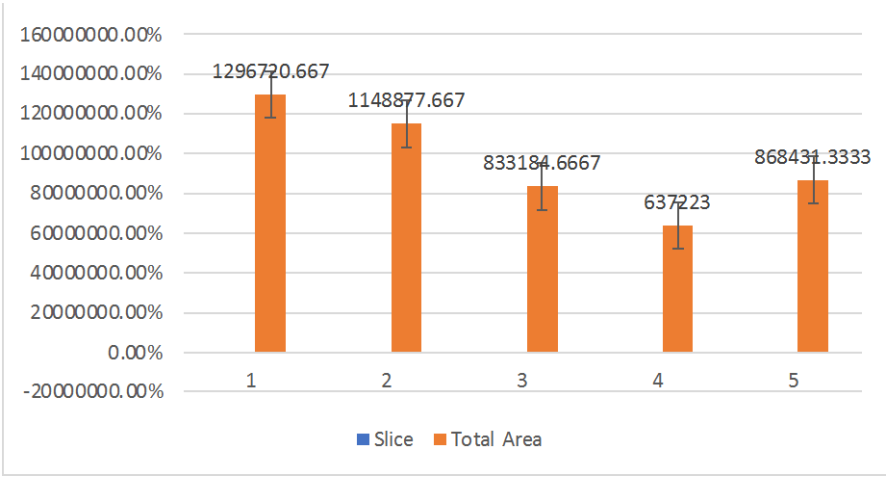


Figure 5. The total area result of the semiquantitative analysis (**Figure 4**) was shown, the transformed plates results 1 represents the sample is treated with 2.5% concentration of erythritol, 2 represents 5% concentration of erythritol, 3 represents 10% of erythritol, 4 represents 20% of erythritol, 5 represents 0% of erythritol.

hemolysis, the more hemoglobin is released, which makes the color of the plate lighter and thus increases its grayscale value. Comparing the median of the four sample to the control group (Figure 6), it is not sufficient to conclude that artificial sweetener increase the level of hemolytic activity. However, the inhibition of hemolysis at 10% erythritol and the promotion at 20% needs further research to conduct.

To better understand the mechanisms underlying erythritol's inhibitory effects on *E. coli* transformation efficiency, further studies about investigating the potential impact of erythritol on specific cellular processes involved in *E. coli* growth and transformation are needed. For instance, it may be useful to explore the effect of erythritol on DNA replication, transcription, or translation, as these processes are critical for *E. coli* transformation. Additionally, further studies may investigate the potential synergistic effects of erythritol in combination with other antimicrobial agents, as such combinations may enhance erythritol's inhibitory effects while reducing the risk of resistance development. Overall, these findings have significant implications for the development of effective antimicrobial strategies against *E. coli* and highlight the potential of erythritol as a promising natural alternative to conventional antibiotics.

Considering the association between artificial sweetener consumption and the likelihood of obesity, a significant number of individuals are currently engaging in weight control practices, often utilizing artificial sweeteners to limit their calorie intake. Despite the widely held belief that calorie restriction is an effective method for achieving successful weight loss, recent research published in *Cell Metabolism* suggests that regular consumption of high-fat and high-sugar foods can alter the brain's reward circuitry and activate the dopaminergic system, leading to increased cravings for similar foods and changes in eating habits [11]. The potential impact of artificial sweeteners on weight control must also be considered, as they too may influence the thalamus, stimulating cravings for sweet

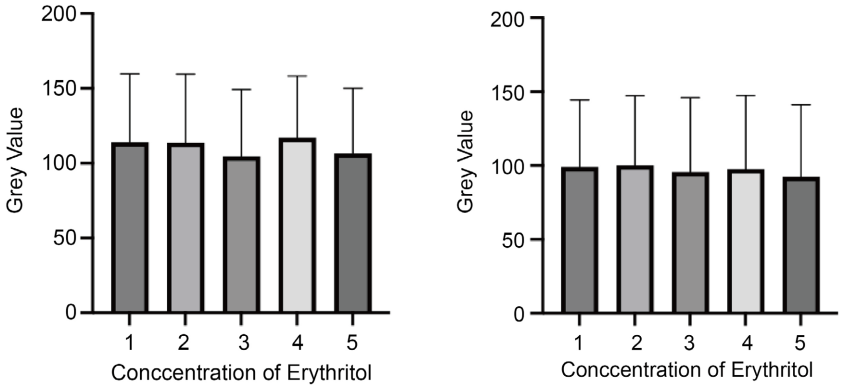


Figure 6. The mean grey value of the sample, (a) and (b) each represents a replicates, and 1 represents the sample is treated with 2.5% concentration of erythritol, 2 represents 5% concentration of erythritol, 3 represents 10% of erythritol, 4 represents 20% of erythritol, 5 represents 0% of erythritol.

foods. Therefore, further research is necessary to determine whether artificial sweeteners can be a viable substitute for individuals experiencing weight control issues. It is imperative that we gain a deeper understanding of the mechanisms underlying artificial sweetener consumption and its potential effects on the body to develop effective strategies for weight management.

Erythritol is a sugar alcohol that is widely used as a low-calorie sweetener and sugar substitute. As a zero-calorie sweetener, it is commonly used in foods and beverages as an alternative to sugar, especially by individuals trying to reduce their calorie intake for weight control purposes. However, recent research challenges the widely held belief that artificial sweeteners have no effect on the human body, including weight management.

A study conducted on artificial sweeteners, including erythritol, found that they can induce individual and specific changes in glycemic response by modifying the gut microbiome. The post-meal spike in blood glucose levels, known as the glycemic response, was influenced by non-nutritive sweeteners like erythritol. This finding implies that erythritol and other artificial sweeteners may not be as metabolically inert as once presumed and may have an impact on weight control practices [12]. As a result, further clinical studies are needed to better understand the potential effects of erythritol consumption on weight management.

The gut microbiota, comprising various microorganisms like bacteria, viruses, and fungi, plays a crucial role in digestion, immune function, and nutrient absorption. Erythritol has been studied for its effects on gut bacteria. Unlike some other sugar alcohols, erythritol is not fermented by gut bacteria, leading to minimal disruption of the gut microbiome.

Studies suggest that erythritol does not promote the growth of harmful bacteria like *Escherichia coli* (*E. coli*) and *Clostridium difficile* (*C. difficile*) in the gut. This is significant as the gut microbiota's balance is essential for overall digestive health and immune function. Therefore, erythritol's minimal impact on gut bacteria makes it a potentially beneficial alternative to other sweeteners in terms of maintaining gut health. (*The Impact of Erythritol on Gut Bacteria: Exploring the Science and Health Benefits-Nao Medical*, n.d.)

The findings of our study offer valuable insights into the metabolic processes of micro-bacteria, and it is important to note that these results should not be extrapolated to make general assumptions about weight control or broader physiological systems. It is worth considering the potential implications of erythritol addition on *E. coli* metabolism, as this could result in significant changes that may impact the digestive system and the body's ability to absorb nutrients. Further research is necessary to determine the potential effects of erythritol and other additives on gut microbiota and the broader implications for human health. A deeper understanding of microbial metabolism is essential for developing effective strategies to promote health and prevent disease. By identifying the mechanisms underlying microbial metabolism and the potential effects of

various compounds on this process, we can gain valuable insights into the intricate workings of the human body and develop new therapies to support overall health and well-being.

5. Conclusion

The general effects we can conclude is that a 20% concentration of erythritol does not have a significant effect on inhibiting *E. coli*'s normal growth at 0 to 48 h, and 10% erythritol has an inhibiting effect during 24 to 48 h. While at low concentration (2.5% and 5%), which is usually the normal concentration added into processed food, it actually enhances *E. coli*'s growth by increasing the optical density compare to the control group (0% erythritol). We can suggest that low amount of erythritol addition can increase *E. coli*'s number and overall population density in each sample, suggesting a positive relationship with *E. coli*'s pathogenicity.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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