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Application of pineapple juice and biofermented pineapple peel juice for the removal of *Escherichia coli* contamination from leaf lettuce

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ABSTRACT

Lettuce is a popular vegetable, primarily for fresh consumption, and is typically grown with manure-based fertilizers. However, this method raises the danger of infection with *Escherichia coli* (*E. coli*), a bacteria present in feces that has been identified as a gastrointestinal tract. The purpose of this study was to evaluate the effectiveness of pineapple juice and biofermented juice obtained from pineapple peel in reducing *E. coli* contamination in lettuce by assessing bacterial levels after immersion in the different treatment solutions. Pineapple juice includes natural acids and enzymes like bromelain, which have been demonstrated to have antibacterial properties. Furthermore, biofermented pineapple peels juices include helpful microbes, that may contribute to an environment that suppresses *E. coli*. *E. coli* was conducted using the spread plate technique on eosin methylene blue (EMB) agar, using sterile distilled water as a control. Biofermented pineapple peels showed significantly greater efficacy than that of pineapple juice ($p < 0.01$). Pineapple juice, regardless of concentration, did not show a statistically significant difference ($p > 0.05$), whereas all tested concentration of biofermented pineapple peel solution produced statistically significant reduction in *E. coli* ($p < 0.01$). Among them, the 25% concentration demonstrated the highest efficacy in reducing *E. coli* levels by 1.98 $\log_{10}$CFU/g more than washing with distilled water ($p < 0.01$). Notably, the 25% concentration of biofermented juice from pineapple peel had the ability to reduce *E. coli* to undetectable levels within 30 minutes. This study demonstrates the potential of biofermented juice from pineapple peel as a natural disinfectant that improves food safety while minimizing organic waste. Its manufacture adheres to circular economic concepts and promotes environmental sustainability. Technology addresses the rising need for chemical-free, clean-label solutions. It offers a practical, low-cost alternative for smallholder farmers and local food producers. Overall, this approach has a significant impact on public health, sustainable innovation, and the effective utilization of waste.

Keywords: biofermented juice, *Escherichia coli*, lettuce, pineapple juice, pineapple peel.

INTRODUCTION

Vegetables are vital for a healthy diet because they contain vitamins, antioxidants, dietary fiber, and minerals that promote general health. The World Health Organization (WHO) set international nutritional guidelines to consume a minimum of five servings of vegetables per day to maximize their health benefits (WHO, 2020; Rippin et al., 2023). Lettuce is a nutritious food source, and pre-packaged, ready-to-eat lettuce in plastic bags has become a popular and simple way to incorporate leafy greens into daily meals (Uhlig et al., 2017). However, eating salad crops such as lettuce, cucumbers, and tomatoes poses a significant danger of microbial infection. This is primarily owing to their high surface-to-weight ratio and slightly alkaline pH, which can favor bacterial growth (Alegbeleye et al., 2022). The typical introduction of common pathogenic pollutants during cultivation or processing, leading to contamination such as *Listeria monocytogenes*, *Salmonella*, and *Escherichia coli* (*E. coli*) O157:H7 (Bhatia et al., 2024). Pathogenic strains of *E. coli* O157 are known to cause major gastrointestinal difficulties, including severe abdominal cramps and bloody diarrhea, as well as life-threatening illnesses like hemolytic uremic syndrome (Haile et al., 2022). According to studies, *E. coli* O157 is typically detected in undercooked meat and leafy greens, making it a serious problem in daily meals. This infection offers a significant public health danger, particularly for vulnerable groups such as small children and the elderly, who are more likely to suffer serious sequelae (Singha et al., 2023). Evidence of *E. coli* contamination in lettuce has been found in various countries. According to the study, *E. coli* counts in lettuce in Trinidad and Tobago ranged from 0.8 to 800,000 CFU/g, with roughly 55% of samples having more than 100 CFU/g (Boodram et al., 2022). According to the Centers for Disease Control and Prevention (CDC)'s Morbidity and Mortality Weekly Report, eating romaine lettuce was linked to a multistate epidemic of *E. coli* O157:H7 infections in the United States. Twenty-three people in 12 states were impacted by the outbreak (Hoff et al., 2021). Furthermore, 300 vegetable and herb samples were collected in Bangkok, Thailand, and the results showed that leaf lettuce had the highest *E. coli* contamination rate, at 36.7% (22 out of 60 samples) (Datta et al., 2024).

To lessen the possibility of *E. coli* O157:H7 and other pathogens contaminating leafy greens, water mixed with chemical disinfectants is routinely employed (Murray et al., 2017; Rosberg et al., 2021). While these disinfectants and water-based procedures are critical for reducing microbial contamination in fresh produce, washing water has been found as a substantial source of cross-contamination (Gombas et al., 2017). Most chemical washing solutions contain chlorine and peroxyacetic acid molecules; nevertheless, their use has been related to the creation of hazardous halogenated byproducts that pose dangers to both human health and the environment (Gil et al., 2009). As a result, numerous recent studies have concentrated on identifying alternative natural compounds for use as wash solutions, because they are abundantly available, cost-effective, and easy for the public to get and prepare (Karntueng et al., 2015).

This study focuses on the effectiveness of pineapple, which is an important commercial crop in Lampang Province, Thailand. According to data from the Office of Agricultural Economics (OAE) for 2023, the total cultivated area for pineapples in Lampang was around 17,902 rai (2864.32 hectares) (Office of Agricultural Economics, 2023). Lampang Province's natural characteristics are ideal for farming the Pattavia pineapple, often known as the Sriracha type. This variety yields the largest fruit among pineapple varieties, has a naturally sweet and juicy flavor, and is the only variety cultivated exclusively for canned pineapple processing (Pongjanta et al., 2011). Previous studies have demonstrated that lime and kaffir lime juice successfully prevent *E. coli* contamination in lettuce, with pH levels measured at 2.02 and 1.87, respectively (Karntueng et al., 2015; Sayamee et al., 2020). Pattavia pineapple juice has a pH of 4.53 to 4.93 (Pongjanta et al., 2011), indicating that it is acidic. Nevertheless, the effectiveness of pineapple juice as a biocidal agent against *E. coli* on fresh vegetables has not yet been examined in any published research. This research presents a novel application of pineapple juice, capitalizing on its enzymatic (bromelain) and acidic properties to serve as a natural antimicrobial wash. This strategy might be included in post-harvest decontamination procedures, providing the fresh produce industry with a sustainable substitute for chemical sanitizers. Previous studies have investigated the antibacterial activities of fermented pineapple peel extract on iceberg lettuce utilizing *Lactobacillus plantarum* M29 (Tanganurat, 2012). The findings show that the fermentation of pineapple peel, an agricultural byproduct, generates a variety of chemicals that promote microbial growth, particularly *Lactobacillus plantarum* (Tanganurat, 2012). This species, a lactic acid bacterium (a beneficial microbe), is usually found with fruits and plays an important role in the fermentation of pineapple peels. The ensuing lactic acid effectively suppresses harmful microbes, such as *E. coli*, which cannot live in severely acidic environments (Tajkarimi and Ibrahim, 2012). The pH of the biofermented solution made from pineapple peel ranges between 0.1 and 3.5. The researchers discovered that a 1.5% concentration of fermented pineapple peel juice efficiently reduced *E. coli* by 2.72 $\log_{10}$ CFU/g. In comparison, soaking iceberg lettuce in water during storage resulted in just a 1.82 $\log_{10}$ CFU/g reduction in *E. coli*. These findings show that the biofermented solution made from pineapple peel is efficient in minimizing *E. coli* contamination in iceberg lettuce (Tanganurat, 2012). Biofermented pineapple peel juice is being researched as a natural, economical, and eco-friendly antibacterial agent. Pineapple peels, which are normally considered agricultural trash, contain bioactive compounds including bromelain, phenolics, and organic acids that have the potential to reduce or eliminate harmful infections (Ortega-Hernández et al., 2023). By generating lactic acid, acetic acid, and bacteriocins during fermentation, probiotic microorganisms such as lactic acid bacteria—found in the probiotic drink that was added—further strengthen the antibacterial qualities (Imade et al., 2021). To reduce surface contamination with *E. coli* and other pathogens, the fermented pineapple peel solution could be used to clean raw fruits or vegetables, such lettuce. Biofermented pineapple peel juice could be useful in real situations, especially on organic farms or in rural areas where commercial disinfectants (like chlorine) could be too costly or unavailable. As a result, researchers sought to investigate the use of pineapple juice and biofermented pineapple peel solution as vegetable-washing agents to minimize bacterial contamination. The study examined their effectiveness in lowering *E. coli* infection in lettuce.

E. coli was chosen as the target microorganism because it belongs to the coliform bacteria group, specifically fecal coliforms, which are often present in the feces of humans and warm-blooded mammals. This bacterium acts as an indication of food and water hygiene. If pineapple juice and biofermented pineapple peel solution prove effective in reducing *E. coli* contamination, they could be developed into an efficient, easily accessible, cost-effective, and safe vegetable-washing solution for consumers.

MATERIALS AND METHODS

Preparation of *E. coli*

The method for preparing *E. coli* was adapted from the procedure described by Karntueng et al. (2015). A loopful of *E. coli* culture, obtained from the Thailand Institute of Scientific and Technological Research (TISTR), was transferred from nutrient agar (NA) to 10 mL of nutrient broth (NB) and incubated at 35 °C for 24 hours. This subculturing process was repeated twice. On the third day, 3 mL of the actively growing culture was pipetted into 300 mL of NB in a 500-mL Erlenmeyer flask. This step was performed for four flasks to obtain a total volume of 1 L. The cultures were then incubated at 35 °C for 18 hours. Subsequently, all *E. coli* cultures were pooled into a sterile 1-L bottle to ensure a uniform concentration before being used as an inoculum for contamination experiments in lettuce (Karntueng et al., 2015).

Preparation of leaf lettuce

Leaf lettuce (*Lettuce sativa* L.) was obtained from a local market in its unwashed state. Leaf lettuce was prepared in accordance with the previous research (Karntueng et al., 2015). The outer 2–3 leaves were removed, and those with similar sizes and petiole lengths were selected. The chosen leaves were first rinsed with tap water to remove soil residues, followed by two rounds of washing with sterilized distilled water. They were then cut into square pieces measuring 5 × 5 cm (Karntueng et al., 2015), with an average weight of approximately 0.55 ± 0.03 g.

Preparation of pineapple juice and biofermented juice from pineapple peel

Pineapple juice

Pineapple juice was prepared at concentrations of 5, 10, 15, 20, and 25% (v/v). Fresh pineapples were peeled, and the flesh was cut using a sterilized knife while the handler wore sterile gloves. The juice was extracted to obtain final volumes of 10, 20, 30, 40, and 50 mL, respectively. Each sample was then adjusted to a total volume of 200 mL by adding distilled water. The pineappl juice was sterilized using ultraviolet (UV) light in a laminar flow cabinet prior to use.

Biofermented juice from pineapple peel

Before fermentation, the equipment, tools, materials, and distilled water were sterilized and also the workspace was disinfected. Pineapple peels were cut into small pieces, totaling 1 kg. The prepared peels (1 kg) were placed into a sterilized 3-L glass jar. Brown sugar (300 g) was evenly distributed over the peels. Then, 1–2 L of water and one bottle of probiotic drink were added, and the mixture was stirred until the brown sugar was completely dissolved. After that, the jar was meticulously sealed and stored for one to two weeks before usage in a dark place away from direct sunlight and frequent handling and opening. To improve the quality, the fermenting time could be prolonged. Sterilized procedures were used for sampling. Fermented pineapple peel solutions were made at varying concentrations of 5, 10, 15, 20, and 25% (v/v). The solution was filtered, yielding final volumes of 10, 20, 30, 40, and 50 mL. Each sample was then adjusted to a total volume of 200 mL using distilled water. During dilution, tools and containers were sterilized. Moreover, biofermented juice from pineapple peel was examined for some properties, including physical characteristics such as color and sediment and chemical characteristics such as pH on days 0, 7, 14, and 28.

Effect of the concentration of pineapple juice and biofermented juice from pineapple peel on color change in leaf lettuce

The leaf lettuce was immersed in pineapple juice and biofermented juice from pineapple peel at concentrations of 5, 10, 15, and 25% (v/v). After 10, 20, and 30 minutes, the soaked leaf lettuce was examined for appearance. These concentrations were chosen to evaluate the dose-dependent antibacterial efficacy of pineapple juice and biofermented juice from pineapple peels against *E. coli*, ensuring both effectiveness and practical applicability. This varies concentration adapted from Karntueng et al. (2015). Lower concentrations (5–10%) represent minimal effective doses suitable for direct application on fresh produce, while higher concentrations (15–25%) assess the maximum antimicrobial potential without compromising safety, cost-effectiveness, or sensory qualities. This approach aligns with previous studies that have demonstrated the dose-dependent antibacterial activity of natural extracts against *E. coli* (Ban et al., 2023; Cahyani et al., 2024).

Effectiveness of pineapple juice and biofermented juice from pineapple peel in reducing *E. coli* contamination in leaf lettuce

The testing approach was based on the procedure given by Karntueng et al. (2015). Leaf lettuce was soaked in distilled water for 5 minutes before draining on a wire rack for an hour. The drained lettuce was immersed in NB containing the prepared *E. coli* culture for 2 minutes, then drained on a rack for 30 minutes. The lettuce was then immersed in 200 mL of each test solution, including fermented pineapple peel solution, pineapple juice, and sterilized distilled water (as a control), for 30 minutes. After treatment, the lettuce was removed using sterile forceps and rinsed once with sterilized distilled water to remove residual test substances. The samples were then placed in beakers containing a 0.85% (w/v) sodium chloride (NaCl) solution. The *E. coli* count was determined using the spread plate technique on EMB Agar, with three replicates per sample. The plates were incubated at 37 °C for 24 hours. Following incubation, colonies that exhibit *E. coli* characteristics are either manually counted or tallied with an automated colony counter. The CFU/g of the original vegetable sample is then used to represent the bacterial burden. CFU levels are converted to a base-10 logarithmic scale ($\log_{10}$ CFU/g) in order to standardize the data and facilitate simpler comparison of results.

Statistical analysis

Before analysis, bacterial counts were logarithmically transformed to compute means and standard deviations, with bacterial growth expressed as $\log_{10}$ CFU/g. Stata software version 14.0 was used for statistical analysis, identifying significant differences ($p < 0.05$) between treatments and controls. The Mann-Whitney U Test was used to compare the *E. coli* count and mean reduction of pineapple juice and biofermented juice from pineapple peels. A one-way analysis of variance (ANOVA) was also used to analyze statistical differences across different treatment groups at a 95% confidence level. The Tukey's honestly significant difference (HSD) post-hoc test was used to evaluate whether pairings of biofermented juice from pineapple peel concentrations showed statistically significant differences.

RESULTS AND DISCUSSION

Physical and chemical properties of biofermented juice from pineapple peel

Table 1 shows the physical and chemical parameters of biofermented juice made from pineapple peels. During the fermenting process, the juice appears clear yellow and sediment-free. However, as the storage period progresses, obvious alterations emerge. A precipitate begins to form on the 14th day, followed by a tiny white blemish on the 28th day. These changes are most likely the result of insufficient sanitation and

Table 1. Physical and chemical properties of biofermented juice from pineapple peel

Period	Physical properties		Chemical characteristic
	Color	Sediment	pH
Day 0	Clear yellow	No sediment	3.64
Day 7	Clear yellow	No sediment	3.22
Day 14	Clear yellow	Sediment	3.14
Day 28	Clear yellow with white patches	No sediment	3.07

improper fermentation conditions. This finding is consistent with prior study on biofermented juice from pineapple peels. In one investigation, fungal contamination was found by the seventh day with a 1% initial inoculum dose, despite the juice's clear yellow look. Furthermore, prior study has shown that fermenting for 21 days increases protease activity, which helps to retain clarity and prevent sediment formation in the biofermented juice (Tanganurat, 2012).

Effect of the concentration of pineapple juice and biofermented juice from pineapple peel on color change in leaf lettuce

Figure 1 depicts the representative experiment images of the color changes in leaf lettuce caused by soaking in pineapple juice at various concentrations. Table 2 shows how different quantities of pineapple juice and biofermented juice from pineapple peel affect the color of leaf lettuce. Concentrations of pineapple juice and pineapple peel biofermentation at 5, 10, 15, 20, and 25% at 10 and 20 minutes showed no change in the color of the lettuce, while at 30 minutes each concentration changed, with the leaf edges turning slightly yellow. The slightly yellow tint may be caused by lettuce carrying polyphenol oxidase (PPO), an enzyme that catalyzes the oxidation of phenolic substances to quinones (Altunkaya and Gökmen, 2009). These quinones can polymerize into pigments that lead to browning. While acidic environments might somewhat block PPO activity, severe acidity or prolonged exposure may disrupt cellular compartments, allowing PPO to interact with its substrates and induce browning (Altunkaya and Gökmen, 2009). Furthermore, a prior study discovered that pineapple shell extract reduced browning values in banana slices more successfully than citric acid solution and distilled water when stored for no more than 1

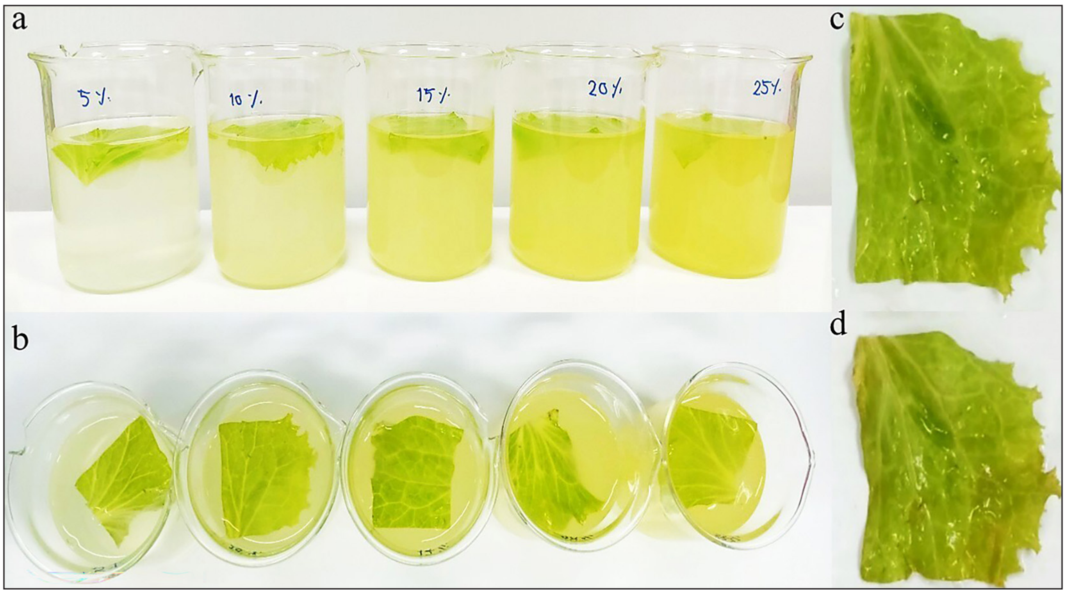

Figure 1. (a) Representative side-view images of the experiment showing color changes in leaf lettuce soaked in pineapple juice at various concentrations; (b) representative top-view images of the same experiment; (c) leaf lettuce treated with 10% pineapple juice at the initial time point (before treatment); and (d) leaf lettuce treated with 10% pineapple juice after 30 minutes (post-treatment)

Table 2. Effect of the concentration of pineapple juice and biofermented juice from pineapple peel on color change in leaf lettuce

Tested substances	Concentration (% v/v)	pH	Soak time (minutes)		
			10	20	30
Pineapple juice	5	3.68	The leaf edge remains unchanged	The leaf edge remains unchanged	The leaf edge changing slightly yellow
	10	3.62			
	15	3.57			
	20	3.51			
	25	3.46			
Biofermented juice from pineapple peel	5	3.37	The leaf edge remains unchanged	The leaf edge remains unchanged	The leaf edge changing slightly yellow
	10	3.31			
	15	3.25			
	20	3.20			
	25	3.16			

hour (Theerakulkait and Saisung, 2006). As a result, the purpose of this study is to determine the efficacy of pineapple juice and biofermented juice from pineapple peels in decontaminating leaf lettuce using a 30-minute washing technique. Although the leaf margins may become slightly yellow, the effect is modest and has no substantial impact on the overall quality of the lettuce.

Efficacy of pineapple juice and biofermented juice from pineapple peel

Table 3 presents the efficacy of pineapple juice and biofermented juice from pineapple peel in reducing *E. coli* contamination in leaf lettuce. The results indicate that washing leaf lettuce by immersing it in either of the test solutions or in distilled water significantly reduced *E. coli* levels, with statistically significant differences observed at the $p < 0.01$ level. Biofermented pineapple peels showed significantly greater efficacy than that of pineapple juice ($p < 0.01$). Pineapple juice, regardless of concentration, did not show a statistically significant difference ($p > 0.05$) in its ability to reduce *E. coli* contamination in lettuce. In contrast, biofermented pineapple peel solution, regardless of concentration, did show a statistically significant difference ($p < 0.01$) in its ability to reduce *E. coli* contamination in lettuce. Biofermented pineapple peel solution at a 25% concentration demonstrated the highest efficacy in reducing *E. coli* levels. It significantly

Table 3. Efficacy of pineapple juice and biofermented juice from pineapple peel at different concentrations on reducing the amount of *E. coli* contaminated in leaf lettuce

Tested substances	Concentration (% v/v)	pH	Mean *E. coli* count ($\log_{10}$ CFU/g)	Mean reduction ($\log_{10}$ CFU/g)
Sterile distilled water (Control)	-	-	4.42 ± 0.04	-
Pineapple juice	5	3.68	4.49 ± 0.21	-0.06 ± 0.21
	10	3.62	4.49 ± 0.04	-0.20 ± 0.04
	15	3.57	4.41 ± 0.12	-0.21 ± 0.12
	20	3.51	4.43 ± 0.01	0.00 ± 0.01
	25	3.46	4.35 ± 0.05	0.08 ± 0.05
Biofermented juice from pineapple peel	5	3.37	$3.60 \pm 0.03^{a,c}$	$0.82 \pm 0.03^{b,e}$
	10	3.31	$3.30 \pm 0.06^{a,d}$	$1.12 \pm 0.06^{b,f}$
	15	3.25	$2.94 \pm 0.21^{a,c}$	$1.48 \pm 0.21^{b,e}$
	20	3.20	$2.70 \pm 0.16^{a,c,d}$	$1.72 \pm 0.16^{b,e,f}$
	25	3.16	$2.45 \pm 0.08^{a,c,d}$	$1.98 \pm 0.08^{b,e,f}$

Note: a, b – indicates $p < 0.01$ compared to concentration in the same column, c,d,e,f – indicates $p < 0.05$ compared to concentration in the same column. Data is presented as $\log_{10}$ CFU/g ± standard deviation.

outperformed distilled water in reducing *E. coli* contamination ($p < 0.01$). Specifically, the 25% biofermented pineapple peel solution reduced *E. coli* levels by 1.98 log_{10} CFU/g more than washing with distilled water. The 5% biofermented pineapple peel juice had a considerably lower *E. coli* mean decrease compared to 15%, 20%, and 25% ($p < 0.05$). The 10% biofermented pineapple peel juice had considerably lower *E. coli* mean decrease compared to 20% and 25% ($p < 0.05$). However, 15% of biofermented pineapple peel juice showed non-significant *E. coli* and a lower mean decrease compared to 20% and 25% ($p > 0.05$). The 20% of biofermented pineapple peel juice showed non-significant *E. coli* and a mean decrease of less than 25% ($p > 0.05$).

Table 4 compares the efficacy of different washing aid solutions for microbial reduction with previous research. This study found that 25% biofermented juice from pineapple peel reduced *E. coli* on iceberg lettuce by 2.72 log_{10} CFU/g, while 15% Kaffir lime juice reduced *E. coli* on leaf lettuce by 6.50 log_{10} CFU/g (Tanganurat, 2012; Sayamee et al., 2020). In contrast, this study found that 25% biofermented juice from pineapple peel decreased *E. coli* more effectively than lemon juice, with a reduction of 0.82 log_{10} CFU/g on iceberg lettuce (Santos et al., 2010). Furthermore, 15% lime juice and rice washing water reduced *E. coli* on leaf lettuce by 0.79 and 0.04 log_{10} CFU/g, respectively (Karntueng et al., 2015). As a result, biofermented pineapple peel juice has the potential to be developed as a vegetable wash due to its efficiency in decreasing bacterial contamination of fresh vegetables. The study results revealed that the kind of test substance, concentration of the test substance, type of microbe, and type of tested vegetable all influence *E. coli* reduction. Therefore, it is difficult to compare the efficacy of decontamination agents. Moreover, factors affecting the efficacy of decontamination include the number of times the produce is washed in the sanitizing solutions (Lang et al., 2004), the characteristics of product surfaces and the location of the tissues (Kondo et al., 2006), the relationship between weight and surface area of the produce (Beuchat et al., 2001), and the rapid deterioration of water quality during washing (Allende et al., 2008).

Optimal washing time for reducing *E. coli* contamination in leaf lettuce using 25% biofermented juice from pineapple peel

Table 5 shows the results of testing the optimal exposure time for 25% biofermented juice from pineapple peel. It was found that a 30-minute exposure time resulted in the greatest reduction of *E. coli* contamination, followed by 25 and 20 minutes, respectively. The mean reduction of 25% biofermented juice from pineapple peel was approximately 2.01 log_{10} CFU/g for 30 minutes. This indicated an antibacterial action that was time dependent. There are several reasons behind this trend. Long-term exposure gives the fermented

Table 4. Comparison of the efficacy of different washing aid solutions for microbial reduction with previous research

Tested substances	Microorganism	Vegetable	Microbial log_{10} reduction	Reference
Lemon juice	*E. coli*	Iceberg lettuce	0.82 log_{10} CFU/g	Santos et al., 2010
	E. coli O157:H7		2.09 log_{10} CFU/g	
Biofermented juice from pineapple peel (1.5%)	*E. coli* O157:H7	Iceberg lettuce	2.72 log_{10} CFU/g	Tanganurat, 2012
Lime juice 15%	*E. coli*	Leaf lettuce	0.79 log_{10} CFU/g	Karntueng et al., 2015
Rice washing water			0.04 log_{10} CFU/g	
Lime juice 15%	Coliform bacteria	Leaf lettuce	1.89 log_{10} CFU/g	
Kaffir lime juice 15%	*E. coli*	Leaf lettuce	6.50 log_{10} CFU/g	Sayamee et al., 2020
Berry pomace extracts (1, 1.5, and 2 gallic acid equivalent, GAE mg/mL)	*E. coli* O157:H7 EDL-933	Spinach leaf	0.5 to 1.6 log_{10} CFU/ spinach leaf	Thapa et al., 2024
Pineapple juice 25%	*E. coli*	Leaf lettuce	0.08 log_{10} CFU/g	This study
Biofermented juice from pineapple peel 25%	*E. coli*	Leaf lettuce	1.98 log_{10} CFU/g	This study

pineapple peel solution's bioactive ingredients, including organic acids such as lactic and acetic acid, phenolic compounds, and antibacterial peptides, more opportunity to interact with bacterial cell membranes (Cueva et al., 2010). These substances change the permeability of membranes, compromise the integrity of microbial cells, and ultimately cause cell lysis (Siroli et al., 2015). Additionally, the fermented solution's acidity decreases the pH of the surrounding environment, which can prevent *E. coli* from growing and increase the effectiveness of antibiotic metabolites. This is consistent with research by Altunkaya and Gökmen (2009), who found that while too much acidity can also compromise tissue integrity, acidic environments may inhibit microbial growth and enzymatic activity. Other natural sanitizers have been shown to reduce *E. coli* in comparable amounts. For instance, Wang et al. (2014) discovered that, depending on exposure duration and acid content, vinegar-based washes could lower *E. coli* O157:H7 on fresh vegetables by as much as 2–3 $\log_{10}$ CFU/g. The reductions in *E. coli* were 2.45, 3.23, and 3.37 $\log_{10}$ CFU/g, respectively, with mean reductions of 1.93, 1.22, and 1.08, respectively. These results align with previous studies indicating that washing time also influences the reduction of *E. coli* O157:H7 and *Salmonella* spp. on lettuce, spinach, and iceberg lettuce (Solomon and Matthews, 2005; Sampedro and Mazzotta, 2013). Therefore, pineapple peel biofermented juice is a promising agricultural waste product that merits more research as a natural vegetable cleanser. The results of this study have significant implications for enhancing food safety, especially when it comes to fresh produce like lettuce, which is vulnerable to microbial infection because of its high surface area and raw consumption. Using pineapple peels to serve as agricultural waste as a product with additional value is one of the greatest advantages. By lowering organic waste, this not only supports environmental sustainability but also suits the ideas of a circular economy and green food processing innovation. Food safety and waste management are two important challenges that are addressed concurrently by turning pineapple waste into a bioactive solution.

Additionally, the study advocates the use of natural antimicrobials rather than widely utilized synthetic chemical sanitizers like chlorine, which may leave hazardous residues or provide health hazards. Consumer preference is shifting toward natural alternatives, particularly in health-conscious markets that prioritize clean-label and environmentally friendly products. This change lowers chemical exposure in home and commercial food preparation settings while also advancing public health objectives. Lowering the amount of *E. coli* on leafy greens is essential from a public health standpoint. In Thailand as well as other countries, outbreaks of foodborne illness caused by *E. coli* O157:H7 in lettuce and other vegetables have resulted in severe sickness and even death (Hoff et al., 2021; Datta et al., 2024). Low-cost and accessible disinfection methods should be provided, especially in developing regions or rural farming communities, which could significantly lower the risk of such outbreaks. Furthermore, the methodology employed in this study could be expanded for local food vendors or smallholder farms, where sophisticated cleaning technology might not be practical or economical. The product is a useful intervention for on-site use in markets or food stalls because of its non-toxic nature, ease of fermentation, and material availability.

Table 5. The optimal washing time for reducing *E. coli* contamination in leaf lettuce using 25% biofermented juice from pineapple peel

Tested substances	Washing time (min)	Mean *E. coli* count ($\log_{10}$ CFU/g)	Mean reduction ($\log_{10}$ CFU/g)
Sterile distilled water (Control)	-	4.45 ± 0.00	-
Biofermented juice from pineapple peel 25 %	10	3.71 ± 0.02^{a}	0.75 ± 0.02^{b}
	15	3.53 ± 0.03^{a}	0.92 ± 0.03^{b}
	20	3.37 ± 0.04^{a}	1.08 ± 0.04^{b}
	25	3.23 ± 0.03^{a}	1.22 ± 0.03^{b}
	30	2.45 ± 0.02^{a}	2.01 ± 0.02^{b}

Note: a – indicates $p \leq 0.001$ compared to concentration in the same column, b – indicates $p \leq 0.001$ compared to concentration in the same column. Data is presented as $\log_{10}$ CFU/g ± standard deviation. The *E. coli* count at time 0 inferred that the initial bacterial load was equivalent to that of the control group (4.45 ± 0.00 $\log_{10}$ CFU/g).

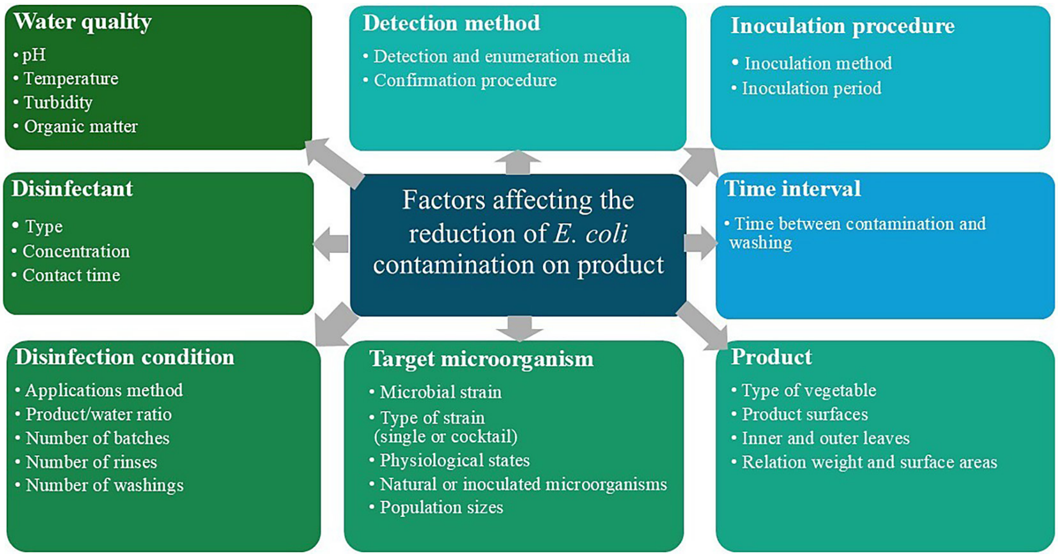

Figure 2. Factors affecting the reduction of *E. coli* contamination on products. (adapted from Gil et al., 2009)

Limitations of the research and further studies

This study provides a preliminary investigation into the effectiveness of pineapple juice and biofermented juice from pineapple peel in reducing *E. coli* contamination on leaf lettuce.

This study evaluated the influence of color change on leaf lettuce at a concentration of pineapple juice and biofermented juice from pineapple peel. This should be used to quantitative approaches such as colorimetry, or a comparison grid based on photographs to provide a clearer picture of the findings (Aekram et al., 2023).The limitation of this study is that we investigate other microorganisms because reduction in *E. coli* levels could indeed result from their inability to compete for nutrients with other microorganisms present in the biofermented juice. Since pineapple peel, brown sugar, water, and a probiotic drink are all used in the fermentation process, a variety of microorganisms—particularly lactic acid bacteria, yeasts, or acetic acid bacteria—are probably present in the system. In order to create an environment that is not conducive to *E. coli*, these organisms may make organic acids or antimicrobial compounds, consume nutrients more quickly than *E. coli*, and lower pH (Hartini et al., 2024; Hosseini et al., 2025). Furthermore, this study only focused on color and pH because of its practical, low-cost, and informative indicators of fermentation progress and microbial activity. Advanced techniques to track the physical and chemical characteristics of biofermented products, such as a spectrophotometer for optical density, turbidity, or specific compound quantification (e.g., flavonoids, phenolics), and chemical assays for sugar content, organic acids, or antioxidant capacity, should be used to better understand changes in bioactive compounds. However, it does not yet address the optimal conditions that influence *E. coli* reduction, such as the time interval between contamination and washing or the inoculation period. Future research should focus on identifying key factors, including water quality, detection methods, disinfection conditions, target microorganisms, and product characteristics, as outlined in Figure 2.

CONCLUSIONS

Biofermented juice from pineapple peel is more effective in reducing *E. coli* contamination in leaf lettuce than pineapple juice after a 30-minute treatment. At concentrations of 20% and 25%, pineapple juice demonstrated only a slight reduction in *E. coli* levels. In contrast, biofermented pineapple peel juice at a 25% concentration achieved the highest reduction, decreasing *E. coli* levels by 1.98 $\log_{10}$ CFU/g. The next most effective concentrations were 20% and 15%, reducing *E. coli* by 1.72 and 1.48 $\log_{10}$ CFU/g, respectively. Higher concentrations of biofermented pineapple peel juice were more effective in reducing *E. coli* contamination than lower concentrations. Although immersing vegetables in biofermented pineapple peel juice does not completely eliminate *E. coil*, it is more effective than washing with distilled water alone. This method helps lower the risk of foodborne illness while also providing a sustainable way to repurpose pineapple peels. Given these benefits, biofermented pineapple peel juice shows promise as a natural vegetable wash for fresh produce. This study highlights the potential of pineapple peel biofermented juice as a natural disinfectant that enhances food safety while reducing organic waste. Its production supports circular economic principles and environmental sustainability. The method aligns with rising demand for clean-label, chemical-free solutions. It offers a practical, low-cost option for smallholder farms and local food vendors. Overall, the approach contributes meaningfully to public health, sustainable innovation, and waste valorization. However, further research is needed to enhance its efficacy in reducing *E. coli* contamination.

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