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MICROBIOLOGICAL ASSESSMENT OF NOODLES SOLD AT RAMADAN BAZAAR IN KUCHING, SARAWAK, MALAYSIA

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ABSTRACT. *The Ramadan bazaar offers a diverse array of food for numerous customers during the month of Ramadan; however, the rising incidence of food poisoning raises concerns about the microbiological safety of these foods. This study aims to evaluate the microbial quality of various noodle types sold at the Ramadan bazaar in Kuching, Sarawak. A total of thirty-three (33) samples were collected from different sites and analyzed for Aerobic Plate Count, coliform bacteria, Escherichia coli, Staphylococcus aureus, Bacillus cereus, and Salmonella spp. Among the samples tested, 88%, 3%, 21%, 6%, and 16% exceeded acceptable limits for APC, coliforms, E. coli, S. aureus, and B. cereus, respectively, while all samples tested negative for Salmonella spp. Additionally, local specialties such as laksa Sarawak, kolo mee, and mee Jawa showed the highest levels of contamination with E. coli, S. aureus, and B. cereus. The presence of these microorganisms indicates cross-contamination likely due to insufficient hygiene practices before and after the cooking process.*

KEYWORDS: Food safety, noodles, microbiological quality, Ramadan bazaar, street food.

INTRODUCTION

The Ramadan bazaar in Malaysia is an annual event that takes place throughout the month of Ramadan. It features a diverse selection of food, ranging from drinks to main courses. Each state in Malaysia showcases unique dishes at their Ramadan bazaars that reflect the local culture, all at affordable prices accessible to all age groups and socioeconomic classes. For instance, Sarawak is renowned for its delicious noodle dishes such as *laksa Sarawak*, *kolo mee*, and *mee jawa*, which can be found at every Ramadan bazaar.

While the food at the Ramadan bazaar is a popular choice, it has often been associated with foodborne illnesses. The food offered is typically ready-to-eat and sold as takeout, intended for immediate consumption or for breaking the fast. Several factors can contribute to the contamination of food at these bazaars, leading to potential foodborne illnesses. Key issues include poor hygiene practices among food handlers, cross-contamination, and improper time and temperature management during food storage. In 2023, the Malaysian Ministry of Health issued 2,188 notices during inspections of 51,849 Ramadan bazaar locations across 640 areas nationwide. Violations included failure to complete food handler training, lack of anti-typhoid vaccinations, and non-compliance with food handler attire requirements. There were also 17 complaints related to food safety at the Ramadan bazaars, but only two reports of food poisoning were associated with food purchased at these events (The Sun, 2023).

Additionally, food samples from the Ramadan bazaar were examined for microbial contamination. The Kelantan State Health Department reported that one sample contained *S. aureus* in duck egg curry, while chicken-based dishes were found to have *Salmonella spp*. (Berita Harian, 2022). Furthermore, Mat Zin *et al*. (2017) conducted a microbiological analysis of meat samples at the Ramadan bazaar in Kelantan, Malaysia, which yielded unsatisfactory results for coliforms, *Staphylococcus spp*., *E. coli*, and *Salmonella spp*. Moreover, in October 2023, a food handler at a Ramadan bazaar in Limbang, Sarawak, was fined RM1,000 for selling food contaminated with pathogenic bacteria during last Ramadan (Utusan Borneo, 2023). Although there have been no reported cases of food poisoning associated with food and drinks sold at Ramadan bazaar stalls across Sarawak, there remains a risk of contamination with pathogenic bacteria, as evidenced by the situation in Limbang. Abdul-Mutalib *et al*. (2015) noted that foodborne diseases are prevalent in Malaysia, yet not all cases are reported, particularly less severe ones. Thus, this study aims to assess the microbial quality of noodle delicacies sold at the Ramadan bazaar in Kuching, Sarawak. Given the popularity of noodles among consumers in Sarawak and their potential for contamination, this research focuses specifically on noodles. The findings of this study may provide valuable insights for regulatory agencies to take appropriate action in the future.

MATERIALS AND METHOD

2.1 Sample Collection and Preparation

The sampling activities took place at six Ramadan bazaars across three locations in Kuching, Sarawak, Malaysia: Kuching North (18 samples), Kuching South (5 samples), and Padawan (10 samples). During the month of Ramadan, a total of 33 samples, representing eight types of noodles (see Figure 1), were collected aseptically for laboratory analysis. Each sample was assigned a serial number, transported in a cool box, and maintained at a temperature of 4 °C. The samples were either examined immediately or stored in a refrigerator to be tested within 24 hours of collection. All analyses were conducted in accordance with standard methods, assessing the samples for APC, coliforms, *E. coli*, *S. aureus*, *B. cereus*, and *Salmonella spp*

Figure 1: Types of noodles collected from the Ramadan bazaar in Kuching, Sarawak.

2.2 Microbiological analysis

A 10-fold serial dilution was carried out on the homogenized samples. Following this, 1 mL of each appropriate dilution was plated in triplicate on the selected media. This study utilized five standard methods for microbiological analysis: (1) AOAC International Official Method 990.12:2002 for Aerobic Count Plate (AOAC, 2002a), (2) AOAC International Official Method 991.14:2002 for Coliform and *E. coli* Plate (AOAC, 2002b), (3) AOAC International Official Method 2003.07 for Staph Express Count Plate (AOAC, 2003), (4) ISO 7932:2004 (E), which describes the horizontal method for the enumeration of presumptive *B. cereus* using the colony-count technique at 30°C, and (5) ISO 6579:2002 (E), detailing the horizontal method for detecting *Salmonella spp*. The contamination levels of APC, coliforms, *E. coli*, *S. aureus*, *B. cereus*, and *Salmonella spp*. were assessed against the standards set by the Food Standards Australia New Zealand (FSANZ) Compendium of Microbiological Criteria for Food (2022).

2.3 Statistical Analysis

All statistical analyses were conducted using IBM's Statistical Package for Social Sciences (SPSS), Version 26. Descriptive statistics were employed to assess the quantities of aerobic plate count (APC), coliforms, *E. coli, S. aureus, B. cereus, and Salmonella spp.* present in the food items sold at the Ramadan bazaar in Kuching, Sarawak, Malaysia. This analysis aimed to provide a comprehensive overview of the microbial contamination levels in the food products, contributing valuable insights into food safety and public health at the bazaar.

RESULTS AND DISCUSSION

3.1 Microbiological Count

The bacterial counts, including aerobic plate count (APC), coliforms, *E. coli*, *S. aureus*, and B. *cereus* in the noodles sold at the Ramadan bazaar in Kuching, are detailed in Table 1. Samples collected from the Ramadan bazaar in Kuching North exhibited the highest levels of APC, coliforms, *E. coli*, and *S. aureus* contamination, with mean values of 5.188 ± 0.445 log CFU/g, 4.246 ± 0.695 log CFU/g, 2.343 ± 0.910 log CFU/g, and 2.205 ± 0.489 log CFU/g, respectively. In contrast, samples from the Ramadan bazaar in Padawan recorded the highest contamination of B. cereus, with a mean value of 3.156 ± 0.848 log CFU/g. Notably, Salmonella spp. was absent in all noodle samples analyzed during this study.

Bacterial loads can vary significantly from one location to another due to a range of factors, including environmental conditions and hygiene practices (Amare *et al*., 2019; Nguendo, 2018). In this instance, the Ramadan bazaar in Kuching South is equipped with adequate handwashing facilities, whereas both Kuching North and Padawan only have a single water source available at each bazaar. This limitation often forces food handlers to retrieve water in buckets or large bottles for handwashing and utensil cleaning, hindering proper hygiene practices. Additionally, it is noteworthy that most food handlers were observed using bare hands when preparing food, which could contribute to cross-contamination.

The presence of APC in all samples, with an overall mean value of 5.035 ± 0.622 log CFU/g, indicates that the noodles sold at the Ramadan bazaar in Kuching, Sarawak, were contaminated with various pathogens. APC is utilized to assess the overall bacterial contamination levels in food and beverage samples, reflecting hygiene and sanitation conditions at the point of sale, as documented by Mohd Nawawee *et al*. (2019) and Salamandane *et al*. (2021). It serves as a valuable tool for monitoring food handling processes, and its results can reveal the cleanliness of food preparation and storage practices (Upadhyaya *et al*., 2017). Furthermore, food items with elevated APC levels are deemed potentially harmful, even in the absence of identified pathogens (Reda *et al*., 2017). Hence, these findings clearly indicate that the noodles sold at the Ramadan bazaar in Kuching, Sarawak, are prepared under inadequate hygiene practices by food handlers.

In this study, coliform bacteria were detected in all samples, yielding a total mean value of 3.974 ± 0.763 log CFU/g. While coliforms are generally harmless to humans, they can occasionally be associated with serious waterborne diseases (Thi *et al*., 2021). The water used for food preparation and utensil cleaning can be a potential contamination source, as food handlers often recycle water for washing and cleaning purposes. As a subgroup of fecal coliform, *E. coli* serves as an indicator of sanitary conditions (Reda *et al*., 2017) and was present across all locations in this study. The mean *E. co*li count observed is consistent with previous research conducted by Mumu et al. (2021), which reported *E. coli* levels in noodles from street food carts ranging between 102 CFU/g to 103 CFU/g. Additionally, *E. coli* was isolated in noodles by Tamilnila *et al*. (2018) and Siddabathuni (2019). The presence of *E. coli* in food suggests either inadequate heat processing or fecal contamination resulting from poor hygiene practices among food handlers (Amare *et al*., 2019).

S. aureus, the most found pathogen on hands (Amare *et al*., 2019; Woh *et al*., 2017), was also detected in this study, indicating cross-contamination likely due to insufficient hand washing practices by food handlers. The washing of hands and utensils was identified as a significant risk factor, especially as food handlers often store water for cleaning in buckets due to the distance from the vending site to the water source. The repeated use of this water can elevate the risk of cross-contamination, resulting in pathogen transfer to food products (Birgen *et al*., 2020). Similarly, S. aureus has been found in noodles by Tamilnila *et al*. (2018) and Siddabathuni (2019), although Mumu *et al*. (2021) reported no S. aureus contamination in noodles, instead isolating other bacteria such as Klebsiella spp. and Pseudomonas spp. Other studies have documented the presence of S. aureus in cooked foods, including meat, rice, and fried snacks (Birgen *et al*., 2020; Vadesh & Neel, 2017; Abd Rahim *et al*., 2019).

Previous research has shown that *B. cereus* is also present in various food items, such as cooked rice and fried snacks (Saba *et al*., 2019; Fahani et al., 2019; Khasnabis *et al*., 2017). In the current study, *B. cereus* was detected in 21% of the samples, with a total mean value of 3.276 ± 0.837 log CFU/g; however, it was absent from samples taken at the Ramadan bazaar in Kuching South. *B. cereus* is a gram-positive, endospore-forming, motile rod-shaped bacterium commonly found in food and soil, and it produces two types of toxins (Yeo *et al*., 2018). According to Fahani *et al*. (2019), leftover food stored in refrigeration for sale the following day can promote toxin formation, leading to food poisoning.

Importantly, no *Salmonella spp*. was detected in any of the noodle samples from the Ramadan bazaar in Kuching, Sarawak. This finding aligns with Mumu *et al*. (2021), which similarly found no *Salmonella spp*. in noodles sampled in Dhaka City, India. Additionally, it corroborates Salamandane *et al*. (2021), who noted that *Salmonella spp.* is rarely found in cooked foods but is often present in street foods due to post-contamination. However, Tamilnila *et al*. (2018) reported potential *Salmonella spp*. contamination in street food noodles in Thanjavur City, India, attributed to poor environmental conditions.

Table 1: Microbiological counts of APC, coliform, *E. coli*, *S. aureus*, *B. cereus* and *Salmonella* spp. in noodles at Ramadan bazaar in Kuching, Sarawak.

Parameter	District	N[1](%)	Mean[2]	SD
APC	Kuching North	18 (100)	5.188	0.445
	Kuching South	5 (100)	4.798	0.944
	Padawan	10 (100)	4.880	0.709
	Total	33 (100)	5.035	0.622
Coliform	Kuching North	18 (100)	4.246	0.695
	Kuching South	5 (100)	3.193	0.478
	Padawan	10 (100)	3.877	0.750
	Total	33 (100)	3.974	0.763

E. coli	Kuching North	7 (39)	2.343	0.910
	Kuching South	1 (20)	2.491	.
	Padawan	1 (10)	2.204	.
	Total	9 (27)	2.344	0.791
S. aureus	Kuching North	13 (72)	2.205	0.489
	Kuching South	4 (80)	2.251	0.169
	Padawan	4 (40)	3.147	0.584
	Total	21 (64)	2.393	0.583
B. cereus	Kuching North	1 (6)	4.000	.
	Kuching South	0(0)	.	.
	Padawan	6 (60)	3.156	0.848
	Total	7 (21)	3.276	0.837
Salmonella spp.	Kuching North	0(0)	.	.
	Kuching South	0(0)	.	.
	Padawan	0(0)	.	.
	Total	0(0)	.	.

[1]Number of positive samples

[2]Mean bacterial counts expressed in Log (CFU/g), SD: standard deviation

3.2 Comparison of Bacterial Count with Microbiology Standard

Table 2 illustrates the contamination levels of APC, coliforms, *E. coli*, *S. aureus*, *B. cereus*, and *Salmonella spp*. in noodles, in comparison with the Food Standards Australia New Zealand (FSANZ) Compendium of Microbiological Criteria for Food (2022). Based on the results, 12% of the total samples were classified as marginal, while 88% were rated as unsatisfactory for APC contamination. The elevated bacterial counts in the noodles may be attributed to the addition of gravy and condiments, such as chopped spring onions and chilies, after the cooking process. Previous studies have also reported high levels of APC contamination exceeding 5.0 Log CFU/g in various food types, including cooked meats, fried snacks, vegetables, and beverages (Mehboob & Abbas, 2019; Mohd Nawawee *et al*., 2019; Alem *et al*., 2020; Thi *et al*., 2021).

In terms of coliform contamination, 97% of the samples were found to fall within the marginal range, while only 3% were deemed unsatisfactory. This finding contradicts several studies that have indicated a high prevalence of unsatisfactory coliform levels in various foods, such as sandwiches, beverages, cooked rice, and fried snacks (Mohd Nawawee *et al*., 2019; Abd Rahim *et al*., 2019; Salamandane *et al*., 2021). As noted by Mohd Nawawee *et al*. (2019), the presence of these bacteria may result from direct contact with the hands or bodies of food handlers or from the

use of unboiled water during food preparation. Furthermore, a significant presence of coliforms in food is indicative of unsanitary conditions during processing, handling, distribution, and post-handling contamination (Abd Rahim *et al*., 2019).

Regarding *E. coli* contamination, 73% of the samples were considered satisfactory, with 6% at a marginal level and 21% classified as unsatisfactory. Abd Rahim *et al.* (2019) reported that only two samples in their research were contaminated with *E. coli*, and both exceeded acceptable standards. The main factors contributing to *E. coli* contamination in their samples were identified as food handlers failing to adhere to proper hygiene practices, such as washing their hands after using the restroom and before handling food. Mumu *et al*. (2021) indicated that *E. coli* counts exceeding permitted food safety standards suggest that many food handlers do not practice appropriate food handling measures, including smoking while working and serving food without gloves or protective coverings for hair and masks. Additionally, a lack of consistent supervision by relevant food safety authorities contributes to the non-compliance of food safety practices among food handlers.

For *S. aureus* contamination, nearly half of the samples achieved satisfactory levels (46%), while 48% were at a marginal level, and only 6% were rated as unsatisfactory. A study by Abd Rahim *et al*. (2019) found that 33% of samples sold in Chow Kit, Kuala Lumpur, were contaminated with *S. aureus*, with mean values surpassing 4 Log CFU/g. Moreover, Afreen et al. (2019) reported that 100% of street drinks tested were classified as unsatisfactory for S. aureus contamination, identifying several risk factors associated with *S. aureus*, including the type of juice, vending location, water source, food covering, serving food with bare hands, and the cleaning of utensils. In this study, 79% of the total samples for *B. cereus* contamination were satisfactory, followed by 6% at a marginal level and 16% that were unsatisfactory. A study conducted in Ghana by Saba *et al*. (2019) reported a higher prevalence of unsatisfactory food samples from enclosed vendors, with fried rice having the highest incidence of unsatisfactory results.

Table 2: Level of contamination level for APC, coliform, *E. coli*, *S. aureus*, *B. cereus* and *Salmonella* spp. in noodles at Ramadan bazaar in Kuching, Sarawak.

Parameter	No. of Satisfactory Samples (%)	No. of Marginal Samples (%)	No. of Unsatisfactory Samples (%)
APC	0 (0%)	4 (12%)	29 (88%)
Coliform	0 (0%)	32 (97%)	1 (3%)
E. coli	24 (73%)	2 (6%)	7 (21%)
S. aureus	15 (46%)	16 (48%)	2 (6%)
B. cereus	26 (79%)	2 (6%)	5 (15%)
Salmonella spp.	33 (100%)	0 (0%)	0 (0%)

3.3 Distribution of *E. coli, S. aureus* and *B. cereus* in Different Types of Noodles

Figure 2 illustrates the distribution of *E. coli* (9 isolates), *S. aureus* (21 isolates), and *B. cereus* (7 isolates) across eight different types of noodles. The popular local noodle, *mee jawa*, showed the highest level of contamination, closely followed by *kolo mee* and *laksa Sarawak*. This pattern indicates that these foods are susceptible to contamination due to the various ingredients used in their preparation. These types of noodles are typically served with gravy and an array of condiments, including meat, sliced spring onions, chilies, bean sprouts, and fried onions. The addition of these condiments increases the likelihood of contamination, particularly from insufficient personal hygiene and poor food handling practices. Food handlers often use their bare hands to add these toppings, raising concerns about their hand hygiene, especially given the limited hand washing facilities available at the Ramadan bazaar. Previous studies have demonstrated that bacterial loads on food handlers' hands can exceed safe limits (Lee *et al*., 2017), and a lack of hand-washing habits is a recognized risk factor for microbial contamination (Afreen *et al*., 2019). While disposable gloves can be effective in preventing cross-contamination if used correctly, they should not replace hand washing (Trafialek *et al*., 2018; Afreen *et al*., 2019). It is also advisable to utilize clean tongs, forks, or spoons when handling food (Birgen *et al*., 2020). Therefore, it is crucial to emphasize proper hand hygiene and effective hand-washing techniques, particularly to new, young, and inexperienced food handlers (Anowai *et al.*, 2019).

Despite the presence of good hand washing facilities at the Ramadan bazaar in Kuching South, other risk factors for microbial contamination were identified, as contamination by *S. aureus* still occurred in nearly all samples. The noodle gravy can also act as a contamination source if not stored and handled correctly by food handlers. Noodle gravy is typically prepared with coconut milk or chili paste and made in advance at home. Improper handling and storage practices of coconut milk may lead to cross-contamination. Previous studies by Nurul *et al*. (2017) and Sari *et al.* (2019) have highlighted the presence of *E. coli* and *S. aureus* in coconut milk due to inadequate personal protective equipment and improper storage practices.

Additionally, fried noodles, such as fried *mee hoon*, fried *kolo mee*, and fried *kuetiau*, were found to contain *E. coli*, *S. aureus*, and *B. cereus*. These fried noodles are often left uncovered during sales, exposing them to flies and dust, which can contribute to contamination. Moreover, cooked foods that remain out for extended periods can create favorable conditions for pathogenic microorganisms to grow and multiply, resulting in potential infections and foodborne illnesses when consumed (Amare *et al*., 2019; Mwove *et al*., 2019).

Previous research by Tamilnila *et al*. (2018) reported the presence of *E. coli*, *S. aureus*, *Salmonella spp*., and *Clostridium* in street food noodles sold in India. They noted a significant increase in contamination risk due to the extremely unsanitary environment in which these foods are prepared and served. Additionally, a lack of adequate garbage disposal facilities and clean water sources for drinking and cleaning further exacerbates the situation. Similarly, Siddabathuni (2019) found 21 bacterial pathogens, including *S. aureus, Proteus spp*., and *E. coli*, in 79% of contaminated noodle samples, linking these findings to poor hand hygiene among vendors, the use of contaminated water in food preparation, and the contamination of utensils by pathogens carried by flies from nearby sewage and garbage. The findings from both Tamilnila *et al*. (2018) and

Siddabathuni (2019) emphasize that extremely poor environmental conditions and inadequate facilities at food premises are significant contributing factors to food contamination.

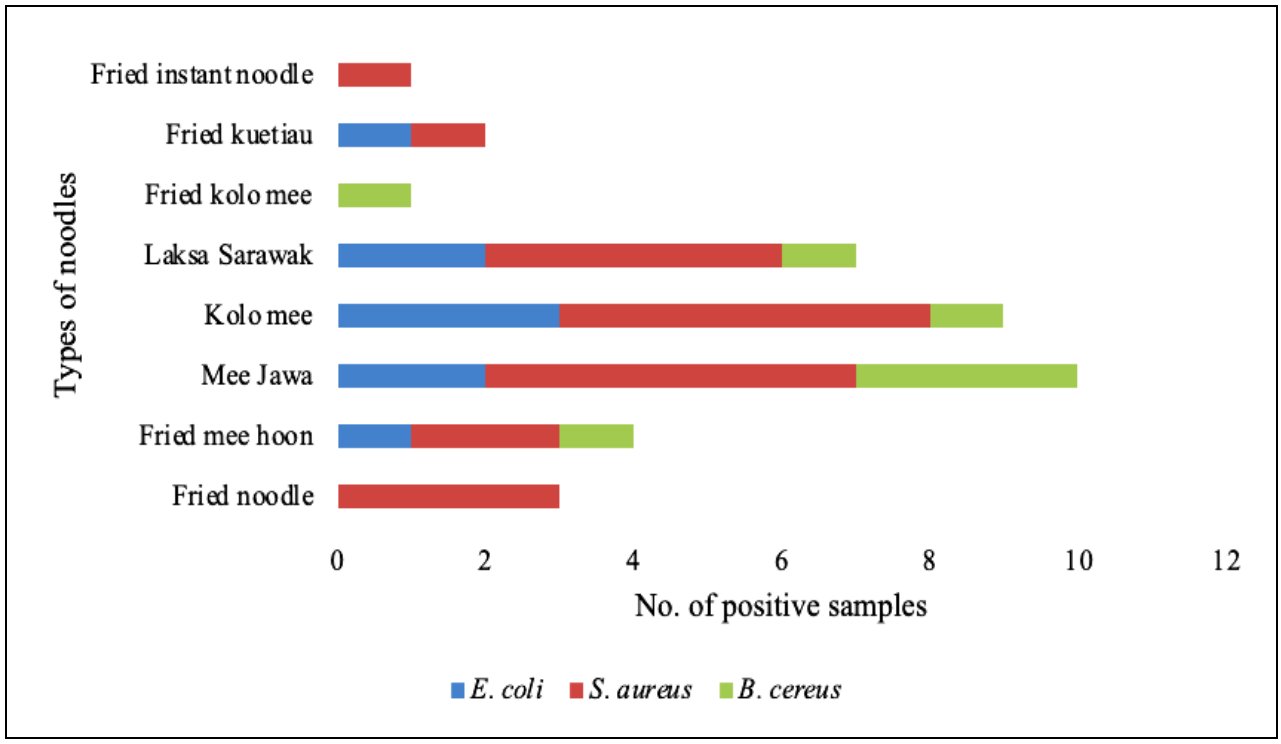

Figure 2: Type of noodles contaminated with *E. coli*, *S. aureus* and *B. cereus*

CONCLUSION

The findings of this study indicate that noodles sold at Ramadan bazaars in Kuching, Sarawak, may have been contaminated with harmful microorganisms, including *S. aureus* and *B. cereus*. The presence of these bacteria in food poses potential health risks for consumers. Furthermore, the microbial analysis showed that some noodle samples had contamination levels that exceeded the acceptable standards. Consequently, this study recommends the implementation of more effective health education and food handling training for food vendors at Ramadan bazaars in Kuching, Sarawak, Malaysia. Additionally, it is essential to establish information and training programs for consumers to raise awareness about food safety, thereby protecting them from potential foodborne illnesses.

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THE SYNTHESIS OF HYDROGEL FROM SELECTIVE NATURAL RESOURCE IN MALAYSIA: A REVIEW

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ABSTRACT. *Hydrogels are hydrophilic polymer materials characterized by three-dimensional (3D) network structures that can absorb and retain significant amounts of water within their interstitial spaces. Due to their potential applications in advanced technologies across various sectors, including biomedical, pharmaceutical, biotechnology, bioseparation, biosensor, livestock, oil recovery, and cosmetics industries, hydrogels—often referred to as smart or hungry networks—are the subject of extensive scientific research. Recently, researchers have focused on creating hydrogels from natural resources to promote environmentally sustainable technologies. This review provides a concise overview of the use of oil palm empty fruit bunch (OPEFB) cellulose, C. asiatica asiaticoside, and cross-linked honey, sourced from Malaysia, in the development of hydrogels.*

KEYWORD. Hydrogel; Natural resources; Eco-Friendly Polymers; OPEFB cellulose; C. asiatica Asiaticoside; Cross-linked honey.

INTRODUCTION

Situated in the heart of Southeast Asia, Malaysia is endowed with a wealth of natural resources that underpin its vibrant and dynamic economy. The country boasts a diverse array of rich resources, including its lush rainforests and fertile agricultural lands, which yield valuable products such as palm oil, *Centella asiatica*, and honey—the latter being a prized by-product collected by bees in the expansive woodlands. As the second-largest producer of palm oil globally, Malaysia generates approximately 22–23 million tons of Oil Palm Empty Fruit Bunch (OPEFB) each year, thanks to its extensive plantations and palm oil mills (Padzil *et al*., 2020). The nation's rich biodiversity includes

an abundant supply of *Centella asiatica*, commonly known as "pegaga," which plays a vital role in the ecosystem (Yusof et al., 2020). Moreover, Malaysia's lush forests are home to a variety of bee species that produce a substantial quantity of honey (Yap and Chin, 2021). This paper explores the various uses of Malaysia's natural resources, particularly their essential role in the development of hydrogels.

Hydrogels are hydrophilic polymer networks capable of absorbing significant amounts of water and undergoing swelling and shrinking. These materials have a three-dimensional (3D) network structure composed of hydrophilic polymer chains that enable them to capture and retain substantial water within their interstitial spaces (Chai et al., 2017; Ebara et al., 2014; Yuan, 2013). Hydrogels swell and form a 3D structure upon contact with water due to the presence of hydrophilic groups, such as -NH2, -OH, -COOH, and -SO3H, in their polymer networks, combined with osmotic strain (Yuan, 2013).

Crosslinking plays a crucial role in hydrogels by preventing them from dissolving in solvents while maintaining their three-dimensional structure during the swelling process. In the case of physical crosslinking, temporary connections are formed through hydrogen bonding, hydrophobic interactions, or electrostatic forces between polar groups (Rizwan et al., 2017). Conversely, smart hydrogels exhibit significant physiochemical changes in response to slight environmental variations, allowing for reversible transformations; when the triggering factor is removed, smart hydrogels can return to their original state (Ebara et al., 2014).

Smart hydrogels are advanced polymeric networks that are highly hydrated and feature intricate three-dimensional microstructures, distinguished by their ability to respond to various environmental stimuli, such as temperature, pH, light, and pressure. This responsiveness is achieved through the incorporation of stimuli-responsive co-monomers into their network architecture. Smart hydrogels boast a high degree of versatility, making them suitable for numerous applications, including drug delivery systems, optical switches, and therapeutic uses (Samal et al., 2014). The relationship between crosslinking and smart hydrogels underscores their capacity for dynamic adaptation to changing conditions, rendering them highly attractive for a wide range of applications.

2.0 CLASSIFICATION OF HYDROGEL

The classification of hydrogels is based on various factors. Hydrogels can be categorized according to their physical properties, swelling behavior, methods of preparation, origin, ionic charges, sources, biodegradation rates, and the nature of crosslinking, as illustrated in Figure 1 (Qiu and Park, 2001; Ullah et al., 2015). While this review does not delve into the specific details of each classification type, it highlights some of the key hydrogels that have garnered significant interest from researchers.

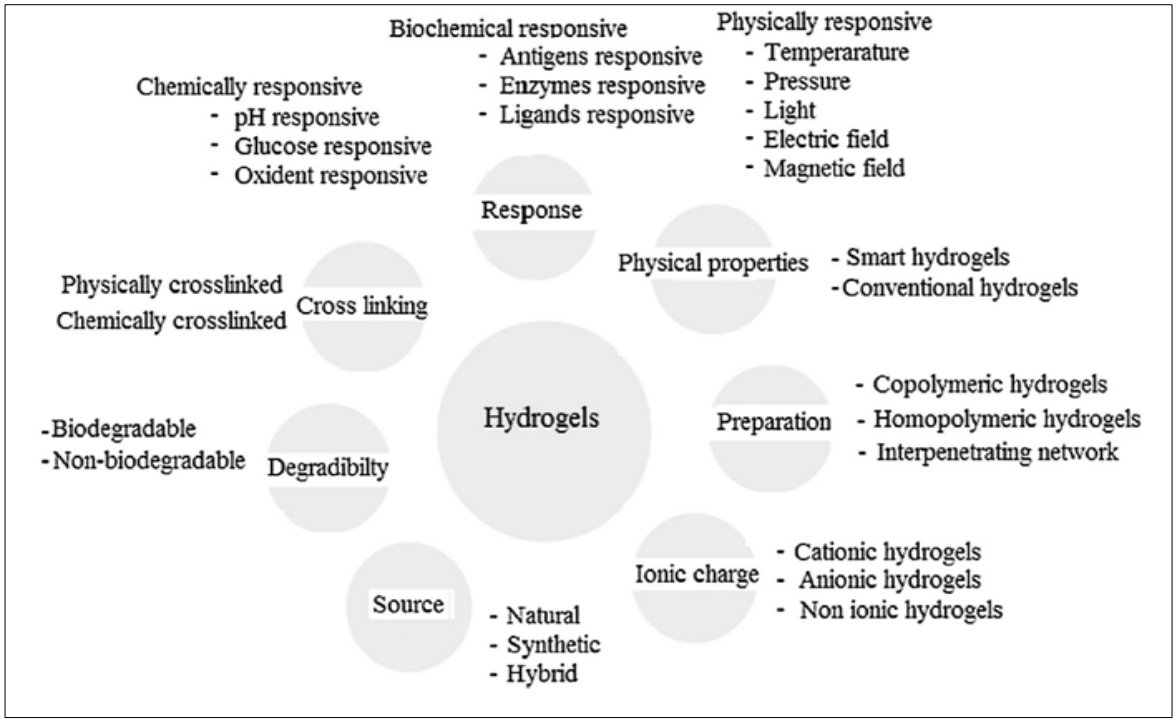

Figure 1. Classification of hydrogels based on different properties (Ullah *et al*., 2015)

The crosslinking mechanism in physical gels is fundamentally physical in nature. Crosslinking typically occurs through processes such as hydrophobic interactions, chain aggregation, crystallization, polymer chain complexation, and hydrogen bonding (Rizwan *et al*., 2017). In contrast, chemical hydrogels are prepared using chemical methods, such as covalent crosslinking, which can occur either simultaneously or after polymerization. Unlike chemical hydrogels, physical hydrogels are reversible due to their configurational changes.

Another type addressed in this review is the dual-network hydrogel, which is formed through electrostatic interactions by integrating both physically and chemically crosslinked hydrogels. This dual-network structure enhances the advantages of both types, offering a high capacity for liquid absorption across a broad pH range and increased sensitivity to pH variations (Qiu and Park, 2001). Recently, another variant of dual-network hydrogels has been reported, consisting of graphene polymer composites that exhibit superior mechanical properties and self-healing capabilities (Cong *et al*., 2013).

3.0 DEVELOPMENT OF HYDROGEL

Hydrogels have emerged as versatile agents renowned for their extensive applications across biological, engineering, and medical fields. Among these, polyelectrolyte hydrogels have garnered considerable attention due to their unique capability to generate or retain charges along their polymer chains, facilitating the formation of complexes with oppositely charged species. This remarkable adaptability is widely utilized in the biological and pharmaceutical sectors (Chai *et al*., 2017). Additionally, cationic polymers have piqued interest as they serve as synthetic carriers that can compact large structures into smaller ones while safeguarding the negatively charged DNA strands. They are employed in the creation of DNA delivery vectors, bile acid sequestration, gene therapy, and cell transfection (Qiu and Park, 2001).

In response to slight environmental variations, hydrogels can undergo substantial volume changes triggered by factors such as electric fields, solvent interactions, pH levels, ionic strength, and temperature. Current research is focused on developing hydrogels synthesized from natural resources to promote a greener environment (Salehi and Moghadam, 2023). This review provides a concise overview of the advancements in hydrogels derived from natural resources previously explored by researchers. Table 1 showcases the methods by which hydrogels were developed using selected natural resources that are readily available and abundant in Malaysia.

Table 1. Hydrogels development from selective natural resources in Malaysia.

Natural Resources	Raw Material Resources	Application	References
Oil palm empty fruit bunch (OPEFB)	Oil palm empty fruit bunch (OPEFB) are produced abundantly as a residue annually in Malaysia. About 15.8 million tonnes of Oil palm empty fruit bunch (EFB), which accounts for 20% of fresh fruit weight, are produced yearly.	The OPEFB contains up to 65% cellulose, making it a promising feedstock for cellulose extraction and manufacturing other cellulose products, such as hydrogel. Because of their enormous amount, nontoxicity, bio-compatibility, and bio-degradability, hydrogels made from natural polymers such as cellulose are excellent for use as biomaterials in the biomedical area. Cellulose-based hydrogels can be made from cellulose derivatives chemically cross-linked and dissolved in water by employing tiny bifunctional molecules as a crosslinking agent.	(Kundu *et al*., 2022) (Padzil *et al*., 2020) (Yiin *et al*., 2019) (Ng *et al*., 2015)
***Cantella asiatica* (Indian pennywort or *Asiatic* pennywort or *pegaga*).**	An herbaceous, perennial plant in the flowering plant family Apiaceae, commonly found commercially produced or plated in Malaysia. It is also grown in the open tropics, common meadows, gardens, farms, and roadsides.	Hydrogel wound dressings can benefit greatly from the addition of *Centella asiatica's Asiaticoside*, which has natural wound-healing qualities. This work investigates the in vivo performance of a hydrogel formulation high in asiaticosides that was created to promote wound healing. The outcomes demonstrate the efficacy of this strategy by demonstrating that the hydrogel rich in asiaticosides effectively promotes angiogenesis and collagen synthesis, hence expediting wound healing.	(Pinthong *et al*., 2023) (Fadzil *et al*., 2020) (Yousaf *et al*., 2020) (Sh Ahmed *et al*., 2019)

Honey	Extracted from floral nectars and other plant secretions, a carbohydrate-rich syrup prepared by honeybees. Honey is also referred to by the geographical location where the honey is produced, the honey's floral source or the trees on which the hives are located.	Studies have shown that adding multiple crosslinkers to crosslinked honey can increase its crosslinking effectiveness. Hydrogels based on alginate, encourage regenerative repair. When it combined with ghee, this mixture results in almost completely scar-free healing and has tissue thickness and build-up similar to normal skin. Additionally, honey-infused hydrogel dressings fulfil important requirements for an efficient burn wound dressing by having remarkable physical qualities like transparency, exudate absorption, and an appropriate acidic pH value.	(Pinthong *et al*., 2023) (Gope *et al*., 2022) (Ahmed and Othman, 2013) (Mohd Zohdi et al., 2012)

3.1 OPEFB Cellulose Hydrogel Development

The Oil Palm Empty Fruit Bunch (OPEFB) is a by-product generated during the processing of crude palm oil (CPO) in palm oil mills. After the fruits are separated from the fresh fruit bunches (FFB), OPEFB is collected from the empty stalks. It is estimated that for every kilogram of palm oil extracted, approximately 4 kilograms of dry biomass is produced (Abdul Khalil *et al*., 2008). In Malaysia, around 22-23 million tonnes of OPEFB are generated annually as a significant residue (Anuar *et al*., 2019). There is a growing global interest in biodegradable and environmentally friendly products, including those made from this entire production stream (Ng *et al*., 2015). Utilizing cellulose derived from OPEFB represents a promising avenue for hydrogel production. In Malaysia, OPEFB is the most affordable natural fibre available and possesses strong properties, making it widely abundant in the country. It holds great potential as an alternative primary raw material to replace woody plants. Additionally, the well-known polymeric hydrogel has attracted considerable attention due to its three-dimensional (3D) cross-linked network and high porosity (Anuar *et al*., 2019).

Despite its advantages, OPEFB-based hydrogels face several efficiency challenges, including issues with weak interfacial connectivity and mechanical strength. To address these concerns, cellulose hydrogels have been introduced. This review examines the potential of utilizing OPEFB as a cellulose source in hydrogel production within Malaysian oil palm plantations. Cellulose can be categorized into three types of nano-structured celluloses based on their processing methods. Currently, 3D printing technology is at the forefront of hydrogel production due to its ability to create complex structures and the need for high-purity products. Additionally, this review discusses some of the latest advanced applications to emphasize the strong commercialization prospects of cellulose hydrogel materials.

3.1.1 Oil Palm Empty Fruit Bunch Cellulose

Cellulose is a linear homopolymer made up of (1-4)-glycosidic bonds linking D-anhydroglucopyranose units (AGUs), as illustrated in Figure 2. Native cellulose, also known as Cellulose I, is a parallel-arranged semi-crystalline polymer and is not the most stable crystalline form (French, 2017). The natural form of cellulose is cellulose I, which consists of two structures, Iα and Iβ. The regenerated cellulose product, commonly referred to as cellulose II, has a similar molecular formula to Cellulose I $(C_6H_{10}O_5)_n$ but is more stable and can be shaped into various products, including membranes, hydrogels, aerogels, and fibres (Mohammad Padzil *et al*., 2018).

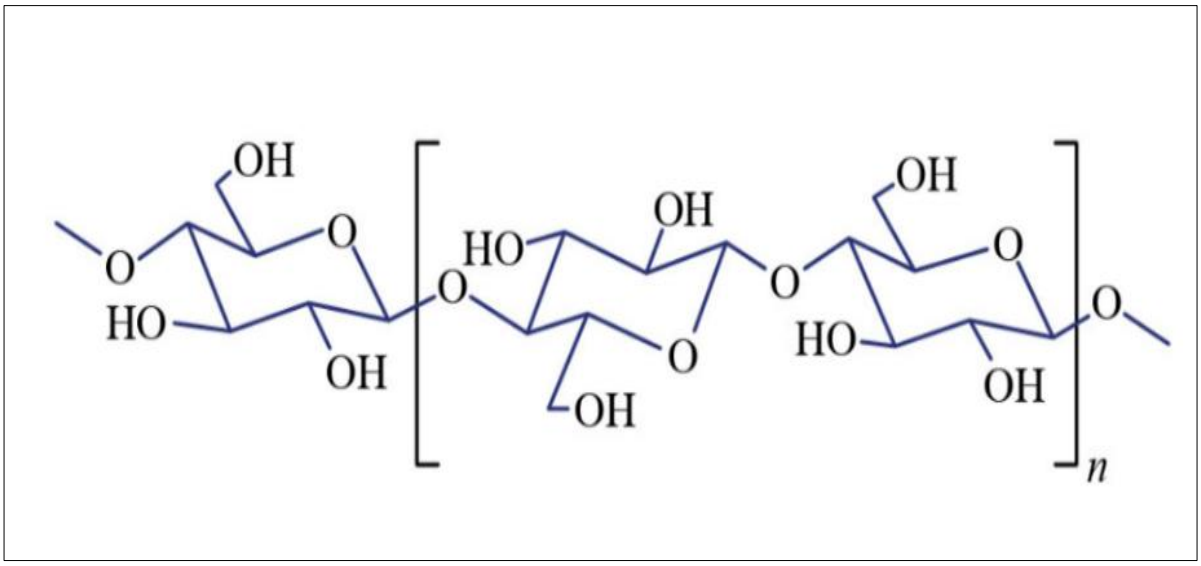

Figure 2 The cellulose structure (Britannica, 2023).

In recent years, several nanomaterials with highly promising properties have emerged, including nanocrystal cellulose (CNC or NCC), bacterial nanocellulose (BNC), and nanofiber cellulose (CNF). These nanocellulose materials are incorporated into polymers as reinforcing agents to create cellulose fibre-reinforced composites. Due to its ultralight and highly porous nature, CNF can be extracted using either chemical or mechanical methods (Gopakumar *et al*., 2020). The following subchapter will explore the potential applications of OPEFB cellulose. Various processing techniques can be employed to develop an OPEFB hydrogel, including homogenization, grafting OPEFB cellulose during the polymerization process, freeze-thaw cycles, and 3D printing technology (Athukoralalage *et al*., 2019).

3.1.2 Potential Application of OPEFB Cellulose Hydrogel

While hydrogels have been utilized across multiple sectors, only a limited number of studies have been conducted on hydrogels made from nano-sized cellulose derived from OPEFB. However, insights from prior research on cellulose-based hydrogels allow for predictions regarding the potential applications of OPEFB cellulose hydrogels. Table 2 presents the hydrogel created from OPEFB cellulose along with its possible applications.

Table 2. Potential Applications of OPEFB and Combination Materials.

Potential Application	Materials	Reference
Alternative medium for constant water supply for plants	OPEFB cellulose + NaOH/urea solvent + NaCMC	(Salleh et al., 2019)
Spikelet MCC—biocomposite, Stalk MCC—food and pharmaceutical products	Microcrystalline cellulose (MCC) extracted from OPEFB, stalks and spikelet	(Xiang et al., 2016)
Tissue engineering and medium for controlled/ slow-release fertilizer	OPEFB cellulose + NaOH/urea solvent + Sodium carboxymethylcellulose (NaCMC)	(Salleh et al., 2018)
Thermal insulating	OPEFB + graphene oxide (GO)	(Gan et al., 2018)

Source: (Padzil *et al*., 2020)

For instance, Xiang et al. (2016) investigated the suitability of microcrystalline cellulose (MCC) derived from OPEFB, including its stalks and spikelets, for use in food products. They found that the cellulose content was highest in the OPEFB stalk fibres and lowest in the spikelet fibres, which also contained the least amount of lignin and residual oil. Comparatively, the spikelet fibre MCC exhibited the highest crystallinity index among the three, suggesting it is a suitable option for load-bearing applications like biocomposites (Nafu *et al*., 2015). Hydrogels made from OPEFB stalk fibre MCC have shown performance equivalent to that of commercial MCC, indicating their potential for use in culinary and medicinal products as a substitute for commercial MCC hydrogels.

In the context of the current industrial revolution (IR4.0), additive manufacturing stands out as a key innovation in production processes. Although research on 3D printing using OPEFB nanocellulose has not yet been conducted, the demonstrated strength enhancements of OPEFB cellulose indicate significant potential for its applications in developing OPEFB nanocellulose hydrogels through 3D printing (Velasco-Hogan et al., 2018). Salleh et al. (2018) produced a regenerated superabsorbent hydrogel by dissolving OPEFB cellulose in a NaOH/urea solution along with sodium carboxymethyl cellulose (NaCMC), which may have applications in tissue engineering. Additionally, Salleh *et al*. (2019) developed a superabsorbent hydrogel leveraging the swelling capacity of OPEFB cellulose, which was found to exceed 80,000%. This type of hydrogel has the potential to maintain optimal hydration for plants. Furthermore, Gan *et al*. (2018) created an aerogel using OPEFB cellulose and graphene oxide, which featured a microporous structure and an equilibrium swelling ratio of 2000 to 3700 percent, making it potentially useful as a thermal insulating material due to its high thermal stability. The previously mentioned studies highlight the ability of OPEFB cellulose to produce superabsorbent hydrogels. Although the hydrogel produced from OPEFB nanocellulose has not yet been reported, it can be inferred that OPEFB cellulose possesses equal or even superior potential for the production of superabsorbent hydrogels suitable for various industries. OPEFB cellulose hydrogel may offer a viable solution for enhancing mechanical strength and bioactivity.

3.2 Asiaticoside-rich Hydrogel Development

Centella asiatica, commonly referred to as Indian pennywort or Asiatic pennywort, is a herbaceous, perennial plant belonging to the Apiaceae family, as depicted in Figure 3. It has traditionally been used to treat minor injuries and various ailments (Joshi and Chaturvedi, 2013). Various extracts of *Centella asiatica* and its active component, asiaticoside, have demonstrated wound-healing properties in multiple in vivo and in vitro studies (Sh Ahmed *et al*., 2019). One study focused on the in vivo effectiveness of an asiaticoside-rich hydrogel formulation in rabbits, aiming to create a formula that promotes accelerated wound healing (Ansell *et al*., 2014).

Figure 3. *Centella asiatica* (Tripathi *et al*., 2015)

3.2.1 Asiaticoside-rich Hydrogel Formulation

The aerial parts of C. asiatica were used to prepare an asiaticoside-rich fraction, which was then incorporated into a hydrogel composed of polyvinyl alcohol and polyethylene glycol (PVA/PEG) (Sh Ahmed *et al*., 2019). This hydrogel was evaluated for its wound-healing properties using an in vivo incision model. According to Sh. Ahmed *et al*. (2019), the C. asiatica PVA/PEG hydrogel was produced using the freeze-thaw technique. Initially, 8% PVA was dissolved in deionized water on a hotplate for one hour while being stirred with a magnetic stirrer. Following this, 5% PEG was added, and the mixture was autoclaved for 15 minutes at 121°C. Subsequently, 24 mg of the asiaticoside-rich fraction was dissolved in the prepared PVA/PEG hydrogel and subjected to five consecutive freeze-thaw cycles. The C. asiatica plant contains three primary compounds—asiaticoside, asiatic acid, and madecassic acid—which are formulated in the hydrogel for wound treatment (Ahmed *et al*., 2018; Ansell *et al*., 2014).

3.2.2 Asiaticoside-rich Hydrogel Potential

Centella asiatica hydrogel incorporates three key components—asiaticoside, asiatic acid, and madecassic acid—for effective wound healing. An effective wound dressing should focus on several important factors, including infection prevention, non-adherence, support for debridement, gas exchange, maintenance of wound moisture, tissue healing, and safety (Dhivya *et al*., 2015). Moreover, the wound healing process involves complex interactions among various cell types, extracellular matrix components, and cytokine mediators (Wang *et al*., 2022).

The wound-healing properties of asiaticosides may be attributed to their regulation of several mechanisms of action. Asiaticoside, in particular, has been shown to enhance antioxidant levels in the early stages of healing, which could play a crucial role in its therapeutic effects (Shukla *et al*., 1999). *C. asiatica* contains various phytochemical constituents, including sesquiterpenes, flavonoids, pentacyclic triterpenoids, plant sterols, caffeoylquinic acids, and eugenol derivatives (Gray *et al*., 2018), which contribute to its ability to maintain a moist wound environment. Given these considerations, wound dressings should also be biocompatible with tissues and blood, non-antigenic, non-toxic, and possess adequate elasticity.

As a review, it is concluded that the Asiaticoside-rich hydrogel formulated in this study is a healthy and biocompatible formulation with good physicochemical properties ideal for topical wound healing applications. Hence, biocompatible polymeric hydrogels may be the most promising wound dressing materials, as they meet the effective wound dressing requirements by providing an easy-to-handle dressing with no irritation and adhesion properties, thereby maintaining or improving patient comfort.

3.3 Cross-linked Honey Hydrogel Development

Honey is a thick, syrupy substance primarily composed of carbohydrates, produced by honeybees from the nectar of flowers (Speer et al., 2021). Its antibacterial, anti-inflammatory, and antioxidant properties are attributed to various components within honey, which also exhibit immunomodulatory effects that can aid in wound healing (Almasaudi, 2021). It is important to recognize that factors such as floral and geographic origin, extraction methods, storage conditions, and the chemical composition and quality of honey can vary by region (Lobos *et al*., 2022).

3.3.1 Cross-linked Honey Hydrogel Formulation

The formulation of honey hydrogel started with obtaining the Polyvinyl pyrrolidone (PVP) based and Polyethylene glycol (PEG), as seen in Figure 4, based on the polymer matrix. Oxoid provided the technical grade agar. Briefly, when the aqueous solution of PVP is prepared, the solution is left at room temperature (25℃) overnight. After dissolving and heating, the mixture was continuously stirred before inserting PEG. In the ultrasonic water bath, the homogenous mixture was left at 37℃, and honey was applied when the solution temperature reached below 45℃. Then, the cross-linked and sterilized electron beam (Ahmed *et al*., 2013) is performed. Thus, the cross-linked honey hydrogel is developed.

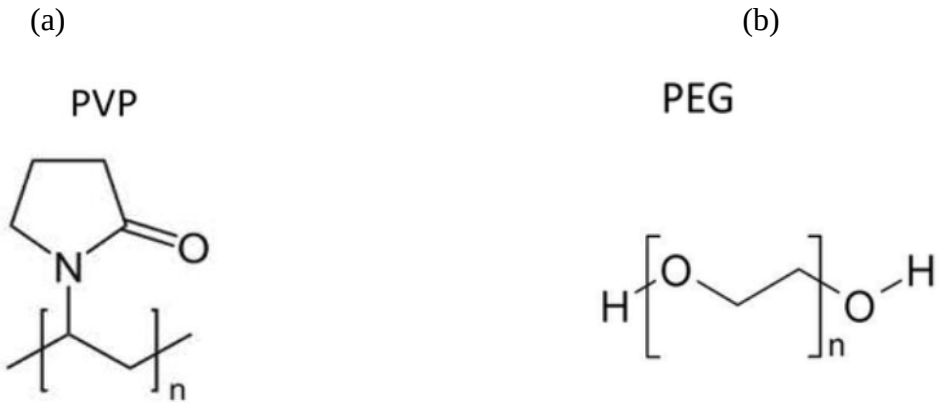

Figure 4. Structure of Polyvinyl pyrrolidone (a) and Polyethylene glycol (b) (Ramkumar *et al.*, 2016)

Studies on the development of honey hydrogels, particularly those focusing on cross-linked varieties, have yielded promising results. Researchers have explored the incorporation of polyvinyl pyrrolidone (PVP) and polyethylene glycol (PEG) as essential components of the polymer matrix to enhance the mechanical properties and bioavailability of these hydrogels (Su *et al*., 2021). When it comes to sterilization, the use of electron beam polymerization is a powerful and versatile method that can enhance the stability of hydrogels, improve crosslinking density, and boost drug incorporation capabilities. This technique facilitates tailored drug delivery and functionalized surfaces for biomaterial applications (Glass *et al*., 2019). As these advanced hydrogels continue to be the subject of ongoing research and technological advancements, they present significant potential for applications in wound healing, tissue engineering, and drug delivery.

3.3.2 Cross-linked Honey Hydrogel Potentials

Manuka honey, pasture honey, jelly bush honey, and African forest honey are well-studied varieties with documented benefits. Recently, Tualang honey (TH), a multifloral jungle honey from Malaysia, has garnered attention for its potential medicinal properties. In contrast, the advantages of Manuka honey are widely recognized across the globe (Ahmed and Othman, 2013). However, only a handful of Malaysian researchers have explored its effects in tissue culture mediums and clinical trials (Ghashm *et al*., 2010). Honey primarily consists of fructose (38%), glucose (31%), and various other sugars, along with over 180 compounds including amino acids, vitamins, minerals, and enzymes (Alnaqdy *et al*., 2005).

Malaysian honey has also demonstrated significant antibacterial properties and is effective in wound treatment. Gelam honey exhibits antioxidant and radical scavenging abilities, largely attributed to its phenolic content (Aljadi and Kamaruddin, 2004). Extracts from this honey have shown inhibitory effects on inflammatory mediators in animal models (Kassim *et al*., 2010), underscoring the considerable potential of Malaysian honey in the pharmaceutical sector. Overall, honey contributes to wound healing by alleviating common issues such as edema, inflammation, and exudation across various wound types. It promotes the proliferation of epithelial cells and fibroblasts. Manuka honey is particularly effective for treating wet burns and other types of wounds (Visavadia *et al*., 2008), and studies have indicated that Tualang honey produces similar results (Nasir *et al*., 2010).

Wounds treated with Tualang honey demonstrate a reduction in size by 32.26% for full-thickness burn wounds when compared to those treated with traditional hydrofibre silver dressings (Khoo *et al*., 2010). Patients tend to prefer Tualang honey hydrogel dressings over traditional ones due to the minimal pain experienced, the soothing effects of the treatment, and the pleasant fragrance of the dressings (Imran *et al*., 2011). Moreover, certain microorganisms are sensitive to specific types of honey. The table below provides a comparative list of microorganisms that are susceptible to both Tualang and Manuka honey.

Table 3. List of microorganisms found to be susceptible to the honey of Tualang and Manuka

Gram-positive strains		**Gram-negative strains**	
Tualang honey	**Manuka honey**	**Tualang honey**	**Manuka honey**
Streptococcus pyogenes	*Streptococcus pyogenes*	*Stenotrophomonas maltophilia*	*Stenotrophomonas maltophilia*
Coagulase-negative Staphylococci	*Coagulase-negative Staphylococci*	*Acinetobacter baumannii*	*Acinetobacter baumannii*
Methicillin-resistant - Staphylococcus aureus (MRSA)	*Methicillin-resistant - Staphylococcus aureus (MRSA)*	*Salmonella enterica Serovar typhi*	*Salmonella enterica Serovar typhi*
Streptococcus agalactiae	*Streptococcus agalactiae*	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
Staphylococcus aureus	*Staphylococcus aureus*	*Proteus mirabilis*	*Proteus mirabilis*
Coagulase-negative-Staphylococcus aureus (CONS)	*Coagulase-negative-Staphylococcus aureus (CONS)* - haemolytic streptococci - *Enterococcus* - *Streptococcus mutans* - *Streptococcus sobrinus* - *Actinomyces viscosus*	*Shigella flexneri*	*Shigella flexneri*
		Escherichia coli	*Escherichia coli*
		Enterobacter cloacae	*Enterobacter cloacae*
		Shigella sonnei	*Shigella sonnei*
		Salmonella typhi	*Salmonella typhi*
		Klebsiella pneumonia	*Klebsiella pneumonia*

	Stenotrophomonas maltophilia	***Stenotrophomonas maltophilia***
		Burkholderia cepacia
		Helicobacter pylori
		Campylobacter spp.
		Porphyromonas gingivalis

Source: Department of Pathology, USM

While Manuka honey is globally recognized for its therapeutic properties, Malaysian honey, particularly Tualang honey, has recently garnered attention for its significant potential in tissue culture, clinical trials, and wound healing applications. These honeys are increasingly valued in the pharmaceutical industry due to their remarkable antibacterial and anti-inflammatory properties, along with their rich presence of bioactive compounds. As further research uncovers their unique benefits, the use of these natural substances may expand, paving the way for innovative approaches to wound care and other areas, thus providing patients with soothing and effective treatment options.

CONCLUSION

Hydrogels are renowned for their ability to absorb water and have a wide range of applications across various fields, making them a focal point of scientific research. Researchers are actively working to develop hydrogels using plentiful Malaysian natural resources such as C. asiatica asiaticoside, cellulose from oil palm empty fruit bunch (OPEFB), and cross-linked honey, aiming to create environmentally sustainable solutions that leverage Malaysia's abundant natural wealth. This selection of resources reflects a strong commitment to resource conservation and eco-friendly technology, paving the way for the development of "green" technologies and innovative products in the rapidly evolving hydrogel research field. By utilizing these renewable resources, researchers can meet market demands for novel materials while promoting the responsible use of advanced technologies. The progress made in natural resource hydrogel research opens up new possibilities for environmentally conscious scientific investigations and applications across various industries. Ultimately, this integration of sustainable practices with scientific research will shape the future of hydrogel applications and their broader implications.

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BACTERIOLOGICAL QUALITY OF SALAD SOLD BY LOCAL FOOD VENDORS WITHIN SELECTED OPEN MARKETS IN ACCRA, GHANA

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ABSTRACT. *This study evaluated the bacterial contamination of salads sold in open markets in Accra, Ghana. Salad samples were gathered from 30 vendors in both Madina and Nima markets. The microbiological analysis included standard plate counts (SPC), Enterobacteriaceae counts (EC), and coliform counts (CC). In total, sixty salad samples were quantitatively assessed for aerobic mesophiles, Enterobacteriaceae, and coliforms using culture-based methods, with identification of bacterial isolates. Data were analyzed using SPSS version 20. It was found that 40% of the samples exceeded acceptable limits for SPC. The mean SPC was significantly higher ($p=0.042$) in unacceptable samples ($3.23x10^8$ cfu/g) compared to acceptable samples ($1.09x10^6$ cfu/g), indicating issues with handling. Both EC and CC means were elevated in both categories but did not differ significantly. Isolated organisms included Salmonella spp. and Staphylococcus spp. Salads served by hand had higher SPC ($3.28x10^8$ cfu/g) and CC ($2.67x10^7$ cfu/g) compared to those served with a spoon, while salads in food flasks exhibited higher counts than those in polythene bags for SPC ($4.53x10^8$ cfu/g, $p=0.021$) and CC ($1.80x10^8$ cfu/g). Enclosed vending areas showed greater microbial loads across all parameters compared to open sites. Poor hygiene practices in salad vending likely contributed to the significant microbial contamination observed. Implementing targeted interventions and regular testing could enhance the safety of street food.*

KEYWORDS: Salad, Bacteriological quality, Local vendor, Contamination, Food safety

INTRODUCTION

Health officials recommend consuming vegetables to bolster the immune system by providing essential nutrients and vitamins, as they are rich in micronutrients vital for biochemical processes that facilitate the formation of antibodies (Calder, 2022; Pecora *et al*., 2020). Salad, typically a combination of raw

vegetables such as lettuce, cabbage, carrots, and onions, is often chopped and sometimes combined with mayonnaise. In contemporary society, salads are convenient and appealing because they require no cooking or preparation. However, children and women of reproductive age in Ghana frequently experience micronutrient deficiencies due to low intake of foods rich in these essential nutrients, adversely affecting their health and hindering social and economic development.

Observational studies have shown that these salads are often stored at inappropriate temperatures, subjected to excessive handling by food vendors, and sold in unsanitary conditions (Kok, 2014; Ramatla *et al*., 2023). Unfortunately, many vegetables are not grown under hygienic conditions, largely due to inconsistent rainfall. Many growers cultivate their crops near polluted water sources within urban areas, where contamination from garbage, as well as human and animal waste, is common. Consequently, these contaminated vegetables make their way to city markets and are prepared into salads for public consumption.

Additionally, the growing preference for fast food, due to its time-saving nature, places strain on fast food operators and local vendors, who often rush to meet consumer demand. This hurried preparation can lead to foodborne illnesses, presenting significant public health challenges that result in human morbidity, mortality, and economic losses. It is crucial to prioritize the hygienic handling and preparation of vegetables, particularly since they are primarily consumed raw. This study focused on evaluating the bacterial quality of salads sold by ready-to-eat food vendors. The research was conducted cross-sectionally and involved samples collected from food vendors in Madina and Nima.

MATREIALS AND METHODS

Samples were collected from vendors in Madina and Nima during mid-morning, using sterile zip-lock bags, and stored in an ice chest for transportation to the laboratory for analysis. A structured questionnaire was employed to gather data on the socio-demographic characteristics of the vendors and the conditions of their surrounding environments.

Sampling technique

A total of 60 vendors were randomly selected, with 30 vendors from each market site in Madina and Nima. All salad vendors at both markets were counted to establish the total number of vendors. The stalls were numbered sequentially, and numbers were assigned by moving through the market following a predetermined pattern, ensuring that each vendor had an equal opportunity for selection. A random number generator was then used to produce 30 random numbers for the Madina market and 30 for the Nima market, aligning with the total vendor count.

Vendors whose numbers matched those generated randomly were approached to secure informed verbal consent for voluntary participation. If a selected vendor was unavailable or chose not to participate, the nearest vendor was then asked instead. After the vendors were chosen, the available salad was thoroughly mixed using sterile spoons. Three composite portions of approximately 100 grams each

were scooped from different areas of the bulk salad to ensure a representative sample. These samples were immediately placed in sterile bags, labeled, and transported in chilled conditions to the analytical laboratory.

Standard Plate Counts

This procedure provides a systematic approach for estimating the bacterial count in food samples. It is an empirical measurement because no single growth medium or set of physical and chemical conditions can fulfill the physiological requirements of all organisms present in a sample. Additionally, organisms may appear individually, in pairs, clusters, or chains. To perform a tenfold serial dilution and analyze it using the pour plate method, ten grams of each salad sample were blended in 20 ml of phosphate buffered saline (PBS) and then adjusted to a final volume of 100 ml (Łepecka *et al*., 2022). A sterile pipette was used to transfer 1 ml of each diluted sample onto the plates for examination. Subsequently, 25 ml of molten, cooled Plate Count Agar was added to each well-labeled plate. The medium and sample were thoroughly mixed, and the plates were allowed to sit on a flat surface. After solidification, the plates were incubated for 18 to 24 hours at 37°C, with cultures performed in duplicate. Following an overnight incubation period, counts were conducted using a colony counting device and expressed as colony-forming units per gram (cfu/g) (Łepecka *et al*., 2022).

Enterobacteriaceae Count

Enterobacteriaceae are part of the intestinal microbiota, and their presence in food and water worldwide signifies fecal contamination and inadequate hygiene practices. An L-rod spreader was employed to evenly distribute the inoculum across the surface of the agar, ensuring uniform growth after applying 0.1 ml of each dilution onto 25 ml of solidified MacConkey agar. The plates were incubated at 37°C for 18 to 24 hours, with cultures conducted in duplicate. After incubation, counts were performed using a colony counting device. Each distinct colony was enumerated whenever possible and reported in colony-forming units per gram (cfu/g) (Łepecka et al., 2022).

Coliform Count

The spread plate technique using MacConkey agar was utilized to accurately quantify the total coliforms present in the salad samples by facilitating discrete colony formation. This method provided a reliable assessment of fecal contamination levels and the overall bacteriological quality of the salads evaluated. Tenfold serial dilutions were carried out in nine additional sterile tubes, each containing 9 ml of sterile water, using the concentrated solution. To promote confluent growth, an L-rod spreader was employed to evenly distribute the inoculum across the surface of the agar after applying 0.1 ml of the sample from each dilution onto 25 ml of solidified MacConkey agar. The plates were then incubated at 37°C for 18 to 24 hours, with cultures conducted in duplicate. Following incubation, counts were made using a colony counting device that allows for visual inspection of individual colonies. Whenever feasible, the distinct lactose-fermenting colonies were enumerated and recorded as colony-forming units per gram (cfu/g) (Łepecka et al., 2022).

Isolation of Organisms

Bacteriological studies commence with the processes of bacterial isolation, purification, and identification. Isolation was carried out to obtain pure bacterial cultures. The supernatant from a 2 ml aliquot of the macerated material was decanted after being spun at 1100 rpm for 30 minutes in a refrigerated centrifuge. The sediment was then inoculated into Selenite F broth and incubated at 37°C for 18 to 24 hours before being sub-cultured on Salmonella/Shigella Agar. To identify *Escherichia coli*, *Klebsiella* spp., and other enterobacteria, a second loop of sediment was placed onto MacConkey Agar. The sediment was also cultured on blood agar and chocolate agar to promote the growth of additional organisms, including Gram-positive bacteria. All incubations were performed at 37°C under aerobic conditions for 18 to 24 hours. In cases of mixed growth, purity plating was employed to identify suspected colonies, and conventional biochemical techniques were utilized to confirm the identities of the colonies (Mridha *et al*., 2020).

Purity plating

A loopful of the inoculum from an isolated colony on the mixed growth culture was used to inoculate both MacConkey agar and blood agar to obtain a pure culture. The inoculum was then streaked on the plates using a four-quadrant method to create parallel overlapping strokes, while flaming the loop between strokes to ensure the formation of isolated colonies. The plates were subsequently incubated at 37°C for 18 to 24 hours in an aerobic environment. Each distinct bacterial colony identified on the mixed growth culture plate underwent the complete procedure again.

Ethical approval and informed consents

The vendors were chosen in consultation with the market leaders at each study site, considering the vendors' willingness to participate. While no ethical approval was necessary, verbal consent was obtained from each vendor during the sample collection process.

Analysis

All collected data was securely locked and coded for confidentiality. The data were analyzed using the Statistical Package for Social Sciences (SPSS) version 20, and results were compiled into tables with Microsoft Office 2010. Two-tailed t-tests were the primary statistical method used to identify quantitative differences between independent categorical groups related to salad vending conditions, storage practices, service methods, and overall microbiological quality in this study, with a significance threshold set at a p-value of <0.05. Descriptive statistics were employed to analyze frequency tables and measures of central tendency, including mean and standard deviation.

RESULTS AND DISCUSSION

According to microbiological standards, the mean standard plate count (SPC), Enterobacteriaceae count (EC), and coliform count (CC) for salad samples were classified as either acceptable or unacceptable (see Table 1). The mean SPC was significantly higher ($p=0.042$) in samples deemed unacceptable compared to those considered acceptable. This substantial difference indicates improper handling and a

lack of hygiene during salad preparation, as elevated SPC levels suggest significant bacterial contamination (Kibret, 2012; Postollec *et al*., 2012; Samapundo *et al*., 2016). An unacceptable SPC value corresponds to counts exceeding 1,000,000 CFU/g (Campos *et al*., 2013; Gilbert, 2000).

Samples with EC of 100 CFU/g or higher are deemed unacceptable for consumption, while samples with CC of 10 CFU/g or more are also considered unacceptable (Campos *et al*., 2013; Gilbert, 2000). In the present study, high levels of EC and CC were observed in both acceptable and unacceptable groups; however, these differences were not statistically significant (see Table 1). Elevated levels of EC and CC, even in acceptable samples, indicate unsanitary practices in production and vending since Enterobacteriaceae and coliforms signify fecal contamination (Figueras & Borrego, 2010; World Health Organization, 2017). Similarly, high levels of EC and CC were reported in salad samples in studies from Saudi Arabia and Ghana (Mensah, 2002). The significant microbial loads highlight the necessity for good agricultural practices during salad vegetable production, as well as hygienic handling and vending, to enhance microbiological quality and safety.

Table 1: Mean count across the various study parameters

1	**Variables**	**Acceptable Mean Count**	**Unacceptable Mean Count**	**p-value**
	SPC	1091363.64	322902631.6	0.042
	EC	1043000.00	242780000.0	0.152
	CC	905945.95	323139130.4	0.36
2	**Mode of serving**	**Hand**	**Spoon**	**p-value**
	SPC	328046666.7	174119791.7	0.43
	EC	1862783333	2065435417	0.89
	CC	266946666.7	88799166.67	0.34
3	**Temperature**	**Warm**	**Cold**	**p-value**
	SPC	239273333.30	124712777.80	0.498
	EC	250900238.10	89534444.44	0.241
	CC	97569523.81	187100000.00	0.589
4	**Nature of site**	**Open**	**Enclosed**	**p-value**
	SPC	103912903.20	312862413.80	0.176
	EC	131878709.70	277972069.00	0.247
	CC	136701935.50	111308965.50	0.868

There were no significant differences between hand and spoon serving methods for any of the parameters assessed. However, the elevated mean levels of SPC and CC associated with hand serving

are concerning, as hands can act as vectors for cross-contamination if proper hygiene is not maintained (Todd *et al*., 2010). Various studies involving street food vendors have detected Staphylococcus aureus in hand swabs, highlighting poor hand hygiene practices (Abdul-Mutalib *et al*., 2012; Kibret, 2012). Spoon-served salads exhibited higher EC counts, potentially due to improper utensil usage or inadequate cleaning. Research underscores the necessity of sanitizing utensils between uses and designating specific serving utensils to prevent cross-contamination (Todd *et al*., 2010).

For SPC, EC, and CC, mean counts were greater in warm samples compared to cold ones, although not significantly. Higher microbial growth is anticipated at elevated temperatures, as bacteria multiply rapidly between 4°C and 60°C (Korajkic *et al*., 2013). Maintaining proper cold storage below 5°C is essential to inhibit pathogen growth (Pham, 2014). The absence of significant differences suggests potential temperature abuse, as samples sold warm would likely have considerably higher counts if they had been adequately refrigerated prior to sale. Although differences were not significant (p=0.758, p=0.161, and p=0.618), mean counts for SPC, EC, and CC were all higher for samples obtained from dirty locations (Figure 1). The environments were categorized as neat or dirty based on a structured scoring system that evaluated the cleanliness and maintenance of food premises (Park *et al*., 2010; Tan *et al*., 2013). Factors such as surfaces, floors, and drainage were assessed, and establishments were classified as dirty or neat based on these evaluations. Contamination of produce in dirty environments can occur through dust, pests, and cross-contamination (Imathiu, 2017). Clean food preparation surfaces are critical for reducing microbial contamination (Kramer & Assadian, 2014; Rusin *et al*., 2002). The lack of significant differences between both groups indicates that sanitation remains inadequate, even in those areas presumed to be clean.

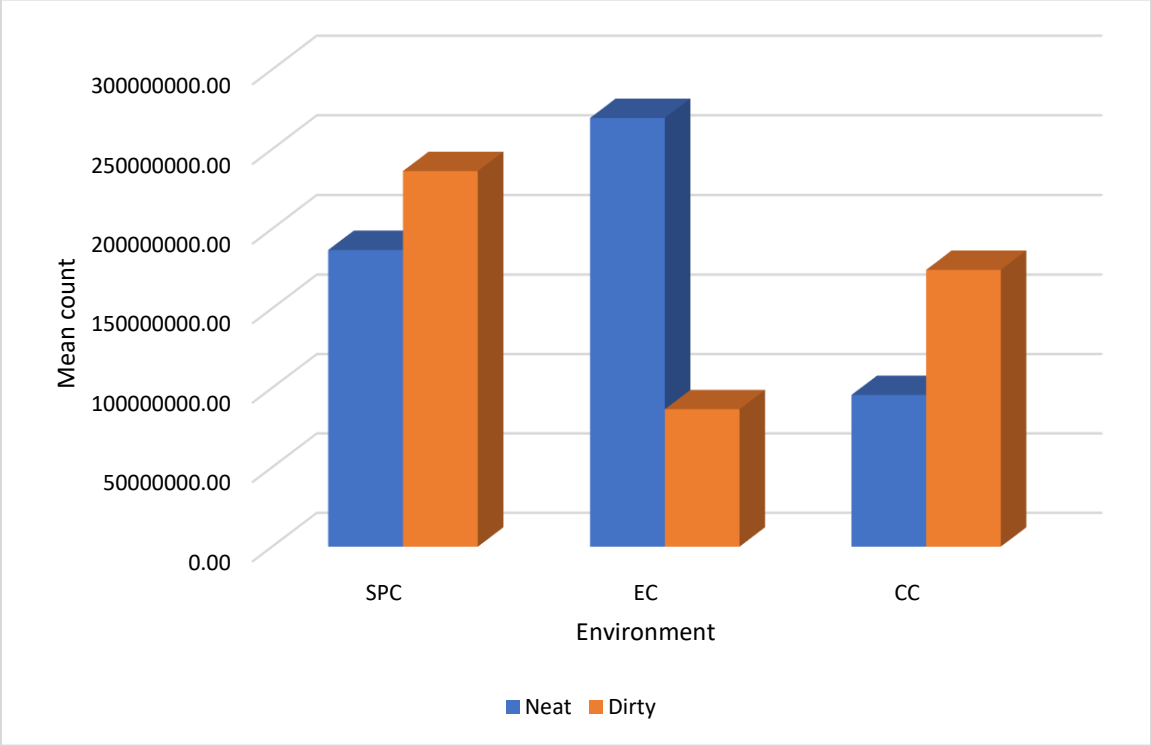

Figure 1: Total bacterial counts of samples per the environment of sampling

For all parameters (SPC, EC, CC), the mean microbial loads were greater in salads sold at enclosed sites compared to those at open sites, although the differences were not statistically significant. The heightened contamination observed in enclosed environments is concerning, as it suggests specific risks associated with closed spaces. Enclosed stalls may be more prone to accumulating dirt, pests, and microbes than open areas, which benefit from better airflow. Vendors operating in enclosed stalls might also engage in riskier practices, such as inadequate washing and refrigeration, compared to their open counterparts who, despite lacking facilities, must keep their produce cool and protected. However, the absence of significant differences between open and enclosed vendors indicates widespread mishandling and poor hygiene practices across both types of vending sites. There is likely a need for all vendors to receive improved food safety training and access to essential sanitation facilities, including running water, refrigeration, and waste disposal (Hill *et al*., 2022; Mosupye & von Holy, 2000; Nkosi & Tabit, 2021). Installing washable surfaces in stalls may also help mitigate contamination risks specifically in enclosed locations.

The high microbial counts observed even in open vending settings highlight potential risks at all sites, not solely those that are enclosed, underscoring the necessity for proper monitoring of microbiological quality across various vending environments. Implementing targeted interventions that consider vending conditions and vendor practices may prove to be more effective than a generalized approach to enhancing food hygiene and safety.

Figure 2 illustrates the microbial counts associated with various types of salad containers—food flasks compared to polythene bags. For both SPC and CC, the mean bacterial loads were significantly higher for salads sold in food flasks than those in polythene bags, with the difference in SPC being statistically significant ($p=0.021$). The increased contamination found in food flasks is likely attributable to cracks, scratches, or worn areas that can harbor microbes even after cleaning. Bacteria such as Listeria species can easily adhere to surfaces of materials like stainless steel, rubber, and glass used in flasks (Soni & Nannapaneni, 2010). Additionally, the humid and enclosed environment within flasks may facilitate bacterial survival and growth compared to more breathable bags. However, the elevated counts observed in polythene bags also point to improper handling practices that result in cross-contamination. While food-grade plastic offers an impermeable barrier, these bags can still facilitate the transmission of pathogens through moisture if they become contaminated during filling and storage (Gilbert, 2000). Vendors should utilize new bags for each batch of salad and exercise caution during the filling process to mitigate risks. The findings emphasize the importance of thorough cleaning and replacing damaged food containers. However, improper handling and hygiene can compromise even the cleanest containers, making it crucial to provide training for workers on safe preparation practices.

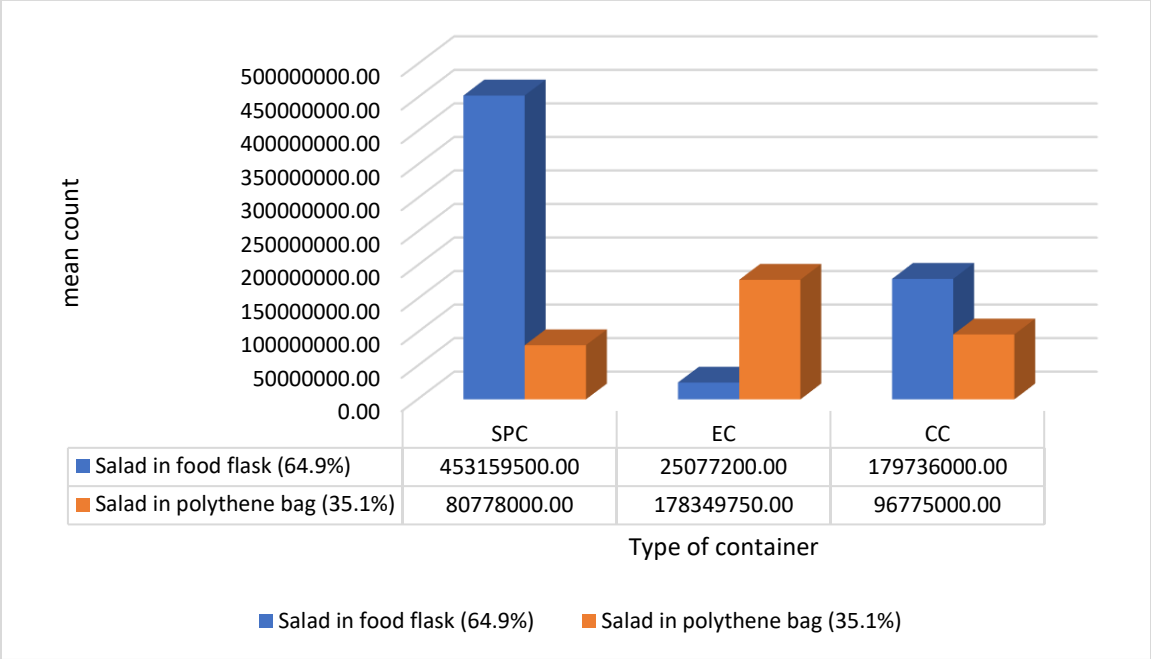

Figure 2: Types of containers used by vendors

The findings of this study highlighted concerning levels of microbial contamination in salad samples collected from street vendors in Accra, Ghana. The average standard plate count surpassed acceptable thresholds in 40% of the samples, suggesting poor microbiological quality. Various factors likely contributed to the elevated contamination levels observed. Salad preparation methods were recognized as a significant source of contamination, with salads served by hand displaying higher microbial loads compared to those served with utensils (Todd *et al*., 2010), indicating risks associated with bare hand contact. Improperly cleaned utensils also served as conduits for microbes (Shayeghi, 2020). Additionally, warm salad samples exhibited higher microbial counts than refrigerated samples, indicating potential temperature abuse (Pham, 2014). Enclosed vending locations were found to contain a greater microbial load, possibly due to inadequate ventilation and hygiene infrastructure (Mosupye & von Holy, 2000). Also, damaged food containers like cracked flasks supported microbial survival and growth more than polythene bags (Soni & Nannapaneni, 2010). The presence of *Salmonella* spp. And *Staphylococcus* spp. Points to fecal contamination and inadequate personal hygiene, respectively (Girma & Aemiro, 2022). Similar pathogens were detected in salads in studies conducted in Saudi Arabia and Thailand (Khiyami *et al*., 2011; Minami *et al*., 2010). Unhygienic handling of produce, washing with contaminated water, and exposure to unsanitary environments likely introduced these pathogens.

In summary, the results underscore the urgent need for vendor training, facility improvements, and enhanced produce handling practices to minimize salad contamination. Interventions should prioritize hand hygiene, temperature regulation, sanitary utensils, protective packaging, and access to clean water. Routine microbiological testing and monitoring of salads sold by street vendors is also crucial to protect public health.

CONCLUSION

The findings of this study highlighted alarming levels of microbial contamination in salad samples from street vendors in Accra, Ghana. The average standard plate count surpassed acceptable food safety limits in 40% of the samples, indicating a significant lack of microbiological quality. Various factors likely contributed to the high levels of contamination, including salad preparation practices, conditions at vending sites, temperature abuses, and damaged food containers. Notably, the method of salad preparation and serving was identified as a primary source of contamination. While no statistical significance was detected, salads served by hand exhibited higher standard plate counts and coliform levels compared to those served with utensils, suggesting potential risks from bare-hand contact. Without thorough handwashing with soap and clean water before and after serving each portion, there is a risk of transmitting pathogens between salads. Furthermore, inadequately cleaned utensils were also found to transmit microbes. The high levels of Enterobacteriaceae in both serving methods highlighted the insufficient hygiene at vending locations.

Temperature abuse was another likely factor contributing to microbial growth. Warm salads generally showed higher bacterial counts than refrigerated ones, although the difference was not statistically significant. If produce had been properly stored below 41°F prior to sale, we would expect substantially lower contamination levels due to inhibited pathogen growth. The lack of a notable difference implies that salads were likely kept at ambient temperatures for extended periods, allowing for microbial proliferation. It is crucial to maintain appropriate temperature controls throughout the entire process, from harvest and transportation to washing, preparation, storage, and serving.

The cleanliness of vending environments also affected produce contamination due to factors such as dust, pests, runoff, and cross-contact. Although the differences were not statistically significant, all microbial parameters showed elevated mean levels in unclean locations. Ensuring proper sanitation is essential to reduce these risks. Maintaining clean food preparation surfaces, washable floors and walls, protected displays for produce, and facilities equipped for cleaning, waste disposal, and ventilation can significantly lower contamination risks.

Additionally, storing salads in damaged containers such as chipped flasks and plastic bags increased bacterial counts, particularly for standard plate counts and coliforms. Cracks or scratches can harbor biofilms even after cleaning, while breathable plastic provides inadequate protection. Nevertheless, high counts in bags indicated that handling practices can overshadow the benefits of proper packaging. Utilizing new food-grade bags for each batch of salad and ensuring careful filling and sealing could mitigate risks more effectively than using pristine containers under poor hygiene practices.

The presence of Salmonella spp. and Staphylococcus spp. suggests inadequate personal hygiene and potential fecal contamination. Possible sources of these pathogens include contaminated irrigation water, soils enriched with manure, and unsanitary practices during post-harvest washing and handling.

Comparable pathogens have also been identified in street-vended salads worldwide. These significant risks highlight the urgent need for interventions throughout the entire supply chain.

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AN ASSESSMENT OF TREE BIODIVERSITY AND CARBON STOCKS IN RANGAN HIRAN SOCIAL FORESTRY AREA, GUNUNG MAS, INDONESIA

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ABSTRACT. *Rangan Hiran Village, located in the upper reaches of the Kahayan River within the Mirimanasa Subdistrict, encompasses an officially designated area of 875 hectares assigned by the state. The necessity of conserving the forest in this region arises from the significant effects of community initiatives on the Kahayan watershed. Instiper Yogyakarta, a forestry educational institution, has launched a program to educate the community about the inherent value of ecosystem services, potential alternative sources of income, and the benefits they offer. The research methodology employed utilizes the Forest Inventory and Analysis Plus (FIA+) method, which was developed by USAID LESTARI in 2018 and is overseen by Michigan State University, USA. An analysis of 10 plots with a sampling intensity of 0.221% indicates a healthy state of the current forest. The carbon stock contributed by Rangan Hiran Forest amounts to 243,998.57 tons and consists of 40 different tree species. Ecologically, the forest exhibits a Menhenick Index of 2.58, a Margalef Index of 7.11, a Shannon Index of 3.43, a Simpson Index of 0.96, and an evenness value of 0.93. These identified potentials create opportunities for collaborative efforts between Rangan Hiran and Instiper Yogyakarta, concentrating on initiatives for forest conservation and the improvement of community well-being.*

KEYWORDS: Biodiversity, Carbon Sequestration, Social Forest; Environmental Partnership Instiper Yogyakarta

INTRODUCTION

Rangan Hiran, situted in the remote upper reaches of the Kahayan River in Gunung Mas Regency, plays a crucial role in the watershed dynamics of the area. Access to the village is notably limited,

especially during the rainy season (Segah *et al*., 2023). A key highlight is the establishment of an 865-hectare Community Forest, recognized by Ministerial Decree Number: 6608/MENLHK-PSKL/PKPS/PSL.0/2016. This Community Forest, adjacent to a similar initiative in Harowu Village, showcases a growing interest in land ownership among residents, reflecting a shift in cultural norms towards more urbanized lifestyles. The rugged terrain surrounding Rangan Hiran, characterized by steep slopes and flourishing forests, is rich in clean water sources, such as mountain springs and waterfalls. These natural features offer potential for ecotourism, hydroelectric energy generation, and bottled water industries, indicating promising avenues for economic development (Harden & Fernández, 2022; Putri *et al*., 2020).

INSTIPER Yogyakarta, alongside its Faculty of Forestry, actively supports local communities involved in social forestry. Through various training programs, they enhance forest management skills and promote community participation in conservation efforts. This partnership, backed by the government and formalized through a Memorandum of Understanding (MoU), exemplifies collaborative forest management. INSTIPER and the Rangan Hiran Village Forest Management Institution are dedicated to conservation and sustainable practices within the designated forest area. This approach aligns with Indonesia's social forestry framework, which encourages community stewardship and reflects a commitment to environmental preservation, with support from the oil palm plantation company.

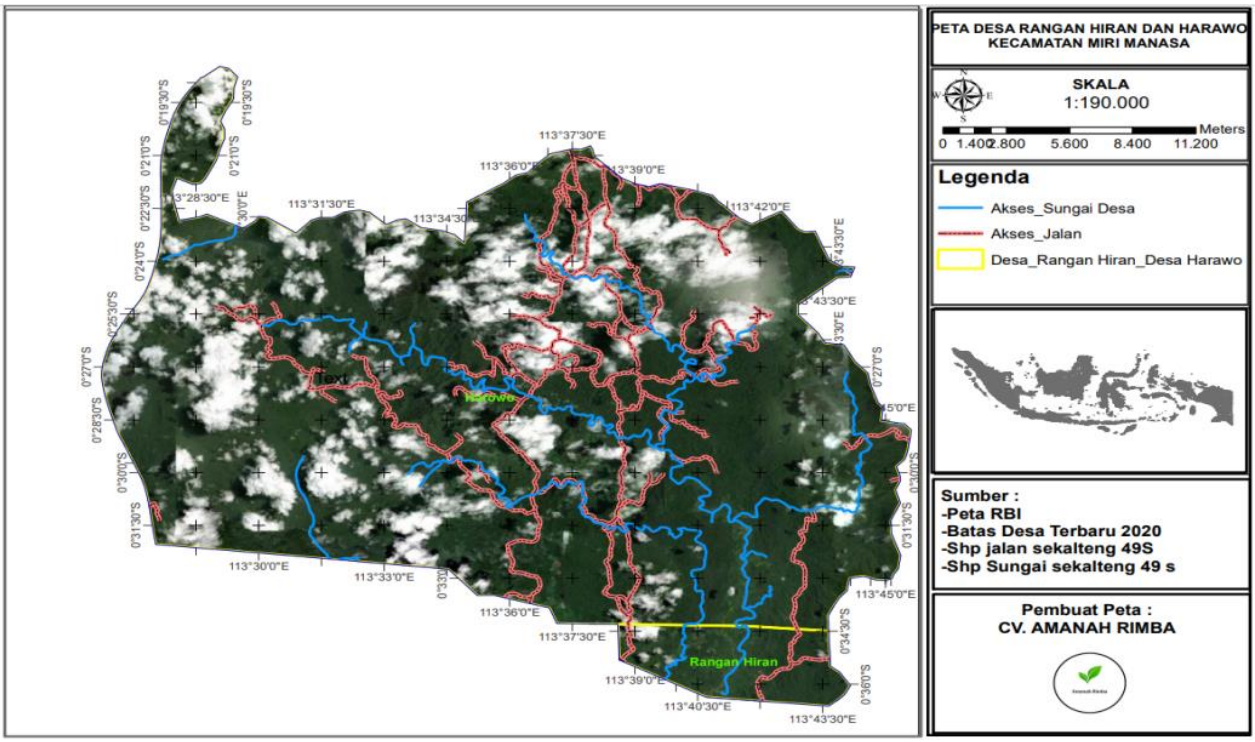
The significance of this activity lies in its foundational role in forest conservation and management. It entails assessing the biodiversity value of flora and the carbon sequestration capabilities within the forest. The biodiversity of forest plants is essential for maintaining ecosystem balance and supporting human livelihoods (Brandon, 2014). A diverse array of plant species creates intricate food webs, affects nutrient cycles, and contributes to ecosystem stability (Bauhus *et al*., 2017; McMeans *et al*., 2015). Furthermore, the biodiversity of forest plants is vital for genetic conservation, which facilitates plant adaptation to environmental changes and enhances resistance to diseases (Isabel et al., 2020). Additionally, forest plants offer environmental advantages by filtering air, preventing soil erosion, and maintaining water quality (Burivalova *et al*., 2019). Simultaneously, the carbon sequestration potential of forest plants is crucial for addressing global climate change (Karimah, 2017; Rajeev & Hukum, 2020). By capturing carbon in their biomass, these plants contribute to lowering atmospheric carbon dioxide levels, thereby alleviating the greenhouse effect and slowing the pace of climate change (Favero *et al.*, 2017; Nunes *et al*., 2020).

MATERIALS AND METHODS

Research Location

The Rangan Hiran Village Forest is a type of social forestry that consists of community-managed plantations located in the Mirimanasa District of Gunung Mas Regency, Central Kalimantan Province, encompassing an area of 865 hectares. The predominant landscape features karst topography with significant elevation changes. Within this forest, one can find various natural elements such as springs, waterfalls, clear rivers, steep rock faces, caves, and large rare tree species, including ironwood (Eusideroxylon zwageri), different types of keruing (Dipterocarpus), and several

varieties of meranti (Shorea montigena Slooten), as well as a diverse array of wildlife. However, the area faces challenges like extensive deforestation due to illegal activities, ineffective law enforcement, and insufficient monitoring and management, highlighting the need for support for local management groups.



Source: Kahayan Hulu Forest Management Unit (2023)

Plant Biodiversity and Carbon Sequestration Measurement

Field inventory data were gathered from sample plots located in two research sites. The inventory plots were arranged in a regular grid pattern, ensuring a minimum distance of 50 meters between each plot. Tree species were recorded within 20 × 20 m plots, while seedlings were noted in 2 × 2 m plots. Measurements included tree diameter at breast height (DBH) and total tree height, using nested plots: trees greater than 20 cm DBH were assessed in 20 × 20 m plots; those larger than 10 cm and up to 20 cm DBH were measured in 10 × 10 m plots; and trees greater than 5 cm and up to 10 cm DBH were evaluated in 5 × 5 m plots.

Data collected from the field were analyzed using Excel-based tools developed by Michigan State University as part of the USAID LESTARI project (2018). The first tool calculated the total tree carbon stock (tC), wood volume (m^3/ha), and stand density (trees/ha). The second tool was utilized to compute various biodiversity indices, including species richness, evenness, and dominance, for trees (greater than 2 cm DBH) and seedlings. Allometric models based on tree DBH were used to estimate total live tree biomass (kg). Carbon content was derived from biomass using a conversion factor of 0.47, which is the standard value provided by the IPCC. The carbon stocks for each tree size class within the nested plots were totaled and converted to tons (tC). These totals were then aggregated to determine the overall carbon per plot and scaled for reporting carbon per hectare (tC/ha). Finally, averages across all plots were calculated and multiplied by the site area to estimate total carbon stocks (tC) (Krisnawati *et al.*, 2012).

$B = 0.75 \times Dt^{2.23}$ Eq. 1

Where

B = total tree biomass (kg)

Dt = diameter at breast height (1.3 m above ground) (cm)

The wood volume (m^3) was determined using measurements of diameter at breast height (DBH) and tree height, applying a numerical equation. The calculations were aggregated for all trees within the plot and converted to a per-hectare basis. Subsequently, averages were calculated for all plots. To ensure conservative estimates for wood volume, an equation incorporating form factor coefficients was utilized.

$Dw = ((1/4) \times ((PI() \times ((Dt/100)^2)) \times Ht) * 0.6)$ Eq. 2

Where

Dw = wood volume (m^3)

Dt = diameter at breast height (1.3 m above ground) (cm)

Ht = total height of the tree (m)

Stand density (trees per hectare) was determined by counting the number of trees within three nested plots of different size classes, with each count scaled to a per-hectare basis. The average density for all plots at the site was then calculated according to tree size class. Biodiversity indices were derived from species counts within designated sample plot areas, allowing for the computation of various biodiversity indices for both trees and seedlings. Table 1 outlines the biodiversity indices, and the methods used for their calculation with the Biodiversity Calculator tool.

Table 1: Plant Biodiversity Indices and measurement methods utilized by the biodiversity calculator tool

Indices	**Computation Method**
Species Richness	S = number of species or taxa
Menhinick's Index (Menhinick, 1964)	D = S/(√N); where S = the number of different species in the sample and N = the total number of individual organisms in the sample
Margalef's Richness Index (Margalef, 1958)	(S-1)/ln(N)
Shannon Index of Species Diversity (Shannon, 1948)	$H = -\sum_{i=1}^{s} p_i \ln p_i$; where pi = the proportion of the total number of individuals
Simpson Index of Diversity (Simpson, 1949)	1 - D = 1 -∑ $(n / N)^2$; where D is the Simpson Index which measures the probability that two individuals randomly selected from a sample will belong to the same species (or some category other than species)
Evenness Index	Evenness = H'/lnS uses Shannon index and species richness values

RESULT AND DISCUSSION

Biodiversity of Rangan Hiran Forest

Table 2 outlines the number of trees and seedlings recorded within each sample plot. A total of 6 plots were surveyed, with the trees distributed evenly across them. The table lists various biodiversity indices calculated for both locations, along with the dominant species for trees greater than 5 cm in diameter at breast height (DBH) and seedlings. As expected in a thriving forest ecosystem, both the species richness of trees and the biodiversity indices reflect moderate values, indicative of a healthy forest system.

Table 2. Plant biodiversity analysis in Rangan Hiran Forest

Indices	**Trees (n=40)**	**Seedlings (n=22)**
Speciess Richness	40	22
Menhinick's Richness Index	2,58	1,69
Margalef's Richness Index	7,11	4,09
Shannon Index	3,43	2,56
Simpson Index of Diversity	0,96	0,90
Evenness	0,93	0,83
Dominant species	Jambu merah (*Syzygium spp.*) Meranti bitik(*Shorea parvifolia*.) Jambu putih (*Syzygium spp.*) Plepek *(Shorea johorensis* Foxw.) Emang (*Hopea dryobalanoides*)	Meranti bitik (*Shorea parvifolia*) Keruing bayan Tuwung (*Dipterocarpus rigidu*s) Plepek *(Shorea johorensis* Foxw.) Benuas (*Shorea xanthophylla*) Timun-timun (*Hopea sericea*)
Other Floras	Lianas (n = 79) Shrubs (n = 90)	Ferns (n = 10) Pandanus (n = 5) Brushwood (n = 30) Other Herbs (n = 24)

The biodiversity indices for the surveyed trees and seedlings, consisting of 40 and 22 individuals respectively, are summarized in Table 2. The species Richness Index stands at 40 for trees and 22 for seedlings, indicating a notable diversity across different life stages within the Rangan Hiran forest ecosystem. Menhinick's Richness Index is 2.58 for trees and 1.69 for seedlings, while Margalef's Richness Index is 7.11 for trees and 4.09 for seedlings. These Richness Indices for both trees and seedlings suggest significant species diversity within the forest. The results from Menhinick's and Margalef's Indices, along with overall species richness, reflect a diverse composition that contributes to the overall biodiversity. This indicates that the surveyed forest area supports a rich array of plant species across various life stages, which is a sign of a healthy and resilient ecosystem (Maimunah et al., 2021).

The biodiversity indices and evenness values offer important insights into the ecological dynamics and health of the surveyed forest ecosystem. The Shannon Index values of 3.43 for trees indicate a high level of species diversity, while the value of 2.56 for seedlings reflects a moderate level within their respective populations. This suggests that the forest sustains a variety of species at different life stages, enhancing overall ecosystem resilience and functionality. The relatively high Shannon Index values further imply that the forest is ecologically rich and diverse, characteristic of a healthy ecosystem. Additionally, the Simpson Index values of 0.96 for trees and 0.90 for seedlings indicate a comparatively high degree of floral biodiversity and heterogeneity. This index also suggests an even distribution of species abundance within the populations, as evidenced by the calculated evenness values of 0.93 for trees and 0.83 for seedlings. The evenness in the forest indicates a balanced representation of different species within the ecosystem. This balanced distribution of species abundance further reinforces the idea of a healthy and resilient forest ecosystem, suggesting that no single species exerts disproportionate influence over the others.

The dominant tree species, including *Syzygium* spp., *Shorea dasyphylla*, *Shorea johorensis* F*oxw*., and *Hopea dryobalanoides*, along with the prevalent seedling species such as *Shorea dasyphylla*, *Dipterocarpus rigidus*, *Shorea scrobiculata*, *Shorea xanthophylla*, and *Hopea sericea*, highlight their ecological importance in shaping the structure of the forest community. Moreover, the presence of other plant groups, including lianas, shrubs, ferns, pandanus, brushwood, and various herbs, further enhances the overall botanical diversity of the area, underscoring its ecological richness. Collectively, these biodiversity metrics illustrate a flourishing and resilient forest ecosystem, marked by a variety of species assemblages and balanced ecological dynamics across different life stages.

Carbon Sequestration of Rangan Hiran Forest

Table 3 presents various site characteristics relevant to data collection and calculations, including the number of plots, total area, and plot size, along with site-level carbon stocks (average tC/ha across all plots, range, and standard deviation of carbon across all plots, as well as total tC for the site). It also documents sample errors and accuracy ranges based on the number of plots at a 95% confidence level. The mean carbon stock is 282.08 tons C/plot, with total carbon within the plots amounting to 1,692.48 tons C/ha, reflecting a total carbon stock of 243,998.57 tons C across all carbon plots. The accuracy of these carbon estimates is somewhat improved and is determined by the number of sample plots, the sample size of each plot, and the carbon variation across all sampled plots.

Table 3. Carbon Stock Measurement Recapitulation in Rangan Hiran Forest

Properties	Value
Number of Plot (n)	6
Forest Area (ha)	865
Total Carbon in Plot (tons)	1692.48
Averaage Carbon in Plot (tons)	282.08
Standard Deviation (tons)	192.71
Plot Size (ha)	0.04
Population (N)	21625
t-student value (α=5%)	2.57
Average default error rate (tons)	78.66
CI95% average (lower limit) (tons)	259.28
CI95% average (upper limit) (tons)	304.88
Sampling Error (%)	8.08
Total carbon stock (tC) – Lower (tons)	224,277.59
Total carbon stock (tC) (tons)	243,998.57
Total carbon stock (tC) – Upper (tons)	263,719.54

The carbon stock data for the Rangan Hiran tropical secondary rainforest offers important insights into the ecosystem's carbon sequestration capabilities. Encompassing a total area of 865 hectares and housing 21,625 trees, the forest exhibits considerable potential for carbon storage. The findings indicate that across six sample plots, the average carbon content per plot is 282.08 tons, with a standard deviation of 192.71 tons, signifying a moderate variation in carbon density throughout the forest. The estimated total carbon stock for the entire forest amounts to 243,998.57 tons, presenting a 95% confidence interval from 224,277.59 tons to 263,719.54 tons. The average default error rate is 78.66 tons, and the sampling error is 8.08%, providing insights into the accuracy and precision of the carbon estimates. Additionally, the t-student value of 2.57, along with the confidence intervals, aids in evaluating the reliability of the calculated carbon stock figures. Overall, this data highlights the significant carbon sequestration potential of the Rangan Hiran tropical secondary rainforest, emphasizing its vital role in combating climate change and maintaining the global carbon balance (Maimunah *et al*., 2023).

Wood Volume and Tree Density

Table 4 provides a summary of the volume calculations for trees based on size classes, including wood volume and tree density. The distribution of tree size classes in the Rangan Hiran forest shows a distinct skew towards the largest size class, which includes trees larger than 15 cm in diameter at breast height (DBH), in comparison to saplings and poles. However, the count of trees

per plot by size class may be somewhat misleading, as data were collected from nested plots measuring 5 x 5 m (for saplings), 10 x 10 m (for poles), and 20 x 20 m (for large trees). The table also provides the sample sizes for saplings and poles in parentheses and estimates the number of trees in the 20 x 20 m plot based on the counts from the nested plots or observed data.

Table 4. Wood Volume and Tree Density of Rangan Hiran Forest

Stage of Development	Wood Volume (m^3 ha^{-1})	Tree Density (tree ha^{-1})
Seedlings	0	0
Saplings	20.81	1,933
Poles	70.73	733
Trees	422.09	242

The data on wood volume and tree density provide valuable insights into the structural composition and dynamics of the Rangan Hiran tropical secondary rainforest. Large trees lead in volume, with 330.55 m^3 per hectare, followed by poles at 70.73 m^3 and saplings at 20.81 m^3, resulting in a total of 422.09 m^3 per hectare. Tree density varies as well, with saplings at 1,933 trees/ha, poles at 733 trees/ha, and large trees at 242 trees/ha. This distribution reflects ongoing forest regeneration and a dynamic ecosystem characterized by active growth. The abundance of saplings and poles emphasizes the forest's regeneration capacity, which is vital for long-term resilience and biodiversity. Therefore, conservation efforts are essential to protect and support these younger tree populations to ensure the forest's sustained vitality (Maimunah *et al*., 2022).

CONCLUSION

The results of this study emphasize the importance of analyzing field inventory data in forests to provide insights into ecosystem service levels. In this research, both biodiversity and carbon stocks were evaluated. The Social Forest of Rangan Hiran showcases a wealth of tree species, as demonstrated by a Richness Index of 2.58 (Menhinick) and 7.11 (Margalef), indicating a diverse range of species. Additionally, the tree plots in Rangan Hiran Forest exhibit a diversity index of 3.43 (Shannon-Wiener) and 0.96 (Simpson), signifying high species diversity with an evenness of 0.93. For the seedling plots, the richness is 1.69 (Menhinick) and 4.09 (Margalef), with a biodiversity index of 2.56 (Shannon-Wiener) and 0.90 (Simpson), along with an evenness value of 0.83. The total estimated carbon stock for Rangan Hiran Forest is 243,998.57 tons.

Ongoing measurements to monitor changes in carbon stocks are vital for further studies. It is essential to track carbon gains from tree growth and losses from tree mortality to formulate effective management plans for the forest area. Lastly, engaging the local community, particularly those who directly benefit from the Rangan Hiran Village forest in Gunung Mas Regency, is crucial for fostering their active participation in conservation efforts and enhancing local economic prosperity.

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AN ASSESSMENT OF TREE BIODIVERSITY AND CARBON STOCKS IN HAROWU SOCIAL FORESTRY AREA, GUNUNG MAS, INDONESIA

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ABSTRACT. *Harowu Village, located in the upper reaches of the Kahayan River within the Mirimanasa Subdistrict, officially oversees a state-granted area of 1,750 hectares. The conservation of the forest in this region is of utmost importance due to its significant influence on the Kahayan watershed. Instiper Yogyakarta, a forestry educational institution, has initiated programs to educate the local community about the importance of alternative income sources and environmental services. Utilizing the Forest Inventory methodology created by USAID LESTARI in 2018 and coordinated by Michigan State University, the study involved analyzing 10 plots with a sampling intensity of 0.221. The Harowu Forest is identified as a major carbon sink, sequestering 626.957,75 tons of carbon in the area. The forest is home to 25 tree species and features a Menhenick Index of 1.57, Margalef Index of 4.34, Shannon Index of 2.98, Simpson index of 0.974, and an evenness value of 0.93. These findings highlight potential opportunities for collaboration between Harowu and Instiper Yogyakarta to promote forest conservation and improve community welfare.*

KEYWORDS: *Biodiversity, Carbon stock, Harowu Forest; Environmental Partnership, Conservation*

INTRODUCTION

Harowu is a village situated at the upper reaches of the Kahayan River, at the northern edge of Gunung Mas Regency, making it a remote location. Access to the village is particularly challenging, especially during the rainy season. Its position is critical as it lies within the primary section of the river basin, significantly affecting the continuity and sustainability of the water catchment area that serves as a source for the Kahayan River. Biodiversity in this area is often overlooked during monitoring efforts, as many species lack apparent economic value to the community. Nevertheless, this diversity is crucial and should be conserved due to its vital role in providing ecosystem services through forests (Maimunah et al., 2022).

Harowu features a social forestry institution in the form of a Village Forest, encompassing an area of 1,750 hectares, as per the Decree of the Minister of Environment and Forestry Number: 6603/MENLHK-PSKL/PKPS/PSL.0/2016. This village forest is located in Harowu Village, Miri Manasa District, Gunung Mas Regency, Central Kalimantan, and is contiguous with another forest area of the same type, though managed differently. There is a strong community interest in land ownership within the Village Forest zone, reflecting a cultural shift from traditional lifestyles to urbanized patterns. The village is surrounded by steep mountain cliffs and healthy forest ecosystems, contributing to an abundant supply of clean water. The presence of numerous mountain springs and waterfalls in the area highlights its potential for regional development in areas such as ecotourism, hydroelectric power generation, and the bottled water industry.

Instiper Yogyakarta is a university dedicated to agricultural sciences in its broadest sense, which includes a Forestry Faculty focused on community assistance programs, particularly in social forestry management. The university aims to develop initiatives and innovations that support communities in effectively managing their forests (social forestry), aligning with the community's strong commitment to nature conservation. This commitment was evident during training sessions on community plantation forest management organized by Instiper Yogyakarta, which inspired participants to actively engage in forest protection.

Instiper Yogyakarta is dedicated to fostering and supporting the community through a collaborative approach to managing social forestry. This effort contributes to sustainable economic development, forest conservation, and the facilitation of social forestry management activities within the Harowu Village Forest, enhancing the value derived from forests managed independently by the community. The objective is to ensure that village forest managers understand biodiversity's potential, as plant life plays a crucial role in maintaining air quality and soil conservation (Raven & Wackernagel, 2020). Additionally, there is a focus on identifying potential alternative income sources from non-timber forest products and environmental services, aiming to facilitate knowledge transfer. This will empower managers to competently identify and manage village forest biodiversity, enabling them to collaboratively protect these areas from threats. It is vital to undertake these activities, as changes in land cover from forest to agricultural use can significantly diminish carbon stocks (Priyadarshini *et al*., 2019).

This collaborative management agreement has been officially documented as a cooperation agreement between the two parties and is recognized by the Government. This partnership serves as a model for the type of social forestry management envisioned by the Indonesian Government, which emphasizes multi-stakeholder support in empowering communities surrounding forests to manage social forestry areas authorized by the government. The partnership between Instiper Yogyakarta and the Harowu village forest management institute focuses on forest conservation and sustainable management of village forests, reflecting a commitment by oil palm plantation companies to preserve nature and the surrounding environment, including forest ecosystems.

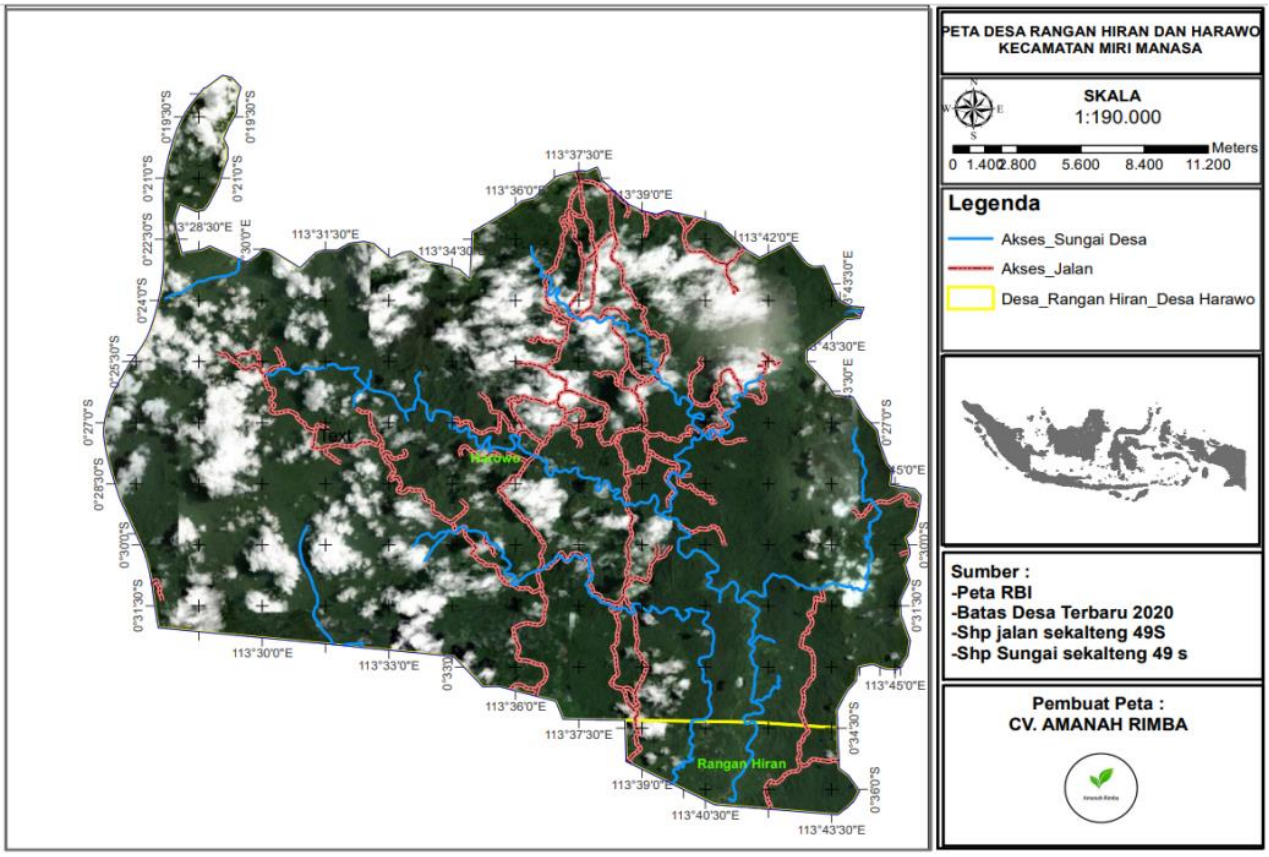
METHODOLOGY

Research Locations

Harowu Village Forest represents a model of social forestry through a community plantation forest situated in the Mirimanasa District of Gunung Mas Regency, Central Kalimantan Province,

covering an area of 1,750 hectares. This forest predominantly consists of karst landscapes with challenging topography, featuring springs, waterfalls, clear rivers, steep rock walls, caves, and a variety of rare tree species such as ironwood (*Eusideroxylon zwageri*), various keruing species (Dipterocarpus), and several types of meranti (*Shorea montigena* Slooten), all coexisting with diverse wildlife. The location presents significant challenges, including widespread illegal land clearing for other uses, while law enforcement and management oversight remain inadequate, emphasizing the need for assistance for area management groups.

Field inventory data was gathered from sample plots distributed across two research locations. The inventory plots were arranged on a regular grid with a minimum distance of 50 meters between them. Tree species were documented within 20 x 20 meter plots, while seedlings were surveyed in 2 x 2 meter plots. Measurements of tree diameter at breast height (DBH) and total tree height were conducted using nested plots: trees with a DBH greater than 20 cm were measured in 20 x 20 meter plots, those between 10 cm and 20 cm DBH were measured in 10 x 10 meter plots, and trees with a DBH greater than 5 cm but less than or equal to 10 cm were recorded in 5 x 5 meter plots.



Source: Kahayan Hulu Forest Management Unit (2023)

Field data was analyzed using a calculator and an Excel-based tool developed by Michigan State University as part of the USAID LESTARI project (2018). One of these tools calculates total tree carbon stock (tC), wood volume (m^3 ha^{-1}), and tree density (trees ha^{-1}). The second tool is designed to compute various biodiversity indices, including species richness, evenness, and dominance for trees (greater than 2 cm DBH) and seedlings. The allometric model employs the tree's DBH to estimate its total live biomass (kg). Carbon is then derived from biomass using a conversion

factor of 0.47, which is the default value provided by the IPCC (Hiraishi *et al*., 2014). Carbon stocks for each tree size class within nested plots are summed and converted to tons (tC). These values are aggregated to determine the total carbon per plot, which is subsequently scaled to report tC per hectare. The averages across all plots are calculated and multiplied by the site area to estimate the overall carbon stock (tC) (Krisnawati *et al*., 2012).

$$B = 0.75 * Dt\text{^}2.23 \quad \text{Eq. 1}$$

Where
B = total tree biomass in kg
Dt = diameter at breast height (1.3 m above ground) in cm

Timber volume (m^3) was determined by applying measurements of diameter at breast height (DBH) and tree height in a numerical equation, then summing the results for all trees within the plot and scaling it to hectares. The average was subsequently calculated across all plots. Using equations modified for shape coefficients yields conservative estimates of wood volume.

$$Dw = ((1/4) * ((PI() * ((Dt/100)\text{^}2)) * Ht) * 0.6) \quad \text{Eq. 2}$$

Where
Dw = wood volume in m^3
Dt = diameter at breast height (1.3 m above ground) in cm
Ht = total height of the tree in m

Tree density (trees ha^{-1}) was determined by counting the number of trees in three nested size class plots, with each size class count scaled to the hectare level. The average density was then calculated for all plots on the site according to tree size class. The biodiversity tool employs species counts from fixed-area sample plots to compute various biodiversity indices for both trees and seedlings. The indices and calculation methods are presented in Table 1.

Table 1: Plant Biodiversity Indices and measurement methods utilized by the biodiversity calculator tool

Indices	**Computation Method**
Species Richness	S = number of species or taxa
Menhinick's Index (Menhinick, 1964)	D = S/(SQRT N); where S = the number of different species in the sample and N = the total number of individual organisms in the sample
Margalef's Richness Index (Margalef, 1958)	(S-1)/ln(N)
Shannon Index of Species Diversity (Shannon, 1948)	$H = -\sum_{i=1}^{s} p_i \ln p_i$; where pi = the proportion of the total number of individuals

Simpson Index of Diversity (Simpson, 1949)	1 - D = 1 -∑ (n / N)2; where D is the Simpson Index which measures the probability that two individuals randomly selected from a sample will belong to the same species (or some category other than species)
Evenness Index	Evenness = H'/lnS uses Shannon index and species richness values

RESULTS AND DISCUSSION

Biodiversity of Harowu Forest

The counts of each tree and seedling recorded in every sample plot are detailed in Table 2. Trees were assessed in six plots, where they were evenly distributed. Table 2 includes various biodiversity indices calculated for both sites, along with the dominant species for trees exceeding 5 cm DBH and for seedlings. As anticipated in a robust forest ecosystem, both tree species richness and biodiversity indices displayed elevated values.

Table 2 Biodiversity of trees and seedlings

Indices	Trees (n=40)	Seedlings (n=22)
Species Richness	25	12
Menhinick's Richness Index	1,57	0,88
Margalef's Richness Index	4,34	2,11
Shannon Index	2,98	2,08
Simpson Index of Diversity	0,94	0,84
Evenness	0,93	0,84
Dominant species	Pisang-pisang/mahawai (*Polyathia lateriflora* King.) Karipak nangka (*Artocarpus kemando*) Mahang kirik (*Macaranga triloba*) Mandarahan merah (*Horsfieldia irya*) Pampaning bitik (*Querqus subsericea*)	Kopi-kopi (*Fragraea racemosa*) Jambu merah (*Syzygium spp*) Meranti bunga (*Shorea parvifolia*) Keruing bayan tuwung (*Dipterocarpus rigidus*) Cangal gading (*Upuna borneensis)*
Others Floras	Lianas: 70 Shrubs: 90	Ferns: 10 Pandan: 5 Small Check: 30 Others Herbal Plants: 24

Table 2 presents that the Richness Value is 25 for trees and 12 for seedlings, with Menhinick's Richness Index at 1.57 for trees and 0.88 for seedlings. Margalef's Richness Index stands at 4.34 for trees and 2.11 for seedlings, while the Shannon Index is 2.98 for trees and 2.08 for seedlings. The Simpson Index of Diversity is 0.94 for trees and 0.84 for seedlings, with Evenness values of 0.93 for trees and 0.84 for seedlings. These high biodiversity indices indicate a healthy forest ecosystem (Maimunah et al., 2021).

The dominant species among trees include Pisang-pisang/mahawai (*Polyathia lateriflora* King.), Karipak nangka (*Artocarpus kemando*), Mahang kirik (*Macaranga triloba*), Mandarahan merah (*Horsfieldia irya*), and Pampaning bitik (*Quercus subsericea*). For seedlings, the dominant species are Kopi-kopi (*Fragraea racemosa*), Jambu merah (*Syzygium* spp), Meranti bunga (Shorea johorensis), Keruing tuwung bayan (*Dipterocarpus rigidus*), and Cangal gading (*Upuna borneensis*). The red Mandarahan (*Horsfieldia irya*) and Jambu merah (*Syzygium* spp) species have also been identified by Mirmanto (2009) and Kalima et al. (2020) in the same province, indicating their status as indigenous species.

Carbon Stock of Harowu Forest

Table 3 presents various site characteristics for data collection and analysis, including the number of plots, total area, and plot size. It also details site-level carbon stocks, which encompass the average tC ha^{-1} across all plots, along with the range and standard deviation of total carbon for the sites. Additionally, the table includes the sampling error and accuracy range based on the number of plots, assessed at a 95% confidence level.

Table 3. Recapitulation of calculation of carbon stocks in the Harowu forest area

Properties	Value
Number of Plot (n)	6
Forest Area (ha)	1750
Total Carbon in Plot (tons)	2149,57
Averaage Carbon in Plot (tons)	358,26
Standard Deviation (tons)	645,97
Plot Size (ha)	0,04
Population (N)	43750
t-student value (α=5%)	2,57
Average default error rate (tons)	263,7
CI95% average (lower limit) (tons)	316,52
CI95% average (upper limit) (tons)	400
Sampling Error (%)	11,65
Total carbon stock (tC) – Lower (tons)	553.907,40
Total carbon stock (tC) (tons)	626.957,75
Total carbon stock (tC) – Upper (tons)	700.008,09

The average carbon stock in community forests is 358.26 tons C ha^{-1}, which is comparable to the average total carbon per hectare found in natural forests within Gunung Mas Regency, as reported by Astuti *et al*. (2020). This indicates that community-managed forests show no signs of carbon reduction. The total carbon observed at the research site exceeds the figures reported by Besar *et al*. (2020), suggesting that the community is effectively managing the village forest, ensuring its sustainability. The total carbon in the plot amounts to 2,149.57 tons C ha^{-1}, and the range of carbon values across all sample plots indicates a total carbon stock of 626,957.75 tons C. Estimating carbon stocks is crucial, as it helps sequester greenhouse gases and mitigate emissions into the atmosphere that contribute to climate change (Maimunah *et al*., 2023). The accuracy of these carbon estimates is enhanced due to the number of sample plots, the size of the plots, and the variability of carbon across the samples.

Wood Volume and Tree Density

Table 4 summarizes the calculations of tree volume by size class, including the number of trees in the sample plot, wood volume, and tree density. The distribution of tree size classes in both locations was notably skewed towards the largest size class, specifically trees with a diameter at breast height (DBH) of more than 15 cm, compared to poles and saplings. However, the number of trees per plot by size class can be somewhat misleading due to data being collected in nested plots of 5 x 5 m (for saplings), 10 x 10 m (for poles), and 20 x 20 m (for large trees). The table includes the sampling and tree counts in brackets, estimating totals for a 20 x 20 m plot based on observed nesting plots or actual counts. The overall average wood density for all trees (m^3 ha^{-1}) was higher at site 2 by one and had a greater volume than site 1. At both sites, large trees contributed to 90% of the total average tree wood volume. Regarding tree density, the number of trees per hectare included a larger proportion of old trees, with the large tree class comprising 66% and 62%, respectively.

Table 4 Wood volume and tree density in Harowu Village Forest

Stage Development	Volume (m^3 ha^{-1})	Average Tree Density (tree ha^{-1})
Saplings	82,31	0
Poles	123,77	2.533
Trees	796,18	717
Total	1002,26	254

Harowu Village Forest features a karst forest ecosystem intermingled with secondary tropical forest, hosting a variety of wildlife, including numerous bird species and rare animals like haruwei, partridges, porcupines, deer, and wild pigs, all of which inhabit the forest alongside several rare tree species such as ironwood, various types of meranti and keruing, gutta-percha, binuang, and several plants used for traditional medicine. The forest's key habitat elements include distinctive caves, rock formations, waterfalls, springs, and mineral lakes, providing essential areas for wildlife. The volume and density of trees in the sapling category are recorded at 82.31 m^3 ha^{-1}, for poles at 123.77 m^3 ha^{-1}, and for larger trees at 796.18 m^3 ha^{-1}, with densities of 2.53 trees ha^{-1} for poles and 717 trees ha^{-1} for larger trees. These figures indicate that the forest is continuously regenerating, thereby preventing degradation (Maimunah *et al*., 2022). The carbon stocks in this area are also significant, totaling 626,957.75 tonnes C/ha, which must be preserved and enhanced despite the challenges posed by

illegal land clearing activities. Potential solutions include raising awareness and creating new job opportunities that promote long-term forest conservation.

CONCLUSION

The findings of this research highlight the importance of analyzing field inventory data in forests to provide insights into ecosystem service levels. In this study, the biodiversity metrics for tree categories are as follows: Richness (25), Menhinick's Richness Index (1.57), Margalef's Richness Index (4.34), Shannon Index (2.98), and Simpson Index of Diversity (0.94). For the seed categories, the values are: Richness (12), Menhinick's Richness Index (0.88), Margalef's Richness Index (2.11), Shannon Index (2.08), Simpson Index of Diversity (9.84), and Evenness (0.84). The carbon stock is measured at 358.26 tons C ha^{-1}. Both carbon stocks and biodiversity serve as critical indicators of forest health. Although the tree biodiversity reported in this area is relatively low, it suggests that the forest functions as a reasonably healthy ecosystem. Expanding biodiversity assessments to include non-tree biota would be beneficial, as these measurements could demonstrate the richness of forest habitats in terms of diverse flora and fauna.

Estimating carbon stocks is vital to illustrate the role these forests play in sequestering greenhouse gases within their biomass, thus preventing emissions that contribute to climate change. Conducting repeated measurements to track changes in carbon stocks is essential for understanding whether these areas are increasing their carbon sequestration over time or emitting carbon. Monitoring carbon additions from tree growth or losses is crucial for developing effective management strategies for these forest areas. Given that the site is designated for ecotourism, informing visitors about the forest's carbon stock will enhance their awareness and may foster contributions to tropical forest conservation efforts. Lastly, the inclusion of local communities, especially those who directly benefit from the forests of Harowu Village in Gunung Mas Regency, is of utmost importance and cannot be overstated.

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