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Evaluating the feasibility of using seaweed and pineapple leaves to develop moisture and blood-absorbent paper for fish packaging

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Abstract Food packaging is crucial for perishable products such as seafood, which can spoil easily. This paper investigates the potential of using cellulose from pineapple leaves, which are generally discarded after harvesting, to produce moisture and blood-absorbing wrapping paper instead of wood fibre, which raises concerns about deforestation. Seaweed powder from *Kappaphycus alvarezii* was used as a bio-preservative to replace synthetic preservatives. A process was developed to produce paper using existing soda pulping methods to produce paper from pineapple leaves. Five different levels of seaweed powder were mixed with 20 g each of pineapple leaf pulp, from two different varieties. The properties of the papers were compared with a commercially available absorbent paper. The cellulose content in the pulps from two pineapple varieties was approximately 65%, indicating the feasibility of using pineapple leaf fibres to produce paper. The developed papers exhibited lower moisture and blood absorption times compared to the commercial paper. However, the results indicated that these properties could be improved in future method developments. The total plate count method revealed fewer colonies as the amount of seaweed increased, demonstrating the effectiveness of *Kappaphycus alvarezii* as a natural antimicrobial agent for use in moisture- and blood-absorbent papers. These findings highlight the potential of utilising pineapple leaf fibre and *Kappaphycus alvarezii* powder in the production of moisture- and blood-absorbent papers, offering a sustainable alternative with added antibacterial benefits.

Keywords: Blood absorbency, Fish, Moisture absorbency, Pineapple leaf waste, Seaweed

INTRODUCTION

Food packaging is a critical factor in the development of safe food products, as it significantly contributes to reducing food loss and contamination. Highly perishable foods, such as seafood are particularly susceptible to spoilage due to factors such as inadequate processing, poor storage and transport conditions. Specialist packaging systems are therefore used in export markets to preserve product freshness and ensure customer satisfaction.

Moisture and blood-absorbing wrapping papers, known for their tensile strength, tear resistance and antimicrobial properties, are commonly used as primary packaging for fresh fish fillet exports. However, selecting the right materials and applying appropriate packaging technologies are essential to maintain the quality and safety of these products. There are different types of packaging materials available worldwide including paper, cardboard, plastic, metal and glass (Marsh and Bugusu, 2007). Among these, a growing trend in environmentally friendly packaging emphasises the potential of using agricultural waste for blood-absorbent papers. This offers a biodegradable alternative to wood fibre and helps reduce deforestation (Jaffur & Jeetah, 2019; Laftah & Rahman, 2016). Current data shows that 45% of industrial wood production is used for pulp production, yet only 10% of the global paper industry utilises non-wood lignocellulosic materials (Sibaly and Jeetah, 2017).

Under such circumstance, pineapple leaves can be introduced as a promising raw material for developing moisture- and blood-absorbent papers. Pineapple leaf fibres, which are composed of 81.27% cellulose, are valued for their high specific strength, stiffness, and hydrophilic properties. (Aremu *et al*., 2015; Arul Marcel Moshi *et al*., 2019). In addition to the cellulose, pineapple leaf fibres contain 12.31% hemicellulose, 3.46% lignin, and 10.52% moisture (Karim *et al.*, 2022; Baghel *et al.*, 2021). The chemical composition of pineapple fibres resembles that of wood, making them an ideal material for paper manufacturing due to their similarity to wood (Azan *et al.*, 2020; Sufian *et al.*, 2020). As such, pineapple leaf fibres hold significant potential as an alternative raw material in the paper industry, owing to their excellent mechanical properties, renewable nature, and low-cost (Mahatme, Kanse and Bandsode, 2018).

Seaweed can be incorporated as a potential ingredient in the production of blood-absorbent papers. Seaweeds are a diverse source of secondary metabolites with a wide range of biological activities, including cytostatic, antiviral, anthelminthic, antifungal, and antibacterial properties, found in the species from Rhodophyceae, Phaeophyceae, and Chlorophyceae (Shekhar, 2013). Given the prevalence of active compounds with antimicrobial properties in these seaweeds (Shekhar, 2013; Prasad *et al*., 2013), incorporating seaweed into wrapping paper offers enhanced protection for food products. While various species of seaweed are found in Sri Lanka, there is a lack of comprehensive studies or systems to evaluate their properties and potential usage (Jayasinghe *et al.*, 2016). In Sri Lanka, seaweed is an underutilised natural resource with significant potential for producing moisture- and blood-absorbent papers. *Kappaphycus alvarezii*, a seaweed found in Sri Lanka, is rich in various bioactive compounds, particularly carrageenan. Carrageenan extracted from this seaweed has demonstrated potent antibacterial properties against several human pathogens (Rupert *et al.*, 2022). Therefore, the present study focused on developing a moisture- and blood-absorbent paper using pineapple leaves and *Kappaphycus alvarezii*, and comparing its performance with a commercially available paper.

MATERIALS AND METHODS

Materials

Fresh samples of pineapple leaves from two pineapple varieties, Kew and Mauritius were used for this study. *Kappaphycus alvarezii* seaweed was sourced from a cultivation site in Jaffna. Fish blood was collected by making a cut in a freshly caught fish. Commercial moisture- and blood-absorbent paper was obtained from a seafood export factory.

Methods

Pineapple leaf preparation

The pineapple leaves were harvested and the prickly edges were trimmed using a knife. The tips, base and any damaged sections of the leaves were then removed and the cut leaves were thoroughly washed to remove soil and other contaminants. The washed leaves were then air dried to remove excess surface water from the leaves, followed by oven drying at 60°C until a constant weight was achieved. Finally, the dried leaves were cut into 1 cm pieces, in length for further processing.

Pulping process

A sample of 20 g of cut pineapple leaves was immersed in 100 mL of 15% sodium hydroxide solution and heated (cooked) at 90±25°C on a hot plate stirring at 300 rpm for 90 min. Afterwards, the mixture was allowed to cool to room temperature and was thoroughly washed with distilled water. The pulp was then separated from the water by filtering through a sieve and subsequently ground to obtain the pulp.

Seaweed powder preparation

The seaweed was cleaned to remove salt and sand, then thoroughly washed with distilled water. They were cut into small pieces and dried in an oven at 40 °C until a constant weight was achieved. The dried seaweed was then ground into a fine powder using a grinder and stored in the refrigerator until further use.

Paper making process

Seaweed powder was mixed with pineapple leaf pulp in five different ratios: 0: 20 (w:w); 1:20 (w:w); 2: 20 (w:w); 3:20 (w:w) and 4: 20 (w:w). A deckle was placed on the mould and clipped in place. The mixture was then spread over the surface of the mould and allowed to drain. The mould was removed from the deckle and placed onto a felt material. The wet sheet was flattened on the felt material using a roller. The mould was then removed and the wet sheets were allowed to dry at room temperature under a pressure of 2 kg for two days. After two days, the paper was peeled off the felt material and flattened under 2 kg pressure for

24 h. The paper was then further dried by placing it between tissue papers.

Cellulose content analysis of paper pulp

A sample of approximately 1 g of pineapple leaf pulp was mixed with 1.5 mL of concentrated nitric acid and 15 mL of 80% acetic acid in test tubes. The tubes were heated in a 100 °C water bath for 20 min, cooled, filtered, and washed thoroughly. The residues were dried on pre-weighed filter papers at 105°C for 24 h. The analysis was performed in triplicate . The cellulose content was calculated using the following equation.

$$\text{Cellulose percentage in the paper pulp} = \frac{\text{Weight of cellulose (g)}}{\text{Weight of pulp (g)}} \text{x } 100\ \%$$

Characterization of the developed paper

Thickness

Paper thickness was measured using a micrometer. Measurements were taken at five different places for each sample and a mean thickness was determined with an accuracy of ±0.001 mm.

Grammage

The weight of the paper was measured using the analytical balance, and the paper grammage was then calculated directly from the weight and area of the paper using the following equation.

$$\text{GSM} = \frac{\text{weight (g)}}{[\text{sample length (m)} * \text{sample width(m)}]}$$

Colour

Colour was measured using a chromometer (Chroma Meter CR-400, Konica Minolta, Japan). After calibrating the colorimeter, the colour difference values (ΔE*) were recorded from five different places in each sample.

Moisture and blood absorbency rate

To measure the moisture absorbency of the paper, a 10 μL drop of water was placed on the paper held on a dry glass surface. The time taken for the water drop to completely disperse was recorded using a stopwatch. This process was repeated ten times at different points on the paper and the average time was calculated to determine the moisture absorbency rate. The same method was used to measure the blood absorbency rate, using the blood taken from a fish.

Microbial test

A piece of approximately 0.1 g of autoclaved paper was homogenised in 60 mL of phosphate buffer. The resulting slurry was serially diluted tenfold through four dilutions on nutrient agar for total plate count. Growth on the agar plates was determined after incubation at 37 °C for 24 h. Colony counts, ranging from 30 to 300, were recorded and the CFU/g of the sample was calculated according to the following equation.

$$\text{CFU/g of sample} = \frac{\text{Average number of colonies} \times \text{Dilution Factor}}{\text{Volume of sample taken}}$$

Statistical analysis

The cellulose content of paper pulps was compared using a two-sample t-test. The thickness and grammage of the developed moisture- and blood-absorbent papers were analysed using regression analysis. Colour, moisture, and blood absorbency rates were compared using two-way ANOVA. These properties of the developed papers were then compared with a commercial paper using a t-test where appropriate. All statistical tests were performed at a 95% confidence level ($P<0.05$) using the Minitab 17 version.

RESULTS AND DISCUSSION

In this study, pulps from the leaves of two pineapple varieties were used to make papers. The cellulose content analysis of the paper pulps revealed no significant difference ($p > 0.05$) between the cellulose content of the pulps from the two pineapple varieties, which was approximately 65% (Table 1). Cellulose content is important for the formation of paper without breaking. Therefore, these results suggest that both pineapple varieties have similar and high cellulose content, making them suitable for paper production.

Table 1. Average cellulose content in the pulp of two pineapple varieties

Pineapple variety	Cellulose content in the paper pulp (%)
Kew	65.2 ± 0.26
Mauritius	65.3 ± 1.59

The photographs of developed papers from the two pineapple varieties at five different seaweed powder: pineapple leaf pulp ratios (w:w) are shown in Figure 1. The thickness and grammage of papers were compared with those of a commercial moisture- and blood-absorbent paper used in fish export. Thickness increased significantly with the addition of seaweed powder for both pineapple varieties, individually (Regression analysis, $P<0.05$) (Figure 2). Moreover, the thickness of the papers made from both pineapple varieties at all five seaweed powder: pineapple leaf pulp ratios were significantly higher ($P<0.05$) compared to the commercial paper. Similarly, grammage also increased significantly with the addition of seaweed powder (Regression analysis, $p<0.05$) (Figure 3). Grammage of all developed papers was also significantly higher ($p<0.05$) compared to the commercial paper. These results suggest that paper thickness and grammage are dependent on the percentage of seaweed powder added. In packaging and exporting fresh fish fillets, these two physicochemical properties are important, as a thicker paper occupies more volume than a thin paper, and wrapping is easier with thinner paper. Weight is also important in export as it accounts for the shipping and airfreight weight.

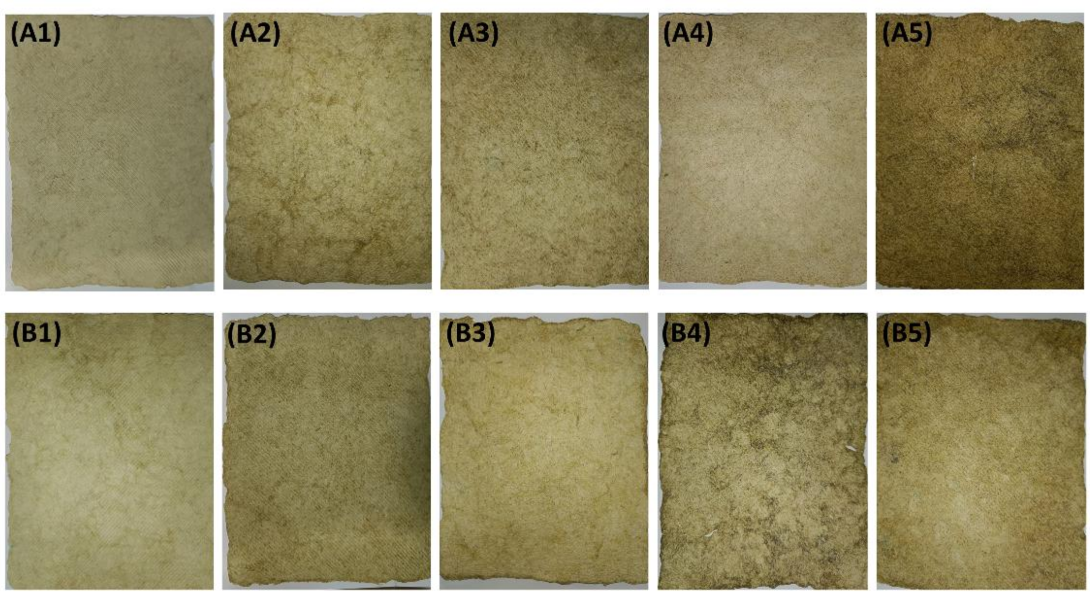

Fig 1. Photographs of papers prepared at five different seaweed powder: pineapple leaf pulp ratios (w:w). A1: Kew variety, 0:20 (w:w); A2: Kew variety, 1:20 (w:w); A3: Kew variety, 2:20 (w:w); A4: Kew variety, 3:20 (w:w); A5: Kew variety, 4:20 (w:w); B1: Kew variety, 0:20 (w:w); B2: Kew variety, 1:20 (w:w); B3: Kew variety, 2:20 (w:w); B4: Kew variety, 3:20 (w:w); B5: Kew variety, 4:20 (w:w).

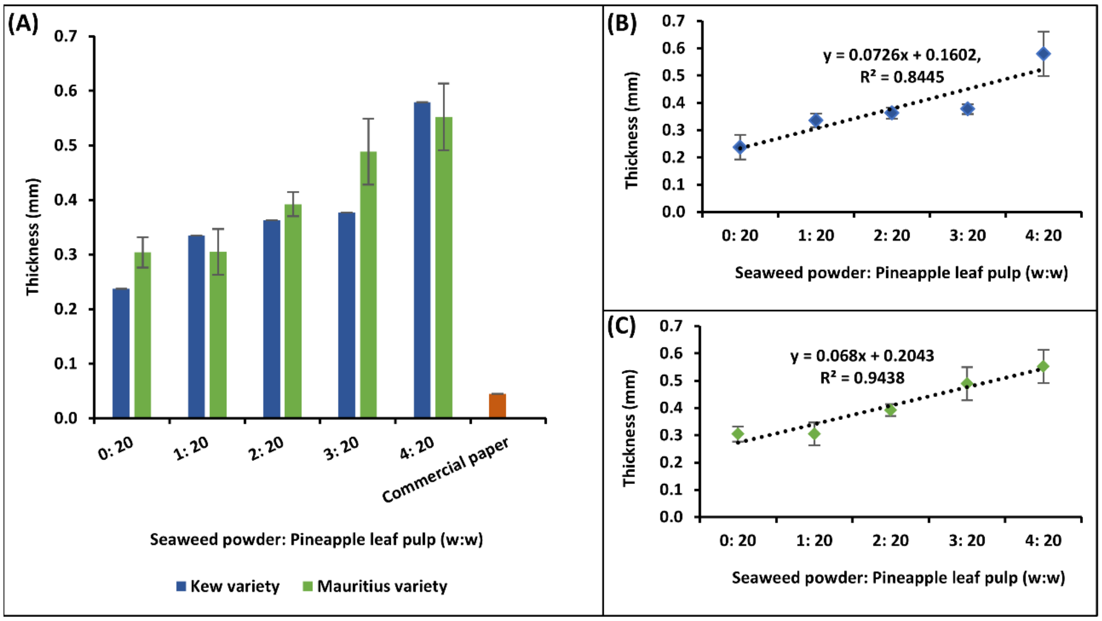

Fig 2. (A) Mean thickness (mm) of developed papers at five different seaweed powder: pineapple leaf pulp ratios (w:w). CP denotes commercial paper; (B) Linear regression analysis for thickness (mm) of papers made with Kew variety vs seaweed powder: pineapple leaf pulp ratio (w:w); (C) Linear regression analysis for thickness (mm) of papers made with Mauritius variety vs seaweed powder: pineapple leaf pulp ratio (w:w).

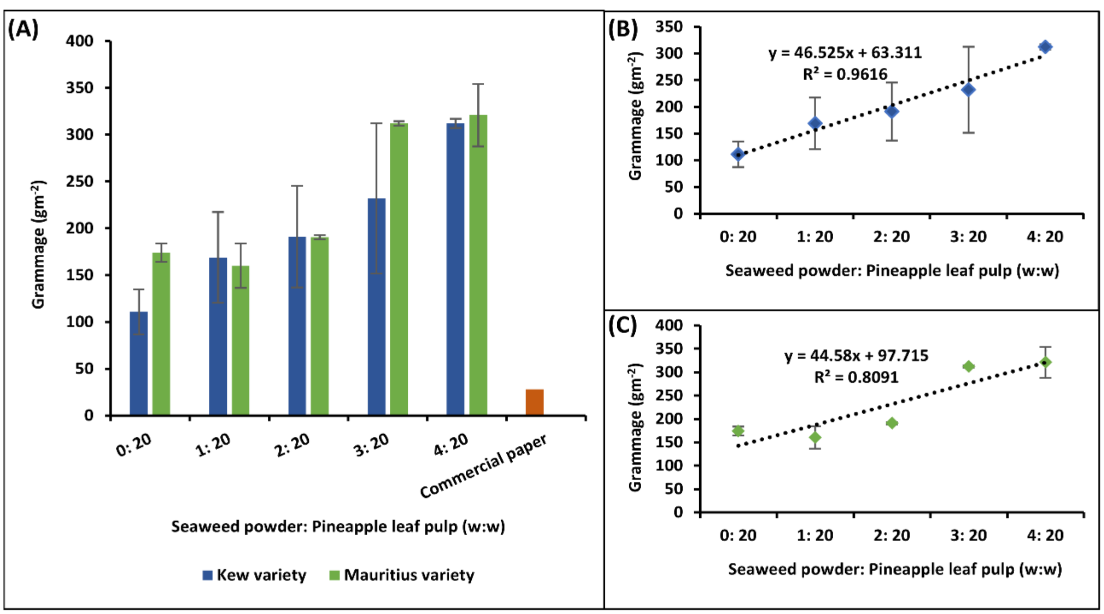

Fig 3. (A) Mean grammage (gm^{-2}) of developed papers at five different seaweed powder: pineapple leaf pulp ratios (w:w). CP denotes commercial paper; (B) Linear regression analysis for grammage (gm^{-2}) of papers made with Kew variety vs seaweed powder: pineapple leaf pulp ratio (w:w); (C) Linear regression analysis for grammage (gm^{-2}) of papers made with Mauritius variety vs seaweed powder: pineapple leaf pulp ratio (w:w).

The colour of the papers was measured as the colour difference from the white standard of the chromameter. According to the two-way ANOVA, both pineapple variety and seaweed powder: pineapple leaf pulp ratios affected the colour of the papers ($P<0.05$), with higher colour values observed for papers made without the addition of seaweed powder (Figure 4). The colour of the papers at all seaweed powder: pineapple leaf pulp ratios from both pineapple varieties were significantly different from the commercial paper ($P<0.05$). However, the colour of the paper is not a significant factor except in terms of the paper's appearance after blood absorption.

The two most important properties of the developed papers were the moisture absorbency rate and blood absorbency rate. These rates were measured by the time taken for a known volume of water and blood to be completely absorbed into the paper, respectively. According to the results of two-way ANOVA ($P<0.05$), moisture absorbency was influenced by the pineapple variety, seaweed powder: pineapple leaf pulp ratio, and the interaction of these two factors (Figure 5). Among the five different seaweed powder: pineapple leaf pulp ratios, papers made without adding seaweed powder took a significantly lower time for moisture absorption (8 s) ($P<0.05$) compared to other ratios. The time taken for moisture absorption by all papers was significantly lower compared to commercial paper which took 18,750±911.81 s.

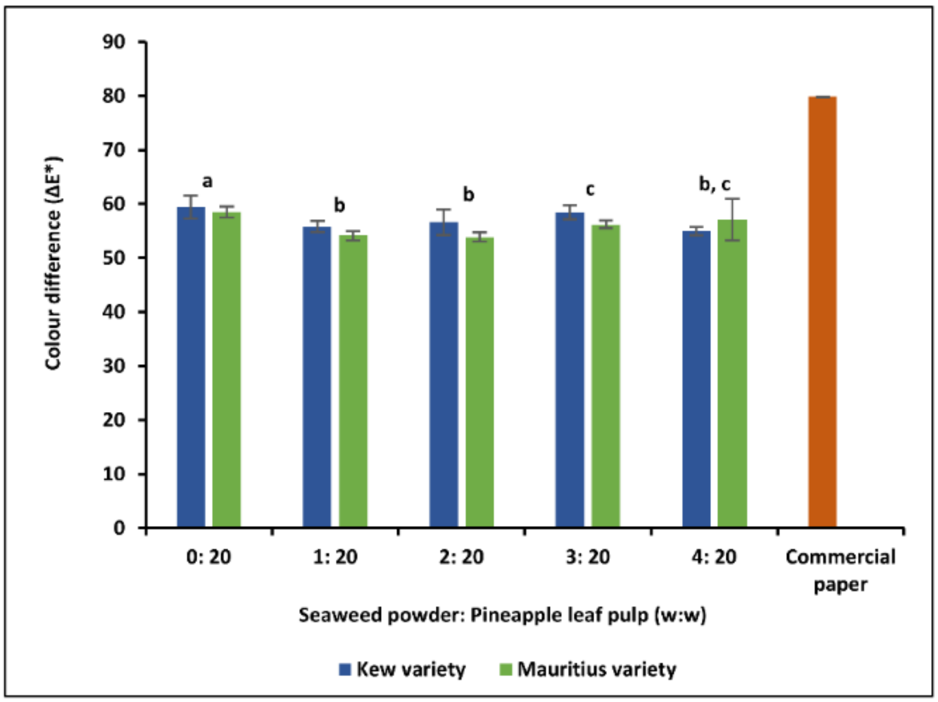

Fig 4. Comparison of mean colour differences (ΔE*) of developed papers at five different seaweed powder: pineapple leaf pulp ratios (w:w). Means with same letters are not significantly different at $P<0.05$. CP denotes commercial paper.

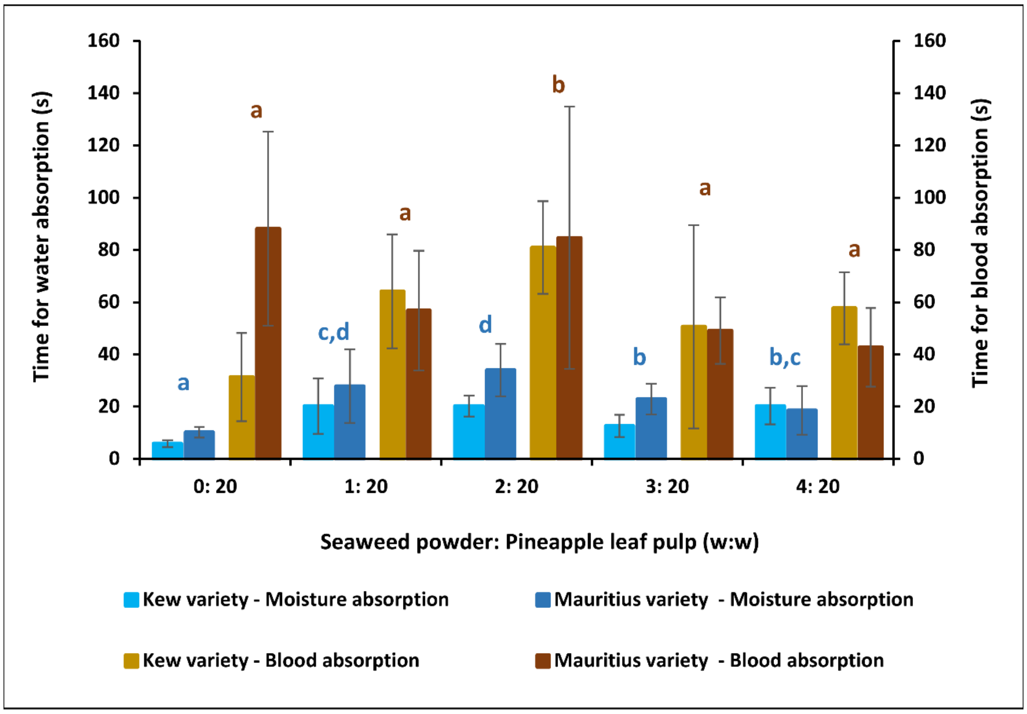

Fig 5. Comparison of mean time for water absorption (s) and blood absorption (s) by developed papers at five different seaweed powder: pineapple leaf pulp ratios (w:w). Means with the same letters are not significantly different at $P<0.05$. CP denotes commercial paper.

Blood absorbency was not affected by the pineapple variety; however, it has been affected by the seaweed powder: pineapple leaf pulp ratio and the interaction of these two factors (two-way ANOVA, $P<0.05$) (Figure 5). Among the five ratios, the seaweed powder: pineapple leaf pulp ratio of 2: 20 (w:w) resulted in a significantly longer blood absorption time ($P<0.05$). Additionally, the blood absorbency time of all developed papers was significantly lower ($P<0.05$) compared to the commercial paper (168.10±15.61). These results depict that the seaweed powder: pineapple leaf pulp ratio is more important than pineapple variety in moisture and blood absorption. The rapid moisture absorption of the developed papers may be due to the absence of water-resistant treatments which are generally applied to commercial papers that facilitate gentle absorption of moisture. The lower moisture and blood absorbency times of the developed papers have both positive and negative impacts. While the rapid absorption of moisture and blood is beneficial for preserving the quality of fish fillets, it may also reduce the strength of the developed paper. .

The total microbial count was used to determine the effect of adding seaweed powder as an antimicrobial agent. With this method, it was anticipated that papers with higher antimicrobial activity would exhibit lower total bacterial counts (Ahmad *et al.*, 2016; Zaidi et *al.*, 2022). Accordingly, the highest antimicrobial activity was shown by the seaweed powder: pineapple leaf pulp ratio of 4: 20 (w:w) (Figure 6). The total plate count was highest in papers without the addition of seaweed powder. These results depict that the antimicrobial properties of *Kappaphycus alvarezii* have been maintained during the paper-making process.

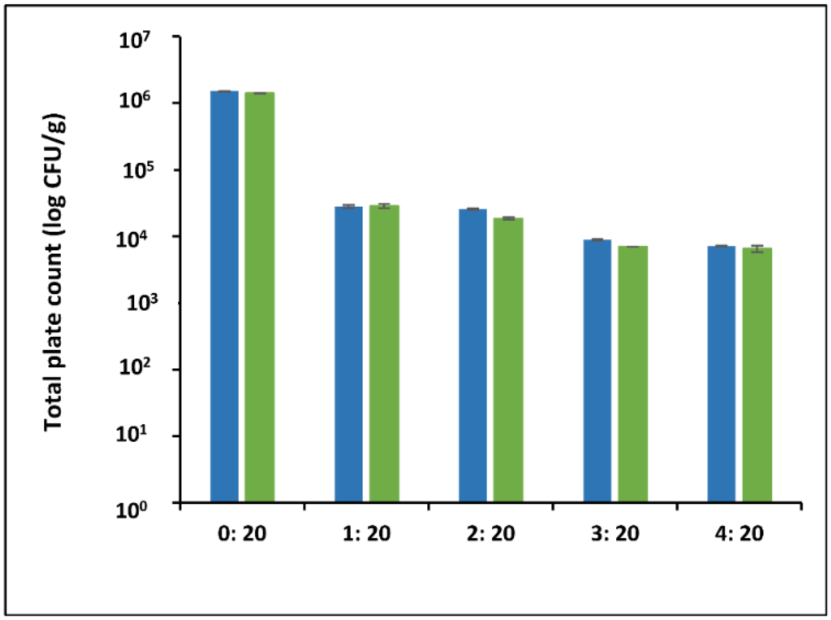

Fig 6. Total plate count (CFU/g) of developed papers at five different seaweed powder: pineapple leaf pulp ratios (w:w).

CONCLUSIONS AND RECOMMENDATIONS

The results demonstrated the potential for developing moisture- and blood-absorbent papers using pineapple leaves as the source of cellulose and *Kappaphycus alvarezii* powder as an antimicrobial agent. While the thickness and grammage of the papers may need to be reduced to meet industry requirements, this adjustment could be achievable at the industrial level of papermaking. Alternatively, there is a scope for further research to modify the paper-making process to produce papers with lower thickness and grammage. Since there was no significant effect of pineapple variety on blood-absorbent properties, any selected commercial variety of pineapple leaves could potentially be used as a source of cellulose. This paper can be further developed into an anti-microbial, moisture- and blood-absorbent paper by selecting the optimum ratio of seaweed powder: pineapple leaf pulp, targeting specific food-borne pathogens.

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