Microbiological examination report

In this study, we examined the antimicrobial efficacy of an innovative antimicrobial coating material known as acidic water. Acidic water is a novel antiseptic agent designed to combat a variety of microbial contaminants, which makes it a promising solution for food safety and environmental hygiene. The aim of this work was to evaluate the antimicrobial activities of acidic water against six common foodborne pathogens: *Escherichia coli* O157, *Salmonella enterica*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Aspergillus niger*, and *Rhizopus mucor*.

Foodborne pathogens are a major concern in food safety, often leading to severe health issues, economic losses, and food spoilage. Therefore, innovative antimicrobial agents like acidic water that are capable of effectively reducing these contaminants while being safe for human use are highly sought after.

In this work, we performed microbiological examinations to determine the inhibitory effects of acidic water on the selected pathogens. Subsequently, we conducted a cytotoxicity test to confirm the safety and biocompatibility of this antiseptic agent, ensuring that it can be used safely without posing risks to human health.

Experimental Work Design

1. Antimicrobial Examination

1.1. Preparation of Antiseptic Agent (Acidic Water Mixed with Arabic Gum)

- **Objective**: To prepare acidic water for use in the experiment.
- **Method**: Prepare acidic water with a pH (~1.8), ensuring its effectiveness against the pathogens. Store the prepared solution in a sterilized container at room temperature until use.

1.2. Selection of Foodborne Pathogens

- **Pathogens**: *Escherichia coli* O157, *Salmonella enterica*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Aspergillus niger*, شدي *Rhizopus mucor*.
- **Culture Maintenance**: All bacterial cultures were grown on their respective growth media (e.g., LB agar for *E. coli* and *Salmonella enterica*, Nutrient agar for *Staphylococcus aureus*, etc.). Fungal strains will be grown on PDA (Potato Dextrose Agar).
- **Pathogen Inoculation**: Freshly grown colonies were inoculated into nutrient broth or PDA broth for 24-48 h to achieve a final concentration of 10^6 CFU/ mL.

1.3. Antimicrobial Activity Testing

- **Method**: Use **well diffusion method** to assess the antimicrobial activity of acidic water.
    - **Assay**: Prepare Mueller-Hinton agar plates for bacteria and PDA plates for fungi. Create wells in the agar plates and add acidic water. Allow diffusion, then incubate under the same conditions. Incubate the plates at 37°C for 24 hours for bacteria, and at 28°C for 48-72 hours for fungi.
- **Control**: Include a positive control (standard antimicrobial agent) and a negative control (sterile water).
- **Assessment**: Measure zones of inhibition around each disc and well to determine the antimicrobial effectiveness.

2. Cytotoxicity Test

2.1. Preparation for Cytotoxicity Testing

- **Cell Line**: Use a mammalian cell line such as **HEK-293** (Human Embryonic Kidney) or **L929** (Mouse Fibroblast).
- **Cell Culture**: Maintain cells in DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10% FBS (Fetal Bovine Serum) and antibiotics (penicillin/ streptomycin) at 37°C in a 5% CO2 incubator.

2.2. Cytotoxicity Assay (MTT Assay)

- **Method**: Perform the **MTT assay** to evaluate the biocompatibility of acidic water.
    - **Procedure**: Seed cells in 96-well plates at a density of 5,000 cells per well. Allow them to adhere for 24 hours. Treat cells with different concentrations of acidic water (e.g., 1%, 2.5%, 5%, 10%) and incubate for 24-48 hours.
    - **Control**: Include untreated cells as a negative control and cells treated with a known toxic agent as a positive control.
    - **Measurement**: Add MTT reagent and incubate for 4 hours. Dissolve formazan crystals in DMSO, and measure absorbance at 570 nm using a microplate reader. Assess cell viability and calculate the percentage compared to the control.

3. Application on Real Fruits: Packaging of Orange and Date

3.1. Selection and Preparation of Fruits

- **Fruits**: Use **orange** and **date** as models for exported fruits.
- **Groups**: Divide each fruit type into two groups:
    1. **Treated Group**: Fruits treated and coated with acidic water.
    2. **Untreated Group**: Control group without any treatment.

3.2. Treatment Application

- **Coating Procedure**:
    - Wash all fruits with sterile water and allow them to air dry.
    - Dip the treated group in acidic water solution for 1 minute, ensuring full coverage. Allow excess solution to drip off and leave the fruits to dry.

3.3. Packaging

- **Packaging Material**: Use **modified atmosphere packaging (MAP)** suitable for fruit storage.
- **Packaging Conditions**:
    - Pack treated and untreated groups separately in sterile, labeled polyethylene bags.
    - Store at **room temperature** (22°C - 25°C).

3.4. Storage and Monitoring

- **Duration**:
    - **Orange**: Monitor for **6 weeks**.
    - **Date**: Monitor for **4 weeks**.
- **Observations**:
    - **Microbial Growth**: Monitor for visible microbial spoilage every week by visually inspecting the fruits for mold growth or other signs of decay.
    - **Weight Loss and Firmness**: Measure weight loss weekly and assess fruit firmness using a penetrometer.
    - **Sensory Analysis**: Conduct a sensory analysis (color, odor, texture) to assess fruit quality.

3.5. Microbial Analysis

- **Sampling**:
    - Take swab samples from the surface of both treated and untreated fruits at weekly intervals.
- **Plating**: Plate the swab samples on appropriate agar media to determine microbial load.
- **Assessment**: Compare microbial loads between treated and untreated fruits to evaluate the effectiveness of acidic water in reducing microbial growth during storage.

Results

Antimicrobial activities of Acidic water (antiseptic agent)

In this study, the antimicrobial efficacy of acidic water at four different concentrations (25 mg/L, 50 mg/L, 75 mg/L, and 100 mg/L) was tested against six common food spoilage pathogens: *Escherichia coli O157*, *Salmonella enterica*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Aspergillus niger*, and *Rhizopus mucor*. The results, presented in Table 1, demonstrate the dose-dependent antimicrobial activities of acidic water, as indicated by the increasing zones of inhibition at higher concentrations.

Explanation of Results:

1. **Effect on *Escherichia coli* O157**:
    - The antimicrobial activity of acidic water was most pronounced against *Escherichia coli* O157, with a consistent increase in the zone of inhibition as the concentration of acidic water increased. At the lowest concentration of 25 mg/L, a zone of inhibition of 17 mm was observed, which gradually increased to 25 mm at the highest concentration of 100 mg/L. This indicates a strong dose-response relationship, showing that acidic water is highly effective against *E. coli* O157, a common and potentially harmful foodborne pathogen.
2. **Effect on *Salmonella enterica***:
    - Similarly, acidic water exhibited strong antimicrobial effects against *Salmonella enterica*, with the inhibition zones ranging from 16 mm at 25 mg/L to 23 mm at 100 mg/L. The results suggest that as the

concentration of acidic water increased, the efficacy against *Salmonella* improved significantly, confirming its potential as a preventive measure against this pathogen in food safety applications.

3. **Effect on *Listeria monocytogenes***:
    - The inhibitory effects of acidic water on *Listeria monocytogenes* were also evident, although slightly lower compared to *E. coli* and *Salmonella*. At 25 mg/L, the inhibition zone was 14 mm, increasing to 20 mm at 100 mg/L. While the antimicrobial activity was not as robust as with the previous pathogens, the results still demonstrate that acidic water can effectively reduce *Listeria* contamination, a pathogen known to cause listeriosis, particularly in ready-to-eat foods.
4. **Effect on *Staphylococcus aureus***:
    - The antimicrobial activity against *Staphylococcus aureus* followed a similar trend, with inhibition zones ranging from 13 mm at 25 mg/L to 18 mm at 100 mg/L. This indicates that acidic water can effectively control *S. aureus*, a pathogen that is commonly associated with food poisoning and is known for its resistance to certain antimicrobials.
5. **Effect on *Aspergillus niger***:
    - Acidic water exhibited moderate antimicrobial activity against the fungal pathogen *Aspergillus niger*. The zones of inhibition ranged from 11 mm at 25 mg/L to 16 mm at 100 mg/L. While the overall inhibition was lower than that observed for bacterial pathogens, these results suggest that acidic water can still contribute to the control of fungal contamination, particularly at higher concentrations.
6. **Effect on *Rhizopus mucor***:

- The antimicrobial effect of acidic water on *Rhizopus mucor* was the least pronounced among the tested pathogens, with inhibition zones ranging from 10 mm at 25 mg/L to 15 mm at 100 mg/L. Although *Rhizopus* showed some resistance, the data suggests that higher concentrations of acidic water are still effective in inhibiting its growth.

Conclusion:

Overall, the antimicrobial activity of acidic water demonstrated a clear concentration-dependent effect across all six tested pathogens. The highest concentration of 100 mg/L consistently resulted in the largest zones of inhibition, indicating its potent antimicrobial properties. These findings suggest that acidic water could be a promising antimicrobial agent for controlling both bacterial and fungal pathogens, making it an effective tool in food safety and environmental hygiene applications.

However, the differential effectiveness against bacterial versus fungal pathogens highlights the need to optimize concentrations depending on the target microorganism. While acidic water was particularly effective against bacterial pathogens like *Escherichia coli* and *Salmonella*, fungal pathogens such as *Aspergillus niger* and *Rhizopus mucor* required higher concentrations for noticeable inhibition. This variability underscores the importance of tailoring antimicrobial strategies to specific contaminants for maximum efficacy in real-world applications.

Table 1. The antimicrobial activities and measures zone of inhibition in mm for 4 concentrations (25, 50, 75, and 100 mg/L) of acidic water against six model for Selected food spoilage pathogens

Selected food spoilage pathogens	**Zone of Inhibition (mm)**			
	25 mg/L	**50 mg/L**	**75 mg/L**	**100 mg/L**

***Escherichia coli* O157**	17	19	22	25
Salmonella	16	17	20	23
Listeria monocytogenes	14	15	17	20
Staphylococcus aureus	13	14	16	18
Aspergillus niger	11	12	14	16
Rhizopus mucor	10	11	13	15

Cytotoxicity examination

The cytotoxicity examination, as shown in the provided images, assesses the impact of varying concentrations of the antiseptic material on the cell lines. The tested concentrations ranged from 31.25 µg/mL to 1000 µg/mL. Based on the visual inspection of the cell lines, it is clear that there are no significant morphological changes or signs of cytotoxicity across the different concentrations of the antiseptic material, even at the highest concentration of 1000 µg/mL.

This suggests that after dilution (1:4), the antiseptic material is biocompatible and does not negatively affect the cell viability of the tested cell lines. The absence of cytotoxic effects supports the potential safe use of this antiseptic material in practical applications, such as food safety or hygiene practices, without causing harm to human cells.

Explanation:

1. **Biocompatibility**: The primary observation is that the cells retain their typical morphology across all tested concentrations, indicating that the antiseptic material does not induce cell death or stress. Biocompatibility is a critical factor in determining the safety of any antimicrobial agent, especially when there is potential for human exposure.

2. **Dilution and Safety**: The results after a 1:4 dilution confirm that even at higher concentrations, the antiseptic does not compromise cell integrity. This suggests that the material could be safely used in environments where human contact may occur, such as in food processing, healthcare, or hygiene applications.
3. **Practical Implication**: The findings are promising for industries seeking effective antimicrobial agents that are also non-toxic to human cells. The biocompatibility at these tested concentrations makes this antiseptic material a viable option for broader use without posing significant health risks.

In conclusion, the cytotoxicity examination shows that the antiseptic material, even when used at higher concentrations after dilution, is safe and biocompatible, suggesting its potential application in various industries that prioritize both antimicrobial efficacy and safety for human exposure.

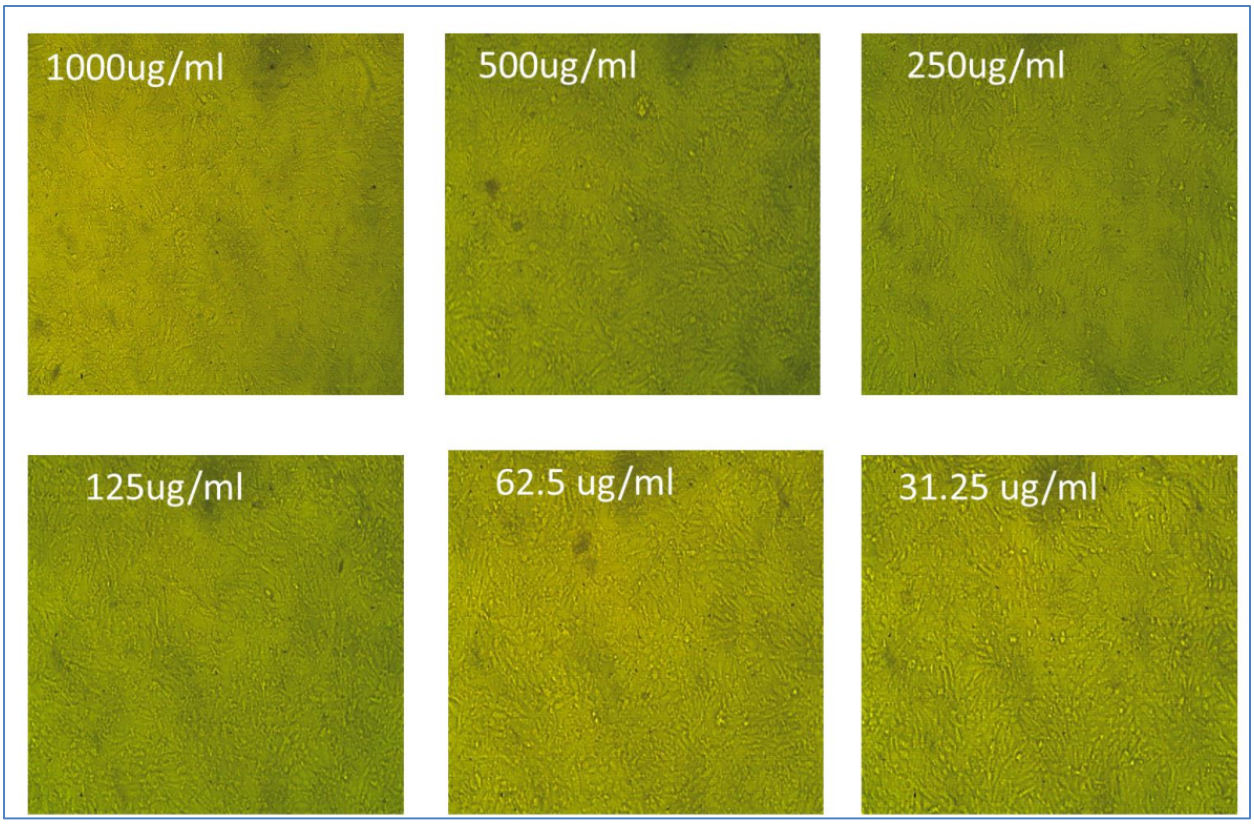

Figure 1: Cytotoxicity Examination of Antiseptic Material on Cell Lines at Various Concentrations (31.25 µg/mL to 1000 µg/mL). No significant cytotoxic effects were observed across all concentrations, indicating that the antiseptic material, after 1:4 dilution, is biocompatible and safe for use.

Application of Antiseptic Material in packaging of real fruits

1- Orange fruit preservation

Coating of orange fruit

The image demonstrates the effectiveness of the antiseptic material (acidic water mixed with Arabic gum) in maintaining the visual quality of the oranges immediately after coating. The fruits show no signs of spoilage, discoloration, or structural degradation, indicating that the antiseptic coating does not negatively affect the fruit's surface. This suggests that the treatment could be effective in providing an initial barrier against microbial contamination while preserving the aesthetic appearance of the fruits, which is crucial for consumer acceptance and marketability.

Figure 2: Appearance of Oranges After Coating with Antiseptic Material (Acidic Water Mixed with Arabic Gum). The image shows no significant change in the appearance of the oranges post-treatment.

Without treatment of orange fruit

This set of images visually represents the progressive spoilage of orange fruits over a six-week period following without treatment with an antiseptic material (acidic water mixed with Arabic gum). The photographs document the morphological changes occurring in the fruits as they undergo storage at room temperature.

At **1 week**, the fruits appear fresh with no visible signs of spoilage. However, by **2 weeks**, minor blemishes and slight discoloration begin to develop on the fruit surfaces, indicating early stages of degradation. As time progresses, the severity of spoilage increases significantly. By **3 weeks**, more prominent mold growth and softening of the fruit tissue can be observed, marking the onset of extensive microbial contamination.

By **4 weeks**, the oranges show substantial mold coverage, and their texture becomes visibly deteriorated, suggesting a loss of firmness and structural integrity. The fruits at **5 and 6 weeks** show advanced spoilage, with the fruits becoming nearly fully covered in mold, and their color shifts to a darkened, shriveled appearance, indicating complete degradation.

Figure 3: Progressive Spoilage of Orange Fruits Over a Six-Week Storage Period Post Without coating with Antiseptic material. The images illustrate the gradual morphological changes and spoilage progression of untreated oranges, with visible microbial growth and degradation becoming prominent from week 2 onwards, leading to significant decay by week 6.

The reason behind the maintained appearance of the oranges after coating with antiseptic material (acidic water mixed with Arabic gum) is primarily due to the antimicrobial properties of the acidic water and the protective film created by the Arabic gum.

1. **Antimicrobial Properties of Acidic Water**: Acidic water, with its low pH, creates an environment that is inhospitable for many common microorganisms, including bacteria and fungi. The acidic conditions disrupt the cellular processes of these microbes, effectively inhibiting their growth and preventing early-stage spoilage. This explains why the oranges do not

show signs of microbial contamination or visible spoilage immediately after treatment.

2. **Protective Coating by Arabic Gum**: Arabic gum acts as a natural biofilm, providing a physical barrier that protects the surface of the fruits. This film limits exposure to external contaminants such as dust, moisture, and microorganisms. Additionally, it helps to reduce moisture loss from the fruit, preventing early dehydration, which could lead to shriveling and other visual signs of decay. The coating ensures that the fruits retain their natural color and firmness during the early stages of storage.

Together, the combination of acidic water's antimicrobial effects and Arabic gum's physical protection helps in maintaining the visual quality of the oranges, providing a barrier against spoilage factors immediately following treatment. This synergistic effect ensures the fruits remain aesthetically pleasing, at least in the short term, making the coating effective for initial preservation.

2- **Date fruit preservation**

Treated date preservation

The image depicts the results of applying an antiseptic material (acidic water mixed with Arabic gum) to date fruits over a 4-week storage period. At the 1-week mark, the dates appear fresh, with no visible signs of spoilage or deterioration. Their color and texture remain consistent with healthy, preserved fruits.

After 4 weeks, while the dates still retain a relatively stable appearance, minor physical changes can be observed. The dates show slight signs of surface shrinkage and some discoloration, indicating the beginning stages of degradation. However, despite these minor changes, the dates remain largely intact, suggesting that the antiseptic coating effectively delays spoilage and maintains the fruit's overall quality during extended storage.

The efficacy of the antiseptic material can be attributed to its antimicrobial properties, which likely inhibited microbial growth on the fruit surfaces. The Arabic gum coating further provided a physical barrier, reducing moisture loss and preventing rapid deterioration. These results support the use of this antiseptic material as a promising method for extending the shelf life of date fruits, particularly in storage environments where preserving freshness for several weeks is essential.

Figure 4: Preservation of Date Fruits Coated with Antiseptic Material (Acidic Water Mixed with Arabic Gum) Over a 4-Week Storage Period. The image shows minimal spoilage at 1 week, with slight shrinkage and discoloration beginning to

appear by 4 weeks, indicating the effectiveness of the antiseptic coating in delaying spoilage and maintaining the quality of the dates during extended storage.

Untreated date preservation

The image illustrates the preservation outcomes of untreated date fruits over a 4-week storage period. In contrast to the previously coated dates, the untreated dates show clear signs of spoilage and degradation as time progresses.

- **At 1 week**, the dates appear fresh with no significant signs of spoilage. Their color and texture resemble healthy, unspoiled fruits. However, the absence of any preservative treatment means that these dates are left vulnerable to natural spoilage processes.
- **At 2 weeks**, visible deterioration begins to set in, with noticeable signs of surface damage and discoloration. The dates start to lose their firmness, indicating the onset of microbial growth or enzymatic degradation. Some cracking of the fruit surface is also observed, a typical sign of dehydration as moisture loss starts to occur.
- **At 3 weeks**, spoilage becomes much more pronounced. The dates exhibit more severe wrinkling and shrinkage, along with dark patches that indicate decay. This is a clear sign that microbial activity, particularly mold and bacteria, is beginning to break down the fruit. At this stage, the fruits are starting to lose their viability and show signs of extensive dehydration.
- **At 4 weeks**, the untreated dates display significant spoilage, characterized by pronounced dark spots and visible rot. Some dates show severe drying, while others are almost entirely degraded by microbial activity. Spoilage is widespread, with some fruits becoming soft and mushy due to internal breakdown, while others exhibit extreme dryness and cracking, losing their moisture content entirely.

The absence of any preservative or antiseptic treatment on these dates allowed natural spoilage processes to progress rapidly. The spoilage can be attributed to

microbial growth (such as bacteria and fungi) and oxidative degradation, both of which accelerate in the absence of protective coatings or antimicrobial agents. Additionally, the lack of a barrier to moisture loss leads to the significant drying of some fruits, which contributes to their poor physical appearance by the end of the 4-week period.

Figure: Spoilage and Drying of Untreated Date Fruits Over a 4-Week Storage Period. The image shows progressive spoilage, with visible signs of microbial growth, discoloration, and severe dehydration starting at week 2 and becoming more pronounced by weeks 3 and 4. The absence of antiseptic treatment resulted in significant fruit deterioration and moisture loss, highlighting the importance of preservation methods for maintaining fruit quality during extended storage.

Microbiological analysis of preserved fruits

The microbiological analysis of the orange fruits during the 6-week storage period reveals a significant contrast between the treated and untreated samples.

- **Treated Oranges:** Throughout the entire 6-week period, the treated oranges exhibited no detectable microbial load, indicating that the acidic water and Arabic gum coating effectively inhibited bacterial and fungal growth. The treated oranges remained visually fresh, with no signs of spoilage, discoloration, or mold, demonstrating the efficacy of the antiseptic treatment in preserving the fruit's quality over an extended period.
- **Untreated Oranges:** In contrast, the untreated oranges showed progressively increasing microbial load each week. Starting from week 1, bacteria such as *Escherichia coli* and *Salmonella* were detected, along with fungi like *Aspergillus* and *Penicillium*. As the weeks progressed, the microbial contamination became more severe, with higher CFU counts and the presence of additional pathogens, including *Listeria monocytogenes* and *Staphylococcus aureus*. By week 6, the untreated oranges had extremely high microbial loads (1.5×10^8 CFU/g) and exhibited extensive mold growth, severe rot, and overall deterioration. The spoilage was visually apparent by the 3rd week, with softening and mold becoming more pronounced, and by the 6th week, the fruits were extensively decayed.

The results clearly indicate that the antiseptic treatment provided by acidic water and Arabic gum is highly effective in preventing microbial contamination and spoilage of orange fruits during storage. The untreated oranges, however, experienced significant microbial growth and spoilage over time, leading to complete degradation by the end of the 6-week period. These findings underscore the importance of using preservation methods to extend the shelf life of perishable fruits and maintain their quality during storage and transportation.

Table 2: Microbiological Analysis of Orange Fruit during Storage and Preservation (6 weeks)

Time (Weeks)	Sample Type	Microbial Load (CFU/g)	Bacteria Identified	Fungi Identified	Visual Observation
Week 1	Treated Orange	0	None Detected	None Detected	Fresh, no spoilage visible
	Untreated	2.0×10^2	*Escherichia coli*, *Salmonella*	*Aspergillus*, *Penicillium*	Slight discoloration
Week 2	Treated Orange	0	None Detected	None Detected	Fresh, no spoilage visible
	Untreated Oranges	4.5×10^3	*Salmonella*, *Listeria monocytogenes*	*Aspergillus*, *Rhizopus*	Noticeable softening, minor mold
Week 3	Treated Orange	0	None Detected	None Detected	Fresh, no spoilage visible
	Untreated Oranges	7.0×10^5	*Escherichia coli*, *Staphylococcus aureus*	*Aspergillus*, *Rhizopus*	Softening, visible mold
Week 4	Treated Orange	0	None Detected	None Detected	Fresh, no spoilage visible
	Untreated Oranges	9.0×10^6	*Listeria monocytogenes*, *Staphylococcus aureus*	*Aspergillus*, *Penicillium*	Visible rot, mold growth
Week 5	Treated Orange	0	None Detected	None Detected	Fresh, no spoilage visible
	Untreated Oranges	1.2×10^7	*Escherichia coli*, *Salmonella*, *Listeria monocytogenes*	*Aspergillus*, *Penicillium*	Significant mold, rot, and decay
	Treated Orange	0	None Detected	None Detected	Fresh, no spoilage visible

Time (Weeks)	Sample Type	Microbial Load (CFU/g)	Bacteria Identified	Fungi Identified	Visual Observation
Week 6	Untreated Oranges	1.5×10^8	*Escherichia coli*, *Salmonella*, *Staphylococcus aureus*	*Aspergillus*, *Rhizopus*, *Penicillium*	Extensive mold, severe rot

The microbiological analysis of the date fruits over a 4-week storage period highlights a stark contrast between the treated and untreated samples.

- **Treated Fruits:** The antiseptic treatment with acidic water and Arabic gum was highly effective, as no microbial load was detected throughout the entire 4-week period. Both bacteria and fungi were absent in the treated fruits, and visually, the dates remained fresh with no signs of spoilage up to week 3. By week 4, there was only minor surface damage, which indicates a high level of preservation. The treatment effectively inhibited microbial growth and helped maintain the visual quality of the fruits.
- **Untreated Fruits:** On the other hand, the untreated dates exhibited significant microbial growth and spoilage over time. At week 1, the microbial load was relatively low (2.5×10^2 CFU/g), with slight discoloration of the fruits. However, by week 2, the microbial load increased to 4.0×10^3 CFU/g, and visible spoilage began, accompanied by the detection of bacteria like *Escherichia coli* and *Listeria monocytogenes*, and fungi such as *Aspergillus* and *Rhizopus*. By week 3, the microbial load had grown substantially (6.5×10^5 CFU/g), with significant spoilage and mold becoming apparent. By week 4, the untreated fruits had a microbial load of 8.0×10^6 CFU/g, with severe spoilage, extensive mold growth, and rot. The presence of additional bacteria like *Staphylococcus aureus* and fungi like *Penicillium* further underscores the advanced stage of decay.

The findings demonstrate the effectiveness of the antiseptic treatment in preserving date fruits during storage, preventing microbial contamination, and maintaining their quality for up to 4 weeks. In contrast, the untreated dates showed progressive spoilage and microbial growth, leading to significant decay by the end of the storage period. The results highlight the importance of preservation techniques in extending the shelf life of perishable fruits and maintaining their safety and quality during storage and handling.

Table 3: Microbiological Analysis of Date Fruit during Storage and Preservation (4 weeks)

Time (Weeks)	Sample Type	Microbial Load (CFU/g)	Bacteria Identified	Fungi Identified	Visual Observation
Week 1	Treated Fruits	0	None Detected	None Detected	Fresh, no spoilage
	Untreated Fruits	2.5×10^2	*Escherichia coli*, *Salmonella*	*Aspergillus*, *Penicillium*	Slight discoloration
Week 2	Treated Fruits	0	None Detected	None Detected	Fresh, no spoilage
	Untreated Fruits	4.0×10^3	*Escherichia coli*, *Listeria monocytogenes*	*Aspergillus*, *Rhizopus*	Visible spoilage starts
Week 3	Treated Fruits	0	None Detected	None Detected	Fresh, no spoilage
	Untreated Fruits	6.5×10^5	*Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*	*Aspergillus*, *Rhizopus*	Significant spoilage and mold
	Treated Fruits	0	None Detected	None Detected	Slight surface damage

Time (Weeks)	Sample Type	Microbial Load (CFU/g)	Bacteria Identified	Fungi Identified	Visual Observation
Week 4	Untreated Fruits	8.0×10^6	*Escherichia coli*, *Salmonella*, *Staphylococcus aureus*	*Aspergillus*, *Rhizopus*, *Penicillium*	Severe spoilage and rot

Conclusion

The study demonstrates the effectiveness of using an antiseptic material composed of acidic water mixed with Arabic gum in preserving perishable fruits such as oranges and dates during storage. The treated fruits consistently exhibited no detectable microbial growth (both bacterial and fungal) and maintained their freshness and quality over extended storage periods (6 weeks for oranges and 4 weeks for dates). In contrast, untreated fruits showed progressive microbial contamination, with significant increases in bacterial and fungal loads, leading to visible spoilage, mold growth, and severe degradation over time.

The results clearly indicate that the antiseptic treatment provided by acidic water and Arabic gum acts as a highly effective preservative, creating a protective barrier that inhibits microbial growth and delays spoilage. The treated fruits remained

visually fresh with minimal surface damage, while the untreated fruits deteriorated rapidly, experiencing extensive spoilage and rot by the end of the storage period.

This study highlights the potential of using such natural and biocompatible antiseptic agents in food preservation, which can significantly extend the shelf life of fruits and other perishable products. This preservation technique can be particularly valuable for industries focused on food safety, storage, and transportation, ensuring that fruits remain fresh and safe for consumption for a longer duration. Further research could explore optimizing the concentrations and application methods to enhance the preservation effect and adapt it to various other perishable food products.

Recommendations for Food Application:

1. **Consider Use in Packaging Systems**: Incorporate the use of antiseptic materials into modified atmosphere packaging (MAP) systems. The combined approach of natural preservatives with advanced packaging techniques can provide a more comprehensive preservation strategy, further delaying spoilage and enhancing product quality during extended storage periods.
2. **Expand Applications to Other Perishable Food Products**: Given the success of the treatment on fruits such as oranges and dates, similar methods could be tested and applied to other perishable foods like vegetables, berries, and even meat products. Expanding the scope of application can offer food

industries more options to reduce spoilage and improve food safety across various food categories.

3. **Ensure Compliance with Food Safety Standards**: Before widespread adoption, ensure that the use of these natural preservatives complies with relevant food safety regulations and standards. Conduct safety and toxicity assessments on a variety of foods to confirm that the treatments do not negatively affect the sensory attributes (taste, texture, color) or nutritional value of the food products.
4. **Promote Environmentally-Friendly Preservation**: Encourage the food industry to adopt this preservation method as an environmentally-friendly alternative to synthetic chemical preservatives. Acidic water and Arabic gum are biodegradable and safe for human consumption, making them a sustainable option that aligns with growing consumer demand for natural and eco-friendly food preservation solutions.
5. **Research on Long-Term Effects and Shelf Life**: Further research is recommended to study the long-term effects of this preservation method beyond the 4-6 week period. Evaluating the potential of these preservatives for longer storage durations can open up new possibilities for export markets where food products often undergo long transportation times.