

Physicochemical Characterization and Preparation of Bacteriological Media Using Coconut Water and Milk

Shilpa S. Ruikar^{*1}, Avadhut Thorat¹, G. R. Pathade¹

¹Krishna Institute of Allied Sciences, Krishna Vishwa Vidyapeeth (Deemed To be University)(Formerly known as Krishna Institute of Medical Sciences, (Deemed To Be University)Malkapur, karad. 415539, MS (India)

Corresponding Author:

Shilpa S. Ruikar

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ABSTRACT

Coconut water and coconut milk have gained attention as promising alternatives for culture media preparation due to their nutrient-rich composition, offering a sustainable and cost-effective substitute for traditional microbial culture media ingredients. These natural substances support the growth of a variety of microorganisms, including bacteria, fungi, and yeast. Their ability to enhance microbial growth makes them valuable in microbiology and biotechnology. This study aimed to explore the physicochemical properties of coconut water and coconut milk, crucial for their application in culture media. The study also included the formulation of nutrient media using coconut water and coconut milk, which was subsequently evaluated for bacterial growth. The analysis covered parameters such as pH, temperature, total dissolved solids, and nutrient content. Coconut water exhibited a pH range between 4.5 and 5.5, while coconut milk had a higher pH range of 6.5 to 7.5, both suitable for microbial growth. Their nutrient content, including sugars, protein content, and fat content, also indicated strong potential for supporting microbial growth. The results of this study highlight coconut water and coconut milk as viable candidates for use in microbiological culture media. Their physicochemical properties not only facilitate microbial proliferation but also offer flexibility in media formulation. This research opens the door for further exploration of coconut-based culture media, offering a sustainable solution in microbiology with potential applications across different microorganisms

Keywords: Coconut water, Coconut Milk, Bacteriological Media, Nutrient Media, Physicochemical analysis.

1. INTRODUCTION

The coconut tree (*Cocos nucifera*) belongs to the family Arecaceae and is the sole species within the genus *Cocos*. Commonly referred to as "coconut," the term can describe the entire tree, the seed, or the fruit, which is botanically classified as a drupe, not a true nut. Coconut trees are widespread in coastal tropical regions and hold cultural significance in many tropical societies.

The coconut tree is highly valued for its diverse applications, providing food, fuel, cosmetics, traditional medicine, and building materials. The edible inner flesh of the mature coconut, along with the coconut milk derived from it, is a staple food source in many tropical and subtropical regions. One of the distinctive features of coconuts is their endosperm, which contains a large volume of nearly clear liquid, known as coconut water. This liquid has numerous culinary and hydration uses. Mature coconuts can serve as edible seeds, or they can be processed for coconut oil and plant-based milk. The hard shell is used to produce charcoal, while the fibrous husk yields coir, which is employed in making various products. Dried coconut flesh, referred to as copra, is a key source of oil and milk, commonly used in cooking, especially frying, as well as in the production of soaps and cosmetics. Additionally, the sap from coconut flowers can be processed into beverages, palm wine, or coconut vinegar. The hard shells, husks, and long leaves are also utilized for crafting furniture and decorative items. Coconuts also possess significant cultural and religious importance in certain societies. (www.wikipedia.org)

Coconut water is a natural, non-fermented beverage extracted from the liquid portion of the coconut fruit (*Cocos nucifera* L.) through a specific technological process (Brasil, 2020)

Table 1 : Nutritional Composition of Coconut water (Tuyekaeet al. ,2021)

Composition	MatureCoconut water	TenderCoconut water
Totalsolids	5.4	6.5
Reducingsugar	0.2	44
Minerals	0.5	0.6
Protein	0.1	0.01
Fat(%)	0.1	0.01
Acidity(mg%)	60	120
PH(%)	5.2	4.5
Pottasium(mg%)	247	290
Sodium(mg%)	48	42
Calcium(mg%)	40	44
Magnesium(mg%)	15	10
Iron(mg%)	79	106
Copper(mg%)	26	26
Phosphorous(mg%)	6.3	9.2

Table 2. Nutritional composition of coconut milk(

Composition	Coconutmilk
Calories	445
Fats(grams)	48
Carbohydrates(grams)	6
Protein(grams)	5
Vitaminsandminerals:	
Vitamins (mg)	5.5
Iron(mg)	7.5
Magnesium(mg)	89
Potassium(mg)	631
Copper(mg)	0.6
Maganese(mg)	2.2
Selenium(mcg)	14.9

Coconut wter and milk are rich in macromolecules microolecules, minerals vitamins, water content (Seow& Gwee,1997), it can serve as a nutrient medium for the cultivation of microbes. Bacteriological culture media are critical in the enrichment, isolation, and cultivation of bacteria for microbiological studies. While commercially available nutrient media are widely used, their high cost poses challenges for researchers, particularly students and laboratories working with limited resources. Given the increasing demand for culture media in academic and research settings, there is a pressing need for the design and

development of cost-effective and sustainable alternatives. Such innovations would enable broader access to essential microbiological tools without compromising the quality and effectiveness of bacterial growth and analysis (Ruikar *et al.*, 2023). The study was aimed at preparation and evaluation of nutrient medium for the use of cultivation of microorganisms.

2. MATERIALS AND METHODS. :

2.1 Collection and preparation of samples. : Coconuts were collected from mature coconut trees and handled under hygienic conditions. The outer husk was removed, and the coconuts were carefully cracked open to obtain the coconut water, which was extracted and filtered to remove any debris.

To prepare the coconut milk, the coconut meat was separated from the shell, washed thoroughly, and grated. The grated coconut was then mixed with warm water in a specific ratio, and the mixture was subjected to mechanical pressing to extract the coconut milk. The obtained milk was filtered to ensure a smooth and uniform consistency, and subjected to physicochemical analysis.

2.2 Study of Physicochemical Properties of coconut water and Coconut Milk :

The following tests were conducted on both coconut water and coconut milk samples: color and odor were assessed visually and organoleptically, respectively, while temperature was measured using a calibrated thermometer. The pH of the samples was determined using a pH meter (HANNA made). Total dissolved solids (TDS) were quantified by the gravimetric method, (Chellapandi, 2007). Protein content was analyzed using the Folin-Lowry method (Jayaraman&Jayaraman, 1981), and sugar content was measured with a refractometer to obtain the Brix value, indicating the concentration of soluble sugars.

2.3Preparation of nutrient Medium Using Coconut water and Coconut Milk :

2.3.1 Preparation of Coconut Water Medium (CWA): (Deshmukh,2001)

Fresh coconut water was collected into a sterile flask. The sample was diluted by adding distilled water to adjust the concentration. The pH was brought to 7 using an appropriate buffering agent. To solidify the medium, 2.5% agar powder was added. The prepared medium was then sterilized in an autoclave at 121°C for 15 minutes. After sterilization, the medium was poured into sterile Petri plates, which were labeled as "Coconut Water Agar" (CWA).

2.3.2 Preparation of Coconut Milk Medium (CMA): (Deshmukh,2001)

Fresh coconut milk was prepared and collected in a sterile flask. The sample was diluted with distilled water to the desired concentration. The pH was adjusted to 7 using a buffering agent. Similarly, 2.5 % agar powder was added as a solidifying agent. The medium was sterilized by autoclaving at 121°C for 15 minutes, and then poured into sterile Petri plates, which were labeled as "Coconut Milk Agar" (CMA).

2.4 Evaluation of Coconut Water Medium (CWA) and Coconut Milk Medium (CMA) for BacterialGrowth Support:

The following commonly used laboratory strains of organisms were selected for the study:

1. *Bacillus subtilis*
2. *Escherichia coli*
3. *Micrococcus luteus*
4. *Pseudomonas aeruginosa*
5. *Staphylococcus aureus*
6. *Serratia marcescens*
7. *Micrococcus roseus*

A loopful suspension of 18-hour-old cultures of the test organisms was streak inoculated on both the coconut water agar (CWA) and coconut milk agar (CMA) using the four-quadrant streaking method. As a control, the same cultures were inoculated on nutrient agar plates. All media, including CWA, CMA, and the control nutrient agar plates, were incubated at 37°C for 24 to 48 hours.(Ruikar *et al.*, 2023)

After incubation, the growth of the organisms on the coconut water and coconut milk media plates was observed and compared with the growth on the control nutrient agar medium. The growth pattern of the organisms on the plates was evaluated using the following point scale (Table 3.)

Table 3 : Growth Evaluation of Microorganisms on Coconut Water Agar (CWA) and Coconut Milk Agar (CMA) Using Point Scale

Growth Pattern	Point Scale
Growth observed only in 1st Quadrant	1
Growth observed only in 2nd Quadrant	2
Growth observed only in 3rd Quadrant	3
Growth observed only in 4th Quadrant	4

The results were recorded based on this evaluation scale.

3. RESULTS AND DISCUSSION :

Table 4 :PhysicochemicalProperties of Coconut Milk and Coconut Water

Attribute	Coconut Water	Coconut Milk
Color	Pale white	White
Odour	Off odour	caramelized aroma
Flavour	Sweet	creamy
pH	5.5 (0.45)	6.5 (0.5)
Temperature	32°C	30°C
Sugar	1%	0.5%
Total Solid	3.1 g.	4 g.

It can be seen from table no 4 that Coconut water was found to be having pale white colour, and sweet taste, pH of the coconut water was 5.5, the Sugar content was found to be 1% and Total solids content was 3.1 g. . Adubofuor et al, (2016) found the pH of the pasteurized preferred green coconut water 4.84(0.01) . Coconut milk was white in colour with caramelized aroma and creamy flavor. The pH of the coconut milk was found to be 6.5 ,Sugar content was 0.5% and total dissolved solids were 4g.

Table 5 : Growth Evaluation of Microorganisms on Coconut Water Agar (CWA) and Coconut Milk Agar (CMA) Using Point Scale

sr.no	Name of Test Bacteria	Growth on CWA on 4 point scale	Pigmentation on 4 point scale	Growth on coconut milk media on 4 point scale	Pigmentation on 4 point scale	Growth on nutrient media on 4 point scale	Pigmentation on 4 point scale
1.	<i>Bacillus subtilis</i>	3	-	1	-	4	-
2.	<i>Escherichia coli</i>	4	-	4	-	4	-
3.	<i>Micrococcus luteus</i>	4	-	1	-	4	-

4.	<i>Pseudomonas aeruginosa</i>	4	Greenish	2	Greenish h	4	Greenish Violet
5.	<i>Staphylococcus aureus</i>	3	-	2	-	3	-
6.	<i>Serratia marcescens</i>	4	Red	4	Red	4	Red
7.	<i>Micrococcus roseus</i>	2	Red	4	Red	4	Red
				4	Red		

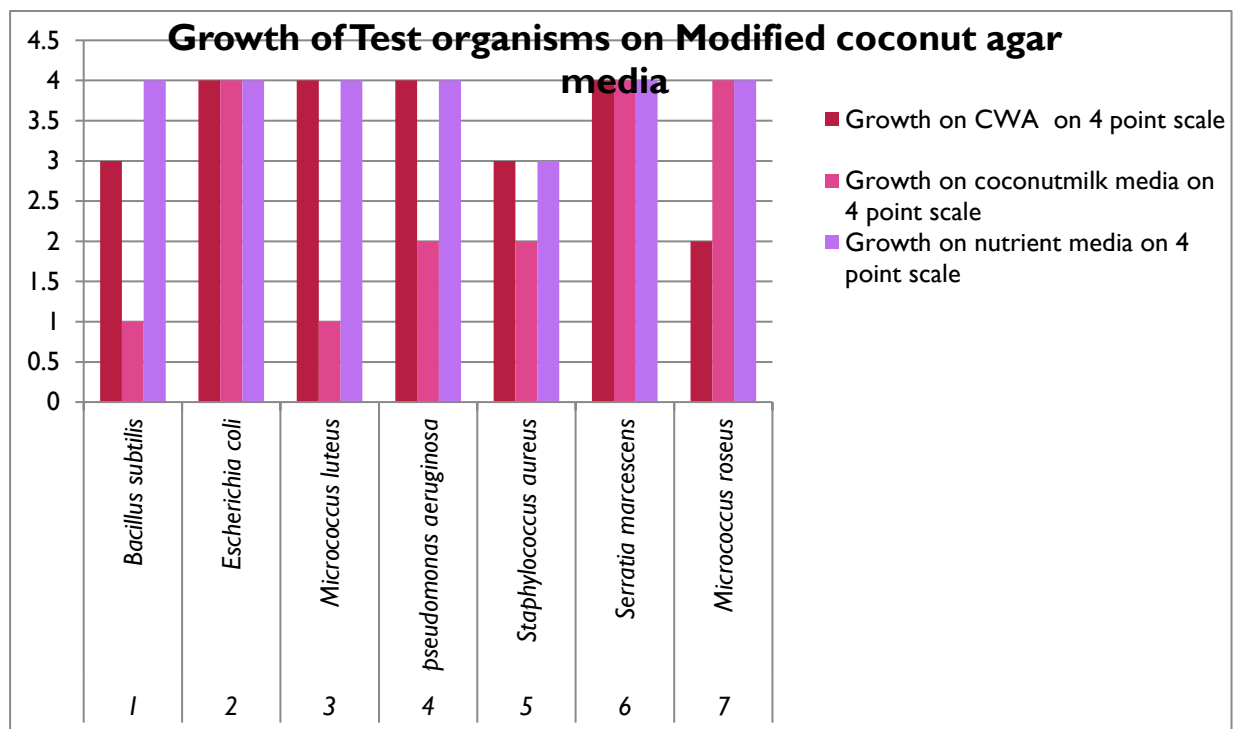
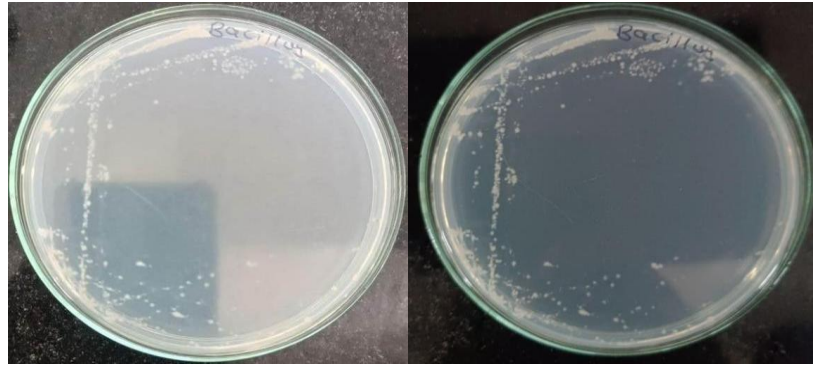


Fig. 1. Growth of Test organisms on Modified coconut agar media

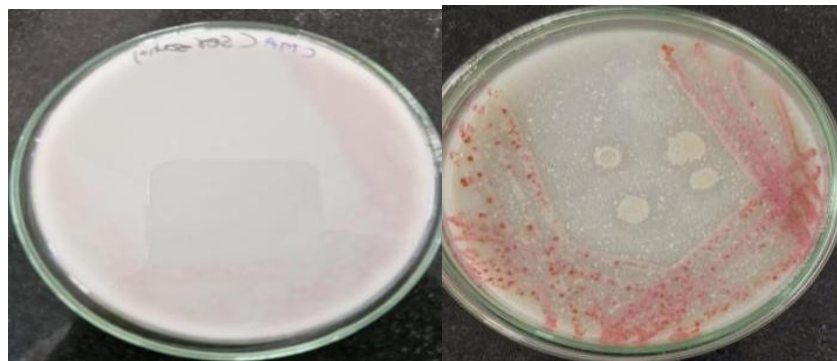
The growth of various bacteria was assessed on coconut water agar (CWA), coconut milk media, and nutrient media using a 4-point scale, with their pigmentation noted. It can be seen from the Table 5 and Fig 1. *Serratia marcescens*, *Escherichia coli*, *Micrococcus luteus* and *Pseudomonas* showed robust growth on CWA, and *Micrococcus roseus*, *E. coli* and *Serratia marcescens* showed Robust growth on Coconut Milk agar. *Pseudomonas aeruginosa* exhibited distinctive greenish pigmentation on Coconut water and coconut milk agar, and violet pigmentation on nutrient media. *Serratia marcescens* and *Micrococcus roseus* demonstrated consistent red pigmentation across all media types, though *Micrococcus roseus* grew better on coconut milk agar.

The use of fruit and vegetable waste as nutrient sources for bacterial culture media, as seen in the work, provides a parallel to the use of coconut water and milk. Both coconut-based media and waste-derived media are grounded in the concept of utilizing readily available, inexpensive raw materials. The study on fruit and vegetable waste emphasizes cost reduction in media formulation, which is a critical advantage of coconut water and milk. Coconut milk, with its rich content of vitamins, minerals, and growth-promoting substances, can be seen as an efficient nutrient base similar to other organic waste. (Jadhav *et al.*, 2018)



Photoplate 1. : Growth of *Bacillus* on Coconut water agar and Nutrient agar

The research by Shantz and Steward (1952) and Caplin and Steward (1948) on coconut milk revealed its growth-promoting properties, particularly due to the presence of cytokinins, auxins, and other plant hormones. These substances, collectively referred to as "coconut milk factor," have been shown to stimulate cellular growth in plant explants. Their ability to promote growth in eukaryotic cells suggests that coconut milk could also enhance the growth of prokaryotic cells, making it a promising component in bacterial media. The growth-promoting effect of coconut milk is largely attributed to its rich composition, which includes sugars, lipids, amino acids, and vitamins, all of which are vital for bacterial metabolism and growth.



Photoplate2 : Growth of *Serratia marcescens* e on Coconut Milk agar and Coconut water agar

The use of temple waste for microbiological media preparation is discussed. This approach mirrors the use of coconut coir and milk, where agricultural and organic waste is repurposed for scientific use. These efforts are aligned with global sustainability goals, as they minimize waste while reducing the reliance on expensive, synthetic media. (Gurav and Pathade, 2011), Coconut coir and coconut water, both byproducts of the coconut industry, can be integrated into similar eco-friendly media formulation strategies.

The study by Nur and Irawan (2015) on the utilization of coconut milk skim effluent (CMSE) as a growth medium for *Spirulina platensis* offers further evidence of the nutritional adequacy of coconut byproducts for microbial cultivation. CMSE is a waste product from coconut milk processing, yet it contains residual nutrients that support the growth of microorganisms. The research shows that CMSE can be optimized for microbial cultures, offering a low-cost, nutrient-rich medium for specific organisms. This finding underscores the versatility of coconut-based media, especially when considering the nutritional diversity of different coconut components, such as milk, water, and coir.

While coconut-based media present several advantages, there are also challenges to be addressed. For instance, ensuring consistency in nutrient composition can be difficult, as the nutrient content of coconut milk and water can vary depending on the source and processing method. Moreover, certain microorganisms may require specific supplements or growth factors that are not adequately provided by coconut alone. Future research could explore the supplementation of coconut-based media with additional nutrients or growth factors to enhance its utility for a broader range of microorganisms.

4. CONCLUSION

The formulation of bacterial growth media using coconut milk, water, and coir provides a sustainable and cost-effective alternative to conventional media. The growth-promoting properties of coconut milk, coupled with the eco-friendly

utilization of coconut milk and water, make these materials valuable resources in microbiological research. Further studies could refine these formulations to maximize their efficiency, making coconut-based media a mainstream option in laboratory settings.

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Conflicts of Interests

The authors declare that there are no conflicts of interests

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