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Impact of endophytic microorganisms on the spoilage of freshly cut muskmelon

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Abstract
At present in India, available technologies offer hardly 4-5 days shelf life in most of the fresh cut fruits, even at refrigerated storage. The recent developments and understanding of the microbiome provide the opportunity to explore new perspectives in the post-harvest biocontrol of fruit and vegetables. The quality maintenance of perishables must be prioritized but is a difficult task. It is crucial to minimize post-harvest losses in an eco-friendly manner. Therefore, endophytic study concisely addresses the importance of fruit residing beneficial microbiome to maintain and extend the post-harvest life of fruit comprising their ecological requirements. The isolates obtained during storage were screened for their enzymatic properties. The data revealed that amylase, cellulose, lipase (esterase) activity was present in majority of the isolates, indicating the role of endophytic fruit microbiome in fruit ripening and senescence changes

Keywords: Epiphytes, endophytes, LAF, pectinolysers, amylase, APC, LAB

Introduction
Intake of fruits and vegetables has been linked with various health benefits. Fruits and vegetables can be consumed either fresh or processed. However, most of the processed foods have less nutritional quality compared to the fresh fruits. Minimal processing is a way to obtain ready- to- eat packed fruits with fresh like characteristics. Production and consumption of minimally processed foods is gaining popularity. Fresh-cut fruits and vegetables are being welcomed by the consumers due to the desire for new and natural products coupled with change in life style of the consumers. However, challenge for fresh-cut industry is to maintain fresh like characteristics of fresh-cut produce for a prolonged storage time (Yousuf *et al.*, 2018) [7].

Fruits host a variety of organisms, including epiphytes and endophytes. While surface washing and peeling operations can manage epiphytes, endophytes pose challenges by affecting the shelf life of cut fruits. Their growth during storage can increase the microbial load, impacting the microbiological safety and quality of the product. This study aimed to deepen understanding of microbial changes in fresh-cut fruits during storage, with musk melon chosen as the model fruit due to its thick rind, which allows effective removal of epiphytic microbes through deep peeling. Kuruppu *et al.* (2024) [1].

Recent advancements in microbiome research present new opportunities for innovative approaches to post-harvest biocontrol of fruits and vegetables. Droby and Wisniewski (2018) [2] highlighted the potential of studying the epiphytic and endophytic microbiomes of fruits as a frontier in post-harvest biocontrol strategies. Ensuring the quality of perishable produce remains a priority, albeit a challenging one, particularly in minimizing post-harvest losses through eco-friendly methods. This review emphasizes the significance of beneficial fruit-residing microbiomes in extending post-harvest shelf life. It addresses their ecological requirements, modes of action, and the role of omics technologies in identifying the fruit holobiont to develop comprehensive biological solutions.

Given the importance, market demand, and the need for suitable minimal processing technologies for fruits, the present study focused on optimizing protocols and investigating the role of endophytic microorganisms in the spoilage of minimally processed musk melon.

Treatment details

Muskmelon fruits were cut in two different process hygiene conditions which includes, non-sterile and sterile

Table 1: Treatment, hygiene, environment, pretreatment, and packaging methods.

Treatments	Process hygiene environment	Pretreatment	Packaging
T_1	Nonsterile (Laboratory bench)	None (control)	PP bag
T_2	Nonsterile (Laboratory bench)	SO_2 pads	PP bag
T_3	Sterile (Laminar air flow cabinet)	None (control)	PP bag
T_4	Sterile (Laminar air flow cabinet)	SO_2 pads	PP bag

Identification of the microbial proliferation pattern at different process hygiene conditions

The different microbiological indicators namely APC, yeasts and molds, Lactic acid bacteria were enumerated on 3rd, 5th and 7th day, which marked the beginning of visual quality spoilage even in the best treatment (SO_2 and PP bags).

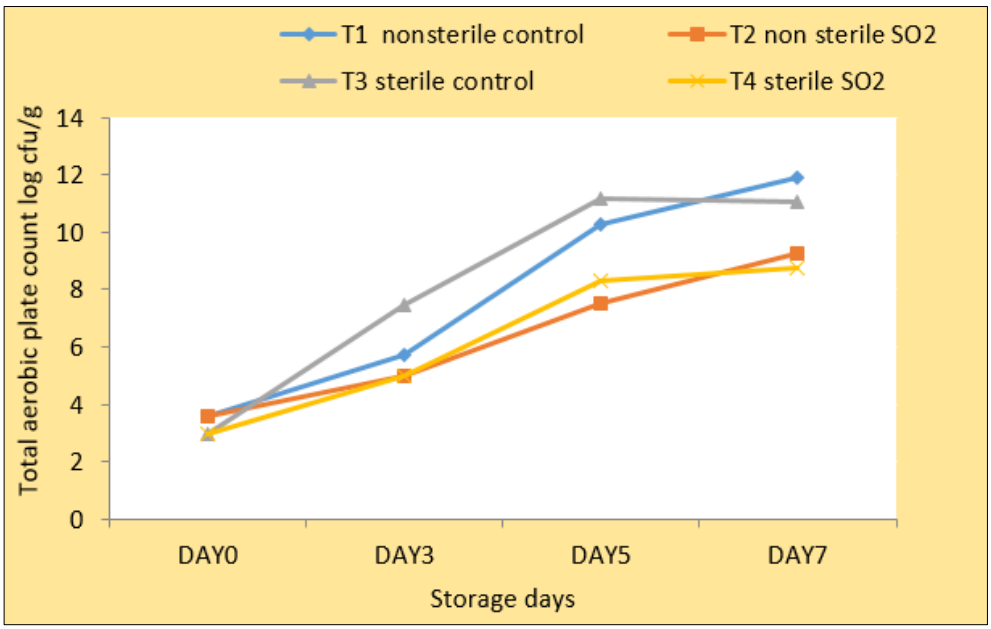
Representative colonies were picked, purified and maintained in the laboratory as slants. 100 such isolates were maintained.

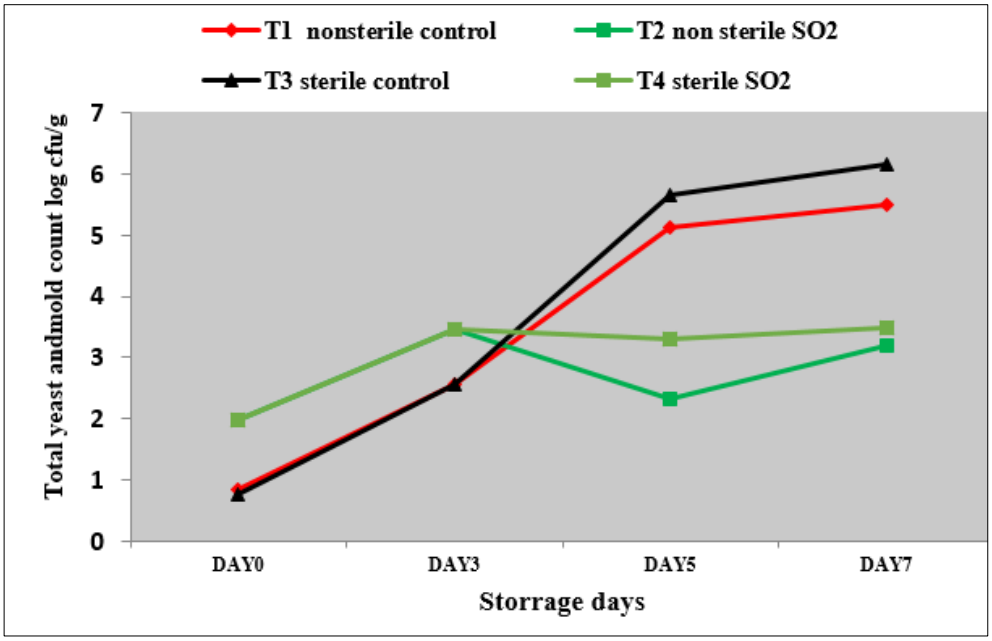
Microbiological quality was assessed by enumerating total aerobes (plate count agar), yeast and mold (PDA, YEPD agar) and coliforms (violet red bile agar) by standard plating method.

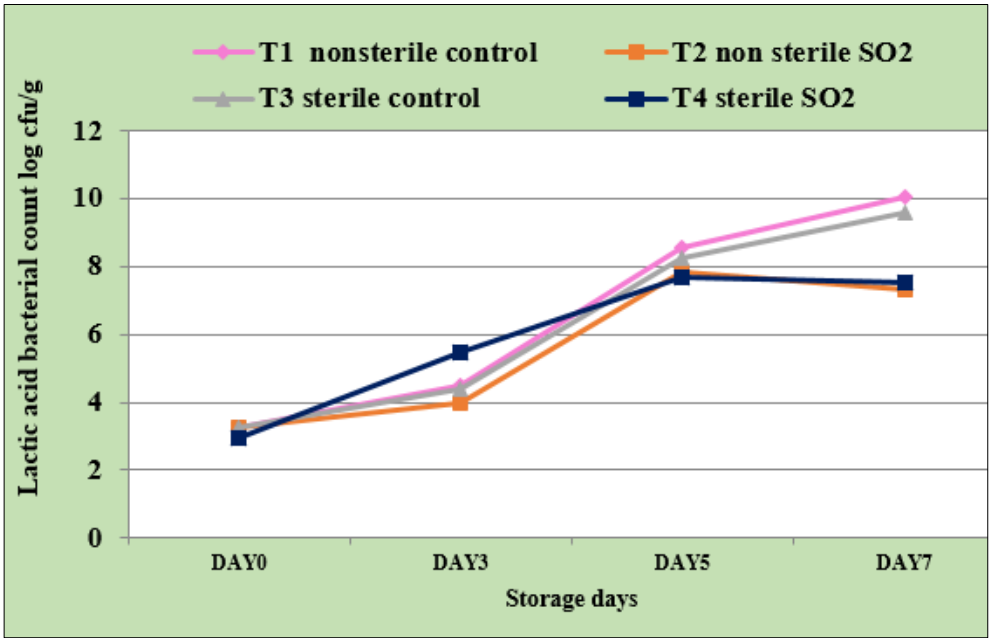
Isolation of endophytic microbes & their enzymatic properties related to fruit ripening (amylase, cellulose esterase and pectinase)

The isolates were then screened for their exogeneous enzyme activities *viz*., cellulase amylase, pectinase and lipase (esterase) using suitable media *viz*. cellulose agar, starch agar, pectolytic agar and lipolytic agar respectively. The clear zone formed due to the breakdown of cellulose starch, pectin and lipids were observed, and the isolates were scored as positive or negative (+/-)

Fig 1: Enzymatic activities of culturable endophytes obtained from ripe muskmelon fruits







Results and discussion

Identification of the microbial proliferation pattern at different process hygiene conditions in minimally processed muskmelon during storage at 5 ºC. Presented in Fig. 31 show the total aerobic plate count (log cfu/g), yeast and mold (log cfu/g), Lactic acid bacterial Growth pattern. On days 0 and 3, there was no significant difference in growth of Total aerobic plate count, yeast and mold and LAB. However, after 5 days of storage, a significant difference in growth was noticed among the treatments. The highest Total aerobic plate count growth was observed in T_1 (11.20 log cfu/g) on par with T_3 (10.29 log cfu/g), whereas the lowest growth was exhibited in T_2 (7.54 log cfu/g). The same trend was continued on day 7 also, the highest count found in T_1 (11.90 log cfu/g) and lowest growth observed in SO_2 treatment T_4: 8.75 log cfu/g and T_2: 9.25 log cfu/g). In case of Yeast and mold the highest count was observed in sterile control (T_4: 4.55 and 6.14 log cfu/g); whereas the lowest growth was noticed in non-sterile T_5 (2.34 and3.2 log cfu/g), and In case of lactic acid bacteria the highest growth was observed in T_1 (3.94 log cfu/g), whereas the lowest growth was exhibited in T_5 (2.36 log cfu/g), observed after 7 days of storage. Among all the treatment, a reduction in microbial growth was observed in SO_2 treatment without influence of different process hygiene includes sterile and non-sterile. This might be attributed to SO_2 and its derivatives can inhibit key enzymes in microbial cells by binding to their functional groups, such as sulfhydryl groups in proteins. This prevents the enzymes from catalyzing essential biochemical reactions, leading to metabolic disruptions and cell death (de Aguiar *et al.*, 2023) [6]. Additionally, (Ahmed *et al.*, 2018) [5] reported SO_2 can alter the permeability of microbial cell membranes. This disrupts the transport of nutrients and waste products across the membrane, impairing cellular function and leading to cell death, also Sulphur dioxide can induce oxidative stress in microbial cells by generating reactive oxygen species (ROS). These ROS can damage cellular components, including lipids, proteins, and nucleic acids, leading to cell dysfunction and death. Similar findings were reported by Daniei *et al.* (2024) [4].

Table 2. Enzymatic activities of culturable endophytes obtained from ripe muskmelon fruits

	% Positives			
Microbe category	**Amylase**	**Cellulase**	**Esterase**	**Pectinase**
Yeasts	32	0	66	0
Bacteria	30	26	50	0
LAB	0	0	45	0

The isolates were then screened for their exogeneous enzyme activities *viz.*, cellulase amylase, pectinase and lipase (esterase) using suitable media. The clear zone formed due to the breakdown of cellulose starch, pectin and lipids were observed, and the isolates were scored as positive or negative (+/-), to represent the enzymatic activities of culturable endophytes obtained from muskmelon fruits **(**Table 2). One hundred such isolates were maintained and screened for their exogenous enzyme activities, including cellulase, amylase, pectinase, and lipase (esterase), using suitable media. Among the 100 isolates, 32% of the yeast isolates exhibited amylase activity and 66% possessed esterase activity; however, no cellulase or pectinase activity was observed from the yeast isolates. Among the Bacterial isolates, 30% showed amylase, 26% cellulase, and 50% esterase activities. Lactic acid bacterial isolates exhibited only esterase activity (45%). No pectinase activity was observed in any of the three types of microbes (yeasts, bacteria, and lactic acid bacteria).

The yeasts and bacteria showed significant amylase activity indicating their possible role in starch hydrolysis during fruit ripening. Majority of the isolates microbes also exhibited high esterase activity, indicating their role in aroma development as well. A recent study on fruit microbiome in raspberry established the correlation of certain bacterial genera in aroma contribution of the raspberry flavor (Sangiorgio *et al.*, 2022) [3]. Future work will aim at clarifying the mechanisms of interaction with the fruit, as well as the optimal conditions for the enhancement of raspberry aroma, safety and overall fruit quality. The absence of pectinase enzymes in the endophytic microbes is also intriguing. Pectin is the first line barrier preventing the weakening of the fruit tissues. The pectinase is produced only by the fruit tissues, showing that most the endophytes have a mutualistic relation with fruit tissue, helping in the fruit development process, and preventing the degradative changes such as middle lamella hydrolysis.

Conclusion

Endophytes within fruit tissues are likely contributors to further spoilage in fresh-cut fruit. Isolates obtained during storage were screened for their enzymatic properties, revealing that most displayed amylase, cellulase, and lipase (esterase) activity. This highlights the role of the endophytic fruit microbiome in ripening and senescence. Future studies on fruit microbiomes could facilitate the identification of beneficial microbes, enabling practical applications such as ripening modulation, biocontrol, and improved flavor development.

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