## Microbial Pathogens and Spoilage Mechanisms in Vegetable: A Review

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## ABSTRACT

The vegetables are an important constituent of human diet, providing essential vitamins, minerals and dietary-fibers. Retailers face challenges in managing the shelf life of perishable commodities such as fruits, vegetables, meat, and dairy products because of the effects of microbial spoilage. This review summarizes the recent knowledge about the kind of microorganisms responsible for vegetable spoilage (bacteria, fungi, yeasts), their ways of contamination and spoilage mechanisms. Intrinsic and extrinsic parameters that affect microbial growth are discussed, and how their neglect during postharvest handling and storage leads to rapid spoilage. Furthermore, this work discusses classical and novel technologies for spoilage prevention like refrigeration, modified atmosphere packaging, LED treatment, ozone, UV-C irradiation and natural antimicrobial coatings. Minimally processed or fresh-cut vegetables, which are gaining in popularity but are more susceptible to contamination from animal or human sources due to the damage of the tissue, also receive special consideration. It highlights the use of integrated approaches that combine hygiene education, increased handling protocols reduction and innovative preservation methods to prolong shelf-life and manifests food safety.

**Keywords:** Microbial Spoilage, Vegetables, Postharvest Loss, Bacteria, Fungi, Fresh-Cut Produce, Food Safety, Shelf Life, Preservation Techniques

1. Introduction: - Vegetables are an essential component in the human diet; they will provide a rich source of essential nutrients, including vitamins (e.g., A, C, K), minerals (e.g., potassium, magnesium), dietary fiber, and health-promoting phytochemicals (Slavin & Lloyd, 2012). They have a well-established association with lower risks of a number of chronic diseases, including heart disease, obesity, certain types of cancer, and digestive diseases (Aune et al., 2017). While old age and cigarette smoking are traditionally recognized as the most two important causes of cancers in general, obesity has emerged as an increasing cause that needs immediate attention in our struggle to prevent certain cancers (Lauby-Secretan et al., 2016). In light of global health trends favoring plant-based diets, vegetable intake has significantly increased across all ages and sociodemographic groups (Medina-Remón et al., 2018; Willett et al., 2019). This demand has driven the growth of agricultural production systems and created room for innovation in the packaging and distribution of fresh and minimally processed produce (Mir et al., 2018; FAO, 2020). But the increasing consumption of vegetable has also laid bare the vulnerabilities in postharvest handling and storage practices, especially the risk of microbial spoilage (Barth et al., 2009; Olaimat & Holley, 2012). Vegetables are excellent substrates for colonization and growth of microorganisms due to their high moisture content, nutrient richness, and nearneutral pH (Ray & Bhunia, 2013; Tauxe et al., 2010). Microbial spoilage of vegetables is caused mainly by different species of bacteria, fungi and yeasts that metabolize cellular components, which eventually leads to observable decay, off-odors, textural breakdown, and discoloration (Lund, 2008; Sharma & Tripathi, 2006). These spoilage organisms can be a source of soil, irrigation water, handling equipment, or the environment during harvesting, packaging, and retail stages (Beuchat, 2002; Allende & Monaghan, 2015). All these steps to preserve food is something that it couldn't be done without; because, according to some reports, we lose almost 40% of fresh produce simply because of microbial activity of food spoilage between the farm and consumer (FAO, 2011; Lipinski et al., 2013). Such losses do more than wreak economic havoc — eroding grower returns and driving up retail prices — they also carry social and environmental costs, helping to fuel food insecurity and wasting scarce resources (FAO, 2019). In regions without cold-chain infrastructure or sanitation standards, spoilage rates may be even higher. Microbial spoilage is not just a question of aesthetics or food waste from a public health perspective; it is a safety issue (WHO, 2015). Some spoilage fungi like Aspergillus and Fusarium are mycotoxin producers known for their resistance to typical cooking; therefore, their consumption can cause serious health consequences (Bennett & Klich, 2003). In addition, the consumption of contaminated or poorly preserved vegetables can cause gastrointestinal infections or exposure to pathogenic microbes especially in risk groups such as children and elderly people (Liu et al., 2015). These health hazards are increased in the case of fresh-cut or minimally processed vegetables, which have no protective outer layers and are susceptible to contamination arising from damage to the surface (Gil et al., 2009). Such challenges call for a

need to devise integrated strategies that tackle microbial spoilage through several dimensions (Alzamora et al., 2021). This involves recognising the most prevalent spoilage organisms, appreciating environmental and handling parameters that promote degradation, and employing conventional and novel control strategies for prolonging shelf-life and protecting the consumer (Vinderola et al., 2019; Jongen, 2007). Thus, this review presents recent findings from literature that together contribute to a broader view of vegetable spoilage by microorganisms (Mahapatra et al., 2020). Through understanding the microbial agents present in spoilage, the mechanisms of spoilage they trigger and what new technologies for preservation are being developed, we aim to assist in shaping best production, processing and distribution practices to combat spoilage and its effects (Gänzle & Hertwig, 2017; Wang et al., 2020).

2. Microorganisms Involved in Vegetable Spoilage: - The microbial spoilage of vegetables is mainly by a wide variety of microorganisms that attack and spoil plant tissues, leading to extensive loss of quality as well as economic loss (Lund et al., 2000). Some of the most wellknown and significant bacterial pathogens include soft rots caused by species from the genera Pseudomonas, Erwinia, Clostridium, and Bacillus, which can lead to tissue maceration and foul-smelling decay (Sharma & Tripathi, 2006). For example, Erwinia carotovora is well known to secrete enzymes that degrade pectin (causing a water-soaked and mushy texture seen in many vegetables including carrot and lettuce, (Swarup et al., 2007). Similarly, Pseudomonas spp. that can multiply at refrigeration temperatures and can lead to spoilage during cold storage (Jørgensen et al., 2011). Fungal spoilage is also common, especially in humid and warm environments, as they are propitious to the development of moulds and yeasts. Examples of vegetable spoilage-related fungal genera include Aspergillus, Penicillium, Rhizopus, Alternaria, and Fusarium (Sharma & Tripathi, 2006). They produce visible colonies and discolorations and commonly secrete secondary metabolites, including mycotoxins, which are harmful to human health (Sharma & Tripathi, 2006). Rhizopus stolonifer (Ehrenburg) Lind however is most commonly associated with soft rot in tomatoes and sweet potatoes, producing flocculent growth and black sporangia on the vegetable surface (Moussa et al., 2013). The Penicillium species specially P. digitatum, which is typically found on citrus fruits and produces blue-green spores, is associated with postharvest decay, and therefore is well studied (Akgül et al., 2010). Yeasts, less often emphasized, are significant opportunistic spoilage microorganisms that tend to grow on the cut surfaces of vegetables, most notably under moist or anaerobic storage circumstances (Müller et al., 2009). They are usually responsible for sliminess and fermentation-like odor in stored produce and are active especially in processed or ready-to-eat vegetable products (Silva et al., 2015). Minimally processed vegetables facilitate their growth due to the large amount of nutrients that are available from cellular fluids released from cutting or shredding the vegetables. Microbial contamination of vegetables has multiple origins, starting from the growing environment through the whole supply chain. Soil is a great reservoir of bacterial and fungal pathogens that attach to saturation surfaces or enter through the root system during growth (Gould, 2013). Microbial contaminants may be transferred to the edible parts of plants from irrigation water, especially if untreated or recycled (Beuchat, 2002). Contamination may occur at the harvesting stage, when damaged tissues become potential entry pathways for microbes, or during handling, washing, and packing as a result of poor sanitation or cross-contamination from tools and surfaces (Barrett et al., 2010). Even packaging materials can carry microbial flora that hasten spoilage in the postharvest environment if they are not adequately sanitized or stored (Kader, 2005). Regional studies give an idea about what type of spoilage microorganisms are present with respect to the climatic and handling conditions (Barth et al., 2009). In Nigeria, for example, studies have shown that a variety of spoilage fungi, such as Aspergillus, Rhizopus and Alternaria, are commonly found on markets due to improper handling and warm environmental conditions (Akinyosoye et al., 2011). In Qatar, 73 fungal isolates were identified from the most consumed fruits and vegetables in supermarkets, where *Penicillium* and *Rhizopus* were the most frequent genera, with the shelf-life and fungal load being dependent on the country from which the sample originated and not on price and market display (Al-Mugheed et al., 2014). India also reported a similar trend, with spoilage microbes like Erwinia, Cladosporium, Fusarium and Botrytis being widely isolated from fresh and stored produce; especially in areas with insufficient refrigeration and storage systems (Chakraborty & Meena, 2015). Such strategies will rely on knowledge of which spoilage organisms can be expected, how these microorganisms behave and how they may originate from the environment (Gram et al., 2002). This knowledge may serve for the planned interventions on farm, in-transit, or market level and will help in checking microbial growth and extending fresh and safe vegetables (Dodd et al., 2015). The microbial spoilage agents were categorized into three primary groups, as depicted in Figure 1. The proportional distribution was accounted for 40% (Bacteria), 45% (Mould), and 15% (Yeast). Table 1 accounted the various microbial species responsible for spoilage of vegetables in farm and post-harvest.

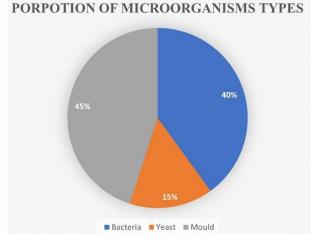


Figure 1: Distribution of Microbial Spoilage Agents in Vegetables (Sandhya et al., 2010).

	Microorganism	Vegetable spoilage	References
Bacteria	P. aeruginosa	Soft rot, biofilm formation on spinach and lettuce.	Zhang et al., 2021
	Erwinia persicina	Pink soft rot in onions and leafy greens.	Li et al., 2022
	Leuconostoc gelidum	Gas production and slime in vacuum- packed carrots and salads	Kim et al., 2021
	Clostridium estertheticum	Anaerobic spoilage of root vegetables in cold storage	Makela et al., 2023
	Lactic acid bacteria	Sour odor, color change, and softening in fermented vegetables	Ahn et al., 2022
	Bacillus cereus	Spoilage of packaged leafy greens with off-odor and slime	Mohamed et al.,2020
Mould	Botrytis cinerea	Gray mold on tomatoes, bell peppers, and leafy greens; soft, water-soaked lesions	Guo et al.,2022
	Alternaria alternata	Black spots on carrots, tomato, and cucumbers; tissue necrosis	Sharma et al.,2023
	Fusarium solani	White or pink rot in root vegetables (e.g., potatoes and carrots)	Chen et al.,2021
	Penicillium expansum	Blue-green mold, soft rotten stored vegetables (e.g., carrots, onions)	Zhang et al.,2020
	Rhizopus stolonifer	Rapid soft rot, watery breakdown in tomato and cucumbers	Lee et al.,2020
Yeast	Candida parapsilosis	Surface slime, fermentation odor in fresh cut carrots and tomato	Silva et al.,2021
	Debaryomyces hansenii	Spoilage in salty or brined vegetables (e.g.,olives ),causes off-flavors and surface film	Lin et al.,2023
	Pichia Kudriavzevii	Gas, production, bloating in packaged leafy vegetables and pickled products	Kumar et al.,2022

Table: 1 Microorganisms Involved in Vegetable Spoilage and Their Associated Effects.

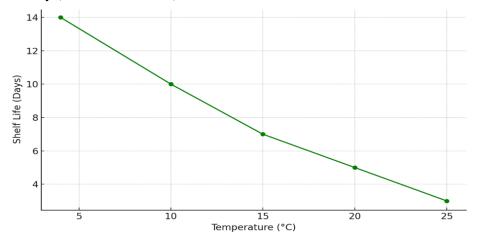
**3.** Mechanisms of Spoilage: Microbial spoilage of vegetables involves a variety of biochemical and physiological processes initiated by the growth and metabolism of spoilage microorganisms such as bacteria, yeasts, and moulds (Sinha et al., 2020). These organisms produce extracellular enzymes including cellulases, pectinases, and proteases that break down the plant's structural components—mainly cell walls and middle lamellae—resulting in tissue softening, liquefaction, and overall degradation of vegetable texture (Barth et al., 2009). In addition to enzymatic action, many spoilage microbes, particularly lactic acid bacteria and members of the Enterobacteriaceae family, ferment available sugars to produce gases such as

carbon dioxide, as well as organic acids and alcohols (Jay et al., 2005). This fermentation process leads to off-flavors, sour odors, and, in packaged products, package bloating (Silva & Gibbs, 2012). Another common spoilage sign is the development of discoloration due to microbial pigment production or enzymatic browning, which makes vegetables visually unappealing (Barth et al., 2009). Additionally, some microorganisms, like Pseudomonas spp., are known for producing exopolysaccharides that result in slime formation on the surface of vegetables, particularly under high-moisture storage conditions (Santo et al., 2018). These spoilage mechanisms significantly reduce the shelf life, market value, and safety of vegetables, leading to both economic loss and potential health risks if consumed (Alvarez-Ordoñez et al., 2019).

- **3.1. Enzymatic Degradation:** Microorganisms produce extracellular enzymes like cellulases, pectinases, and proteases that break down plant cell walls, leading to softening, tissue breakdown, and liquefaction of vegetable tissues.
- **3.2. Fermentation and Gas Production:** Some bacteria, particularly lactic acid bacteria and coliforms, ferment sugars present in vegetables, producing gases like CO<sub>2</sub> and organic acids. This results in swelling of packaging, off-flavors, and sour odors (Parvez et al., 2013).
- **3.3. Pigment and Odor Formation:** Spoilage microbes may produce pigments (e.g., black, green, or pink discolorations) and volatile organic compounds (VOCs), leading to off-color and foul odor that make vegetables unpalatable and unacceptable for consumption (Lund, 2008).
- **3.4. Slime Production:** Certain spoilage bacteria, such as *Pseudomonas* spp., secrete exopolysaccharides, creating a slimy layer on vegetable surfaces, especially in high-moisture environments. This is common in leafy greens and pre-cut vegetables (Martínez-Romero et al., 2005)
- 4. Factors Influencing Spoilage and Spoilage Mechanisms: Vegetable spoilage is regulated by multifaceted intrinsic and extrinsic conditions creating an environment that is conducive or non-conducive for microbial growth. These factors are classified as intrinsic (individual to the vegetable) or extrinsic (related to its surroundings) (Ray & Bhunia, 2013). Intrinsic factors include the moisture content, pH, and availability of nutrients. These high-water activities make vegetables a suitable environment for several microorganisms, particularly fungi, and bacteria (Beuchat, 2002). A pH that is neither too acidic nor too alkaline, common in many vegetables, especially leafy greens and root crops, also favourably conditions the growth and proliferation of spoilage (E.g., Pseudomonas and Erwinia) organisms (such as Pseudomonas and Erwinia (Bari & Inatsu, 2013). Spoilage microbes use the carbohydrate and other soluble nutrients abundant in vegetables as an energy source, which appears to speed up the spoilage process once contamination has taken place (Barth et al., 2009). Other extrinsic factors such as temperature, humidity, storage conditions and packaging methods are also important determinants of the rate and extent of spoilage (Chong et al., 2019). Microbial proliferation

occurs quickly at high ambient temperatures and humidity levels, especially in tropical and subtropical regions with little or no cold-chain logistic availability (Beuchat, 2002). The reason for this is that suboptimal storage conditions (excess storage, contamination surfaces, poor ventilation, etc.) promote the formation of anaerobic microenvironments that facilitate the growth of facultative and obligate anaerobes such as Clostridium (Kader, 2005). If not matched to the respiration patterns of the vegetable to be packed, this could result in creating the conditions for mould development by increasing condensation or internal humidity in the packaging (Parth et al., 2009). Minimal processing as well as the increased consumption of fresh-cut products is a critical issue in contemporary vegetable processing (Kader, 2008). These types of processing include physical damage to plant tissues causing the release of intracellular fluids, peeling, slicing, shredding, or trimming, which increase the vulnerability of the surfaces to microbial invasion (Allende et al., 2006). The injured tissues not only become more nutritive than normal tissue, but also lose their natural defence barriers, making them prey for the colonization by bacteria, yeasts, and moulds (Francis et al., 2012). Also, fresh-cut vegetables skip thermal processing steps and are kept at low temperatures and subjected to mild sanitizations, which is not always enough to inhibit microorganisms. The symptoms of spoilage depend on the type of vegetable and the responsible microorganism (Chaves-López et al., 2015). A typical manifestation involves soft rot, caused by Erwinia species, which causes a mushy, water-soaked appearance and heads up with the malodor (Barth et al., 2009). Gray mould, which is most commonly caused by Botrytis cinerea, develop fuzzy grey spores on grapes, tomatoes, and other soft fruits under humid storage conditions (Elad et al., 2007). Rhizopus soft rot, which is characterized by cottony growth with black sporangia, is often seen on sweet potatoes and tomatoes, whereas black mould rot caused by Aspergillus niger produces dark, powdery spores and is common in onions and similar bulbs (Pitt & Hocking, 2009). Both visual and olfactive determinants are visible quality losses that will lower marketability and consumer acceptance. Indeed, postharvest conditions are a major contributor to the acceleration of microbial spoilage (Wolfe et al., 2014). If harvested produce is not cooled immediately, temperature accumulation can occur, accelerating its respiration rate and accelerating the decaying process of plant tissues (Kader, 2005). Furthermore, mechanical injuries during transport or manipulation are concern in spoilage as they provide sites for pathogenic microorganisms to proliferate, breaking cell integrity (Barth et al., 2009). Even short-term storage can allow for considerable microbe growth, provided that ambient temperature and sufficient sanitization are not rigorously maintained, especially in developing world contexts with limited infrastructure (Beuchat, 2002). Understanding spoilage mechanisms is crucial to developing effective postharvest solutions. The modification of storage conditions, improvement of packaging technologies, and minimization of mechanical injury might slow

microbial growth and increase vegetable shelf-life, minimizing economic losses and enhancing food safety (Francis et al., 2012).



**Figure 2:** Effect of Storage Temperature on Vegetable Shelf Life (Sandhya et al., 2010). The Figure: 2 clearly shows the inverse relationship between temperature and vegetable shelf life. As temperature increases from 5°C to 25°C, shelf life decreases from ~14 days to ~4 days, which is scientifically accurate and expected due to faster microbial activity at higher temperatures.

5. Spoilage Control and Prevention Strategies: - The management of microbial spoilage in vegetables necessitates a multi-level approach that includes good hygiene practices and effective storage systems, technological innovations, and continued education at each level of the supply chain (Dodd et al., 2014). One of the strategies on which the foundations are laid is hygienic handling at harvesting, transportation and retail level (Durán et al., 2012). Significantly, initial microbial loads on vegetable surfaces are reduced when tool, containers and storage environments are kept clean and wash water is filtered (Beuchat, 1998). Washing treatments with chlorinated water or mild disinfectants can reduce surface contaminants even further; though their effectiveness depends on contact time, water quality, and the extent of physical damage of the produce (Gil et al., 2009). Methods of storage are crucial for interrupting the growth and development of microorganisms and maintaining vegetable quality (Kader & Rolle, 2004). The most popular technology is refrigeration, which inhibits the metabolic processes of both vegetable tissues and spoilage microbes (Kader, 2005). Another strategy, modified atmosphere packaging (MAP), involves modifying the level of oxygen (O2) and carbon dioxide (CO2) present in the packaging, which is also useful for preventing microbial growth and delaying senescence, especially in fresh-cut vegetables (Sandhya, 2010). Recent studies have also proven that light-emitting diodes (LEDs) can extend postharvest quality through the inhibition of microbial growth and modification of ethylene biosynthesis, both important factors affecting shelf life (Nguyen et al., 2020).) Various novel technologies applied independently have demonstrated efficacy against vegetable decay inducing microorganisms (Balta et al., 2020). Ozone treatment is a strong oxidizing agent that damages microbial cell

membranes and DNA to decrease bacterial and fungal loads on fresh produce, for instance (Vinas et al., 2008). Its use as a bactericide is appealing for the inactivation of surface microorganisms without chemical residues, making it a fit to minimize microbial load in freshcut vegetables (Zhao et al., 2012). Cold plasma is a novel non-thermal technique able to produce reactive species to sterilize surfaces and packaging materials without affecting the produce itself (Misra et al., 2016). Natural polymer-based edible coatings like chitosan, alginate, or protein isolates marginally retards vegetables respiration by forming semipermeable barriers on the surface of vegetables and limiting microbial penetration (Xie et al., 2018). In addition to physical treatments, preventive measures are equally paramount. Awareness about hygiene standards and spoilage prevention measures among farmers, handlers, and vendors is paramount, and education and training programs are critical in this context (Ghazali et al., 2014). Setting standardized postharvest practices (time-temperature management, packaging requirements, microbial testing conditions, etc.) can help harmonize practices along the supply chain, which in return can contribute to a decrease in spoilage (Sani et al., 2018). In addition, further studies on spoilage mechanisms, microbial resistance, and sustainable preservation technologies are needed to combat these emerging issues especially since there is an increasing trend towards organic and minimally processed vegetables (Ravishankar et al., 2018). By adopting these approaches within a holistic postharvest management model, stakeholders can ensure better food security, economic return and public health outcome through remarkable reduction in microbial spoilage of vegetables (Kader, 2005).

- **5.1. Detection and Identification Techniques:** Accurate detection and identification of microbial spoilage agents are crucial for monitoring vegetable quality and ensuring food safety. Several techniques, ranging from conventional to advanced molecular approaches, are employed for this purpose (Peterz et al., 2017).
- **5.2. Traditional Culturing Methods:** Conventional microbiological techniques involve the use of selective and differential media to isolate and identify spoilage microorganisms based on their morphological and biochemical characteristics (Ray & Bhunia, 2013). These methods are relatively inexpensive and can provide viable cell counts. However, they are often time-consuming, labor-intensive, and may not detect viable but non-culturable (VBNC) organisms. (Oliver, 2010)
- **5.3. Molecular Methods (PCR, and qPCR):** Polymerase Chain Reaction (PCR) and quantitative PCR (qPCR) offer highly sensitive and specific detection of spoilage organisms by targeting species-specific DNA sequences (Postollec et al., 2011). These methods significantly reduce detection time and can identify pathogens even in mixed microbial populations. qPCR additionally quantifies microbial load, which is valuable for assessing contamination severity and progression.

- **5.4. Rapid Detection Kits:** Commercially available rapid detection kits, such as lateral flow assays and biosensors, provide on-site, user-friendly alternatives for screening microbial contaminants. These kits are designed for quick results, often within minutes to hours, and are suitable for routine monitoring in supply chains and retail environments. While convenient, their sensitivity and specificity may vary depending on the target organism and kit design.
- 6. Conclusion: Microbial spoilage is a major challenge to postharvest management of vegetables, contributing to global food security, public health and economic sustainability. Due to their high-water content, high nutrient density, and low processing, vegetables provide an excellent habitat for microbial attachment and spoilage especially by bacteria of the genus Pseudomonas and Erwinia and fungi of the genus Aspergillus and Rhizopus. These microorganisms can degrade quality but even more importantly pose a significant health hazard by producing toxins and allergenic substances, particularly when vegetables are ingested raw or with minimal cooking. This becomes even more problematic in areas where sanitation, cold storage, and standard handling practices are not in place, with irreversible spoilage rates 3-4xhigher. Effective spoilage control can be achieved through a two-prong approach combining old school hygiene with new technologies. Strategies for solution like refrigeration, modified atmosphere packaging, or LED exposure have shown their potential in improving shelf life and reduction of microbial loads. Recently, new approaches have emerged that include ozone treatment, UV-C irradiation, cold plasma applications, and edible coatings giving rise to new avenues in spoilage control without using chemicals. Nonetheless, the effective implementation of these approaches requires not only functional technological development but also education, awareness, and compliance with standardized procedures on the producer, distributor, and consumer sides (i.e., throughout the supply chain). The growing preference of the consumers towards less processed or organic vegetables has thus created a demand for safer, more effective preservation methods. Research on microbiological ecology, spoilage dynamics, and novel preservation techniques needs to be continued to achieve the twin objectives of safety and quality. Overall, a long-term strategy requires participation of researchers, producers, retailers, and policy makers working together to minimize microbial spoilage, food waste, while supplying the consumer with safe, high-quality vegetables.

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