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Nutritional, phytochemical, and microbial changes in leafy greens across developmental stages

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Abstract

Leafy greens are vital dietary components due to their rich nutritional and phytochemical profiles and are consumed at various developmental stages, from sprouts and microgreens to baby and mature leaves. This review synthesizes current knowledge on the ontogenic changes in nutritional composition, bioactive compounds, and microbial safety across these stages. Early developmental forms, especially microgreens, exhibit superior concentrations of vitamins, antioxidants, and phytochemicals like polyphenols and carotenoids, whereas mature leaves contribute more fiber and structural carbohydrates. Phytochemical content fluctuates significantly with growth, with microgreens often showing peak antioxidant potential. However, microbial safety varies inversely; sprouts, due to moist and warm growth conditions, pose the highest contamination risk, while mature leaves tend to host more diverse but less pathogenic microbial communities. Importantly, certain phytochemicals have antimicrobial properties, suggesting synergies between nutritional quality and food safety. The review emphasizes the influence of genotype, environment, and cultivation practices, including controlled-environment agriculture on quality and safety outcomes. It highlights the need for integrated research on underexplored species and stages, and calls for standardized protocols to better inform harvest timing, breeding strategies, and regulatory frameworks. This holistic understanding is essential for producing safe, nutrient-dense, and functionally beneficial leafy greens for modern diets.

Keywords: Leafy greens, developmental stages, microgreens, nutritional composition, phytochemicals, microbial safety

1. Introduction

Leafy greens constitute an essential component of the human diet worldwide due to their rich nutritional profile and health-promoting properties. They provide a valuable source of vitamins, minerals, dietary fiber, and a diverse array of bioactive phytochemicals, including antioxidants and polyphenols, which contribute to reducing the risk of chronic diseases such as cardiovascular ailments, cancer, and diabetes (Boeing *et al*., 2012; Slavin & Lloyd, 2012) [3, 28]. Increasing consumer awareness regarding healthy eating habits has driven demand for fresh, minimally processed leafy vegetables that retain high nutritional and functional qualities (Jones, 2016; Khan *et al*., 2024a) [11, 14]. Microgreens have emerged as an ideal superfood choice for health-conscious and younger populations due to their compact nutrient density, visual appeal, and ease of incorporation into modern diets **(**Khan *et al*., 2024a**)** [14].

Leafy greens undergo distinct developmental stages from seed germination to mature leaf formation. These stages typically include sprouts (germinated seeds with emerging radicles), microgreens (young seedlings with cotyledons and first true leaves), baby leaves (immature leaves harvested before full maturity), and mature leaves (fully developed leaves) (Xiao *et al*., 2012; Khan *et al*., 2025c) [31, 18]. Each developmental phase represents a unique physiological and biochemical status that affects the concentration and composition of nutrients and phytochemicals, as well as the microbial communities associated with the plant surface and tissues (Kyriacou and Rouphael, 2018; Pirlak *et al*., 2021) [22, 26].

Studying ontogenic variation, that is, changes occurring throughout development, in leafy greens is critical for several reasons. Firstly, the nutritional and phytochemical content can vary significantly with growth stage, influencing the health benefits of the produce (Zhao *et al*., 2018) [32].

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For example, microgreens have been shown to contain higher concentrations of certain vitamins and antioxidants compared to mature leaves (Xiao *et al*., 2012; Khan *et al*., 2025a**;** Khan *et al*., 2025c) [31, 15, 18]. Secondly, microbial safety concerns differ across developmental stages. Sprouts, for instance, have been repeatedly linked to foodborne outbreaks due to favorable conditions for pathogen growth during germination (Taormina *et al*., 1999; De Jesus and Whiting, 2020) [29, 7]. Understanding how microbial populations fluctuate with plant development can help in designing better safety protocols.

Despite the importance of these aspects, most studies tend to focus on either nutritional or safety aspects in isolation, and comprehensive reviews integrating nutritional, phytochemical, and microbial changes across all developmental stages are limited. This review aims to fill this gap by providing a holistic synthesis of current knowledge on the dynamic changes in nutritional quality, bioactive phytochemicals, and microbial safety risks of leafy greens from seed germination to maturity. It draws particular emphasis on the emerging role of microgreens as a class of highly nutrient-dense, functional vegetables (Khan *et al*., 2024b; Khan *et al*., 2025a; Khan *et al*., 2025b) [16, 15, 17], and discusses implications for producers, consumers, and food safety regulators, highlighting areas for future research and innovation.

2. Developmental Stages of Leafy Greens: Definitions and Characteristics

Leafy greens undergo several distinct developmental stages, each characterized by specific morphological and physiological traits that influence their nutritional and safety profiles. The key developmental stages include seed, sprout, microgreen, baby leaf, and mature leaf. The seed stage represents the dormant phase containing the embryonic plant, which upon imbibition initiates germination. Sprouts emerge within 2-7 days after germination and consist mainly of the radicle and hypocotyl with emerging cotyledons; this stage is characterized by rapid metabolic activity and cell division (Xiao *et al*., 2012) [31]. Microgreens are young seedlings harvested shortly after the cotyledons have fully expanded and often after the first true leaves appear, typically within 7-21 days post-germination (Kyriacou *et al*., 2016) [23]. The baby leaf stage refers to small, immature leaves harvested before full maturity, usually between 14-30 days depending on species, while the mature leaf stage corresponds to fully expanded, physiologically mature leaves capable of supporting reproduction (Kader, 2008) [13].

This developmental trajectory is clearly illustrated in Figure 1, which depicts the sequential growth stages of fenugreek (*Trigonella foenum-graecum*), from sprouts and microgreens to baby greens and fully mature leaves. Such visual representation underscores the morphological transitions that occur with age, each accompanied by shifts in nutritional and biochemical properties.

Fig 1: Four developmental stages of fenugreek: Sprouts, Microgreens, Baby-greens, and Mature-greens. Each stage exhibits distinct morphological features and nutritional profiles, influencing its culinary use and health benefits

Morphologically, these stages differ markedly. Sprouts are mostly stem and root tissues with undeveloped leaf structures, whereas microgreens have visible cotyledons and early leaf primordia. Baby leaves exhibit juvenile leaf structures, smaller and more tender than mature leaves, which show fully developed leaf lamina, veins, and trichomes (Jones & Kubota, 2019) [12]. Physiologically, photosynthetic capacity, nutrient transport, and secondary metabolite synthesis evolve with leaf age. For example, chlorophyll content and antioxidant enzyme activities typically increase from sprout to mature leaf stages, reflecting enhanced metabolic functions (Pirlak *et al*., 2021) [26].

Commonly studied leafy green species across these developmental stages include lettuce (Lactuca sativa), spinach (*Spinacia oleracea*), kale (Brassica oleracea var. acephala), arugula (Eruca vesicaria), and mustard greens (*Brassica juncea*). These species are selected due to their nutritional value, consumer popularity, and adaptability to controlled environment agriculture (Xiao *et al*., 2012; Kyriacou *et al*., 2016) [31, 23]. Each species displays specific growth rates and biochemical profiles, which influence the optimal harvest time for maximizing nutritional quality and safety.

3. Nutritional Changes Across Developmental Stages

Leafy greens exhibit significant variation in nutritional composition throughout their developmental stages, reflecting the changing physiological and metabolic processes from seed germination to maturity. Understanding these changes is crucial for optimizing harvest timing to maximize nutritional benefits. This section explores the key nutritional components affected by development, including macronutrients, micronutrients, antioxidant capacity, and enzyme activities, followed by a comparative analysis of nutrient density across growth stages and the factors influencing these variations.

Macronutrient Content

The content of proteins, carbohydrates, and fats varies considerably as leafy greens develop. Sprouts and microgreens generally show higher protein content on a fresh weight basis compared to mature leaves, owing to their high metabolic activity and rapid cell division during early growth (Xiao *et al*., 2012; Kyriacou *et al*., 2016; Khan *et al*., 2025a) [31, 23, 15]. Carbohydrate levels, predominantly in the form of simple sugars and starch, also fluctuate, often peaking during early stages to support growth energy requirements and declining as leaves mature and allocate carbon towards structural components (Pirlak *et al*., 2021) [26]. Fat content in leafy greens is generally low but may increase slightly in mature leaves, reflecting the accumulation of membrane lipids and lipophilic compounds (Zhao *et al*., 2018) [32].

Micronutrients

Vitamins and minerals show dynamic changes during plant development. Microgreens often exhibit higher concentrations of vitamin C, carotenoids (pro-vitamin A), and some B vitamins compared to mature leaves (Xiao *et al*., 2012; Kyriacou *et al*., 2016; Khan *et al*., 2024b; Khan *et al*., 2025b) [31, 23, 16, 17]. Mineral content, including potassium, calcium, magnesium, and iron, also tends to be elevated in younger tissues, although accumulation patterns are species-dependent (Pirlak *et al*., 2021) [26]. These variations are influenced by nutrient uptake, translocation, and partitioning during growth.

Antioxidant Capacity and Enzyme Activities

Antioxidant capacity, measured by assays such as DPPH radical scavenging or ORAC, generally increases from sprouts to microgreens and baby leaves but may decline or plateau at full maturity (Xiao *et al*., 2012; Chen *et al*., 2020) [31, 4]. This pattern is attributed to the biosynthesis and accumulation of phenolic compounds and flavonoids, which protect young tissues from oxidative stress. Enzymatic antioxidants such as superoxide dismutase (SOD), catalase, and peroxidases also show developmental stage-dependent activity, peaking in stages with high metabolic flux (Chen *et al*., 2020) [4]. A consolidated overview of these nutritional trends across stages, from macronutrient shifts to antioxidant changes, is presented in Table 1. This comparative summary aids in visualizing the ontogenic variations that influence harvest decisions and functional food value.

Table 1: Nutritional Changes in Leafy Greens Across Developmental Stages (Sprouts to Mature Leaves)

Nutritional Component	Sprouts	Microgreens	Baby Leaves	Mature Leaves
Protein Content	High (metabolic activity)	High (cell division and growth)	Moderate	Low to moderate
Carbohydrate Content	High (energy reserves)	Moderate to high	Decreasing	Lower (more structural)
Fat Content	Very low	Low	Low	Slight increase (membrane lipids)
Vitamin C	Moderate	High	Moderate	Lower
Carotenoids (Pro-vitamin A)	Moderate	High	Moderate	Lower to moderate
B Vitamins	Moderate	High	Moderate	Lower
Minerals (K, Ca, Mg, Fe)	Moderate	High (species-dependent)	Moderate	Variable
Antioxidant Capacity	Moderate	High	High	Declines or plateaus
Enzymatic Antioxidants (SOD, CAT, POX)	Moderate	High	Moderate to high	Variable or reduced

Sources: Xiao *et al*. (2012) [31]; Kyriacou *et al*. (2016) [23]; Pirlak *et al*. (2021) [26]; Zhao *et al*. (2018) [32]; Chen *et al*. (2020) [4]; Khan *et al*., 2024b [16]; Khan *et al*., 2025a [15]; Khan *et al*., 2025b [17]

Comparative Nutrient Density

Comparative studies have demonstrated that microgreens often provide superior nutrient density compared to baby and mature leaves when analyzed on a fresh weight basis (Xiao *et al*., 2012; Khan *et al*., 2024b; Khan *et al*., 2025a**)** [31, 16, 15]. For example, microgreens of lettuce and kale have been reported to contain 4-6 times higher vitamin C and carotenoid levels than their mature counterparts (Xiao *et al*., 2012) [31]. Sprouts, while nutritionally rich, may have lower total biomass and thus require consumption in larger amounts to meet dietary needs. Mature leaves, although sometimes lower in certain micronutrients, contribute bulk dietary fiber and other health benefits (Kyriacou *et al*., 2016; Khan *et al*., 2024a) [32, 14].

Factors Influencing Nutritional Quality

Genotype plays a significant role in determining nutrient profiles, with some cultivars inherently richer in specific vitamins or minerals (Bergquist *et al*., 2021) [2]. Environmental factors such as light intensity and quality, temperature, and nutrient availability can alter the synthesis and accumulation of nutrients and phytochemicals during growth (Pirlak *et al*., 2021; Chen *et al*., 2020) [26, 4]. Cultivation methods, including soil versus hydroponic systems and organic versus conventional farming, also influence nutritional quality, often mediated through stress responses and nutrient supply (Kyriacou & Rouphael, 2018) [22].

4. Phytochemical Dynamics During Leafy Green Ontogeny

Leafy greens are renowned for their rich phytochemical content, which contributes significantly to their health-promoting properties (Bergquist *et al*., 2021; Khan *et al*., 2025a) [2, 15]. The major phytochemical classes include polyphenols, flavonoids, carotenoids, and glucosinolates, among others. These compounds serve critical roles in plant defense and human nutrition, acting as antioxidants, anti-inflammatory agents, and modulators of cellular signaling pathways (Dragsted *et al*., 2019; Khan *et al*., 2025b) [8, 17].

Key Phytochemical Classes

Polyphenols constitute a broad group of secondary metabolites including phenolic acids, flavonoids, and tannins. Flavonoids such as quercetin and kaempferol are abundant in leafy greens and contribute to antioxidant activity (Kim & Kim, 2020) [21]. Carotenoids, including beta-carotene and lutein, provide pigmentation and play a role as vitamin A precursors. Glucosinolates, predominantly found in Brassica vegetables like kale and mustard greens, are sulfur-containing compounds with known chemoprotective effects (Verkerk *et al*., 2009) [30].

Biosynthesis and Accumulation Patterns

Phytochemical biosynthesis is tightly regulated and varies with developmental stage. During early growth, biosynthetic pathways are often highly active, leading to rapid accumulation of phenolics and flavonoids to protect vulnerable young tissues from oxidative stress and UV damage (Pirlak *et al*., 2021; Khan *et al*., 2024b) [26, 16]. Carotenoid accumulation generally increases as leaves mature and chloroplasts develop fully (Zhao *et al*., 2018) [32]. Glucosinolate content can peak at intermediate growth stages before declining or stabilizing at maturity (Fahey *et al*., 2001) [9].

Influence of Growth Stage on Bioactive Compound Concentration and Diversity

The concentration and diversity of bioactive phytochemicals fluctuate significantly with growth stage. Microgreens often contain higher total phenolic content and antioxidant capacity than mature leaves (Xiao *et al*., 2012; Khan *et al*., 2025a) [31, 15]. For example, red cabbage microgreens can have up to five times more anthocyanins than mature leaves (Palada & Chang, 2003) [25]. This ontogenic variation is visually summarized in Figure 1, which illustrates the relative concentrations of major phytochemical classes, phenolics, carotenoids, anthocyanins, and glucosinolates, across different stages of leafy green development. Notably, microgreens show peak levels of phenolics and anthocyanins, while glucosinolates are more concentrated in mature tissues, highlighting stage-specific health-promoting potential. However, compound-specific trends may vary depending on the species and environmental factors (Verkerk *et al*., 2009) [30].

Health Implications of Phytochemical Variation

Variation in phytochemical profiles across developmental stages has important health implications. Higher levels of antioxidants in microgreens and baby leaves may enhance their protective effects against oxidative stress-related diseases (Bergquist *et al*., 2021) [2]. Glucosinolates and their hydrolysis products have been linked to cancer chemoprevention, making the developmental stage crucial for maximizing functional benefits (Verkerk *et al*., 2009) [30]. Understanding these dynamics can guide consumers and producers to select the optimal harvest time for functional foods.

Impact of Pre and Post-Harvest Factors on Phytochemical Stability

Phytochemical content is not only affected by ontogeny but also by pre-harvest conditions such as light quality, temperature, and nutrient availability, which can modulate biosynthesis pathways (Kyriacou & Rouphael, 2018) [22]. Post-harvest handling and storage conditions significantly influence phytochemical stability; for instance, carotenoids and polyphenols may degrade under prolonged storage or exposure to light and oxygen (Rickman *et al*., 2007) [27]. Minimizing these losses requires optimized harvesting and storage protocols.

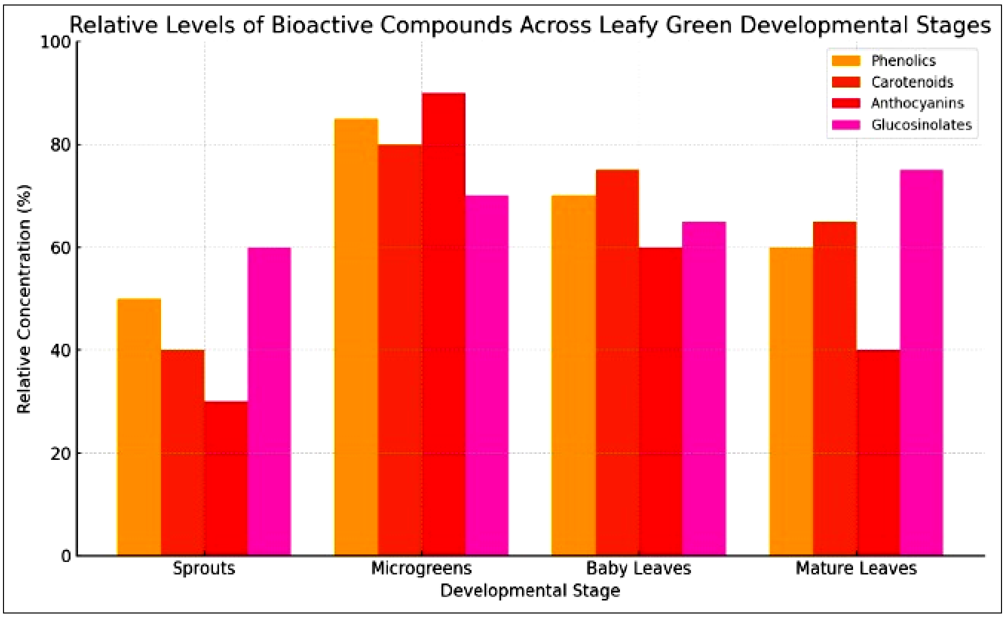

Fig 2: Relative levels of bioactive compounds (phenolics, carotenoids, anthocyanins, and glucosinolates) across the developmental stages of leafy greens. Data illustrate the generalized trend from sprouts to mature leaves, emphasizing the high antioxidant potential of microgreens and glucosinolate accumulation in mature tissues

5. Microbial Safety and Changes Across Developmental Stages

Leafy greens are highly susceptible to microbial contamination due to their large surface area, high moisture content, and consumption often in raw form. Common microbial contaminants include a range of bacteria, fungi, and viruses that can pose significant food safety risks.

Common Microbial Contaminants

Bacterial pathogens such as *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* are frequently implicated in outbreaks linked to leafy greens (Heaton & Jones, 2008; Olaimat & Holley, 2012) [10, 24]. Fungal contaminants include molds and yeasts that contribute to spoilage and potential mycotoxin production, while viruses such as norovirus and hepatitis A virus can contaminate produce through irrigation water or handling (Khatri *et al*., 2020) [19].

Variation in Microbial Load and Diversity Across Developmental Stages

Microbial populations on leafy greens vary significantly from seed germination through to mature leaves. Seeds themselves may carry epiphytic or endophytic microbes, some beneficial and others potentially pathogenic (Barak & Liang, 2008) [1]. Sprouts, grown in warm, moist conditions, provide an ideal environment for rapid microbial proliferation, making this stage especially vulnerable to contamination and outbreaks (Taormina *et al*., 1999) [29].

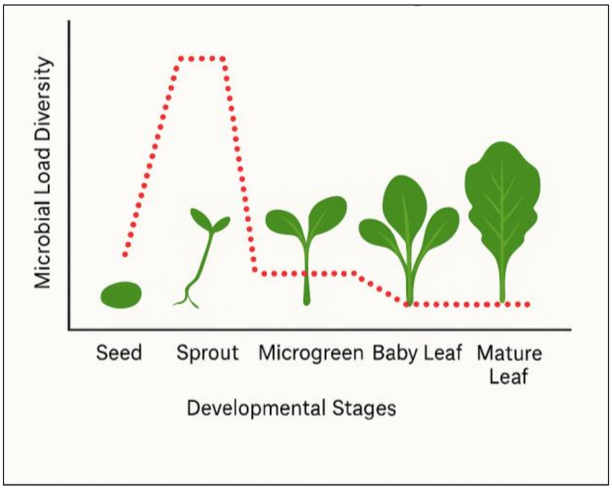

Fig 3. Variation in microbial load and diversity across developmental stages

As the plant develops into microgreens, baby leaves, and mature leaves, microbial load and diversity shift depending on environmental exposure, plant surface characteristics, and physiological changes (Pirlak *et al.*, 2021) [26]. Mature leaves may host more diverse microbial communities, including beneficial epiphytes that can outcompete pathogens (Heaton & Jones, 2008) [10]. These dynamic changes in microbial load and diversity across developmental stages are visually summarized in Figure 3, that highlight the microbial risk at the sprouting stage due to favorable growth conditions, then declines as the plant matures.

Factors Influencing Microbial Colonization and Contamination Risk

Several factors affect microbial colonization on leafy greens. The extensive surface area and natural openings such as stomata and trichomes provide niches for microbes (Heaton & Jones, 2008) [10]. Moisture from irrigation or condensation supports microbial survival and growth. Tissue susceptibility varies with leaf age; younger tissues may lack fully developed physical and chemical defenses, while mature leaves produce more antimicrobial compounds (Kim *et al.*, 2018) [20]. Environmental factors like temperature, humidity, and handling practices also influence contamination risks (Olaimat & Holley, 2012) [24].

Microbial Safety Concerns Specific to Developmental Stages

The sprouting stage is the highest-risk period for microbial contamination due to conditions favoring pathogen growth and the consumption of raw sprouts (Taormina *et al.*, 1999) [29]. Microgreens and baby leaves, while generally less risky than sprouts, still require careful production practices to prevent contamination. Mature leaves often have lower pathogen loads but can still transmit microbes if contaminated during harvest or processing (Khatri *et al.*, 2020) [19].

Strategies for Minimizing Microbial Contamination

To mitigate risks, good agricultural and manufacturing practices are essential. These include using pathogen-free seeds, sanitizing irrigation water, and controlling environmental conditions during sprouting and growth (Olaimat & Holley, 2012) [24]. Post-harvest interventions such as washing with sanitizers, proper cold storage, and minimizing handling reduce microbial loads (Khatri *et al.*, 2020) [19]. Emerging technologies like biocontrol agents, edible coatings, and UV-C treatments show promise in enhancing microbial safety without compromising nutritional quality (Kim *et al.*, 2018) [20].

6. Interrelationships Between Nutrition, Phytochemicals, and Microbial Safety

The interplay between nutritional components, phytochemicals, and microbial safety in leafy greens is complex and multifaceted. Phytochemicals not only contribute to the health benefits of these vegetables but also play an important role in influencing microbial colonization and contamination risks.

Phytochemicals and Microbial Colonization

Many phytochemicals found in leafy greens exhibit antimicrobial properties that can inhibit the growth of foodborne pathogens and spoilage organisms. Polyphenols and flavonoids, for example, disrupt bacterial cell membranes and interfere with microbial enzymes, reducing pathogen survival on leaf surfaces (Cowan, 1999; Cushnie & Lamb, 2011) [5, 6]. Glucosinolates and their hydrolysis products, such as isothiocyanates in Brassica species, show broad-spectrum antimicrobial activity against bacteria and fungi (Verkerk *et al.*, 2009) [30]. These natural defense compounds contribute to the plant's innate ability to limit microbial colonization, which in turn may reduce contamination risks during production and post-harvest handling.

Trade-offs and Synergies Between Maximizing Nutrition/Phytochemicals and Microbial Safety

While phytochemicals can enhance microbial safety, trade-offs may exist in certain contexts. For example, the accumulation of specific bioactives may coincide with increased moisture retention or softer leaf tissues that favor microbial attachment and growth (Pirlak *et al.*, 2021) [26]. Conversely, higher phytochemical levels may correlate with reduced pathogen colonization, suggesting synergy between nutritional quality and safety (Kim *et al.*, 2018) [20]. These dynamics highlight the importance of selecting cultivars and growth conditions that optimize both nutritional value and microbial resistance. Furthermore, the use of biostimulants or elicitors to enhance phytochemical content has shown promise in simultaneously improving food safety outcomes (Kyriacou & Rouphael, 2018) [22]

Role of Cultivation Practices and Controlled Environment Agriculture

Cultivation methods significantly influence the balance between nutrition, phytochemicals, and microbial safety. Controlled environment agriculture (CEA), including hydroponics and vertical farming, allows precise management of light, temperature, humidity, and nutrient supply, which can enhance phytochemical accumulation while reducing microbial contamination risks (Kyriacou *et al.*, 2016; Olaimat & Holley, 2012) [23, 24]. For instance, UV-B supplementation has been demonstrated to increase flavonoid content and simultaneously reduce bacterial loads on leaves (Kim *et al.*, 2018) [20]. Organic versus conventional farming practices may also impact this balance, with organic systems sometimes favoring higher phytochemical levels but potentially higher microbial loads due to manure use if not properly managed (Bergquist *et al.*, 2021) [2]. Integrating optimized cultivation protocols with good agricultural practices is essential to produce leafy greens that are both nutritionally superior and microbiologically safe.

7. Current Knowledge Gaps and Research Needs

Despite considerable progress in understanding the ontogenic changes in leafy greens, several knowledge gaps persist that limit the comprehensive utilization of these crops for nutrition and safety.

Insufficient Data on Certain Developmental Stages or Species

Most research has focused on a limited number of species such as lettuce, kale, and brassicas, and on select developmental stages like sprouts and microgreens (Xiao *et al*., 2012; Kyriacou *et al*., 2016) [31, 23]. Data on baby leaves and mature stages, particularly for less-studied species, remain sparse. This limits the generalizability of findings and hinders the identification of species-specific or stage-specific nutritional and safety profiles (Bergquist *et al*., 2021) [2]. Expanding studies to diverse leafy greens across all developmental stages is necessary to develop comprehensive guidelines.

Need for Integrated Studies Combining Nutritional, Phytochemical, and Microbial Analyses

Current studies often examine nutritional, phytochemical, or microbial attributes in isolation, missing the opportunity to understand their interrelationships fully. Integrated approaches that simultaneously assess macronutrients, micronutrients, bioactive compounds, and microbial communities across developmental stages would provide a more holistic understanding of quality and safety (Pirlak *et al*., 2021; Olaimat & Holley, 2012) [26, 24]. Such multi-disciplinary studies are critical to identify trade-offs and synergies, enabling optimization of cultivation and harvest strategies.

Standardization Issues in Sampling and Analysis Across Stages

Variability in sampling protocols, growth conditions, and analytical methods complicates comparisons across studies and developmental stages. Differences in units of measurement (fresh weight vs. dry weight), extraction techniques, and microbial detection methods create inconsistencies (Kyriacou & Rouphael, 2018; Chen *et al*., 2020) [22, 4]. Establishing standardized protocols for sampling, biochemical assays, and microbial quantification is imperative to generate reliable and comparable data that can inform best practices and regulatory standards.

Addressing these research needs will enhance our ability to harness the full potential of leafy greens as nutritious, phytochemical-rich, and safe foods.

8. Practical Implications and Recommendations

Optimizing the harvest timing of leafy greens is critical to balance maximizing nutritional quality, phytochemical content, and microbial safety. The dynamic changes occurring across developmental stages necessitate evidence-based guidelines for growers, consumers, and regulators.

Guidelines for Optimal Harvest Timing

Research consistently shows that microgreens and baby leaves often contain higher concentrations of vitamins, antioxidants, and bioactive phytochemicals compared to mature leaves, making these stages ideal for harvesting when the goal is maximal nutritional benefits (Xiao *et al*., 2012; Kyriacou *et al*., 2016) [31, 23]. However, the microbial safety risks are notably higher during the sprout stage due to favorable conditions for pathogen proliferation (Taormina *et al*., 1999) [29]. Therefore, avoiding raw consumption of sprouts without rigorous safety measures is recommended. Harvesting microgreens and baby leaves under controlled conditions with strict hygiene protocols can minimize microbial risks while maximizing nutrition (Olaimat & Holley, 2012) [24]. Mature leaves, while somewhat lower in certain micronutrients, provide bulk and fiber and are typically safer from a microbial standpoint, particularly when subjected to proper washing and handling (Khatri *et al*., 2020) [19].

Implications for Consumers, Growers, and Food Safety Regulators

Consumers should be informed about the nutritional benefits and potential microbial risks associated with different developmental stages, encouraging appropriate preparation and handling, especially for high-risk stages such as sprouts (Taormina *et al*., 1999) [29]. Growers are advised to implement good agricultural practices, including seed sanitation, irrigation water quality management, and post-harvest hygiene to reduce contamination (Olaimat & Holley, 2012) [24]. Food safety regulators must establish and enforce guidelines that address stage-specific risks, such as microbial testing requirements for sprouts and microgreens, and provide clear labeling standards to inform consumers (Khatri *et al*., 2020) [19].

Potential for Breeding and Biotechnological Interventions

Breeding programs can focus on cultivars with enhanced phytochemical profiles and intrinsic antimicrobial properties to improve both nutrition and microbial safety. Genotypes that naturally accumulate higher levels of glucosinolates, flavonoids, or other bioactives may offer dual benefits of health promotion and pathogen resistance (Bergquist *et al*., 2021) [2]. Biotechnological approaches, including CRISPR/Cas-mediated gene editing, can further optimize biosynthetic pathways for phytochemicals or enhance plant immune responses (Chen *et al*., 2020) [4]. Additionally, elicitor treatments and controlled environment cultivation can be leveraged to modulate phytochemical synthesis while minimizing microbial risks (Kyriacou & Rouphael, 2018) [22].

Integrating these approaches with comprehensive risk assessment and consumer education will support the production and consumption of leafy greens that are both nutritious and safe.

9. Conclusion

Leafy greens, across their developmental continuum from sprouts to mature leaves, exhibit dynamic shifts in their nutritional composition, phytochemical richness, and microbial safety profile. Nutritionally, earlier stages such as microgreens often concentrate higher levels of vitamins, minerals, and antioxidants, while mature leaves provide increased dietary fiber and biomass. Phytochemical content, including flavonoids, glucosinolates, and carotenoids, also follows distinct accumulation patterns that reflect underlying physiological and metabolic changes. Concurrently, microbial risks vary significantly-sprouts and microgreens are particularly vulnerable due to high humidity, warmth, and minimal processing barriers, while baby and mature leaves may face contamination risks from irrigation water, soil contact, and postharvest handling. These findings underscore the importance of developmental stage as a critical determinant of both quality and safety in leafy greens.

To fully harness the nutritional potential and ensure safety, a holistic and integrative approach is essential, one that considers not only the biochemical and microbial changes but also the complex interactions among plant genotype, environmental factors, and cultivation practices. Importantly, the antimicrobial roles of specific phytochemicals suggest opportunities for synergistic improvement of both quality and microbial resistance. Controlled-environment agriculture and precision harvesting technologies offer promising avenues for optimizing crop output while minimizing health risks.

However, critical research gaps persist. Many species and growth stages remain underexplored, particularly with respect to combined assessments of nutrition, phytochemicals, and microbiomes. The lack of standardized sampling and analytical methodologies further hinders meaningful comparisons and meta-analyses. To address these gaps, future research must prioritize interdisciplinary studies with harmonized protocols across stages and species. This will be instrumental for developing evidence-based guidelines for growers, policy makers, and the food industry, informing decisions on harvest timing, breeding targets, and postharvest handling to deliver safe, nutrient-rich, and phytochemically diverse leafy greens to consumers.

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