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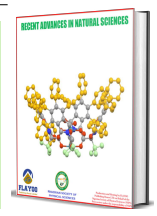

Microbial properties of *Hibiscus sabdariffa* processed through different thermal methods

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ABSTRACT

A comparative analysis of the microbial properties of *Hibiscus sabdariffa* calyces and leaves (red variety) subjected to different thermal drying methods—electric oven, infrared oven, and sun drying—was conducted using standard analytical and molecular techniques. The study revealed significant differences ($p < 0.05$) in microbial loads, with sun drying exhibiting the lowest heterotrophic bacterial load at 3.56 ± 0.13 log10 CFU/g, compared to the electric and infrared oven methods, which recorded bacterial loads of 3.82 ± 0.35 and 3.85 ± 0.14 log10 CFU/g, respectively. Similarly, sun drying resulted in a fungal count of 3.60 ± 0.43 log10 CFU/g, indicating the varying impact of drying methods on fungal contamination. Notably, oven-dried leaf samples identified *Vibrio* at 2.00 ± 0.1 log10 CFU/g, highlighting potential safety concerns associated with this method. Sequencing analysis revealed a diverse microbial spectrum, including *Proteus vulgaris* strain SI-9, *Acinetobacter baumannii* strain ZHOU, *Providencia rettgeri* strain AR_0082, and *Pseudomonas aeruginosa* strain DBH3. These findings underscore the necessity of selecting appropriate drying methods to effectively mitigate microbial risks. Phylogenetic analysis elucidated the genetic relationships among the isolates, reflecting their ecological origins and the influence of anthropogenic activities on microbial diversity in processed food products. This comparative assessment advocates for enhanced processing protocols to ensure the microbial safety of *Hibiscus sabdariffa*, aligning with global food safety standards and promoting consumer confidence in the quality of dried botanicals.

1. INTRODUCTION

Roselle (*Hibiscus sabdariffa* L.) is grown extensively in Nigeria's woodland and savannah ecological zones. As an industrial crop, it has a lot of promise. Roselle is cultivated for its fiber content as well as its leaves, calyces, roots, and seeds, which have industrial, medicinal, commercial, or domestic applications. Maintaining the microbiological safety of fresh food, such as the harvestable component of Roselle (Leaf and Calyces), requires a systemic strategy that includes all phases of production, process-

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ing, distribution, and consumption. Understanding the behavior of microbial pathogens and spoilage organisms on such fresh produce is necessary for the development of techniques to remove them from these foods in order to prevent outbreaks of foodborne disease due to their consumption [1].

Calyces are the most utilized component of a roselle plant. Calyces are obtained by separating the flower's calyces or petals from the seed-containing capsules. Calyces are used to make herbal drinks, cold and warm beverages, jams and jellies [1]. Around the world, fresh calyces are used to produce jelly wine, gelatin, syrup, pudding, beverages and cakes, while dried calyces are used to make tea, ice cream, jelly, butter etc. Calyces are also utilized as coloring and flavoring agents in rum in the West Indies. The bloom and fleshy fruit are used in the pharmaceutical sector to treat bronchitis and coughs in non-food applications. Additionally, calyces are utilized to treat hypertension, diarrhea, Ceylon mouth, and several other conditions [2, 3]. In Nigeria, the calyces of cultivated roselle are often crimson, green, or dark red. The red calyces are often used to prepare caffeine-free drinks like tea, but the green calyces are utilized as soup vegetables.

Despite the quality and food/industrial value of *Hibiscus sabdariffa* (HS), it is necessary to conduct microbiological quality evaluations at many crucial stages in the production line. Numerous pre- and post-processing contamination routes and sources exist for agricultural and horticultural crop products, and much research has been done to pinpoint the precise processes by which infections are introduced into them [4, 5]. Various manufacturing zones have different produce contamination sources and pathways. This is due to the unique combinations of environmental risk variables that each farm possesses, including terrain, interactions between different land uses, and climate. The incidence and spread of foodborne pathogens are influenced by combinations of these unusual environmental risk variables, which in turn affect the risk of produce contamination [6]. Many different methods exist for pathogens to contaminate produce "on-field," including deposition from the atmosphere, absorption from polluted groundwater and soil [5, 7]. The use of untreated or badly treated raw compost and manure, exposure to polluted water (via irrigation or flooding), transmission by insects, and fecal contamination caused by domesticated animals or wild animals all contribute to the spread of disease [8, 9]. During the harvesting process, which involves human handling, harvesting tools, transport containers, wild or domestic animals, the air, a vehicle for transportation, ice, and water; during the processing process, which involves equipment, human handling, and raw materials such as water; and during the period of storage, which involves wild or domestic animals, the air, a vehicle for transportation, ice, and water.

Foodborne contamination prevention in fresh-cut processing begins in the field with the identification and removal of likely contamination sources. This is done in order to reduce the risk of foodborne illness. This is done to lessen the possibility of getting sick from consuming contaminated food. When it comes to addressing pre-harvest concerns, "Good Agricultural Practices" (GAP) and "Good Handling Practices" (GHP) continue to be the fundamental cornerstones of food safety management systems. Both "good agricultural practices" and "good handling procedures" are abbreviated as "GAP" and "GHP," respectively.

Recent years have seen significant advancements in a number of thermal and non-thermal approaches for the decontamination of fresh vegetables [10, 11]. In the laboratory, some of these technologies have been shown to be beneficial, but they have not yet been marketed on a large scale. This is because of variables such as high costs (high pressure and pulsed electric fields), a lack of public acceptance (irradiation), and, in certain instances, the requirement for the process to be refined or validated (UV and pulsed white light). Other limits, such as the potential impact on the organoleptic and sensory profile of treated produce and the potential hazards to personnel (particularly chemical treatment procedures), may cause certain treatments to experience a delay in the process of commercialization [10]. The aim of this study was to explore the impact of various thermal drying methods (electric oven, infrared oven, and sun drying) on the microbial properties of *Hibiscus sabdariffa*, with a focus on calyces and leaves. These methods were investigated for their effectiveness in reducing microbial contamination, thereby enhancing food safety [10, 11]. By examining the microbial load post-different drying processes, this research contributes to optimizing drying practices for microbial safety, aligning with the Codex Alimentarius standards for food safety [8, 9].

2. MATERIALS AND METHODS

Hibiscus sabdariffa calyces and leaves (Red variety) were collected and subjected to different thermal drying processes: electric oven, infrared oven, and sun drying. Prior to drying, 200 grams of calyces were meticulously washed with distilled water to remove extraneous materials, adhering to the protocol outlined by Adebayo-tayo and Samuel [12].

The thermal treatments applied were electric oven drying, infrared drying, and sun drying, selected for their common use in food preservation and their differing impacts on microbial viability [10, 11]. The specific conditions for each method were optimized as shown in Table 1 based on preliminary trials to achieve consistent drying while potentially minimizing microbial survival. To optimize sun drying, preliminary trials were conducted. Samples were spread uniformly on trays in an open area with maximum sun exposure, monitored for daily sunlight hours, ambient temperature, and humidity. Multiple trials (1-3 days) identified 2 days with 8 hours of sunlight as optimal for consistent drying and microbial reduction. Samples were periodically rotated to ensure uniform drying.

Following the drying processes, samples were prepared for microbial analysis. A 50 g portion of each dried sample was homogenized in 450 mL sterile 0.1% peptone water, employing the technique described by Cheesbrough [13] to enumerate the total culturable heterotrophic microbial loads via pour plate methods.

Pure colonies were isolated through repeated sub-culturing using the streak method, and preserved in McCarthy bottles containing agar slants at 30 ± 2°C for 18 to 24 hours before refrigeration at 4°C for future analyses, following established microbiological practices [13].

DNA was extracted from bacterial cell broth using a comprehensive lysis and purification procedure, as detailed by Adebayo-tayo and Samuel [12]. Polymerase Chain Reaction (PCR) amplification of the extracted DNA utilized primers 27F and 1429R, under conditions specified by Tamura and Nei [14], to amplify

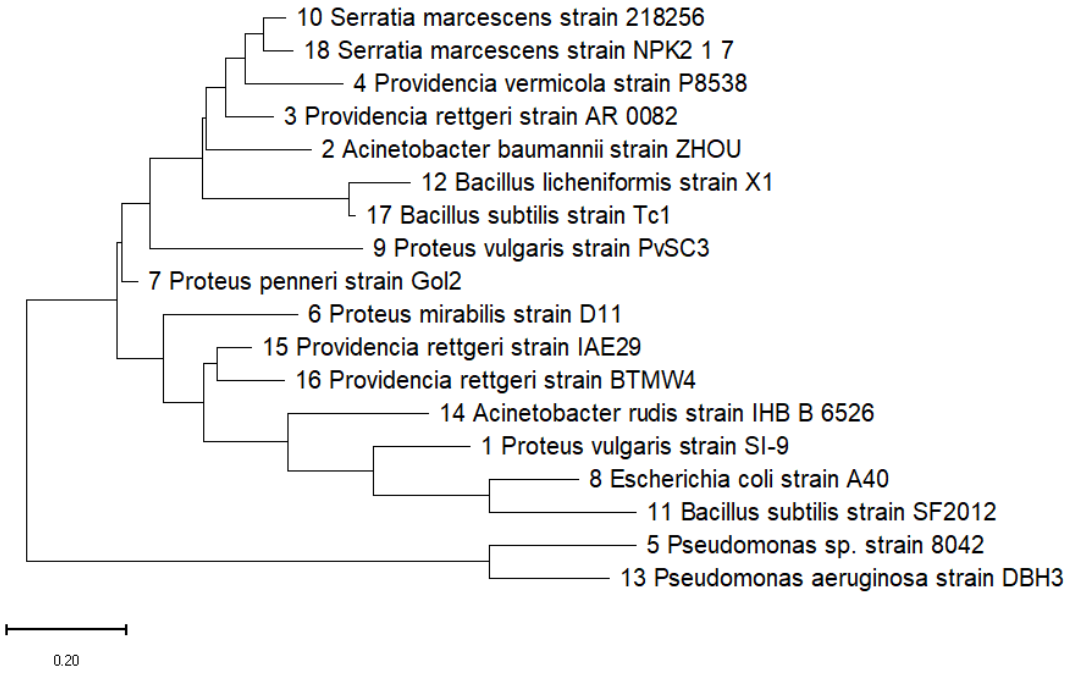

Figure 1. Dendogram showing the relatedness of the bacterial isolates using the Neighbor-Joining method.

Table 1. Optimization conditions for electric oven drying, infrared drying, and sun drying methods.

S/N	Drying Method	Temperature (° C)	Duration (hours)	Outcome
1	Electric Oven Drying	60	6	Consistent drying with minimal moisture content and some microbial viability.
2	Infrared Drying	70	4	Rapid, uniform drying with reduced microbial viability.
3	Sun Drying	Direct sunlight	2 days (average 8 hours/day)	Adequate drying with higher variability in moisture content and microbial viability.

bacterial 16S rRNA genes.

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Sequencing analysis was performed in accordance with Tamura and Nei [14], using the ABI 3130 x l genetic analyzer. The BLAST tool from the NCBI database was employed to identify sequences, and phylogenetic trees were constructed using MEGA 7 software, illustrating the genetic relationships among the isolates.

Data analysis was carried out using XLSTAT PREMIUM Software (Evaluation 2023.1.5.1409 Version), with Welch's ANOVA employed to assess the significance of differences between drying methods on microbial loads.

3. RESULTS AND DISCUSSION

The microbial analysis of dried Hibiscus sabdariffa samples across different drying methods revealed variations in microbial loads, as summarized in Table 2. The heterotrophic bacterial counts for calyx samples subjected to electric oven drying at 60°C for a consistent period were observed at 3.82 ± 0.35 log10 CFU/g, indicating a moderate level of bacterial presence. Infrared drying of calyx samples, performed at 70°C for a fixed duration, resulted in similar bacterial loads, with counts at 3.85 ± 0.14 log10 CFU/g. Notably, oven drying of calyx samples yielded slightly higher bacterial counts at 3.90 ± 0.36 log10 CFU/g, alongside a fungal count of 3.15 ± 0.23 log10 CFU/g, suggesting a differential impact of drying methods on fungal contamination levels. For leaf samples, oven drying resulted in the highest heterotrophic bacterial count at 3.99 ± 0.35 log10 CFU/g

Table 2. Optimization conditions for electric oven drying, infrared drying, and sun drying methods.

Sample Type	Drying Method	Heterotrophic Bacteria	Fungal Count	Salmonellae/Shigellae	Vibrio count	Clostridial Count	Total Coliform Count	Faecal Coliform Count
Calyx	Electric Oven Drying	3.82 ± 0.35[a]	NG	NG	NG	NG	3.61 ± 0.15 [a]	NG
Calyx	Infrared Drying	3.85 ± 0.14 [a]	NG	NG	NG	2.60± 0.51 [a]	3.49 ± 0.37 [a]	NG
Calyx	Oven Drying	3.90 ± 0.36 [a]	3.15± 0.23 [a]	NG	NG	2.85± 0.39 [a]	3.48 ± 0.47 [a]	NG
Leaves	Oven Drying	3.99 ± 0.35 [a]	NG	NG	2.00± 0.1 [a]	3.18± 0.04 [b]	3.66 ± 0.19 [a]	NG
Leaves	Infrared Drying	3.61 ± 0.26 [b]	NG	NG	NG	2.48± 0.08 [a]	3.36 ± 0.17 [a]	NG
Leaves	Infrared Drying	3.77 ± 0.18 [a]	3.04± 0.05 [a]	NG	NG	NG	3.52 ± 0.27 [a]	NG
Calyx	Sun Drying	3.56 ± 0.13 [b]	3.60± 0.43 [b]	NG	NG	2.78± 0.12 [a]	3.15 ± 0.21 [b]	NG
Codex Alimentarius Standard		3.00	<1	0	0	0	0	0

Means with the same superscript along the same column are not significantly different (p = 0.05), ± = standard error, NG = No Growth.

Table 3. Identification and Accession Numbers of Microbial Isolates.

Isolate Codes	Identity	Percentage identity	Accession Number
1	*Proteus vulgaris* strain SI-9	85.71	KP059136.1
2	*Acinetobacter baumannii* strain ZHOU	92.24	CP104297.1
3	*Providencia rettgeri* strain AR_0082	99.73	CP029736.1
4	*Providencia vermicola* strain P8538	93.01	CP048796.1
5	*Pseudomonas* sp. 8042	71.02	AM111077.1
6	*Proteus mirabilis* strain D11	87.70	MK806682.1
7	*Proteus penneri* strain Gol2	99.56	MT263017.1
8	*Escherichia coli* strain A40	82.46	CP028736.1
9	*Proteus vulgaris* strain PvSC3	69.83	CP034668.1
10	*Serratia marcescens* strain 218256	95.62	OK432495.1
11	*Bacillus subtilis* strain SF2012	79.22	MN588235.1
12	*Bacillus licheniformis* strain X1	90.02	MH622750.1
13	*Pseudomonas aeruginosa* strain DBH3	81.88	MN416905.1
14	*Acinetobacter rudis* strain IHB B 6526	83.72	KF475856.1
15	*Providencia rettgeri* strain IAE29	84.05	MK414972.1
16	*Providencia rettgeri* strain BTMW4	89.00	ON740910.1
17	*Bacillus tequilensis* strain A4	98.00	GU391355.1
18	Serratia marcescens strain NPK2_1_7	97.58	MN691619.1

and was the only method to register a Vibrio count of 2.00 ± 0.1 log10 CFU/g, indicating potential inadequacies in controlling Vibrio contamination. Infrared drying of leaf samples produced lower heterotrophic bacterial counts at 3.61 ± 0.26 log10 CFU/g and 3.77 ± 0.18 log10 CFU/g, with one set showing a fungal load of 3.04 ± 0.05 log10 CFU/g. Sun drying of calyx samples, which involved exposure to natural sunlight until a constant weight was achieved, exhibited the lowest bacterial load at 3.56 ± 0.13 log10 CFU/g and a fungal count of 3.60 ± 0.43 log10 CFU/g.

The clostridial counts varied across the samples, with sun-dried calyx samples and infrared-dried leaf samples displaying lower counts, suggesting these drying methods might be more effective in reducing Clostridium levels. Total coliform counts across the samples showed slight variations, with the lowest counts observed in sun-dried calyx samples at 3.15 ± 0.21 log10 CFU/g, indicating a potential variance in the effectiveness of drying methods in reducing coliform bacteria.

When compared to the Codex Alimentarius standard, which recommends no presence of pathogenic microorganisms and a limit of <1 log10 CFU/g for fungal contaminants, our results demonstrate a higher microbial load in dried *Hibiscus sabdariffa* samples. The absence of Salmonellae/Shigellae and faecal coliforms across all samples aligns with the standard, yet the presence of heterotrophic bacteria and fungi in certain samples necessitates attention to improve drying methods to ensure microbial safety.

Sequencing analysis, aligned with the Basic Local Alignment Search Tool (BLAST) Table 3 and further phylogenetic analysis (Figure 1), identified a diverse array of bacterial species. This diversity included strains of *Proteus vulgaris*, *Acinetobacter baumannii*, *Providencia rettgeri*, *Pseudomonas aeruginosa*, among others, each with specific accession numbers denoting their genetic identity. The identified bacterial isolates exhibit a range of functionalities, from potential pathogens to species with environmental or biotechnological significance. The dendrogram analysis utilizing the Neighbor-Joining method revealed notable clustering among the bacterial isolates, specifically: *Escherichia coli* strain A40 and *Serratia marcescens* strain 218256 formed a distinct cluster; *Providencia vermicola* strain P8538, *Providencia rettgeri* strain AR_0082, and *Acinetobacter baumannii* strain ZHOU were closely related; *Bacillus licheniformis* strain X1 and *Bacillus tequilensis* strain A4 were grouped together; *Proteus vulgaris* strain PvSC3, *Proteus penneri* strain Gol2, and *Proteus mirabilis* strain D11 were identified as a coherent unit; *Providencia rettgeri* strain IAE29 and *Providencia rettgeri* strain BTMW4 also clustered together; *Acinetobacter rudis* strain IHB B 6526, *Proteus vulgaris* strain SI-9, and *Bacillus subtilis* strain SF2012 were linked; and finally, *Pseudomonas sp.* 8042 and *Pseudomonas aeruginosa* strain DBH3 were paired, illustrating the phylogenetic relationships and genetic similarity among these isolates (Figure 1).

The investigation into the microbial properties of dried *Hibiscus sabdariffa*, subjected to different thermal drying methods, yielded insightful findings on microbial reduction and food safety. The differential microbial reduction observed across drying techniques—electric oven, infrared, and sun drying—underscores the nuanced efficacy of these methods in controlling microbial load. Notably, infrared and sun drying methods demonstrated superior reduction in microbial contaminants, aligning with recent studies that emphasize the potential of specific drying methodologies to enhance food safety [15, 16].

The presence of fungal contaminants and Vibrio in samples

processed via electric oven drying signals a need for improved handling and storage strategies to mitigate such microbial risks. These findings echo the concerns raised by Hassan *et al.* [17] who noted the susceptibility of dried botanicals to fungal invasion post-processing. The detection of Vibrio in oven-dried leaf samples raises particular concerns regarding the adequacy of this method in safeguarding against microbial hazards, suggesting a potential area for methodological refinement [18].

Clostridium and coliform bacteria reductions in samples subjected to sun and infrared drying illuminate the partial efficacy of these methods. However, the consistent absence of fecal coliforms across all samples signifies a positive outcome, adhering to food safety standards. Yet, the overall microbial load in some samples surpassing Codex Alimentarius recommendations signals a pressing need for improved drying practices to effectively mitigate microbial contamination risks [5, 19, 20].

Advancements in microbial assessment techniques, as employed in our study, offer new avenues for accurately characterizing and understanding the microbial ecology of food products. The use of comprehensive analytical and molecular techniques facilitates a deeper insight into the microbial dynamics at play, supporting the development of targeted strategies for microbial control. These advancements are crucial for ensuring the microbiological safety of dried botanicals. Sequencing and phylogenetic analysis revealed a diverse array of bacterial species, including potential pathogens and environmentally significant species. This diversity highlights the necessity of rigorous microbial assessment to ensure the safety and quality of dried botanicals [21]. The dendrogram analysis using the Neighbor-Joining method provided a clear illustration of the phylogenetic relationships among the isolates, suggesting their derivation from similar ecological niches or anthropogenic sources [22, 23].

The study's findings emphasize the importance of selecting appropriate drying methods to ensure microbial safety in dried *Hibiscus sabdariffa*. Furthermore, it accentuates the role of advanced microbial analysis techniques in identifying potential risks and ensuring compliance with food safety standards. Future research should focus on exploring innovative drying technologies and microbial control strategies that can be integrated into processing lines to enhance the safety and quality of dried plant products [24, 25].

4. CONCLUSION

The comprehensive analysis conducted in this study on *Hibiscus sabdariffa* subjected to various thermal drying methods has elucidated critical insights into the influence of these methods on microbial properties, underscoring their significant implications for food safety and quality. The findings reveal that the selection of drying methods plays a pivotal role in microbial reduction, with infrared and sun drying methods demonstrating superior efficacy in mitigating microbial loads compared to electric oven drying. This variance in microbial safety among the drying techniques accentuates the necessity for adopting optimized drying practices to ensure the microbial integrity of dried botanical products. Moreover, the presence of potential pathogenic microorganisms in some dried samples highlights the ongoing challenges in maintaining food safety standards and underscores the imperative of continuous improvement in drying practices. The utilization of advanced analytical and molecular techniques in assessing microbial diversity further illustrates the complexity of microbial ecosystems in food products and the importance of employing comprehensive assessment strategies to safeguard consumer health. Given the paramount importance of microbial safety in the food industry, this study advocates for further research into innovative drying technologies and microbial control strategies that could be integrated into existing processing lines. Such advancements are essential not only for enhancing the microbial safety of dried botanical products but also for maintaining their quality, thereby fostering consumer confidence in the safety and integrity of food products. The pursuit of innovative research in this domain will undoubtedly contribute to setting new benchmarks for the food processing industry, ensuring that food safety and quality are maintained at the highest standards.

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References

[1] P. J. Tsai, J. McIntosh, P. Pearce, B. Camden & B. R. Jordan, "Anthocyanin and antioxidant capacity in Roselle (*Hibiscus Sabdariffa* L.) extract", Food Research International **35** (2002) 351. https://www.infona.pl/resource/bwmeta1.element.elsevier-154b7284-9e72-330c-b3d6-6929a1ffe9e7.

[2] T. Chewonarin, T. Kinouchi, K. Kataoka, H. Arimochi, T. Kuwahara, I. Init & Y. Ohnishi, "Effects of roselle (*Hibiscus sabdariffa* Linn), a Thai medicinal plant, on the mutagenicity of various known mutagens in Salmonella typhimurium and on formation of aberrant crypt foci induced by the colon carcinogens azoxymethane and 2-amino-methyl-6-phenylimidazo (4,5-b) pyridine in F344 rats", Food and Chemical Toxicology **37** (1999) 591. https://www.sciencedirect.com/science/article/pii/S0278691599000411.

[3] M. H. Faraji & A. Haji Tarkhani, "The effect of sour tea (Hibiscus sabdariffa) on essential hypertension", Journal of Ethnopharmacology **65** (1999) 231. https://www.sciencedirect.com/science/article/abs/pii/S0378874198001573.

[4] P. Kotzekidou, "Food hygiene and toxicology in ready to eat foods", Academic Press, 2016. https://www.sciencedirect.com/book/9780128019160/food-hygiene-and-toxicology-in-ready-to-eat-foods.

[5] O. O. Alegbeleye & A. S. Sant'Ana, "Risks associated with the consumption of irrigation water contaminated produce: On the role of quantitative microbial risk assessment", Current Opinion in Food Science **41** (2021) 88. https://www.sciencedirect.com/science/article/abs/pii/S221479932100062X.

[6] L. K. Strawn, Y. T. Grohn, S. Warchocki, R. W. Worobo, E. A. Bihn, & M. Weidmann, "Risk factors associated with Salmonella and *Listeria monocytogenes* contamination of produce fields", Applied and Environmental Microbiology **79** (2013) 7618. https://journals.asm.org/doi/abs/10.1128/aem.02831-13.

[7] J. Mei Soon, L. Manning, W. Paul Davies & R. Baines, "Fresh produce-associated outbreaks: a call for HACCP on farms?", British Food Journal **114** (2012) 553. https://www.emerald.com/insight/content/doi/10.1108/00070701211219568/full/html?journalCode=bfj.

[8] M. B. Cooley, D. Chao, & R. E. Mandrell, "Escherichia coli O157 survival and growth on lettuces is altered by the presence of epiphytic bacteria", Journal of Food Protection **69** (2006) 2329. https://www.sciencedirect.com/science/article/pii/S0362028X22075974.

[9] M. Uyttendaele, L. A. Jaykus, P. Amoah, A. Chiodini, D. Cunliffe, L. Jacxsens, K. Holvoet, L. Korsten, M. Lau, P. McClure & G. Medema, "Microbial hazards in irrigation water: Standards, norms, and testing to manage use of water in fresh produce primary production", Comprehensive Reviews in Food Science and Food Safety **14** (2015) 336. https://doi.org/10.1111/1541-4337.12133.

[10] M. Barth, T. R. Hankinson, H. Zhuang & F. Breidt, "Microbiological spoilage of fruits and vegetables", Compendium of the microbiolog-

ical spoilage of foods and beverages (2009) 135. https://doi.org/10.1007/978-1-4419-0826-1_6.

[11] I. Castro-Ibáñez, M. I. Gil, J. A. Tudela, R. Ivanek, & A. Allende, ‘‘Assessment of microbial risk factors and impact of meteorological conditions during production of baby spinach in the Southeast of Spain", Food Microbiology **49** (2015) 173. https://doi.org/10.1016/j.fm.2015.02.004.

[12] B. C. Adebayo-Tayo & U. A. Samuel, ‘‘Microbial quality and proximate composition of dried *Hibiscus sabdariffa* calyxes in Uyo, Eastern Nigeria", Malaysian Journal of Microbiology **5** (2009) 12. http://dx.doi.org/10.21161/mjm.12608.

[13] M. Cheesbrough, ‘‘District Laboratory Practice in Tropical Countries, Part 2 (2nd ed.)", Cambridge University Press, IJMS, 2006, pp. 65–68. https://www.cambridge.org/core/books/district-laboratory-practice-in-tropical-countries/6F6FE267756B004A4826209C692BD5B3.

[14] K. Tamura & M. Nei, ‘‘Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees", Molecular Biology and Evolution **10** (1993) 512. https://doi.org/10.1093/oxfordjournals.molbev.a040023.

[15] R. Khiari, H. Zemni & D. Mihoubi, ‘‘Raisin processing: Physicochemical, nutritional and microbiological quality characteristics as affected by drying process", Food Reviews International **35** (2019) 246. https://doi.org/10.1080/87559129.2018.1517264.

[16] F. M. Allai, Z. A. A. Azad, N. A. Mir & K. Gul, ‘‘Recent advances in non-thermal processing technologies for enhancing shelf life and improving food safety", Applied Food Research **3** (2023) 100258. https://doi.org/10.1016/j.afres.2022.100258.

[17] M. M. Hasan, M. R. Islam, A. R. Haque, M. R. Kabir, K. J. Khushe & S. K. Hasan, ‘‘Trends and challenges of fruit by-products utilization: insights into safety, sensory, and benefits of the use for the development of innovative healthy food: a review", Bioresources and Bioprocessing **11** (2024) 10. https://doi.org/10.1186/s40643-023-00722-8.

[18] J. W. Buck, R. R. Walcott & L. R. Beuchat, ‘‘Recent trends in microbiological safety of fruits and vegetables", Plant Management Network **4** (2003) 25. https://doi.org/10.1094/PHP-2003-0121-01-RV.

[19] A. S. Sant’Ana, M. S. Barbosa, M. T. Destro, M. Landgraf & B. D. Franco, ‘‘Growth potential of Salmonella spp. and Listeria monocytogenes in nine types of ready-to-eat vegetables stored at variable temperature conditions during shelflife", International Journal of Food Microbiology **157** (2012) 52. https://doi.org/10.1016/j.ijfoodmicro.2012.04.011.

[20] D. Aruscavage, S. A. Miller, M. L. Ivey, K. Lee & J. T. LeJeune, ‘‘Survival and dissemination of Escherichia coli O157 on physically and biologically damaged lettuce plants", Journal of Food Protection **71** (2008) 2384. https://doi.org/10.4315/0362-028X-71.12.2384.

[21] O. Nwaiwu, C. C. Aduba, V. C. Igbokwe, C. E. Sam & M. U. Ukwuru, ‘‘Traditional and artisanal beverages in Nigeria: Microbial diversity and safety issues", Beverages **6** (2020) 53. https://doi.org/10.3390/beverages6030053.

[22] V. Vrbovská, I. Sedláček, M. Zeman, P. Švec, V. Kovařovic, O. Šedo, M. Laichmanová, J. Doškař & R. Pantůček, ‘‘Characterization of *Staphylococcus intermedius* group isolates associated with animals from Antarctica and emended description of *Staphylococcus delphini*", Microorganisms **8** (2020) 204. https://doi.org/10.3390/microorganisms8020204.

[23] B. Gawrońska, M. Marszałek, P. Kosiński, M. Podsiedlik, L. Bednorz & J. Zeyland, ‘‘No wonder, it is a hybrid. Natural hybridization between *Jacobaea vulgaris* and *J. erucifolia* revealed by molecular marker systems and its potential ecological impact", Ecology and Evolution **13** (2023) e10467. https://doi.org/10.1002/ece3.10467.

[24] S. K. Sharma, A. Ramesh, M. P. Sharma, O. P. Joshi, B. Govaerts, K. L. Steenwerth & D. L. Karlen, ‘‘Microbial community structure and diversity as indicators for evaluating soil quality", Sustainable Agriculture **5** (2011) 317. https://link.springer.com/chapter/10.1007/978-90-481-9513-8_11.

[25] P. Trivedi, C. Mattupalli, K. Eversole & J. E. Leach, ‘‘Enabling sustainable agriculture through understanding and enhancement of microbiomes", New Phytologist **230** (2021) 2129. https://doi.org/10.1111/nph.17319.