HUNGARIAN UNIVERSITY OF
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Microbiota analysis of dried fruits and stress modelling in *Escherichia coli*

Viktória Dobó

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The PhD School
Name: Doctoral School of Agricultural and Food Sciences

Discipline:
Food Sciences

Head of the Doctoral School:
Prof. Dr. Melinda Kovács
University Professor, Full Member of the Hungarian Academy of Sciences

Supervisors:
Dr. Ágnes Belák
Associate professor, PhD
Department of Food Microbiology, Hygiene and Safety
Institute of Food Science and Technology
Hungarian University of Agriculture and Life Sciences

Dr. Csilla Mohácsi-Farkas
University Professor, PhD
Department of Food Microbiology, Hygiene and Safety
Institute of Food Science and Technology
Hungarian University of Agriculture and Life Sciences

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Approval of the Head of Doctoral School

....

Approval of the Supervisor Approval of the Supervisor

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LIST OF ABBREVIATIONS

AFLP - Amplified Fragment Length Polymorphism

a_w - Water activity

CC strain- *E. coli* strain (ATCC B.02031) from the culture collection

CFU - Colony forming unit

D-value - Decimal radiation dose

FAO - Food and Agriculture Organization

FI strain - *E. coli* strain from a low water activity food (buckwheat flour)

FISH - Fluorescent In Situ Hybridisation

IAEA - International Atomic Energy Agency

LMFs - Low-moisture foods

MMGM - Mineral Modified Glutamate Medium

NA - Nutrient Agar

OD- Optical density

PCR – Polymerase Chain Reaction

PFGE - Pulsed Field Gel Electrophoresis

PMA – Propidium Monoazide

PMA - qPCR - Propidium Monoazide–quantitative Polymerase Chain Reaction

RAPD - Random Amplified Polymorphic DNA

rep-PCR - Repetitive Element Polymerase Chain Reaction

RFLP - Restriction Fragment Length Polymorphism

TBARS - Thiobarbituric Acid Reactive Substances

TBX - Tryptone Bile X-glucuronide

TSA - Tryptic Soy Agar

VBNC - Viable but non-culturable

VRBD - Violet Red Bile Glucose Agar

WHO - World Health Organization

1 INTRODUCTION

In recent years, dried fruits have gained importance in modern diets due to growing consumer interest in healthy food products. Dried fruits are concentrated forms of their fresh counterparts, processed either by traditional methods, such as sun-drying or by using advanced mechanical dryers (Ringeisen et al., 2014). According to the European Food-Based Dietary Guidelines (FBDG), individuals are advised to consume approximately 400–500 grams of fruits and vegetables daily, with the emphasis on fresh, seasonal products. However, in several countries, including Lithuania and the United Kingdom, dried fruits are also recommended to be consumed daily as part of the balanced diet (Web 1). Due to climatic limitations in most of the European countries, year-round availability of fresh fruits is limited, thus, dried fruits serve as a great alternative throughout the year. These snacks are rich in essential nutrients, including dietary fibres, various vitamins (C, E, K, B-complex), minerals (Ca, Mg, Fe, K), and bioactive compounds such as phytochemicals and antioxidants. Their regular consumption supports the digestive health, enhances the immune functions, contributes to bone health, and lowers the risk of cardiovascular diseases (Jeszka-Skowron et al., 2017; Rybicka et al., 2021; Alasalvar et al., 2023; Zeng et al., 2023).

The drying process reduces the moisture content in fruits and consequently lowers their water activity (a_w). This inhibits microbial growth and significantly extends the shelf life of the products, making dried fruits more suitable for storage and distribution (Średnicka-Tober et al., 2020). Traditional dried fruits, such as raisins, apricots, peaches, apples, prunes, dates and figs, typically contain no added sugar. However, some dried fruits, such as cranberries, mangoes, and blueberries, are often sweetened with sugar syrup before drying (Alasalvar et al., 2020). In addition to enhancing sweetness, the added sugar further reduces water activity thereby extending the shelf-life. Despite dried fruits having low water activity levels, typically around or even below 0.60, these products are not entirely free from microbial contamination (Beuchat et al., 2011). Although bacterial growth is generally inhibited at a_w values below 0.87, several microorganisms, including certain bacteria, yeasts, and moulds, are capable of surviving these conditions. Studies have shown that common pathogens such as *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis*, furthermore *Salmonella* spp., *Enterobacter* spp., and *Streptococcus* spp. can persist on dried products at room and refrigeration temperatures. These contaminants have been isolated from dates, dried tomatoes, and other dried fruits, even including home-dried samples (Risiquat, 2013; Ntuli et al., 2017; Canakapalli et al., 2021; Shah et al., 2022, Abed, 2025). Notably, Ntuli et al. (2017) reported that more than half of the home-dried fruit and vegetable samples tested were positive for faecal coliforms, indicating potential hygiene lapses. As dried fruits are often consumed without further processing, such as cooking, microbial and mycotoxin contamination remain major safety concerns (Zakaria et al., 2015). Bacterial and fungal contamination may occur during production, such as drying, handling, packaging, or

storage due to poor hygienic practices. As a result, understanding the microbiological profile of these products is critical to food safety.

Microbial detection in food products traditionally relies on culture-based methods, including different selective media, biochemical tests (IMViC, oxidase, or urease tests), and advanced identification tools, such as Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS). Although MALDI-TOF MS allows rapid and accurate species-level identification of the cultured isolates, culture-based methods are labour-intensive, time-consuming and may underestimate microbial diversity, as not all organisms can grow under standard laboratory conditions (Biswas and Rolain, 2013). In contrast, culture-independent methods, such as the 16S rDNA amplicon sequencing, or quantitative PCR enable the detection of both culturable and unculturable microorganisms directly from food, environmental or clinical samples. These newer methods bypass the need for cultivation and can provide a broader overview of microbial communities (Cuscó et al., 2017; Kanatani et al., 2024; Ge et al., 2025). However, biases in DNA extraction, the choice of primers, target genes and reference databases significantly can affect the accuracy of taxonomic identification (O'Sullivan et al., 2011; Quigley et al., 2013; Schoonbroodt et al., 2023). Combining culture-dependent and culture-independent methods therefore yield the most comprehensive characterisation of the food microbiota.

In response to environmental stress, bacteria employ several adaptive mechanisms, including the upregulation of stress-response proteins, adaptive mutations, or the entry into a dormant state. The dormant state can be triggered by various food preservation methods, such as heat treatment (pasteurization), low temperature storage (refrigeration, freezing), drying (dehydration), chemical preservation (addition of acids), high osmolarity (salt or sugar), as well as new preservation methods, including irradiation (gamma, UV) or high hydrostatic pressure, or also the combination of these methods (freeze-drying) (Fu et al., 2020; Li et al., 2020b; Cai et al., 2022; Jayeola et al., 2022; König et al., 2023; Pazos-Rojas et al., 2023; Hu et al., 2024; Shangguan et al., 2025). Several dormant sates exist, including sporulation, persistent cells, and the viable but non-culturable (VBNC) state (Harriott, 2019; Kapinusova et al., 2023). While bacterial spores are metabolically inactive, but can germinate and be quantified by standard methods, VBNC cells remain metabolically active, yet fail to grow under standard laboratory conditions making detection and identification challenging. The detection of VBNC cells therefore requires culture-independent approaches, such as qPCR or 16S rRNA sequencing. Because VBNC cells may retain pathogenic potential, and have been linked to delayed infections, they pose food safety concerns (Muela et al., 2008; Li et al., 2014; Ramamurthy et al., 2014).

In high-osmolarity environments (hyperosmotic stress), bacteria must adapt quickly to maintain turgor pressure and intracellular hydration. Osmotic stress causes water to move out of the cytoplasm across

the cell membrane, leading to cell shrinkage and disruption of normal cellular functions. To counter this, cells accumulate compatible solutes (osmoprotectants), such as glycine betaine, carnitine, proline betaine, and trehalose (Pilizota and Shaevitz, 2012; Breisch and Averhoff, 2020). In *E. coli*, osmotic stress triggers a complex response involving gene regulation, metabolic shifts, and solute accumulation (Sévin and Sauer, 2014; Sun et al., 2019; Tempelhagen et al., 2020). Genes such as *rpoB*, *rpoS*, and *osmC* are activated (Shin et al., 2004; Weber et al., 2006; Xiao et al., 2017b). Osmotic stress also alters metabolism in *E. coli* (Arense et al., 2010). The *talA* gene is upregulated to support trehalose synthesis and oxidative stress resistance (Song et al., 2006; Gu et al., 2009; Fuentes-Lemus et al., 2023). To manage energy under changing conditions, *E. coli* not only accumulates but also degrades trehalose when osmotic pressure decreases. Trehalose serves as both a compatible solute and a carbon source. Its catabolism is mediated by *treA*, which encodes a periplasmic trehalase (Ells and Hansen, 2011; Pavanelo et al., 2018).

Gamma irradiation is an emerging, non-thermal food preservation method that uses high-energy photons, that are typically emitted by radio nuclides such as Cobalt-60 or Cesium-137 to inactivate microorganisms and extend the shelf life of products (Kebede, et al., 2015; Singh et al., 2015; Jarrett, 2018). Other ionizing radiation that is permitted to be used for food processing include X-rays with energies up to 5 MeV, and accelerated electrons with energies up to 10 MeV. At sufficient doses, radiation damages microbial DNA and other critical cellular components, preventing replication and thus effectively reducing the load of pathogenic and spoilage organisms. However, at lower doses, some microorganisms may survive and activate stress response pathways, or enter the VBNC state, which only delays replication rather than preventing it (Bruckbauer et al., 2020; Gaougaou et al., 2020; Gallyamova et al., 2024). Importantly, at lower doses ($\geq$ 4 kGy) gamma radiation has only minimal effect on food macronutrients, micronutrients, flavour, and texture (Hing et al., 2022; Rafiepour et al., 2024). Higher doses can be responsible for the degradation of lipids, causing undesirable odours and rancid flavours (Kebede et al., 2015; Tomac et al., 2015; Huang et al., 2023). It can be applied to packaged products, thereby preventing post-treatment contamination (Inamura et al., 2012; Pelicia et al., 2015). In addition to microbial control, gamma irradiation can delay ripening, inhibit sprouting, and control insect infestation (Arthur and Albano, 2021; Mshelia et al., 2023; Yoon et al., 2023). While gamma irradiation is widely recognized as safe by the WHO and FAO, its acceptance and application vary by country and is influenced by consumer perceptions and awareness (Web 2; Web 3; Galati et al., 2019; D'Souza et al., 2021). Given that osmotic stress in dried fruits might promote the VBNC state in bacteria, understanding how gamma irradiation interacts with these pre-stressed microbial populations is critical for ensuring food safety.

2 OBJECTIVES

While fresh fruits have been extensively studied for their microbial communities, less is known about the microbiota of dried fruits. Several studies have demonstrated that unhygienic conditions at any point in the food chain can lead to contamination with pathogenic bacteria, including *Escherichia coli*, *Enterobacter*, *Salmonella*, *Listeria*, and *Staphylococcus*, raising food safety concerns. *Escherichia coli* was selected for this thesis as an indicator of faecal contamination in dried fruits, and as a model organism for studying osmotic stress. To investigate the composition of the microbiota and evaluate how sugar induced osmotic stress influences *Escherichia coli*, I conducted a series of experiments with the following objectives:

- The first part of the thesis focuses on characterizing the microbial composition of commercially available dried fruits (dried apricot, prune, and raisin) from Hungary and Austria with special emphasis on finding Enterobacteriaceae, and more specifically, *E. coli*. The complex microbiota was investigated using both culture-dependent methods combined with MALDI-TOF MS for identification and culture-independent approach with 16S rDNA amplicon sequencing. The goal was to enable a more complete analysis with this dual approach, yielding valuable results about the microbiological safety and quality of these popular food products. Additional analyses were further conducted to obtain a complete profile about the pH, water activity, sugar content, and added preservatives of the dried fruits examined in this thesis.
- Limited research has focused on the behaviour of *E. coli* in sugar-rich environments, as most osmotic stress-related studies focus on salt-induced conditions. The second part of the thesis therefore investigates the effects of sugar-induced osmotic stress, similar to those found in dried fruits. To mimic these environments, media containing glucose, fructose and sucrose were created at different concentrations and combinations. All these sugars serve as metabolizable carbon sources for *E. coli*. Prior to initiating the osmotic stress experiments, growth studies were conducted using a Bioscreen system to determine whether *E. coli* is capable of proliferating under such sugar-rich conditions.
- The osmotic stress experiments were further divided into two parts. The first part was aimed to investigate whether the osmotic environment found in dried fruits induce a viable but non-culturable-like state in *E. coli*. This state poses food safety risk, as conventional laboratory techniques fail to detect VBNC cells, even though they retain metabolic activity and potential virulence.
- The second part of the osmotic stress experiments aimed to obtain a broader view of *E. coli*'s adaptation to sugar-based osmotic stress in dried fruit-mimicking environment. Three target

genes were selected to represent different aspects of the osmotic stress response: the *osmC* (involved in general stress resistance), the *talA* (encoding transaldolase A and participates in the pentose phosphate pathway, having a role in osmotic adaptation), and *treA* (encoding periplasmic trehalase, which hydrolyzes trehalose into glucose).

- The final part of the thesis investigates the effect of ionizing radiation under osmotic stress conditions. To mimic these environments, culture media containing glucose, fructose and sucrose were created at varying concentrations, along with sugary media resembling the conditions of dried prune. Different radiation sources (linear accelerator, gamma radiation) were used at various doses to assess the effect of different sugars on the response of *E. coli* under ionizing radiation.

3 LITERATURE REVIEW

3.1 *Escherichia coli*

Escherichia coli was first isolated in 1885 by Theodor Escherich, a German paediatrician, from an infant faecal sample. It belongs to the coliform group of Gram-negative bacteria, which are commonly used as indicators of possible food or water contamination by environmental microorganisms. Faecal coliforms are a subgroup of coliforms, originating from the intestines of warm-blooded animals and therefore serve as a stronger indicator of faecal contamination. *E. coli* is the most frequently used indicator organism, as its presence almost always signals recent faecal contamination and potential risk of other enteric pathogens. Furthermore, *E. coli* can be easily grown under standard laboratory conditions, without the need for expensive media and equipment.

Morphologically, the cells are typically straight cylindrical rods that occur singly or in pairs, with parameters such as 1.1–1.5 × 2.0–6.0 μm. Most strains are motile, which contribute to their ability to grow, reproduce, and persist in diverse environments. The motility is due to the peritrichous flagella, that rotate counterclockwise in a bundle (Odonkor and Ampofo, 2013; Scheutz and Strockbine, 2015; Sosa-Hernández, 2017; Grognot and Taute, 2021; Basavaraju and Gunashree, 2022).

As *E. coli* is a facultative anaerobic bacterium, it conducts mixed-acid fermentation under oxygen-limited conditions, leading to the production of ethanol, lactate, acetate, formate and succinate, along with gaseous byproducts such as hydrogen (H_2) and carbon dioxide (CO_2). It can utilize a broad range of carbon sources such as glucose, fructose, lactose, glycerol, and acetate (Blankschien et al., 2010; Wang et al., 2011; Trchounian and Trchounian, 2013; Förster and Gescher, 2014; Leone et al., 2015; Yaratha et al., 2017; Metcalfe et al., 2020).

For the biochemical identification of *E. coli*, a series of classical biochemical tests are used, among which the IMViC reactions play an important role. These include the indole production, methyl red, Voges-Proskauer, and citrate utilization tests. Typical *E. coli* isolates are indole-positive, methyl-red-positive, Voges-Proskauer-negative, and citrate-negative. However, for an accurate identification, additional biochemical tests, such as oxidase, urease, and triple iron sugar (TSI) reactions, moreover confirmation with modern methods, such as MALDI-TOF MS or molecular methods are needed (Hsu et al., 2010; Panda et al., 2014; Widodo et al., 2022).

The rapid growth rate, which enables its division once every 30 minutes, combined with its well-characterized genetics and minimal nutritional requirements, highlights its adaptability, and makes it a well-liked model organism in microbiology and biotechnology (Taj et al., 2014).

3.1.1 Pathogenic strains of Escerichia coli

Most of the *E. coli* strains inhabiting the gastrointestinal tract are non-pathogenic and contribute to maintaining a healthy gut microbiota (Martinson and Walk, 2020; Pokharel et al., 2023). However, several pathotypes have been classified based on their pathogenetic profile, which is defined by specific virulence factors and the types of infections they cause in humans. The pathotypes are divided into two broad groups: intestinal pathogenic *E. coli* (IPEC), which cause infections of the gastrointestinal tract, and extraintestinal pathogenic *E. coli* (ExPEC), which cause disease outside the intestine. IPEC pathotypes include the enteropathogenic *E. coli* (EPEC), that causes infant diarrhoea and sporadic diarrhoea in children and adults; the enterotoxigenic *E. coli* (ETEC), a major cause of traveller's diarrhoea; the enteroinvasive *E. coli* (EIEC), which invades epithelial cells, leading to watery diarrhoea and fever; enterohemorrhagic *E. coli* (EHEC), that produces the Shiga-like toxin and verocytotoxin, leading to haemorrhagic colitis and haemorrhagic uremic syndrome (HUS); enteroaggregative *E. coli* (EAEC), which forms mucoid biofilms leading to chronic diarrhoea, vomiting and abdominal pain; diffusely adherent *E. coli* (DAEC), which is associated with chronic diarrhoea; and adherent invasive *E. coli* (AIEC), responsible for causing inflammatory bowel disease and chronic diarrhoea (Govindarajan et al., 2020; Jaybhaye and Deb, 2021; Sora et al., 2021). The ExPEC group contains the uropathogenic *E. coli* (UPEC), which colonizes the urinary tract and causes urinary tract infections; neonatal meningitis *E. coli* (NMEC), associated with meningitis in newborns; sepsis-associated *E. coli* (SEPEC), which causes septicaemia; avian pathogenic *E. coli* (APEC), a potential foodborne zoonotic pathogen; and mammary pathogenic *E. coli* (MPEC), associated with bovine mastitis (Agarwal et al., 2012; Wijetunge et al., 2015; Kathayat et al., 2021; Sora et al., 2021; Jung et al., 2025).

Most individuals with no underlying health condition typically recover from *E. coli* infection within approximately one week, without the need for medical treatment. However, symptoms, including persistent diarrhoea for more than three days, high fever, bloody stools and severe vomiting requires medical intervention due to the elevated risk of dehydration. Treatment plans are strain-dependent and symptom-specific, and usually involve oral or intravenous rehydration, and antidiarrheal agents to alleviate the symptoms. The use of antibiotics is not recommended as a first-line approach because of the adverse side effects, and the promotion of antimicrobial resistance, however, in the cases of severe infection, antibiotic therapy may be appropriate (Jaybhaye and Deb, 2021; Mueller and Tainter, 2023).

3.1.2 Conditions needed for culturing Escherichia coli in laboratory

E. coli can grow in both liquid and in solid culture media under laboratory conditions. As it is easily cultured, common media, such as Nutrient, Luria-Bertani (LB), and Minimal media are commonly used (Noor et al., 2013; Basavaraju and Gunashree, 2022). In applied microbiological analysis,

standardized methods are available for the detection and enumeration of *E. coli*. These include ISO 16649-1:2018, ISO 16649-2:2001, or ISO 9308-1:2014, all of which are widely used in food and water testing (Teramura et al., 2019; Paramitadevi et al., 2025). These methods rely on the detection of β-glucuronidase activity characteristic of *E. coli* and are based on colony-count or most probable number (MPN) techniques. For the differentiation of coliforms and fecal coliforms, selective and differential media are utilized. Eosin Methylene Blue (EMB) agar distinguishes coliform colonies based on characteristic colour changes, while MacConkey (MAC) agar selectively supports the growth of Gram-negative bacteria and is used to differentiate them according to their lactose metabolism (Lal and Cheeptham, 2007; Web 4). Tryptone-Bile-X-Glucuronide (TBX) agar differentiates *E. coli* from other coliforms based on the presence of the glucuronidase enzyme, and the Mineral-Modified Glutamate Medium (MMGM) serves as a liquid enrichment medium for isolating glucuronidase-positive *E. coli* from food, water, and environmental samples (Web 5; Web 6).

As *E. coli* lives in the intestines of warm-blooded organisms, the optimal growth temperature is between 36 and 40 °C, classifying it as a mesophilic bacterium, though certain strains were reported to grow at temperatures up to 49 °C (Kumar and Libchaber, 2013; Odonkor and Ampofo, 2013; Jang et al., 2017). It is important to note that the ability of *E. coli* to survive in various environmental conditions is strain and genotype dependent (Jang et al., 2017).

3.1.3 Outbreaks associated with Escherichia coli

Contamination of food by pathogenic microorganisms poses a significant public health concern and leads to economic losses throughout the world. Pathogenic *E. coli* is a frequent contaminant of food products, and not just in developing countries. Bacterial contamination can happen at any stage of the production chain, originating from environmental, animal, or human sources, and can result in foodborne illness. The most common route of *E. coli* infection is the faecal-oral route, but cross-contamination in processing facilities, poor temperature control during storage or transport, and contamination risks in retail or delivery settings all enable bacterial growth and spread. While meat, lettuce-based salads, dairy products, and water are the predominant sources of *E. coli* infections (Supplementary Table 1), the bacterium has also been detected in low–water activity products, including dried spinach and pumpkin leaves (Blyton et al., 2014; Ntuli et al., 2017; Yang et al., 2017; Alhadlaq et al., 2024). Infections associated with low-water activity food products are detailed in Chapter 3.2.3.

3.2 Dried fruits and low-moisture food safety

Nutrition plays an important role in our everyday life. National dietary guidelines, such as the OKOSTÁNYÉR® Dietary Guidelines for the General Population in Hungary, recommend a daily consumption of at least 500 g of vegetables and fruits, three portions of grains, 500 mL of milk or dairy products, limited red meat consumption (500–700 g/week), and eight glasses of fluids, with at least five glasses being water. These guidelines further emphasize the consumption of fresh, seasonal fruits and vegetables (Web 7). In Lithuania and the United Kingdom, alongside the consumption of fresh fruits, the consumption of dried fruits is also advised, whereas in Norway and Denmark dried fruits are excluded from the recommended daily portions of fruits (Web 8).

Fresh fruits typically contain over 80 % moisture, making them easily perishable. Moreover, fresh fruits of all categories are not available all year around, thus dried fruits offer a good alternative. Dried fruits are a concentrated form of their fresh counterparts. Drying reduces water content, while increasing the shelf-life, making the product available throughout the year (Shah et al., 2022).

Water activity (a_w) describes how much water in a food product is available to support microbial growth, which is not equal to the total moisture content. The total moisture content refers to the total amount of water present in the food product including free, absorbed and chemically bound water, and is typically expressed as a percentage of the product's total mass. The a_w is defined as the ratio of the food's vapor pressure (p) at an equilibrium with the surrounding air, to the vapor pressure of pure water(p_o) at the same temperature, expressed as decimals ($aw = (\frac{p}{p0})$). For example, the a_w of 0.80 means that the food has 80 % of the vapor pressure of pure water. The a_w can also be used to calculate the equilibrium relative humidity (ERH) ($ERH = 100\ x\ \left(\frac{p}{p0}\right) = 100\ x\ aw$).

Most fresh foods, including fruits, vegetables, meat, milk have a_w values above 0.95, providing enough moisture for bacterial, yeast and mould growth (Bradley, 2010; Labuza and Altunakar, 2020; Caushaj et al., 2024). In contrast, dried fruits typically have a_w values between 0.03 and 0.70 (Chitrakar et al. 2019).

According to literature, the minimum water activity for bacterial growth is around 0.87, while an a_w of 0.60 or lower is required to completely inhibit microbial activity. Food products below the a_w of 0.85 are classified as low-moisture foods (LMFs) (Beuchat et al., 2011; Syamaladevi et al., 2016). This category includes cereals, chocolate, cocoa powder, dried fruits and vegetables, seeds, rice and other grains, egg powder, flour, herbs and spices, honey, meat powder, dried meat, fermented dry sausage, pasta, milk powder, powdered infant formula, peanuts and tree nuts and peanut butter. Although LMFs have long been perceived as microbiologically stable, the increasing number of reports on foodborne outbreaks have begun to question this assumption (see Chapter 3.2.3 for details).

The resilience of pathogens in these products highlights a critical safety issue, that the processing of LMFs usually relies on hygiene during harvesting, transportation, and subsequent manufacturing steps. For dried fruits and vegetables, spices and herbs, in cases when after harvest and during storage no further bacterial reduction step is involved, the drying step might be the only decontamination step. As many dried products are consumed directly without heat treatment or incorporated into ready-to-eat foods, they can act as vehicles for bacterial transmission to nutrient-rich foods (Niemira, 2014; Bourdoux et al., 2016; Sánchez-Maldonado et al., 2018). The survival of microorganisms on dried fruits is well-documented. Common pathogens, such as *E. coli*, *Salmonella*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Proteus mirabilis* are able to survive on dates, sundried tomatoes, dried pluots at refrigerated and ambient temperatures (Risiquat, 2013; Al Hazzani et al., 2014; Canakapalli et al., 2022; Shah et al., 2022).

The most commonly consumed dried fruits are apricots, prunes, raisins, dates and figs, though a wide range of other fruits such as dried apples, pineapples, berries, mangoes, papayas and others are also available (Chang et al., 2016). Dried apples, apricots, raisins, prunes, figs, pears and peaches do not contain added sugar. In contrast, blueberries, cranberries, cherries, strawberries and mangoes are usually sweetened with fruit juice concentrate or sugar solution prior to drying (Alasalvar et al., 2020).

Over the decade from 2009/10 to 2019/20, the global dried fruit production increased by about 976,000 metric tons, reaching 3.2 million metric tons. Dried grapes (raisins, sultanas, currants) remained dominant with 1.3 million tons, which is 41 % of total output. This was followed by dates with 1.1 million tons, which represents 35 % of the world share. The remaining share included prunes (7 %), sweetened dried cranberries (6 %), dried apricots (6 %), and figs (5 %). The USA dominated prune and cranberry production and was the second-largest dried grape producer. Turkey was the top supplier of raisins, dried apricots, and figs. Iran ranked second in date and dried fig production, and was among the top producers of apricots and grapes (Web 9).

These naturally sweet snacks are a great source of dietary fibre, with average values ranging from 3.7 to 9.8 g/100g, depending on the fruit category (Alasalvar et al., 2023). They also provide essential minerals, such as calcium, iron, magnesium, natrium, potassium, copper (Rybicka et al., 2021; Shah et al., 2022). Even though the vitamin content degrades during drying, choosing the optimal drying method can help preserve the various vitamins in these products, such as vitamin A, B-complex, C, E and K (Chang et al., 2016; Donno et al., 2019; Radojčin et al., 2021, Shah et al., 2022). Dried fruits are naturally rich in sugar such as glucose, fructose, with glycaemic indices (GI) ranging from low (< 55) to moderate (from 56 to 69). The glycaemic index ranks carbohydrate-rich food products based on how quickly they raise blood sugar levels. Foods with low GI contain slowly digestible carbohydrates that elicit a reduced postprandial blood glucose response (Sagili et al., 2022). The presence of bioactive compounds such as flavonoids, phenolic acids, carotenoids, stilbenes,

phytoestrogens, tannins and fibre contributes to moderated glycaemic and insulin responses (Donno et al., 2019, Alasalvar et al., 2023). The antioxidant capacity of dried fruits is high but varies depending on the type of dried fruits, as well as on the cultivar (Alasalvar et al., 2023).

The beneficial effect of dried fruits cannot be neglected. Due to the high polyphenol content, they exhibit strong antioxidant, anti-inflammatory, anti-aging, and anti-carcinogenic properties while also enhancing endothelial function and reducing oxidative damage (Han et al., 2007). The high fiber content supports digestive health, improves glycaemic control, and lowers the risk of obesity, type 2 diabetes, and colorectal cancer (Silva et al., 2013; Ötles and Ozgoz, 2014; Song et al., 2018). Potassium helps reduce blood pressure and supports heart health, while magnesium contributes to better glycaemic control and a reduced risk of type 2 diabetes (Binia et al., 2015; Santos et al., 2023). Iron prevents anaemia and is involved in collagen synthesis, while copper supports iron metabolism, collagen metabolism and bone tissue health (Lal, 2020; Ciosek et al., 2023). Calcium is essential for bone health, (Vannucci et al., 2018). The high beta-carotene (precursor to vitamin A) content in dried apricots aids in preventing anaemia, supports vision, and protects against oxidative stress (Kasperczyk et al., 2014; Abrego-Guandique et al., 2023; Pawar et al., 2023). Vitamin B complexes are essential for red blood synthesis and maintaining nervous system health (Shakoor et al., 2021). Vitamin C contributes to immune defence by supporting both the innate and the adaptive immunity, and vitamin E protects against oxidative stress (Carr and Maggini, 2017). Vitamin K has been linked to blood calcium regulation and to maintaining bone and vascular health (Palmer et al., 2020).

The microbial composition of dried fruits is less studied. Most research focuses on the mycotoxin content of commercial dried fruits as moulds tolerate lower water activity levels better than most bacteria. Bacterial growth is inhibited below the water activity (a_w) of 0.87, while mycotoxigenic mould growth is limited around water activity of 0.78, although the required a_w for mycotoxin production is higher than the minimal values for growth. Xerophilic moulds grow between a_w values of 0.65–0.75 and osmophilic yeasts as low as a_w 0.61–0.65 (Tapia et al., 2020). Therefore, legal limits have been set for mycotoxins produced by moulds in dried fruits, while bacterial contamination is regulated only through non-mandatory guidelines issued by the German Society for Hygiene and Microbiology (Web 10).

Farahbakhsh et al. (2015) investigated the microbial quality of raisins dried by either shade or solar drying. *E. coli* was not detected in the samples, but significant coliform contamination and high yeast and mould levels were observed. The comparison of the two drying methods revealed that shade-dried raisins had lower fungal contamination due to reduced contamination possibility from the environment, and shorter processing time. Alghamdi et al. (2023a) combined the culturing and molecular analysis methods to determine the fungal contamination of dried fruits, including dried apricots, raisins and dates. The combination of the two methods revealed 13 genera of fungi from

dried dates and raisins, with the most predominant genera being *Aspergillus* sp. (3.67×10^2 Colony Forming Unit, CFU), *Penicillium* sp. (7×10^1 CFU), and *Rhizopus* sp. (2.7×10^1 CFU). The only detected yeast from dried apricots were *Zygosaccharomyces* sp. (2.9×10^3 CFU). Another study by Alghamdi et al. (2023b) focused on the molecular characterization of bacterial isolates from 180 dried fruit samples, including raisins, dates and apricots, from Saudi Arabia. The researchers identified 31 bacterial species from three genera, including *Bacillus*, *Micrococcus* and *Staphylococcus*, using the 16S rRNA sequencing. *Bacillus* was the dominant genus. A study on dried figs, apricots, raisins and plumes purchased in Egypt revealed that both home-dried and commercially purchased dried fruits contained aerobic mesophilic bacteria, moulds, yeasts and spore-forming bacteria. Raisins had the highest aerobic mesophilic bacterial count ($6.3\times10^4 \pm 2.3\times10^4$ CFU/g), but figs and plums also reached the 10^4 CFU/g levels. Raisins also had the highest spore-forming contamination ($7.8\times10^2 \pm 2.8\times10^2$ CFU/g), and the highest total aflatoxin (2.5 ± 1.1 µg/kg) and Ochratoxin A (2.6 ± 1.6 µg/kg) contamination among the tested samples. Fungi were detected in 67% of the dried fruit samples (Guirguis, 2018). A study of 20 dried fruit (raisins, dates, dried plums, figs and apricot) samples from Jeddah, Saudi Arabia, identified seven fungal genera, with *Aspergillus* (80.6 %), *Rhizopus* (13.6 %), and *Penicillium* (3.3 %) being dominant. Only *Aspergillus flavus* and *A. parasiticus* produced mycotoxins, particularly aflatoxins (Al Ghamdi et al., 2019). A study on hard and soft dates purchased from a market in Nigeria, found bacterial loads ranging from 8×10^5 to 1.9×10^6 CFU/g in hard dates and 10^6 to 2×10^6 CFU/g in soft dates. Isolated bacteria included *Staphylococcus aureus*, *Streptococcus* sp., *Proteus mirabilis*, *Enterobacter* sp., *Escherichia coli*, and *Salmonella* sp. Coliform counts ranged from 3 to 9 in hard dates and 11 to 23 in soft dates. Mould counts were 1.0×10^2 to 2.8×10^2 CFU/g in hard dates and 4.8×10^3 to 7.2×10^3 CFU/g in soft dates, while yeast counts were 1.6×10^2–8.2×10^3 CFU/g in hard dates and 5×10^4–1.6×10^5 CFU/g in soft dates. The study recommends developing preservation methods to prevent the microbial contamination and ensure storage under hygienic conditions (Risiquat, 2013).

3.2.1 Drying techniques throughout the ages

The Middle East and Oriental culture were the first to use dehydration for fish and meat preservation back in 12,000 BC. They only had the sun as an energy source. Roman people and Mesopotamians were fond of sweets and therefore consumed dried fruits, mostly dried dates and figs. Moreover, Mesopotamians also made sugar syrup from dates that was used for cooking, baking, and as an ingredient in the making of barley beer. During the Iron Age, people were experimenting with meat drying. In the eleventh century, in Italy and Arabia, people were experimenting with the drying of processed food such as pasta. During the Middle Ages, people used fires to generate enough heat to dry foods due to the lack of sunlight. As technology advanced, people used different mechanical techniques to speed up the drying process. In 1795, the first dehydrator was established for drying

fruits and vegetables. Nowadays, modern dehydrators generate constant heat and temperature, making the process more reliable and quicker (Widell et al., 2013; Joardder and Masud, 2019; Jena et al. 2022).

Drying and dehydration both remove moisture from the fresh fruits, however, the definitions differ. Drying refers to the removal of unbound water, while dehydration is employed to remove the moisture to reach 2% to 5% of the initial weight. Fresh fruits, such as grapes, apricots and berries can be dried as whole. Fruits such as kiwis, papayas and mangoes are dried in slices, while peaches and plums can be dried in halves (Bourdoux et al., 2016, Shah et al., 2022). Drying preserves food by lowering its moisture content and water activity, which limits the amount of water available for microorganisms to grow. As a result, dried fruits with increased shelf-life become easier to handle, store, and transport, (Średnicka-Tober et al., 2020).

The most used method for the drying of fruits, vegetables, herbs and spices is the convective hot air drying. This method uses chambers, belts, or tunnels with controlled airflow and temperature. The drying process can be carried out at atmospheric pressure. It is faster and more efficient than solar drying, but the typical drying time still ranges from a few hours to a day (Saravacos and Kostaropoulos, 2002). Apart from the drying time, the other main disadvantage is that it changes the physical, mechanical, chemical and nutritional values. The heat-sensitive nutrients such as phenolics, flavonoids, anthocyanins, and ascorbic acid are degraded during hot air drying. In contrast, techniques such as vacuum drying or freeze-drying operate at low temperatures or under reduced pressure, which helps in preserving heat-sensitive bioactive compounds, and flavour. Combining hot air with techniques such as microwave, infrared, or ultrasonic pretreatments can improve product quality and reduce drying time (Bourdoux et al., 2016; Radojčin et al., 2021).

Other techniques used for the drying of fruits include osmotic, vacuum, solar, microwave and freeze-drying. Osmotic drying involves immersing the food product in a hypertonic solution to remove water without phase change, and often used as a pretreatment to other drying methods. It improves energy efficiency, flavour, texture, and pigment stability but may cause minor nutrient losses due to solute diffusion (Akbarian et al., 2014; Bikić and Mitrevski, 2015). Subsequent drying or preservation steps, such as freezing or vacuum drying, are usually required for shelf stability (Radojčin et al., 2021). Vacuum drying removes moisture by creating low-pressure conditions inside the drying chamber, which decreases the saturation vapor pressure at a given temperature and allows water vapor to be constantly eliminated. The drying takes place at reduced temperatures, thus, heat transfer occurs primarily through conduction or radiation, minimizing thermal damage to the product. This technique is suitable for preserving the quality of heat-sensitive food products (Saberian et al., 2014; Shah et al., 2022). Solar builds on traditional sun drying by using different systems to capture solar energy more efficiently, particularly in tropical and subtropical regions. Natural convection solar dryers, such as

the cabinet type solar dryer, are inexpensive and require no external power, however, they often have unsatisfactory drying rates. To improve performance, forced convection solar dryers, including tunnel, greenhouse, roof-integrated, and solar-assisted systems have been developed and tested for drying a wide range of products such as fruits, vegetables, grains, spices, fish, and meat, offering better control and scalability (Janjai and Bala, 2012; Natarajan et al., 2022). Microwave drying uses electromagnetic waves to rapidly heat food volumetrically, which provides fast drying with low energy consumption. The most commonly used frequency is 2450 MHz. However, uneven heating can occur due to product size or composition, so it is often combined with hot air or vacuum drying to improve uniformity and efficiency (Arballo et al., 2010; Shah et al., 2022). Freeze-drying, also known as lyophilization, removes water by sublimation at low temperatures. Therefore, it is the preferred method for drying plant-based foods, such as apple, guava, strawberry, blackberry, pumpkin, tomato, asparagus, coffee, tea, garlic and ginger, as the bioactive compounds, flavour, and structure is preserved well. Its main drawbacks are high cost and long processing time, though pretreatments such as pulsed electric fields (PEF) can shorten drying time significantly (Fan et al., 2019; Bhatta et al., 2020; Shah et al., 2022).

3.2.2 Outbreaks associated with low-water activity food products (LMFs)

Dried fruits belong to the low water activity food product category. As mentioned in Chapter 3.2.2 the water activity of dried fruits (dry foods in general) should be 0.60 or lower to ensure food safety and quality, as microbial growth is inhibited under this value. However, in the last decades, several outbreaks have been linked to the consumption of dry food products. Table 1 presents a list of pathogen infections associated with the consumption of contaminated dry food products.

Table 1 Foodborne outbreaks associated with low-water activity food products (Source: Beuchat et al., 2011; Bourdoux et al., 2016; Dobó et al., 2023).

Food product	Year	Pathogen	Location	Number affected	Remarks
Almond	2000	S*almonella enteritidis* PT30	USA, Canada	168	Outbreak strain was detected in raw almonds collected from orchards and distribution, warehouse, retail, and home locations, and from processing equipment 6 - 7 months after being used. Frequent and prolonged recovery suggests diffuse and persistent contamination.
	2003	*S. enteritidis* PT9c	USA, Canada	29	Raw almonds from an opened package, one environmental sample collected at the manufacturer, and tree samples from two huller-shellers that supplied the manufacturer were positive for Salmonella.
	2005	*S. enteritidis*	Sweden	15	Phage type of isolates from patients was the same as the phage type of isolates from the 2000 almond-associated outbreak.
Black pepper	1981	*Salmonella enterica* serovar Oranienburg	Norway	126	*S.* oranienburg was isolated from six samples of black, ground pepper from the patients' households and from 15 samples of black, ground pepper from unopened, original packings.
Black and red pepper	2009-2010	*S. enterica* serovar Montevideo	USA	272	The outbreak strain was isolated from salami products made with contaminated black and red pepper.
Cereal (toasted)	1998	*S. enterica* serovar Agona	USA	209	An opened box of cereal yielded a S. Agona isolate with a PFGE pattern indistinguishable from the predominant PFGE pattern among outbreak-associated clinical isolates. Cereal from unopened boxes was also positive for S. Agona.
Cereal (puffed)	2008	*S. enterica* serovar Agona	USA	28	Puffed rice and puffed wheat cereals implicated in the outbreak were manufactured at the same plant that manufactured toasted oat cereal implicated in a 1998 outbreak of S. Agona infections. S. Agona was isolated from the plant and from bags of puffed rice cereals.
Chocolate	1973	*S. enterica* serovar Eastbourne	Canada, USA	95	Levels of 2 - 9 salmonellae per chocolate bar were estimated. Deficiencies in plant operations coupled with inadequate quality control contributed to spread of Salmonella to different areas of the plant.
	1982	*S. enterica* serovar Napoli	UK	245	Chocolate bars produced on at least 11 days over a 6-week period contained Salmonella. Bars containing a low number of Salmonella caused illness at least 7 months after manufacture. S. Napoli was isolated from bars 12 months after manufacture.
	1985	*S. enterica* serovar Nima	Canada, USA	33	Suspect chocolate contained 4.3 - 24 S. Nima per 100 g, suggesting that small numbers can cause clinical symptoms. Samples were positive for S. Nima at least 19 months after manufacture.
	1987	*Salmonella typhimurium*	Norway, Finland	361	Levels of ≤ 10 *S.* Typhimurium per 100 g were detected in 91% of positive samples, suggesting that low numbers can cause infections. Other serotypes were isolated from dust collected from rooms in which cocoa beans were stored or rinsed.
	2022	*Salmonella enterica* serovar Typhimurium	Belgium, France, Germany, Ireland, Luxembourg, the Netherlands, Norway, Spain,	151	The outbreak was linked to chocolate products from Belgium. These products were distributed to at least 113 countries and territories across all WHO Regions.

Food product	Year	Pathogen	Location	Number affected	Remarks
Coconut	1953	*Salmonella enterica* serovar Typhi *Salmonella enterica* serovar Senftenberg *Salmonella enterica* serovar Potsdam *Salmonella enterica* serovar Orion	Australia	>50	Salmonellae were isolated from packets of desiccated coconut obtained from households and from unopened cartons at retail and wholesale.
	1999	Salmonella enterica serovar Java phage type Dundee	UK	18	Seventy-one percent (128 of 181 samples) of desiccated coconut obtained from retail packets and sacks in warehouses yielded the outbreak strain.
Curry	2009	*Bacillus cereus*	Belgium	7	The causative agent was detected in curry.
Dried fruit/ice cream served in open containers	2005	Hepatitis A	Denmark	4	The ice cream was served in large plastic containers, open for self-service with communal spoon placed in adjacent container of water.
Exotic dried fruits	2018	*Salmonella enterica* serovar Agbeni	Norway	56	*S.* Agbeni was isolated from the fruit mix that originated from different countries.
Flour	2016	Shiga toxin–producing *Escherichia coli* (STEC) serogroups O121 and O26	USA	63	Epidemiologic, laboratory, and traceback evidence indicated that flour produced at a General Mills facility in Kansas City, Missouri was the likely source of this outbreak.
	2018	Shiga toxin–producing *E. coli*	Germany	50	Investigations of flour samples from mills in Germany found that 10% to 21% of the samples tested positive for STEC.
	2019	*E. coli* O26	USA	21	Samples were collected at a bakery where an ill person reported eating raw dough. Records indicated that the bakery used Baker's Corner All Purpose Flour from ALDI. The outbreak strain was isolated from an unopened bag of Baker's Corner All Purpose Flour collected at the bakery. WGS results showed that the *E. coli* O26 strain identified in the Baker's Corner All Purpose Flour sample was closely related genetically to the *E. coli* O26 strain identified in ill people.
Ground cumin	2011	*B. cereus*	Finland	3	The causative agent was detected in a food product.
Halva (helva)	2001	*Salmonella enterica* serovar Typhimurium phage type 104	Australia, Germany, Norway, Sweden, UK	>70	*S.* Typhimurium DT104 was isolated from jars of halva (plain, pistachio, and chocolate flavors) and other sesame seed-based products.

Food product	Year	Pathogen	Location	Number affected	Remarks
Infant dried milk product	1985	*Salmonella enterica* serovar Ealing	UK	76	Source traced to defective factory spray drier. S. Ealing was isolated from scrapings taken from a silo into which waste powder and dust (sweepings) were deposited.
Infant food/ cereal	1995	*Salmonella enterica* serovar Senftenberg	UK	5	Cleaning remains from milling machinery were implicated as sources of contamination. The HACCP system was evaluated and highlighted this hazard.
In-shell hazelnut	2011	*E. coli* O157:H7	USA	8	Most of the in-shell hazelnuts were purchased from bulk bins at retail food stores. Source tracing identified a common distributor for the hazelnuts consumed by ill people: DeFranco & Sons in Los Angeles, California.
Paprika and paprika-powdered potato chips	1993	*Salmonella enterica* serovar Saintpaul, *Salmonella enterica* serovar Rubislaw, *Salmonella enterica* serovar Javiana	Germany	>1000	Pathogens were isolated at the same time period from paprika powder, spice mixtures, snacks and patients.
Powdered milk formula	1998	*Enterobacter sakazakii*	Belgium	12	10 out of 12 patients were fed orally with the same brand of powdered milk formula. *Enterobacter sakazakii* was isolated from the milk formula, as well as from several unopened cans of a single batch.
Peanuts	2001	*Salmonella enterica* serovar Stanley, *Salmonella enterica* serovar Newport	Australia, Canada, UK	109	Asian-style, dry-flavoured or roasted peanuts in their shell were implicated as contaminated sources. Unopened packets were positive for S. Stanley, S. Newport, S. Lexington, S. Kottbus, and an unnamed serotype.
Pepper (black)	1981	*Salmonella enterica* serovar Oranienburg	Norway	126	Consumption of minced meat and/or minced fish products containing ground black pepper was associated with infection. *S.* Oranienburg was isolated from an unopened package of pepper. Counts of 10 - > 240/100 g were found in 12 samples positive for *S.* Oranienburg.
	2011	*B. cereus*	Denmark	52	The causative agent was detected in the food product.
Potato crisps (chips)	1993	*Salmonella enterica* serovar Saintpaul, *Salmonella enterica* serovar Rubislaw, *Salmonella enterica* serovar Javiana	Germany	1000	Paprika powder and chips seasoned with powder contained salmonellae. Analysis of paprika powder yielded 2.5 salmonellae/g; a second count 8 months later revealed 0.7 salmonellae/g. Snacks with as few as 0.04 salmonellae/g caused infection. The importance of HACCP, including production of paprika powder, was emphasized.
Powdered infant formula	1986	*C. sakazakii*	Iceland	3	Four strains of *Enterobacter sakazakii* (*Cronobacter* spp.) isolated from infected neonates were indistinguishable from 22 strains isolated from formula.
	1988	*C. sakazakii*	USA	4	Blender used to prepare formula was contaminated with *C. sakazakii*. Pathogen was found in the powdered milk formula.
	1993	*Salmonella enterica* serovar Tennessee	USA, Canada	3	In addition to powdered infant formula, other spray-dried products (medical food supplement, protein supplement, medical meal replacement, powdered milk, diet beverage, and weaning formula) manufactured at the same plant were recalled.

Food product	Year	Pathogen	Location	Number affected	Remarks
Powdered infant formula	1998	*C. sakazakii*	Belgium	12	*C. sakazakii* was isolated from the implicated prepared milk formula as well as from unopened cans of powdered milk formula. Recommendations for preparing and handling infant milk formula were made, with the goal of enhancing safety.
	2001	*C. sakazakii*	USA	11	*C. sakazakii* was isolated from unopened and opened cans of powdered infant formula. PFGE patterns of these isolates were indistinguishable from a clinical isolate.
	2008	*Salmonella enterica* serovar Give	France	8	All cases were associated with consumption of a single brand of powdered infant milk formula.
Powdered milk	1973	*Salmonella enterica* serovar Derby	Trinidad	3000	Imported dried milk products were packaged in a single plant. Workers may have been vehicles of S. Derby.
	2004	*S. aureus*	China	150	Old milk powder was suspected to have been repackaged. Powder contained *S. aureus* toxin.
Raw cake batter	2021	*E. coli*	USA	16	Whole genome sequencing (WGS) showed that bacterial isolates from infected individuals are closely related, indicating a likely exposure to the same food source.
Raw cookie dough	2009	*E. coli* O157:H7	USA	72	A culture of a sample of prepackaged Nestle Toll House refrigerated cookie dough yielded *E. coli* O157:H7.
Salami	1994	*E. coli* O157:H7	USA	4	Isolates from intact packages of dry fermented salami collected from the plant warehouse, at retail, and from patients had identical PFGE patterns. Estimated infectious dose was 2 - 45 cells.
Semi-dried tomatoes	2011	Hepatitis A virus	England	2	All patients consumed semi-dried tomatoes, which were contaminated with multiple strains of the virus.
Snack (rice/ corn)	2007	*Salmonella enterica* serovar Wandsworth, *Salmonella enterica* serovar Typhimurium	USA	75	S. Wandsworth and S. Typhimurium were isolated from sealed bags of a rice/corn vegetable-coated snack intended for children.
Spices and herbs	2007	*Salmonella enterica* serovar Senftenberg	Denmark	3	The causative agent was detected in the food product.
	2007	*B. cereus*	France	146	The causative agent was detected in the food product.
Spice mix containing dried vegetables	2015	*Salmonella enterica* serovar Enteritidis	Sweden	174	The outbreak strain was isolated from two different brands of imported dried-vegetable spice mix.
Tea (aniseed)	2003	*Salmonella enterica* serovar Agona	Germany	42	S. Agona was isolated from six brands of tea containing aniseed. Various serotypes were isolated from 61 (11%) of 575 tea and other products containing aniseed. S. Agona survived when exposure to hot water during tea-making.
Turmeric	2011	*B. cereus*	Finland	23	The causative agent was detected in the food product.

3.3 Food irradiation

The modern food industry is facing complex challenges as the population increases, including food safety, food quality, while also focusing on food waste reduction and environmental sustainability. The growing middle class demands a constat supply of diverse, conveniently packaged, fresh and chemical free food products. In years to come, conventional food preservation methods won't be able to suffice all these needs, giving opportunities for more modern and innovative technologies that deliver minimally processed and additive-free products all year around. Moreover, these conventional techniques are mostly used due to their easy applicability and lower costs. Thermal treatments, such as cooking, pasteurization, sterilization, cooling, freezing, however, have negative effects on the nutritional, functional and sensory properties of food products. The photo-oxidation leads to the degradation of fat-soluble vitamins, while water-soluble vitamins are affected by leaching (Petruzzi et al., 2017; Herrera-Ardila et al., 2022). Off-odours and off-taste often develop during heat treatments, while colour changes, loss of freshness and nutrients remain a problem for the food industry (Ling et al., 2015). To counteract the negative effects of heat treatments, and to offer a new perspective, new, non-thermal technologies have been developed. These include ionizing irradiation, high pressure process (HPP), pulse electric field (PEF), and cold plasma (CP) (Li et al., 2025a).

One of these new and emerging technologies is food irradiation that has been used for food preservation for more than 100 years, however in the early 20th century, food irradiation research was mostly theoretical and not widely applied in practice. However, Eisenhower's program called the "Atoms for Piece" motivated the U.S. institutions to invest in studies of irradiation not only for military but for civilian uses as well. This encouraged other countries to follow this model, leading to global collaboration with focus on determining whether irradiated food is safe to consume (Ehlermann, 2016; Farkas and Mohácsi-Farkas, 2011; Pillai and Shayanfar, 2016).

3.3.1 Introduction to irradiation

Electromagnetic radiation refers to the stream of photons with different energies. It can be divided to non-ionizing such as heat, visible light, radio waves, microwaves, or ionizing such as gamma rays, electron beam or X-rays (Figure 1). Ionizing radiation can remove electrons from atoms or molecules, creating charged particles (ions). These radiations have much shorter wavelengths, higher frequencies, and higher energies than non-ionizing radiation. Ionization occurs when electrons gain enough energy to escape the nucleus's control, which usually requires 4–20 eV depending on the atom. Gamma and X-rays exceed this energy threshold. When radiation energy is below the ionization potential, the excitation energy generates heat, as seen in microwave or radio frequency applications. For food processing, three types of radiation can be used, including gamma rays from radio nuclides (Cobalt-60 or Cesium-137), electron beam (e-beam) irradiation from linear accelerators (LINAC), and X-rays

also from X ray tubes or LINAC (Pillai and Shayanfar, 2016; Fan and Niemira, 2020). The statement is based on the Codex Alimentarius standard, which represents the joint position of the Food and Agriculture Organization (FAO) and World Health Organization (WHO) and specifies energy limits for safe food irradiation. The International Atomic Energy Agency (IAEA) guidelines also highlight these three technologies, and together, these three organizations jointly recognise them as acceptable for safe food irradiation (Web 11).

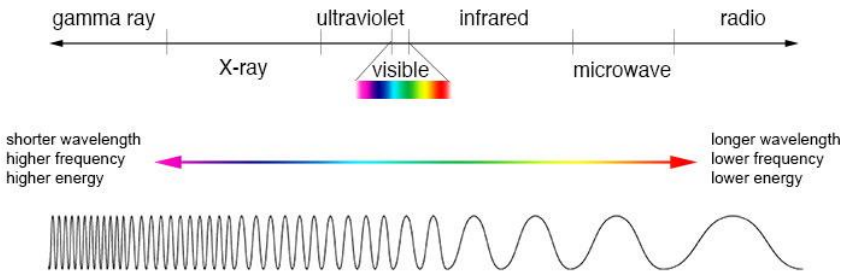

Figure 1 The electromagnetic spectrum (From: Fan and Niemira, 2020).

Gamma irradiation uses radioactive sources which emit high energy photons with no mass. Due to this, gamma rays can penetrate materials even with high density. The penetration depth depends on their energy (electron volts or mega electron volts), as Cobalt-60 emits photons at 1.17-1.33 MeV, while Cesium-137 emits at 0.662 MeV. Since Cobalt-60 has better penetrating power, it became the main source of commercial food irradiator facilities. The energy of gamma photons is typically expressed in electron volts (MeV), while the absorbed radiation dose in the target material is measured in Grays (Gy) (Kavaz et al., 2022). The energy from the sources is neither enough to induce radioactivity in the target, nor in any other material, thus making it a safe technology for improving food safety (Farkas and Mohácsi-Farkas, 2011; Fan and Niemira, 2020). Another important factor is the dose rate, which is the speed at which radiation energy is deposited in the target material. Gamma irradiation has a much lower dose rate than e-beam processes or X-rays, which affects the speed of the process on an industrial scale. The unit Gray (Gy) represents the amount of radiation energy absorbed in the target material. The absorption of 1 kilojoule (kJ) of radiation energy per kilogram material equals 1 Gray (Amit et al., 2017). Depending on the doses, three categories can be distinguished, such as low doses ($\leq$ 1 kGy), medium doses (1-10 kGy), and high doses ($>$ 10 kGy). Low doses of gamma radiation, also called radurization, are used to extend shelf life by inhibiting sprouting, and delaying ripening, while also controlling insects and pests in different types of food products such as tubers, fresh fruits and vegetables, dried vegetables and spices, and grains. Medium doses are called radicidation, which reduces the levels of non-spore forming pathogen bacteria, yeasts and moulds, and even parasites in fresh seafood, poultry, fresh meat, dried fruits, spices and herbs, and animal products. The third and most severe category is radappertization, a high-dose radiation,

which eliminates spore forming pathogens in dried spices, animal-based ready-to-eat food products. This method is also used to create food suitable for immune compromised patients and astronauts. Food treated this way is similar to canned food (Stefanova et al., 2010; Mostafavi et al., 2012; Park et al., 2012; Mohácsi-Farkas, 2016; King and Josephson, 2018; Mshelia et al., 2023). A study by Balakrishnan et al. (2021) shows that gamma radiation at doses of 4-5 kGy could effectively eliminate *E. coli*, *Salmonella enterica* Typhimurium and *Aspergillus niger* from dried chilies. Rico et al. (2010) compared the effects of steaming and gamma radiation on the microbial properties of dried red pepper that was stored for 6 months at either refrigerated or room temperature. Compared to steam treatment, gamma radiation could reduce the microbial load from 10^6 CFU/g to 10^1 CFU/g, while causing minimal changes to its physiochemical properties. In another study, 6 kGy decreased the fungal contamination of chili samples by 6 log, while another study states that mould growth was not visible after irradiation at 6 kGy and after storage of 3 months (Iqbal et al., 2012, Iqbal et al., 2013). Song et al. (2014) reported that gamma irradiation of dried capsicum at doses between 1-5 kGy was able to inactivate *E. coli* O157:H7 and *Salmonella Typhimurium*. The reduction of pathogens was dose-dependent and showed minimal impact on the colour.

Gamma irradiation is a non-thermal technology with several advantages for maintaining food quality, making it a promising choice for food preservation. Notably, it can be used to preserve packaged, frozen, or fresh foods without chemical additives, while inducing nutritional changes comparable to other preservation methods (Mostafavi et al., 2012). A study by Hashem et al (2022) investigated how gamma irradiation at doses of 1.5, 2 and 4 kGy affected fresh mutton stored at -20 °C for 60 days. Researchers evaluated the nutritional, physicochemical, sensory and microbial properties. They concluded that irradiation increased oxidation and peroxide values, as well as cooking loss, while the pH was slightly lowered. The colour and flavour change were dose dependent, but tenderness, juiciness and overall acceptability remained unaffected. Another study revealed that the carbohydrate content, antioxidant capacity and protein/lipid profiles remained unchanged in wheat flour after gamma irradiation up to 1 kGy, although higher doses (5-10 kGy) resulted in irregular granule morphology and increased crystallinity (Manupriya et al., 2020). Additionally, gamma irradiation has been shown to have no negative impact on the nutritional and sensory parameters of fruits and vegetables (Bhatnagar et al., 2022). Structural proteins, enzymes and vitamins are not damaged at lower doses (Pillai and Shayanfar, 2016). However, as every technology, this also comes with downsides. Establishing gamma irradiator facilities requires high initial costs, for example, due to the need for heavy shielding. Food irradiation is conducted in a chamber, surrounded by thick concrete walls to protects personnels from ionizing radiation. When in use, the source is raised above the water, and the products usually bypass the source on a conveyor. However, the absorbed dose depends on both the exposure time, and the distance from the source. In practice, products are positioned on the

conveyor at distances ranging from approximately 0.5 to 3 meters, however, according to Farah et al. (2006) the optimal distance of the conveyor around the source is 1.70, as products must be positioned close to the source in order to capture as many gamma rays as possible. Nevertheless, the energy absorbed by the product ranges between 15 and 40 % depending on its density and positioning within the irradiation chamber (Dethier, 2016). Cobalt-60 sources are stored under water when not in use. Management of the radioactive waste also increases the operational costs, while regular dosimetry is needed to ensure accurate dose delivery. Furthermore, Cesium-137 has longer half-life compared to Cobalt-60 with 30.17 and 5.27 years respectively. Therefore, to maintain similar strength of radiation, frequent source replacement is needed. Establishing the correct dose is also challenging, as a given dose might kill certain microorganisms, others may survive (Dethier, 2016; Fan and Niemira, 2020). The appropriate dose should be determined based on the initial microbial load, the D-values of the target microorganisms, and the characteristics of the irradiated products such as the composition, water activity, and packaging. Although low irradiation doses generally do not affect the nutritional values of food products, higher doses, usually above 10 kGy, can however degrade vitamins, decompose carbohydrates and promote lipid rancidity (Danyo et al., 2024). To add to the list of disadvantages, consumer acceptance still remains a significant barrier, as consumers remain reluctant to accept irradiated food products. More information about consumer acceptance is provided in Chapter 3.3.5.

Electron beam irradiation uses high energy electron beams for the inactivation of microorganisms and viruses, with the maximum energy of 10 MeV. One of the main advantages is that it does not require radioactive isotopes thus offering economic benefits, as radioactive waste disposal is not needed. The high dose rate (~ 36,000 kGy/h) allows for a faster and more efficient irradiation compared to gamma and X-rays. Moreover, the dose, the generation and disappearance of the electrons are easy to control, the machine can be switched off as required, rendering this process an alternative to gamma and X-ray radiation (Tahergorabi et al., 2012; Fan and Niemira, 2020; Fertey at al., 2020). Various food products can be irradiated with e-beam, such as fruits (strawberry, mango, papaya), rice grains, meat, shell egg, milk, flour and cereal based products (Ward, 2017; Arshad et al., 2020; Khaneghah et al., 2020; Yoon et al., 2020b; Nguyen et al., 2021; Lin et al, 2024; Li et al., 2025b; de Oliveira et al., 2025; Tesfai et al., 2025). Moreover, in agriculture it is used to inhibit sprouting and control pathogens. Pieces of medical equipment and pharmaceuticals can also be irradiated to ensure their safety (McCoy, 2020; Xu et al., 2021, Peng et al., 2025). Despite the advantages, several studies have found drawbacks. For example, e-beam radiation has lower penetration depths thus thicker or more dense food products can be irradiated less effectively (Lucas et al., 2023). According to Hewitt and Leelawardana (2014), in high moisture food products, the practical penetration is only 3.9 cm for 10 MeV electrons. To increase dose uniformity packaged food products can be irradiated on both sides

(Pillai and Shayanfar, 2016). Another study investigated how different e-beam doses (0.3 and 7 kGy) affect frozen duck meat regarding fatty acid composition, microbiological and chemical properties. The 3 kGy dose reduced the total bacteria by more than two orders of magnitude and coliforms by 1 log cycle. The radiation slightly increased indicators of oxidation such as peroxide value, TBARS (Thiobarbituric Acid Reactive Substances, an indicator of lipid oxidation), and volatile nitrogen, but did not affect the taste or the smell. However, the meat became less light and red; vitamin A and E, and the overall fatty acid content slightly decreased after irradiation. Despite the slight change in the physiochemical properties, an electric nose could clearly tell irradiated and non-irradiated samples apart (Arshad et al., 2020). Kang el al. (2013) reported that a dose of 1 kGy or lower reduced the microbial populations of leafy vegetables by 2.0-2.5 log CFU/g. Higher doses, such as 3 kGy eliminated all microorganisms in the samples. In another study it was reported that e-beam irradiation of fresh-cut cantaloupe at doses of 0.7 and 1.5 kGy immediately reduced *Salmonella enterica* serovar Poona by 1.1 CFU/g and 3.6 CFU/g, respectively. After 21 days of storage at refrigerated temperatures (5 °C), the samples showed a 4 log reduction, compared to the control samples (Palekar et al., 2015). Shayanfar et al. (2017) concluded that using 1 kGy e-beam irradiation to strawberries could significantly reduce the *E. coli* infection risks, achieving about a 4-log reduction of different non-O157 Shiga toxin producing *E. coli* serotypes (O26:H11, O45:H2, O103:H2, O111:NM, O121:H19, and O145) in strawberry purée.

X-rays are produced when high energy electrons strike a metal target such as tantalum, tungsten or gold with energies up to 5 or 7.5 MeV. This form of radiation is an alternative to gamma radiation or e-beam radiation, furthermore, X-ray machines can be switched off, offering operational flexibility and improved safety compared to radioisotope sources. Other benefits include a more cost-effective equipment compared to gamma irradiators, and they avoid the use of hazardous radioactive isotopes. Additionally, X-rays penetrate deeper than e-beams, making it a promising option for targeting complex structures such as biofilms (Kebede et al., 2015; Kasmudin et al., 2022; Indiarto et al., 2020; Cha and Ha, 2022). Furthermore, the dose rate of X-rays (100 kGy/h) is greater than that of gamma rays (10 kGy/h) (Pillai and Shayanfar, 2016). According to several studies, X-rays can be applied to various food products such as strawberry with the dose of 0-1 kGy, spinach leaf with the dose of 0.3 Gy plus 1 % citric acid, sliced cheese with doses of 0.2, 0.4, 0.6, and 0.8 kGy, lettuce with the dose of 0.05-0.3 kGy plus 0.5 % gallic acid, and rice with the maximum dose of 1-5 kGy (Park and Ha, 2019; Begum et al., 2020; Jeon and Ha, 2020a; Jeon and Ha, 2020b; Yoon et al., 2020a). This type of radiation can also be used to clean food contact surfaces (Cha and Ha, 2022). However, this technology presents some limitations, including lower dose uniformity compared to gamma irradiation, and high energy requirements for the generation of X-rays (Web 12).

The advantages and disadvantages of the discussed radiation types are presented in Table 2.

Table 2 Advantages and disadvantages of gamma, electron and X-ray.

Radiation type	Advantages	Disadvantages
Gamma	Non-thermal	High initial costs
	High penetration depth →suitable for dense products	Cobalt-60 source must be frequently replaced
	Can be applied to various food products, including packaged and frozen	Management of radioactive waste is costly
	Preserves food quality (nutritional and sensory parameters)	Lower dose rate compared to electron beam
	Inhibits sprouting, delays ripening, controls insects and pests	Regular dosimetry is needed
	Reduces the levels of non-spore forming pathogen bacteria, yeasts and moulds, and even parasites; eliminates spore-forming pathogens	Limited consumer acceptance
	-	Higher doses (5-10 kGy) can degrade vitamins, carbohydrates, and promote lipid oxidation
Electron beam	Non-thermal	Limited penetration depth→not suitable for dense or bulk products
	No radioactive isotopes	Limited consumer acceptance
	High dose-rate, more efficient irradiation than gamma and X-rays	High energy requirement
	Machine can be switched off→operational flexibility	-
	The dose is easy to control	-
	Applicable to various food products	-
	Inhibits sprouting, delayes ripening, controls insects and pests	-
	Reduces the levels of non-spore forming pathogen bacteria, yeasts and moulds, and even parasites; eliminates spore-forming pathogens	-
	Preserves food quality (nutritional and sensory parameters)	-
X-ray	Non-thermal	Lower dose uniformity compared to gamma
	No radioactive isotopes	High energy requirement
	Higher penetration depth than electron beam	Limited consumer acceptance
	Machine can be switched off→operational flexibility	-
	Greater dose rate than gamma	-
	Applicable to food products and surfaces	-
	Reduces pathogenic and spoilage bacteria, viruses and parasites	-

3.3.2 Microbial cell inactivation mechanisms of irradiation

Irradiation inactivates microorganisms through both direct and indirect mechanisms. Direct effects occur when ionising radiation damages sensitive molecules, such as DNA, proteins and cell membranes, directly through e-beams, gamma and X-rays. Ionising radiation transfers energy to these critical cell components, resulting in their ionization and molecular bond disruption that interferes with essential metabolic functions. For example, in the case of DNA, the absorbed energy can not only cause single and double-strand breaks, but also base oxidation and cross linking between DNA and proteins (Douki et al., 2006; Nakano et al., 2017). All of these compromise the integrity of the genetic material and replication.

Indirect damage happens when radiation ionizes water (radiolysis), generating free radicals such as hydroxyl radicals (•OH), H^+ ions, hydrated protons, and hydrogen peroxide (H_2O_2) that attack DNA, cell membrane and other cell components indirectly. These reactive species cause oxidative stress, leading to the oxidation of lipids and proteins and to the damage of nucleic acids. They also damage the purine and pyrimidine bases and the deoxyribose sugar which results in the breakage of sugar-phosphate bonds. While different enzymes and DNA repair systems can repair single and double strand DNA breaks, complex double-stranded breaks and oxidative base lesions are usually irreparable, resulting in the loss of viability. At typical doses (1 kGy), 10 to 100 of such breaks can occur, reducing microbial populations and thereby extending food shelf life. Higher doses can also affect plasmid DNA, RNA, membranes, and enzymes (Eccles et al., 2011; Pillai and Shayanfary, 2016; Fan and Niemira, 2020; Kim et al., 2024).

3.3.3 Microbial resistance and factors influencing food irradiation

Microbial resistance to ionizing radiation is expressed by the D-value, which represents the radiation dose needed to reduce the microbial population by one logarithmic unit, or in other words, the dose needed to inactivate 90 % of the bacterial population. Processing doses for food irradiation are chosen based on the desired log reduction and vary with factors influencing irradiation efficiency, such as the organism type (e.g., *E. coli* D ≈ 0.1 kGy, while *Salmonella* sp. ≈ 0.2 kGy), food composition (high moisture or dry), temperature (fresh or frozen), oxygen level, packaging atmosphere, and the physiological state of the cells (Pillai and Shayanfar, 2016; Pi et al., 2021). Radiation sensitivity is inversely proportional to the size of the genome, thus bacteria are more sensitive to irradiation than viruses (Shahi et al., 2021). The susceptibility of most fungi to radiation is comparable to that of the vegetative bacterial cells, however, moulds tend to be more sensitive to irradiation than yeasts, and yeasts are more sensitive than bacteria (Farkas, 2006; Arapcheska et al., 2020). Bacterial spores are more resistant than vegetative bacteria and fungi. This resistance comes from multiple protective mechanisms. For example, the high concentrations of calcium-dipicolinic acid (Ca-DPA) protect against desiccation, DNA damage, and helps maintain dormancy. The cortex of the endospores contributes to the resistance by reducing its internal water content through dehydration, thus it reduces indirect radiation damage. Moreover, small acid-soluble proteins (SASPs) bind to the endospore DNA, shielding it from radiation damage. The thick coat, crust and inner membrane further act as a physical shield (Cortesão et al., 2019). Generally, Gram-negative bacteria are more sensitive to gamma radiation than Gram-positive bacteria, and rods are more sensitive than cocci (Beauchamp and Lacroix, 2012; Arapcheska et al., 2020).

Both direct and indirect irradiation destroy microorganisms, however direct radiation works better if the surface is uniformly exposed, while indirect action, generating free radicals is more effective in

water-rich food products (Li et al., 2025a). Furthermore, oxygen amplifies these reactions, promoting peroxide and ozone formation, which can further effect microbial viability (Fan and Niemira, 2020).

3.3.4 Regulations for labelling

Most countries follow the Codex General Standard on Labelling of Pre-packaged Foods, but the interpretation may vary among countries (CAC, 2010). The Radura symbol is an international symbol indicating that a food product has been treated with ionizing radiation. It is displayed in green, consisting of a plant (central dot with two leaves) within a circle, with the upper dashed arc representing irradiation by ionizing rays (Ehlermann, 2009). The EU, Australia and New Zealand require clear text labelling for all irradiated foods and ingredients, with no minimum concentration limits (labelling is required, regardless of the proportion of irradiated ingredients). In the EU, the wording is specified but the Radura logo (Figure2) is not required, while in Australia/New Zealand the wording is not fixed by law, there are only suggestions, and the Radura symbol is optional (EU, 1999; ANZFSC, 2016). In the U.S., whole foods must display the Radura logo and wording such as "treated with radiation" or "treated by irradiation", but irradiated ingredients in non-irradiated products need not be labelled (USFDA, 2015). Malaysia and Canada only require labelling if irradiated components exceed 5% and 10% of product weight, respectively (MOHM, 2011; CFIS, 2014). Some nations, like Indonesia, mandate stating the purpose of irradiation, whereas others do not address it (Roberts, 2014).

Figure 2 Radura symbol (From: Web 36).

3.3.5 Consumer acceptance

The food industry is facing great challenges regarding the consumer acceptance of irradiated food products. According to Castell-Perez and Moreira (2020) consumer decisions are often guided by emotions rather than scientific evidence. Typically, concerns are derived from fears about potential health risks, despite several studies confirming that irradiated foods are safe to consume and do not become radioactive (Feliciano, 2018; Ravindran and Jaiswal, 2019; Satin, 2020). Acceptance levels depend on different demographic factors, such as place of habitat, age and education. For example, U.S. consumers are more likely to accept irradiated food products than Europeans, while acceptance in Asia is growing (Hohl and Gaskell, 2008; Roberts and Henon, 2015; Galati et al., 2019). Elderly consumers are more likely to reject irradiated food products than younger ones (Spaulding et al., 2007). Other studies suggest that consumers with adequate background information on irradiation are more likely to buy irradiated products (Shahbaz et al., 2016; Finten et al., 2017). Providing helpful information on social media has been shown as a potentially effective approach to increase irradiated food acceptance (Fan and Niemira, 2020).

Several myths exist about irradiated food products. Common misconceptions include that irradiation makes food radioactive and thus can cause cancer. Interestingly, e-beam radiation is more accepted by consumers as it does not require radioisotopes (Pi et al., 2021). Other studies also confirmed that consumers prefer machine generated ionizing radiation to radioactive sources (Tahergorabi et al., 2012). Other consumers are afraid that the process of irradiation is only used to cover up poor hygiene practices in food processing facilities (Pillai and Shayanfar, 2016; Castell-Perez and Moreira, 2020). Another common misconception is that irradiated foods have less nutritional value. Consumers are not aware that all food processing technologies have an effect on the micro-and macrocomponents to different extents. Prakash (2020) stated that as a non-thermal technology, irradiation does not heat up food products, preserving nutrients better than thermal processes. Furthermore, irradiation does not cause changes in the organoleptic properties of food products (Nyamakwere et al., 2022; Mashak and Abbasi, 2023). Another barrier is consumer resistance to purchasing irradiated foods, along with concerns for high prices. Establishing irradiation facilities comes with great costs and food producers are likely to pass the cost of these treatments to consumers (Roberts, 2014). Moreover, consumers like to have personal gains from the products they buy. However, they fail to see personal benefits, such as the extended shelf-life, or the ability to process foods in packages or large containers (Castell-Perez and Moreira, 2020).

According to Figure 3, food irradiation is perceived negatively among consumers, as it is harmful and dangerous, while conventional techniques such as canning or freezing were placed positively. Regarding the axis ranging from well understood to unknown/uncertain, most food processing technologies fall to the negative sided, including the previously mentioned canning and freezing. Researchers believe that in the future, the Radura symbol might become highly valuable, as it could distinguish companies that use advanced pathogen-elimination technologies from those that do not. Food traceability is becoming more important to consumers, but it also helps in identifying food adulteration, mislabelling, counterfeiting, and food safety (Pillai and Shayanfar, 2016).

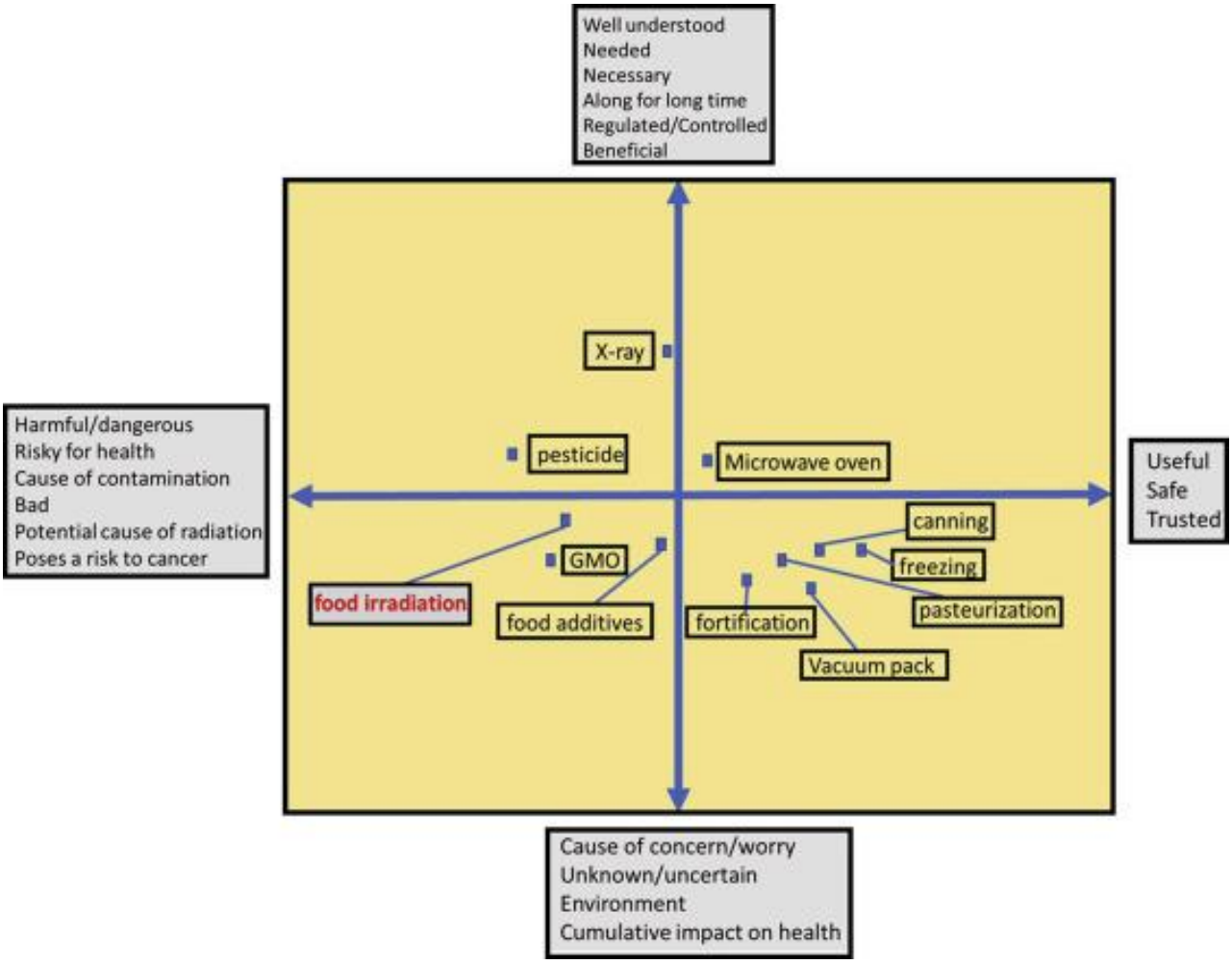

Figure 3 Consumer perception of food preservation techniques (From: Castell-Perez and Moreira, 2020).

3.4 Stress responses and cross protection mechanisms in *Escherichia coli* under food-related stress conditions

Researchers have observed that microorganisms exposed to sub-lethal stress can often withstand subsequent stresses that would otherwise be lethal. Furthermore, stress-adapted bacteria can withstand both similar and unrelated stresses. In microbiology, stress is defined as a harmful factor or condition that negatively impacts microbial growth or survival. According to this definition, a wide range of food processing treatments can be regarded as stressors. During food processing, the most frequently encountered stresses include physical treatments (heat, pressure, electric pulses, irradiation, osmotic shock, and ultrasonic waves), addition of chemicals (acids, salts, oxidants), and biological stresses (competition, microbiological metabolites, antagonism). Stress may be mild, moderate, or severe and can range from sub-lethal levels of stress, that do not affect viability, to lethal levels that reduce microbial populations (Yousef and Courtney, 2003; Bucur et al., 2018). To withstand stress, bacteria

use different mechanisms that either eliminate the stressor; repair or remove damaged DNA, proteins and lipids; increase tolerance to the stressor; facilitate the entry to a dormant state such as VBNC or spore formation; or undergo beneficial adaptive mutations (Hottes et al., 2013; Sokolova , 2013; Kołodziej et al., 2018; Wagley et al., 2021; Dawan and Ahn, 2022; Kapinusova et al., 2023). Traditional food processing techniques are more likely to lethally injure the cells, than minimal-processing techniques such as food irradiation (Sahoo et al., 2023).

Bacterial stress response is tightly regulated to ensure that the protective proteins are produced only under stress. This regulation can occur at multiple levels, including transcription, translation, and control of the mRNA or protein stability. This regulation depends both on the organism and on the type of stress. One of the main strategies in stress response is the transcriptional regulation through sigma factors. While housekeeping genes are transcribed under the control of constitutive sigma factors (for example σ^{70} in *E. coli*), the alternative sigma factors redirect RNA polymerase to transcribe stress-response genes. Their activity can be modulated by anti-sigma factors, as seen in the regulation of σ^{S} in *E. coli*. Anti-sigma factors bind sigma factors, until stress sensors triggers their release, resulting in the transcription of the stress related genes. Repressor proteins also regulate stress genes by preventing transcription by blocking the promoter region of specific gene. At the translational level, mRNA secondary structures can prevent ribosome binding, until stress disrupts these structures. The heat shock sigma factor (σ^{32}) is regulated like this. Another level of control is the stability of mRNA and proteins. For example, the CspA mRNA involved in cold tolerance is only stable at lower temperatures, while the σ^{S} is degraded by ClpXP protease under non-stress conditions (Yousef and Courtney, 2003).

Stress sensing is crucial for adaptation. For example, cold stress affects the mRNA secondary structure, thus enabling enhanced translation. This is aided by a surveillance system involving RNase R and cold shock proteins (Csps) that dynamically modulate mRNA conformation, thus resulting in changes in the protein expression levels (Zhang et al., 2018b). Proteomic analysis of *E. coli* showed that various stressors, such as oxidizing and denaturing agents induced significant upregulation of heat shock proteins/foldases, transcription/translation-related proteins, and metabolic enzymes (Han et al., 2008). Ribosomes can act as stress sensors that promote structural modifications, such as the cleavage of 43 nucleotides at the 3′-end of 16S rRNA, leading to selective translation (Shen et al., 2025). The heat sensitivity of ribosomes makes them vulnerable to heat shock (Yousef and Courtney, 2003). Bacteria adapt membrane fluidity under stress by modifying fatty acid content. Low temperature can reversibly decrease membrane fluidity, turning fluid membranes into gels. In this phase, the protein functions are impaired, affecting cell wall synthesis, cell division and energy production (Bramkamp, 2022).

In *E. coli*, the general stress response under diverse environmental stresses is controlled by the alternative sigma factor RpoS (σ^S), that controls a large set of genes, which comprises 10 % of the *E. coli* genome. During the exponential growth phase, RpoS levels are normally low due to tight control at transcriptional and translational levels. However, upon stress, including nutrient limitation, osmotic shock, oxidative stress, and DNA damage, RpoS-dependent genes are induced while RpoS degradation is inhibited, inducing broad stress resistance. This global induction explains cross-resistance. Cross-resistance suggests that RpoS regulated genes are activated by diverse stresses, while also protecting from other stressors (Battesti et al., 2011). Cross resistance is well documented. For example, starvation can lead to increased tolerance to heat or UV-C radiation, while osmotic stress can also increase heat tolerance in *E. coli*. Similarly, alkaline pH improved the UV-C radiation tolerance, while acid adaptation led to increased heat resistance (Rangel, 2011; Horn and Bhunia, 2018). Supporting the concept of stress-induced cross-protection, Zorraquino et al. (2017) found that *E. coli* exposed to acidic and osmotic stress developed increased resilience through cross-stress protection, while Dragosits et al. (2013) reported that pre-exposed to acidic or oxidative environments improved survival under hyperosmotic stress. Additional studies have shown that initial heat and acid stress increased the resistance of *E. coli* to UV and gallic acid treatment, and UV and gallic acid resistant cells had higher resistance to heat or acid treatments (Wang et al., 2019). Other stressors such as gamma radiation were found to induce heat shock proteins including DnaK, GroES in *E. coli*, indicating an increased tolerance to subsequent heat exposure (Caillet et al., 2008). Furthermore, high salt concentrations were shown to induce cross-protection against antibiotics such as tetracycline and chloramphenicol in the antibiotic treatment of *E. coli* (Zhu and Dai, 2018). These findings emphasize that stress-induced cross-protection, which is largely mediated by RpoS, plays a critical role in bacterial persistence under diverse food-related stresses.

From a food safety point of view, cross-protection impairs the hurdle concept, which relies on the combination of preservation technologies to inactivate pathogens in food products. Sub-lethal stress exposures during processing may unintentionally enhance bacterial resilience to subsequent hurdles and thus reduce the overall effectiveness of these control measures. This highlights the need to consider cross-protection when designing food safety measures, especially in the context of fresh and minimally processed foods (Begley and Hill, 2015; Akritidou et al., 2023).

3.4.1 Osmotic stress response and adaptation mechanisms in Escherichia coli

In the food industry, bacteria may encounter osmotic stress pre-harvest, in the form of irrigation water and soil salinity, and during food processing due to food additives such as salt or sugars, or due to dehydration such as dried fruits (Albarracín et al., 2011; Yan et al., 2015; Kaur et al., 2022; Loganathachetti et al., 2022).

In *E. coli*, osmotic stress triggers a two-phase response. Initially, the cell rapidly accumulates potassium ions via the Kdp and Trk transport systems, accompanied by glutamate synthesis to balance the charge. For longer-term adaptation, potassium is gradually replaced by compatible solutes such as proline, glycine betaine, carnitine, choline and trehalose. Compatible solutes, also known as osmoprotectants are small, non-toxic molecules that help counterbalance osmotic pressure. Beyond this, these molecules also help to preserve protein structure and membrane integrity, protecting cellular functions from osmotic stress-induced damage. These osmoprotectants are taken up or synthesized through transporters such as ProP, ProU, and BetT, or through pathways encoded by otsA and otsB, many of which are regulated by RpoS. Membrane proteins also contribute to osmoregulation, with porins OmpF and OmpC that adjust permeability under the EnvZ-OmpR control. The choice of compatible solute can influence the metabolism. For example, proline reduces metabolic efficiency under high NaCl, while glycine betaine or choline can improve it up to 4.5% NaCl. At 5% of NaCl, glycine betaine even drives a shift from aerobic to fermentative metabolism, resembling responses seen during oxidative stress (Hussain Wani et al., 2013; Begley and Hill, 2015; Burgess et al., 2016). It was also demonstrated, that elevated trehalose production increased the thermal tolerance of *E. coli*, while also increasing the tolerance to high salt and sugar concentrations (Purvis et al., 2005). In *E*. coli, grown under high glucose (12%, weight per volume [wt/vol]) conditions, supplementation with osmoprotectans improved bacterial growth under osmotic pressure (Xiao et al., 2017a).

3.4.1.1 Changes at transcriptional level

Under osmotic stress induced by high sugar concentrations, E. coli activates multiple transcriptional and regulatory mechanisms to counteract the negative effects of high osmotic pressure. One key adaptation mechanism is the regulation of the potassium homeostasis, as prokaryotes lack the Na^+ / K^+ pumps. *E. coli* uses special potassium uptake systems, that include three transporter classes: Kdp, Kup, and Trk. The *E. coli* genome has genes responsible for all three classes (*kdp, kup*, and *trk*), while also encoding a potassium channel homolog (*kch*). Together, these systems maintain intracellular potassium levels under osmotic stress (Tanudjaja et al., 2023). Compatible solutes are another major defence. Osmolytes such as glycine beatine or proline beatine are regulated by the proU operon which encodes a high-affinity glycine betaine/proline betaine transport system in *E. coli.* It consists of three genes, such as *proV*, *proW* and *proX*. The gene *proV* codes for the nucleotide binding protein, *proW* forms the transmembrane channel, and *proX* codes for the periplasmic substrate-binding protein (Gul and Poolman, 2013). Choline uptake is similarly regulated by ProU and also by the BetT system. Under lower osmotic pressure, choline is mainly taken up by BetB, under higher concentrations ProU also transports choline (Aktas et al., 2011). Trehalose is an important compatible solute, which is synthetized in the cytoplasm by the trehalose-6-phosphate synthase (*otsA*) and trehalose-6-phosphate

phosphatase (*otsB*). It helps in maintaining membrane fluidity and overall cellular protection (Joseph et al., 2010). The general stress sigma factor (σ^S) is responsible for a broader osmotic stress response by regulating genes, such as *osmC*, and *osmY*. This sigma factor accumulates due to the increased translation of *rpoS* mRNA (Wood, 2010). *OsmC*, also an RpoS-regulated lipoprotein, is induced by osmotic stress and also provides protection against oxidative damage (Charoenwong et al., 2011). The transcription of the *osmC* gene is controlled by two promoters, $osmCp_1$ and $osmCp_2$. While $osmCp_2$ is activated by the general stress sigma factor, $osmCp_1$ is σ^S independent and is activated in response to osmotic upshifts (Davalos-Garcia et al., 2001). A study profiling early stress responses caused by high osmotic pressure by Weber and Jung (2002) revealed that *osmC* was induced under osmotic stress due to NaCl. The *talA* gene encodes transaldolase A, which is an enzyme in the pentose phosphate pathway (PPP). By facilitating NADPH production, it is essential for maintaining redox balance, providing precursors for nucleotide and nucleic acid synthesis, and even supplying metabolites for CO_2 fixation in photosynthetic organisms, thereby contributing to metabolic adaptation under high osmotic pressure. This gene is also regulated by the general stress sigma factor and was found to be induced under osmotic stress in the early stationary phase to support trehalose synthesis and increase NADPH production, which aids in oxidative stress defense (Song et al., 2006; Gu et al., 2009; Samland and Sprenger, 2009; Kocharunchitt et al., 2014; Fuentes-Lemus et al., 2023). Glucose-6-phosphate is an important precursor for trehalose production. As osmotic and oxidative stresses are potentially linked, proteins like OsmC, Dps, KatE, and AhpC are co-induced under salt stress (Weber et al., 2006). RyfA, a small non-coding RNA, also plays a role in tolerance to both stress types, and its absence reduces bacterial survival (Bessaiah et al., 2021). Phadtare et al. (2002) further confirmed *talA* upregulation under oxidative stress. The periplasimc trehalase (TreA) also plays a significant role when the osmotic pressure is high. While, under these conditions, the normal trehalose uptake pathway is blocked, *treA* hydrolyzes exogenous trehalose into glucose, which can then enter the cytoplasm via the glucose-PTS system. Additionally, treA helps recycle any trehalose that was leaked from the cytoplasm back into the periplasm, conserving this important osmoprotectant (Shrestha et al., 2024). Its transcription was found to be upregulated in response to osmotic stress, reflecting its role in cellular adaptation (Purvis et al., 2005).

3.4.2 Stress caused by ionizing radiation

Bacteria can be exposed to radiation stress at both pre-harvest and post-harvest stages. Pre-harvest exposure mostly involves non-ionizing ultraviolet (UV) radiation, while post-harvest exposure typically arises from food processing methods that use ionizing radiation, such as gamma rays or electron beam (e-beam) treatments (Gautam and Tripathi, 2016; Peng et al., 2022). Machine-generated irradiation such as e-beam works by accelerating electrons that collide with target molecules at a high

speed, resulting in changes in the molecular structure of microorganisms. Similarly, radiation emitted by radioisotopes causes changes in the microorganisms.

To overcome radiation stress *E. coli* employs a combination of transcriptional regulation and DNA repair mechanisms. Under sub-lethal irradiation, the SOS response is activated, upregulating genes such as *recA*, *recN*, and *recJ*, along with DNA repair proteins including MutM (base excision repair), DinG (recombinational DNA repair), and DnaC (chromosome replication). In addition, several other genes such as UV resistance, heat shock, acid stress and virulence genes are also induced post-irradiation (Bolsunovsky et al., 2016; Gaougaou et al., 2020). A study by Byrne et al. (2014) focused on how *E. coli* can evolve extreme resistance to ionizing radiation. They found that mutations in classical DNA repair genes such as *recA* and *dnaB* enhanced the repair of double-strand DNA breaks without requiring major changes in global gene expression. Wintenberg et al. (2023) further reported that the exposure to low-dose ionizing radiation alters the expression of genes involved in RpoS-mediated stress response, oxidative stress pathways, cell envelope biosynthesis, and amino acid metabolism.

Radiation can not only enhance DNA repair mechanisms but can also induce the viable but non-culturable (VBNC) state of bacteria. Several studies have shown that *E. coli* enters the VBNC state upon UV radiation, a form of non-ionizing radiation (Zhang et al., 2015; Zhang et al., 2018a; Alvear-Daza et al., 2021, Zhang et al., 2025). However, research on how gamma radiation triggers VBNC formation is scarce. Only one report describes the entry of bacteria into the VBNC state following gamma radiation, while another one describes a VBNC-like state based on indirect observations. For example, Koschnitzki et al. (2021) measured the integrity of the genome of *Ignicoccus hospitalis*, after exposure to high dose of gamma radiation (> 27.2 kGy) and assessed survival using RAPD (Random Amplified Polymorphic DNA) and qPCR techniques. The metabolic activity was confirmed by H_2S production, detected on lead acetate paper, while the ability of the cells to reproduce was lost. The separation of metabolic activity from cell division indicates a potential VBNC state. Further serial dilution experiments confirmed that although the cells remained metabolically active, they did not increase in number supporting the conclusion that VBNC cells were induced upon gamma irradiation. Another study investigated the potential induction of VBNC-like cells of *Staphylococcus aureus* upon gamma irradiation. Researchers have irradiated the samples at three different doses (1.2 kGy, 2.9 kGy, and 3.5 kGy), to either cause sub-lethal damage, induce the VBNC-like state, and to cause lethal inactivation, respectively. VBNC cells were not directly found, but the protein expression profiles were analysed using capillary electrophoresis. It was observed that at dose intended to induce the VBNC-like state (2.9 kGy), specific proteins were differentially expressed, suggesting that the cells were damaged but not fully inactivated. Their work supports that gamma radiation can generate

bacterial cells with characteristics of the VBNC state, however direct evidence of metabolic activity with the loss of culturability was not shown (Trudeau et al., 2012).

3.5 The viable but non-culturable (VBNC) state: a survival strategy employed by bacteria in response to stress

Upon harsh environmental conditions, bacteria activate stress response mechanisms that help them survive the stress. One of these mechanisms is the transition into the viable but non-culturable (VBNC) state. In this state, bacteria are alive, with low metabolic activity that is still measurable, with intact membranes, yet lost culturability on media that normally supports their growth, making them difficult to detect by standard microbiological methods (Zhang et al., 2018a; Liu et al., 2023). This phenomenon poses a serious challenge in food safety, as several foodborne pathogens, such as *E. coli*, *Salmonella*, *Listeria monocytogenes*, and *Staphylococcus aureus* can enter the VBNC state in response to a wide range of stresses, while also maintaining pathogenicity (Highmore et al., 2018; Fu et al., 2020; Li et al., 2020a; Ou et al., 2021). These stressors include extreme temperatures (low and high temperatures), nutrient limitation/starvation, extreme pH (acidic, alkaline), osmotic stress (sugars, salt, desiccation), drying, irradiation (UV, gamma rays, e-beam), oxidative damage, heavy metals, low oxygen levels, high carbon dioxide levels, food preservatives, and disinfectants such as chlorin/chloramin (Darcan et al., 2009; Zhao et al., 2016; Vilhena et al., 2019; Fu et al., 2020; İzgördü et al., 2022; Kovács et al., 2024; Zhang et al., 2025). VBNC bacteria have been isolated from different food products, including seafood, pork, poultry, fruit juices, milk, wine and beer according to studies summarized in a review by İzgördü et al. (2022).

Another problem for the food industry is that VBNC bacteria can be resuscitated under favourable conditions, while also regaining virulence, potentially leading to outbreaks. When VBNC cells were induced under high temperature, *E. coli* O157:H7 was successfully resuscitated by removing the stressor (Fu et al., 2020). Another study (Liu et al., 2009) found that *E. coli* O157:H7, following exposure to chloraminated tap water entered the VBNC state, and was later resuscitated. Zhang et al (2015) could successfully resuscitate *E. coli* that enter the VBNC state following UV disinfection treatment. Key virulence factors, such as toxins, adhesion proteins, and stress-response genes, remain active or are even upregulated in VBNC state. In *E. coli* O157:H7, the virulence genes such as *eae* and *hlyA*, as well as Shiga toxin genes (*stx1, stx2*) showed increased expression in the VBNC state (İzgördü et al., 2022).

Since these bacteria have the potential to regain virulence, stricter control measures need to be applied to prevent VBNC-related foodborne illnesses (Oliver, 2010; Zhang et al., 2018a). Unfortunately, food safety is often overlooked at the household level. For example, overnight oats can be sweetened with dried fruits. These are popular, convenient and nutrient rich meals that offer enough moisture and

nutritional content to potentially enable the recovery of VBNC bacteria. If consumers leave the oats at room temperature instead of refrigerating, bacteria that initially entered the VBNC state upon osmotic shock may recover, leading to health hazards. Similar risks exist in other products, such as fruit cakes, energy bars, granolas, and pastries, which are commonly paired with jams or fruit purées. Under such conditions, bacteria can adapt to hyperosmotic environments and can be later transferred to dried fruits through direct contact during processing or by cross-contamination from shared equipment and handling surfaces. For example, residual contamination on conveyors, or packaging lines may deposit osmotically pre-adapted *E. coli* onto dried fruits, where their prior adaptation provides a survival advantage. Together these scenarios highlight how everyday consumer habits and food processing practices can unintentionally create favourable conditions for VBNC bacteria to resuscitate and regain pathogenicity.

The induction of the VBNC state is linked to the regulatory systems such as the histidine kinase EnvZ, the stress-related alternative sigma factor RpoS, and the polyphosphate kinase PPK1. Recent transcriptomic studies revealed that VBNC induction is associated with a vast amount of gene expression changes. In VBNC state of *E. coli*, out of the 2298 genes detected, 1735 genes were upregulated, and the expression of 563 genes were decreased (Zhong and Zhao, 2019). Similarly, Ye et al. (2020) revealed that a total of 362 genes changed significantly in *E. coli* after chlorine stress to induce VBNC state.

Bacterial viability is not defined by a single mechanism, but rather by various processes such as respiration, membrane integrity, ATP production, DNA/RNA synthesis, and protein translation (Manina and McKinney, 2013). Several methods have been developed to assess these functions in VBNC bacteria. For example, respiratory activity can be measured with CTC (5-cyano-2,3-ditoyl tetrazolium chloride) staining, where red fluorescence indicates active respiration, while CTC-flow cytometry (CTC-FCM) can be used for single-cell analysis. Membrane integrity is often evaluated with the LIVE/DEAD BacLight kit or Propidium Monoazide–quantitative Polymerase Chain Reaction (PMA-qPCR). Meanwhile, RT-qPCR (reverse transcription qPCR) enables the monitoring of gene expression and RNA-based viability and is sometimes combined with plate counts to detect VBNC state pathogens in complex samples (Zhang et al., 2018a). These various processes highlight the need for culture-dependent methods for improving food safety, by monitoring VBNC pathogens during production.

3.6 Laboratory practices to detect, isolate, identify and characterise microorganisms from food products

This chapter provides an overview of the different laboratory practices used to detect, isolate, identify, and characterise microorganisms in food products. Both culture-dependent and culture-independent methods are introduced to highlight their roles, advantages, and limitations in the field of food microbiology.

3.6.1 Culture-dependent detection and identification methods

In the past, microbiological assays have relied on culture-dependent methods, commonly referred to as conventional laboratory practices. These include a series of steps such as sample collection, sample preparation, incubation, followed by enumeration, and evaluation of the colonies. Before testing, all required consumables, such as sterile materials and culture media need to be prepared in advance. Sample preparation consists of serial dilution and inoculation onto agar media. When the presence of stressed or injured cells is suspected, an enrichment step may be included prior to inoculation to enhance recovery (Pundir et al., 2013; Park, 2016). The incubation period generally ranges from one to seven days but can last up to 14 days (Nemati et al., 2016). Choosing the right culture media is important. While general media, such as Tryptic Soy Agar (TSA) or Nutrient Agar (NA) supports the growth of a broad spectrum of microorganisms, selective media only enhance the growth of the target microbe. For example, MacConkey agar and Violet Red Bile Glucose Agar (VRBD) are used for the detection of Enterobacteriaceae, while Tryptone Bile X-glucuronide (TBX) agar is used for the selective enumeration of *E. coli*. If presumably injured *E. coli* cells are present in a sample, a selective enrichment medium, such as the Mineral Modified Glutamate Medium (MMGM) can be used for improved detection (Çoçoli et al., 2012; Sandle, 2014; Jozić et al., 2019; Atmanto et al., 2022; Terrones-Fernandez et al., 2023). Following the isolation of the microorganism on agar medium, their colony morphology such as the size, shape, colour, texture and the edge is assessed to obtain enough information for preliminary identification. These observations are typically complemented with biochemical tests for further characterization (Sousa et al., 2013; Hameed et al., 2018; Saravanan et al., 2021). However, a limitation of these approaches is their inability to detect VBNC bacteria (Trinh and Lee, 2022).

As can be seen from the description, culture-based methods are laborious, time-consuming, and have a high demand for consumables (Temmerman et al., 2004). The generated waste is regarded as biohazard, and can pose risks to human health (Ge et al., 2017). Furthermore, the methods always need to be optimized to detect the relevant microorganisms (Vaz-Moreira et al., 2011). Despite the disadvantages, several advantages are present. The direct observation of colony morphology offers an initial phenotypic characterization. Pure cultures can be isolated for genomic analysis. Microbial load

can be easily estimated through the enumeration of the colonies on agar plates, which only allows the detection of viable microorganisms. Thus, culturing offers the direct assessment of viability. These methods require relatively inexpensive consumables and standard laboratory equipment, making them accessible and widely used (Sousa et al., 2013; Nemati et al., 2016; Cross et al., 2019; Trinh and Lee, 2022). Although conventional culture-based methods remain reliable for detecting and enumerating diverse microorganisms, they can be identified at the species level using several methods. These include traditional biochemical tests (catalase, coagulase, oxidase, indole, sulphur, urease, triple sugar iron, nitrate, starch hydrolysis, carbohydrate fermentation, citric acid utilization, methyl red, Voges-Proskauer tests, analytical profile index (API) tests), molecular and proteomic techniques (PCR, 16S rRNA sequencing, Pulsed Field Gel Electrophoresis (PFGE), Randomly Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP)), and mass spectrometry-based approaches (MALDI-TOF MS, Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry) (Temmerman et al., 2004; Talaiekhozani, 2013; França et al., 2015; Srinivasan et al., 2015; Quintilla et al., 2018; AL-Joda and Jasim, 2021). These molecular methods can be applied directly to food or environmental samples, but they are often used in combination with culture-based isolation (Parizad et al., 2016; Baselga et al., 2017; Forbes et al., 2017). MALDI-TOF MS characterise microorganisms through their unique protein profiles, offering a rapid, precise, robust, and cost-efficient approach to microbial classification (Li et al., 2022). According to Ge et al. (2017) using MALDI-TOF MS reduced the processing time of aerobes from 32.5 hours to 4.1 hours, and of anaerobes from 71.5 to 46 hours. This method also cut the costs of detection, as it was decreased by US $0.9 per isolate, saving US $94,500 annually. The amount of biohazard waste was also decreased by 350 kg/ month.

3.6.2 Culture-independent methods for the detection of microbes

The number of bacterial cells might be underestimated using culture-dependent approaches, as it only detects viable and culturable microorganisms. However, upon a diverse range of environmental conditions, microbes are able to enter the viable but non-culturable state, which poses significant health risks, due to their ability to resuscitate when conditions become favourable (Dong et al., 2020; Li et al., 2020a). The phenomenon known as the “great plate count anomaly” refers to the fact that fewer bacteria form colonies on conventional laboratory media than are observed under a microscope. Many bacteria cannot grow under standard laboratory conditions or exist in a dormant or slow-growing state that makes them difficult to detect. Factors contributing to this include specialized nutrient requirements, special pH, temperature, or oxygen levels. Furthermore, they might have a dependence on environmental signals or microbial interactions that are absent in artificial media and can only be found in their natural habitat. Moreover, some organisms are inhibited by competitors and

toxic substances, such as bacteriocins. As a result, many naturally abundant microbial species can only be cultivated using highly specialized or nontraditional techniques (Harwani, 2013).

The limitations of the culture-dependent techniques have led to the development of culture-independent molecular methods. Molecular approaches identify and differentiate microorganisms by analysing their genetic material rather than relying on cultivation. Most of these methods are based on the Polymerase Chain Reaction **(**PCR**)**, which selectively amplifies the targeted DNA regions using specific primers, allowing bacterial detection at various taxonomical levels. For example, quantitative PCR (qPCR) is a PCR-based method, that monitors the amplification of DNA in real time using fluorescent dyes (SYBR Green) or probes (TaqMan probe) (Artika et al., 2022). The culture-independent methods typically involve sampling, DNA extraction, gene amplification, fragment separation, data analysis, and community profiling. Genomic fingerprinting techniques, including PFGE and AFLP analyse DNA directly, while RNA-targeting or sequence-targeting methods, such as microarrays and Fluorescent In Situ Hybridisation (FISH) detect specific rRNA sequences or genes in situ (Peplies et al., 2006; Bertani et al., 2019, Neoh et al., 2019; Gu et al., 2022). These techniques differ mainly in how they separate amplified DNA fragments, using electrophoresis-based methods or restriction digestion. While PCR-based approaches are prone to amplification bias, probe-based methods such as microarrays and FISH detect specific rRNA or gene sequences without the need for PCR, thus reducing this bias. One of the main advantages of these methods is that they bypass the need for cultivation, thus bacterial identification is possible directly from food, clinical, and environmental samples (Lee et al., 2007; Iacumin et al., 2009; Rogers et al., 2009). Their ability to identify microorganisms that are otherwise unculturable under normal laboratory conditions enable new opportunities in microbial identification (McIlwaine et al., 2024). Moreover, these methods offer rapid, sensitive and reproducible results, while also allowing the identification of microbes without prior knowledge on the specific growth requirements (Cocolin et al., 2013; Wang and Salazar, 2016). The cost of these methods relies on the equipment and reagents used but are generally more cost-effective than culture-based methods (Wang and Salazar, 2016). However, culture-independent methods have their limitations. The correct protocol for DNA extraction must be selected to minimize biases, alongside the correct primer selection which could improve accuracy (Cocolin et al., 2013). Standard molecular methods detect DNA from both live and dead cells potentially yielding misleading results, especially when viability is relevant (Zampieri et al., 2021). To overcome this, propidium monoazide (PMA), a photoreactive dye, is used that makes high affinity bonds with the DNA. Upon exposure to intense visible light, PMA forms covalent bonds with the double-stranded DNA, preventing its amplification by PCR. Because this dye cannot penetrate intact cell membranes, they can be used with qPCR to differentiate between live and dead microorganisms. The PMA-qPCR method is widely applied for rapid microbial detection from food, clinical, and environmental samples

(Liu et al., 2022a; Zheng et al., 2022). Accurate analysis also depends on the use of the correct reference databases as contamination, taxonomic errors, inappropriate inclusion or exclusion of the sequences, and sequence content errors can compromise the results (Chorlton, 2024). Furthermore, while culture-independent methods reveal a great number of organisms that cannot be cultured using culture-dependent methods, these two methods not always identify the same bacteria. For the most comprehensive characterization of the microbial communities, combining both approaches is recommended (Vaz-Moreira et al., 2011; Su et al., 2012; Zampieri et al., 2021).

3.6.2.1 Metagenomic approaches for microbial community analysis

Currently, two primary approaches are used to study microbial communities with next-generation sequencing (NGS) techniques. These are the 16S rDNA amplicon sequencing and the shotgun metagenomics. The 16S rDNA amplicon sequencing amplifies a specific region of the 16S gene through PCR amplification, using primers designed to amplify rDNA from as many species as possible. The amplified fragments are then sequenced, and the variation within the gene's less-conserved regions allows for taxonomic identification based on reference rDNA sequence databases. On the other hand, shotgun metagenomics involves sequencing all the DNA extracted from a microbial community. The resulting reads are then compared against metagenomic reference databases to assign each sequence to a particular taxon (Laudadio et al., 2018). In the past, traditional short-read platforms such as Illumina were used for microbial analysis, which have only sequenced a part of the 16S gene, which limited the identification only to genus level. In contrast, full-length 16S sequencing that uses long-read technologies, such as the Oxford Nanopore MinION device, overcomes this limitation by reading the entire fragment. This leads to better accuracy, a higher proportion of reads, better replicability and reduced amplification noise (Low et al., 2021; Szoboszlay et al., 2023). The MinION device is an affordable sequencer, that was launched by Oxford Nanopore Technologies in 2014. Its low startup cost, small size and portability make it practical for laboratory and field applications, while also enabling same-day, on-site analyses (Marin et al., 2022; Werner et al., 2022). However, the raw read error rate is generally high, ranging between 10-20%, which could interact with identifying closely related species (Baloğlu et al., 2021).

3.6.2.2 Gene expression assays

Gene expression is the process by which the genetic information in the DNA is transcribed into RNA and then is translated into proteins which determine cellular structure and function (Bervoets and Charlier, 2019). The gene expression ought to change rapidly in bacteria, in response to different environmental stresses. This rapid change allows their survival under adverse conditions such as dehydration, high osmolarity, high or low temperatures, which the bacteria might encounter during food processing (Chen and Goulian, 2018; Bremer and Krämer, 2019; Gregory and Boyd, 2021;

Wiktorczyk-Kapischke et al., 2023). A common method to measure the gene expression is by the quantitative real-time PCR (qPCR) method, which quantifies the relative abundance of specific transcripts (Kosari et al., 2020; Schütze et al., 2020). The most commonly used approach for the interpretation of the qPCR data is the ΔΔCt method. For this, the cycle threshold (Ct) values of both the target and reference (housekeeping) genes should be first determined by qPCR. Then the average Ct values have to be calculated. The difference between the Ct of the target genes and the Ct of the reference genes ($\Delta Ct = Ct_{target\ gene} - Ct_{reference\ gene}$) represents the normalized expression for each sample. The ΔΔCt value compares the ΔCt of the treated sample to the ΔCt of the control sample. The last step is to convert the logarithmic Ct values to linear ratio. The fold change is calculated as $2^{-\Delta\Delta Ct}$. This method provides a reliable measure of relative gene expression, and allows for the comparison of transcriptional activity across different conditions (Adnan et al., 2011). In food microbiology, gene expression studies help identify bacterial responses to preservation stresses (Sihto et al., 2014). Correct data normalization and data visualization using heatmaps or volcano plots allow researchers to interpret expression changes, which help in understanding bacterial adaptation and survival mechanisms in food products (Zhao et al., 2014; Goedhart and Luijsterburg, 2020).

4 MATERIALS AND METHODS

4.1 Collection of dried fruit samples

To investigate the microbiota of dried fruits, a total of 30 samples from Austrian and Hungarian markets and supermarkets were purchased (Supplementary Table 2). An attempt was made to cover a diverse range of product categories. Thus, different packages (e.g., paper, nylon), organic and non-organic, packaged and unpackaged fruits, fruits with or without sulphur-dioxide were chosen. The selected fruits were: dried apricots, prunes and raisins. All products were stored at room temperature (23 ± 2° C) to mimic the household environments.

Researchers from the Hungarian University of Agriculture and Life Sciences (MATE), Department of Food Microbiology, Hygiene and Safety and from the University of Natural Resources and Life Sciences, Vienna (BOKU) Department of Food Science and Food Technology collaborated in a CEEPUS and a Hungarian Austrian bilateral project. As part of these projects, I have spent several months in Vienna, that is why samples were collected in both countries.

4.2 Selection of the tested *Escherichia coli* strains

Two *E. coli* strains were used in this thesis. One strain (ATCC B.02031) was kindly provided by the National Collection of Agricultural and Industrial Microorganisms (NCAIM, Institute of Food Science and Technology, Hungarian University of Agriculture and Life Sciences, Budapest). The strain is hereafter referred to as culture collection strain and indicated as CC). The other strain, referred to as the food isolate (designated as FI), was previously isolated from a low water activity food product.

The isolation of the food (buckwheat flour) derived *E. coli* strain occurred incidentally during a parallel study in the Department of Biotechnology and Food Science and Technology, BOKU, Vienna. The aim of that study was to isolate *Cronobacter* spp. from low water activity food products according to the ISO 22964:2017 standard (ISO, 2017a), with modifications. Initially, 90 mL of buffered peptone water (BPW; Merck KGaA, Darmstadt, Germany) was added to 10 g of food sample. Following homogenization for two minutes at 230 rpm, and incubation at 37 °C for 18-24 hours, 0.1 mL of the pre-enriched sample was added to 10 ml Cronobacter Selective Broth supplemented with vancomycin (10 mg/L) (CSB) and incubated at 41.5 °C for 24 hours. Subsequently, a loop full of enriched sample in CSB was streaked onto a Chromogenic Cronobacter Isolation agar (CCI) plate and incubated at 41.5 °C for an additional 24 hours. Presumptive and non-presumptive colonies were subcultured on tryptic soy agar (TSA; Merck KGaA, Darmstadt, Germany) and incubated at 37 °C for 24 hours. Finally, Matrix-Assisted Laser Desorption Ionization–time of Flight Mass Spectrometry (MALDI-TOF MS) was used to identify the isolates, which resulted in the detection of *E. coli* in addition to

Cronobacter sp. To verify this result, the *E. coli* isolate was re-inoculated onto both selective and non-selective media, followed by a repeated MALDI-TOF MS analysis.

4.3 Determination of growth potential using turbidity-based methodology

The growth potential of *E. coli* strains (CC and FI) was evaluated using the growth curves generated by Bioscreen C Pro device (Oy Growth Curves Ab Ltd., Turku, Finland). Each experiment was conducted in three technical replicates, following the device settings detailed in Supplementary Table 3.

For statistical analyses, non-parametric tests were used because the data were not normally distributed. The Kruskal–Wallis test was used to compare the differences between three or more groups (growth in different media). When the Kruskal–Wallis test indicated a significant difference, pairwise Wilcoxon rank-sum tests were performed to compare two groups (one medium versus another, or the two strains). Differences were considered statistically significant at $p < 0.05$. All analyses were performed using JMP statistical software (version 18.2.1; SAS Institute Inc., Cary, NC, USA).

In a preliminary experiment, the measurement was carried out over a 24-hour period at 37 °C. Freshly grown overnight cultures of both *E. coli* strains were prepared in Luria-Bertani broth (LB; 10 g tryptone, 5 g yeast extract, and 10 g NaCl per liter of water; Merck KGaA, Darmstadt, Germany), in three parallels per each strain. The following day, cultures were adjusted to an initial OD_{600} value of 0.01 in three different media types: fresh LB (control), a medium containing reduced sugar content compared to that of dried fruits, however higher than that of standard microbiological media (hereafter referred to as low-sugar medium), and an LB based medium mimicking the sugar compositions of dried fruits (dried apricot, prune, and raisin). The sugar composition is detailed in Table 3. The composition of the media mimicking the conditions of different dried fruits was based on published data of the typical sugar types and contents of dried apricot, prune and raisin (Web 13). The amount of glucose, fructose and sucrose were calculated according to these data, and the corresponding quantities were dissolved in water. Subsequently, the appropriate amount of LB medium (as indicated on the label) was added.

For each condition, 300 µl sample was pipetted into the wells of the honeycomb microplate, in triplicates. Blank controls for each medium type were also included in triplicates. The growth potential measurement settings of the Bioscreen C Pro device are detailed in Supplementary Table 3.

In the next step, the setup of the first round was used with only modifying the incubation period form 24 hours to 72 hours. This extension allowed the assessment of growth over a longer period compared to the initial 24-hour-long experiment.

Later on, osmotically pre-adapted cells were tested. The cells were pre-adapted in either LB medium (as control) or in prune-mimicking medium for 192 hours at 37 °C, prior to the growth potential experiment. For the experiment, the OD_{600} value of these cultures, in both cases were set to 0.1 in LB (control), as well as in dried apricot, prune and raisin-mimicking media. The device settings did not change (Supplementary Table 3). However, the incubation period was increased to 96 hours. As an additional control, pre-adapted cells from both the LB and prune-mimicking media were plated on LB plates, in triplicates. The plates were incubated at 37° C for three days to assess viability.

Table 3 Composition of low-sugar medium and dried fruits mimicking media.

Osmotic media (dried fruit-mimicking media of diverse sugar content)	Glucose (mol/L)	Fructose (mol/L)	Sucrose (mol/L)
Dried apricot	1.83	0.69	0.23
Prune	1.41	0.68	0.004
Golden raisin	1.72	1.92	-
Low-sugar	0.35	-	-

4.4 Determination of microbiota of dried fruits using a culture-dependent approach

In this section, the microbiota of dried fruits was characterised using the culture-dependent method. The aim was to identify and quantify the cultivable microorganisms present.

4.4.1 Sample preparation

In this subsection, the procedures used for preparing the dried fruit samples prior to microbiological analysis are described.

4.4.1.1 General detection of microorganisms

To determine the microbiota of the selected dried fruit samples with culture-based screening approach, two separate experiments were conducted. The first was a qualitative approach with an enrichment step, as the physiological state of the microorganisms in the dried fruits was unknown, and the cells might have been injured or stressed. Therefore, this experiment was conducted to initially screen the samples in order to detect aerobic mesophilic microorganisms (bacteria, fungi and yeast) with focus on Enterobacteriaceae and non-pathogenic *E. coli*. The second experiment involved a quantitative analysis to determine the original microbial load of the samples without enrichment, and another qualitative analysis to further characterise the microbiota.

The detection of Enterobacteriaceae was based on ISO 21528-1:2017 (ISO, 2017b) and ISO 21528-2:2017 (ISO, 2018), while the detection of *E. coli* followed the ISO 16649-1:2018 (ISO, 2016)

standard with modifications to all applied protocols. *E. coli* was specifically targeted in this study as it is an indicator organism for hygiene and microbial quality.

For the detection of bacteria, 25 g of each sample was measured into a sterile stomacher bag with filter and 100 mL of buffered peptone water (BPW) was added. The samples were homogenized with a stomacher (Seward, Worthing, West Sussex, UK) for 2 mins at 230 rpm, followed by an enrichment step, where 1 mL of homogenized sample was transferred into 9 mL Minerals Modified Glutamate Medium (MMGM; Thermo Fisher Scientific Inc., Waltham, MA, USA) broth and 9 ml LB broth for the detection of *E. coli*, and into 9 mL Tryptic Soy Broth (TSB; Merck KGaA, Darmstadt, Germany) for the detection of other Enterobacteriaceae. The enrichment step was thought to be necessary, based on the assumption that the dried fruit matrix offers a stressful environment for microorganisms, potentially resulting in low bacterial counts or undetectable levels. All enrichment cultures were incubated at 37 °C for 24 h.

After the enrichment, the samples were streaked on *E. coli* selective Trypton Bile X-glucuronide (TBX; Thermo Fisher Scientific Inc., Waltham, MA, USA) agar for the detection of *E. coli*, and spread plated with Violet Red Bile Agar with Glucose (VRBG; Sigma-Aldrich) overlay for the detection of other Enterobacteriaceae. All agar plates were incubated aerobically, except for VRBG plates, as these were incubated under semi-anaerobic conditions. Selective TBX plates were incubated at 44 °C for 24 h, selective VRBD plates and non-selective TSA plates at 37 °C for 24 h.

The schematic process of detecting and identifying the microbiota of dried fruits is depicted in Supplementary Figure 1.

4.4.1.2 Quantitative and additional qualitative analyses of the samples

In the second experiment, the aim was to quantify and further characterize the microbiota present in the dried fruit samples. The methods were the same as described above with two differences. First, in order to enumerate the initial bacterial load, the enrichment step was left out. Second, following the homogenization step, a 10-fold dilution of the samples was prepared in each case by transferring 1 mL of the homogenized sample into 9 mL buffered peptone water (BWP). The first three dilutions were plated on TBX for the detection of *E. coli*, and on VRBD with overlay to detect other Enterobacteriaceae. These samples were further used to characterise the isolated microbiota.

To determine the total aerobic mesophilic bacterial count, Plate Count Agar (PCA; Oxoid Ltd., Basingstoke, Hampshire, UK) was used with the addition of 4 % Actidion (Merck KGaA, Darmstadt, Germany). This is an antifungal agent that inhibits the growth of yeasts and moulds, thereby helping the enumeration of bacterial colonies. To determine the yeast and mould counts, Dichloran-Glycerol (DG18; Merck KGaA, Darmstadt, Germany) agar was used. All agar plates were incubated aerobically, except for VRBD, which were incubated under semi-anaerobic conditions. The selective

TBX plates were incubated at 44 °C for 22 h, VRBD plates at 37 °C for 24 h, PCA + Actidion plates at 30 °C for 72 h, and DG18 plates at 25 °C for 120 h prior to enumeration.

The statistical analysis of the microbial load between the fruit categories was carried out using JMP statistical software (Version 18.2.1). The differences were assessed with the non-parametric Wilcoxon/Kruskal-Wallis test.

4.4.2 Identification of the isolates by MALDI-TOF MS

The bacteria isolated from the dried fruit samples were identified using Matrix-Assisted Laser Desorption/Ionization-Time-Of-Flight Mass Spectrometry (MALDI-TOF MS; Bruker Daltonik GmbH, Bremen, Germany) according to the manufacturer's recommendations (Web 14).

For most of the samples, from each plate (prepared in duplicates), four morphologically distinct colonies were chosen. However, in the case of samples where a greater colony diversity (> 4 distinct colonies) was observed, six colonies were chosen per plate (prepared in duplicates). In contrast, for samples where uniform colony morphology was observed, one colony per plate was chosen. This ensured, that based on visual inspection, morphologically distinct colonies were chosen, while also trying to minimize repetition. Selected colonies were sub-cultured onto fresh agar plates, and subsequently picked to be transferred onto a spot on the MALDI target plate.

Each spot was overlaid with 1 µL of 70% formic acid (Merck KGaA, Darmstadt, Germany) and left to dry at room temperature. Subsequently, 1 µL of HCCA matrix (a 10 mg/mL solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile, 2.5% trifluoroacetic acid and 47.5% ultra-pure water, Bruker) (Wei et al., 2020) was immediately applied to each spot and allowed to dry at room temperature. Bacterial test standard (BTS; Bruker Daltonik GmbH, Bremen, Germany) was used to calibrate the instrument. The Bruker main spectra libraries for filamentous fungi, Listeria, SR_BBFV, BDAL and mycobacteria were used for identification. MBT Compass software was used to generate mass spectra within the mass range of 2000-20,000 Da at a laser frequency of 20 Hz.

To identify the isolated yeasts and moulds, an extraction method for filamentous fungi grown on agar plates was employed. For that, 300 µL UHQ water (Thermo Fisher Scientific Inc., Waltham, MA, USA) was transferred to a tube. Prior to analyzation, a fresh culture of the filamentous fungi was prepared in each case, and a small portion of the culture was added to the tube containing the water. Following vortexing for 10 seconds, 900 µL ethanol was added and vortexed again, followed by centrifugation for 2 minutes at 13000-15000 rpm, and lastly, the supernatant was discarded. Another centrifugation step ensured the removal of any leftover ethanol from the samples. The pellets were left to dry at room temperature and 70% formic acid was added. For small pellets 20 µl of 70 % formic acid was used and for large pellets 100 µL of 70 % formic acid was added. The same volume of acetonitrile as the 70% formic acid was added and the solution was mixed to fully resuspend the pellet.

Following centrifugation for 2 minutes at 13000-15000 rpm, 1 µL of the prepared extract was applied onto the MALDI target plate. The HCCA matrix was added to each spot was air dried at room temperature. The identification was performed using the "MBT Filamentous Fungi Library". The settings were the same as those described above for the previous method.

4.5 Additional tests for the characterization of the microbiota of dried fruits

To gain a more comprehensive understanding of the bacterial microbiota in dried fruits and the factors influencing these characteristics, additional tests were conducted. These include pH and water activity measurements, as well as total sugar characterization.

4.5.1 pH measurements

The pH of the dried fruits samples was measured using the Lab 845 pH meter (SI Analytics GmbH, Mainz, Germany). Before the analysis, the samples were homogenized using a grinder.

For statistical analysis, JMP statistical software (Version 18.2.1) was used. Data between the fruit sample categories were evaluated with the non-parametric Wilcoxon/Kruskal-Wallis Test, while differences within the fruit categories were evaluated using the Student's t-test.

4.5.2 Water activity measurements

The water activity of the samples was determined using the LabMaster Neo Water Activity Meter (Neutec Group Inc., Farmingdale, New York, USA). The two goals were to measure both the internal and surface water activity of the dried fruits. For the internal water activity measurements, the samples were cut, and the inner part was transferred to the measuring chamber. For surface water activity, the whole intact fruits were placed directly into the measuring chamber. All measurements were conducted at 20 °C according to ISO 18787:2017 standard (Web 15).

The statistical analysis was carried out with JMP statistical software (Version 18.2.1), using the non-parametric Wilcoxon/Kruskal-Wallis Test.

4.5.3 Sugar determination of the samples

Sugar concentrations of the dried apricot, prune and raisin samples were determined using the Rida Cube system (R-Biopharm AG, Darmstadt, Germany) in triplicates. Before analysis, 5 g of each sample was soaked in 10 mL of sterile deionized water at 6 ± 1° C overnight. The soaked samples were then homogenized for two minutes at the rate of 8000 rpm using Ultra-Turrax homogenizer (IKA-Werke GmbH & Co. KG Staufen, Germany), followed by centrifugation at 4000 rpm for 10 minutes.

The sugar concentrations were measured using the Rida Cube Sucrose/D-glucose/D-fructose RCS4190 kit (Web 16). From the prepared sample, 20 µl of the supernatant was added to the tube provided by the manufacturer.

4.6 16S rDNA amplicon sequencing as a culture-independent method for the determination of bacterial community of dried fruits

The bacterial diversity in the dried fruit samples was determined by a culture-independent method using 16S rDNA amplicon sequencing besides the culture-dependent (based on the application of ISO standards and MALDI-TOF MS) approach to ensure comprehensive characterization.

4.6.1 Sample preparation and DNA extraction

The genomic DNA extraction was carried out with the DNeasy PowerFood Microbial Kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's instructions (Web 17). Briefly, the samples were homogenized in BPW, followed by cell lysis using chemical treatment combined with bead-beating, and a purification step. After washing, DNA was eluted in Elution Buffer (EB) and stored at - 20 °C (short term) to –80 °C (long term) until sequencing.

4.6.2 PCR amplification of the 16S rDNA gene

The 16S rDNA gene was amplified by PCR using the 16S Barcoding Kit 24 V14 (Oxford Nanopore Technologies, Oxford, UK) and the AccuStart II PCR SuperMix (2x) (Quanta Biosciences, Inc., Beverly, MA, USA) according to the manufacturer's protocols. The total amount of the PCR reaction was 25 µl, and included 12.5 µl of AccuStart II PCR SuperMix, 1 µl of template DNA, 1 µl of forward primer and 1 µl of reverse primer, and 9.5 µl of PCR grade water.

The PCR cycling conditions were based on the description of the kit: an initial denaturation at 94 °C for 180 s, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, and extension at 72 °C for 120 s, followed by final extension for 420 s at 72°C and hold at 4 °C.

4.6.3 Gel electrophoresis and visualization of the PCR products

The size of the amplified 16S rDNA amplicons (~1,500 bp) was verified by gel electrophoresis. A 2 % agarose gel was prepared in TAE (Tris-acetate-EDTA (ethylenediaminetetraacetic acid), pH 8.0) buffer. The PCR products were loaded with a loading dye alongside a DNA ladder (100 kb) to help estimate the fragment size. The electrophoresis was performed at 80 V, for 45 minutes.

Following electrophoresis, the gel was stained with GelRed dye (Biotium, Inc., Fremont, CA, USA) according to the manufacturer's post-staining protocol (Web 18). Briefly, the gel was submerged in a pre-prepared GelRed solution and was incubated for 30 minutes on a shaker. The DNA bands were visualized using the Gel Doc imaging system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). A

photo was taken using the ImageLab software, with default exposure settings compatible with GelRed fluorescence.

4.6.4 Library preparation and the amplification of the barcoded 16S rDNA for Nanopore sequencing

Genomic DNA (gDNA) was quantified using a Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). The integrity of the DNA was further confirmed with the Agilent 4150 TapeStation system and Genomic DNA ScreenTape (Agilent, Vienna, Austria), ensuring a minimum of 10 ng of high molecular weight gDNA per barcode for sequencing.

To prepare the sequencing library, the rapid sequencing DNA - 16S barcoding kit 24 V14 (Oxford Nanopore Technologies, Oxford, UK), the MK1C MinION sequencing device (Oxford Nanopore Technologies, Oxford, UK) and the Flow Cell (R10.4.1) (Oxford Nanopore Technologies, Oxford, UK) were used according to the manufacturer's instructions and recommendations (Web 19). The barcodes were then thawed at room temperature, briefly centrifuged and kept on ice. The LongAmp Hot Start Taq 2X Master Mix (New England Biolabs, Inc., Ipswich, MA) was also thawed, briefly centrifuged, and placed on ice.

The next step was to prepare the gDNA in nuclease free water. Since the amount of DNA in each sample was different, the formula below was used to calculate the volumes of DNA and nuclease free water needed to achieve the requested volume of 15 µl.

$$C1\ x\ V1 = C2\ x\ V2$$

where, V1=The amount of DNA needed, C1=known DNA concentration (Qubit), C2= 10ng/15 µl (concentration of 1 µl) and V2=final volume of 15 µl.

After mixing, and a brief centrifugation, the PCR master mix was prepared according to the kit protocol, containing the DNA template, and the LongAmp Hot Start Taq 2X Master Mix. Then 10 µl of each 16S barcode was transferred into the corresponding sample tubes. The PCR cycling conditions were the following: initial denaturation at 95 °C for 60 s, followed by 25 cycles of denaturation at 95 °C for 20 s, annealing at 55 °C for 30 s, and extension at 65 °C for 120 s, followed by final extension at 65°C for 300 s and hold at 4 °C.

After amplification, 4 µl of EDTA was added to each barcoded sample to stop the reaction. The samples were incubated for 5 minutes at room temperature, then 1 µl of each barcoded sample was quantified using a Qubit fluorometer. Barcoded samples were pooled in equimolar ratios based on the sample with the lowest concentration. The pooled DNA was purified using 0.6 x AMPure XP beads, then were washed twice with 80 % ethanol, and eluted in 15 µl elution buffer. The eluate (1 µl) was quantified using a Qubit fluorometer.

The final concentration was adjusted to 50 fmol per barcode, taking into account the length of the amplicon (1.5 kb). The requested mass was calculated using an online calculator (Web 20). The Rapid Adapter was diluted and added to the pooled DNA, then incubated for 5 minutes at room temperature. This mix was loaded into the flow cell of the MinION (Oxford Nanopore technologies) device to conduct the sequencing.

4.6.5 Nanopore sequencing and data analysis

The metabarcoding analysis and the taxonomic sample profiling were carried out by the BOKU Core Facility Bioinformatics.

Basecalling and demultiplexing were carried out using Guppy (version 2.2.3). The raw reads were exported as FASTQ files and subsequently concatenated by sample. Raw reads were processed in R (version 4.3.0), using the *filterAndTrim* function from the dada2 package (version 1.28.0; Callahan et al., 2016). The cleaning steps were performed sequentially, starting with discarding reads longer than 1800 bp, followed by truncating 100 bp from both ends, and finally removing reads shorter than 1000 bp. A quality control analysis of both raw and cleaned reads was performed using longQC.py v1.2.1 (Fukasawa et al., 2020) and the results were manually inspected.

Cleaned Nanopore reads were taxonomically classified using EMU v3.4.5 (parameters: --keep-counts, --keep-files, --keep-read-assignments, --output-unclassified), which implements as error correction step and the expectation–maximization algorithm (Curry et al., 2022). The EMU database used for the taxonomic classification was last updated on 2023-03-13 (Web 21). The relative abundance estimates produced by EMU were compiled into a genera abundance table, using taxonomy as the mapping key. A phyloseq object was then constructed by integrating both the raw abundance data and corresponding taxonomy information using the phyloseq package v1.46 (McMurdie and Holmes, 2013).

The data analysis was conducted using R (version 4.3.0; Web 22). Alpha diversity was examined with the plot_richness function (Web 23), while beta diversity was assessed through multidimensional scaling (MDS) visualizations using the plot_ordination function (Web 24). Bar plots were created using the ggplot2 package (version 3.5.1) (Wickham et al., 2007).

4.7 Stress induction by ionizing radiation

4.7.1 Comparison of e-beam and gamma irradiation

To test the response of *E. coli* to ionizing radiation, a preliminary experiment was conducted. The initial objectives were to compare the effects of radiation from a linear accelerator (LINAC, electron beam) and gamma radiation, and to determine the D-value of *E. coli*. The schematic overview of the irradiation process is portrayed in Supplementary Figure 2.

An overnight culture of the CC strain of *E. coli* was prepared in LB medium and incubated at 37 °C, in triplicate. On the day of the experiment, the OD_{600} value of the cultures was adjusted to 0.1 using fresh LB medium. The samples were then transferred into sterile 50 mL Falcon tubes without further dilution, in triplicates. The irradiation was performed at the Wigner Research Centre for Physics (formerly known as the Central Research Institute for Physics, KFKI) in Budapest using both e-beam and gamma radiation sources, with doses of 0.2, 0.4, 0.6, 0.8, and 1 kGy. Following irradiation, the samples were serially diluted and plated on LB agar plates using the spread plating method, in triplicates. The plates were incubated at 37 °C for 24 hours.

4.7.2 Determining the D-value of culture collection (CC) strain of Escherichia coli with e-beam irradiation

In the next experiment, the aim was to determine the D-value of e-beam radiation for the CC strain of *E. coli*, in LB medium based on results from the initial trial. The sample preparation followed the same protocol as described previously (Chapter 4.7.1), with irradiation doses of 0.5, 1, 1.5, and 2 kGy. Following irradiation, the samples were serially diluted and plated onto LB agar plates. The plates were then incubated at 37 °C for 24 hours. All steps were performed in triplicates.

4.7.3 Assessing the behaviour of Escherichia coli in osmotic environments resembling dried fruit conditions upon gamma irradiation

In another experiment, the aim was to assess the behaviour of *E. coli* in media containing different types and concentrations of sugars. An overnight culture of the CC strain was prepared in LB medium, in triplicates, and incubated at 37 °C. On the following day, the bacterial cultures were adjusted to an OD_{600} value of 0.1 using freshly prepared sugar-containing media. These media included either glucose, fructose, or sucrose at concentrations of 0%, 10%, 30%, and 50%. The samples were then transferred into sterile Falcon tubes and irradiated with gamma radiation at doses of 1, 1.5, 1.8, 2, and 2.5 kGy. After irradiation, the samples were serially diluted and plated on LB agar for colony count determination. The plates were incubated at 37 °C for 24 hours. Each experimental condition was tested in triplicates.

4.7.4 Determination of the D-values of the culture collection and low water activity food isolate strains of Escherichia coli using gamma irradiation

In an experiment involving both CC and FI strains, the radiation doses were modified to 1.3, 1.6, 1.9, 2.2, and 2.5 kGy. However, these samples were irradiated with gamma radiation in LB and in prune-mimicking medium as well. Sample preparation and post-irradiation procedures remained the same as described in the previous experiments. The prune mimicking medium was selected based on the results of experiments, described in chapter 4.3.

4.8 Primer design for quantitative PCR (qPCR) studies

The primers (Microsynth AG, Balgach, Switzerland) used in the qPCR reactions were designed for the amplification of *rpoB* (a housekeeping gene), *osmC, treA* and *talA* genes. Their sequences are listed in Table 4. For qPCR analysis the specific primers were designed using the Primer3web tool (version 4.1.0, Web 25). The aim was to design oligos with length of 18-22 bp, melting temperature of 55-61 °C and a maximum of 250 bp product size.

From the potential oligonucleotides selected for synthesis, those with the lowest likelihood of forming secondary structures were selected. This was checked by OligoAnalyzer Tool (Integrated DNA Technologies, Coralville, Iowa, USA, Web 20). The specificity of the designed oligonucleotides was verified through NCBI's Nucleotide Blast (Nucleotide Blast, NCBI, Web 26). To identify the optimal annealing temperature for each primer pairs, gradient PCR was performed using the DNA of the CC *E. coli* strain as the template. Each 25 µl reaction contained 12.5 µl of 2X AccuStart II PCR SuperMix (Quanta Biosciences, Inc., Beverly, MA, USA), 1 µl of forward and 1 µl reverse primer (*rpoB*, 10 µM each), 1 µl of DNA template and 9.5 µl of PCR grade water.

The PCR program consisted of an initial denaturation step at 95° C for 180 seconds, 40 cycles of denaturation at 95° C for 15 seconds, annealing at 55° C, 57° C, 59° C and 61° C for 30 seconds, and extension at 72° C for 30 seconds, with a final extension step at 72°C for 300 seconds. The cycling conditions were based on previously established protocols. The range of annealing temperatures was chosen according to the technical datasheet provided by Microsynth to identify the optimal temperature for each primer pair, and optimise primer binding efficiency. All PCR products were analysed by agarose gel electrophoresis described in Chapter 4.6.3.

Table 4 Sequences of designed oligonucleotide primers used in the amplification processes.

Name of the oligonucleotide	Sequence (5' -> 3')	Amplicon length (bp)
***rpoB*_ forward**	GCCGGTAGACATCGTACTGA	172
***rpoB*_ reverse**	GTACGCACGCTGGATGAATT	
***osmC*_forward**	CCCTGAAGAACTGATTGGCG	197
***osmC*_reverse**	TAGAGGCATCAATACCCGGC	
***talA*_ forward**	CAAATCCTCGCCTTAACCGG	225
***talA*_ reverse**	TTGATCAACGGCGAACAGAC	
***treA*_ forward**	AACGTCAATTTCACCCTGCC	227
***treA*_ reverse**	TCGGCAAGTCCTAACATGGT	

4.9 Detection of viable but non-culturable (VBNC)-like cells, and the investigation on the effect of pre-adaptation

To determine whether *E. coli* cells enter a viable but non-culturable (VBNC)-like state under different periods of osmotic stress, first a protocol was established. The osmotic stress experiments consisted of two parts. The schematic overview is presented in Supplementary Figure 3.

4.9.1 The induction of osmotic stress without pre-adaptation period (viability and culturability assay)

In the first part of the osmotic stress experiments, no pre-adaptation period was used in order to monitor changes induced solely by osmotic stress. Freshly grown cultures of the CC and FI *E. coli* strains were prepared in LB broth, in duplicate, and incubated at 37 °C for overnight. Following the incubation, the cultures were serially diluted and plated on LB agar, in triplicates, as controls. The OD_{600} values of the overnight cultures were set to 0.1 in LB medium, and in media containing varying types and concentrations of sugars that resemble the sugar concentrations of dried fruits. The cells were then exposed to different osmotic conditions by incubation in these media for 1, 2, 3, 4, 5, 24, and 96 hours, respectively.

For stress induction, the cells were grown in media designed to mimic different osmotic environments, including dried, apricot, prune, and raisin mimicking media. LB medium was used as a control. The compositions of different fruit-mimicking media, as well as low-sugar LB medium are shown in Table 3. After each stress period, the stressed cultures were plated on LB agar plates, and incubated at 37 °C for 24 hours. Additionally, 1 mL of each stressed sample was stored at -20 °C for subsequent DNA extraction.

4.9.2 The induction of osmotic stress with pre-adaptation period

In the second part, a pre-adaptation period was included to observe its effect on the cell's response to subsequent stress exposure. In the course of this study, LB medium served as control, while low-sugar medium containing 0.35 M glucose was used to induce moderate osmotic stress.

Initially, a freshly grown culture of the CC strain was prepared in LB medium and incubated at 37 °C overnight, in duplicates. On the next day, the samples were serially diluted, and were plated on LB agar plates, in triplicates, as controls. The plates were then incubated at 37 °C for 24 hours.

To determine potential differences between LB and low-sugar medium (0.35 M glucose), the optical density at 600 nm (OD_{600}) of the freshly grown cultures were adjusted to 0.1 in both media (in duplicates), followed by incubation at 37 °C for 2.5 hours as a pre-adaptation period. After the pre-adaptation, the samples were serially diluted and plated on LB agar plates in triplicates. The plates

were incubated at 37 °C for 24 hours, as controls of pre-adaptation. Additionally, 1 mL of each pre-adapted sample was placed to -20 °C for DNA extraction.

After the pre-adaptation period in either LB or low-sugar medium, the cells were subjected to osmotic stress using media mimicking the environments of dried fruits (dried apricot, prune, and raisin) at 37 °C for 4 and 24 hours, in duplicates. After each stress period, the samples were serially diluted and plated onto LB agar plates, in triplicates. Moreover, one mL of each sample was stored at -20 °C for DNA extraction.

4.9.3 DNA extraction from stressed cells

The DNA was extracted using the peqGOLD Bacterial DNA Kit (VWR International, LLC, Radnor, Pa, USA) according to the manufacturer's protocol (Web 27), with the inclusion of a propidium monoazide (PMA; Biotium Inc., Fremont, CA, USA) treatment step before DNA extraction in order to differentiate between the live and dead cells. Briefly, PMA (10 mM) was added to each sample in an Eppendorf tube, that was wrapped in aluminium foil to prevent light exposure. This was followed by 10 min of incubation on a shaker and 15 min of photoactivation in an ice bath under strong visible light. Following this, the DNA extraction proceeded according to the kit protocol. The cell lysis step was performed using lysozyme (10 µL of lysozyme-containing elution buffer, 37 °C, 10 min), followed by proteinase K (50 µg/mL, New England Biolabs, Inc., Ipswich, MA) treatment for protein degradation and RNase treatment (Qiagen GmbH, Hilden, Germany) to remove RNA. The sample then was further processed using peqGOLD DNA mini column (VWR) with several elution and washing steps. The DNA was eluted twice in preheated (65 °C) elution buffer to increase the yield and was stored at -20 °C until further analysis.

4.9.4 Propidium monoazide quantitative PCR (PMA-qPCR)

Viability PCR was performed using a SYBR Green-based master mix (Thermo Fisher Scientific, Waltham, MA, USA). Each reaction mixture (final volume: 25 µL) contained 12.5 µL of SYBR Green Mix; 2.5 µL of *rpoB* (target gene) forward and reverse primers (2.5 µM each); 2 µL of template DNA; and 5.5 µL of nuclease free water. A negative control (no-template control, NTC, containing nuclease free water instead of DNA) was included in each assay. Amplification was carried out using a Rotor-Gene Q instrument (Qiagen GmbH, Hilden, Germany), with the following thermal conditions: initial denaturation at 95 °C for 60 seconds, followed by 40 cycles of denaturation at 95 °C for 15 seconds, annealing at 57 °C for 30 seconds, and extension at 72 °C for 30 seconds. Fluorescence detection was performed at the end of each extension step. At the end of the run, a melt curve analysis was performed to confirm the specificity of the amplification. During this step, the temperature was gradually increased from 57 °C to 95°C while monitoring the fluorescent changes. The optimal value of the annealing temperature (Ta) was determined with gradient PCR described in Chapter 4.8.

For every PCR assay, a five-point standard curve was prepared in duplicates using the ten-fold serial dilution series of purified DNA obtained from an overnight *E. coli* culture grown in LB medium. The DNA concentrations in the standards were predetermined. Mean cycle threshold (Ct) values were plotted against the logarithm of the cell counts, and the resulting linear regression equation was used to calculate the log cell counts of the samples.

Culturability was assessed in triplicates, while viability was assessed in duplicates. Statistical tests were conducted using JMP statistical software (Version 18.2.1). Data were evaluated with the non-parametric Wilcoxon/Kruskal-Wallis Test, followed by pairwise non-parametric comparisons using the Wicoxon method as a post-hoc analysis.

4.10 Gene expression study

The gene expression was studied using the prune-mimicking medium, selected based on the results of preliminary experiments. The schematic overview of the method used to assess gene expression is shown in Supplementary Figure 4.

4.10.1 Induction of osmotic stress

A freshly grown culture was prepared in LB broth for both *E. coli* strains (CC and FI), in duplicate, and the cultures were incubated at 37 °C. The next day, the OD_{600} (optical density at 600 nm) value of the samples were adjusted to 0.1 in sterile LB broth and the cells were further incubated at 37 °C for 4 hours to reach the mid-logarithmic growth phase. Then, the cells were vortexed and centrifugated (4000 rpm, 10 minutes, room temperature) to harvest them. The resulting pellets were resuspended in LB medium and prune mimicking LB medium, then subjected to different stress periods (0, 0.5, 1, 3, and 16 hours) to simulate osmotic stress conditions. To create the dried fruit mimicking media, LB broth was prepared supplemented with the appropriate amount of sugar (see Table 3).

4.10.2 Harvesting and stabilizing the cells

Following the designated stress periods, the cells were harvested using centrifugation at 4000 rpm for 10 minutes at 4 °C. After discarding the supernatant, the resulting pellet was resuspended in 1000 µL of RNA Protect Solution (Qiagen) and stored at 4 °C until further use.

4.10.3 Cell lysis

The cell lysis was performed to release RNA. First, the samples were centrifuged at 7500 rpm for 10 minutes to remove the RNA Protect solution. A TE (Tris-EDTA) buffer (VWR International, LLC, Radnor, Pa, USA) solution containing lysozyme was prepared according to the producer's recommendations (Web 28). Proteinase K was then added to this solution at a final concentration of 50 µg/mL to digest proteins. Dithiothreitol (DTT) was also included as a reducing agent instead of β-

mercaptoethanol, due to its lower toxicity, reduced volatility and minimal odour. In the final step, the ethanol was added to promote the RNA precipitation.

4.10.4 RNA isolation

The RNA isolation procedure followed the RNeasy Mini Kit (Qiagen GmbH, Hilden, Germany) protocol (Web 28). Briefly, 700 µL of the lysate was transferred to an RNeasy spin column placed in a 2 mL collection tube and was centrifuged at ≥8000 × g for 15 seconds. The flow-through was discarded, and the column was transferred to a new collection tube. Two washes were performed with 500 µL of RNA Pre-Elution (RPE) buffer, followed by centrifugation. RNA was eluted twice in 50 µL of RNase-free water to maximize yield.

4.10.5 Reverse transcription

For the synthesis of the complementary DNA (cDNA), the QuantiTect Reverse Transcription Kit (Qiagen GmbH, Hilden, Germany) was used according to the manufacturer's protocol (Web 29). All reagents were kept on ice during preparation. First, the genomic DNA was eliminated by an incubation at 42° C for 2 minutes. Samples were then returned to ice immediately. Then, the reverse transcription reaction proceeded at 42° C for 15 minutes, followed by an inactivation step at 95° C for 3 minutes. The resulting cDNA samples were stored at -20 °C until qPCR analysis.

4.10.6 Quantitative PCR (qPCR)

The primer sequences used in the reactions are presented in Chapter 4.8. The PCR reactions were performed using the qTower (Analytik Jena GmbH+Co. KG, Jena, Germany) instrument. The SYBR Green dye was used for detection. Each 25 µL reaction contained 12.5 µL SYBR Green master mix, 2.5 µL forward primer (2.5 µM), 2.5 µL reverse primer (2.5 µM), 5.5 µL PCR grade water and 2 µl of template cDNA.

The PCR cycling conditions included an initial denaturation at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing at 57°C for 30 seconds, and extension at 72°C for 30 seconds. No template controls were included by substituting the template DNA with PCR-grade water. All reactions were run in duplicates. Fluorescence detection was performed at the end of each extension step. To verify product specificity, a melt curve analysis was performed following amplification.

The quantification cycle (Ct) values were generated automatically by the software of the instrument, and the relative gene expression was calculated using the ΔΔCt method, with *rpoB* as the reference housekeeping gene. First, the Ct values of both the target gene and the reference gene were determined by the software for each sample. Next, the Ct of the target gene was normalized to the Ct of reference

gene to correct the potential differences in RNA input and reverse transcription efficiency. For that the following equation was used:

$$\Delta Ct = Ct_{target} - Ct_{reference}$$

This step gave the ΔCt value for each sample. Then, the ΔCt of each stressed sample was compared to the ΔCt of the corresponding non-stressed control in order to calculate the ΔΔCt. For this, the following equation was used:

$$\Delta\Delta Ct = \Delta Ct_{sample} - \Delta Ct_{control}$$

Finally, the fold change in gene expression was determined by the following equation:

$$Fold\ change = 2^{-\Delta\Delta Ct}$$

A fold change greater than 1 indicates upregulation of the gene, while a value less than 1 indicates downregulation. All fold change values were averaged across duplicates and are presented as mean ± standard deviation.

5 RESULTS AND DISCUSSION

5.1 Determination of growth potential using turbidity-based methodology

The ability of two *E. coli* strains (CC and FI) to grow in the presence of diverse sugar concentration in dried apricot, prune and raisin-mimicking media was assessed. These experiments gave the fundamentals for the sugar stress experiments conducted in this thesis.

5.1.1 Results of growth experiments conducted for 24 and 72 hours

In the first experiment of growth, the two *E. coli* strains (CC and FI) were grown in LB medium (as control), low-sugar medium (0.35 M glucose), and in dried apricot, prune and raisin-mimicking media for 24 hours at 37 °C.

Figure 4 (A) presents the mean OD values of three parallel measurements in each case of the media with both *E. coli* strains. In the case of the CC strain, the control sample grown in LB medium reaches the highest optical density (OD) value (around 1.37) amongst all samples. The curve presents a classic bacterial growth curve with lag, exponential and stationary phase. The stationary phase starts around 11 hours into the experiment and does not end until the end of the experiment. The FI strain reaches the same optical density value as the CC strain, but at the end of the experiment. However, it does not enter the stationary phase during the experiment, moreover, the OD values are still increasing at the end.

The OD value of the bacterial strains in the low-sugar medium changed along with the control samples (CC and FI strain in LB medium) in the first two hours, but while the growth rate of the control samples slowed down in the next three hours, the bacteria in the low-sugar media proliferated till an average OD value of 0.8 was reached.

The OD values of the strains in sugar-mimicking media are different than the OD values of the low-sugar and the control media (Figure 4 (B)). No growth was visible until the end of the experiment with one exception. In the case of FI strain in prune medium, a slight increase can be seen starting from the 7-hour time-point. This increase does not stop until the end of the experiment, almost reaching the OD value of 0.2. At the 18-hour time-point, the OD value of the CC strain in the prune medium also starts to slowly increase but does not reach that of the FI strain.

The blank samples showed no growth in either case.

This experiment can indicate that *E. coli* has the potential to proliferate over time in sugary conditions resembling prune.

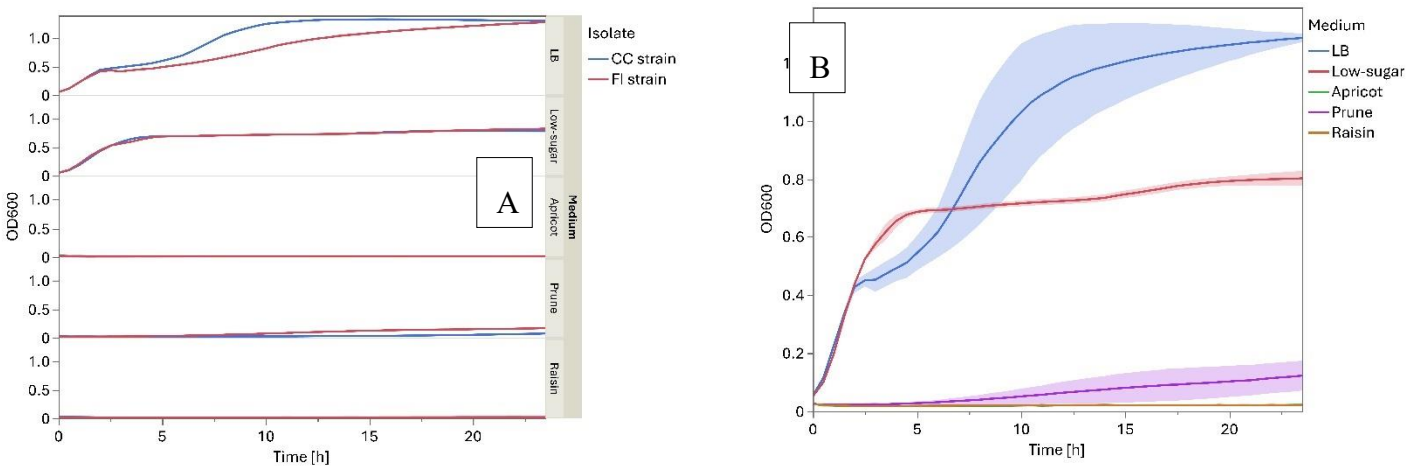

Figure 4 Comparison of the growth curves of (A) CC and FI strains of *E. coli* at 37 °C for 24 hours in LB medium (control), low-sugar medium and dried fruit (dried apricot, prune, and raisin) mimicking media, and (B) of the mean OD_{600} values of each medium calculated by combining the measurements of both strains. In figure B, the OD_{600} values represent the average of all six replicates (3 CC + 3 FI) for each medium. The shaded areas indicate the standard deviation.

In the next experimental experiment, the incubation of the cells was done for 72 hours at 37 °C. The results are similar in a way that three groups can be distinguished amongst the media regardless of the strains (Figure 5 (B)). The first group is the control group in LB medium, which reached the highest OD value (1.3). The second group is the low-sugar medium with OD values reaching 0.9, while the third cluster contains the dried fruit-mimicking media. In this case, no growth was visible in dried apricot and raisin-mimicking media, however, the OD values in prune-mimicking medium reached 0.2.

Not only were the OD values of the control (LB medium) group similar to those experienced in the first experiment, but the growth curve also portrayed a similar pattern. In Figure 5 (A) the OD values of the CC strain in LB medium started to decline at 24 hours, which is also visible in Figure 4 (A). This decline does not stop until the end of the three-day-long experiment. In the case of the FI strain, a slow increase of the OD values can be detected in the first experiment, which is visible in the second study as well. This does not stop until the end of the experiment.

The OD values of the bacteria in low-sugar medium were similar to those of in the first study. The two strains had similar OD values, both reaching 0.9.

In this case of prune-mimicking medium, the OD values of the FI strain started to increase after 5 hours of incubation at 37 °C and slowly increased to the value of 0.21. The OD value of the CC strain also increased to 0.21, however this increase started around 36 hours into the experiment and reached the value of 0.21 after 65 hours into the experiment. After this point, the OD values of both strains were the same till the end of the experiment.

The OD values of the blank samples did not change throughout the experiment.

This second round of experiments yielded the same result as the first one, indicating the growth potential of both CC and FI *E. coli* strains in an environment where the sugar content is optimal for their proliferation.

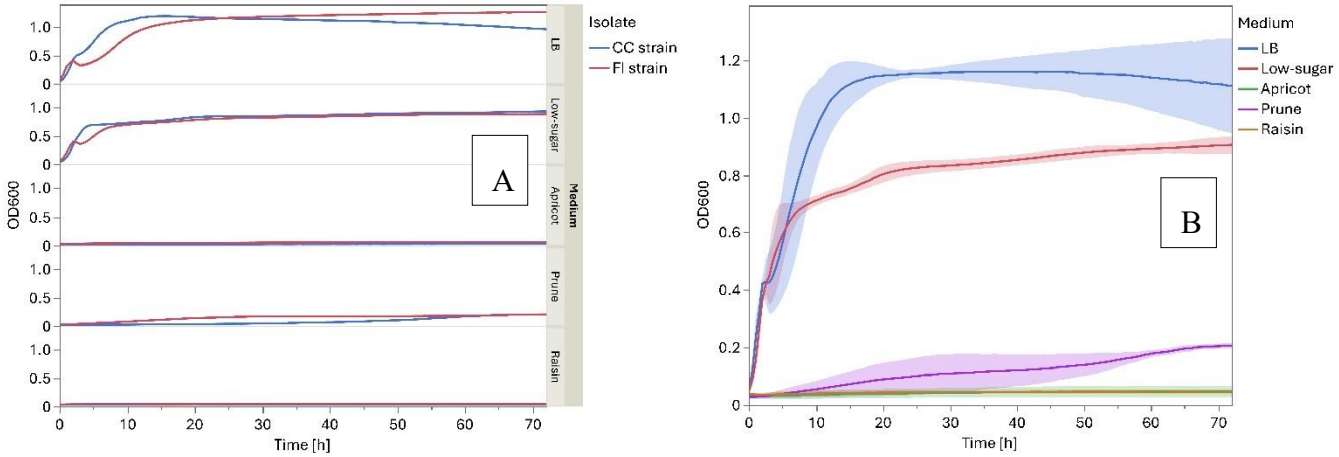

Figure 5 Comparison of the growth curves of (A) CC and FI strains of *E. coli* at 37° C for 72 hours in LB medium (control), low-sugar medium and dried fruit (dried apricot, prune, and raisin) mimicking media, and (B) of the mean OD_{600} values of each medium calculated by combining the measurements of both strains. In figure B, the OD_{600} values represent the average of all six replicates (3 CC + 3 FI) for each medium. The shaded areas indicate the standard deviation.

For statistical analysis, based on the similar results of the 24-hour-long and the 72-hour-long growth experiments, the data of the two rounds for the first 24 hours were combined. Thus, the first 24-hour values represent the merged mean of both rounds (Supplementary Figure 5). The Wilcoxon Kruskal Wallis tests were used to detect differences among groups, followed by a pairwise comparison using the Wilcoxon rank-sum method to determine significant differences between the two strains and between the different media. These non-parametric tests were chosen because the data did not meet normality assumptions. The results show that in the first 5 hours of the experiment, in LB medium, the two *E. coli* strains had significantly OD values different ($S = 21$, $Z = -2.80$, $p = 0.0050$ at both 2.5 and 5 hours, $\alpha = 0.05$) (Supplementary Figure 6, Supplementary Figure 7). The significant difference lasted till 17.5 hours, after which the two strains did not differ significantly ($S = 30$, $Z = -1.36$, $p = 0.1735$, $\alpha = 0.05$). Similarly, at 5 hours, the OD values of the CC and FI strains in prune medium were significantly different ($S = 57$, $Z = 2.81$, $p = 0.0049$ at 5 hours, $\alpha = 0.05$), which lasted until the end of the experiment ($S = 57$, $Z= 2.7$, $p = 0.0051$ at 22.5 hours, $\alpha = 0.05$) (Supplementary Figure 8, Supplementary Figure 9). In all the other media, the two strains did not differ significantly (Supplementary Figure 10, Supplementary Figure 11, Supplementary Figure 12, Supplementary Figure 13).

Analysing the effects of the media, regardless of the strains, after 5 hours, the OD values in LB medium were significantly different from the OD values measured in the sugary media ($Z = 4.12$, $p < 0.0001$, $\alpha = 0.05$), but the LB and low-sugar media had no significant difference ($Z = 1.29$, $p = 0.1938$, $\alpha = 0.05$) (Supplementary Figure 14, Supplementary Figure 15 (A)). The sugar-mimicking media also showed no significant differences among each fruit (prune-apricot: $Z= 1.79$, $p = 0.073$, raisin-apricot: $Z = 0.43$, $p = 0.6648$, raisin-prune: $Z = -1.24$, $p = 0.2140$, $\alpha = 0.05$). At the 7.5-hour point, LB and low-sugar media still had unsignificant differences ($Z = 0.00$, $p = 1.000$, $\alpha = 0.05$), although they differed significantly from all the fruit media ($p < 0.0001$, $\alpha = 0.05$). Among the fruit media, the prune medium became significantly different from the dried apricot-mimicking medium ($Z = 2.40$, $p = 0.0163$, $\alpha = 0.05$) Supplementary Figure 15 (B). At 10 hour, dried apricot and raisin-mimicking media were similar ($Z = 0.63$, $p = 0.5247$, $\alpha = 0.05$), however LB ($Z = 4.13$, $p < 0.0001$, $\alpha = 0.05$), low-sugar medium ($Z = 4.13$, $p < 0.0001$, $\alpha = 0.05$), and prune-mimicking media ($Z = - 4.13p < 0.0001$, $\alpha = 0.05$) were statistically different from all other media Supplementary Figure 15 (C).

5.1.2 Results of the growth experiment studies, where the cells were pre-adapted in LB medium for 192 hours prior to OD measurements

In this growth experiment, the cells were pre-adapted in LB media (control) for 192 hours, and then the OD values were set to 0.01 in LB (control) and dried apricot, prune and raisin-mimicking media applying both strains. The 192-hour pre-adaptation period was chosen to provide an extended incubation that could induce mild physiological stress or adaptation in the cells. The experiment assessing the growth potential of the strains lasted for 96 hours. This experiment is meant to mimic what would happen if *E. coli* from optimal conditions contaminated dried fruits with diverse sugar conditions.

The control samples which were pre-adapted in LB medium and transferred to LB medium with both *E. coli* strains showed the highest OD values amongst all the other media (Figure 6). The highest OD value belonged to the FI strain, nearly reaching 1.2. The highest OD value of the CC strain was 1.07. In the case of the FI strain, after reaching the highest OD value, the values start to slowly decline, with a harsh decline at 88 hours, after which the values slowly start to increase again. After reaching the highest OD value, the OD values of the CC strain also start to decline till the 48-hour time-point, after which the values did not change for twelve hours, but then the values began to rise again.

The lag phase differed depending on the medium. Among the dried fruit-mimicking media, only the prune-mimicking medium supported the growth of *E. coli*. The lag phase in this medium lasted approximately 32 hours for the FI strain, and 85 hours for the CC strain, after which slow growth was observed. After the lag phase, the OD values of the CC strain nearly reached that of the FI strain. On the contrary, in LB medium, the lag phase was much shorter, lasting only 3 hours.

Similarly to previous growth experiments, no growth was observed in either blank sample, indicating an effectively indefinite lag phase under these conditions.

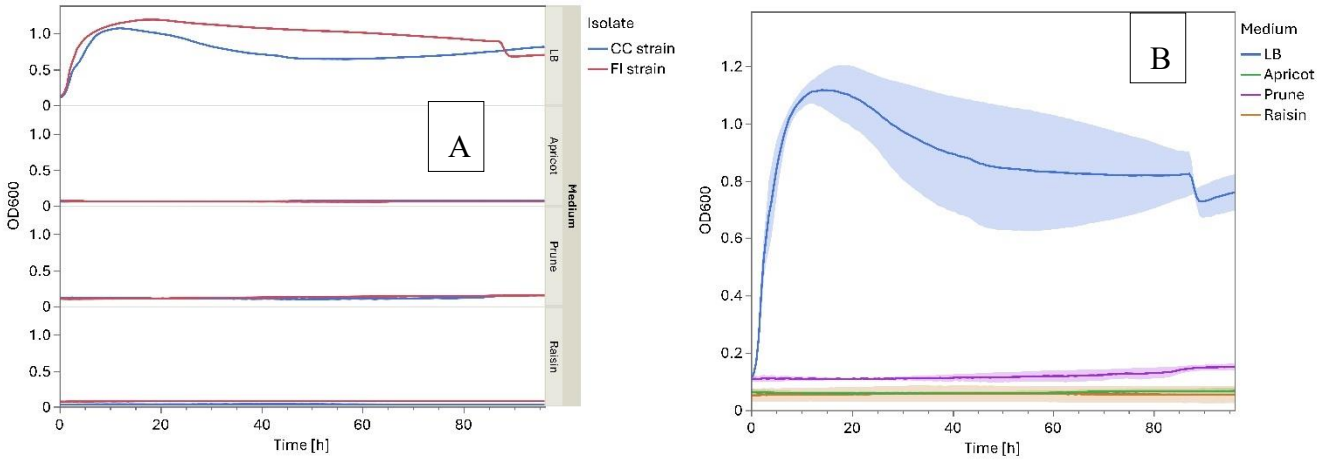

Figure 6 Comparison of the growth curves of (A) CC and FI strains of *E. coli* at 37° C pre-adapted for 192 hours in LB medium (control) and transferred to dried fruit (dried apricot, prune, and raisin) mimicking media for 96 hours at 37°C, and (B) of the mean OD_{600} values of each medium calculated by combining the measurements of both strains. In figure B, the OD_{600} values represent the average of all six replicates (3 CC + 3 FI) for each medium. The shaded areas indicate the standard deviation.

5.1.3 Results of growth experiment studies, where the cells were pre-adapted in prune medium for 192 hours prior to OD measurements

This experiment depicts a scenario where stressed bacteria (from prune-mimicking medium) enter an environment with optimal conditions (LB medium) for growth. In this experiment, both *E. coli* strains were pre-adapted in prune-mimicking medium for 192 hours and then transferred to various media, including LB medium designed to be beneficial for the growth of *E. coli*, and to apricot, prune and raisin-mimicking media.

The highest OD values were observed with the FI strain in LB medium, reaching 0.61, which are lower than the usual OD values observed previously in this medium with the same strain (Figure 7). A short lag phase can be observed in the first three hours of the experiment followed by a quick increase. A drop in the OD values can be observed till 24 hours, after which the values begin to rise till the end of the 4-day-long experiment. The same pattern can be observed with the CC strain, although its lag phase lasted for six hours, which is double the time it lasted for the FI strain.

The difference in the OD values among the tested media are shown in Figure 7B, where the values represent the average of all six replicates (3 CC + 3 FI) for each medium. The only dried fruit-mimicking medium, where growth was observed, was the prune, and only in the case of the FI strain.

In this case, the already stressed cells could grow in a medium with yet again suboptimal conditions. After a 13-hour long lag phase, the OD values of the FI strain started to slowly increase, which did not stop until the end of the experiment. In contrast, Figure 7 (A) shows the strain-specific growth curves in LB, dried apricot, prune and raisin medium. In LB where the lag phases were much shorter, lasting about 4 hours for the CC strain, and 7 hours for the FI strain.

The results of this last growth experiment mimic what would happen if *E. coli* from sub-optimal growth conditions (for example from dried fruits) contaminates a product where conditions for proliferation are optimal. For example, nowadays, overnight oats are preferred with flavourful fruits or dried fruits. These products offer optimal water activity, pH and carbohydrate source for stressed bacteria. If temperature control (refrigerator) is neglected (consumer left the overnight oats on the counter for a night), the healthy breakfast could easily become a nightmare.

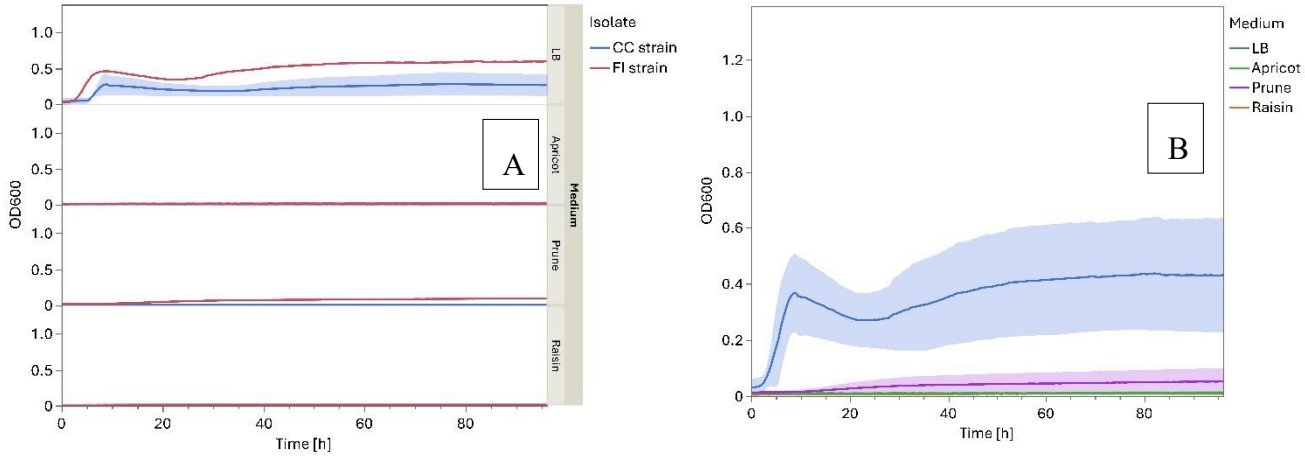

Figure 7 Comparison of the growth curves of (A) CC and FI strains of *E. coli* at 37° C pre-adapted for 192 hours in prune medium and transferred to dried fruit (dried apricot, prune, and raisin) mimicking media for 96 hours at 37°C, and (B) of the OD_{600} values of each medium calculated by combining the measurements of both strains. In figure B, the OD600 values represent the average of all six replicates (3 CC + 3 FI) for each medium. The shaded areas indicate the standard deviation.

For statistical analysis the combination of the two different stress media (LB as the control, and prune-mimicking medium) regarding the strains is presented in Supplementary Figure 16. The connective letter reports summarising the statistical differences between the strains in LB, dried apricot, prune, and raisin media are shown in Supplementary Figure 17, Supplementary Figure 18, Supplementary Figure 19, and Supplementary Figure 20, respectively. The corresponding p-values are porvided in Supplementary Figure 21, Supplementary Figure 22, Supplementary Figure 23, and Supplementary Figure 24. To generate the connecting letters reports, the OD values measured at each sampling time point were analysed using the Kruskal–Wallis test, followed by pairwise Wilcoxon rank-sum post hoc

comparisons with $\alpha = 0.05$. The connecting letters represents statistically homogenous groups, where groups sharing the same letter are not statistically different. Although in this case, the OD values were used to distinguish bacterial responses across the different media, other growth curve characteristics, such as lah-phase duration, growth rate or maximum OD could have also been applied.

According to Supplementary Figure 21 (A), after 192 hours of pre-adaptation in LB medium, followed by a transfer to LB medium as the control, 10 hours after starting the experiment, no significant difference was detected between the CC and FI strains ($p = 0.3052$, $\alpha = 0.05$). In contrast, when pre-adapted in prune medium for the same period followed by a transfer to LB medium, the strains displayed significant differences ($p < 0.0051$, $\alpha = 0.05$). Across the first 40 hours of incubation, cells pre-adapted in either LB or prune medium showed differences in LB medium (Supplementary Figure 17, Supplementary Figure 21). In the next hours, the significant differences persisted between the CC and FI strain pre-adapted in prune medium, and the strains pre-adapted in LB medium, indicating that food isolates might show different adaptive mechanisms (Supplementary Figure 21). The type of the media also influenced the growths, as overall the CC strain pre-adapted in LB medium displayed higher OD values throughout the experiment than when pre-adapted in prune medium (Supplementary Figure 16).

Upon transfer to dried apricot-mimicking medium, the OD values of the strains pre-adapted in LB were different form the OD values of the strains pre-adapted in prune-mimicking medium, in the first 40 hours of the experiment (Supplementary Figure 18). The corresponding p-values are presented in Supplementary Table 22. Between 50 and 60 hours, significant differences were found between all strain and media combinations. At the end of the experiment, the strains in prune media acted similar ($p = 0.0539$, $\alpha = 0.05$), whereas in LB medium, the CC strain remained significantly different ($p = 0.0072$, $\alpha = 0.05$) from the FI strain.

Following a 192-hour pre-adaptation period in either LB or prune medium, upon transfer to prune medium for a 96-hour incubation period, significant differences were observed in almost all time points and strain-medium combinations, except in the 20th and 90th hours (Supplementary Figure 19, Supplementary Figure 23). In both cases, the difference was only significant between strains pre-adapted in prune medium, while in LB pre-adaption medium the strains behaved similar upon transfer to prune medium.

In contrast to what was observed in Supplementary Figure 19, the raisin medium yielded opposite results. The prune pre-adapted CC and FI strains were similar throughout the incubation period in raisin medium, whereas both strains exhibited significant differences when pre-adapted in LB (Supplementary Figure 20). The exact values are presented in Supplementary Figure 24.

Overall, the observed differences between the CC and FI strains following prolonged pre-adaptation in either LB or prune-mimicking medium were strongly medium and time dependent. The strains pre-adapted in prune medium for 192 hours exhibited no differences at the end of the experiment when incubated for 96 hours in dried apricot or raisin media, whereas the opposite trend was observed in prune medium itself. In contrast, LB-pre-adapted strains behaved similarly in prune medium after 96 hours of incubation but showed differences in the other two dried fruit-mimicking media. This suggests that studying and understanding bacterial behaviour in conditions reflecting food matrices may provide valuable information for improving food hygiene and safety practices.

The pre-adaptation medium also strongly influenced the lag phase of both strains. Following the pre-adaptation in LB, both CC and FI strains displayed a short lag phase of approximately 2 hours, followed by a 5-hour exponential phase. In contrast, the pre-adaptation in prune the lag phase increased to 3 hours in the FI strain and to 6 hours in the CC strain, although the exponential phase duration remained similar (3 hours).

In all the growth experiments, prune-mimicking medium acted differently from all the other dried fruit-mimicking media, indicating a potential threat if this delicious snack is not handled hygienically.

Several studies have proved that foodborne pathogens can persist on dried fruits for extended periods. Liu et al. (2021) investigated how long foodborne pathogens (Shiga-toxin producing *Escherichia coli*, known as STEC, *Salmonella* spp., and *L. monocytogenes*) can survive on dried apricots. In the study, either wet (phosphate-buffered saline, PBS) or dry (sand) caried were used for the inoculation of the samples. When the samples were inoculated with the wet carrier, all pathogens survived the initial 48-hour drying. However, during the three-month storage period, pathogen levels declined, with STEC surviving in the highest numbers and the longest. Using dry carrier, *Salmonella* survived in higher numbers, suggesting increased stress tolerance after being exposed to stress conditions before inoculation. Another study on the survival of common foodborne pathogens on dried fruits, *Salmonella* showed the greatest persistence among *E. coli* and *L. monocytogenes*. On dates, up to 5.31 log CFU/g of *Salmonella* could be recovered after 180 days or refrigerated storage (Canakapalli et al., 2021). Beuchat and Mann (2014) reported in their study that *Salmonella* can survive in dried strawberries for up to six months, and in dried cranberries, raisins and date paste for up to eight months when stored at 4° C. Liu et al. (2022) observed that *Enterococcus faecium* and *Salmonella* survived on dried peaches for up to three months at ambient temperature storage. Cuzzi et al. (2021) investigated the long-term survival of *L. monocytogenes* on dried apples, raisins, and dried strawberries over a 336-day period under different storage conditions (4 °C, 25–81 % relative humidity (RH), and 23 °C, 30–35 % RH). Researchers performed dry inoculation, using sand as a carrier and applied a four-strain *L. monocytogenes* cocktail to reach numbers of 4.0 to 4.6 CFU/g. *Listeria* was unrecoverable from dried apples immediately after inoculation (on day 0), suggesting

rapid inactivation, or incompatibility with the dried apple matrix. *Listeria* populations declined rapidly at 23 °C on the raisins and dried strawberries, however survival was enhanced at 4 °C with linear population declines of 0.1 log CFU/g/month on raisins, and 0.2 log CFU/g/month on dried strawberries. Their key finding is that *L. monocytogenes* is rapidly inactivated on raisins and strawberries at ambient temperature but can persist for nearly a year at refrigerated temperature.

These findings, in agreement with previous studies, demonstrate that *E. coli* can persist on dried fruit surfaces for extended periods and suggest that prunes may provide more favourable conditions for its survival than other dried fruits.

5.2 Microbial diversity of dried fruits assessed via culture-dependent method

In this thesis work, 20 different bacterial genera were identified from 30 dried fruit samples via culture-dependent approach (Table 5). For isolates, where no identification result is presented in Table 5, culturing did not yield any visible colonies prior to MALDI-TOF MS analysis. The detection of a wide range of bacteria, predominantly Gram-positive (e.g., *Priestia megaterium*), along with Gram-negative (e.g., *Pantoea agglomerans*) genera underscores the microbial diversity present in dried fruit products.

The MALDI-TOF MS generates a log score value ranging from 0.000 to 3.000, which reflects the likelihood of correctly identifying an isolate. This score is derived by comparing the peak list of an unknown isolate with the reference spectra in the database. Scores ranging from 2.300 to 3.000 are indicative of a highly reliable species level identification. Scores falling between 2.000 and 2.299 indicate secure genus level identification with a likelihood of species-level identification. A score from 1.700 to 1.999 suggests probable genus-level identification. Scores below 1.700 are considered unreliable for accurate identification (Gasper et al., 2017).

Bacillus was present in all types of dried fruit analysed - dried apricots, prunes, and raisins. *Priestia* was detected in six raisin samples, five dried apricot samples, and one prune sample. *Peribacillus* appeared in six dried apricot samples and four raisin samples but was absent in prunes. *Micrococcus* was identified from three raisin samples and only one dried apricot and prune sample each. *Paenibacillus* was identified in four dried apricot samples and one prune sample. *Pantoea* was found in one dried apricot and two raisin samples. *Staphylococcus* was present in two prune samples and one dried apricot sample. *Cytobacillus* was identified in one sample of each dried apricot, prune, and raisin. *Lysinibacillus* was present in one dried apricot and one prune sample. *Niallia* was identified from two raisins. The frequent detection of these bacterial genera in dried fruits suggests that similar environmental conditions and processing practices contribute to a shared microbiota.

Raisins showed the highest diversity, containing an average of 4.81 bacterial species and 3.36 genera. Dried apricots harboured an average of 3.7 bacterial species and 2.7 genera, and prunes exhibited the lowest microbial density, with an average of 1.87 bacterial species and 1.62 genera.

The low bacterial diversity detected in prunes may be largely influenced by their chemical composition, notably their high concentrations of phenolic acids and flavonoids, which are known for their antioxidant properties. A study reported that plums have higher total phenolic content (TPC) and higher total antioxidant capacity (TAC) than other fruits such as peaches (Cevallos-Casals, 2006). Similarly, Wu et al. (2004) found that prunes have greater TPC and TAC values compared to other dried fruits such as dates, figs, and raisins. According to Najafabad and Jamei (2014), the TPC of various plum cultivars ranges between 130 and 626 mg gallic acid equivalents (GAE)/100 g, while the TAC ranges from 2.67 to 16.64 mg α-tocopherol/g. Raisin samples analysed by Meng et al. (2011) showed TPC values ranging from 193 to 678 mg GAE/100 g dry weight, flavonol content from 18 to 1370 mg catechin equivalents (QE)/100 g dry weight, and found flavonoid content between 177 and 552 mg QE/100 g dry weight. Dried apricots also contain considerable amounts of phenolic compounds, with TPC values reported between 60 and 498 mg GAE/100 g, and flavonoid levels from 481 to 812 mg QE/100 g (Igual et al., 2012; Čanadanović-Brunet et al., 2013; Vega-Gálvez et al., 2019). It's important to mention that fruit composition can vary significantly, even within the same fruit type, due to factors such as cultivar, agricultural practices, environmental conditions, harvest timing, and genetic differences (Pawłowska et al., 2010).

Several bacterial species identified through MALDI-TOF MS are typically associated with agricultural or soil environments. These include *Bacillus amyloliquefaciens*, *B. licheniformis*, *B. pseudomycoides*, *B. mojavensis*, *B. thuringiensis*, and *B. velezensis*, which have been previously described for their beneficial roles in plant growth promotion (biofertilizer) and pathogen suppression (biocontrol) (Pramanik et al., 2019; Diabankana et al., 2021; Kumar et al., 2021; Muras et al., 2021; Luo et al., 2022; Rabbee et al., 2023). The detection of *Curtobacterium flaccumfaciens*, a species with dual functionality as both a pathogen and a biocontrol/biofertilizer agent, further supports the likelihood of environmental or soil-derived contamination (Chebotar et al., 2023). Additionally, the presence of *Lysinibacillus* (Miwa et al., 2009; Lee et al., 2010; Jeon et al., 2021) and *Metabacillus* (Mao et al., 2020; Jeong et al., 2022; Yin et al., 2022; Fang et al., 2023), both of which are known soil inhabitants, points to potential contamination during harvesting, handling, or inadequate processing conditions.

In addition to bacteria originating from agricultural and soil environments, several potentially pathogenic microorganisms were identified from dried fruit samples, many of which have likely been introduced during post-harvest handling (Samrot et al., 2015; Ray et al., 2020). *Staphylococcus epidermidis*, an opportunistic pathogen, and a common inhabitant of the human skin microbiota was

detected in several samples, and its presence likely reflects contamination due to improper hygiene during manual processing (Otto, 2009; Schoenfelder et al., 2010). While it can be opportunistic, this bacterium also contributes to skin health, by producing antimicrobial peptides that inhibit the colonization of other harmful bacteria (Qi et al., 2025). Similarly, the presence of environmental bacteria such as *Pseudomonas* spp. (some species are phytopathogens and human pathogens) and *Pantoea agglomerans*, known as a plant pathogen and emerging opportunistic human pathogen, may indicate contamination due to poor sanitation, improper washing, or contact with contaminated surfaces. Even the genus name *Pantoea* means omnipresent, which highlights its widespread occurrence across various sources including seeds, vegetables, water, and diverse plant hosts (Mardaneh and Pouresmaeil, 2024; Dutkiewicz et al., 2016). However, *Pantoea* spp. can have beneficial effects in the agriculture as biocontrol agents and plant growth promoters, while certain *Pseudomonas* strains can be used for biodegradation and for the production of bioactive compounds (Lv et al., 2022; Alattas et al., 2024). Although *Pseudomonas cichorii*, identified in this study from a raisin sample, is not recognized as a human pathogen, its presence reflects conditions that could facilitate the survival or introduction of other harmful microorganisms (Hikichi et al., 2013).

The identification of lactic acid bacteria (LAB), namely *Enterococcus* sp., raises concerns due to their ability to survive in low water activity environments such as dried fruits, with implications for both spoilage and safety. LAB occurrence in dried fruits is not well-documented. To date, Säde et al. (2016) reported their presence mainly in spices and dried vegetables, identifying genera such as *Weissella*, *Pediococcus*, *Enterococcus*, and *Leuconostoc*. Notably, *Enterococcus* is highly resistant to environmental stressors and is a known human pathogen (Higashide et al., 2005; García-Solache and Rice, 2019). However, certain strains produce bacteriocins that inhibit spoilage organisms and pathogens, presenting probiotic potential (Fugaban et al., 2021).

Additional contamination indicators include airborne and soil-associated genera such as *Brevibacillus* and *Micrococcus*, which may originate from dust, air, or poorly sanitized equipment. *Micrococcus luteus*, in particular, shows resilience to desiccation and low water activity, which conditions are typical of dried fruits. While generally considered non-pathogenic, it may pose a risk to immunocompromised individuals (Mauclaire and Egli, 2010). *Brevibacillus* spp., often studied for their agricultural benefits as plant growth-promoting rhizobacteria (PGPR) and bioremediation agents, have also been isolated in rare clinical cases. For example, *Brevibacillus brevis* was associated with meningitis and bacteraemia that was successfully treated with vancomycin (Parmar et al., 2020).

The identification of *Mixta calida* and *Priestia flexa* in dried fruits further highlights the importance of monitoring emerging or under-characterized bacterial species in food matrices. *Mixta calida* has been sporadically linked to serious infections including meningitis, bacteraemia, osteitis, implantable defibrillator infection, and skin necrosis (Bhopalwala et al., 2022; Blairon et al., 2024; Van Hees et

al., 2024), although the clinical significance of this organism is not yet explored. *Priestia flexa* was recently isolated from human faeces, and has been associated with urinary tract infections (Gbégbé et al., 2024).

Dried fruits offer an ideal environment for the growth of yeasts and moulds (See Table 6), as these microorganisms exhibit greater tolerance to osmotic stress and low water activity than most bacteria. Due to this, regulatory limits have been established for mycotoxins produced by moulds in dried fruit products. In contrast, bacterial contamination is only addressed through non-mandatory guidelines, such as those issued by the German Society for Hygiene and Microbiology (Web 10). However, low water activity food products have been linked with bacterial outbreaks, such as *E. coli* linked with dough mixes and hazelnut, as well as *Salmonella* associated with dried exotic fruits and chocolate (Johansen et al., 2021; Web 30, Web 31, Web 32). These incidents may result from inadequate microbial inactivation during processing or contamination occurring post-production. The current findings highlight the vulnerability of dried fruits, as they are usually sold unpackaged in open air market settings, and typically consumed without further heat treatment or washing steps.

Table 5 Identified bacteria at the genus and species level by MALDI-TOF MS. "N/A" indicates not available.

Sample code	Sample category	Country of origin	Identified genus	Identified species	MALDI score
1	Dried apricot	Hungary	*Paenibacillus*	*Paenibacillus glucanolyticus*	1.89
2	Dried apricot	Hungary	*Bacillus*	*Bacillus mojavensis*	2.03
			Bacillus	*Bacillus subtilis*	1.99
			Bacillus	*Bacillus licheniformis*	2.12
3	Dried apricot	Hungary	*Solibacillus*	*Solibacillus silvestris*	1.86
			Paenibacillus	*Paenibacillus glucanolyticus*	1.92
4	Dried apricot	Hungary	*Bacillus*	*Bacillus cereus*	2.34
			Bacillus	*Bacillus thuringiensis*	2
			Micrococcus	*Micrococcus luteus*	2.28
			Paenibacillus	*Paenibacillus glucanolyticus*	1.94
5	Dried apricot	Hungary	*Peribacillus*	*Peribacillus simplex*	2.1
6	Dried apricot	Austria	*Bacillus*	*Bacillus cereus*	2.3
			Bacillus	*Bacillus thuringiensis*	2
7	Dried apricot	Austria	*Peribacillus*	*Peribacillus simplex*	2.09
			Priestia	*Priestia megaterium*	2.15
			Bacillus	*Bacillus subtilis*	2.04
			Peribacillus	*Peribacillus lautus*	1.99

Sample code	Sample category	Country of origin	Identified genus	Identified species	MALDI score
8	Dried apricot	Austria	*Bacillus*	*Bacillus cereus*	2.21
			Bacillus	*Bacillus thuringiensis*	2.42
			Peribacillus	*Peribacillus simplex*	1.97
			Priestia	*Priestia megaterium*	2.22
			Staphylococcus	*Staphylococcus epidermidis*	2.01
			Bacillus	*Bacillus pseudomycoides*	2.17
			Pantoea	*Pantoea agglomerans*	1.92
			Bacillus	*Bacillus weihenstephanensis*	1.92
			Enterococcus	*Enterococcus mundtii*	2.5
9	Dried apricot	Austria	*Peribacillus*	*Peribacillus simplex*	2.01
			Priestia	*Priestia endophytica*	1.99
			Bacillus	*Bacillus mycoides*	1.91
10	Dried apricot	Austria	*Peribacillus*	*Peribacillus simplex*	1.99
			Bacillus	*Bacillus cereus*	2.31
			Bacillus	*Bacillus pumilus*	2.03
			Cytobacillus	*Cytobacillus firmus*	2.13
11	Dried apricot	Austria	*Peribacillus*	*Peribacillus simplex*	1.98
			Priestia	*Priestia megaterium*	2.16
			Lysinibacillus	*Lysinibacillus xylanilyticus*	2.1
			Paenibacillus	*Paenibacillus tylopili*	1.97
			Priestia	*Priestia endophytica*	1.9
12	Prune	Hungary	N/A	N/A	N/A
13	Prune	Hungary	N/A	N/A	N/A
14	Prune	Hungary	*Staphylococcus*	*Staphylococcus epidermidis*	2.28
			Bacillus	*Bacillus amyloliquefaciens*	2.22
			Micrococcus	*Micrococcus luteus*	2.17
15	Prune	Hungary	*Bacillus*	*Bacillus mycoides*	1.94
16	Prune	Hungary	*Bacillus*	*Bacillus licheniformis*	2.17
			Brevibacillus	*Brevibacillus fluminis*	2.08
			Priestia	*Priestia megaterium*	2.22
			Bacillus	*Bacillus weihenstephanensis*	1.93
17	Prune	Hungary	*Priestia*	*Priestia megaterium*	1.92
			Paenibacillus	*Paenibacillus lautus*	2.25
			Lysinibacillus	*Lysinibacillus xylanilyticus*	1.96
			Paenibacillus	*Paenibacillus tylopili*	2.1
			Cytobacillus	*Cytobacillus horneckiae*	1.89
			Staphylococcus	*Staphylococcus epidermidis*	1.93
18	Prune	Hungary	*Paenibacillus*	*Paenibacillus tylopili*	2.05
19	Prune	Austria	N/A	N/A	N/A

Sample code	Sample category	Country of origin	Identified genus	Identified species	MALDI score
20	Raisin	Hungary	*Priestia*	*Priestia megaterium*	2.23
			Bacillus	*Bacillus amyloliquefaciens*	2.34
			Bacillus	*Bacillus licheniformis*	2.01
			Peribacillus	*Peribacillus simplex*	1.97
			Micrococcus	*Micrococcus luteus*	2.23
			Bacillus	*Bacillus cereus*	1.91
			Bacillus	*Bacillus velezensis*	2.1
			Niallia	*Niallia circulans*	2.05
			Alkalihalobacillus	*Alkalihalobacillus clausii*	1.76
			Priestia	*Priestia flexa*	2.34
			Ureibacillus	*Ureibacillus sinduriensis*	2.01
21	Raisin	Hungary	*Priestia*	*Priestia megaterium*	2.2
			Bacillus	*Bacillus velezensis*	2.15
			Niallia	*Niallia circulans*	1.89
			Cytobacillus	*Cytobacillus horneckiae*	1.7
22	Raisin	Hungary	*Bacillus*	*Bacillus licheniformis*	2.04
			Bacillus	*Bacillus pumilus*	2.16
			Peribacillus	*Peribacillus muralis*	1.86
23	Raisin	Hungary	*Peribacillus*	*Peribacillus simplex*	2.1
			Bacillus	*Bacillus cereus*	2.03
			Bacillus	*Bacillus velezensis*	2.05
			Bacillus	*Bacillus pumilus*	2.09
			Bacillus	*Bacillus mycoides*	2.09
24	Raisin	Hungary	*Bacillus*	*Bacillus pumilus*	2.1
			Bacillus	*Bacillus altitudinis*	1.72
			Metabacillus	*Metabacillus idriensis*	1.81
25	Raisin	Hungary	*Bacillus*	*Bacillus mycoides*	2
			Bacillus	*Bacillus cereus*	2.29
			Priestia	*Priestia megaterium*	1.92
			Bacillus	*Bacillus amyloliquefaciens*	2.11
26	Raisin	Austria	*Bacillus*	*Bacillus licheniformis*	1.84
27	Raisin	Austria	*Priestia*	*Priestia megaterium*	2.25
			Bacillus	*Bacillus pumilus*	2.2
			Pantoea	*Pantoea agglomerans*	2.14
			Pseudomonas	*Pseudomonas cichorii*	1.84
			Micrococcus	*Micrococcus luteus*	2.36
			Kocuria	*Kocuria rosea*	2.47

Sample code	Sample category	Country of origin	Identified genus	Identified species	MALDI score
28	Raisin	Austria	*Priestia*	*Priestia megaterium*	2.17
			Bacillus	*Bacillus pumilus*	1.9
			Pantoea	*Pantoea agglomerans*	2.31
			Mixta	*Mixta calida*	2.44
			Curtobacterium	*Curtobacterium flaccumfaciens*	2.2
			Peribacillus	*Peribacillus simplex*	1.94
			Bacillus	*Bacillus pseudomycoides*	1.91
			Bacillus	*Bacillus subtilis*	2.05
29	Raisin	Austria	*Priestia*	*Priestia megaterium*	2.31
			Bacillus	*Bacillus pumilus*	2.09
			Bacillus	*Bacillus mojavensis*	2.14
30	Raisin	Austria	*Pantoea*	*Pantoea agglomerans*	2.31
			Micrococcus	*Micrococcus luteus*	2.35
			Bacillus	*Bacillus thuringiensis*	1.9
			Bacillus	*Bacillus cereus*	2.02
			Bacillus	*Bacillus mycoides*	2.02

5.2.1 Most prevalent bacterial genera among all samples

The most frequently identified bacterial genera among all samples, in descending order of prevalence were *Bacillus*, *Priestia, Peribacillus, Micrococcus, Paenibacillus, Pantoea, Staphylococcus*, *Cytobacillus*, *Lysinibacillus* and *Niallia*. Ten genera were only found sporadically (Figure 8). These results are consistent with findings from recent studies (Al Hazzani et al., 2014; Prosekov et al., 2016; Mathot et al., 2021).

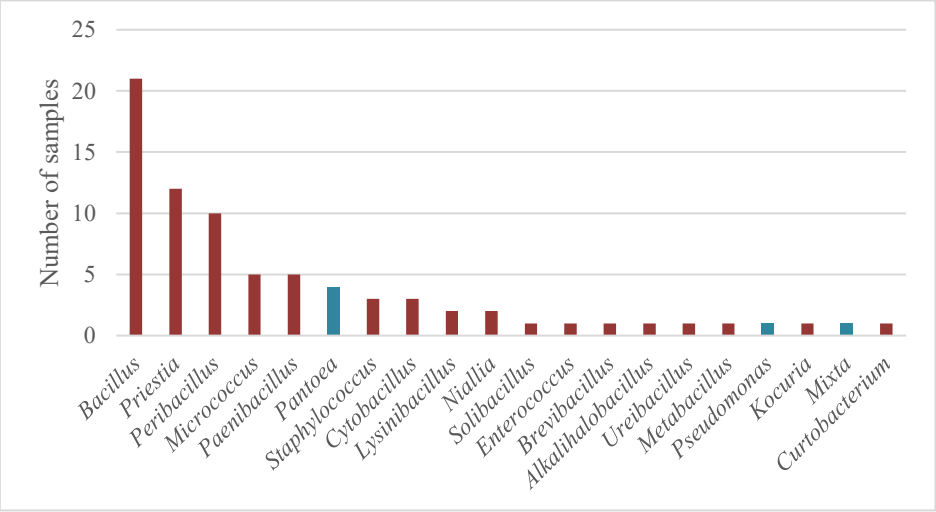

Figure 8 The bacterial genera identified from dried fruit samples using culture-dependent approach, listed in descending order of prevalence. Red bars represent Gram-positive bacteria, while blue bars represent Gram-negative bacteria.

5.2.2 Identified bacteria at the genus level, divided according to sample type

Bacillus, *Priestia*, *Micrococcus*, and *Cytobacillus* were present in all three fruit types (Figure 9, Figure 10, Figure 11). *Paenibacillus*, *Staphylococcus*, and *Lysinibacillus* were found predominantly in dried apricots and prunes, while *Peribacillus* and *Pantoea* appeared in raisins and dried apricots. *Enterococcus* and *Solibacillus* were uniquely identified from dried apricot samples, while *Brevibacillus* was found only in prune. Compared to dried apricots and prunes, raisins harboured the greatest number of uniquely identified bacterial genera with *Pseudomonas*, *Mixta*, *Curtobacterium*, *Kocuria*, *Niallia*, *Alkalihalobacillus*, *Ureibacillus* and *Metabacillus* identified in these samples.

The consistent prevalence of *Bacillus* species across all samples indicates their role as typical inhabitants of dried fruit-based products. Their capacity to form spores allows them to withstand drying processes and other extreme conditions encountered during dried fruit production. Certain species, such as *Bacillus cereus*, are noteworthy due to their potential to produce toxins and cause foodborne illness, even in low-moisture foods like dried fruits (Reyes et al., 2007; Jovanovic et al., 2021).

Among the 30 dried fruit samples analysed, 14 (46.7 %) exhibited relatively high bacterial diversity, with the presence of four or more bacterial species identified via MALDI-TOF MS (Table 5). When broken down by fruit type, five out of the 11 dried apricot samples (45.5 %) and seven out of the 11 raisin samples (63.6 %) were found to be bacterially diverse, compared to only two out of the eight prune samples (25 %).

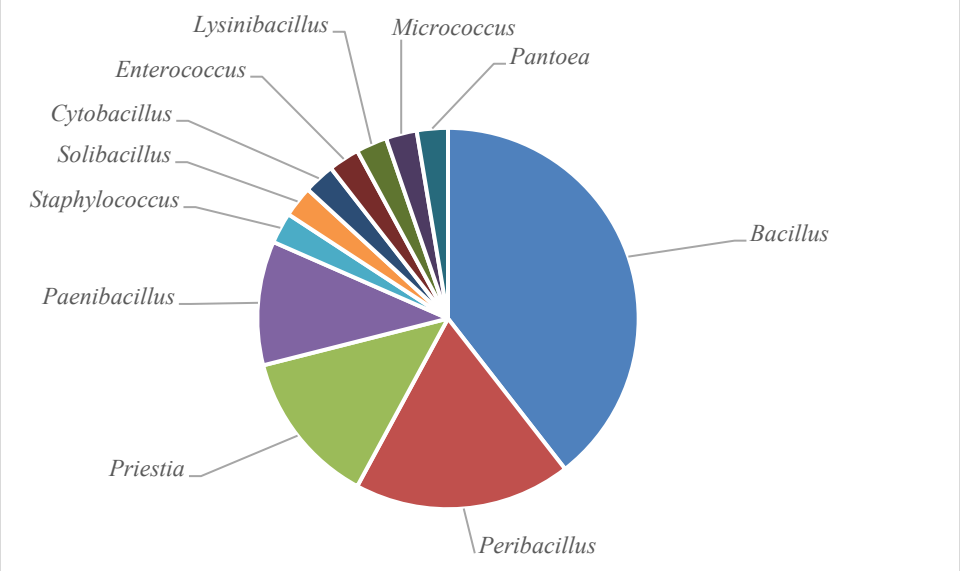

Figure 9 Bacteria at the genus level identified by MALDI-TOF MS, in all dried apricot samples.

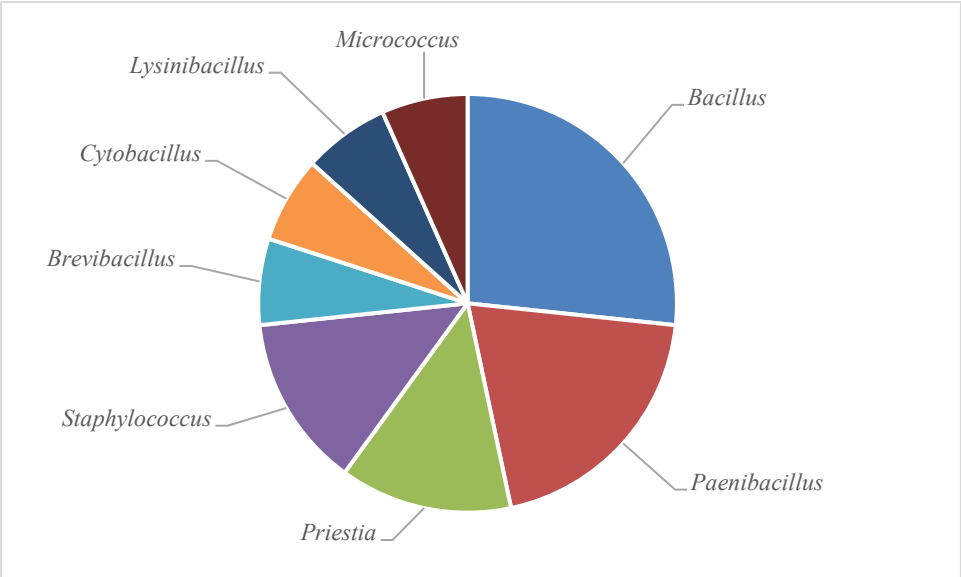

Figure 10 Bacteria at the genus level identified by MALDI-TOF MS, in all prune samples.

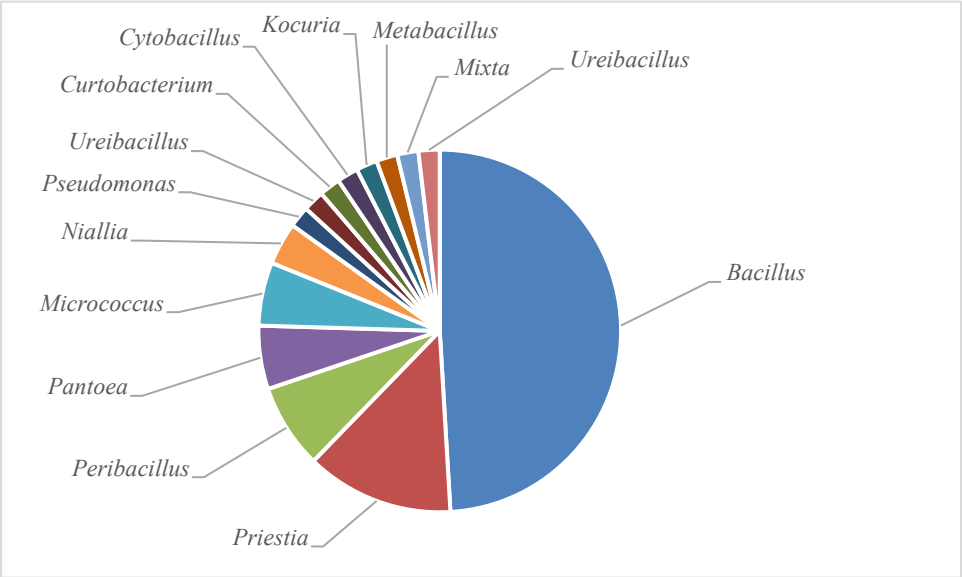

Figure 11 Bacteria at the genus level identified by MALDI-TOF MS, in all raisin samples.

5.2.3 Bacterial diversity and microbial load of individual samples

Raisins harboured the most diverse bacterial communities, followed by dried apricots and prunes. Diverse samples were defined as containing four or more bacterial species. On the other hand, dried apricots had the highest average bacterial count (6.65 x 10^3 CFU/g), followed by raisins (1.13 x 10^3 CFU/g) and prunes (4.96 x 10^1 CFU/g) (Supplementary Figure 25).

Regarding the sample category, the bacterial counts ranged from 6.5 x 10^1 CFU/g to 5 × 10^4 CFU/g in dried apricots, from < 5 x 10 CFU/g to 2.9 x 10^2 CFU/g in prunes, and from 4 x 10^1 CFU/g to

4.74 x 10^3 CFU/g in raisins. On average, dried apricots had the highest bacterial count (Supplementary Table 4).

Notably, one dried apricot sample with high diversity also had the highest overall microbial load (5×10^4 CFU/g), implying a potential link between higher bacterial diversity and increased microbial counts. On average, the microbial loads of raisins and apricots were statistically comparable (dried apricot-raisin: $Z = 0.032$, $p = 0.9738$, $\alpha = 0.05$), but both exceeded that of prunes, which shows significant difference (raisin-prune: $Z = 2.93$, $p = 0.0033$; dried apricot-prune: $Z = -2.93$, $p = 0.0033$; $\alpha = 0.05$) from the other two groups (Supplementary Figure 26). The connecting letters report is presented in Supplementary Figure 27. Despite this, all raisin samples remained below the 10^4 CFU/g threshold. Three prune samples (Sample 12, 13, 19) had bacterial counts below the quantification limit, yet genus-level identification was still possible in two cases with 16S rDNA amplicon sequencing. More information about the identified microbiota by this culture-independent method is provided in Chapter 5.3. These findings suggest that diversity is not necessarily proportional to microbial load.

Enterobacteriaceae counts could only be determined in one raisin sample (Sample 28), with counts remaining below 10^3 CFU/g (8.6 x 10^2 CFU/g). A correlation was observed between the enumeration results and the MALDI-TOF MS identification, as *Mixta calida* (a member of the Enterobacteriaceae family) was identified in this sample.

A pattern emerged regarding sample packaging and exposure. Out of the 14 samples with high bacterial diversity, 11 were purchased from open or partially open containers, suggesting environmental exposure as a contributing factor to diversity. However, three of these high-diversity samples were pre-packaged, indicating that packaging and product handling alone does not fully determine the microbial profile of dried fruits.

The remaining 16 samples (53.3%) exhibited lower diversity, with three or fewer bacterial species detected. Interestingly, nine of these were also kept in open or semi-open containers, while seven were pre-packaged. These observations suggest that factors beyond packaging, such as harvesting practices, processing, and post-harvest handling also influence the microbial diversity of dried fruits. This correlates with other recent findings that highlight that environmental conditions, agricultural practices, storage conditions or transport influence microbial communities on fruits (Bösch et al., 2021; Zhang et al., 2021b; Wassermann et al., 2022).

5.2.4 Identified fungi from dried fruit samples

Although the presence of yeasts and moulds was visible in the dried fruit samples, due to the limitations of the MALDI-TOF MS database, identification was not possible from sample 2, 3, 4, 5, 7, 9, 10 (dried apricot), sample 11, 12, 13, 14, 15, 18, 19 (prune) and sample 23, 26, 30 (raisin). Fungal

identification was only possible in 27 % of dried apricot samples, 25 % of prune samples, however, it was possible in 72% of raisin samples (Table 6).

Within dried apricot category, *Zygosaccharomyces rouxii*, an osmotolerant yeast known for spoiling sugar-rich products, was detected in two out of three dried apricot samples. Other previous findings are consistent with this result (Martorell et al., 2007; Alghamdi et al., 2023a). In contrast, a single apricot sample (Sample 8) yielded *Arthrinium phaeospermum*, *Aspergillus niger*, *Alternaria alternata*, and *Neoscytalidium dimidiatum*. Interestingly, this sample also displayed high bacterial diversity, suggesting inadequate storage or handling conditions.

In prune samples, *Zygosaccharomyces bailii*, another osmotolerant yeast, was found in one sample, while *Arthrinium phaeospermum*, previously found in dried apricot, was identified in another.

Across 11 raisin samples, a total of 11 fungal species were identified, highlighting the variability of fungal contamination in raisins. *Scopulariopsis brevicaulis*, *Microsporum audouinii*, *Aspergillus tritici*, *Cladosporium macrocarpum*, *Aspergillus lentulus*, and *Z. rouxii* were exclusive to Hungarian samples, whereas *Penicillium olsonii*, *Aspergillus montevidensis*, *Rhizopus stolonifera*, and *Penicillium chrysogenum* were only observed in Austrian samples. Interestingly, *Aspergillus niger* was identified in samples from both countries, indicating potential common environmental sources.

The genus *Zygosaccharomyces* was present in all fruit types, indicating its frequent occurrence in dried fruit products. The genus *Arthrinium* was detected in dried apricot and prune samples, but was absent in raisin samples, suggesting either fruit-specific susceptibility or differences in post-harvest handling. *Aspergillus* species were also identified across all three fruit categories, reflecting their common environmental distribution and the capacity to survive desiccated conditions. Notably, the pathogenic species *Aspergillus niger* was identified in 46 % of the samples, in which identification was possible. Other fungal genera were identified in fewer samples. *Alternaria* and *Neoscytalidium* were both detected in only one sample (Sample 8), while *Scopulariopsis* and *Microsporum* were detected in another (Sample 20). *Cladosporium* was identified in one raisin sample (Sample 21). *Penicillium* species were detected in two raisin samples (Sample 27 and Sample 29). *Penicillium olsonii* was identified in Sample 27, and *Penicillium chrysogenum* was identified from Sample 29.

The detection of *Aspergillus niger* and *Arthrinium phaeospermum* may raise potential safety concerns, as *A. niger* is known to produce mycotoxins such as ochratoxin A in certain food matrices, while both fungi have been associated with opportunistic infections in humans (Gautam et al., 2011; Li et al., 2020b). However, *A. niger* is commonly used in wastewater bioremediation and as an industrial host for producing food-grade enzymes (Li et al., 2020c; Subhash et al., 2022). In dried fruits, fungal contamination is generally regarded as more crucial than bacterial contamination due to the ability of certain moulds to produce chemically stable mycotoxins that can remain in the products even after

drying or other preservation steps. *Neoscytalidium dimidiatum*, apart from being a plant pathogen is an emerging human pathogen, and can cause not only skin infections but also systematic diseases (da Silva et al., 2016; Derviş et al., 2024). *Scopulariopsis brevicaulis*, a soil-associated saprophyte and the leading cause of dermatomycosis in birds, points to a potential contamination source from the environment (Tunç et al., 2022). Likewise, *Microsporum audouinii*, a dermatophyte responsible for infections of the scalp, skin, and nails, also supports the idea of environmental exposure (Sacheli et al., 2016). *Cladosporium macrocarpum* has been linked to mild allergic reactions (Hasnain et al., 2004). The identification of *Penicillium olsonii*, considered a potential biocontrol agent in agriculture, in dried fruit samples suggests contamination from agricultural sources (Rojas et al., 2022).

Table 6 Identified fungi with MALDI-TOF MS.

Sample code	Sample category	Country of origin	Identified genus	Identified species	MALDI Score
1	Dried apricot	Hungary	*Zygosaccharomyces*	*Zygosaccharomyces rouxii*	2.05
6	Dried apricot	Austria	*Zygosaccharomyces*	*Zygosaccharomyces rouxii*	2.07
8	Dried apricot	Austria	*Arthrinium*	*Arthrinium phaeospermum*	1.92
			Aspergillus	*Aspergillus niger*	2.31
			Alternaria	*Alternaria alternata*	2.50
			Neoscytalidium	*Neoscytalidium dimidiatum*	1.97
16	Prune	Hungary	*Zygosaccharomyces*	*Zygosaccharomyces bailii*	1.89
17	Prune	Hungary	*Arthrinium*	*Arthrinium phaeospermum*	2.10
20	Raisin	Hungary	*Scopulariopsis*	*Scopulariopsis brevicaulis*	1.88
			Microsporum	*Microsporum audouinii*	2.41
21	Raisin	Hungary	*Aspergillus*	*Aspergillus tritici*	2.24
			Cladosporium	*Cladosporium macrocarpum*	1.99
22	Raisin	Hungary	*Aspergillus*	*Aspergillus lentulus*	2.31
			Aspergillus	*Aspergillus niger*	1.97
24	Raisin	Hungary	*Aspergillus*	*Aspergillus tritici*	1.92
			Aspergillus	*Aspergillus niger*	2.29
25	Raisin	Hungary	*Aspergillus*	*Aspergillus niger*	2.47
			Zygosaccharomyces	*Zygosaccharomyces rouxii*	2.22
27	Raisin	Austria	*Aspergillus*	*Aspergillus niger*	1.96
			Penicillium	*Penicillium olsonii*	2.08
28	Raisin	Austria	*Aspergillus*	*Aspergillus montevidensis*	1.86
			Rhizopus	*Rhizopus stolonifer*	1.87
29	Raisin	Austria	*Aspergillus*	*Aspergillus niger*	2.31
			Penicillium	*Penicillium chrysogenum*	1.92

5.2.5 Additional tests for the characterization of the microbiota of dried fruits

The following chapter will present the results of additional tests, including pH, water activity, and sugar content, that were conducted to comprehensively characterise the microbiota of dried fruits.

Regarding the preservative content, the conclusions were drawn based on the information present on the label of the products. Experiments regarding the preservative levels were not conducted.

5.2.5.1 pH results of dried fruit samples

The pH values of commercially purchased dried fruits are presented in Figure 12, and the exact pH values are presented in Supplementary Table 5. The pH of the dried fruits played a role in the microbial loads of the samples.

The pH ranged from 3.89 to 5.02 in the dried apricot category with an average pH of 4.21, from 3.62 to 4.09 in the prune category with an average pH of 3.84, and from 3.29 to 4.24 in the raisin category with an average pH of 3.83. The Kruskal Wallis test concluded that the pH values of dried apricot samples differed significantly from the pH values of prune ($Z = -5.55$, $p < 0.0001$, $\alpha = 0.05$) and raisin ($Z = -3.81$, $p = 0.0001$, $\alpha = 0.05$) samples. However, there was no significant difference between the pH values of prunes and raisins ($Z = 1.01$, $p = 0.3080$) (Supplementary Figure 28). On average, dried apricots had the highest pH (Figure 12 (A)).

The pH values varied within the dried fruit categories (Figure 12 (B)). According to the Students t-test, among dried apricots, almost every sample differed significantly in pH, except for example Sample 1 and 7 ($p = 0.3937$, $\alpha = 0.05$), or Sample 3 and 5 ($p = 0.9022$, $\alpha = 0.05$), or Sample 9 and 10 ($p = 0.1500$, $\alpha = 0.05$) (Supplementary Table 29). The connecting letter report is presented in Supplementary Figure 32. Sample 1 and 7 were purchased in two different countries but both were pre-packaged, while Sample 3 and 5 were sourced from different markets, but were all stored in open container accessible only to the seller, while Sample 9 and 10 were purchased at the same market from different vendors, also stored in open containers. In contrast, Sample 2 and 8, both pre-packaged, had significantly different pH ($p< 0.0001$, $\alpha = 0.05$) (Supplementary Figure 29). Sample 3 and Sample 5 had the lowest pH (both 3.89). In both cases low bacterial counts were found ($< 3x10^2$ CFU/g). Using MALDI-TOF MS, *Solibacillus silvestris* and *Paenibacillus glucanolyticus* were found in Sample 3, and *Peribacillus simplex* in Sample 5. The highest pH was found in Sample 2 (5.02), yet only three *Bacillus* species (*B.mojavensis*, *B. subtilis, B. licheniformis*) could be identified with the culture-dependent method, despite the high pH. Despite being pre-packaged, Sample 2 had a relatively high bacterial load (2×10^4 CFU/g), which could indicate insufficient microbial reduction during processing or post-production contamination. This sample had the highest pH among all samples.

Regarding prune samples, only Sample 13, 15, and 16, and Sample 17 and 18 were similar in pH (Figure 18 (B), Supplementary Figure 30). Sample 13 was pre-packaged, while Sample 15 and 16 were purchased from open containers. Sample 17 and 18 derived from the same market but from different vendors. Sample 15 had the lowest (3.62) and Sample 17 had the highest (4.09) pH. This case shows that pH plays an important factor in bacterial counts, as while using culture-dependent

methods, only one bacterial strain (*Bacillus mycoides*) could be identified from the lowest pH sample, the sample with the highest pH harboured six types of bacterial strains (*Priestia megaterium, Paenibacillus lautus, Lysinibacillus xylanilyticus, Paenibacillus tylopili, Cytobacillus horneckiae, Staphylococcus epidermidis*), as well as a fungal species (*Arthrinium phaeospermum*).

Among raisin samples, for example, only Sample 23 and 30, or 24 and 28 had similar pH (Supplementary Figure 31). Sample 23 and 24 were purchased in Hungary, while Sample 28 and 30 were sourced from Austria. Sample 29, which was statistically different from all the other raisin samples ($p = 0.0001$, $\alpha = 0.05$) had the lowest pH (3.29) among all samples, yet, still contained bacterial species such as *Priestia megaterium*, *Bacillus pumilus* and *B. mojavensis* and fungal species such as *Aspergillus niger* and *Penicillium chrysogenum* were identified using MALDI-TOF MS. This sample was purchased from a partially open container, accessible to customers. The highest pH within this category belonged to Sample 21 (4.24), with four identified bacterial strains by MALDI-TOF MS, such as *Priestia megaterium, Bacillus velezensis, Niallia circulans and Cytobacillus horneckiae*, and two fungal strains such as *Aspergillus tritici* and *Cladosporium macrocarpum*. Only *Priestia* and *Bacillus* could be identified with the 16S rDNA amplicon sequencing. This sample was purchased from a covered container, accessible to customers, but the microbial load remained under 2×10^3 CFU/g. These results indicate that while low pH potentially leads to lower microbial diversity and microbial loads, pH alone is not enough for the determination of the complex microbiota of dried fruits.

Recent studies have also investigated how pH effects the bacterial survival in dried fruits. Canakapalli et al. (2021) evaluated the long-term survival of *E. coli* O157:H7, *Salmonella* and *Listeria monocytogenes* on various dried fruits – low and high moisture Medjool dates, sundried tomatoes and dried plouts. Their experiment lasted 180 days at a refrigerated and ambient temperature. Two different carriers were used. Dates were inoculated using a dry (sand) carrier, while dried tomatoes and plouts were inoculated using wet (PBS) carrier to ensure even distribution without altering the fruit's physiochemical properties. The pH and water activity did not change throughout the storage. The researchers have observed that pathogens survived longer and at higher levels for longer time on dates (pH 5.59-5.83) than sundried tomatoes (pH 3.80) and plouts (pH 3.45), indicating that lower acidity reduces survival. Gurtler et al. (2020) investigated the survival of desiccation resistant *Salmonella* on dehydrated apple slices from six cultivars with different pH. Cultivars with lower pH showed greater *Salmonella* inactivation, indicating that apple acidity influences pathogen survival.

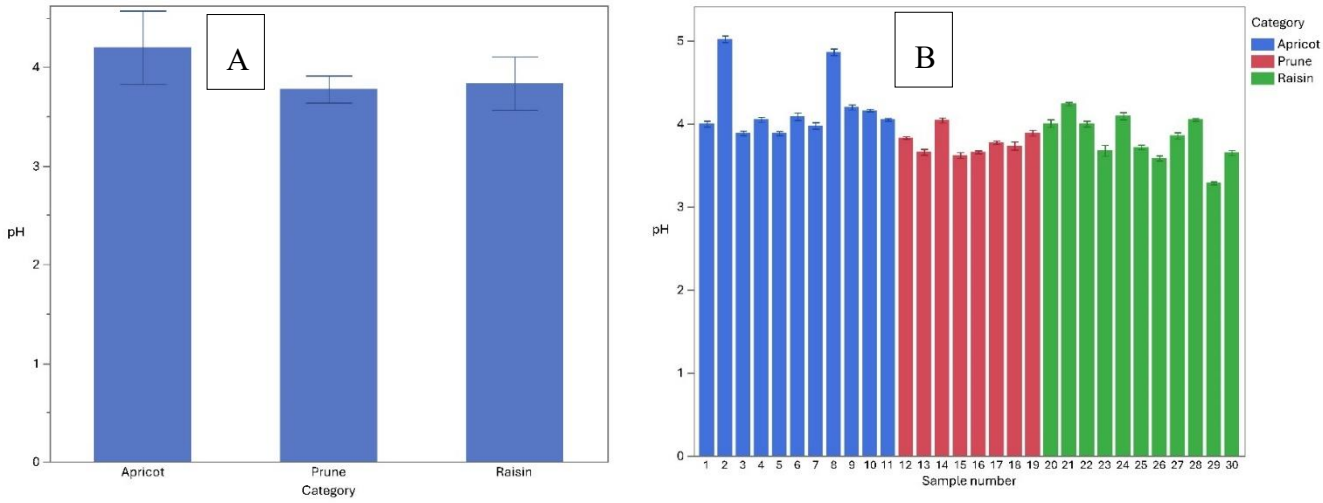

Figure 12 The average pH of dried fruit categories (A), and the pH within the sample categories (B).

5.2.5.2 Water activity results of dried fruit samples

Water activity (a_w) was also found to be an important factor in the microbial diversity of the samples. The average water activity values, along with the values of the individual samples are presented in Figure 13. The exact values are provided in Supplementary Table 5. The a_w values of the whole fruits, in the dried apricot category ranged from 0.416 to 0.735, with an average value of 0.64; in the prune category ranged from 3.62 to 4.09, with an average of 0.74; and in the raisin category ranged from 3.29 to 4.24, with an average of 0.46 (Supplementary Table 5). The a_w values of the cut fruits, in the dried apricot category ranged from 0.411 to 0.742, with an average of 0.64; in the prune category ranged from 0.691 to 0.853, with an average of 0.74; and in the raisin category from 0.349 to 0.536, with an average of 0.45. The a_w values of whole and cut fruit samples did not differ significantly ($Z = 0.10901$, $p = 0.9132$ in dried apricots, $Z = 0.1752$, $p = 0.8609$ in prunes, and $Z = 0.5386$, $p = 0.5901$ in raisins, $\alpha = 0.05$) (Supplementary Figure 33). The connecting letters report is presented in Supplementary Figure 34 (A). Significant differences (prune-apricot: $Z = 4.86$, $p < 0.0001$; raisin-prune: $Z = -6.39$, $p < 0.0001$; raisin-apricot: $Z = -6.10$, $p < 0.0001$; $\alpha = 0.05$) were observed between the dried fruit categories (Supplementary Figure 35)). The connecting letters report is presented in Supplementary Figure 36. Within the sample categories, only a few samples showed no differences in their a_w values. In contrast, Sample 8 had a significantly lower a_w value ($Z = -2.80$, $p = 0.0050$, $\alpha = 0.05$) (0.411) than other dried apricots. The corresponding p-values are presented in Supplementary Figure 37. The connecting letters report is presented in Supplementary Figure 40 (A). Despite the low a_w, nine bacterial species and five genera in total (*Peribacillus, Priestia, Staphylococcus, Pantoea,* and *Enterococcus*) could be identified using MALDI-TOF MS. Interestingly, this sample also harboured four fungal genera, namely *Arthrinium, Aspergillus, Alternaria* and *Neoscytalidium*. Notably, this sample was purchased from a partially open container

which was also accessible to customers (Supplementary Table 2). Furthermore, this sample also had the highest microbial load ($5x10^4$ CFU/g) (Supplementary Table 4). The highest a_w in this group belonged to Sample 4, where only four bacterial strains (*Bacillus cereus, B. thuringiensis, Micrococcus luteus, Paenibacillus glucanolyticus*) could be identified using the same method, indicating that other internal and external factors could play a role in the microbial loads of the dried fruits. Sample 2 had similar water activity to Sample 4, with three bacterial species (*B. mojavensis, B. subtilis, B. licheniformis*) identified with MALDI-TOF MS (Table 5). However, regarding the bacterial count, Sample 4 and 8 had similar bacterial load.

Among prune samples, Sample 17 had significantly lower a_w value (0.691) than other prune samples, yet five bacterial strains belonging to five different genera were identified via MALDI-TOF MS, namely *Priestia megaterium*, *Paenibacullus lautus*, *Lysinibacillus xylanilyticus, Cytobacillus horneckiae*, *Staphylococcus epidermidis*. The corresponding p-values are presented in Supplementary Figure 38. The connecting letter report is presented in Supplementary Figure 40 (B). Interestingly, this sample was purchased from a partially covered container, accessible only to the seller, meaning that customers did not play a role in the relatively high bacterial diversity. On the other hand, Sample 16 had statistically the highest a_w (0.853) ($Z = 2.80$, $p = 0.0051$, $\alpha = 0.05$) in this group, where only four bacterial species representing three genera were identified with MALDI-TOF MS, such as *Bacillus licheniformis*, *B. weihenstephanensis, Brevibacillus fluminis* and *Priestia megaterium*. The yeast species *Zygosaccharomyces bailii* was also identified. The bacterial load of this sample did not reach 10^2 CFU/g. Despite having significantly higher a_w values than the other groups (prune-apricot: $Z = 4.86$, $p < 0.0001$; raisin-prune: $Z = -6.39$, $p < 0.0001$; $\alpha = 0.05$), prune samples exhibited lower microbial diversity compared to dried apricots and raisins. No identification was possible with the culture-dependent method from Sample 12, 13, and 19, as no bacterial colonies were successfully cultivated prior to MALDI-TOF MS analysis. These samples were pre-packaged.

In the raisin category, in Sample 21, with significantly the lowest a_w (0.349) ($Z = 2.80$, $p = 0.0051$, $\alpha = 0.05$), *Priestia megaterium*, *Bacillus velezensis*, *Niallia circulans* and *Cytobacillus horneckiae* were identified via MALDI-TOF MS. The p-values are presented in Supplementary Figure 39. The connecting letters report is presented in Supplementary Figure 40 (C). Interestingly, Sample 28 had similar a_w value, however, six different bacterial genera (*Bacillus*, *Priestia*, *Pantoea*, *Mixta*, *Curtobacterium*, *Peribacillus*) were identified with MALDI-TOF MS. These two samples also had similar bacterial counts (1×10^3 CFU/g, 1.3×10^3 CFU/g, respectively). In contrast, Sample 24 had the highest a_w, but only three bacterial species, such as *Bacillus pumilus*, *B. altitudinis* and *Metabacillus idriensis* were identified using the culture-dependent method. Additionally, two species of *Aspergillus* (*Aspergillus niger*, *A. tritici*) were also detected. Interestingly, the bacterial load in Sample 24 (2.6×10^2 CFU/g) was lower than that of observed in the low a_w samples. While these

samples were purchased from partially covered containers, accessible to customers, Sample 24 was purchased from an open container, accessible only to the seller. The different handling of the products may give an explanation to the different microbiota and microbial loads.

These results indicate that bacteria can persist in low-a_w food products, even below the well-known threshold of 0.60. Furthermore, it highlights that low a_w alone is not the only factor influencing bacterial survival, which correlates with other studies. Beuchat and Mann (2014) assessed the survival of *Salmonella* on dried cranberries, raisins, strawberries and in date paste. Researchers have used mist inoculation to apply the acid-adapted *Salmonella* onto the fruits. They observed that at an ambience temperature (25 °C) *Salmonella* survived on cranberries (a_w 0.47) and raisins (a_w 0.46) for up to 21 days, on strawberries (a_w 0.21) for 42 days, and in date paste (a_w 0.69) for 84 days. The study demonstrates that water activity is a key factor in pathogen persistence on dried fruits. In their study, as water activity increases, *Salmonella* survives longer, making dried fruits with higher water activity a greater food safety concern. However, other factors such as pH, antimicrobial compounds and surface properties of the dried fruits also greatly influence bacterial survival. This could explain why *Salmonella* survived longer on dried strawberries with a very low water activity than on cranberries and raisins with higher a_w.

It is important to note that pH and water activity of the dried fruits, even within the same category, is influenced by several other factors even pre-harvest and post-harvest. The influencing factors pre-harvest could be the growing conditions and environmental factors (radiation, temperature), the geographical factors, harvest maturity or even genetic variations. Post harvest factors influencing the chemical composition of dried fruits are, method of harvesting, time of harvesting, storage conditions (temperature, relative humidity), packaging and packaging materials (Tyagi et al., 2017; Bekele, 2018).

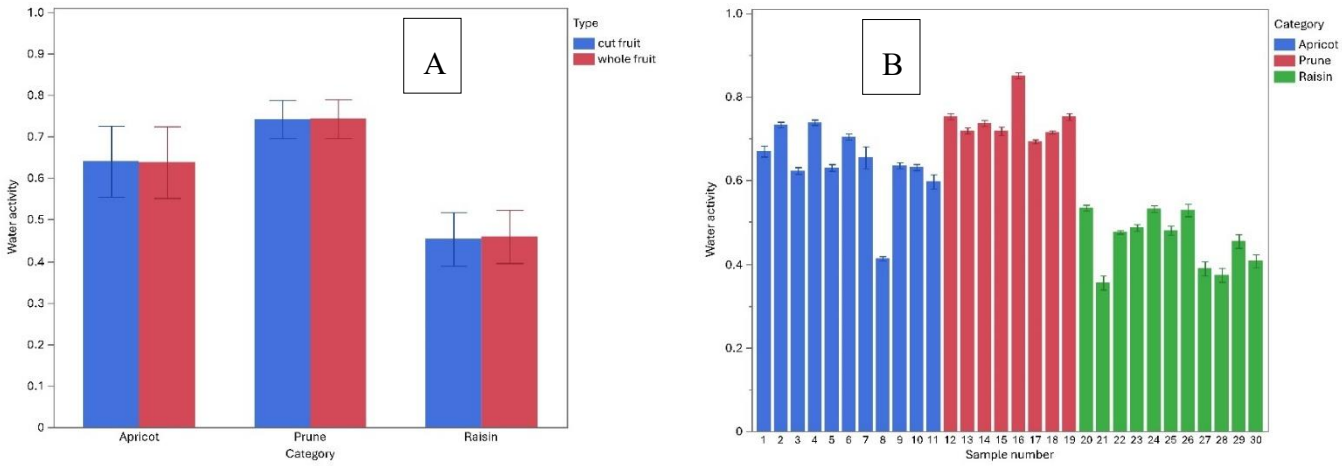

Figure 13 The average water activity values of cut and whole dried fruits divided by dried fruit categories (A); water activity values of whole and cut combined presenting the differences within the dried fruit categories (B).

5.2.5.3 Sugar concentrations of dried fruit samples

The Rida Cube system was used to determine the sugar (glucose, fructose, sucrose) concentrations of dried fruit samples. The results are presented in Supplementary Table 5.

The sugar concentration ranged from 1.32 to 17.34 g/100g in dried apricots, with an average concentration of 6.85 g/100g; from 1.89 to 19.43 g/100g in prunes, with an average concentration of 10.52 g/100g; and from 4.90 to 28.53 g/100g in raisins, with an average concentration of 17.74 g/100g. According to literature, the total sugar content of dried apricot, prune and raisin is 53.4 g/100g, 38.13 g/100 g, and 65.7 g/100 g, respectively (Web 33; Web 34; Web 35). The results differ from these literature values. Even though Naryal et al. (2019) observed that genetic factors and geographical elevation can influence the sugar content of fruits, the cause of this alteration might rather be due to the age of fruits as the samples were measured after almost one year of storage.

5.2.5.4 Added preservatives

Among the 30 dried fruit samples, seven samples (Sample 1, 2, 7 – dried apricots; Sample 12, 14, 19 – prunes, Sample 20 – Raisin) contained preservatives according to their label (potassium sorbate, sorbic acid, sulphur oxide) (Supplementary Table 2). For the remaining samples, the preservative content is unknown, as it was not listed on the label, or not provided by the seller. The preservative content was not analytically measured within this thesis, therefore the conclusions regarding the effect of the preservatives on the microbiota of these samples are made with this limitation.

Other samples might have contained preservatives; however, no information was provided regarding that. Sample 1 (pre-packaged) was treated with sulphur dioxide and yielded only one bacterial genus (*Paenibacillus*) and one yeast genus (*Zygosaccharomyces*) using MALDI-TOF MS. Furthermore,

Sample 7 (pre-packaged) containing the same preservative exhibited similar levels of bacterial diversity with three bacterial (*Bacillus*, *Priestia*, *Peribacillus*) and one fungal (*Zygosaccharomyces*) genus identified using culture-dependent methods. As these samples were pre-packaged, this microbiota level could indicate unhygienic processing environments. Interestingly, in Sample 2 (pre-packaged, treated with potassium sorbate) only *Bacillus* was identified with the culture-dependent method. These findings indicate that based on preservative treatment, different bacterial presences can be observed in dried apricots. However, dried apricot samples not known to have been treated with preservatives (Sample 4, 8, 9, 10, 11) yielded diverse microbiota based on the identification results by MALDI-TOF MS and. This correlates with other findings. In a study by Liu et al. (2021) pathogens (STEC, *Salmonella* spp., *L. monocytogenes*) survived longer or at higher levels on dried apricot samples not treated with sulphur dioxide. In another study, greater reduction of *Salmonella* and *Enterococcus faecium* was observed on dried peaches made with sulphur dioxide treatment (Liu et al., 2022b). These studies show that sulphur dioxide has antimicrobial effects and can be used effectively for the preservation of dried fruit products.

The bacterial presence also varied on prune samples depending on the preservative treatment or identification method. Bacterial identification in Sample 14 (pre-packaged, treated with sorbic acid) revealed three bacterial genera (*Bacillus*, *Staphylococcus*, *Micrococcus*) using the culture-dependent method. From Sample 12 (pre-packaged, treated with potassium sorbate) and Sample 19 (pre-packaged, treated with sorbic acid) no bacterial and fungal genera could be cultivated prior to MALDI-TOF MS analysis. Interestingly, despite not being treated with preservatives, Sample 13 (pre-packaged, organic) yielded no bacteria using either identification method. Similarly to Sample 13, Sample 17 (purchased from a partially open container, accessible only to seller) was not treated with either potassium sorbate, sorbic acid, or sulphur oxide, however, five bacterial genera (*Priestia*, *Paenibacillus*, *Lysinibacillus*, *Cytobacillus*, Staphylococcus) and one fungal genus (*Arthrinium*) could be identified via MALDI-TOF MS. Despite this diversity, the bacterial load was found to be below 3×10^2 CFU/g (Supplementary Table 4). This high level of bacterial diversity highlights that proper storage conditions are important factors influencing the microbiota of dried fruits. Cárdenas et al. (2024) evaluated the effect of potassium sorbate and storage temperature on the quality and microbial stability of prunes over 12 months. They observed that while storage at 37° C significantly affected the quality of prunes (reduced moisture content, increased firmness), no significant differences were observed in mould and yeast presence between prunes treated with or without potassium sorbate.

Among raisin samples, only Sample 20 (treated with sulphur dioxide) was knowingly treated with preservatives. However, based on the identification results, this sample was found to be one of the most diverse within this fruit group, as seven bacterial genera (*Bacillus*, *Priestia*, *Peribacillus*,

Micrococcus, *Niallia*, *Alkalihalobacillus*, *Ureibacillus*) and two fungal genera (*Scopulariopsis*, *Microsporum*) were identified using the culture-dependent method.

Sample 21, 27, 28 and 30 are not known to have been treated with preservatives, suggesting potentially high levels of microbial diversity. Consistent with this, several bacterial and fungal genera were detected in these samples using both methods.

In a recent study, Gala apple slices were pretreated with eight different antimicrobial solutions (potassium sorbate, sodium benzoate, ascorbic acid, propionic acid, lactic acid, citric acid, fumaric acid, and sodium bisulphate) applied prior to dehydration (60 °C for 5 hours). The goal was to identify methods to reduce *Salmonella* contamination in dried apples, as repeated contamination incidents have led to multiple product recalls in recent years. The most effective treatments were the fumaric acid and sodium bisulphate rinses with *Salmonella* survival of 2.64 log CFU and 0.00 log CFU respectively (Gurtler et al., 2020). This study supports our results that the correct choice of preservative treatment influences bacterial survival in dried fruit products.

In contrast to preservative treated samples that exhibited diverse microbiota, several samples (Sample 3, 6, 13, 15, 18, 22, 23, 24, 25, 26, 29) not known to have been treated with preservatives demonstrated low bacterial diversity with only one or two genera detected using the culture-dependent method. This low level of diversity may indicate effective hygiene practices during processing or storage, or intrinsic properties of the samples that inhibit microbial colonization or proliferation. It is also possible, that some of these samples contained preservatives that limited microbial growth. Overall, these results suggest that while potassium sorbate and sorbic acid might effectively inhibit certain bacterial and fungal genera, their efficacy depends on the fruit type. Sulphur dioxide had no impact on the overall microbial diversity of the samples.

5.3 Microbial diversity assessed via culture-independent method

Among the 30 commercially purchased dried fruit products, a total of seven bacterial genera were identified using the culture-independent method via 16S rDNA amplicon sequencing (Supplementary Table 6). Bacterial identification was not possible in one sample (Sample 13) due to insufficient amount or quality DNA.

Bacillus was also identified as the most predominant genus using the culture-independent approach. All the other genera detected via this method were also observed with the culture-dependent approach, with the exception of *Neobacillus*, *Mesobacillus*, *Tumebacillus*. Notably, *Neobacillus* and *Mesobacillus* were recently proposed as separate genera, reclassified from the genus *Bacillus*, as phylogenomic analyses revealed distinct monophyletic clades within this genus (Patel and Gupta; 2020). *Neobacillus* were identified from two dried apricot samples (Sample 7, 10). *Tumebacillus* was detected in one dried apricot (Sample 10) and in one raisin sample (Sample 23), while *Mesobacillus*

was identified from a dried apricot sample (Sample 10). The samples were purchased from different environments. Sample 7 was pre-packaged, while in contrast, Sample 10 was obtained from an open-air market, where fruits were stored in a partially open container, accessible only to the seller. Sample 23 also originates from a market where it was stored in an open container.

In the literature, *Neobacillus* species have been isolated from diverse environments, including mangrove sediment, soil, citrus rhizosphere soil, and from a Mars 2020 spacecraft assembly facility (Tang et al., 2021; Liu et al., 2022a; Yuki et al., 2022; Hameed et al., 2025). *Mesobacillus* species have been reported from extreme or unique environments such as sandy soil of a cold desert, hot spring water, and an orange-coloured pond (Rai et al., 2021; Zhang et al., 2021a; Burhan and Al-Rawi, 2025). Similarly, *Tumebacillus* species have also been isolated from soil, water and from the roots of cottonwood (Her et al., 2015; Carper et al., 2020; Kang et al., 2022; Zhang et al., 2023).

These findings suggest that some members of these genera can withstand low-water activity and high temperature habitats, conditions similar to the desiccation process in dried fruits. As the number of newly identified species within these genera increases, the need for proper hygiene practices and post-harvest handling arises, especially given the potential emergence of pathogenic strains in the future.

5.3.1 Most prevalent bacteria among all samples

The metabarcoding analysis and the taxonomic sample profiling were carried out by the BOKU Core Facility Bioinformatics.

Amplicon sequencing of the 16S rDNA gene revealed that *Bacillus*, *Priestia*, *Pantoea*, and *Neobacillus* were the most prevalent bacterial genera identified across the dried fruit samples (Figure 14). *Bacillus* was consistently detected in all samples, except in one case (Sample 13) where identification was not possible, though its relative abundance varied widely. In most samples, it accounted for 64 % and 100 % of the bacterial community, underscoring its dominance in the dried fruit microbiota. This widespread occurrence is due to related to the genus's ability to form spores, which enhances its survival in desiccated environments (Checinska et al., 2015). However, two dried apricot samples (Sample 7 with 47 %; Sample 10 with 36 %) and two raisin samples (Sample 21: 28 %; Sample 27: 29 %) showed notably lower abundance of *Bacillus*, indicating that microbial competition or even environmental conditions may influence its relative abundance.

Priestia was the second most dominant genus, detected in 14 samples in total, including six dried apricot, two prune, and six raisin samples (Supplementary Table 6). It was especially abundant in two raisin samples (Sample 21 with 71 %; Sample 27 with 72 %). Although these two samples were obtained from different storage conditions, they showed nearly identical bacterial compositions. Sample 21 was openly displayed and accessible to customers, while Sample 27 was pre-packaged. In other raisin samples, *Priestia* was present in varying proportions, from less than 10% (Samples 20,

22, and 28) to moderate levels such as 24 % in Sample 24. Similarly, in dried apricot samples, *Priestia* ranged from low abundance (Samples 1, 3, and 4, each below 10 %) to higher levels, such as 33 % in Sample 5 and 15 % in Sample 10. The genus was not detected in prune samples.

Pantoea was present at relatively low levels in all samples where it was detected, always remaining below 15 % of the total microbiota. Its lowest detected proportion was in Sample 10, where it represented only 1 % amidst a diverse bacterial community.

Neobacillus was detected at high abundance in two dried apricot samples, in Sample 7 with 53%, and in Sample 10 with 31 %. These samples came from different market settings, with Sample 7 purchased pre-packaged and Sample 10 collected from a partially open container. This difference in storage and handling conditions may have influenced the microbial composition, suggesting a potential link between exposure and the prevalence of certain bacterial taxa.

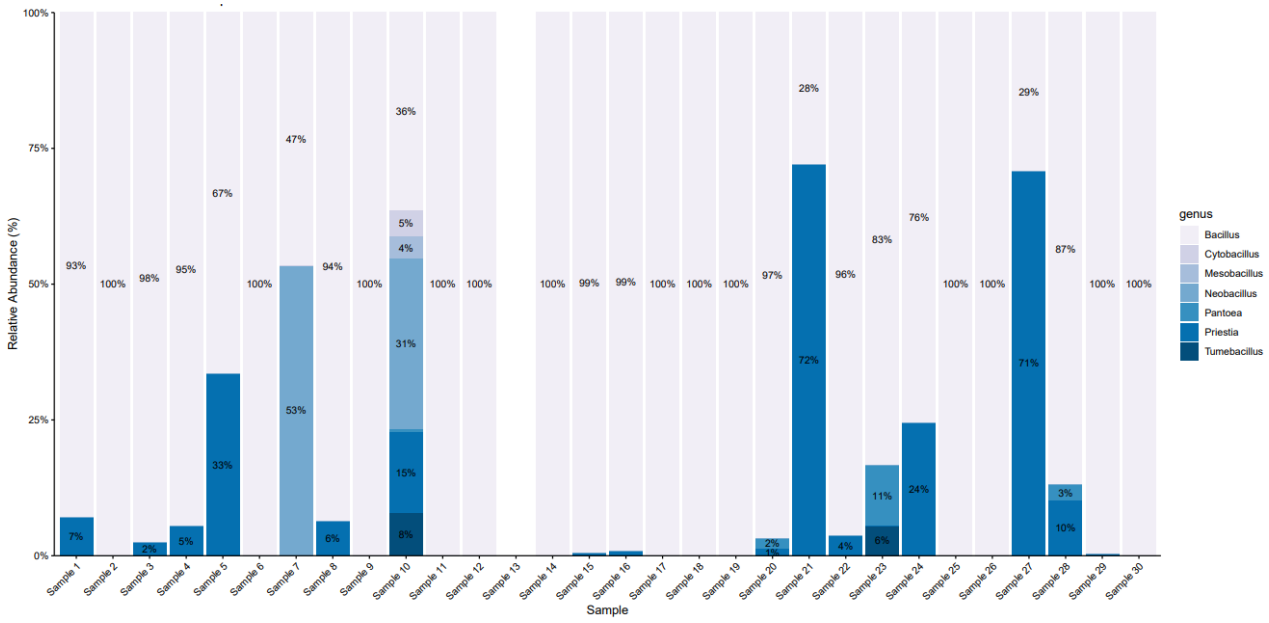

Figure 14 Relative abundance of bacterial genera identified from dried apricot, prune and raisin samples by 16S rDNA amplicon sequencing.

Bacillus was identified in 96.7 % of the samples, while *Priesta* could only be identified in 46.7 % of the samples. *Pantoea* appeared in 13.3 % of the samples, *Neobacillus* and *Tumebacillus* were detected in 6.7 % of the samples, while *Cytobacillus*, and *Mesobacillus* were only identified from a single sample (3.3 % each) (Figure 15).

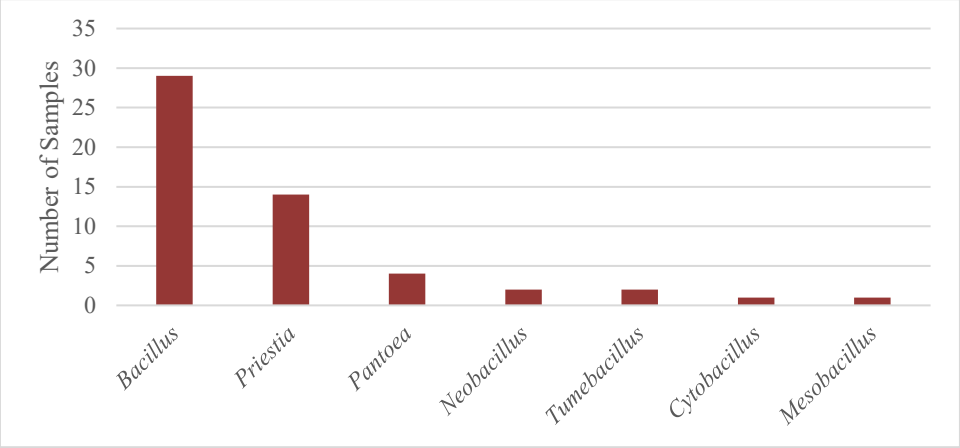

Figure 15 The bacterial genera identified from dried fruit samples using culture-independent approach.

When comparing the dried fruit categories, *Bacillus* and *Priestia* were identified in all three dried fruit categories (Table 7). *Pantoea* and *Tumebacillus* were detected in both dried apricots and raisins. *Neobacillus*, *Cytobacillus* and *Mesobacillus* were uniquely identified from dried apricot samples.

Table 7 Bacteria at the genus level identified by 16S rDNA amplicon sequencing, divided according to sample types.

Types of dried fruits	Identified bacteria
Dried apricot	*Bacillus*
	Priestia
	Neobacillus
	Cytobacillus
	Mesobacillus
	Pantoea
	Tumebacillus
Prune	*Bacillus*
	Priestia
Raisin	*Bacillus*
	Priestia
	Pantoea
	Tumebacillus

5.3.2 Bacterial diversity of individual samples

Based on the results of the 16S rDNA amplicon sequencing, bacterial diversity appeared lower compared to results obtained via MALDI-TOF MS. Among the 30 dried fruit samples, 12 samples (40 %) contained two bacterial genera, three samples (10 %) contained three genera, and one sample

(3.3 %) showed the presence of seven genera (Figure 14). Conversely, 13 samples (43.3 %) revealed only a single bacterial genus.

The most diverse bacterial community was found in a dried apricot sample (Sample 10), where seven genera were identified in descending order of relative abundance: *Bacillus* (36 %), *Neobacillus* (31 %), *Priestia* (15 %), *Tumebacillus* (8 %), *Cytobacillus* (5 %), *Mesobacillus* (4 %), and *Pantoea* (1 %). This sample was purchased from a market where it had been stored in a partially open container accessible only to the vendor, minimizing direct customer contact. The presence of multiple genera in a single sample highlights the potential impact of suboptimal storage conditions on bacterial diversity, even in products with low water activity such as dried fruits. However, other dried apricot samples (Sample 3, 4, 5, 6, 8, 9, 11), also sourced from markets demonstrated lower diversity, with only two genera identified. Notably, Sample 8 was directly accessible to customers, yet its microbial profile only consisted of two bacterial genera, indicating that additional factors beyond storage, such as initial microbial load, handling, fruit type may also play a role in microbial diversity.

In contrast, all prune samples displayed a highly uniform bacterial profile, with *Bacillus* as the sole detected genus. In most cases, it accounted for 100 % of the relative abundance, except in Samples 15 and 16, where *Priestia* was present in negligible amount (1 %). These samples were also obtained from open market containers.

Raisin samples showed microbial diversity patterns similar to those observed in dried apricots. In most samples *Bacillus* was the predominant genus across most samples, followed by *Priestia*. Other genera, including *Tumebacillus*, *Cytobacillus*, *Mesobacillus*, and *Pantoea* appeared infrequently and at low relative abundances, suggesting that these taxa might be transient environmental contaminants or have lower competitive ability in dried fruit matrices. In pre-packaged samples (Sample 20, 26, 27), differences in their microbial composition were observed. Sample 20 harboured three genera (*Bacillus*, *Priestia*, *Pantoea*), with *Bacillus* dominating with 97 %. In contrast, Sample 27 contained two genera, with *Priestia* being the predominant genus with 71 %, while *Bacillus* was present only in 29 %. Sample 26 yielded only a single genus, *Bacillus*.

5.3.3 Alpha diversity analysis of microbial communities

Two commonly applied mathematical indicators for assessing alpha diversity are the Chao1 and the Shannon index. Chao1, introduced by Anne Chao, is a non-parametric method focusing solely on species richness, with estimating the total number of species in a sample. It assumes that rare species give the best indication of undetected species, placing more emphasis on those found in low numbers. It relies on species represented only once (singletons) or twice (doubletons) to estimate the number of undetected species. This makes Chao1 useful in communities with high amounts of low-abundance

organisms. High Chao1 values indicate a high number of undetected or rare species, suggesting a more diverse community than observed (Kim et al., 2017). It is calculated as

$$S_{Chao1} = S_{obs} + \frac{n_1^2}{2n_2},$$

where the S_{obs} is the observed number of species, n_1 is the number of singleton species, and n_2 is the number of doubleton species (Huang et al., 2016).

In contrast, the Shannon index, developed by Claude Shannon, considers both species richness and the evenness of the distribution, thus, offering more information on the diversity of a community. Higher Shannon values reflect communities with not only more species (richness) but also more uniform species abundance (evenness) (Spellerberg et al., 2003; Avalos-Fernandez et al., 2022). It is calculated as

$$H' = -\sum_{i=1}^{S} p_i \ln(p_i),$$

where H' is the diversity index, S is the number of species, p_i is the proportion of individuals belonging to species i ($pi = n_i/N$), $\sum$ is the summation of $[p_i * \ln(p_i)]$ (Shazali et al., 2024). Both the Chao1 and the Shannon indices were calculated in R (version 4.3.0).

Analysis of the alpha diversity in this thesis work showed that most samples exhibited low Chao1 values, with only five (Samples 10, 18, 19, 20, 23) showing elevated values (Figure 16). This indicates a potentially greater presence of rare taxa. Similarly, Shannon indices were generally low, ranging between 0 and 2, with only five samples (Samples 10, 11, 19, 20, 23) exceeding a value of 2. This pattern suggests that even in samples with high Chao1 values, a few dominant species likely shape the community, resulting in low evenness.

Among the samples with higher Chao1 values, Sample19 (prune) and Sample20 (raisin) were pre-packaged, while the other high value samples were obtained from markets where the dired fruits were stored in open or partially open containers. Notably, samples with lower Chao1 values were also purchased under similar conditions. Similarly, all samples with higher Shannon indices, except Sample 19 and 20, were sourced from marked vendors.

It is important to note, that a high Chao1 value does not necessarily translate to high Shannon value, and vice versa. For example, Sample 18 exhibited a Chao1 value exceeding 100, yet its Shannon index was identical to that of Sample 29 with the value of 1.5. This sample had a much lower Chao1 value of less than 25. This implies that this sample may contain a number of different species, including some rare or low-abundance ones, however the community is uneven, as small number of species make up most of the population. This correlates with the relative abundance profile (Figure 14), which showed *Bacillus* as the dominated genus, however, several low-abundance taxa could possibly be present below threshold levels of detection or visualization. On the contrary, Sample 11 had higher

Shannon index, but its Chao1 value remained low, indicating that smaller number of species contribute relative evenly to the total community. Although only *Bacillus* was visualized in the relative abundance plot (Figure 14), the observed difference may reflect multiple *Bacillus* species present, as well as the presence of rare taxa below the limit of visualization. These findings highlight the limitations of relying solely on relative abundance graphs for analysing the diversity of the microbial community.

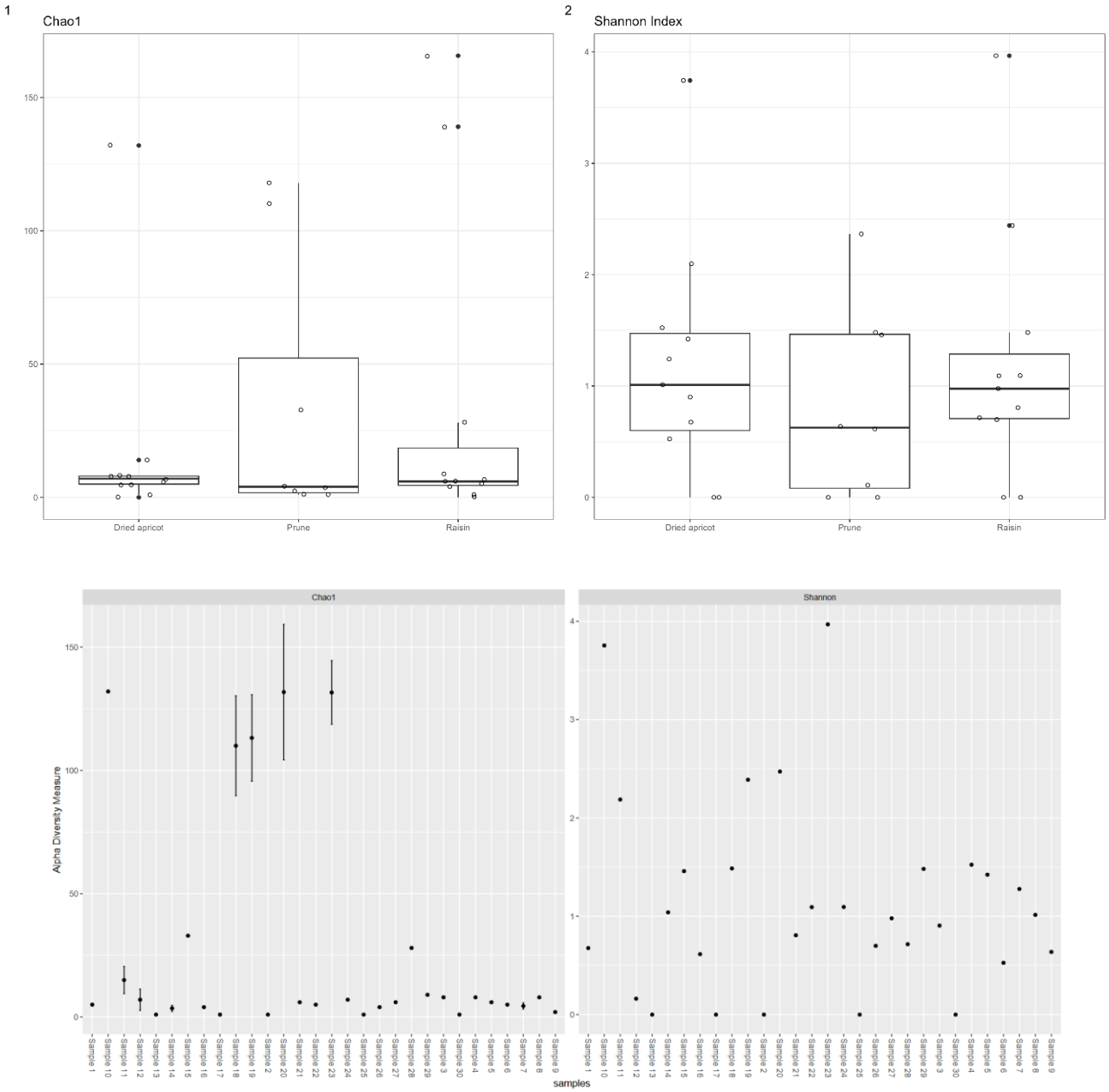

Figure 16 Alpha diversity (Chao1, Shannon) of dried fruits.

5.3.4 Beta diversity analysis

Beta diversity assesses the variations in the composition of the microbial community across different samples (Hopfner et al., 2017). In this thesis work, the beta diversity ordination plot shows that the two axes account for 99.8 % of the total variance, indicating that differences among the microbial communities are well represented. As illustrated in Figure 17, certain phyla cluster closely together, indicating co-occurrence or similarity in the community structure, whereas others are more widely spread, reflecting greater ecological differences among the samples. Additionally, a statistical comparison was performed to evaluate microbial diversity across the different fruit types. The analysis of similarities (ANOSIM) yielded a p-value of 0.043, indicating that the observed differences were not statistically significant. The R value of 0.082 suggests that the overall composition of the microbial community is relatively similar across samples.

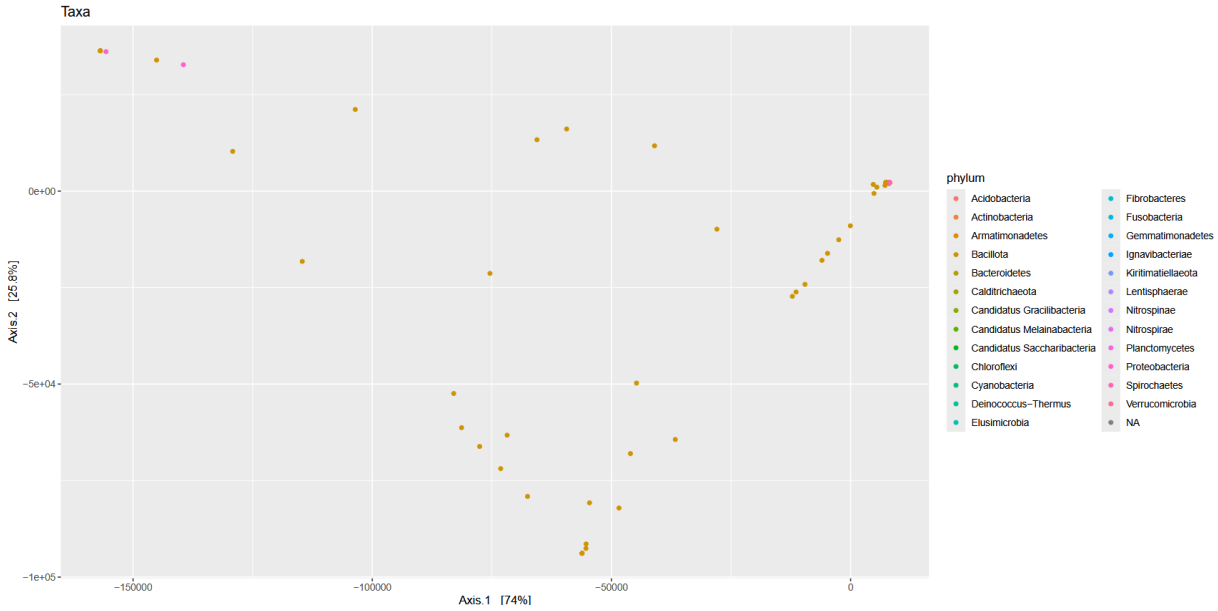

Figure 17 Beta diversity of dried fruit samples.

5.4 Microbial diversity in dried fruits: culture-dependent and culture-independent approaches

This section compares the microbial communities in different dried fruit types using both culture-dependent and culture-independent methods.

5.4.1 Overall comparison of the microbiota across the dried fruit types

The comparative analysis of culture-dependent and culture-independent approaches revealed similarities but also great differences in the microbial profiles of the dried fruit samples.

By analysing the dried fruit categories individually, a trend emerged. Among the 11 dried apricot samples, *Bacillus* was identified in seven samples by both MALDI-TOF MS and 16S rDNA amplicon

sequencing (Table 8). However, *Priestia* (Sample 8), and *Cytobacillus* (Sample 10), were identified by both methods in only one sample each. In the remaining dried apricot samples, the microbial composition varied depending on the method used. In Sample 4, 7, 8, 9 and 11, the culture-dependent approach revealed greater microbial diversity, whereas in Sample 1, 5 and 10, the culture-independent method identified a broader range of bacterial genera. In Sample 3, both methods revealed similar levels of diversity, but different genera were identified. MALDI-TOF MS identified *Solibacillus* and *Paenibacillus*, while 16S rDNA amplicon sequencing revealed the presence of *Bacillus* and *Priestia*. In Sample 2 and 6, *Bacillus* was determined as the dominant genus by both methods. *Paenibacillus*, *Solibacillus*, *Peribacillus*, *Staphylococcus*, *Enterococcus* and *Lysinibacillus* were detected only by the culture-dependent approach, while *Neobacillus*, *Mesobacillus* and *Tumebacillus* were solely identified by the culture-independent method.

Across the eight prune samples, *Bacillus* was identified by both methods in three samples. However, 16S rDNA amplicon sequencing detected *Bacillus* in all prune samples except for Sample 13, which was unique as no bacteria were detectable by either method. Notably, this sample was an organic, pre-packaged product with no added preservatives. The culture-dependent method identified *Bacillus* only in Sample 14, 15, and 16. Furthermore, apart from *Bacillus*, it also revealed six additional genera not detected by the culture-independent approach, including *Staphylococcus*, *Micrococcus, Brevibacillus, Paenibacillus*, *Lysinibacillus*, and *Cytobacillus*. Conversely, in Sample 12 and 19 no bacteria were identified with MALDI-TOF MS, while the 16S rDNA amplicon sequencing confirmed the presence of *Bacillus* in both prunes. Sample 17 exhibited the highest bacterial diversity among the prune samples based on the results of the culture-dependent approach, with five genera identified using MALDI-TOD MS. However, this diversity was not reflected in the 16S rDNA amplicon sequencing results, as only *Bacillus* was identified.

All 11 raisin samples tested positive for *Bacillus* using both approaches, highlighting its widespread presence in dried fruits. *Priestia* was detected in four samples (Sample 10, 21, 27, 28) using both methods, and *Pantoea* was identified uniquely in Sample 28 by both techniques. Other genera, including *Peribacillus, Micrococcus, Niallia, Alkalihalobacillus, Ureibacillus, Cytobacillus, Metabacillus, Pseudomonas, Kocuria, Mixta* and *Curtobacterium* were exclusively found using the culture-dependent method. In contrast, *Tumebacillus* was only detected via 16S rDNA amplicon sequencing.

These findings suggest that the culture-dependent approach may reveal different or even more diverse microbiota in some cases. However, the identification of certain taxa within the dried fruit categories solely by 16S rDNA amplicon sequencing approach suggests the possible presence of viable but non-culturable (VBNC) bacteria, potentially induced by the osmotic stress associated with dried fruit matrices.

Importantly, *E. coli* was not detected in any of the dried fruits samples by either method. However, this does not necessarily prove its absence in these products. If VBNC *E. coli* was present in very low abundance compared to the dominant genera, its presence might have been below the detection limit of sequencing.

Taken together, these findings emphasize that dried fruits, though generally considered low-risk, due to their low water activity, may still harbour a range of microbial contaminants introduced at various stages of the supply chain. This underlines the critical need for good hygiene practices and regular microbiological monitoring throughout processing and storage to ensure microbial safety and quality of dried fruit products.

Table 8 Comparison of the identified bacterial genus by culture-dependent and culture-independent methods. "N/A" indicates not available.

Sample code	Sample category	Country of origin	Culture-dependent Identified genus	Culture-independent Identified genus
1	Dried apricot	Hungary	*Paenibacillus*	*Bacillus* *Priestia*
2	Dried apricot	Hungary	*Bacillus*	*Bacillus*
3	Dried apricot	Hungary	*Solibacillus* *Paenibacillus*	*Bacillus* *Priestia*
4	Dried apricot	Hungary	*Bacillus* *Micrococcus* *Paenibacillus*	*Bacillus* *Priestia* -
5	Dried apricot	Hungary	*Peribacillus* -	*Bacillus* *Priestia*
6	Dried apricot	Austria	*Bacillus*	*Bacillus*
7	Dried apricot	Austria	*Bacillus* *Priestia* *Peribacillus*	*Bacillus* *Neobacillus*
8	Dried apricot	Austria	*Bacillus* *Priestia* *Peribacillus* *Staphylococcus* *Pantoea* *Enterococcus*	*Bacillus* *Priestia* - - - -
9	Dried apricot	Austria	*Bacillus* *Priestia* *Peribacillus*	*Bacillus* - -

Sample code	Sample category	Country of origin	Culture-dependent Identified genus	Culture-independent Identified genus
10	Dried apricot	Austria	*Bacillus*	*Bacillus*
			Cytobacillus	*Cytobacillus*
			Peribacillus	*Neobacillus*
			-	*Priestia*
			-	*Mesobacillus*
			-	*Pantoea*
			-	*Tumebacillus*
11	Dried apricot	Austria	*Peribacillus*	*Bacillus*
			Priestia	-
			Lysinibacillus	-
			Paenibacillus	-
12	Prune	Hungary	N/A	*Bacillus*
13	Prune	Hungary	N/A	N/A
14	Prune	Hungary	*Bacillus*	*Bacillus*
			Staphylococcus	-
			Micrococcus	-
15	Prune	Hungary	*Bacillus*	*Bacillus*
			-	*Priestia*
16	Prune	Hungary	*Bacillus*	*Bacillus*
			Priestia	*Priestia*
			Brevibacillus	
17	Prune	Hungary	*Priestia*	*Bacillus*
			Paenibacillus	-
			Lysinibacillus	-
			Cytobacillus	-
			Staphylococcus	-
18	Prune	Hungary	*Paenibacillus*	*Bacillus*
19	Prune	Austria	N/A	*Bacillus*
20	Raisin	Hungary	*Bacillus*	*Bacillus*
			Priestia	*Priestia*
			Peribacillus	*Pantoea*
			Micrococcus	-
			Niallia	-
			Alkalihalobacillus	-
			Ureibacillus	-
21	Raisin	Hungary	*Bacillus*	*Bacillus*
			Priestia	*Priestia*
			Niallia	-
			Cytobacillus	-

Sample code	Sample category	Country of origin	Culture-dependent Identified genus	Culture-independent Identified genus
22	Raisin	Hungary	*Bacillus*	*Bacillus*
			Peribacillus	*Priestia*
23	Raisin	Hungary	*Bacillus*	*Bacillus*
			Peribacillus	*Pantoea*
			-	*Tumebacillus*
24	Raisin	Hungary	*Bacillus*	*Bacillus*
			Metabacillus	*Priestia*
25	Raisin	Hungary	*Bacillus*	*Bacillus*
			Priestia	-
26	Raisin	Austria	*Bacillus*	*Bacillus*
27	Raisin	Austria	*Bacillus*	*Bacillus*
			Priestia	*Priestia*
			Pantoea	-
			Pseudomonas	-
			Micrococcus	-
			Kocuria	-
28	Raisin	Austria	*Bacillus*	*Bacillus*
			Pantoea	*Pantoea*
			Priestia	*Priestia*
			Mixta	-
			Curtobacterium	-
			Peribacillus	-
29	Raisin	Austria	*Bacillus*	*Bacillus*
			Priestia	-
30	Raisin	Austria	*Bacillus*	*Bacillus*
			Micrococcus	-
			Pantoea	-

5.4.2 Comparison of the microbiota based on the country of purchase

Table 9 compares the bacterial genera identified in dried fruit samples from Hungary and Austria, using both culture-dependent and culture-independent approaches.

The dried apricot samples from Hungary showed lower microbial diversity compared to Austrian samples. Using MALDI-TOF MS for identification, genera such as *Bacillus*, *Paenibacillus*, and *Peribacillus* were shared between both countries. However, *Solibacillus* and *Micrococcus* were exclusive to Hungarian samples, while six genera including *Priestia*, *Cytobacillus*, *Pantoea*, *Staphylococcus*, *Lysinibacillus*, and *Enterococcus* were uniquely detected in Austrian samples. The sequencing approach also revealed a broader diversity in Austrian dried apricot samples, with genera such as *Cytobacillus*, *Pantoea*, *Neobacillus*, *Mesobacillus*, and *Tumebacillus* detected only in these samples, while *Bacillus* and *Priestia* were common in both countries. In a few cases an overlap

between the two methods was observed. For example, *Bacillus* was identified by both approaches in Hungarian samples, and *Bacillus*, *Priestia*, *Cytobacillus*, and *Pantoea* were detected in Austrian samples by both methods. Among the dried apricot samples purchased in Hungary, for which product origin was available, Sample 1 (Hungarian origin) contained only *Paenibacillus*, while Sample 2 (Turkish origin) contained the genus *Bacillus*. Both samples were originally packaged products with expiration dates extending to February and March of the following year, respectively.

Regarding prunes, only one sample originated from Austria, thus limiting the comparability. In Hungarian prune samples, the culture-dependent approach identified eight bacterial genera, while the culture-independent method confirmed the presence of *Bacillus* in samples from both countries and additionally identified *Priestia* in Hungarian samples. MALDI-TOF MS identification was not possible from the Austrian prune sample. For the prune samples purchased in Hungary with known origin, Sample 12 (Hungarian origin) yielded no identifiable genera with either method, while Sample 13 (Bulgarian origin) yielded the genus *Bacillus* upon 16S rDNA amplicon sequencing. These samples were originally packaged, and the expiration dates were comparable to the apricot samples, although the expiration date of Sample 13 was August 2024.

Raisin samples from both countries demonstrated similar levels of overall bacterial diversity, with nine genera detected in each country. However, the composition of the genera was different. *Bacillus*, *Priestia*, *Peribacillus*, and *Micrococcus* were detected in both countries, whereas *Niallia*, *Alkalihalobacillus*, *Ureibacillus*, *Cytobacillus*, and *Metabacillus* were exclusive to Hungarian samples. In contrast, *Pantoea*, *Pseudomonas*, *Kocuria*, *Mixta*, and *Curtobacterium* were found only in Austrian samples. Importantly, *Bacillus* and *Priestia* were detected in samples from both countries using both methods. Among the raisin samples with known origin, from Sample 21 (purchased in Hungary of Iranian origin), *Bacillus*, *Priestia*, *Niallia* and *Cytobacillus* were detected, while from Sample 26 (purchased in Austria, originating from India) only *Bacillus* was detected. Sample 21 was purchased from a covered container, accessible to consumers, while Sample 26 was originally packaged. The expiration date of Sample 21 was unknown, while Sample 26 expired in September of the same year the sample was purchased (April 2023).

These findings suggest regional differences in the microbiota of dried fruits, which are likely influenced by factors such as origin, handling, and packaging. Austrian dried apricot samples generally exhibited greater microbial diversity than their Hungarian counterparts, especially when assessed by the 16S rDNA amplicon sequencing. However, there are limitations to the interpretation of these results. Although information such as origin, packaging type and expiration dates (only for packaged samples) was available in some cases, the age of the products at the time of the purchase, the storage conditions prior to sampling, and the specific methods and the location of the drying were unknown (Supplementary Table 2). Nonetheless, the consistency in detecting *Bacillus* and *Priestia*

with both methods, across all sample types and countries highlights their adaptability to the dried fruit matrix, as well as their potential resistance to environmental stress. Moreover, using both culture-dependent and culture-independent methods gives a more complete understanding of the microbial communities present in dried fruit products, which highlights the value of combining different approaches when evaluating the safety and diversity of low water activity foods such as dried fruits.

Table 9 Comparison of the microbiota of dried fruits based on the country of purchase.

Country	Apricot		Prune		Raisin	
	Identification by MALDI-TOF MS	16S rDNA amplicon sequencing	Identification by MALDI-TOF MS	16S rDNA amplicon sequencing	Identification by MALDI-TOF MS	16S rDNA amplicon sequencing
Hungary	*Bacillus*	*Bacillus*	*Bacillus*	*Bacillus*	*Bacillus*	*Bacillus*
	Paenibacillus	*Priestia*	*Priestia*	*Priestia*	*Priestia*	*Priestia*
	Peribacillus	-	*Staphylococcus*	-	*Peribacillus*	*Pantoea*
	Solibacillus	-	*Micrococcus*	-	*Micrococcus*	*Tumebacillus*
	Micrococcus	-	*Brevibacillus*	-	*Niallia*	-
	-	-	*Paenibacillus*	-	*Alkalihalobacillus*	-
	-	-	*Lysinibacillus*	-	*Ureibacillus*	-
	-	-	*Cytobacillus*	-	*Cytobacillus*	-
	-	-	-	-	*Metabacillus*	-
Austria	*Bacillus*	*Bacillus*	-	*Bacillus*	*Bacillus*	*Bacillus*
	Priestia	*Priestia*	-	-	*Priestia*	*Priestia*
	Cytobacillus	*Cytobacillus*	-	-	*Pantoea*	*Pantoea*
	Pantoea	*Pantoea*	-	-	*Micrococcus*	-
	Staphylococcus	*Neobacillus*	-	-	*Peribacillus*	-
	Lysinibacillus	*Mesobacillus*	-	-	*Pseudomonas*	-
	Enterococcus	*Tumebacillus*	-	-	*Kocuria*	-
	Paenibacillus	-	-	-	*Mixta*	-
	Peribacillus	-	-	-	*Curtobacterium*	-

5.5 Irradiation experiments

This section presents the irradiation experiments conducted to assess the effects of radiation on *E. coli* strains in different media.

5.5.1 Comparison of electron beam (e-beam) and gamma irradiation

Two irradiation sources, electron beam and gamma rays, were compared to select the most suitable for further irradiation experiments. Literature analysis revealed that gamma irradiation is more frequently used for food preservation than e-beam irradiation. Thus, the initial goal was to determine and compare the decimal radiation dose (D-value) for both cases. Figure 18 presents the mean values of log cell counts from three parallel measurements with radiation doses of 0.2, 0.4, 0.6, 0.8 and 1 kGy. The samples were irradiated at room temperature. As the time of irradiation, the FI strain had not yet

been isolated from the low water activity food sample, therefore this experiment was only conducted with the CC strain. The D-value can be determined from the scatter chart by choosing two points on each regression line of the survival curves that differ by one logarithmic unit on the y-axis (log cell count). The corresponding difference on the x-axis (radiation dose) will represent the D-value. The D-value of *E. coli* was 0.2 kGy for e-beam irradiation, and 0.21 kGy for gamma irradiation. This means that a 0.2 kGy dose is required to reduce the bacterial population by 1 log (90 %). Since the two D-values had negligible difference, the e-beam irradiation was selected to be the irradiation source for subsequent experiments.

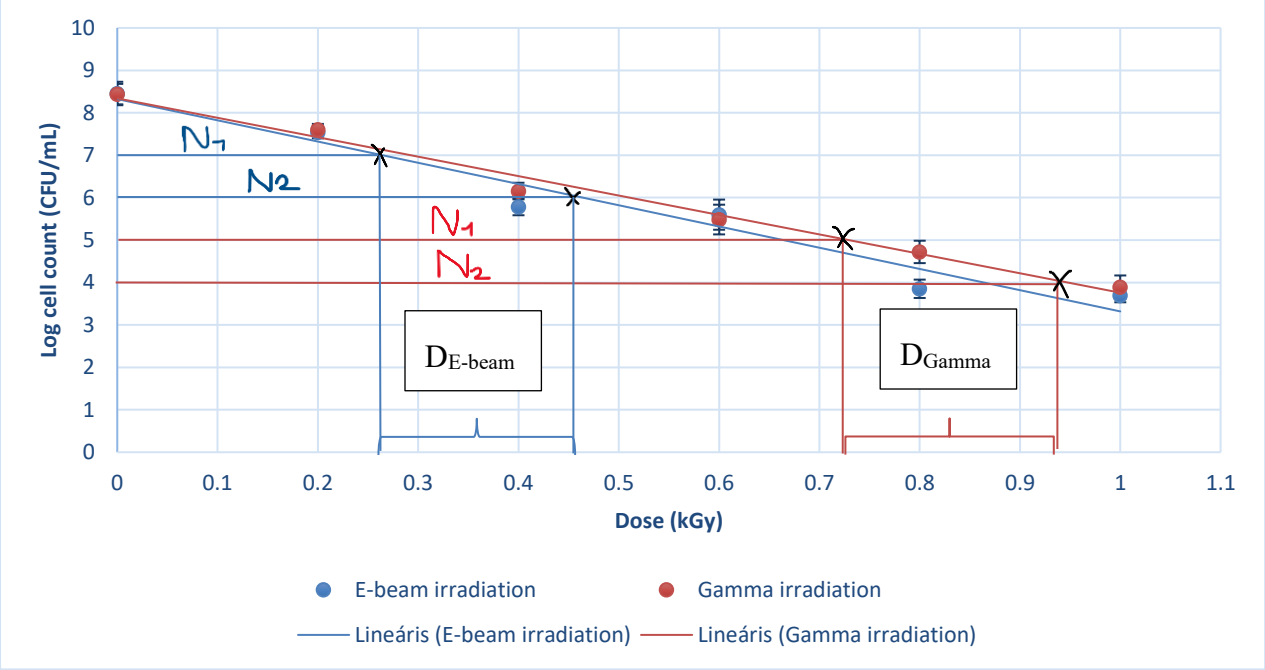

Figure 18 Comparison of electron beam and gamma irradiation with *Escherichia coli* strain from the culture collection.

5.5.2 Determining the D-value of an Escherichia coli strain from the culture collection using electron beam radiation

Figure 19 presents the survival curve of the CC strain of *E. coli* irradiated with e-beam radiation at room temperature in LB medium. The decimal reduction dose (D-value) was calculated using the previously described method, which was the determination of the radiation dose required to achieve a 1-log reduction in the cell count. According to the regression line of the scatter plot, the D-value was 0.24 kGy. At a dose of 2 kGy, the surviving population decreased to just above 1 log CFU/mL. In this case it meant an average of 1.67 colony on the LB agar plates among the three parallels. This represents an overall of 8 log reduction of the population, resulting in a nearly complete inactivation.

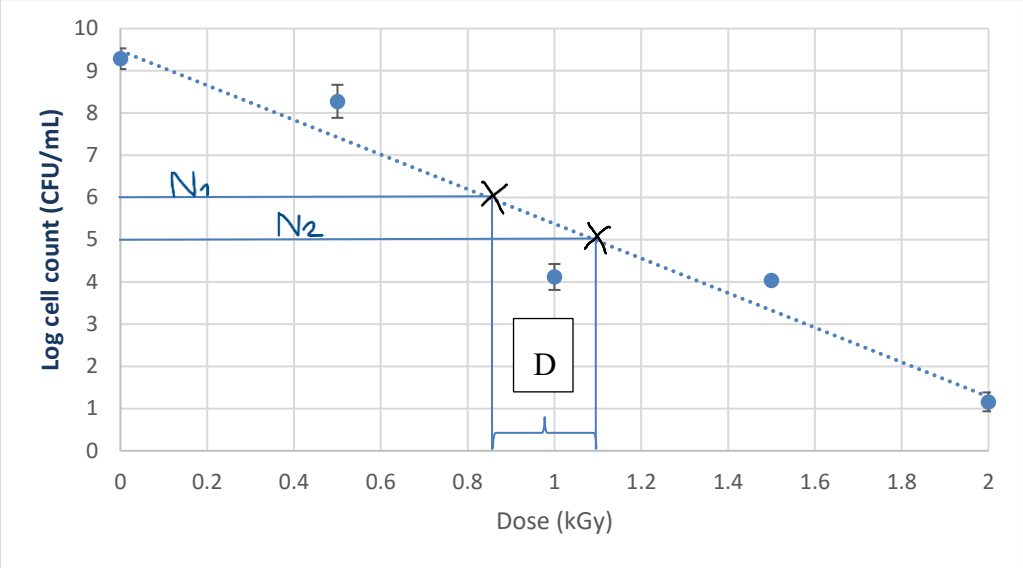

Figure 19 Survival curve of the *Escherichia coli* strain from the culture collection, irradiated with electron beam.

5.5.3 Assessing the behaviour of Escherichia coli in osmotic environments using gamma irradiation

The aim of this experiment was to evaluate the influence of different sugars in different concentrations on the D-value of the CC *E. coli* strain. At the time of this experiment, the FI strain had not yet been isolated from the low water activity food product. As shown in Figure 20, the regression curve for the medium containing 50 % glucose exhibited the least steep slope, while the control (sugar-free) medium displayed the steepest slope. The D-values were calculated by the previously described method. These values increased with the glucose concentration and were 0.32, 0.38, 0.41 and 0.74 kGy in media containing 0, 10, 30 and 50 % glucose, respectively. The increase between 10-30 % was moderate, while the sudden rise at the 50 % indicates a stronger protective effect of high glucose concentration.

These results indicate that dried fruits with high glucose concentration may protect *E. coli* cells from gamma irradiation used for food preservation.

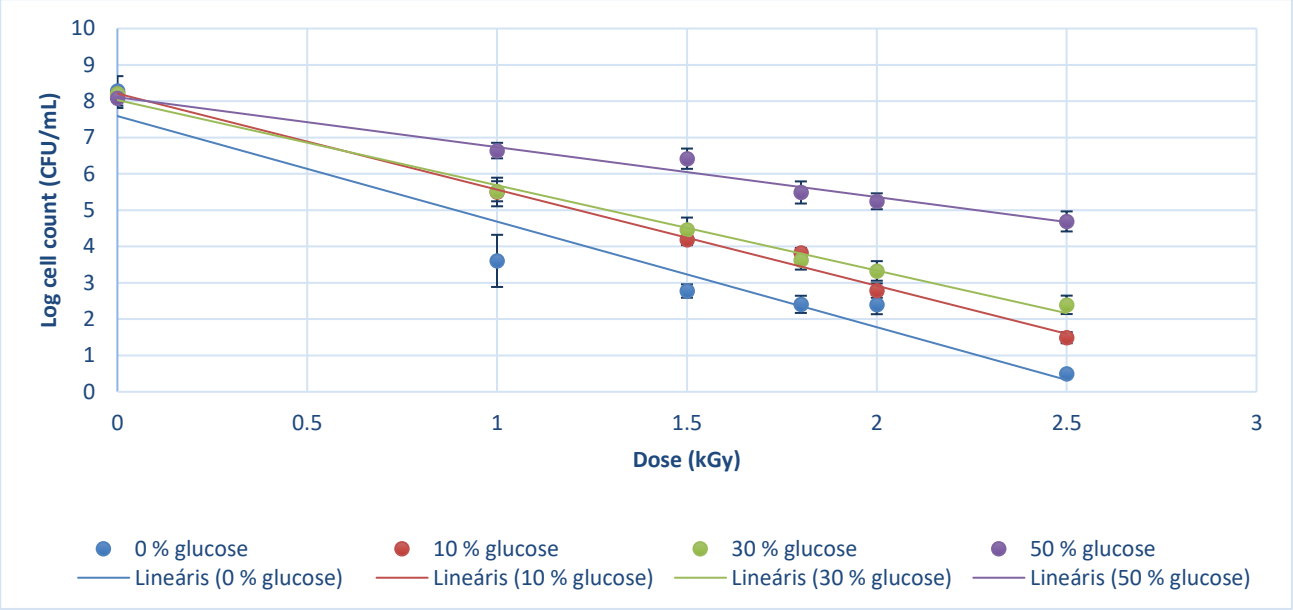

Figure 20 Survival curves of the *Escherichia coli* strain from the culture collection in media containing different concentrations of glucose.

The next experiment investigated the effect of different concentrations of fructose. The D-values were calculated by the previously described method. According to Figure 21, these values in media containing 0, 10, 30, and 50 % fructose, in increasing order were 0.32, 0.33, 0.41 and 0.43 kGy, respectively. The values of the control and the medium containing 10 % fructose were similar, suggesting no protection at low concentrations. At 30 and 50 %, the D-values were higher, indicating some protective effect of fructose, although this effect was weaker than that observed with glucose.

This highlights that although it is important to investigate bacterial behaviour in individual sugars, to understand its behaviour in complex food matrices, the combination of different sugars is essential.

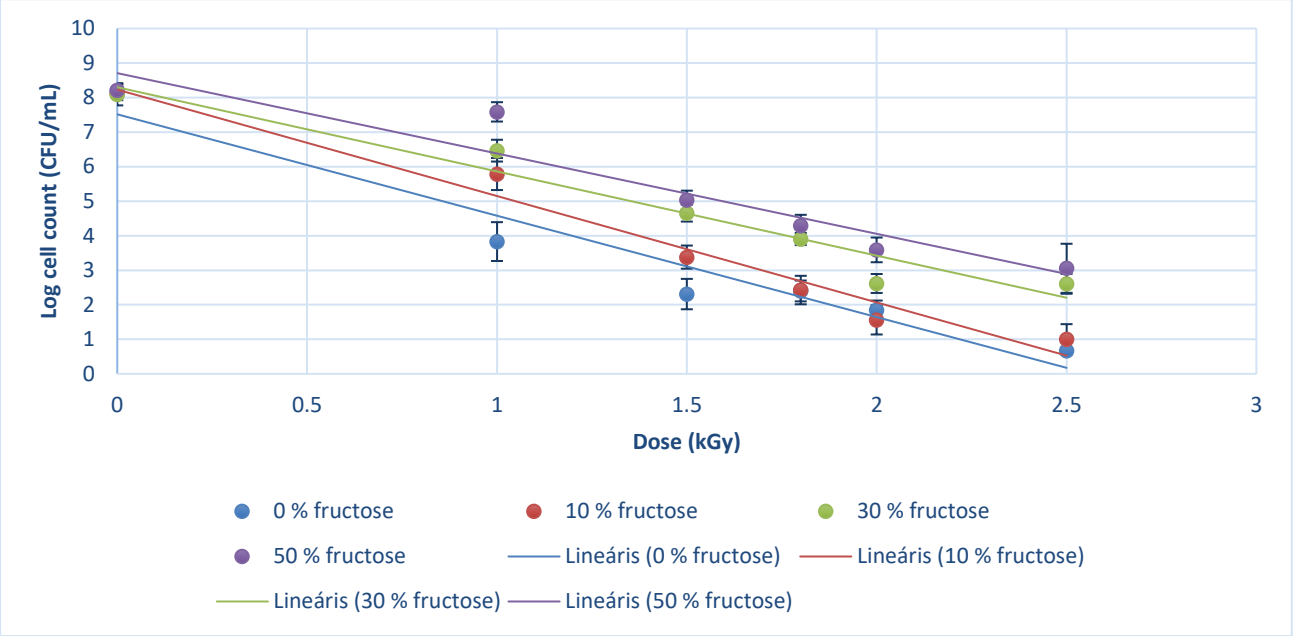

Figure 21 Survival curves of the *Escherichia coli* strain from the culture collection in media containing different concentrations of fructose.

In the last experiment assessing the behaviour of *E. coli* in osmotic environments, sucrose concentrations were tested. The D-values, calculated by the same method as mentioned previously, in media containing 0, 10, 30 and 50 % sucrose were 0.32, 0.36, 0.39 and 0.42 kGy, respectively (Figure 22). These values were similar to those observed with the medium containing fructose and lower than those observed with the media containing glucose at the same concentration. This indicates that the 50 % sucrose provided a moderate protective effect against gamma irradiation compared to glucose. Together, these results also underline the need to study bacterial behaviour in mixed-sugar systems to obtain relevant knowledge in real food matrices.

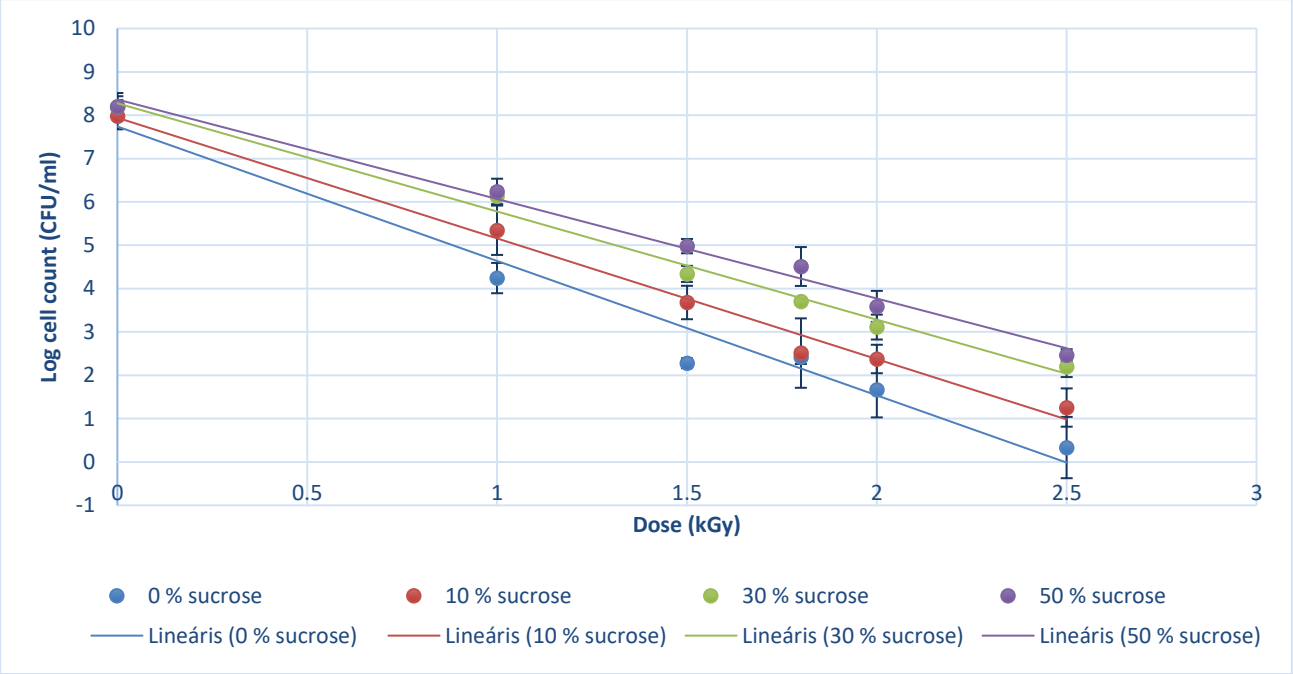

Figure 22 Survival curves of the *Escherichia coli* strain from the culture collection in media containing different concentrations of sucrose.

5.5.4 Determination of the D-values of the Escherichia coli strains from the culture collection and low water activity food using gamma irradiation

This experiment involved both the culture collection (CC) and the low water activity food isolate (FI) strains of *E. coli*. For economic reasons, gamma irradiation was used as the radiation source instead of e-beam. The aim was to find the D-values of both strains using gamma irradiation not only in LB medium, but also in prune-mimicking medium. The prune-mimicking medium was selected based on the results of the growth experiments.

Figure 23 presents the irradiation results. The D-values were determined using the previously described method. The D-values in LB medium for the CC and FI strains were both 0.31 kGy, while in prune-mimicking medium, the D-values increased slightly to 0.34 and 0.36 kGy, respectively. This indicates a modest protective effect against gamma radiation. Although these increases are small, they suggest that the components of the food matrix composition influences bacterial radiation tolerance.

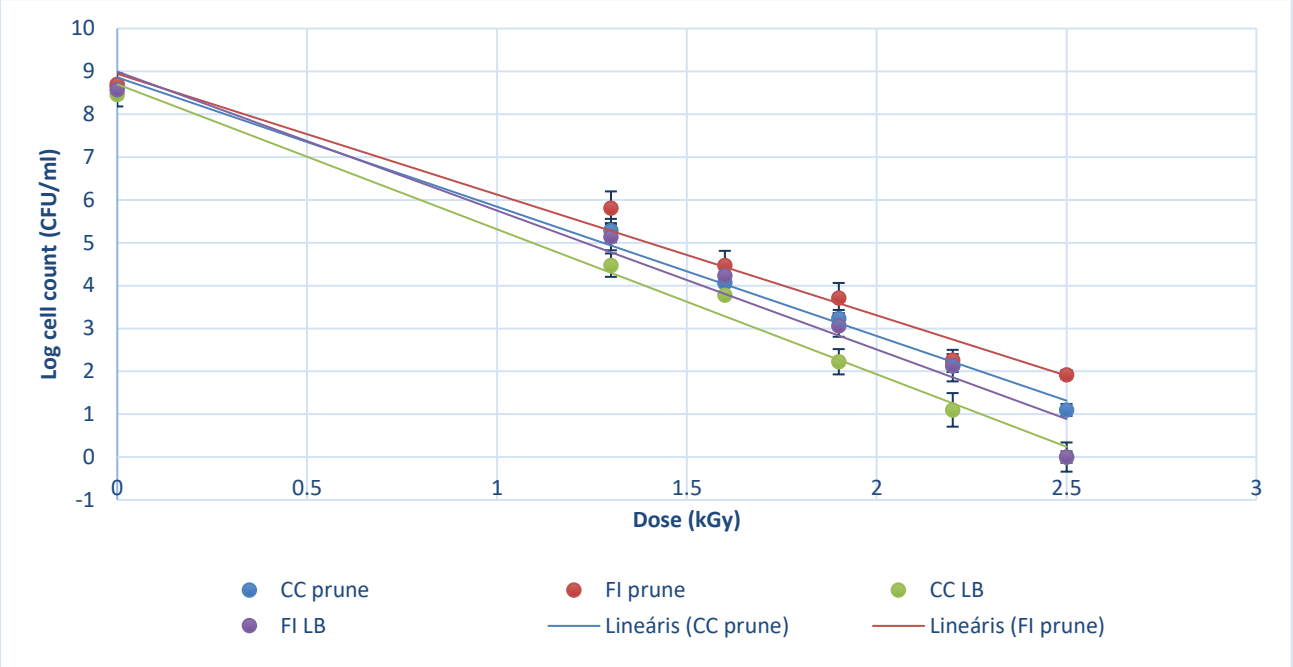

Figure 23 Survival curves of the *Escherichia coli* strains from the culture collection and low water activity food, irradiated in LB and prune-mimicking media.

5.6 Osmotic stress simulations

Osmotic stress simulations were performed to evaluate how high-sugar, low water activity environments resembling the environments of different dried fruits (dried apricot, prune, raisin) influence the viability and culturability of *E. coli*. These experiments also assessed whether prior exposure to mild osmotic conditions alters bacterial tolerance during subsequent high-osmotic stress.

5.6.1 Potential viable but non-culturable (VBNC)-like cells detected upon osmotic stress in dried fruit-mimicking media (viability and culturability assay)

The induction of the viable but non-culturable (VBNC) state in bacteria can be triggered by various environmental stresses, including nutrient deprivation, extreme temperatures, osmotic pressure, oxidative stress, acidification, desiccation, and irradiation (Zhang et al., 2015; Pienaar et al., 2016; Zhao et al., 2016; Wei and Zhao, 2018; Fu et al., 2020; Se et al., 2021; Pan et al., 2023). In dried fruits, *E. coli* may enter the VBNC state in response to desiccation, elevated osmolarity, or heat stress during the drying process.

To evaluate the potential entry of *E. coli* into a VBNC-like state, fruit-mimicking media containing different types and amounts of sugar to simulate dried apricot, prune, and raisin conditions were prepared. The viability and culturability of two *E. coli* strains (culture collection, CC, and food isolate, FI) were monitored over 96 hours using plate counts (culturability) and PMA-qPCR (viability). LB medium was included as the control. Viability assays were performed in technical duplicates, while

culturability assessments were conducted in technical triplicates. In Figure 24, the values of the individual fruit mimicking media were combined, as no difference was observed among them. This allowed for a clearer graphical representation.

Bacterial counts in the fruit-mimicking media were consistently lower under every tested condition (PMA-qPCR, plating, and for both *E. coli* strains) than observed in the control media. After 96 hours, the viability measurements showed significantly higher (CC strain of *E. coli* $Z = 2.16$, $p = 0.0304$; FI strain of *E. coli*: $Z = 2.16$, $p = 0.0304$; $\alpha = 0.05$) cell counts in the control medium compared to the 1-hour measurement for both *E. coli* strains, whereas counts in the fruit-mimicking media remained relatively unchanged (CC strain of *E. coli* $Z = -1.20$, $p = 0.2290$; FI strain of *E. coli*: $Z = 1.52$, $p = 0.1282$; $\alpha = 0.05$) over the same period (Supplementary Figure 41).The connecting letters report for viability measurements is shown in Supplementary Figure 42. In contrast, culturability in fruit-mimicking media has started to decline after 24 hours, resulting in a significant reduction (CC strain of *E. coli* $Z = -2.95$, $p = 0.0031$; FI strain of *E. coli*: $Z = -2.16$, $p = 0.0304$; $\alpha = 0.05$) in culturable cells by 96 hours in both *E. coli* strains. No significant changes (CC strain of *E. coli* $Z = -0.56$, $p = 0.5752$; FI strain of *E. coli*: $Z = -1.36$, $p = 0.1735$; $\alpha = 0.05$) in culturability were observed in the control medium in either *E. coli* strains, where counts remained stable throughout the incubation period (Supplementary Figure 43). The connecting letters report for culturability is presented in Supplementary Figure 44.

These results are important from the food safety perspective, as the difference between viability and culturability in fruit-mimicking media suggests that *E. coli* may enter a VBNC-like state after prolonged exposure to media mimicking the conditions found in dried-fruits. However, in the pre-adaptation experiments, cells pre-adapted in prune-mimicking medium for 192 hours were able to regain their growth capacity upon transfer to LB medium. This observation suggests that the apparent loss of culturability observed in this experiment at 96 hours in the fruit-mimicking media might not represent a true VBNC state, but rather an extended lag phase or prolonged metabolic dormancy. Overall, while the difference between viability and culturability indicates a potentially VBNC-like behaviour, additional experiments including resuscitation steps, flow cytometry and metabolic activity assays would be required to conclusively determine whether the cells had truly entered the VBNC state.

It is established that osmotic and desiccation stresses might induce VBNC formation in *E. coli* (Zhao et al., 2016). This presents a food safety concern, as certain ready-to-eat foods may provide optional conditions for resuscitation. For example, overnight oats prepared with dried fruits have sufficient moisture and nutrients to support the revival and growth of VBNC bacteria (Wolever et al., 2019; Biel et al., 2020). If such meals are stored at room temperature rather than under refrigeration, VBNC cells may resuscitate and proliferate, increasing the risk of a potential for foodborne illness. These findings

underscore the importance of monitoring VBNC state bacteria when assessing microbial safety in minimally processed or ready-to-eat foods.

The literature also suggests that other foodborne pathogens can enter the VBNC state under dried fruit conditions. Jayeola et al. (2022) examined how *Salmonella* can enter the VBNC state on ready-to-eat dried fruits due to environmental stress. Researchers inoculated dried apples, strawberries and raisins with high concentration (10^9 CFU/g) of a five-strain cocktail of *Salmonella*. The fruits were then dried at 25 °C in desiccators to match their natural water activity (aw). They found that *Salmonella* could not be recovered from any of the fruits after drying, not even after enrichment, indicating a reduction of around 8 log CFU/g. When dried apples were spot-inoculated with the *Salmonella* cocktail, air dried at 25 °C, *Salmonella* could still be recovered after drying. During storage, *Salmonella* levels decreased more quickly at 25 °C (undetectable by day 46) than at 4 °C (still detectable until day 82). However, even when *Salmonella* could no longer be grown from the dried fruit, fluorescent microscopy using the LIVE/DEAD BacLight viability assay showed 56-85 % of cells on the dried fruit were still alive. Their study highlights that environmental stressors in dried fruits can induce the VBNC state of *Salmonella*. This finding has implications for food safety, as VBNC bacteria may remain undetected in dried fruit products.

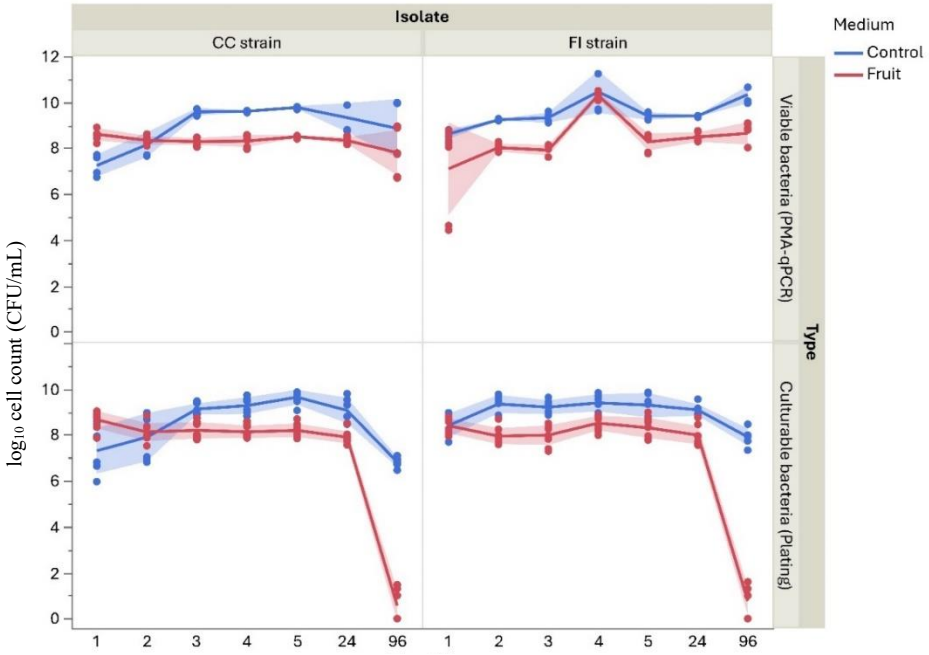

Figure 24 Change in viability and culturability of the *E. coli* strain from culture collection (ATCC B.02031, CC) and the strain from the low water activity food isolate (buckwheat flour, FI) over a 96-hour period in dried fruit mimicking media. LB medium was used as the control, while the fruit-mimicking medium represents the combined values of the individual dried fruit-mimicking media (dried apricot, prune, raisin).

5.6.2 Osmotic stress simulations, investigating the effects of pre-adaptation in low-sugar environment

To investigate whether prior exposure to a low-sugar (0.35 M glucose) environment influences the response of *E. coli* to subsequent high osmotic conditions, cells were pre-adapted in LB medium (control) and low-sugar medium for 2.5 hours. The pre-adapted cultures were inoculated at an initial OD_{600} value of 0.1. After pre-adaptation, the samples were transferred to dried fruit-mimicking media that was prepared to match the sugar content of dried apricot, prune, and raisin, and incubated for 4 and 24 hours. At each stage (freshly grown cultures, after pre-adaptation, after stress exposure) the culturability was assessed by plating the samples on LB agar plates.

During osmotic drying, exposure to hypertonic solutions could allow *E. coli* to pre-adapt, enabling the bacteria to survive the otherwise inhibitory or even lethal hyperosmotic conditions found in dried fruits. This pre-adaptation could activate osmotic stress response pathways, including the uptake of compatible solutes that could improve cell integrity under high sugar concentrations.

Figure 25 presents the log cell counts (CFU/mL) of *E. coli* cells after the preadaptation, in dried apricot-, prune- and raisin-mimicking media. After 4 hours, pre-adapted cells exhibited significantly higher counts in prune-mimicking medium compared to the non-adapted control ($S = 40$, $Z = 2.5067$, $p = 0.0122$, $\alpha = 0.05$), though this difference was no longer evident at 24 hours (Supplementary Figure 45). In contrast, in raisin-mimicking medium, the advantage of low-sugar pre-adaptation became significant ($S = 38$, $Z = 2.0889$, $p = 0.0367$, $\alpha = 0.05$) after 24 hours, suggesting enhanced persistence or growth in this environment upon pre-adaptation (Supplementary Figure 46). For apricot-mimicking medium, log counts of pre-adapted cells were higher at both 4 and 24 hours compared to the non-adapted control, though the differences were not statistically significant (at 4 hours: $S = 36$, $Z = 1.6711$, $p = 0.0947$; at 24 hours: $S = 36$, $Z = 1.6711$, $p = 0.0947$, $\alpha = 0.05$) (Supplementary Figure 47). Overall, these result highlight that pre-adaptation to low-sugar conditions promotes higher cell counts in prune-mimicking medium at 4 hours and in raisin-mimicking medium at 24 hours.

Considering the widespread consumption of these products in Europe, these findings emphasize the need to consider osmotic pre-adaptation when assessing microbial survival and potential food safety risks. These results are relevant considering the fact that dried fruits are widely consumed for their nutritional value and natural sweetness. The sugar concentrations in these products can sustain bacterial survival, including *E. coli*, while not always reaching bactericidal levels. Routine food processing and consumption practices may inadvertently facilitate the recovery and reactivation of VBNC bacteria into their vegetative forms. These findings suggest that the pre-adaptation to low-sugar (0.35 M glucose) environment can enhance the survival of *E. coli* under hyperosmotic conditions, highlighting a potential risk for bacterial persistence in dried fruit products. This

mechanism of the stress cross-protection aligns with observations in other foodborne pathogens, such as *Salmonella* and *Listeria*, where researchers found that mild pre-stress enhances the survival under osmotic stress (Pittman et al., 2014; Gómez-Baltazar et al., 2025). However, in these studies NaCl was used to induce hyperosmotic conditions.

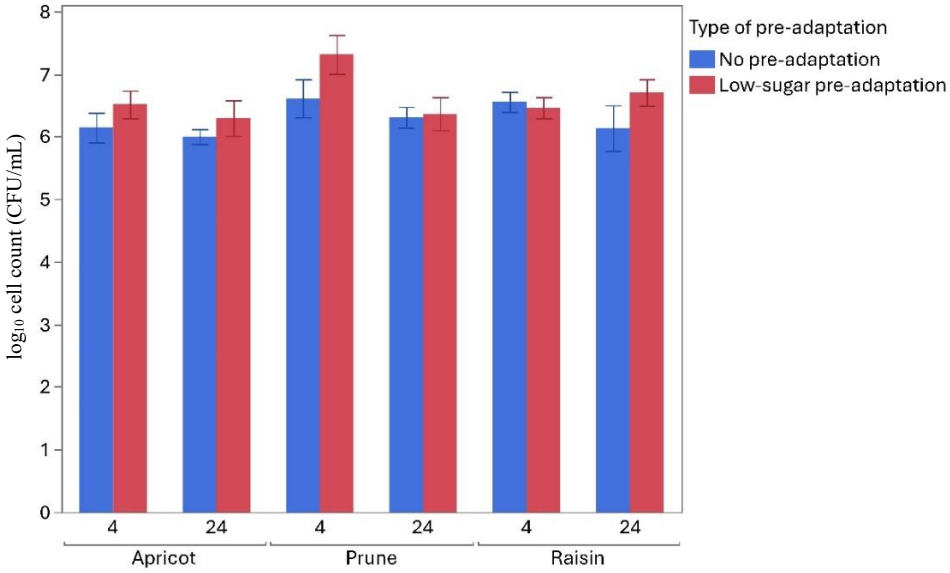

Figure 25 Effect of low sugar pre-adaptation on the viability of *E. coli*. Each bar represents five measurements, comprising triplicate plate counts and duplicate qPCR values. The integration of data from both viability and culturability assays was justified by the similarity of the values obtained from both methods over the 24-hour experimental period.

5.7 Gene expression under osmotic stress

The gene expression analyses were performed using qPCR to examine the transcriptional response of three stress-associated genes (*osmC*, *talA*, and *treA*) in CC and FI strains of *E. coli* under osmotic stress (Figure 26). The relative expression levels were calculated using the ΔΔCt method with *rpoB* as the reference gene. The results are presented as fold changes, and the values represent the mean of duplicate reactions. This method enabled the comparison of the gene expression between two different media: LB as the control, and prune-mimicking medium to induce osmotic stress. Each condition was analysed in two technical replicates, but no independent biological replicates were performed. Samples were collected at 0, 0.5, 1, 3, and 16 hours of incubation. The gene expression levels were normalized to the values of the housekeeping gene (*rpoB*), which served as the reference. A reference value of 1 represents the baseline expression level. Relative expression values above 1 indicate an increase in the expression of the target gene, while values below 1 indicate a decrease.

In the CC strain, *osmC* expression in prune-mimicking medium rose sharply within 30 minutes, and declined after 1 hour. It was then stabilized throughout the experiment. A similar trend occurred in

the FI strain, though the overall expression was approximately 50 % lower than observed with the CC strain, suggesting greater osmotic tolerance in the FI strain. At every time point, *osmC* expression in prune-mimicking medium was consistently lower in the FI strain compared to the CC strain.

For the CC strain, *talA* expression also peaked strongly at 30 minutes, similarly to the *osmC* expression, supporting their involvement in early osmotic stress adaptation in. The expression of this gene then decreased after 1 hour and remained constant. In the FI strain, *talA* showed a transient increase at 30 minutes but reached only about 30% of the CC strain peak value, indicating a more rapid transcriptional adjustment. Beyond this point, the expression remained unchanged in both strains.

In the contrary, *treA* expression showed only a slight increase at 30 minutes in both strains, followed by stable, low-level expression throughout the incubation period.

Overall, these results indicate that *osmC*, *talA* and *treA* respond rapidly within the first 30 minutes of exposure to prune-mimicking medium, with *treA* showing a more moderate response. The expression levels stabilized after 1 hour and remained constant through the 16-hour long experiment. The consistently lower expression of the stress genes in the FI strain suggests that it is more efficiently adapted to hyperosmotic conditions. This might be due to its origin from a food product with reduced water activity. Moreover, the peak in the expression of all three investigated genes observed at 30 minutes (that was not observed at later time points), might be due to the rapid activation of the stress responses in *E. coli* after transferring to sugar-rich medium. The adaptive mechanisms reduce the need for continuous high-level gene expression.

The observed rapid transcriptional shifts highlight the need for shorter sampling intervals such as every five minutes in the first 30 minutes, to better capture early gene expression responses.

Researchers have found that *osmC* is upregulated under high osmotic pressure. A proteomic analysis of *E. coli* subjected to osmotic stress by high NaCl levels revealed that OsmC and TalA was among those 12 proteins (Dps, HchA, HdhA, InfB, OsmC, OsmY, ProX, KatE, PspA, TalA, TktB, and TreF) whose levels significantly increased (Weber et al., 2006). The transcription of *treA* was found to be upregulated in response to osmotic stress (Purvis et al., 2005). Moreover, in uropathogenic *E. coli* (UPEC), exposure to osmotic stress induced by NaCl resulted in changed gene expression in 318 genes and osmotic stress led to the upregulation of 160 genes (Withman et al., 2013). Gunasekera et al. (2008) revealed that osmotic stress triggers a broad gene expression changes in the oxidative-stress regulation in *E. coli*, indicating cross-tolerance. They also found that numerous genes, such as that of the *proU operon, proP, otsAB, osmC, osmY* that are induced in the early stage of osmotic stress adaptation are overexpressed under continuous osmotic stress.

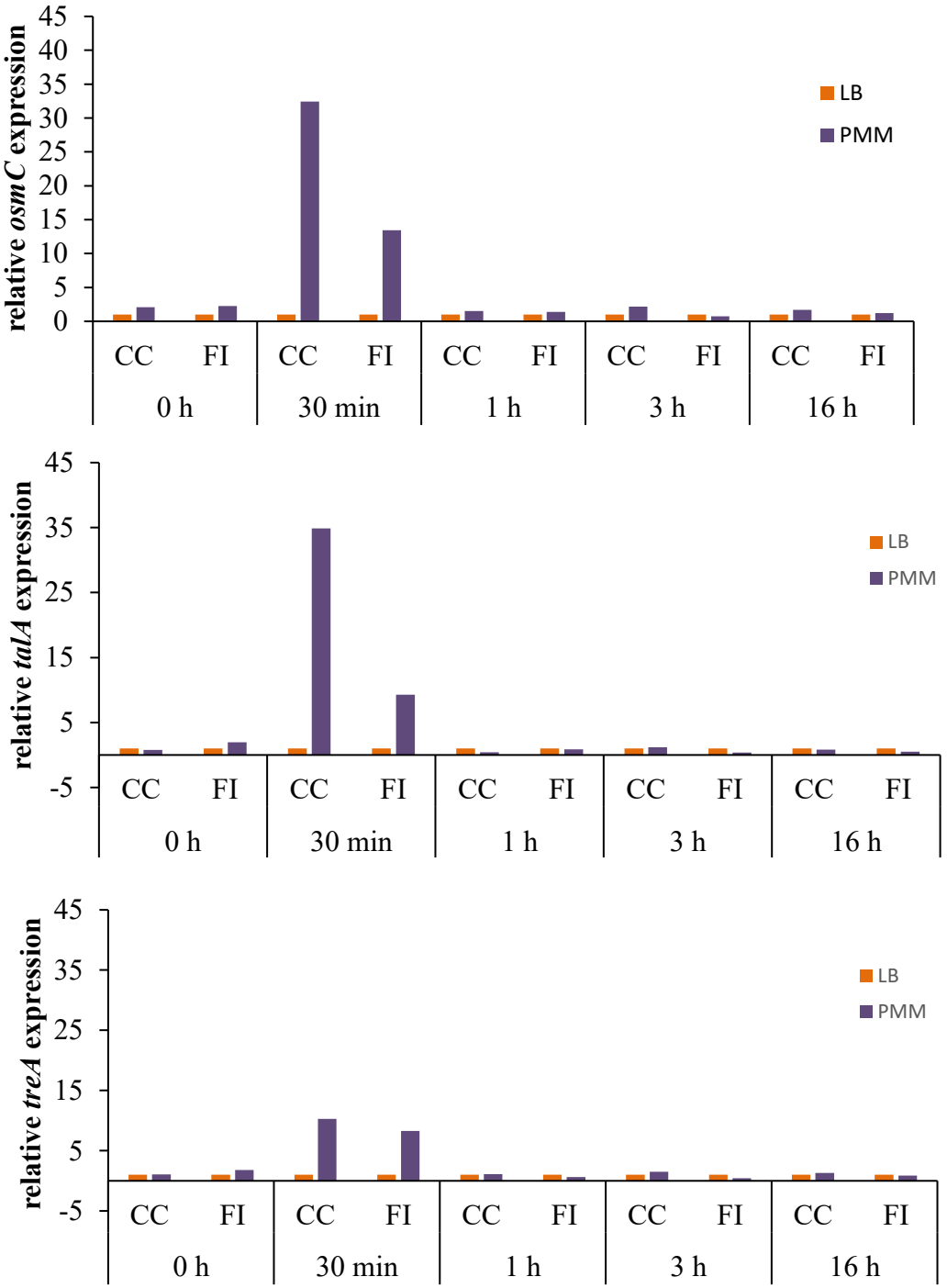

Figure 26 Relative gene expression levels of the three stress-related genes *osmC*, *talA*, and *treA* in the culture collection (ATCC B.02031, CC) and low water activity food isolate (buckwheat flour, FI) strains of *E. coli*. The expression levels were normalized to that of the housekeeping gene *rpoB*, which was used as the reference. The reference value of 1 in each case indicates consistent baseline expression. Relative gene expression values greater than 1 indicate an increase in the expression of the target gene, and values below 1 indicate a decrease. LB= Luria–Bertani (LB) medium, PMM= Prune-mimicking medium.

6 CONCLUSIONS AND RECOMMENDATION

With the culture-dependent approach using MALDI-TOF MS for colony identification, 20 different genera were identified from 30 dried fruit samples. The three most frequently identified genera were *Bacillus* (from 21 samples), *Priestia* (from 12 samples), and *Peribacillus* (from 10 samples). Most of the isolates were environmental species such as *Bacillus amyloliquefaciens, B. licheniformis*, *B. pseudomycoides*, *B. mojavensis*, *B. thuringiensis*, *B. velezensis,* and *Curtobacterium flaccumfaciens*. However, potentially pathogenic bacteria, including *Staphylococcus epidermidis, Pantoea agglomerans, Enterococcus mundtii, Micrococcus luteus, Mixta calida* and *Priestia flexa* were also identified. Some members of the genera *Pantoea* and *Enterococcus*, on the other hand, can have beneficial roles in foods or natural environments, such as promoting plant growth, producing antimicrobial compounds, or contributing to fermentation processes, reflecting their dual potential to act as opportunistic pathogens or useful organisms. The culture-independent 16S rDNA amplicon sequencing identified *Bacillus* across 29 fruits samples, indicating broader detection coverage compared to culture-dependent approach. This method additionally revealed the presence of environmental contaminants (*Mesobacillus*, *Neobacillus* and *Tumebacillus*) not identified using MALDI-TOF MS, which could indicate the potential presence of VBNC bacteria in the dried fruit samples. Notably, *E. coli* was not detected with either method. In recent studies *E. coli* has been previously isolated from dried fruits, which demonstrates its ability to survive in low water activity environments, and has also been implicated in multiple outbreaks associated with low-moisture food products (Ntuli et al., 2017; Abed, 2025). Therefore, its detection would have allow the assessment of the microbiological safety of the samples, and the identification of potential post-processing contamination routes during handling and packaging. However, its presence in VBNC state or at levels below the detection limit cannot be excluded, as high abundance species could mask its presence, especially if its DNA was present in small amounts. In conclusion, the culture-dependent method revealed a more diverse microbiota, while the culture-independent provided complementary information. The results highlight that the comprehensive understanding of microbial diversity requires the combination of the culture-dependent and independent methods, as well as the combined interpretation of both richness and evenness metrics. The fungal presence was only assessed by the culture-dependent approach, which identified, by MALDI-TOF MS, two yeast species (*Zygosaccharomyces rouxii* and *Z. bailii*) and 13 species of moulds, belonging to 9 genera, including *Arthrinium*, *Aspergillus*, *Alternaria, Neoscytalidium, Scopulariopsis, Microsporum, Cladosporium, Penicillium,* and *Rhizopus.* The detection of pathogenic fungi (*Aspergillus niger* and *Arthrinium phaeospermum*) is concerning, as *A. niger* is known to produces mycotoxins, and both species have been associated with opportunistic human infections. However, *A. niger* is also widely used industrially for enzyme production. Notably, in dried fruits, fungal contamination is often of greater

concern than bacterial contamination, as many fungi are capable of producing mycotoxins that can persist throughout processing and storage.

To investigate the behaviour of *E. coli* under osmotic stress, dried fruit-mimicking media (dried apricot, prune, raisin) with varying sugar compositions were prepared. The growth experiment demonstrated that *E. coli* could grow in prune mimicking medium at 37 °C. The lag phase, however, was extended, lasting approximately 32 hours for the FI strain and 85 hours for the CC strain in the case where LB-pre-adapted cells were transferred to prune-mimicking medium. Moreover, the cells that were pre-adapted in prune-mimicking medium and then transferred to the nutrient-rich LB medium exhibited much shorter lag phases (~3 hours for FI and ~6 hours for CC), and *E. coli* regained growth capacity. A similar scenario in everyday life would be the transfer of *E. coli* from dried fruits to yogurt or oats prepared with either milk or water.

The previously described model media were used to assess the induction of a potentially VBNC-like state of *E. coli* under osmotic stress. After 96 hours of exposure, culturability decreased significantly via the plate count method, whereas the viability, measured by PMA-qPCR, remained unchanged, indicating a VBNC-like behaviour. However, the actual viability was not verified, and no resuscitation step was performed, therefore the presence of VBNC cells cannot be confirmed, only inferred. Moreover, cells pre-adapted in prune-mimicking medium for 192 hours were able to regain their growth capacity upon transfer to LB medium, potentially indicating an extended lag phase, rather than true VBNC state.

The effect of pre-adaptation to osmotic environments was assessed in order to observe if the adaptation changes bacterial behaviour in media containing high sugar concentrations. It was observed that *E. coli* pre-exposed to moderate osmotic stress (0.35 M glucose) for 2.5 hours exhibited significantly higher cell counts in prune-mimicking medium after 4 hours of stress compared to the non-adapted cells ($p = 0.0122$, $\alpha = 0.05$). However, after 24 hours, this difference diminished. In raisin mimicking medium, after 24 hours of stress, the pre-adapted cells had significantly higher cell counts compared to the non-adapted cells ($p = 0.0367$, $\alpha = 0.05$). In apricot-mimicking medium, there was a considerable but not significant difference between the pre-adapted cells and the non-adapted cells at both 4 and 24 hours. This experiment revealed that the pre-adaptation gives a survival advantage to *E. coli* compared to non-adapted cells. Given the widespread consumption of dried fruits and the survival potential of *E. coli* in high-sugar matrices, osmotic pre-adaptation should be considered when evaluating microbial risks, as it may increase the likelihood of pathogen persistence in ready-to-eat food products.

Gene expression studies in the prune-mimicking medium demonstrated rapid transcriptional responses to osmotic stress. Within 30 minutes, the expression of all three tested genes (*osmC*, *talA*, and *treA*)

increased. The comparison of the strains from the culture collection and low water activity food revealed that the food isolate had greater tolerance to the high osmotic pressure. The observed spike in gene expression at 30 minutes might be due to the rapid activation of stress response mechanisms which stabilize cell homeostasis in *E. coli* upon transfer to sugar-enriched media, while the long-term adaptation mechanisms are initiated. As the cells adapt to the osmotic conditions, the expression of these genes declines. Alternatively, it is possible that the cells may not rely on these specific genes as prerequisite for their long-term survival.

The D-value of *E. coli* under gamma irradiation was assessed in media containing different concentrations of glucose, fructose or sucrose, as well as in the prune-mimicking medium. The results showed that high sugar concentrations increased the decimal radiation dose of *E. coli*, indicating that sugars might have protective effect. This has important implications for designing effective treatments for dried fruits and other high-sugar food products.

Based on the conclusions above, the following recommendations are proposed for future studies:

- Increase the sample size for microbiota characterisation,
- Optimize sample preparation protocols for both culture-dependent and culture-independent methods,
- Sequence the Internal Transcribed Spacer (ITS) region to enable fungal identification,
- Include PMA-qPCR analysis for improved culture-independent microbiota characterisation,
- Utilise 30 % glucose agar to facilitate the detection of osmotolerant microbiota,
- Investigate the matrix-effect in osmotic media, including pH adjustment and antioxidant content to better mimic dried fruit conditions,
- Conduct VBNC induction experiments under extended stress exposure (e.g., 2 weeks or longer), and include a resuscitation step and viability assays to confirm the presence of VBNC cells,
- Employ different pre-adaptation media to mimic other dried fruit products,
- Apply tighter sampling intervals to monitor early gene expressions (e.g., every 5 minutes during the first 30 minutes),
- Analyse broader stets of genes for transcriptomic analysis of *E. coli*,
- Investigate the effects of gamma irradiation on the gene expression of *E. coli* under osmotic stress
- Determine whether gamma irradiation induces the VBNC state in *E. coli*,
- Include multiple biological replicates in each experiment,
- Conduct experiments (e.g., growth measurement, stress experiments) at room temperature to more accurately reflect household storage conditions.

7 NEW SCIENTIFIC RESULTS

1. This is the first investigation on the microbiota of commercially available dried fruit products (dried apricot, prune, raisin) from Hungary and Austria using both culture-dependent (plating and MALDI-TOF MS) and culture-independent (16S rDNA amplicon sequencing) approaches.
   - Using the culture-dependent approach, several bacterial and fungal species were identified, including *Pantoea agglomerans*, *Enterococcus mundtii*, *Mixta calida*, *Priestia flexa*, *Aspergillus niger*, *Arthrinium phaeospermum* were identified from dried fruits samples, while the culture-independent approach revealed the presence of *Mesobacillus, Neobacillus,* and *Tumebacillus* genera. Some of the detected species, such as *Pantoea agglomerans*, *Enterococcus mundtii* and *Aspergillus niger* are known opportunistic pathogens, highlighting potential food safety concerns in dried fruit products.
   - Dried apricots and raisins exhibited higher bacterial diversity compared to prunes (on average 3.36 genera per sample in raisins, 2.70 genera per sample in dried apricots, and 1.62 genera per sample in prunes), likely due to the suppressive effects of phenolic compounds and antioxidants in prunes.
   - Raisins harboured the greatest number of unique bacterial genera (*Pseudomonas*, *Mixta*, *Curtobacterium*, *Kocuria*, *Niallia*, *Alkalihalobacillus*, *Ureibacillus* and *Metabacillus*), reflecting fruit-specific microbial niches.
   - The combined use of MALDI-TOF MS and 16S rDNA amplicon sequencing revealed the presence of both culturable and low-abundance taxa, demonstrating the importance of multi-approach profiling in low water activity food products (Dobó et al., 2025).
2. It was demonstrated for the first time that the pre-adaptation to a 0.35 M glucose containing medium influences the growth potential of *E. coli* strains.
   - Both a food isolate from a low-water activity product (buckwheat flour, FI strain) and a strain originated from culture collection (ATCC B.02031, CC strain), are able to grow in osmotic media containing 0.35 M glucose, and also in prune-mimicking medium (LB supplemented with 25.5 g/100 g glucose, 12.4 g/100 g fructose and 0.15 g/100 g sucrose). Furthermore, cells pre-adapted in prune-mimicking medium for 192 hours regained their growth capacity after transferring to LB medium, which provides a nutrient rich and low-osmotic environment, indicating resuscitation potential.
   - The pre-adaptation to 0.35 M glucose was shown to enhance the survival of *E. coli* (ATCC B.02031, CC strain) in dried fruit-mimicking media. The cell counts of the pre-adapted sample were significantly higher compared to the non-adapted controls in prune-mimicking medium after 4 hours of osmotic stress ($Z = 2.5067$, $p = 0.0122$, $\alpha = 0.05$) and in raisin-mimicking medium after 24 hours of osmotic stress ($Z = 2.0889$, $p = 0.0367$, $\alpha = 0.05$).

3. Exposure to diverse sugar concentrations resembling those found in dried apricot, prune and raisin may have induced a viable but non-culturable (VBNC)-like state in both the culture collection strain (ATCC B.02031, CC strain) and the low water activity food isolate (buckwheat flour, FI strain) of *E. coli*, over a 96-hour period. This presents the first evidence suggesting that conditions similar to those of dried fruits may trigger VBNC-like behaviour in *E. coli*. The potential VBNC-like state was inferred by comparing the results of the PMA-qPCR based viability assessments with the results of the conventional culturing (plate count) method. The significant difference between these two approaches observed between 24 and 96 hours in the dried fruit-mimicking media (CC strain of *E. coli* $Z = -2.95$, $p = 0.0031$; FI strain of *E. coli*: $Z = -2.16$, $p = 0.0304$; $\alpha = 0.05$) indicates the presence of metabolically active cells that were non-culturable under the tested conditions.
4. Gene expression studies revealed that the expression of three osmotic stress-related genes (*osmC*, *talA*, and *treA*) abruptly increased in the *E. coli* strains (culture collection: ATCC B.02031, CC; low water activity food isolate, from buckwheat flour, FI) after 30 minutes of osmotic stress in prune-mimicking medium. The food-originated (FI) strain presented greater tolerance to osmotic stress than the strain from culture collection (CC), presumably due to its origin from a low water activity environment.
5. It was demonstrated for the first time, that media with high osmotic pressure, containing high concentrations of glucose (30 %, 50 %), fructose (30 %, 50 %) or sucrose (50 %) showed a protective effect on the strain of *E. coli* from the culture collection (ATCC B.02031) under gamma irradiation. The decimal radiation dose increased compared to the control medium:
   - in the case of glucose: from 0.32 kGy (control) to 0.41 kGy and 0.74 kGy (30 % and 50 % glucose, respectively),
   - in the case of fructose: from 0.33 kGy (control) to 0.41 kGy and 0.43 kGy (30 % and 50 %, respectively),
   - in the case of sucrose. from 0.32 kGy (control) to 0.42 kGy (50 %)

   The strongest protective effect was observed with the 50 % glucose. Similarly, the prune-mimicking medium with sugar concentrations and composition resembling the sugary environment of prunes also had a protective effect (moderate) on both the strain from the culture collection (ATCC B.02031) and the strain from the low water activity food isolate (buckwheat flour):

   - The decimal radiation doses were increased from 0.31 kGy (control) to 0.34 kGy for the culture collection strain, and to 0.36 kGy for the low water activity food isolate strain.

8 SUMMARY

Dried fruits play an important role in our everyday life. They are widely recognized as healthy snacks that have gained popularity due to increasing consumer demand for nutritious food products. Their extended shelf life, ease of storage and transport, and naturally sweet flavour contributed to their widespread consumption. These products are rich in dietary fibre, phytochemicals, bioactive compounds, vitamins, and minerals, all of which contribute to potential health benefits and may help reduce the risk of various chronic diseases (Średnicka-Tober et al., 2020; Sandhu et al., 2023; Juhnevica-Radenkova et al., 2024; Bu et al., 2024). However, as dried fruits are typically consumed without further processing that could reduce the microbial load, such as heat treatment or washing, both pre-harvest and post-harvest contamination pose risks to their microbiological quality and safety. Characterizing their microbial communities is important for identifying spoilage and potentially pathogen microorganisms. Furthermore, investigating bacterial behaviour, such as VBNC formation or changes in the gene expressions towards the stress conditions encountered in dried fruits may contribute to enhanced food safety and quality.

In the first part of the thesis, the aim was to isolate and characterize the microbial populations present in commercially available dried apricots, prunes, and raisins from markets in Hungary and Austria. An attempt was made to cover a diverse range of product categories. Thus, different packages (e.g. paper, nylon), organic and non-organic, packaged and unpackaged fruits, fruits with and without sulphur-dioxide were chosen. Two approaches were used, including a culture-dependent method and a culture-independent approach based on 16S rDNA amplicon sequencing using the MinION device. The majority of the identified microorganisms were environmental or spoilage-related, including *Bacillus amyloliquefaciens, B. licheniformis*, *B. pseudomycoides*, *B. mojavensis*, *B. thuringiensis*, *B. velezensis, Curtobacterium flaccumfaciens, Lysinibacillus xylanilyticus,* and *Metabacillus idriensis*. However, some samples also harboured potentially pathogenic bacteria, such as *Pantoea agglomerans*, *Mixta calida*, *Micrococcus luteus*, *Enterococcus mundtii* and *Staphylococcus epidermidis*. It is worth mentioning, that certain species, including *Pantoea* or *Enterococcus*, can also play beneficial roles in specific food products or natural environments, highlighting that their presence is not always harmful. The presence of non-pathogenic bacteria, such as *Pseudomonas cichorii* is noteworthy, as insufficient hygiene practices could also contribute to the growth of other human pathogens (Hikichi et al., 2013). Additionally, *Priestia flexa*, recently associated with urinary tract infections, highlights the importance of monitoring emerging or non-traditional species in the food industry. Moreover, pathogenic moulds such as *Aspergillus niger*, *Arthrinium phaeospermum*, *Neoscytalidium dimidiatum*, *Scopulariopsis brevicaulis,* and *Microsporum audouinii* were also detected, raising concerns regarding overall food safety and quality.

The genus *Bacillus* was consistently detected across all fruit types with the 16S rDNA amplicon sequencing. Furthermore, genera such as *Mesobacillus*, *Neobacillus*, and *Tumebacillus* were exclusively detected using the culture-independent approach, possibly indicating the presence of bacteria in the viable but non-culturable state. The culture-dependent method combined with MALDI-TOF MS for identification provided broader genus level diversity in the dried fruit samples. Species level identification was only possible using MALDI-TOF MS, as the sequencing approach was limited to genus-level resolution. These findings underscore the value of combining both methods to obtain a more complete view of the microbial composition of dried fruits. It is important to note that 16S rDNA amplicon sequencing only targets bacterial DNA and does not detect yeasts or moulds, as these organisms lack the 16S rRNA gene. For fungal identification, the amplification and sequencing of the Internal Transcribed Spacer (ITS) region would be required. Additionally, a limitation of the sequencing method is its inability to differentiate between DNA from live and dead cells. While it successfully detected several genera not identified by MALDI-TOF MS, suggesting the potential presence of VBNC bacteria, some of the detected DNA may have originated from dead cells. The use of viability-based methods such as propidium monoazide quantitative PCR (PMA-qPCR) could help resolve this issue by selectively amplifying DNA from live cells. To completely characterise fungal communities of dried fruits, the amplification and sequencing of the ITS (Internal Transcribed Spacer) region of the rRNA gene cluster is recommended. The physical characteristics of dried fruits, such as their wrinkled and uneven surfaces, may also affect the accuracy and consistency of microbial detection in both culture-dependent and independent approaches. Sample preparation is therefore a critical step that may influence the results. Moreover, a larger sample size would offer greater understanding of the microbiota of dried fruits, with potentially involving other popular dried fruits such as apples, cranberries, mangoes. In conclusion, this part of the thesis revealed that dried fruit products harbour diverse microbial communities, including potential spoilage and pathogenic organisms. These findings highlight the importance of strict hygiene practices, appropriate packaging, and controlled storage and distribution conditions to reduce contamination risks and ensure product safety.

The second part of the thesis focused on assessing bacterial behaviour under osmotic stress. Monitoring bacterial growth is important to determine whether *E. coli* can grow in media mimicking the conditions of dried fruits. For this, two strains of *E. coli* were used (CC, FI). Growth experiments were performed in media mimicking the osmotic environments of dried fruits (dried apricot, prune, raisin), based on their sugar characteristics and concentrations. Additionally, a low-sugar medium containing 0.35 M glucose was also prepared. The selected concentration was based on the evidence that *E. coli* grows optimally at approximately 0.3 osmol/L, and changing this balance could impair bacterial growth (Scheidle et al., 2011).

The ability of the two *E. coli* strains to grow under osmotic pressure was assessed using the Bioscreen system. It was observed that both *E. coli* strains were able to grow in the low-sugar and the prune-mimicking medium. However, in the prune-mimicking medium the lag phase was prolonged, lasting approximately 32 hours for the FI strain and 85 hours for the CC strain after the LB-preadapted cells were transferred into this high-osmotic-pressure environment. Furthermore, another experiment demonstrated that cells previously exposed to stress could regain their growth potential following a transfer to nutrient and water rich medium. In this case, prune-preadapted cells showed much shorter lag phases upon transfer to nutrient rich LB medium (~3 hours for FI and ~6 hours for CC), indicating rapid recovery under favourable growth conditions. This is relevant for food safety as this result reflects the potential for *E. coli* persisting in dried fruits to resume growth under favourable conditions Previous studies have proven that microbial cells pre-exposed to sub-lethal doses of stress could improve their ability to survive higher otherwise lethal doses of the same or different stressors. This phenomenon is called cross-tolerance (Bae and Lee, 2010; Shah and Bergholz, 2020; Martin et al., 2025). Therefore, future studies should focus on monitoring the growth potential of *E. coli* at room temperature to better represent household environments. Moreover, to solely investigate the effect of diverse sugars, the media were not adjusted to match the pH or antioxidant content of dried fruits, thus the potential influence of these matrix-specific factors were not investigated. Future studies integrating these parameters with osmotic pressure would offer valuable insight into bacterial growth dynamics in these environments.

Viable but non-culturable bacteria offers food safety risks as they cannot be cultured using conventional laboratory methods, despite maintaining metabolic activity, and having the potential to regain pathogenicity upon resuscitation. To study the transition of *E. coli* into a VBNC-like state, model media were created. The culturability, using the plate count method, and the viability, using PMA-qPCR were monitored over 96 hours of two strains of *E. coli* (CC and FI). The results showed a decrease in culturability by 96 hours in both strains of *E. coli*, while PMA-qPCR indicated no change in the viability of the cells, suggesting that sugar concentrations mimicking the conditions of dried fruits may have induced VBNC-like behaviour in *E. coli*. However, no resuscitation experiments or additional viability assays were performed, and the observed loss of culturability could alternatively reflect an extended lag phase rather than a true VBNC state. Future studies should include resuscitation steps and complementary viability assessments to confirm VBNC formation. Additionally, since these experiments were conducted at the optimal growth temperature of *E. coli*, studies under room temperature conditions are needed to better reflect typical household environments.

Pre-adaptation to osmotic conditions has implications on food safety. For example, during osmotic drying the food is immersed in hypertonic solutions, which, if contaminated with *E. coli* can provide

an opportunity for bacterial adaptation. Upon transfer to dried fruits, the pre-adapted cells can withstand the hyperosmotic environment of dried fruits that otherwise would be lethal, as the pre-adaptation gives the bacteria a survival advantage once present on dried fruits. To test the pre-adaptation effect, *E. coli* strains (CC and FI) were pre-adapted in low-sugar medium (0.35 M glucose) for 2.5 hours, and subsequently transferred to media mimicking the osmotic conditions of dried fruits (dried apricot, prune, raisin) for 4 and 24 hours. After 4 hours of adaptation, cells pre-adapted to low sugar had significantly higher counts in the prune-mimicking medium than non-adapted cells ($S = 40$, $Z = 2.5067$, $p = 0.0122$, $\alpha = 0.05$), but this difference disappeared after 24 hours. In the raisin-mimicking medium, low-sugar pre-adapted cells maintained significantly higher counts after 24 hours ($S = 38$, $Z = 2.0889$, $p = 0.0367$, $\alpha = 0.05$), suggesting improved persistence or growth. In the apricot-mimicking medium, pre-adapted cells also showed higher counts at both 4 and 24 hours, though the differences were not statistically significant. However, similarly to VBNC experiments, the pre-adaptation assay was performed at 37 °C. Repeating this experiment at lower temperatures would provide valuable insight into bacterial behaviour under household storage conditions. Furthermore, pre-adaptation medium with different sugar composition and concentration would allow for a more comprehensive assessment of bacterial adaptation to osmotic environments.

When exposed to stress, bacterial responses can change on the transcriptional or translational level to help their adaptation. Gene expression is a regularly studied response to stresses encountered during food processing. In my thesis, the expression of three stress related genes, such as *osmC*, *talA*, and *treA*, were studied under osmotic stress after 0.5, 1, 3, and 16 hours. The analysis showed that within 30 minutes, both the CC and FI strains increased the expression of the osmotic stress associated genes, notably *osmC* and *talA*, although strain dependent differences were observed across all three genes. The FI strain consistently exhibited lower expression levels, indicating that it is better adapted to osmotic conditions, possibly due to its foodborne origin from buckwheat flour. The peak observed at 30 minutes could reflect a rapid stress response of *E. coli* leading to adaptation that reduces the need for continuous high-level expression. These results highlight that *E. coli* can activate diverse survival strategies in challenging food-related environments. Since the adaptation occurs rapidly after the induction of the stress, tighter sampling intervals are suggested, such as every five minutes up to 30 minutes, followed by every 10 minutes until 60 minutes to better characterize early adaptive response. A larger set of genes would allow for a more comprehensive understanding of the gene expressions of *E. coli* under osmotic stress induced by high sugar concentrations.

Ionizing radiation is an emerging, non-thermal food processing method that allows the preservation of fresh or packaged food products, without substantially changing the appearance, aroma and the texture. The reason for comparing e-beam and gamma radiation was that during the time of my experiments, a parallel research was being conducted at the Department of Food Microbiology,

Hygiene and Safety, which focused on the effects of e-beam on bacterial contaminants in sewage water. As both radiation sources are approved for food irradiation purposes, and operate within the short-wave, high-energy region of the electromagnetic spectrum, it was found to be essential to first determine the suitable radiation source for subsequent experiments. Consumer acceptance tends to favor machine-generated ionizing radiation over isotopic sources (Tahergorabi et al., 2012). However, e-beam radiation has lower penetration depth, higher installation and maintenance costs, and less uniform dose distribution compared to gamma radiation (Park et al., 2010; Chou et al., 2018). Microbial inactivation is dose-dependent, thus selecting the correct those is crucial for effectively eliminating spoilage organisms or pathogens. In my thesis work, the aim was to identify the D-value of *E. coli* in different media, including LB (as a general control), and in media supplemented with varying concentrations of glucose, fructose or sucrose, as well as in media reflecting the sugar composition of prunes. The experiment involving the prune-mimicking medium was performed with both *E. coli* strains (CC and FI). The results showed a protective effect of high sugar concentrations against irradiation. The highest D-value was observed in media with 50% glucose (0.74 kGy), followed by 50% fructose (0.43kGy), and 50% sucrose (0.42 kGy). Media containing 30% glucose and 30% fructose both had a D-value of 0.41 kGy, while 10% glucose (0.38 kGy) showed a similar effect to 30% sucrose (0.39 kGy). In the prune-mimicking medium, the D-values were 0.34 kGy for the CC strain, and 0.36 kGy for the FI strain. These results indicate that food products with higher sugar content need higher radiation doses to eliminate *E. coli*, as higher sugar concentrations enhance bacterial stress resistance to irradiation. Thus, when designing effective food irradiation doses, the sugar content of the product should be taken into consideration. Future studies should integrate molecular approaches, such as the PMA-qPCR to determine whether irradiation induces the VBNC state. Additionally, monitoring irradiation induced gene expression could provide deeper insight into stress responses. Investigating bacterial behaviour by combining irradiation with osmotic pressure may also serve as a valuable insight for designing food preservation processes.

9 LIST OF PUBLICATIONS IN THE FIELD OF STUDY

9.1 Journal articles

First author publications

Dobó, V., Homlok, R., Mohácsi-Farkas, C., and Belák, Á. (2023). Effect of gamma irradiation, high sugar content and antimicrobials on survival of Escherichia coli: A review. Czech Journal of Food Sciences, 41(4), 231-247. https://doi.org/10.17221/235/2022-CJFS

Dobó, **V**., Wagner, E., Belák, Á., Peham, T., and Domig, K. J. (2025). Deciphering the microbial composition of dried fruits purchased from Austrian and Hungarian markets using culture-dependent and culture-independent methods. *Food Control*, 111845. https://doi.org/10.1016/j.foodcont.2025.111845

Co-authored publications

Kiskó, G., Bajramović, B., Elzhraa, F., Erdei-Tombor, P., **Dobó, V**., Mohácsi-Farkas, C., Taczman-Brückner, A., Belák, Á. (2025). The Invisible Threat of Antibiotic Resistance in Food. *Antibiotics*, *14*(3), 250. https://doi.org/10.3390/antibiotics14030250

Kovács, M., Pomázi, A., Taczman-Brückner, A., Kiskó, G., **Dobó, V**., Kocsis, T., Mohácsi-farkas, C., Belák, Á. (2025). Detection and Identification of Food-Borne Yeasts: An Overview of the Relevant Methods and Their Evolution. *Microorganisms*, *13*(5), 981. https://doi.org/10.3390/microorganisms13050981

9.2 Conferences

Cefood 2024, Szeged, Hungary. **Dobó, V.,** Belák, Á., Mohácsi-Farkas, C. (poster: Microbiota of dried fruits and the osmotic stress of *E. coli*) 13-16 October 2024

The Miller Online Workshop on Radiation Chemistry. Homlok R., Kiskó G., Kovács A., Tóth T., **Dobó V**., Takács E., Mohácsi-Farkas Cs., Wojnárovits L., Szabó L. (poszter: Effect of electron beam irradiation and the presence of antibiotics on the population dynamics of resistant/sensitive bacterial cultures in model wastewater matrix). 10-12 February 2022

Third Research Coordination Meeting for CRP "Radiation Inactivation of Bio-hazards Using High Powered Electron Beam Accelerators" (International Atomic Energy Agency). Homlok R., Kiskó G., Kovács A., Tóth T., **Dobó V**., Takács E., Mohácsi-Farkas Cs., Wojnárovits L., Szabó L. (online. előadás: Effect of electron beam irradiation and the presence of antibiotics on the population ratio of resistant/sensitive bacterial in model wastewater matrix). 31 January - 04 February 2022

27th International Symposium on Analytical and Environmental Problems. Szeged, Magyarország. Homlok R., Kiskó G., Kovács A., Tóth T., **Dobó V**., Takács E., Mohácsi-Farkas Cs., Wojnárovits L., Szabó L. (poszter: Effect of electron beam irradiation and the presence of antibiotics on the population ratio of resistant/sensitive bacteria added prior to advanced oxidation treatment). 22-23 November 2021

A Magyar Tudomány Ünnepe. "Fiatal Talajbiológusok az élhető jövőért" Homlok R., Kiskó G., Kovács A., Tóth T., **Dobó V**., Takács E., Mohácsi-Farkas Cs., Wojnárovits L., Szabó L. (online előadás: Nagyhatékonyságú oxidációs kezelés és antibiotikumok hatása rezisztens/szenzitív baktériumkultúrák populációs arányára). 12 November 2021

Őszi Radiokémiai Napok, Balatonszárszó. Homlok R., Kiskó G., Kovács A., Tóth T., **Dobó V**., Takács E., Mohácsi-Farkas Cs., Wojnárovits L., Szabó L. (előadás: A környezeti szempontból jelentős koncentrációjú antibiotikumok hatása a rezisztens/szenzitív baktériumkultúrák populációs arányára, valamint kezelés gyorsított elektronokkal szennyvízmátrixban) 18-20 October 2021

MTA Élelmiszer-tudományi Bizottság 382. Tudományos kollokvium, Budapest. Homlok R., **Dobó V**., Kiskó G., Kovács A., Tóth T., Takács E., Mohácsi-Farkas Cs., Wojnárovits L., Szabó L. (előadás: Antibiotikum rezisztencia kialakulásának megelőzése szennyvízmátrixokban nagyenergiájú sugárzással). 28 May 2021

The Miller Online Workshop on Radiation Chemistry. Homlok R., Kiskó G., Kovács A., Tóth T., **Dobó V**., Takács E., Mohácsi-Farkas Cs., Wojnárovits L., Szabó L. (Online Flash prezentáció (3 perc előadás + 2 perc kérdés): Effect of electron beam irradiation and the presence of antibiotics on the population dynamics of resistant/sensitive bacterial cultures in model wastewater matrix with antibiotics and bacteria prior to advanced oxidation treatment). 10-12 February 2022

The Miller Online Workshop on Radiation Chemistry. Homlok R., Kiskó G., Kovács A., Tóth T., **Dobó V**., Takács E., Mohácsi-Farkas Cs., Wojnárovits L., Szabó L. (Flash video: Effect of electron beam irradiation and the presence of antibiotics on the population dynamics of resistant/sensitive bacterial cultures in model wastewater matrix with antibiotics and bacteria prior to advanced oxidation treatment). 10-12 February 2022

10 APPENDICES

10.1 A1 Bibliography

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10.3 A2 Supplementary

Supplementary Table 1 Foodborne outbreaks caused by pathogenic *Escherichia coli* (From: Yang et al., 2017; Alhadlaq et al., 2024).

Pathogen	Source	Date	Country	Number of cases	Species/serotypes	Remarks
Enteropathogenic *E. coli* (EPEC)	Egg soup and tuna bibimbap	2013	South Korea	33	aEPEC O157:H45	-
	Dining room	2010	China	112	aEPEC O127a:K63	18–23 years olds
Shiga toxin-producing *E. coli*/ Enterohemorrhagic *E. coli*) (STEC/EHEC)	Chipotle Mexican Grill restaurant	2015	USA	55	O26	From 11 states
	Costco Rotisserie Chicken salad	2015	USA	19	O157:H7	From 7 states
	Flour	2016-2017	Canada	29	O121	-
	Raw clover sprouts	2014	USA	19	O121	From 6 states
	Ground beef	2014	USA	12	O157:H7	1.8 million pounds of ground beef were recalled
		2019	USA	209	O103	-
	Romaine lettuce	2011	USA	58	O157:H7	From 9 states
		2018-2019	USA	234	O157:H7	From 33 states
	Sprouts	2011	USA	6	O104:H4	4 HUS, 1 death
		2011	Germany	3816	O104:H4	810 HUS, 54 deaths
	Store-made guacamole	2024	USA	5	O157:H7	-
	Fenugreek seeds	2011	France	24	O104:H4	7 HUS
	Frozen ground beef products	2011	France	18	Sorbitol-fermenting *E. coli* O157:H7	Children aged 6 months to 10 years old
	Raw beef dishes	2011	Japan	181	O111 and O157	-
	Raw leeks and potatoes	2010	England, Wales and Scotland	252	O157 PT8	1 Death
	Raw prepackaged cookie dough	2009	USA	72	O157:H7	From 30 states
	Restaurant	2008	USA	341	O111:NM	1 Death
	Prepackaged spinach	2006	USA	205	O157:H7	3 Deaths, involving 26 states

Pathogen	Source	Date	Country	Number of cases	Species/serotypes	Remarks
Enteroinvasive *E. coli* (EIEC/*Shigella*)	Bridge club	2012	USA	43	*Shigella sonnei*	-
	Basil pesto	2011	Norway	46	*Shigella sonnei*	-
	Canteen (cooked vegetables)	2012	Italy	109	O96:H19 (*ipaH*$^{+}$)	-
	Canteen (food handler)	2009	Belgium	52	*Shigella sonnei*	-
	Cold meat (school)	2006	China	937	*Shigella sonnei*	-
	Food on religious place	2010	India	About 150	*Shigella sonnei*	-
Enteroinvasive *E. coli* (EIEC/*Shigella*)	Food on wedding party	2009	India	>300	*Shigella sonnei*	2–70 years old
	Suspected to cooked food and ice block	2013	Papua New Guinea	About 1200	*Shigella flexneri* serotype 2	5 Deaths
	Water	2009	China	118	*Shigella flexneri* 2b	-
Enterotoxigenic *E. coli* (ETEC)	Fresh basil	2006	Denmark	About 200	ETEC O92:H- and O153:H2	-
	Imported chives and scrambled eggs	2012	Norway	>300	O78 (LT1 positive)	-
	Japanese restaurant	2012	Japan	102	O169:H41	-
	Kimchi	2012	Korea	230	O169	From 7 schools
	Lettuce	2010	Denmark	264	ETEC O6:K15:H16 and Norovirus	-
Enteroaggregative *E. coli* (EAEC)	Cheese (unsterilized raw milk)	2006	Italy	125	O92:H33	-
	Food festival (multi-pathogen)	2013	England	592	O131:H27, O104:H4, O20:H19	-

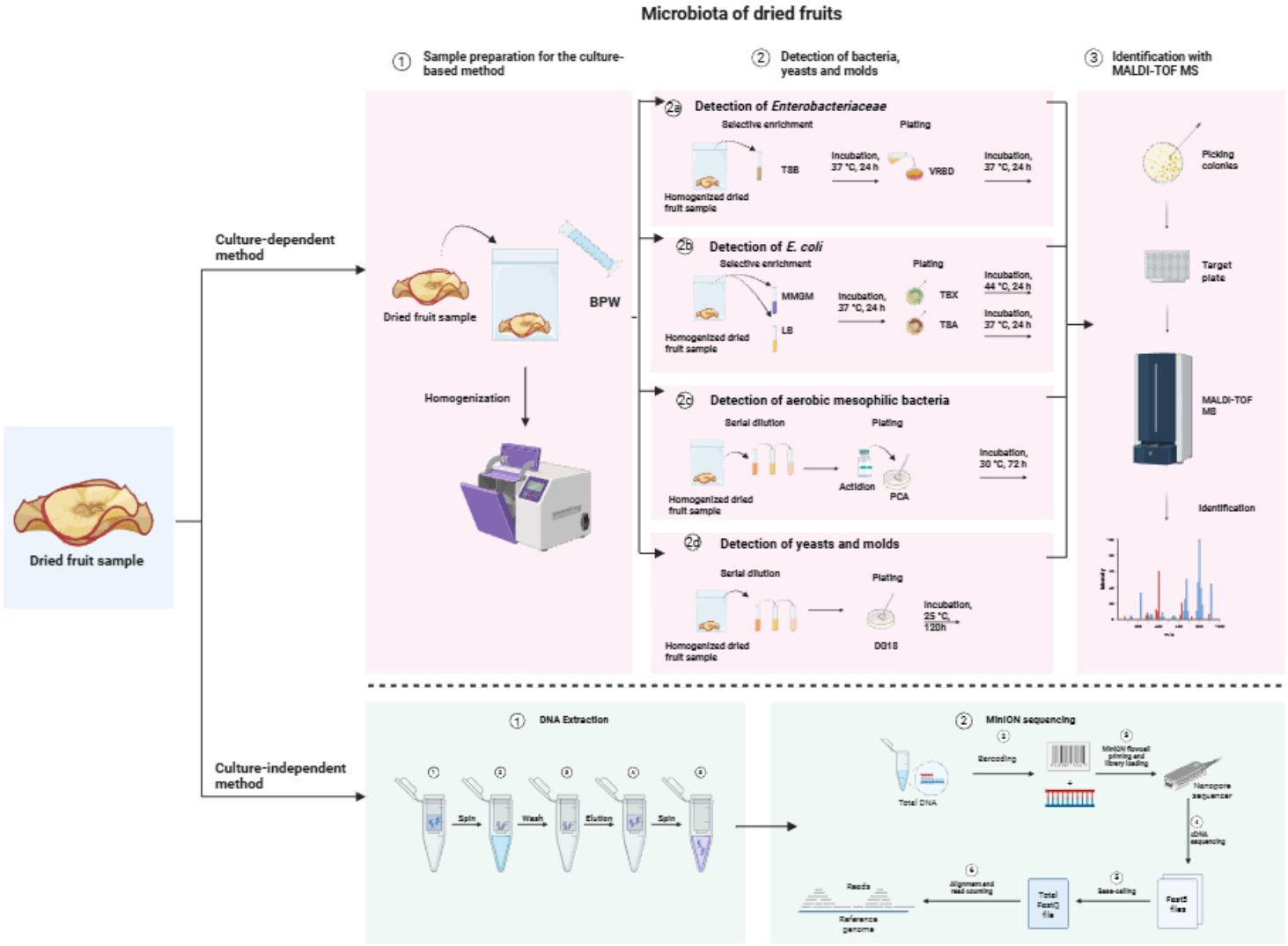
Supplementary Table 2 Overview of sample codes, purchase locations, potential preservative treatment, and comments on purchasing and storage conditions. "N/A." indicates not available. The samples were collected in April 2023.

Sample code	Sample category	Place of purchase	Treated with SO_2	Other preservative	Country of origin	Expiration date	Comments
1	Dried, pitted apricot	Interspar, Budapest	Yes	N/A	Hungary	22 Fenruary 2024	Sample was purchased pre-packaged in a plastic bag (non-transparent), stored in the same packaging
2	Dried apricots	TESCO, Budapest	N/A	N/A	Turkey	28 March 2024	Sample was purchased pre-packaged in a zip lock paper bag, stored in the same packaging
3	Dried apricots	Fehérvári Road Market, Vendor 1, Budapest	N/A	N/A	N/A	N/A	Sample was purchased from a container, accessible only to the seller, and stored in a nylon bag (transparent)
4	Dried apricots	Fehérvári Road Market, Vendor 2, Budapest	N/A	N/A	N/A	N/A	Sample was purchased from a container, accessible only to the seller, and stored in a nylon bag (transparent)
5	Dried apricots	Great Market Hall, Budapest	N/A	N/A	N/A	N/A	Sample was purchased from a container, accessible only to the seller, and stored in a nylon bag (transparent)
6	Dried apricots	Great Market Hall, Budapest	N/A	N/A	N/A	N/A	Sample was purchased from a partially covered container, accessible only to the seller, and stored in a nylon bag (transparent)
7	Dried apricots	Billa, Vienna	Yes	N/A	N/A	21 March 2024	Sample was purchased pre-packaged in a plastic bag (transparent), stored in the same packaging
8	Dried apricots	Baran Supermarkt, Vienna	N/A	N/A	N/A	N/A	Sample was purchased from a partially covered container, accessible to customers, and stored in a nylon bag (transparent)
9	Dried apricots	Naschmarkt, Vienna	N/A	N/A	N/A	N/A	Sample was purchased from a partially open container, accessible only to the seller, and stored in a nylon bag (transparent)
10	Dried apricots	Naschmarkt, Vendor 1, Vienna	N/A	N/A	N/A	N/A	Sample was purchased from a partially open container, accessible only to the seller, and stored in a nylon bag (transparent)
11	Dried apricots	Naschmarkt, Vendor 2, Vienna	N/A	N/A	N/A	N/A	Sample was purchased from a partially open container, accessible only to the seller, and stored in a nylon bag (transparent)
12	Pitted prunes	Interspar, Budapest	N/A	Potassium sorbate	Hungary	16 February 2024	Sample was purchased pre-packaged in a plastic bag (non-transparent), stored in the same packaging
13	Organic dried pitted prunes	Interspar, Budapest	No	No	Bulgaria	28 August 2024	Sample was purchased pre-packaged in a plastic bag (transparent), stored in the same packaging
14	Pitted prunes	TESCO, Budapest	N/A	Sorbic acid	N/A	12 December 2023	Sample was purchased pre-packaged in a plastic bag (non-transparent), stored in the same packaging
15	Pitted prunes	Fehérvári Road Market, Budapest	N/A	N/A	N/A	N/A	Sample was purchased from a partially covered container, accessible only to the seller, and stored in a nylon bag (transparent)
16	Prunes	Fehérvári Road Market, Budapest	N/A	N/A	N/A	N/A	Sample was purchased from a partially covered container, accessible only to the seller, and stored in a nylon bag (transparent)
17	Pitted prunes	Great Market Hall, Vendor 1, Budapest	N/A	N/A	N/A	N/A	Sample was purchased from a partially covered container, accessible only to the seller, and stored in a nylon bag (transparent)
18	Pitted prunes	Great Market Hall, Vendor 2, Budapest	N/A	N/A	N/A	N/A	Sample was purchased from a partially covered container, accessible only to the seller, and stored in a nylon bag (transparent)
19	Prunes	Billa, Vienna	N/A	Sorbic acid	N/A	20 October 2023	Sample was purchased pre-packaged in a plastic bag (transparent), stored in the same packaging
20	Sultana raisins	TESCO, Budapest	Might contain SO_2	N/A	N/A	24 February 2024	Sample was purchased pre-packaged in a plastic bag (transparent), stored in the same packaging

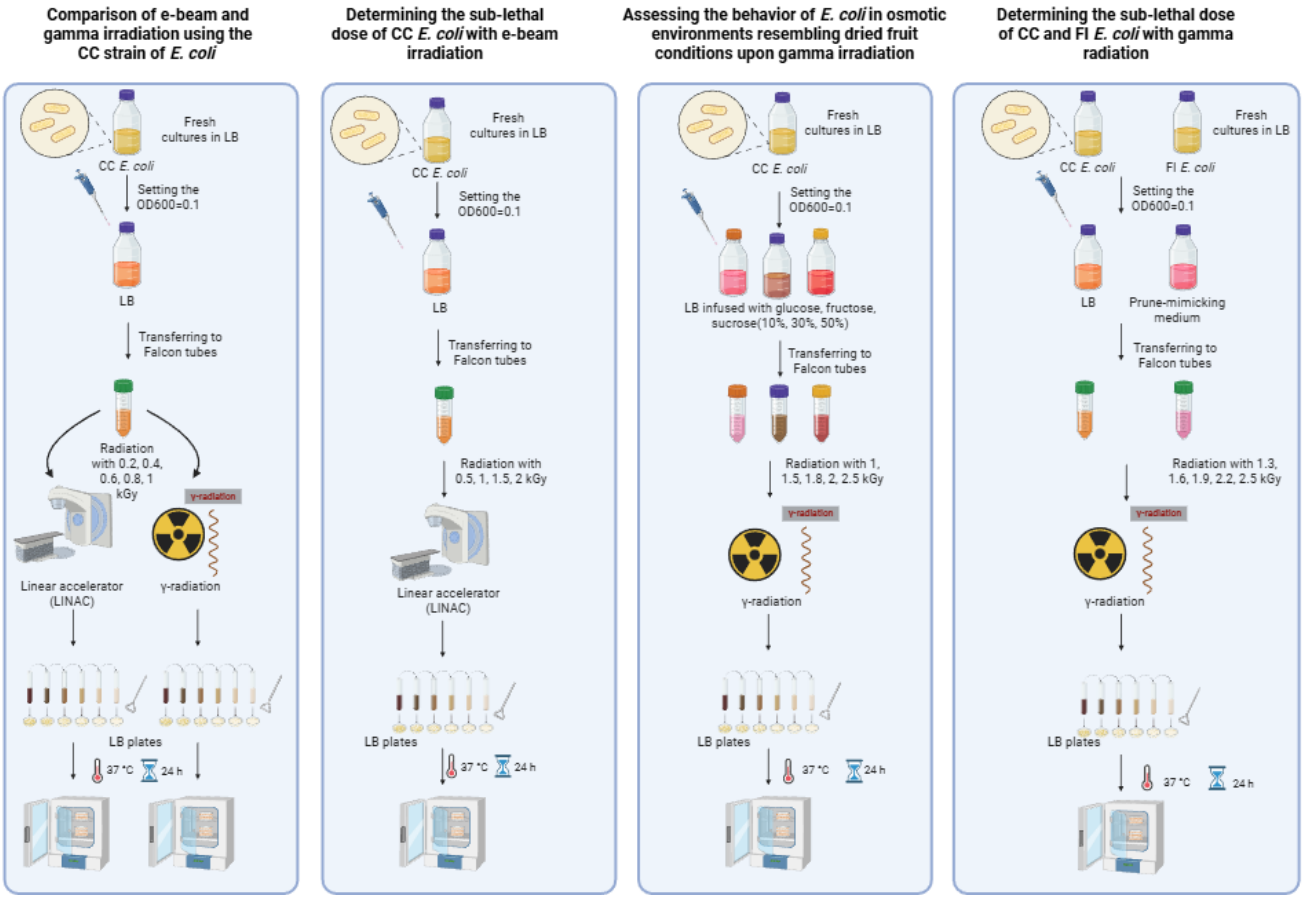
Sample code	Sample category	Place of purchase	Treated with SO_2	Other preservative	Country of origin	Expiration date	Comments
21	Raisins	TESCO, Budapest	N/A	N/A	Iran	14 March 2024	Sample was purchased from a covered container, accessible to customers, and stored in a nylon bag (biodegradable)
22	Raisins	Fehérvári Road Market, Budapest	N/A	N/A	N/A	N/A	Sample was purchased from an open container, accessible only to the seller, and stored in a nylon bag (transparent)
23	Golden raisnis	Fehérvári Road Market, Budapest	N/A	N/A	N/A	N/A	Sample was purchased from an open container, accessible only to the seller, and stored in a nylon bag (transparent)
24	Raisnis	Great Market Hall, Budapest	N/A	N/A	N/A	N/A	Sample was purchased from an open container, accessible only to the seller, and stored in a nylon bag (transparent)
25	Raisins	Great Market Hall, Budapest	N/A	N/A	N/A	N/A	Sample was purchased from a partially covered container, accessible only to the seller, and stored in a nylon bag (transparent)
26	Raisins	Prosi, Vienna	N/A	N/A	India	03 September 2023	Sample was purchased pre-packaged in a plastic bag (transparent), stored in the same packaging
27	Raisins	Baran Supermarkt, Vienna	N/A	N/A	N/A	05 November 2023	Sample was purchased pre-packaged in a plastic bag (transparent), stored in the same packaging
28	Raisins	Baran Supermarkt, Vienna	N/A	N/A	N/A	N/A	Sample was purchased from a partially open container, accessible to customers, and stored in a nylon bag (transparent)
29	Golden raisnis	Baran Supermarkt, Vienna	N/A	N/A	N/A	N/A	Sample was purchased from a partially open container, accessible to customers, and stored in a nylon bag (transparent)
30	Golden raisnis	Naschmarkt, Vienna	N/A	N/A	N/A	N/A	Sample was purchased from a partially covered container, accessible only to the seller, and stored in a paper bag

Supplementary Table 3 Growth potential measurement settings using the Bioscreen C Pro device.

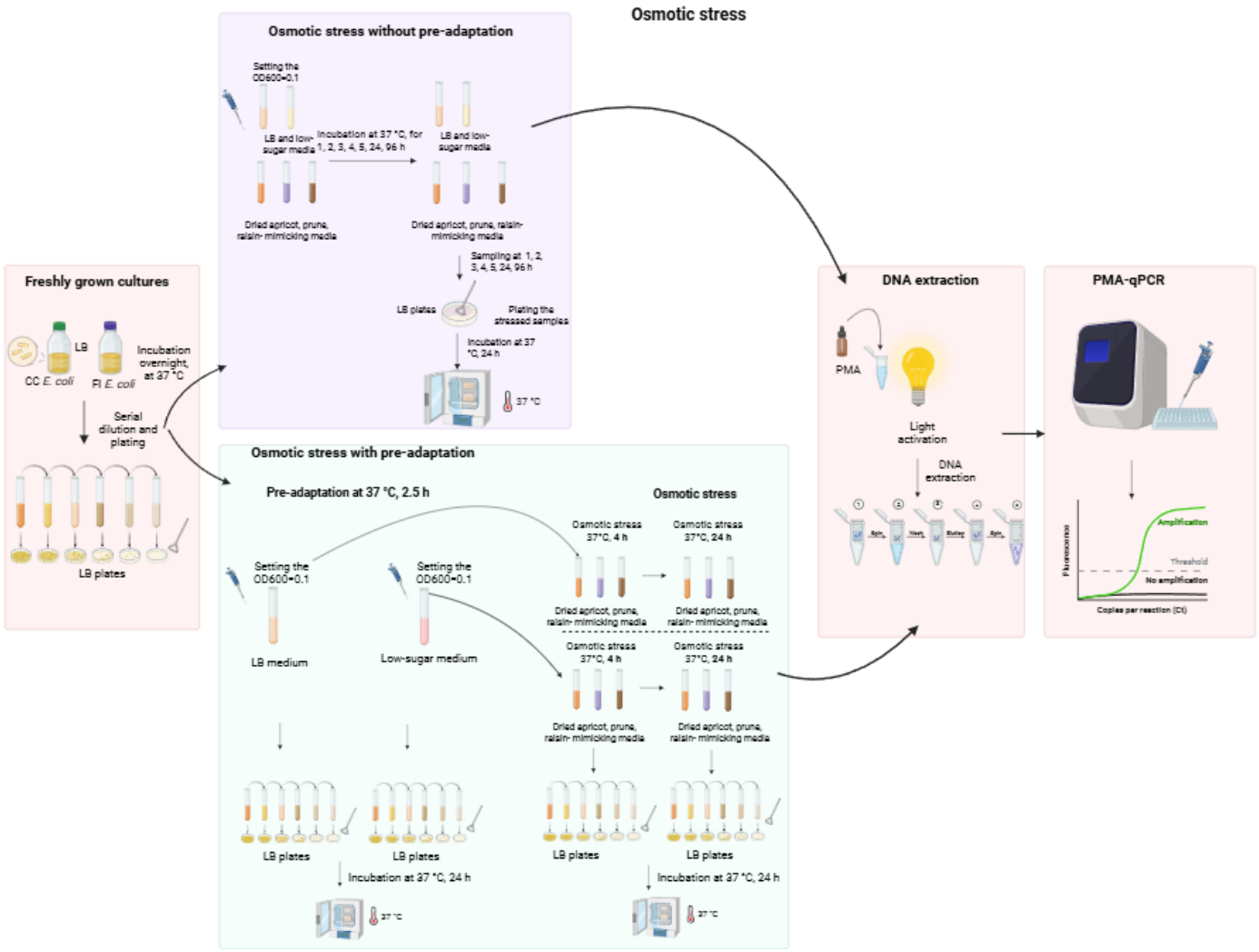
Sample volume	**300 µl**
Temperature	37 °C
Filter	600 nm
Shaking	Before the measurement, linear pattern, low amplitude
Shaking duration	15 seconds
Measurement interval	Every 30 minutes
Duration of experiment	24 hour (first round), 72 hour (second round), 96 hour (third round)



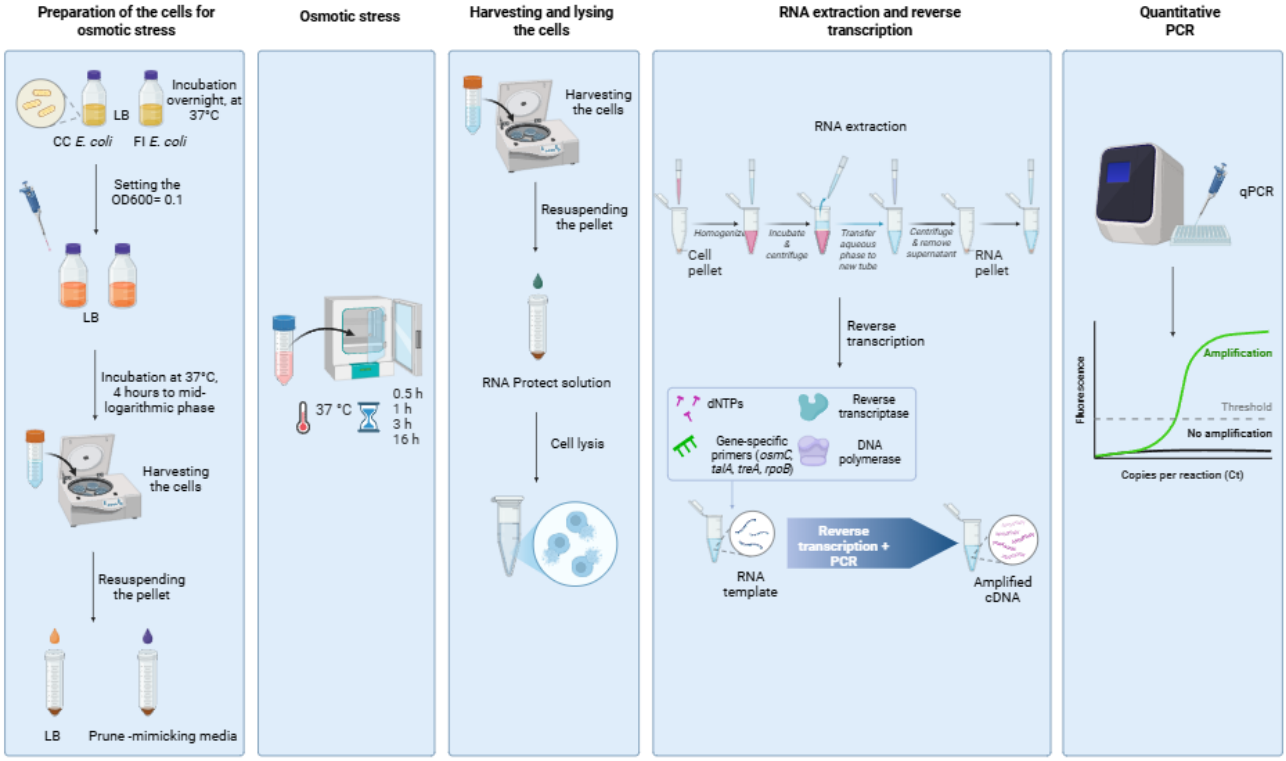
Supplementary Figure 1 The schematic process of detecting and identifying the microbiota of dried fruits. Created with BioRender.com (2025).



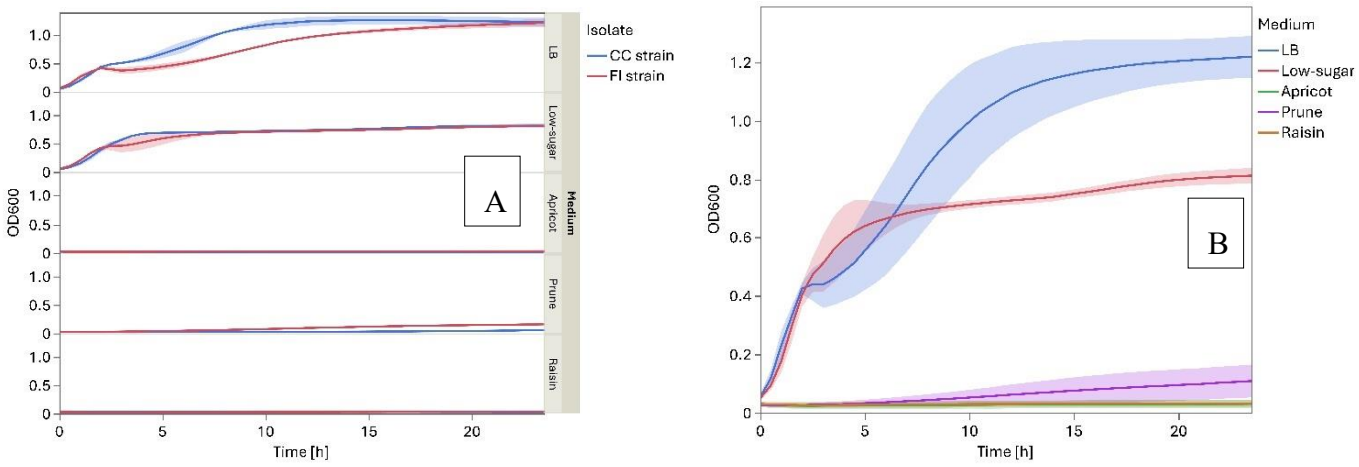
Supplementary Figure 2 The schematic overview of the irradiation assays. Created with BioRender.com (2025).



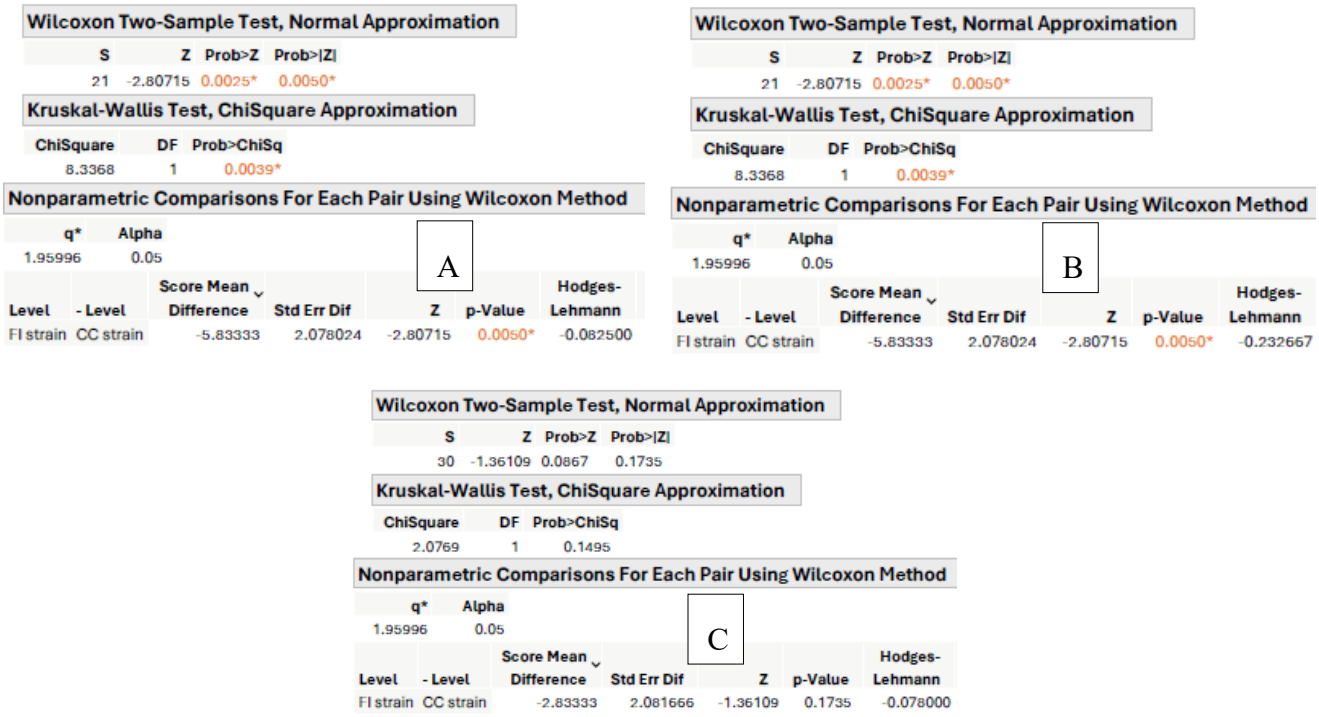
Supplementary Figure 3 Schematic overview of the viability and culturability assessment. Created with BioRender.com (2025).



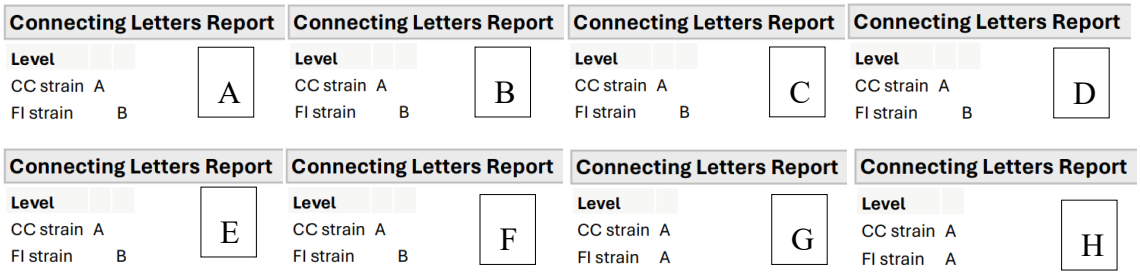
Supplementary Figure 4 Schematic overview of the gene expression study. Created with BioRender.com (2025).



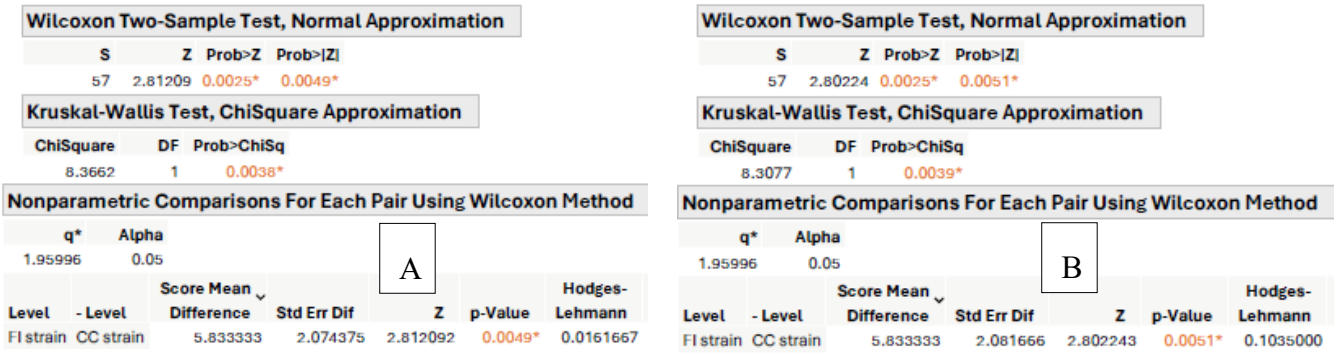
Supplementary Figure 5 Combination of the growth curves obtained in the first two rounds of growth curve experiments, where (A) presents the CC and FI strains of *E. coli* at 37° C for 24 hours in LB medium (control), low-sugar medium and dried fruit (dried apricot, prune, and raisin) mimicking media, and (B) presents the OD_{600} values of each media regardless of the bacterial strains in LB medium (control), low-sugar medium and dried fruit (dried apricot, prune, and raisin) mimicking media, and (B) presents the OD_{600} values of each media regardless of the bacterial strains. The figure shows the mean values of the measurements, with the standard deviation represented by the shaded regions (B).



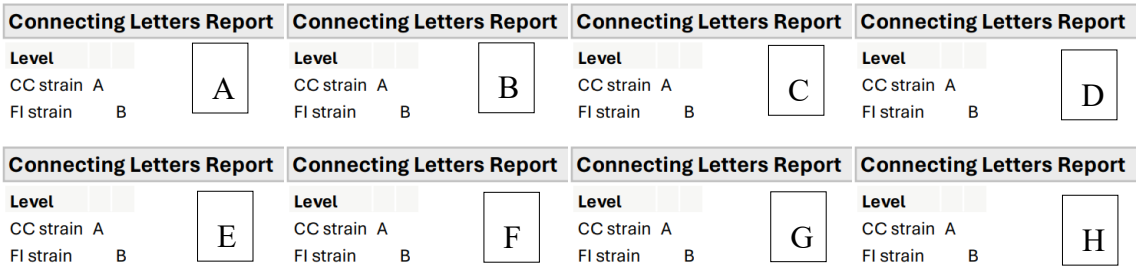
Supplementary Figure 6 Values of the differences in LB medium at (A) 2.5 hours, (B) at 5 hours, and (C) at 20 hours.



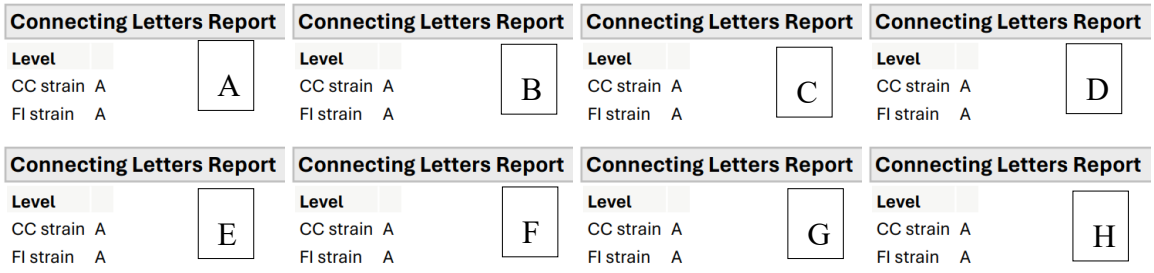
Supplementary Figure 7 Connecting letters report on the differences among the two strains (CC, FI) of *E. coli* in LB medium, (A) at 5 hours, (B) at 7.5 hours, (C) at 10 hours, (D) at 12.5 hours, (E) at 15 hours, (F) at 17.5 hours, (G) at 20 hours, and (H) at 22.5 hours. Levels not connected by the same letter are significantly different.



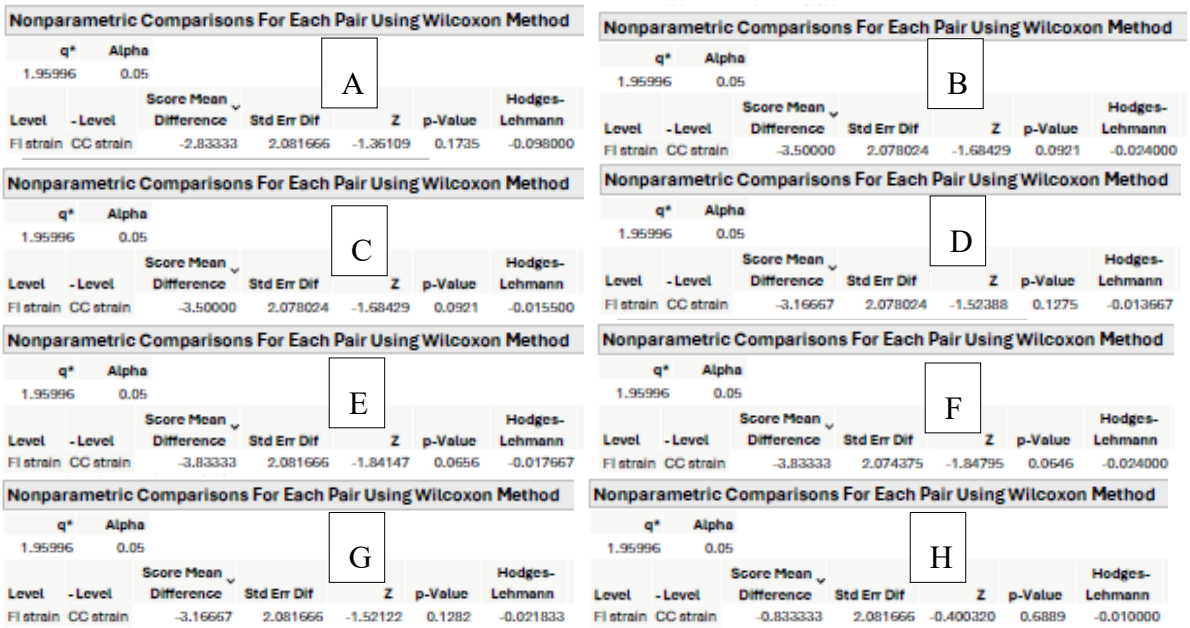
Supplementary Figure 8 Values of the differences observed in prune-mimicking medium at (A) 5 hours, and (B) at 22.5 hours.



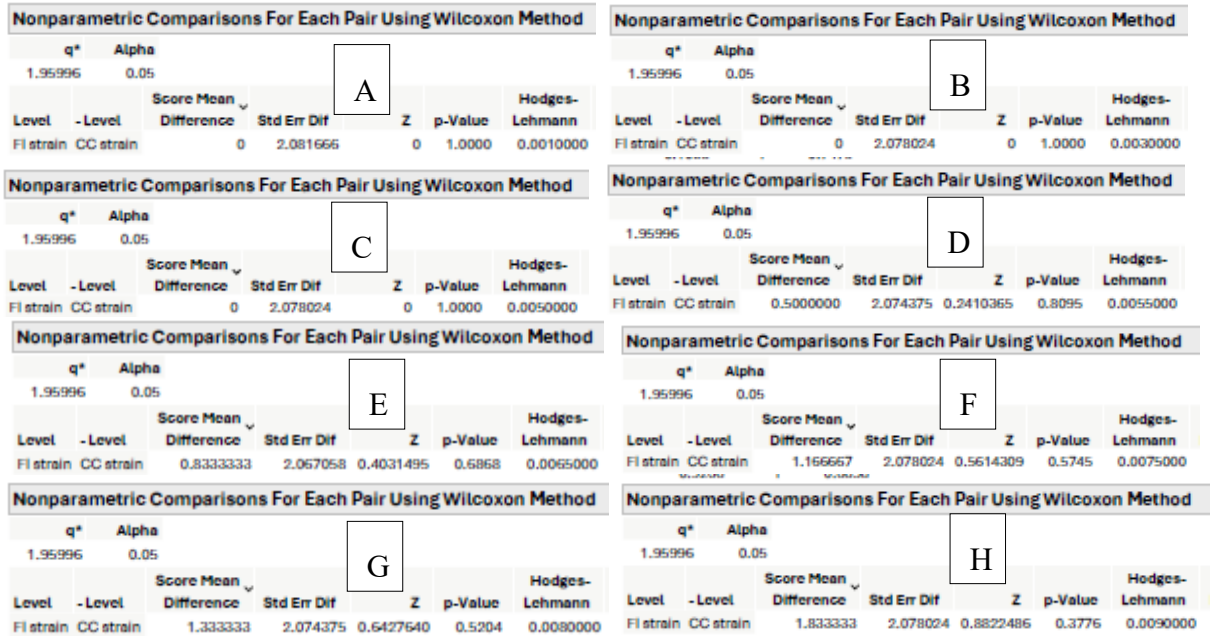
Supplementary Figure 9 Connecting letters report on the differences among the two strains (CC, FI) of *E. coli* in prune-mimicking medium, (A) at 5 hours, (B) at 7.5 hours, (C) at 10 hours, (D) at 12.5 hours, (E) at 15 hours, (F) at 17.5 hours, (G) at 20 hours, and (H) at 22.5 hours. Levels not connected by the same letter are significantly different.



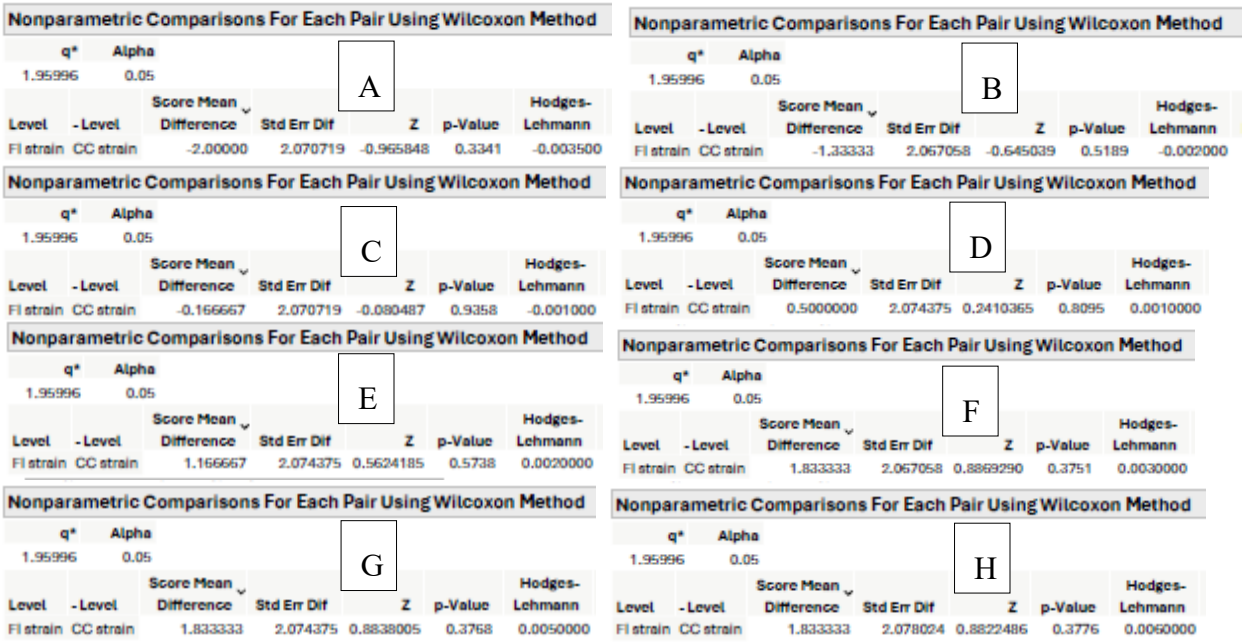
Supplementary Figure 10 Connecting letters report on the differences among the two strains (CC, FI) of *E. coli* in low-sugar, dried apricot and raisin media, (A) at 5 hours, (B) at 7.5 hours, (C) at 10 hours, (D) at 12.5 hours, (E) at 15 hours, (F) at 17.5 hours, (G) at 20 hours, and (H) at 22.5 hours. Levels not connected by the same letter are significantly different.



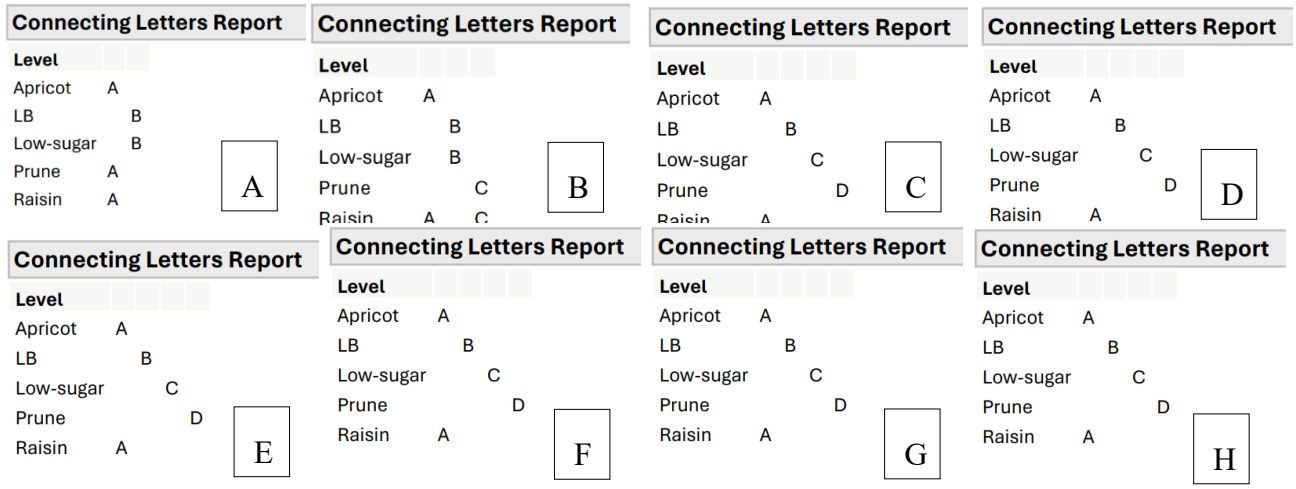
Supplementary Figure 11 Values of the differences observed in low-sugar medium at (A) at 5 hours, (B) at 7.5 hours, (C) at 10 hours, (D) at 12.5 hours, (E) at 15 hours, (F) at 17.5 hours, (G) at 20 hours, and (H) at 22.5 hours.



Supplementary Figure 12 Values of the differences observed in apricot-mimicking medium at (A) at 5 hours, (B) at 7.5 hours, (C) at 10 hours, (D) at 12.5 hours, (E) at 15 hours, (F) at 17.5 hours, (G) at 20 hours, and (H) at 22.5 hours.



Supplementary Figure 13 Values of the differences observed in raisin-mimicking medium at (A) at 5 hours, (B) at 7.5 hours, (C) at 10 hours, (D) at 12.5 hours, (E) at 15 hours, (F) at 17.5 hours, (G) at 20 hours, and (H) at 22.5 hours.



Supplementary Figure 14 Connecting letters report on the significant differences among the media, (A) at 5 hours, (B) at 7.5 hours, (C) at 10 hours, (D) at 12.5 hours, (E) at 15 hours, (F) at 17.5 hours, (G) at 20 hours, and (H) at 22.5 hours. Levels not connected by the same letter are significantly different.

A

Nonparametric Comparisons For Each Pair Using Wilcoxon Method

q*	Alpha
1.95996	0.05

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann
LB	Apricot	11.9167	2.886124	4.12895	<.0001*	0.509000
Low-sugar	Apricot	11.9167	2.886751	4.12805	<.0001*	0.659333
Prune	Apricot	5.1667	2.884868	1.79095	0.0733	0.007000
Low-sugar	LB	3.7500	2.886124	1.29932	0.1938	0.092000
Raisin	Apricot	1.2500	2.884868	0.43330	0.6648	0.001667
Raisin	Prune	-3.5833	2.883612	-1.24265	0.2140	-0.003667
Prune	LB	-11.9167	2.884868	-4.13075	<.0001*	-0.506000
Prune	Low-sugar	-11.9167	2.885496	-4.12985	<.0001*	-0.651333
Raisin	LB	-11.9167	2.884240	-4.13165	<.0001*	-0.507333
Raisin	Low-sugar	-11.9167	2.884868	-4.13075	<.0001*	-0.656333

B

Nonparametric Comparisons For Each Pair Using Wilcoxon Method

q*	Alpha
1.95996	0.05

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann
LB	Apricot	11.9167	2.886124	4.12895	<.0001*	0.754667
Low-sugar	Apricot	11.9167	2.885496	4.12985	<.0001*	0.672333
Prune	Apricot	6.9167	2.879840	2.40175	0.0163*	0.009667
Raisin	Apricot	1.2500	2.879840	0.43405	0.6643	0.002000
Low-sugar	LB	0.0000	2.886124	0.00000	1.0000	-0.110333
Raisin	Prune	-5.4167	2.877952	-1.88213	0.0598	-0.008833
Prune	LB	-11.9167	2.880469	-4.13706	<.0001*	-0.745000
Prune	Low-sugar	-11.9167	2.879840	-4.13796	<.0001*	-0.649167
Raisin	LB	-11.9167	2.884240	-4.13165	<.0001*	-0.753833
Raisin	Low-sugar	-11.9167	2.883612	-4.13255	<.0001*	-0.670500

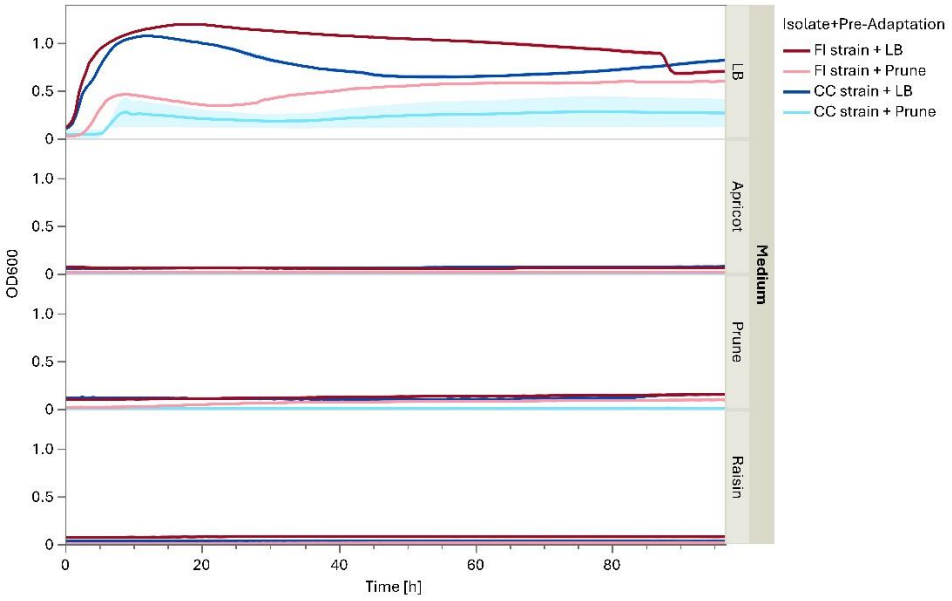
C

Nonparametric Comparisons For Each Pair Using Wilcoxon Method

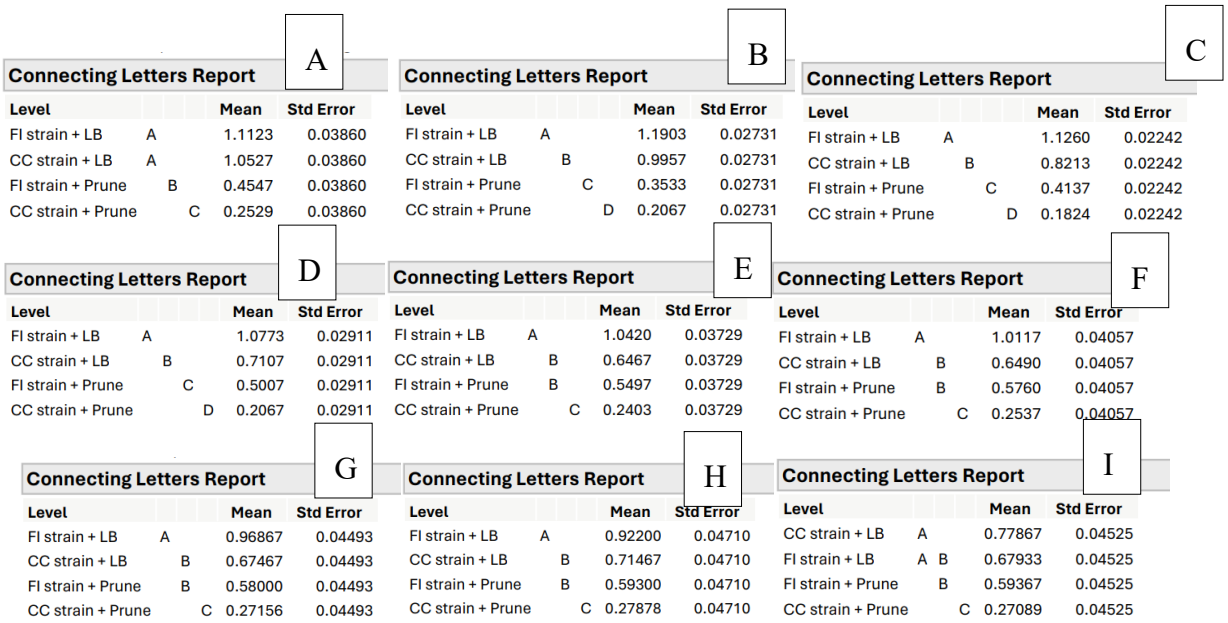
q*	Alpha
1.95996	0.05

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann
LB	Apricot	11.9167	2.882984	4.13345	<.0001*	0.933167
Low-sugar	Apricot	11.9167	2.885496	4.12985	<.0001*	0.687667
Prune	Apricot	8.2500	2.881098	2.86349	0.0042*	0.014167
Raisin	Apricot	1.8333	2.881727	0.63619	0.5247	0.002000
Raisin	Prune	-5.9167	2.879840	-2.05451	0.0399*	-0.013333
Low-sugar	LB	-11.9167	2.882984	-4.13345	<.0001*	-0.260333
Prune	LB	-11.9167	2.878582	-4.13977	<.0001*	-0.916500
Prune	Low-sugar	-11.9167	2.881098	-4.13615	<.0001*	-0.659000
Raisin	LB	-11.9167	2.881727	-4.13525	<.0001*	-0.932833
Raisin	Low-sugar	-11.9167	2.884240	-4.13165	<.0001*	-0.685833

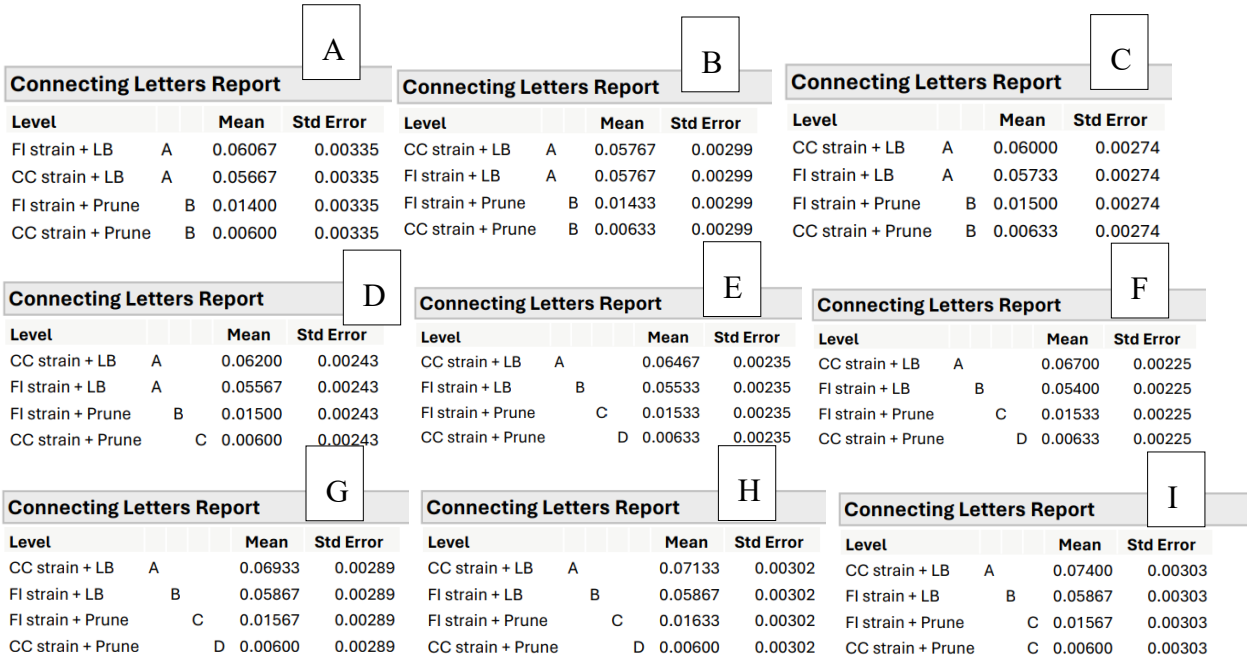
Supplementary Figure 15 Values representing the differences among the media at (A) at 5 hours, (B) at 7.5 hours, and (C) at 10 hours.



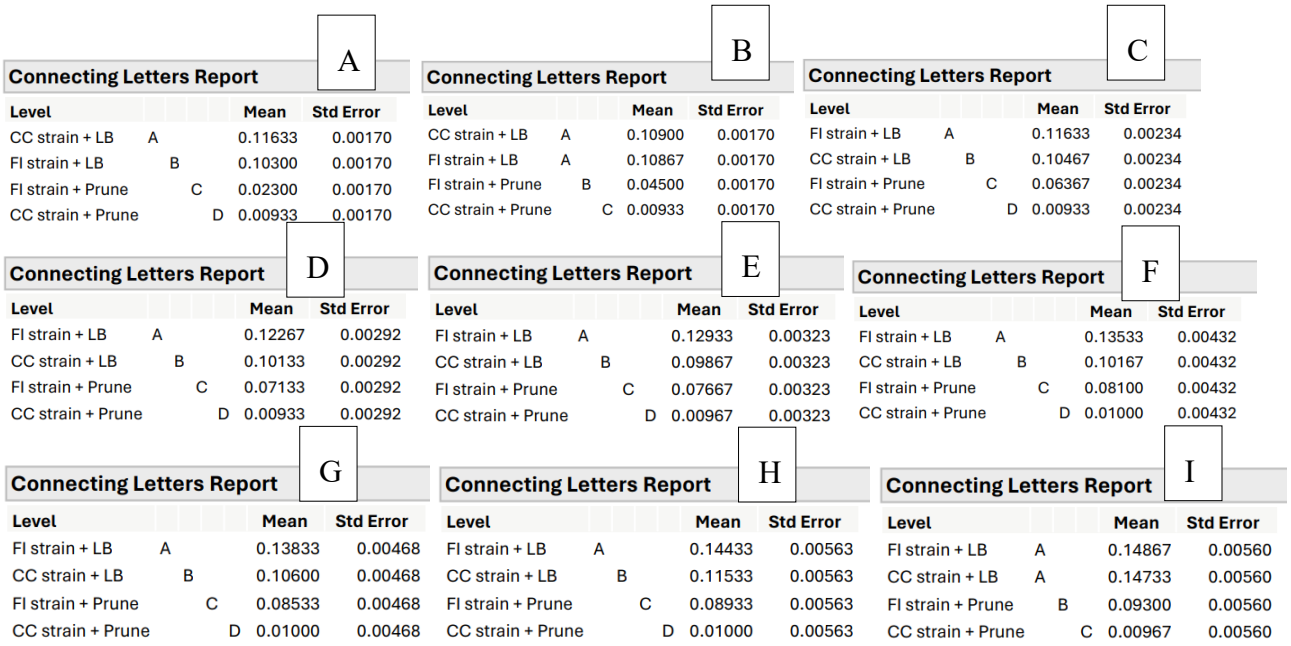
Supplementary Figure 16 Combined OD_{600} values of culture collection (CC) and food isolate (FI) strains of *E. coli* pre-adapted in either LB or prune-mimicking medium, followed by a transfer to LB medium and dried fruit-mimicking media. The figure shows the mean OD_{600} values of the measurements (3-3 technical replicates), with the standard deviation represented by the shaded regions.



Supplementary Figure 17 Connecting letter report on the significant differences observed at (A) 10 hours, (B) 20 hours, (C) 30 hours, (D) 40 hours, (E) 50 hours, (F) 60 hours, (G) 70 hours, (H) 80 hours, and (I) 90 hours in LB medium, between the strains pre-adapted in either LB or prune-mimicking medium. Levels not connected by the same letter are significantly different.



Supplementary Figure 18 Connecting letter report on the significant differences observed at (A) 10 hours, (B) 20 hours, (C) 30 hours, (D) 40 hours, (E) 50 hours, (F) 60 hours, (G) 70 hours, (H) 80 hours, and (I) 90 hours in dried apricot-mimicking medium, between the strains pre-adapted in either LB or prune-mimicking medium. Levels not connected by the same letter are significantly different.



A

Connecting Letters Report

Level		Mean	Std Error
CC strain + LB	A	0.11633	0.00170
FI strain + LB	B	0.10300	0.00170
FI strain + Prune	C	0.02300	0.00170
CC strain + Prune	D	0.00933	0.00170

B

Connecting Letters Report

Level		Mean	Std Error
CC strain + LB	A	0.10900	0.00170
FI strain + LB	A	0.10867	0.00170
FI strain + Prune	B	0.04500	0.00170
CC strain + Prune	C	0.00933	0.00170

C

Connecting Letters Report

Level		Mean	Std Error
FI strain + LB	A	0.11633	0.00234
CC strain + LB	B	0.10467	0.00234
FI strain + Prune	C	0.06367	0.00234
CC strain + Prune	D	0.00933	0.00234

D

Connecting Letters Report

Level		Mean	Std Error
FI strain + LB	A	0.12267	0.00292
CC strain + LB	B	0.10133	0.00292
FI strain + Prune	C	0.07133	0.00292
CC strain + Prune	D	0.00933	0.00292

E

Connecting Letters Report

Level		Mean	Std Error
FI strain + LB	A	0.12933	0.00323
CC strain + LB	B	0.09867	0.00323
FI strain + Prune	C	0.07667	0.00323
CC strain + Prune	D	0.00967	0.00323

F

Connecting Letters Report

Level		Mean	Std Error
FI strain + LB	A	0.13533	0.00432
CC strain + LB	B	0.10167	0.00432
FI strain + Prune	C	0.08100	0.00432
CC strain + Prune	D	0.01000	0.00432

G

Connecting Letters Report

Level		Mean	Std Error
FI strain + LB	A	0.13833	0.00468
CC strain + LB	B	0.10600	0.00468
FI strain + Prune	C	0.08533	0.00468
CC strain + Prune	D	0.01000	0.00468

H

Connecting Letters Report

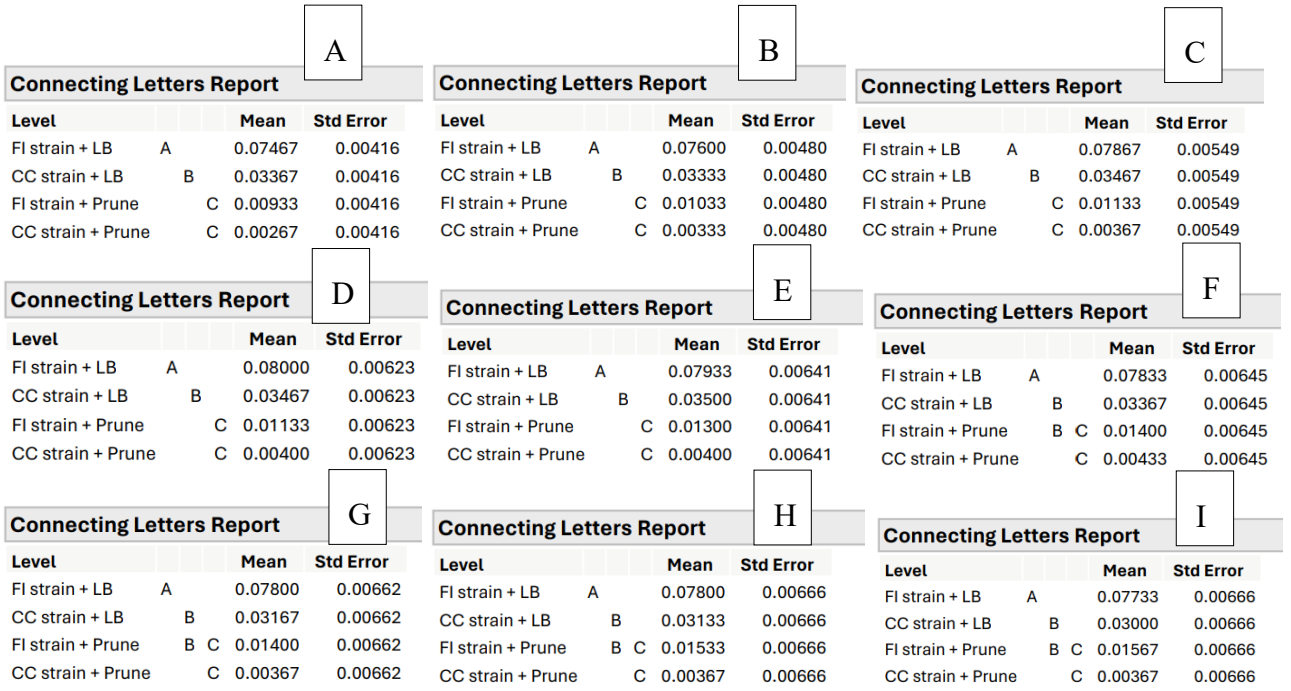
Level		Mean	Std Error
FI strain + LB	A	0.14433	0.00563
CC strain + LB	B	0.11533	0.00563
FI strain + Prune	C	0.08933	0.00563
CC strain + Prune	D	0.01000	0.00563

I

Connecting Letters Report

Level		Mean	Std Error
FI strain + LB	A	0.14867	0.00560
CC strain + LB	A	0.14733	0.00560
FI strain + Prune	B	0.09300	0.00560
CC strain + Prune	C	0.00967	0.00560

Supplementary Figure 19 Connecting the letter report on the significant differences observed at (A) 10 hours, (B) 20 hours, (C) 30 hours, (D) 40 hours, (E) 50 hours, (F) 60 hours**,** (G) 70 hours, (H) 80 hours, and (I) 90 hours in prune-mimicking medium, between the strains pre-adapted in either LB or prune-mimicking medium. Levels not connected by the same letter are significantly different.



A

Connecting Letters Report

Level		Mean	Std Error
FI strain + LB	A	0.07467	0.00416
CC strain + LB	B	0.03367	0.00416
FI strain + Prune	C	0.00933	0.00416
CC strain + Prune	C	0.00267	0.00416

B

Connecting Letters Report

Level		Mean	Std Error
FI strain + LB	A	0.07600	0.00480
CC strain + LB	B	0.03333	0.00480
FI strain + Prune	C	0.01033	0.00480
CC strain + Prune	C	0.00333	0.00480

C

Connecting Letters Report

Level		Mean	Std Error
FI strain + LB	A	0.07867	0.00549
CC strain + LB	B	0.03467	0.00549
FI strain + Prune	C	0.01133	0.00549
CC strain + Prune	C	0.00367	0.00549

D

Connecting Letters Report

Level		Mean	Std Error
FI strain + LB	A	0.08000	0.00623
CC strain + LB	B	0.03467	0.00623
FI strain + Prune	C	0.01133	0.00623
CC strain + Prune	C	0.00400	0.00623

E

Connecting Letters Report

Level		Mean	Std Error
FI strain + LB	A	0.07933	0.00641
CC strain + LB	B	0.03500	0.00641
FI strain + Prune	C	0.01300	0.00641
CC strain + Prune	C	0.00400	0.00641

F

Connecting Letters Report

Level		Mean	Std Error
FI strain + LB	A	0.07833	0.00645
CC strain + LB	B	0.03367	0.00645
FI strain + Prune	B C	0.01400	0.00645
CC strain + Prune	C	0.00433	0.00645

G

Connecting Letters Report

Level		Mean	Std Error
FI strain + LB	A	0.07800	0.00662
CC strain + LB	B	0.03167	0.00662
FI strain + Prune	B C	0.01400	0.00662
CC strain + Prune	C	0.00367	0.00662

H

Connecting Letters Report

Level		Mean	Std Error
FI strain + LB	A	0.07800	0.00666
CC strain + LB	B	0.03133	0.00666
FI strain + Prune	B C	0.01533	0.00666
CC strain + Prune	C	0.00367	0.00666

I

Connecting Letters Report

Level		Mean	Std Error
FI strain + LB	A	0.07733	0.00666
CC strain + LB	B	0.03000	0.00666
FI strain + Prune	B C	0.01567	0.00666
CC strain + Prune	C	0.00367	0.00666

Supplementary Figure 20 Connecting letter report on the significant differences observed at (A) 10 hours, (B) 20 hours, (C) 30 hours, (D) 40 hours, (E) 50 hours, (F) 60 hours, (G) 70 hours, (H) 80 hours, and (I) 90 hours in raisin-mimicking medium, between the strains pre-adapted in either LB or prune-mimicking medium. Levels not connected by the same letter are significantly different.

A

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
FI strain + LB	CC strain + Prune	0.8594444	0.0545906	0.733558	0.9853306	<.0001
CC strain + LB	CC strain + Prune	0.7997778	0.0545906	0.673892	0.9256640	<.0001
FI strain + LB	FI strain + Prune	0.6576667	0.0545906	0.531780	0.7835529	<.0001
CC strain + LB	FI strain + Prune	0.5980000	0.0545906	0.472114	0.7238862	<.0001
FI strain + Prune	CC strain + Prune	0.2017778	0.0545906	0.075892	0.3276640	0.0061
FI strain + LB	CC strain + LB	0.0596667	0.0545906	-0.066220	0.1855529	0.3062

B

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
FI strain + LB	CC strain + Prune	0.8594444	0.0545906	0.733558	0.9853306	<.0001
CC strain + LB	CC strain + Prune	0.7997778	0.0545906	0.673892	0.9256640	<.0001
FI strain + LB	FI strain + Prune	0.6576667	0.0545906	0.531780	0.7835529	<.0001
CC strain + LB	FI strain + Prune	0.5980000	0.0545906	0.472114	0.7238862	<.0001
FI strain + Prune	CC strain + Prune	0.2017778	0.0545906	0.075892	0.3276640	0.0061
FI strain + LB	CC strain + LB	0.0596667	0.0545906	-0.066220	0.1855529	0.3062

C

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
FI strain + LB	CC strain + Prune	0.9836667	0.0386250	0.8945973	1.072736	<.0001
FI strain + LB	FI strain + Prune	0.8370000	0.0386250	0.7479306	0.926069	<.0001
CC strain + LB	CC strain + Prune	0.7890000	0.0386250	0.6999306	0.878069	<.0001
CC strain + LB	FI strain + Prune	0.6423333	0.0386250	0.5532640	0.731403	<.0001
FI strain + LB	CC strain + LB	0.1946667	0.0386250	0.1055973	0.283736	0.0010
FI strain + Prune	CC strain + Prune	0.1466667	0.0386250	0.0575973	0.235736	0.0053

D

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
FI strain + LB	CC strain + Prune	0.9435556	0.0317097	0.8704328	1.016678	<.0001
FI strain + LB	FI strain + Prune	0.7123333	0.0317097	0.6392106	0.785456	<.0001
CC strain + LB	CC strain + Prune	0.6388889	0.0317097	0.5657661	0.712012	<.0001
CC strain + LB	FI strain + Prune	0.4076667	0.0317097	0.3345439	0.480789	<.0001
FI strain + LB	CC strain + LB	0.3046667	0.0317097	0.2315439	0.377789	<.0001
FI strain + Prune	CC strain + Prune	0.2312222	0.0317097	0.1580995	0.304345	<.0001

E

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
FI strain + LB	CC strain + Prune	0.8706667	0.0411724	0.7757229	0.9656104	<.0001
FI strain + LB	FI strain + Prune	0.5766667	0.0411724	0.4817229	0.6716104	<.0001
CC strain + LB	CC strain + Prune	0.5040000	0.0411724	0.4090563	0.5989437	<.0001
FI strain + LB	CC strain + LB	0.3666667	0.0411724	0.2717229	0.4616104	<.0001
FI strain + Prune	CC strain + Prune	0.2940000	0.0411724	0.1990563	0.3889437	<.0001
CC strain + LB	FI strain + Prune	0.2100000	0.0411724	0.1150563	0.3049437	0.0009

F

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
FI strain + LB	CC strain + Prune	0.7580000	0.0573701	0.625704	0.8902958	<.0001
FI strain + LB	FI strain + Prune	0.4356667	0.0573701	0.303371	0.5679625	<.0001
CC strain + LB	CC strain + Prune	0.3953333	0.0573701	0.263038	0.5276291	0.0001
FI strain + LB	CC strain + LB	0.3626667	0.0573701	0.230371	0.4949625	0.0002
FI strain + Prune	CC strain + Prune	0.3223333	0.0573701	0.190038	0.4546291	0.0005
CC strain + LB	FI strain + Prune	0.0730000	0.0573701	-0.059296	0.2052958	0.2390

G

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
FI strain + LB	CC strain + Prune	0.6971111	0.0635396	0.550588	0.8436337	<.0001
CC strain + LB	CC strain + Prune	0.4031111	0.0635396	0.256588	0.5496337	0.0002
FI strain + LB	FI strain + Prune	0.3886667	0.0635396	0.242144	0.5351893	0.0003
FI strain + Prune	CC strain + Prune	0.3084444	0.0635396	0.161922	0.4549671	0.0013
FI strain + LB	CC strain + LB	0.2940000	0.0635396	0.147477	0.4405226	0.0017
CC strain + LB	FI strain + Prune	0.0946667	0.0635396	-0.051856	0.2411893	0.1746

H

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
FI strain + LB	CC strain + Prune	0.6432222	0.0666099	0.489620	0.7968249	<.0001
CC strain + LB	CC strain + Prune	0.4358889	0.0666099	0.282286	0.5894916	0.0002
FI strain + LB	FI strain + Prune	0.3290000	0.0666099	0.175397	0.4826027	0.0011
FI strain + Prune	CC strain + Prune	0.3142222	0.0666099	0.160620	0.4678249	0.0015
FI strain + LB	CC strain + LB	0.2073333	0.0666099	0.053731	0.3609360	0.0144
CC strain + LB	FI strain + Prune	0.1216667	0.0666099	-0.031936	0.2752693	0.1052

I

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
CC strain + LB	CC strain + Prune	0.5077778	0.0639893	0.360218	0.6553375	<.0001
FI strain + LB	CC strain + Prune	0.4084444	0.0639893	0.260885	0.5560041	0.0002
FI strain + Prune	CC strain + Prune	0.3227778	0.0639893	0.175218	0.4703375	0.0010
CC strain + LB	FI strain + Prune	0.1850000	0.0639893	0.037440	0.3325597	0.0202
CC strain + LB	FI strain + LB	0.0993333	0.0639893	-0.048226	0.2468930	0.1592
FI strain + LB	FI strain + Prune	0.0856667	0.0639893	-0.061893	0.2332264	0.2174

Supplementary Figure 21 The values of differences observed at (A) 10 hours, (B) 20 hours, (C) 30 hours, (D) 40 hours, (E) 50 hours, (F) 60 hours, (G) 70 hours, (H) 80 hours, and (I) 90 hours in LB medium, between the *E. coli* strains pre-adapted in either LB or prune-mimicking medium.

A

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
FI strain + LB	CC strain + Prune	0.0546667	0.0047376	0.043742	0.0655915	<.0001
CC strain + LB	CC strain + Prune	0.0506667	0.0047376	0.039742	0.0615915	<.0001
FI strain + LB	FI strain + Prune	0.0466667	0.0047376	0.035742	0.0575915	<.0001
CC strain + LB	FI strain + Prune	0.0426667	0.0047376	0.031742	0.0535915	<.0001
FI strain + Prune	CC strain + Prune	0.0080000	0.0047376	-0.002925	0.0189248	0.1298
FI strain + LB	CC strain + LB	0.0040000	0.0047376	-0.006925	0.0149248	0.4230

B

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
CC strain + LB	CC strain + Prune	0.0513333	0.0042230	0.041595	0.0610715	<.0001
FI strain + LB	CC strain + Prune	0.0513333	0.0042230	0.041595	0.0610715	<.0001
CC strain + LB	FI strain + Prune	0.0433333	0.0042230	0.033595	0.0530715	<.0001
FI strain + LB	FI strain + Prune	0.0433333	0.0042230	0.033595	0.0530715	<.0001
FI strain + Prune	CC strain + Prune	0.0080000	0.0042230	-0.001738	0.0177381	0.0948
FI strain + LB	CC strain + LB	0.0000000	0.0042230	-0.009738	0.0097381	1.0000

C

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
CC strain + LB	CC strain + Prune	0.0536667	0.0038730	0.044736	0.0625978	<.0001
FI strain + LB	CC strain + Prune	0.0510000	0.0038730	0.042069	0.0599311	<.0001
CC strain + LB	FI strain + Prune	0.0450000	0.0038730	0.036069	0.0539311	<.0001
FI strain + LB	FI strain + Prune	0.0423333	0.0038730	0.033402	0.0512644	<.0001
FI strain + Prune	CC strain + Prune	0.0086667	0.0038730	-0.000264	0.0175978	0.0556
CC strain + LB	FI strain + LB	0.0026667	0.0038730	-0.006264	0.0115978	0.5106

D

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
CC strain + LB	CC strain + Prune	0.0560000	0.0034400	0.048067	0.0639326	<.0001
FI strain + LB	CC strain + Prune	0.0496667	0.0034400	0.041734	0.0575992	<.0001
CC strain + LB	FI strain + Prune	0.0470000	0.0034400	0.039067	0.0549326	<.0001
FI strain + LB	FI strain + Prune	0.0406667	0.0034400	0.032734	0.0485992	<.0001
FI strain + Prune	CC strain + Prune	0.0090000	0.0034400	0.001067	0.0169326	0.0308
CC strain + LB	FI strain + LB	0.0063333	0.0034400	-0.001599	0.0142659	0.1029

E

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
CC strain + LB	CC strain + Prune	0.0583333	0.0033166	0.0506852	0.0659815	<.0001
CC strain + LB	FI strain + Prune	0.0493333	0.0033166	0.0416852	0.0569815	<.0001
FI strain + LB	CC strain + Prune	0.0490000	0.0033166	0.0413518	0.0566482	<.0001
FI strain + LB	FI strain + Prune	0.0400000	0.0033166	0.0323518	0.0476482	<.0001
CC strain + LB	FI strain + LB	0.0093333	0.0033166	0.0016852	0.0169815	0.0227
FI strain + Prune	CC strain + Prune	0.0090000	0.0033166	0.0013518	0.0166482	0.0265

F

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
CC strain + LB	CC strain + Prune	0.0606667	0.0031798	0.0533340	0.0679993	<.0001
CC strain + LB	FI strain + Prune	0.0516667	0.0031798	0.0443340	0.0589993	<.0001
FI strain + LB	CC strain + Prune	0.0476667	0.0031798	0.0403340	0.0549993	<.0001
FI strain + LB	FI strain + Prune	0.0386667	0.0031798	0.0313340	0.0459993	<.0001
CC strain + LB	FI strain + LB	0.0130000	0.0031798	0.0056674	0.0203326	0.0035
FI strain + Prune	CC strain + Prune	0.0090000	0.0031798	0.0016674	0.0163326	0.0221

G

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
CC strain + LB	CC strain + Prune	0.0633333	0.0040825	0.0539191	0.0727476	<.0001
CC strain + LB	FI strain + Prune	0.0536667	0.0040825	0.0442524	0.0630809	<.0001
FI strain + LB	CC strain + Prune	0.0526667	0.0040825	0.0432524	0.0620809	<.0001
FI strain + LB	FI strain + Prune	0.0430000	0.0040825	0.0335858	0.0524142	<.0001
CC strain + LB	FI strain + LB	0.0106667	0.0040825	0.0012524	0.0200809	0.0310
FI strain + Prune	CC strain + Prune	0.0096667	0.0040825	0.0002524	0.0190809	0.0454

H

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
CC strain + LB	CC strain + Prune	0.0653333	0.0042753	0.0554746	0.0751921	<.0001
CC strain + LB	FI strain + Prune	0.0550000	0.0042753	0.0451413	0.0648587	<.0001
FI strain + LB	CC strain + Prune	0.0526667	0.0042753	0.0428079	0.0625254	<.0001
FI strain + LB	FI strain + Prune	0.0423333	0.0042753	0.0324746	0.0521921	<.0001
CC strain + LB	FI strain + LB	0.0126667	0.0042753	0.0028079	0.0225254	0.0181
FI strain + Prune	CC strain + Prune	0.0103333	0.0042753	0.0004746	0.0201921	0.0420

I

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
CC strain + LB	CC strain + Prune	0.0680000	0.0042817	0.058126	0.0778737	<.0001
CC strain + LB	FI strain + Prune	0.0583333	0.0042817	0.048460	0.0682071	<.0001
FI strain + LB	CC strain + Prune	0.0526667	0.0042817	0.042793	0.0625404	<.0001
FI strain + LB	FI strain + Prune	0.0430000	0.0042817	0.033126	0.0528737	<.0001
CC strain + LB	FI strain + LB	0.0153333	0.0042817	0.005460	0.0252071	0.0072
FI strain + Prune	CC strain + Prune	0.0096667	0.0042817	-0.000207	0.0195404	0.0539

Supplementary Figure 22 The values of differences observed at (A) 10 hours, (B) 20 hours, (C) 30 hours, (D) 40 hours, (E) 50 hours, (F) 60 hours, (G) 70 hours, (H) 80 hours, and (I) 90 hours in dried apricot-mimicking medium, between the *E. coli* strains pre-adapted in either LB or prune-mimicking medium.

A

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
CC strain + LB	CC strain + Prune	0.1070000	0.0024037	0.1014571	0.1125429	<.0001
FI strain + LB	CC strain + Prune	0.0936667	0.0024037	0.0881237	0.0992096	<.0001
CC strain + LB	FI strain + Prune	0.0933333	0.0024037	0.0877904	0.0988763	<.0001
FI strain + LB	FI strain + Prune	0.0800000	0.0024037	0.0744571	0.0855429	<.0001
FI strain + Prune	CC strain + Prune	0.0136667	0.0024037	0.0081237	0.0192096	0.0005
CC strain + LB	FI strain + LB	0.0133333	0.0024037	0.0077904	0.0188763	0.0005

B

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
CC strain + LB	CC strain + Prune	0.0996667	0.0024037	0.094124	0.1052096	<.0001
FI strain + LB	CC strain + Prune	0.0993333	0.0024037	0.093790	0.1048763	<.0001
CC strain + LB	FI strain + Prune	0.0640000	0.0024037	0.058457	0.0695429	<.0001
FI strain + LB	FI strain + Prune	0.0636667	0.0024037	0.058124	0.0692096	<.0001
FI strain + Prune	CC strain + Prune	0.0356667	0.0024037	0.030124	0.0412096	<.0001
CC strain + LB	FI strain + LB	0.0003333	0.0024037	-0.005210	0.0058763	0.8931

C

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
FI strain + LB	CC strain + Prune	0.1070000	0.0033082	0.0993712	0.1146288	<.0001
CC strain + LB	CC strain + Prune	0.0953333	0.0033082	0.0877045	0.1029621	<.0001
FI strain + Prune	CC strain + Prune	0.0543333	0.0033082	0.0467045	0.0619621	<.0001
FI strain + LB	FI strain + Prune	0.0526667	0.0033082	0.0450379	0.0602955	<.0001
CC strain + LB	FI strain + Prune	0.0410000	0.0033082	0.0333712	0.0486288	<.0001
FI strain + LB	CC strain + LB	0.0116667	0.0033082	0.0040379	0.0192955	0.0078

D

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
FI strain + LB	CC strain + Prune	0.1133333	0.0041298	0.1038099	0.1228568	<.0001
CC strain + LB	CC strain + Prune	0.0920000	0.0041298	0.0824766	0.1015234	<.0001
FI strain + Prune	CC strain + Prune	0.0620000	0.0041298	0.0524766	0.0715234	<.0001
FI strain + LB	FI strain + Prune	0.0513333	0.0041298	0.0418099	0.0608568	<.0001
CC strain + LB	FI strain + Prune	0.0300000	0.0041298	0.0204766	0.0395234	<.0001
FI strain + LB	CC strain + LB	0.0213333	0.0041298	0.0118099	0.0308568	0.0009

E

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
FI strain + LB	CC strain + Prune	0.1196667	0.0045704	0.1091272	0.1302061	<.0001
CC strain + LB	CC strain + Prune	0.0890000	0.0045704	0.0784606	0.0995394	<.0001
FI strain + Prune	CC strain + Prune	0.0670000	0.0045704	0.0564606	0.0775394	<.0001
FI strain + LB	FI strain + Prune	0.0526667	0.0045704	0.0421272	0.0632061	<.0001
FI strain + LB	CC strain + LB	0.0306667	0.0045704	0.0201272	0.0412061	0.0002
CC strain + LB	FI strain + Prune	0.0220000	0.0045704	0.0114606	0.0325394	0.0013

F

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
FI strain + LB	CC strain + Prune	0.1253333	0.0061146	0.1112329	0.1394337	<.0001
CC strain + LB	CC strain + Prune	0.0916667	0.0061146	0.0775663	0.1057671	<.0001
FI strain + Prune	CC strain + Prune	0.0710000	0.0061146	0.0568996	0.0851004	<.0001
FI strain + LB	FI strain + Prune	0.0543333	0.0061146	0.0402329	0.0684337	<.0001
FI strain + LB	CC strain + LB	0.0336667	0.0061146	0.0195663	0.0477671	0.0006
CC strain + LB	FI strain + Prune	0.0206667	0.0061146	0.0065663	0.0347671	0.0096

G

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
FI strain + LB	CC strain + Prune	0.1283333	0.0066207	0.1130660	0.1436006	<.0001
CC strain + LB	CC strain + Prune	0.0960000	0.0066207	0.0807327	0.1112673	<.0001
FI strain + Prune	CC strain + Prune	0.0753333	0.0066207	0.0600660	0.0906006	<.0001
FI strain + LB	FI strain + Prune	0.0530000	0.0066207	0.0377327	0.0682673	<.0001
FI strain + LB	CC strain + LB	0.0323333	0.0066207	0.0170660	0.0476006	0.0012
CC strain + LB	FI strain + Prune	0.0206667	0.0066207	0.0053994	0.0359340	0.0142

H

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
FI strain + LB	CC strain + Prune	0.1343333	0.0079687	0.1159575	0.1527092	<.0001
CC strain + LB	CC strain + Prune	0.1053333	0.0079687	0.0869575	0.1237092	<.0001
FI strain + Prune	CC strain + Prune	0.0793333	0.0079687	0.0609575	0.0977092	<.0001
FI strain + LB	FI strain + Prune	0.0550000	0.0079687	0.0366242	0.0733758	0.0001
FI strain + LB	CC strain + LB	0.0290000	0.0079687	0.0106242	0.0473758	0.0066
CC strain + LB	FI strain + Prune	0.0260000	0.0079687	0.0076242	0.0443758	0.0115

I

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
FI strain + LB	CC strain + Prune	0.1390000	0.0079197	0.120737	0.1572629	<.0001
CC strain + LB	CC strain + Prune	0.1376667	0.0079197	0.119404	0.1559296	<.0001
FI strain + Prune	CC strain + Prune	0.0833333	0.0079197	0.065070	0.1015963	<.0001
FI strain + LB	FI strain + Prune	0.0556667	0.0079197	0.037404	0.0739296	0.0001
CC strain + LB	FI strain + Prune	0.0543333	0.0079197	0.036070	0.0725963	0.0001
FI strain + LB	CC strain + LB	0.0013333	0.0079197	-0.016930	0.0195963	0.8705

Supplementary Figure 23 The values of differences observed at (A) 10 hours, (B) 20 hours, (C) 30 hours, (D) 40 hours, (E) 50 hours, (F) 60 hours, (G) 70 hours, (H) 80 hours, and (I) 90 hours in prune-mimicking medium, between the *E. coli* strains pre-adapted in either LB or prune-mimicking medium.

A

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
FI strain + LB	CC strain + Prune	0.0720000	0.0058878	0.058423	0.0855774	<.0001*
FI strain + LB	FI strain + Prune	0.0653333	0.0058878	0.051756	0.0789107	<.0001*
FI strain + LB	CC strain + LB	0.0410000	0.0058878	0.027423	0.0545774	0.0001*
CC strain + LB	CC strain + Prune	0.0310000	0.0058878	0.017423	0.0445774	0.0008*
CC strain + LB	FI strain + Prune	0.0243333	0.0058878	0.010756	0.0379107	0.0033*
FI strain + Prune	CC strain + Prune	0.0066667	0.0058878	-0.006911	0.0202441	0.2903

B

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
FI strain + LB	CC strain + Prune	0.0726667	0.0067864	0.057017	0.0883162	<.0001*
FI strain + LB	FI strain + Prune	0.0656667	0.0067864	0.050017	0.0813162	<.0001*
FI strain + LB	CC strain + LB	0.0426667	0.0067864	0.027017	0.0583162	0.0002*
CC strain + LB	CC strain + Prune	0.0300000	0.0067864	0.014350	0.0456495	0.0022*
CC strain + LB	FI strain + Prune	0.0230000	0.0067864	0.007350	0.0386495	0.0095*
FI strain + Prune	CC strain + Prune	0.0070000	0.0067864	-0.008650	0.0226495	0.3325

C

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
FI strain + LB	CC strain + Prune	0.0750000	0.0077675	0.057088	0.0929118	<.0001*
FI strain + LB	FI strain + Prune	0.0673333	0.0077675	0.049422	0.0852451	<.0001*
FI strain + LB	CC strain + LB	0.0440000	0.0077675	0.026088	0.0619118	0.0005*
CC strain + LB	CC strain + Prune	0.0310000	0.0077675	0.013088	0.0489118	0.0040*
CC strain + LB	FI strain + Prune	0.0233333	0.0077675	0.005422	0.0412451	0.0170*
FI strain + Prune	CC strain + Prune	0.0076667	0.0077675	-0.010245	0.0255784	0.3525

D

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
FI strain + LB	CC strain + Prune	0.0760000	0.0088160	0.055670	0.0963298	<.0001*
FI strain + LB	FI strain + Prune	0.0686667	0.0088160	0.048337	0.0889964	<.0001*
FI strain + LB	CC strain + LB	0.0453333	0.0088160	0.025004	0.0656631	0.0009*
CC strain + LB	CC strain + Prune	0.0306667	0.0088160	0.010337	0.0509964	0.0083*
CC strain + LB	FI strain + Prune	0.0233333	0.0088160	0.003004	0.0436631	0.0294*
FI strain + Prune	CC strain + Prune	0.0073333	0.0088160	-0.012996	0.0276631	0.4296

E

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
FI strain + LB	CC strain + Prune	0.0753333	0.0090676	0.054423	0.0962434	<.0001*
FI strain + LB	FI strain + Prune	0.0663333	0.0090676	0.045423	0.0872434	<.0001*
FI strain + LB	CC strain + LB	0.0443333	0.0090676	0.023423	0.0652434	0.0012*
CC strain + LB	CC strain + Prune	0.0310000	0.0090676	0.010090	0.0519100	0.0091*
CC strain + LB	FI strain + Prune	0.0220000	0.0090676	0.001090	0.0429100	0.0414*
FI strain + Prune	CC strain + Prune	0.0090000	0.0090676	-0.011910	0.0299100	0.3500

F

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
FI strain + LB	CC strain + Prune	0.0740000	0.0091257	0.052956	0.0950438	<.0001*
FI strain + LB	FI strain + Prune	0.0643333	0.0091257	0.043290	0.0853772	0.0001*
FI strain + LB	CC strain + LB	0.0446667	0.0091257	0.023623	0.0657105	0.0012*
CC strain + LB	CC strain + Prune	0.0293333	0.0091257	0.008290	0.0503772	0.0123*
CC strain + LB	FI strain + Prune	0.0196667	0.0091257	-0.001377	0.0407105	0.0633
FI strain + Prune	CC strain + Prune	0.0096667	0.0091257	-0.011377	0.0307105	0.3204

G

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
FI strain + LB	CC strain + Prune	0.0743333	0.0093630	0.052742	0.0959246	<.0001*
FI strain + LB	FI strain + Prune	0.0640000	0.0093630	0.042409	0.0855912	0.0001*
FI strain + LB	CC strain + LB	0.0463333	0.0093630	0.024742	0.0679246	0.0011*
CC strain + LB	CC strain + Prune	0.0280000	0.0093630	0.006409	0.0495912	0.0173*
CC strain + LB	FI strain + Prune	0.0176667	0.0093630	-0.003925	0.0392579	0.0959
FI strain + Prune	CC strain + Prune	0.0103333	0.0093630	-0.011258	0.0319246	0.3018

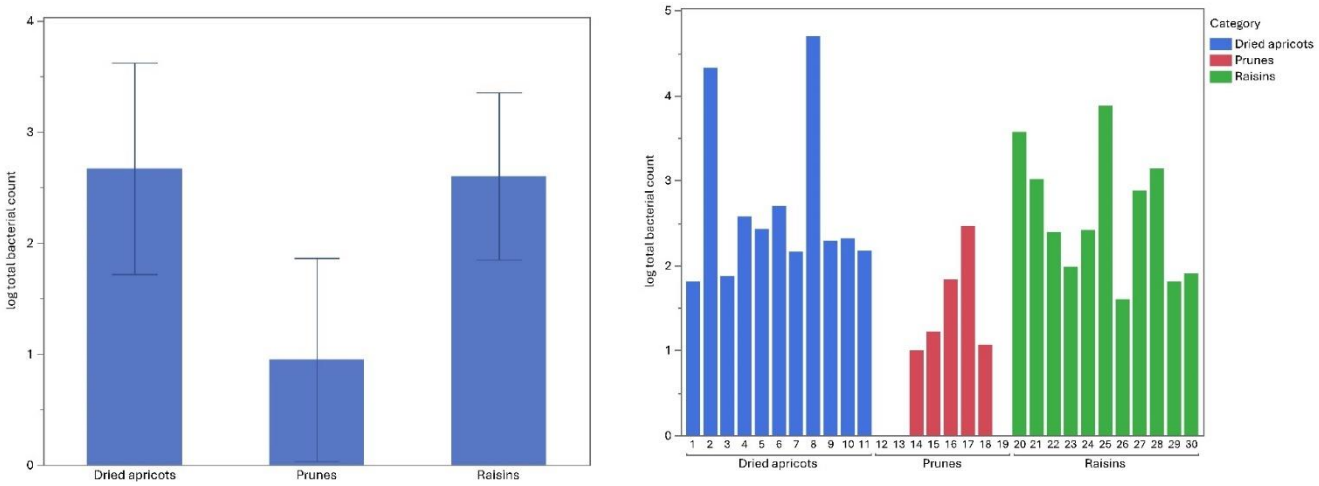
H

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
FI strain + LB	CC strain + Prune	0.0743333	0.0094251	0.052599	0.0960678	<.0001*
FI strain + LB	FI strain + Prune	0.0626667	0.0094251	0.040932	0.0844011	0.0002*
FI strain + LB	CC strain + LB	0.0466667	0.0094251	0.024932	0.0684011	0.0011*
CC strain + LB	CC strain + Prune	0.0276667	0.0094251	0.005932	0.0494011	0.0188*
CC strain + LB	FI strain + Prune	0.0160000	0.0094251	-0.005734	0.0377344	0.1280
FI strain + Prune	CC strain + Prune	0.0116667	0.0094251	-0.010068	0.0334011	0.2509

I

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
FI strain + LB	CC strain + Prune	0.0736667	0.0094163	0.051953	0.0953807	<.0001*
FI strain + LB	FI strain + Prune	0.0616667	0.0094163	0.039953	0.0833807	0.0002*
FI strain + LB	CC strain + LB	0.0473333	0.0094163	0.025619	0.0690474	0.0010*
CC strain + LB	CC strain + Prune	0.0263333	0.0094163	0.004619	0.0480474	0.0233*
CC strain + LB	FI strain + Prune	0.0143333	0.0094163	-0.007381	0.0360474	0.1665
FI strain + Prune	CC strain + Prune	0.0120000	0.0094163	-0.009714	0.0337140	0.2383

Supplementary Figure 24 The values of differences observed at (A) 10 hours, (B) 20 hours, (C) 30 hours, (D) 40 hours, (E) 50 hours, (F) 60 hours, (G) 70 hours, (H) 80 hours, and (I) 90 hours in raisin-mimicking medium, between the *E. coli* strains pre-adapted in either LB or prune-mimicking medium.



Supplementary Figure 25 Bacterial count of the dried apricot, prune, and raisin samples.

Nonparametric Comparisons For Each Pair Using Wilcoxon Method

q*	Alpha
1.95996	0.05

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann
Raisins	Prunes	7.66477	2.610198	2.93647	0.0033*	1.80455
Raisins	Dried apricots	0.09091	2.768093	0.03284	0.9738	0.02803
Prunes	Dried apricots	-7.66477	2.610198	-2.93647	0.0033*	-1.66409

Supplementary Figure 26 The values representing the differences in bacterial cell counts among the dried fruit categories.

Connecting Letters Report

Level		
Dried apricots	A	
Prunes		B
Raisins	A	

Supplementary Figure 27 Connecting letters report on the significant differences among the dried fruit sample categories. Levels not connected by the same letter are significantly different.

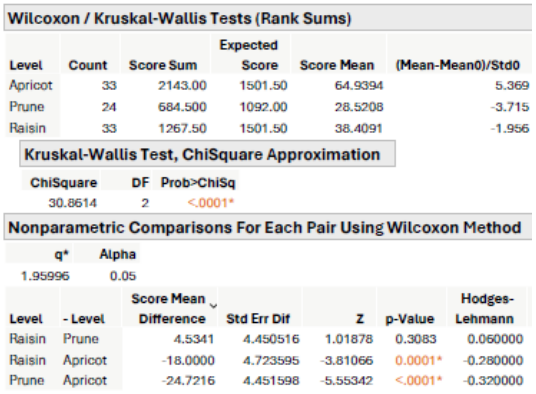
Supplementary Table 4 Total bacterial counts (mean ± SE) of dried fruit samples expressed in CFU/g. SE = standard error.

Sample code	Sample category	Country of origin	Total bacterial count (CFU/g)
1	Dried apricot	Hungary	6.50E+01 ± 1.41E+00
2	Dried apricot	Hungary	2.12E+04 ± 2.05E+00
3	Dried apricot	Hungary	7.50E+01 ± 8.16E-01
4	Dried apricot	Hungary	3.67E+02 ± 9.43E-01
5	Dried apricot	Hungary	2.68E+02 ± 1.70E+00
6	Dried apricot	Austria	4.98E+02 ± 5.31E+00
7	Dried apricot	Austria	1.45E+02 ± 1.63E+00
8	Dried apricot	Austria	5.00E+04 ± 1.19E+01
9	Dried apricot	Austria	1.95E+02 ± 3.74E+00
10	Dried apricot	Austria	2.08E+02 ± 2.05E+00
11	Dried apricot	Austria	1.48E+02 ± 1.70E+00
12	Prune	Hungary	< 5.00E+00
13	Prune	Hungary	< 5.00E+00
14	Prune	Hungary	1.00E+01 ± 8.16E-01
15	Prune	Hungary	1.67E+01 ± 4.71E-01
16	Prune	Hungary	6.83E+01 ± 9.43E-01
17	Prune	Hungary	2.90E+02 ± 3.74E+00
18	Prune	Hungary	1.17E+01 ± 4.71E-01
19	Prune	Austria	< 5.00E+00
20	Raisin	Hungary	3.72E+03 ± 4.11E+00
21	Raisin	Hungary	1.06E+03 ± 1.31E+01
22	Raisin	Hungary	2.47E+02 ± 5.73E+00
23	Raisin	Hungary	9.67E+01 ± 1.25E+00
24	Raisin	Hungary	2.60E+02 ± 2.94E+00
25	Raisin	Hungary	4.74E+03 ± 1.26E+01
26	Raisin	Austria	4.00E+01 ± 8.16E-01
27	Raisin	Austria	7.77E+02 ± 2.35E+01
28	Raisin	Austria	1.33E+03 ± 7.87E+00
29	Raisin	Austria	6.50E+01 ± 1.41E+00
30	Raisin	Austria	8.00E+01 ± 2.45E+00

Supplementary Table 5 pH, water activity (whole and cut) and sugar concentration parameters (mean ± SE) of commercially purchased dried apricots, prunes and raisins. SE=standard error.

Sample code	Sample category	Country of origin	pH	Water activity (whole)	Water activity (cut)	Sugar concentration g/100g
1	Dried apricot	Hungary	4.00 ± 0.03	0.668 ± 0.014	0.673 ± 0.008	9.12 ± 0.46
2	Dried apricot	Hungary	5.02 ± 0.03	0.733 ± 0.007	0.734 ± 0.006	4.39 ± 0.21
3	Dried apricot	Hungary	3.89 ± 0.02	0.623 ± 0.002	0.624 ± 0.010	1.415 ± 0.08
4	Dried apricot	Hungary	4.05 ± 0.02	0.735 ± 0.004	0.742 ± 0.005	8.11 ± 0.34
5	Dried apricot	Hungary	3.89 ± 0.02	0.629 ± 0.005	0.632 ± 0.009	6.38 ± 0.25
6	Dried apricot	Austria	4.09 ± 0.04	0.704 ± 0.005	0.705 ± 0.008	5.34 ± 0.24
7	Dried apricot	Austria	3.98 ± 0.03	0.678 ± 0.004	0.632 ± 0.005	11.6 ± 0.49
8	Dried apricot	Austria	4.87 ± 0.03	0.416 ± 0.001	0.411 ± 0.006	1.32 ± 0.07
9	Dried apricot	Austria	4.20 ± 0.02	0.631 ± 0.001	0.641 ± 0.006	17.34 ± 0.75
10	Dried apricot	Austria	4.16 ± 0.01	0.630 ± 0.007	0.633 ± 0.007	3.59 ± 0.17
11	Dried apricot	Austria	4.05 ± 0.01	0.599 ± 0.020	0.596 ± 0.009	6.74 ± 0.26
12	Prune	Hungary	3.83 ± 0.01	0.757 ± 0.003	0.750 ± 0.007	11.77 ± 0.51
13	Prune	Hungary	3.66 ± 0.03	0.716 ± 0.007	0.721 ± 0.006	7.69 ± 0.36
14	Prune	Hungary	4.04 ± 0.02	0.733 ± 0.004	0.741 ± 0.006	23.33 ± 0.94
15	Prune	Hungary	3.62 ± 0.03	0.713 ± 0.005	0.724 ± 0.010	3.96 ± 0.21
16	Prune	Hungary	3.66 ± 0.01	0.848 ± 0.005	0.853 ± 0.007	8.07 ± 0.30
17	Prune	Hungary	4.09 ± 0.02	0.695 ± 0.000	0.691 ± 0.006	8.00 ± 0.34
18	Prune	Hungary	3.89 ± 0.04	0.717 ± 0.002	0.715 ± 0.004	1.89 ± 0.10
19	Prune	Austria	3.98 ± 0.03	0.756 ± 0.008	0.750 ± 0.005	19.43 ± 0.84
20	Raisin	Hungary	4.01 ± 0.04	0.533 ± 0.006	0.536 ± 0.006	4.90 ± 0.22
21	Raisin	Hungary	4.24 ± 0.02	0.362 ± 0.018	0.349 ± 0.008	23.16 ± 0.97

Sample code	Sample category	Country of origin	pH	Water activity (whole)	Water activity (cut)	Sugar concentration g/100g
22	Raisin	Hungary	4.00 ± 0.03	0.475 ± 0.003	0.478 ± 0.004	28.53 ± 1.12
23	Raisin	Hungary	3.68 ± 0.05	0.483 ± 0.008	0.491 ± 0.005	20.11 ± 1.04
24	Raisin	Hungary	4.10 ± 0.03	0.532 ± 0.010	0.532 ± 0.004	15.83 ± 0.76
25	Raisin	Hungary	3.72 ± 0.02	0.475 ± 0.010	0.487 ± 0.006	14.30 ± 0.60
26	Raisin	Austria	3.59 ± 0.02	0.524 ± 0.017	0.534 ± 0.006	12.31 ± 0.68
27	Raisin	Austria	3.86 ± 0.03	0.378 ± 0.013	0.400 ± 0.007	18.77 ± 0.76
28	Raisin	Austria	4.05 ± 0.01	0.363 ± 0.013	0.385 ± 0.006	17.08 ± 0.71
29	Raisin	Austria	3.29 ± 0.01	0.449 ± 0.017	0.461 ± 0.009	19.83 ± 0.99
30	Raisin	Austria	3.65 ± 0.02	0.416 ± 0.015	0.399 ± 0.006	20.25 ± 0.94



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
Apricot	33	2143.00	1501.50	64.9394	5.369
Prune	24	684.500	1092.00	28.5208	-3.715
Raisin	33	1267.50	1501.50	38.4091	-1.956

Kruskal-Wallis Test, ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
30.8614	2	<.0001*

Nonparametric Comparisons For Each Pair Using Wilcoxon Method

q*	Alpha
1.95996	0.05

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann
Raisin	Prune	4.5341	4.450516	1.01878	0.3083	0.060000
Raisin	Apricot	-18.0000	4.723595	-3.81066	0.0001*	-0.280000
Prune	Apricot	-24.7216	4.451598	-5.55342	<.0001*	-0.320000

Supplementary Figure 28 The values representing the differences in pH among the dried fruit categories.

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
2	5	1.136667	0.0268178	1.08105	1.192283	<.0001*
2	3	1.133333	0.0268178	1.07772	1.188950	<.0001*
2	7	1.043333	0.0268178	0.98772	1.098950	<.0001*
2	1	1.020000	0.0268178	0.96438	1.075617	<.0001*
8	5	0.980000	0.0268178	0.92438	1.035617	<.0001*
8	3	0.976667	0.0268178	0.92105	1.032283	<.0001*
2	4	0.970000	0.0268178	0.91438	1.025617	<.0001*
2	11	0.970000	0.0268178	0.91438	1.025617	<.0001*
2	6	0.933333	0.0268178	0.87772	0.988950	<.0001*
8	7	0.886667	0.0268178	0.83105	0.942283	<.0001*
8	1	0.863333	0.0268178	0.80772	0.918950	<.0001*
2	10	0.860000	0.0268178	0.80438	0.915617	<.0001*
2	9	0.820000	0.0268178	0.76438	0.875617	<.0001*
8	4	0.813333	0.0268178	0.75772	0.868950	<.0001*
8	11	0.813333	0.0268178	0.75772	0.868950	<.0001*
8	6	0.776667	0.0268178	0.72105	0.832283	<.0001*
8	10	0.703333	0.0268178	0.64772	0.758950	<.0001*
8	9	0.663333	0.0268178	0.60772	0.718950	<.0001*
9	5	0.316667	0.0268178	0.26105	0.372283	<.0001*
9	3	0.313333	0.0268178	0.25772	0.368950	<.0001*
10	5	0.276667	0.0268178	0.22105	0.332283	<.0001*
10	3	0.273333	0.0268178	0.21772	0.328950	<.0001*
9	7	0.223333	0.0268178	0.16772	0.278950	<.0001*
6	5	0.203333	0.0268178	0.14772	0.258950	<.0001*
9	1	0.200000	0.0268178	0.14438	0.255617	<.0001*
6	3	0.200000	0.0268178	0.14438	0.255617	<.0001*
10	7	0.183333	0.0268178	0.12772	0.238950	<.0001*
4	5	0.166667	0.0268178	0.11105	0.222283	<.0001*
11	5	0.166667	0.0268178	0.11105	0.222283	<.0001*
4	3	0.163333	0.0268178	0.10772	0.218950	<.0001*
11	3	0.163333	0.0268178	0.10772	0.218950	<.0001*
10	1	0.160000	0.0268178	0.10438	0.215617	<.0001*
2	8	0.156667	0.0268178	0.10105	0.212283	<.0001*
9	4	0.150000	0.0268178	0.09438	0.205617	<.0001*
9	11	0.150000	0.0268178	0.09438	0.205617	<.0001*
1	5	0.116667	0.0268178	0.06105	0.172283	0.0003*
9	6	0.113333	0.0268178	0.05772	0.168950	0.0003*
1	3	0.113333	0.0268178	0.05772	0.168950	0.0003*
6	7	0.110000	0.0268178	0.05438	0.165617	0.0005*
10	4	0.110000	0.0268178	0.05438	0.165617	0.0005*
10	11	0.110000	0.0268178	0.05438	0.165617	0.0005*
7	5	0.093333	0.0268178	0.03772	0.148950	0.0021*
7	3	0.090000	0.0268178	0.03438	0.145617	0.0029*
6	1	0.086667	0.0268178	0.03105	0.142283	0.0038*
4	7	0.073333	0.0268178	0.01772	0.128950	0.0121*
11	7	0.073333	0.0268178	0.01772	0.128950	0.0121*
10	6	0.073333	0.0268178	0.01772	0.128950	0.0121*
4	1	0.050000	0.0268178	-0.00562	0.105617	0.0757
11	1	0.050000	0.0268178	-0.00562	0.105617	0.0757
9	10	0.040000	0.0268178	-0.01562	0.095617	0.1500
6	4	0.036667	0.0268178	-0.01895	0.092283	0.1854
6	11	0.036667	0.0268178	-0.01895	0.092283	0.1854
1	7	0.023333	0.0268178	-0.03228	0.078950	0.3937
3	5	0.003333	0.0268178	-0.05228	0.058950	0.9022
11	4	0.000000	0.0268178	-0.05562	0.055617	1.0000

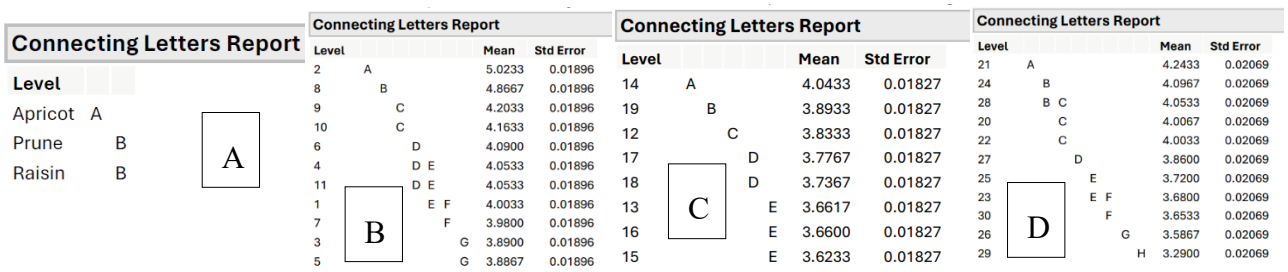
Supplementary Figure 29 The values representing the differences in pH among the dried apricot samples.

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
14	15	0.4200000	0.0258333	0.365236	0.4747642	<.0001*
14	16	0.3833333	0.0258333	0.328569	0.4380976	<.0001*
14	13	0.3816667	0.0258333	0.326902	0.4364309	<.0001*
14	18	0.3066667	0.0258333	0.251902	0.3614309	<.0001*
19	15	0.2700000	0.0258333	0.215236	0.3247642	<.0001*
14	17	0.2666667	0.0258333	0.211902	0.3214309	<.0001*
19	16	0.2333333	0.0258333	0.178569	0.2880976	<.0001*
19	13	0.2316667	0.0258333	0.176902	0.2864309	<.0001*
12	15	0.2100000	0.0258333	0.155236	0.2647642	<.0001*
14	12	0.2100000	0.0258333	0.155236	0.2647642	<.0001*
12	16	0.1733333	0.0258333	0.118569	0.2280976	<.0001*
12	13	0.1716667	0.0258333	0.116902	0.2264309	<.0001*
19	18	0.1566667	0.0258333	0.101902	0.2114309	<.0001*
17	15	0.1533333	0.0258333	0.098569	0.2080976	<.0001*
14	19	0.1500000	0.0258333	0.095236	0.2047642	<.0001*
17	16	0.1166667	0.0258333	0.061902	0.1714309	0.0004*
19	17	0.1166667	0.0258333	0.061902	0.1714309	0.0004*
17	13	0.1150000	0.0258333	0.060236	0.1697642	0.0004*
18	15	0.1133333	0.0258333	0.058569	0.1680976	0.0005*
12	18	0.0966667	0.0258333	0.041902	0.1514309	0.0018*
18	16	0.0766667	0.0258333	0.021902	0.1314309	0.0091*
18	13	0.0750000	0.0258333	0.020236	0.1297642	0.0104*
19	12	0.0600000	0.0258333	0.005236	0.1147642	0.0337*
12	17	0.0566667	0.0258333	0.001902	0.1114309	0.0434*
17	18	0.0400000	0.0258333	-0.014764	0.0947642	0.1411
13	15	0.0383333	0.0258333	-0.016431	0.0930976	0.1573
16	15	0.0366667	0.0258333	-0.018098	0.0914309	0.1750
13	16	0.0016667	0.0258333	-0.053098	0.0564309	0.9494

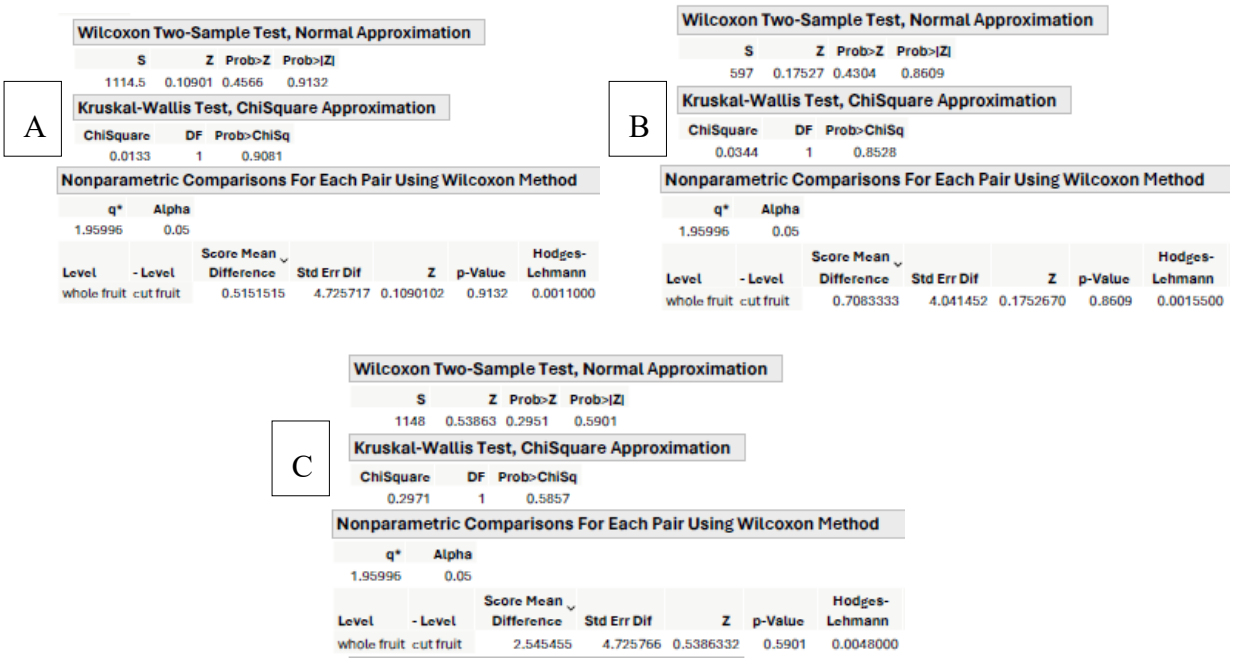
Supplementary Figure 30 The values representing the differences in pH among the prune samples.

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
21	29	0.9533333	0.0292671	0.892637	1.014030	<.0001*
24	29	0.8066667	0.0292671	0.745970	0.867363	<.0001*
28	29	0.7633333	0.0292671	0.702637	0.824030	<.0001*
20	29	0.7166667	0.0292671	0.655970	0.777363	<.0001*
22	29	0.7133333	0.0292671	0.652637	0.774030	<.0001*
21	26	0.6566667	0.0292671	0.595970	0.717363	<.0001*
21	30	0.5900000	0.0292671	0.529304	0.650696	<.0001*
27	29	0.5700000	0.0292671	0.509304	0.630696	<.0001*
21	23	0.5633333	0.0292671	0.502637	0.624030	<.0001*
21	25	0.5233333	0.0292671	0.462637	0.584030	<.0001*
24	26	0.5100000	0.0292671	0.449304	0.570696	<.0001*
28	26	0.4666667	0.0292671	0.405970	0.527363	<.0001*
24	30	0.4433333	0.0292671	0.382637	0.504030	<.0001*
25	29	0.4300000	0.0292671	0.369304	0.490696	<.0001*
20	26	0.4200000	0.0292671	0.359304	0.480696	<.0001*
22	26	0.4166667	0.0292671	0.355970	0.477363	<.0001*
24	23	0.4166667	0.0292671	0.355970	0.477363	<.0001*
28	30	0.4000000	0.0292671	0.339304	0.460696	<.0001*
23	29	0.3900000	0.0292671	0.329304	0.450696	<.0001*
21	27	0.3833333	0.0292671	0.322637	0.444030	<.0001*
24	25	0.3766667	0.0292671	0.315970	0.437363	<.0001*
28	23	0.3733333	0.0292671	0.312637	0.434030	<.0001*
30	29	0.3633333	0.0292671	0.302637	0.424030	<.0001*
20	30	0.3533333	0.0292671	0.292637	0.414030	<.0001*
22	30	0.3500000	0.0292671	0.289304	0.410696	<.0001*
28	25	0.3333333	0.0292671	0.272637	0.394030	<.0001*
20	23	0.3266667	0.0292671	0.265970	0.387363	<.0001*
22	23	0.3233333	0.0292671	0.262637	0.384030	<.0001*
26	29	0.2966667	0.0292671	0.235970	0.357363	<.0001*
20	25	0.2866667	0.0292671	0.225970	0.347363	<.0001*
22	25	0.2833333	0.0292671	0.222637	0.344030	<.0001*
27	26	0.2733333	0.0292671	0.212637	0.334030	<.0001*
21	22	0.2400000	0.0292671	0.179304	0.300696	<.0001*
24	27	0.2366667	0.0292671	0.175970	0.297363	<.0001*
21	20	0.2366667	0.0292671	0.175970	0.297363	<.0001*
27	30	0.2066667	0.0292671	0.145970	0.267363	<.0001*
28	27	0.1933333	0.0292671	0.132637	0.254030	<.0001*
21	28	0.1900000	0.0292671	0.129304	0.250696	<.0001*
27	23	0.1800000	0.0292671	0.119304	0.240696	<.0001*
20	27	0.1466667	0.0292671	0.085970	0.207363	<.0001*
21	24	0.1466667	0.0292671	0.085970	0.207363	<.0001*
22	27	0.1433333	0.0292671	0.082637	0.204030	<.0001*
27	25	0.1400000	0.0292671	0.079304	0.200696	<.0001*
25	26	0.1333333	0.0292671	0.072637	0.194030	0.0002*
23	26	0.0933333	0.0292671	0.032637	0.154030	0.0042*
24	22	0.0933333	0.0292671	0.032637	0.154030	0.0042*
24	20	0.0900000	0.0292671	0.029304	0.150696	0.0055*
30	26	0.0666667	0.0292671	0.005970	0.127363	0.0328*
25	30	0.0666667	0.0292671	0.005970	0.127363	0.0328*
28	22	0.0500000	0.0292671	-0.010696	0.110696	0.1016
28	20	0.0466667	0.0292671	-0.014030	0.107363	0.1251
24	28	0.0433333	0.0292671	-0.017363	0.104030	0.1529
25	23	0.0400000	0.0292671	-0.020696	0.100696	0.1855
23	30	0.0266667	0.0292671	-0.034030	0.087363	0.3721
20	22	0.0033333	0.0292671	-0.057363	0.064030	0.9104

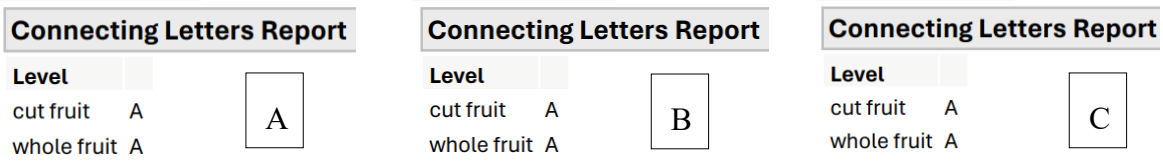
Supplementary Figure 31 The values representing the differences in pH among the raisin samples.



Supplementary Figure 32 Connecting letter report on the significant differences in the pH of the (A) sample categories, (B) dried apricot samples, (C) prune samples, and (D) raisin samples. Levels not connected by the same letter are significantly different.



Supplementary Figure 33 The values representing the differences in the water activity of whole and cut (A) dried apricot, (B), prune, (C) raisin samples.



Supplementary Figure 34 Connecting letters report on the significant differences in the cut and whole water activity values of (A) dried apricots, (B) prunes, and (C) raisins. Levels not connected by the same letter are significantly different.

A

Nonparametric Comparisons For Each Pair Using Wilcoxon Method

q*	Alpha
1.95996	0.05

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann
Prune	Apricot	21.6629	4.452681	4.86513	<.0001*	0.086950
Raisin	Prune	-28.4640	4.452825	-6.39235	<.0001*	-0.280250
Raisin	Apricot	-28.8485	4.725766	-6.10451	<.0001*	-0.196900

B

Nonparametric Comparisons For Each Pair Using Wilcoxon Method

q*	Alpha
1.95996	0.05

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann
Prune	Apricot	22.0587	4.452825	4.95387	<.0001*	0.092700
Raisin	Prune	-28.4640	4.452753	-6.39245	<.0001*	-0.270500
Raisin	Apricot	-29.0303	4.725766	-6.14298	<.0001*	-0.187400

Supplementary Figure 35 The values representing the differences in the water activity of each dried fruit category in the case of (A) cut, and (B) whole dried fruit samples.

Connecting Letters Report

Level	
Apricot	A
Prune	B
Raisin	C

A

Connecting Letters Report

Level	
Apricot	A
Prune	B
Raisin	C

B

Supplementary Figure 36 Connecting letters report on the significant differences in the water activity values among each dried fruit group, in the case of (A) cut, (B) whole dried fruit samples. Levels not connected by the same letter are significantly different.

Nonparametric Comparisons For Each Pair Using Wilcoxon Method

q*	Alpha
1.95996	0.05

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann
2	1	5.83333	2.081666	2.80224	0.0051*	0.060800
4	1	5.83333	2.081666	2.80224	0.0051*	0.066300
4	3	5.83333	2.081666	2.80224	0.0051*	0.115150
6	1	5.83333	2.081666	2.80224	0.0051*	0.031800
6	3	5.83333	2.081666	2.80224	0.0051*	0.080850
6	5	5.83333	2.081666	2.80224	0.0051*	0.074150
9	8	5.83333	2.078024	2.80715	0.0050*	0.220950
10	8	5.83333	2.078024	2.80715	0.0050*	0.219750
11	8	5.83333	2.078024	2.80715	0.0050*	0.186350
7	3	4.83333	2.081666	2.32186	0.0202*	0.034200
9	3	4.83333	2.081666	2.32186	0.0202*	0.011550
7	5	3.50000	2.081666	1.68135	0.0927	0.023850
10	3	3.16667	2.081666	1.52122	0.1282	0.009150
5	3	2.83333	2.081666	1.36109	0.1735	0.006750
4	2	2.50000	2.081666	1.20096	0.2298	0.005500
9	5	2.33333	2.078024	1.12286	0.2615	0.005700
10	5	0.50000	2.081666	0.24019	0.8102	0.001450
10	9	-1.16667	2.081666	-0.56045	0.5752	-0.004050
9	7	-1.50000	2.081666	-0.72058	0.4712	-0.016400
7	1	-1.83333	2.081666	-0.88070	0.3785	-0.009000
10	7	-3.16667	2.081666	-1.52122	0.1282	-0.026200
11	3	-5.16667	2.081666	-2.48199	0.0131*	-0.024650
9	1	-5.50000	2.081666	-2.64211	0.0082*	-0.037000
3	1	-5.83333	2.081666	-2.80224	0.0051*	-0.049400
3	2	-5.83333	2.081666	-2.80224	0.0051*	-0.110550
5	1	-5.83333	2.081666	-2.80224	0.0051*	-0.040900
5	2	-5.83333	2.081666	-2.80224	0.0051*	-0.103050
5	4	-5.83333	2.081666	-2.80224	0.0051*	-0.108650
6	2	-5.83333	2.081666	-2.80224	0.0051*	-0.028500
6	4	-5.83333	2.081666	-2.80224	0.0051*	-0.034150
7	2	-5.83333	2.081666	-2.80224	0.0051*	-0.075750
7	4	-5.83333	2.081666	-2.80224	0.0051*	-0.084050
7	6	-5.83333	2.081666	-2.80224	0.0051*	-0.047200
8	1	-5.83333	2.078024	-2.80715	0.0050*	-0.259300
8	2	-5.83333	2.078024	-2.80715	0.0050*	-0.319900
8	3	-5.83333	2.078024	-2.80715	0.0050*	-0.209600
8	4	-5.83333	2.078024	-2.80715	0.0050*	-0.324900
8	5	-5.83333	2.078024	-2.80715	0.0050*	-0.216500
8	6	-5.83333	2.078024	-2.80715	0.0050*	-0.291500
8	7	-5.83333	2.078024	-2.80715	0.0050*	-0.244700
9	2	-5.83333	2.081666	-2.80224	0.0051*	-0.097850
9	4	-5.83333	2.081666	-2.80224	0.0051*	-0.103350
9	6	-5.83333	2.081666	-2.80224	0.0051*	-0.068850
10	1	-5.83333	2.081666	-2.80224	0.0051*	-0.041150
10	2	-5.83333	2.081666	-2.80224	0.0051*	-0.102200
10	4	-5.83333	2.081666	-2.80224	0.0051*	-0.106650
10	6	-5.83333	2.081666	-2.80224	0.0051*	-0.073200
11	1	-5.83333	2.081666	-2.80224	0.0051*	-0.073000
11	2	-5.83333	2.081666	-2.80224	0.0051*	-0.135300
11	4	-5.83333	2.081666	-2.80224	0.0051*	-0.141650
11	5	-5.83333	2.081666	-2.80224	0.0051*	-0.034050
11	6	-5.83333	2.081666	-2.80224	0.0051*	-0.107100

Supplementary Figure 37 The values representing the differences in water activity among the dried apricot samples.

Nonparametric Comparisons For Each Pair Using Wilcoxon Method

q*	Alpha
1.95996	0.05

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann
14	13	5.83333	2.081666	2.80224	0.0051*	0.017200
16	12	5.83333	2.081666	2.80224	0.0051*	0.097150
16	13	5.83333	2.081666	2.80224	0.0051*	0.131800
16	14	5.83333	2.081666	2.80224	0.0051*	0.113150
16	15	5.83333	2.081666	2.80224	0.0051*	0.132900
18	17	5.83333	2.081666	2.80224	0.0051*	0.021950
19	13	5.83333	2.081666	2.80224	0.0051*	0.033400
19	15	5.83333	2.081666	2.80224	0.0051*	0.035500
19	17	5.83333	2.081666	2.80224	0.0051*	0.057750
19	18	5.83333	2.081666	2.80224	0.0051*	0.035000
19	14	5.16667	2.081666	2.48199	0.0131*	0.015150
15	13	0.16667	2.081666	0.08006	0.9362	0.000250
18	15	-0.16667	2.081666	-0.08006	0.9362	-0.000900
19	12	-1.16667	2.081666	-0.56045	0.5752	-0.003800
18	13	-1.50000	2.081666	-0.72058	0.4712	-0.002500
14	12	-5.16667	2.081666	-2.48199	0.0131*	-0.016850
15	14	-5.16667	2.081666	-2.48199	0.0131*	-0.020150
13	12	-5.83333	2.081666	-2.80224	0.0051*	-0.035150
15	12	-5.83333	2.081666	-2.80224	0.0051*	-0.036450
17	12	-5.83333	2.081666	-2.80224	0.0051*	-0.062300
17	13	-5.83333	2.081666	-2.80224	0.0051*	-0.024350
17	14	-5.83333	2.081666	-2.80224	0.0051*	-0.043550
17	15	-5.83333	2.081666	-2.80224	0.0051*	-0.024500
17	16	-5.83333	2.081666	-2.80224	0.0051*	-0.159350
18	12	-5.83333	2.081666	-2.80224	0.0051*	-0.040150
18	14	-5.83333	2.081666	-2.80224	0.0051*	-0.020850
18	16	-5.83333	2.081666	-2.80224	0.0051*	-0.136000
19	16	-5.83333	2.081666	-2.80224	0.0051*	-0.099550

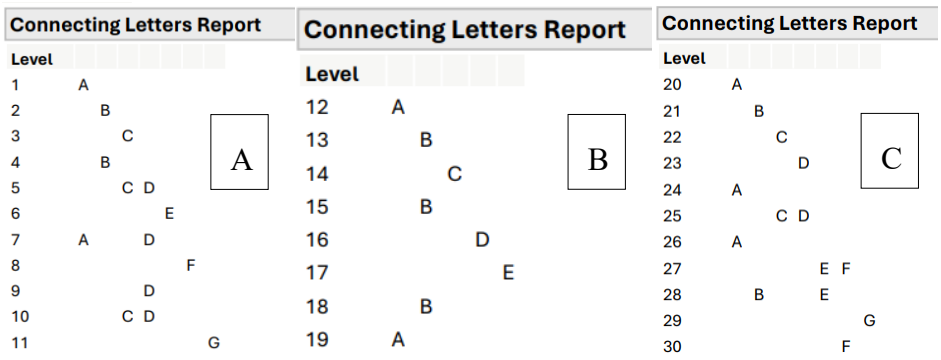
Supplementary Figure 38 The values representing the differences in water activity among the prune samples.

Nonparametric Comparisons For Each Pair Using Wilcoxon Method

q*	Alpha
1.95996	0.05

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann
22	21	5.83333	2.081666	2.80224	0.0051*	0.126100
23	21	5.83333	2.081666	2.80224	0.0051*	0.133800
24	21	5.83333	2.081666	2.80224	0.0051*	0.179500
24	22	5.83333	2.081666	2.80224	0.0051*	0.057000
24	23	5.83333	2.081666	2.80224	0.0051*	0.045300
25	21	5.83333	2.081666	2.80224	0.0051*	0.127600
26	21	5.83333	2.081666	2.80224	0.0051*	0.178400
26	22	5.83333	2.081666	2.80224	0.0051*	0.057800
26	23	5.83333	2.081666	2.80224	0.0051*	0.044500
26	25	5.83333	2.081666	2.80224	0.0051*	0.050550
29	21	5.83333	2.081666	2.80224	0.0051*	0.105650
29	27	5.83333	2.081666	2.80224	0.0051*	0.062200
29	28	5.83333	2.081666	2.80224	0.0051*	0.079150
30	21	5.83333	2.081666	2.80224	0.0051*	0.052250
30	28	5.83333	2.081666	2.80224	0.0051*	0.032150
27	21	5.16667	2.081666	2.48199	0.0131*	0.035650
23	22	4.16667	2.081666	2.00160	0.0453*	0.012750
28	21	3.16667	2.081666	1.52122	0.1282	0.020150
25	22	2.83333	2.081666	1.36109	0.1735	0.006350
30	27	2.83333	2.081666	1.36109	0.1735	0.016750
26	24	-0.50000	2.081666	-0.24019	0.8102	-0.001700
26	20	-0.83333	2.081666	-0.40032	0.6889	-0.002550
24	20	-1.00000	2.078024	-0.48123	0.6304	-0.001950
25	23	-2.16667	2.081666	-1.04083	0.2980	-0.007000
28	27	-2.83333	2.081666	-1.36109	0.1735	-0.015400
29	25	-5.16667	2.081666	-2.48199	0.0131*	-0.026250
29	22	-5.50000	2.081666	-2.64211	0.0082*	-0.021150
29	23	-5.50000	2.081666	-2.64211	0.0082*	-0.032250
30	29	-5.50000	2.081666	-2.64211	0.0082*	-0.052850
21	20	-5.83333	2.081666	-2.80224	0.0051*	-0.182250
22	20	-5.83333	2.081666	-2.80224	0.0051*	-0.059200
23	20	-5.83333	2.081666	-2.80224	0.0051*	-0.047000
25	20	-5.83333	2.081666	-2.80224	0.0051*	-0.052850
25	24	-5.83333	2.081666	-2.80224	0.0051*	-0.052100
27	20	-5.83333	2.081666	-2.80224	0.0051*	-0.142450
27	22	-5.83333	2.081666	-2.80224	0.0051*	-0.082900
27	23	-5.83333	2.081666	-2.80224	0.0051*	-0.095750
27	24	-5.83333	2.081666	-2.80224	0.0051*	-0.140450
27	25	-5.83333	2.081666	-2.80224	0.0051*	-0.088900
27	26	-5.83333	2.081666	-2.80224	0.0051*	-0.138350
28	20	-5.83333	2.081666	-2.80224	0.0051*	-0.157100
28	22	-5.83333	2.081666	-2.80224	0.0051*	-0.097350
28	23	-5.83333	2.081666	-2.80224	0.0051*	-0.110450
28	24	-5.83333	2.081666	-2.80224	0.0051*	-0.155950
28	25	-5.83333	2.081666	-2.80224	0.0051*	-0.104400
28	26	-5.83333	2.081666	-2.80224	0.0051*	-0.153850
29	20	-5.83333	2.081666	-2.80224	0.0051*	-0.077850
29	24	-5.83333	2.081666	-2.80224	0.0051*	-0.075950
29	26	-5.83333	2.081666	-2.80224	0.0051*	-0.073850
30	20	-5.83333	2.081666	-2.80224	0.0051*	-0.130800
30	22	-5.83333	2.081666	-2.80224	0.0051*	-0.074550
30	23	-5.83333	2.081666	-2.80224	0.0051*	-0.082400
30	24	-5.83333	2.081666	-2.80224	0.0051*	-0.127250
30	25	-5.83333	2.081666	-2.80224	0.0051*	-0.078600
30	26	-5.83333	2.081666	-2.80224	0.0051*	-0.127250

Supplementary Figure 39 The values representing the differences in water activity among the raisin samples.



Supplementary Figure 40 Connecting letters report on the significant differences in the water activity values among (A) dried apricots, (B) prunes, and (C) raisins. Levels not connected by the same letter are significantly different.

Supplementary Table 6 Identified bacteria at the genus level by 16S rDNA amplicon sequencing. Identification was not possible in Sample 13. "N/A." indicates not available

Sample code	Sample category	Country of origin	Identified genus
1	Dried apricot	Hungary	*Bacillus* *Priestia*
2	Dried apricot	Hungary	*Bacillus*
3	Dried apricot	Hungary	*Bacillus* *Priestia*
4	Dried apricot	Hungary	*Bacillus* *Priestia*
5	Dried apricot	Hungary	*Bacillus* *Priestia*
6	Dried apricot	Austria	*Bacillus*
7	Dried apricot	Austria	*Bacillus* *Neobacillus*
8	Dried apricot	Austria	*Bacillus* *Priestia*
9	Dried apricot	Austria	*Bacillus*
10	Dried apricot	Austria	*Bacillus* *Cytobacillus* *Neobacillus* *Priestia* *Mesobacillus* *Pantoea* *Tumebacillus*
11	Dried apricot	Austria	*Bacillus*

Sample code	Sample category	Country of origin	Identified genus
12	Prune	Hungary	*Bacillus*
13	Prune	N/A	N/A
14	Prune	Hungary	*Bacillus*
15	Prune	Hungary	*Bacillus* *Priestia*
16	Prune	Hungary	*Bacillus* *Priestia*
17	Prune	Hungary	*Bacillus*
18	Prune	Hungary	*Bacillus*
19	Prune	Austria	*Bacillus*
20	Raisin	Hungary	*Bacillus* *Priestia* *Pantoea*
21	Raisin	Hungary	*Bacillus* *Priestia*
22	Raisin	Hungary	*Bacillus* *Priestia*
23	Raisin	Hungary	*Bacillus* *Pantoea* *Tumebacillus*
24	Raisin	Hungary	*Bacillus* *Priestia*
25	Raisin	Hungary	*Bacillus*
26	Raisin	Austria	*Bacillus*
27	Raisin	Austria	*Bacillus* *Priestia*
28	Raisin	Austria	*Bacillus* *Pantoea* *Priestia*
29	Raisin	Austria	*Bacillus*
30	Raisin	Austria	*Bacillus*

A

Nonparametric Comparisons For Each Pair Using Wilcoxon Method

q*	Alpha
1.95996	0.05

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann
3	1	3.750000	1.732051	2.165064	0.0304*	2.30907
3	2	3.750000	1.732051	2.165064	0.0304*	1.41280
4	1	3.750000	1.732051	2.165064	0.0304*	2.33454
4	2	3.750000	1.732051	2.165064	0.0304*	1.43827
5	1	3.750000	1.732051	2.165064	0.0304*	2.49494
5	2	3.750000	1.732051	2.165064	0.0304*	1.59867
5	4	3.750000	1.732051	2.165064	0.0304*	0.17707
24	1	3.750000	1.721710	2.178067	0.0294*	2.11451
24	2	3.750000	1.721710	2.178067	0.0294*	1.19832
96	1	3.750000	1.732051	2.165064	0.0304*	1.63333
2	1	2.750000	1.732051	1.587713	0.1124	0.91620
5	3	2.750000	1.732051	1.587713	0.1124	0.16040
96	2	1.750000	1.732051	1.010363	0.3123	0.71714
4	3	0.000000	1.732051	0.000000	1.0000	5.329e-15
24	3	0.000000	1.721710	0.000000	1.0000	-0.23317
24	4	0.000000	1.721710	0.000000	1.0000	-0.25864
24	5	0.000000	1.721710	0.000000	1.0000	-0.41903
96	3	0.000000	1.732051	0.000000	1.0000	-0.71435
96	4	0.000000	1.732051	0.000000	1.0000	-0.73982
96	5	0.000000	1.732051	0.000000	1.0000	-0.90021
96	24	0.000000	1.721710	0.000000	1.0000	-0.45987

B

Nonparametric Comparisons For Each Pair Using Wilcoxon Method

q*	Alpha
1.95996	0.05

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann
2	1	3.75000	1.732051	2.16506	0.0304*	0.56063
3	1	3.75000	1.732051	2.16506	0.0304*	0.74912
4	1	3.75000	1.732051	2.16506	0.0304*	1.90579
4	2	3.75000	1.732051	2.16506	0.0304*	1.22941
4	3	3.75000	1.732051	2.16506	0.0304*	1.11394
5	1	3.75000	1.732051	2.16506	0.0304*	0.81143
24	1	3.75000	1.732051	2.16506	0.0304*	0.74868
24	2	3.75000	1.732051	2.16506	0.0304*	0.16937
96	1	3.75000	1.732051	2.16506	0.0304*	1.79166
96	2	3.75000	1.732051	2.16506	0.0304*	1.11528
96	3	3.75000	1.732051	2.16506	0.0304*	0.99981
96	5	3.75000	1.732051	2.16506	0.0304*	0.95348
96	24	3.75000	1.732051	2.16506	0.0304*	0.96236
5	2	2.25000	1.732051	1.29904	0.1939	0.16180
5	3	0.75000	1.732051	0.43301	0.6650	0.10237
24	3	0.75000	1.732051	0.43301	0.6650	0.09349
3	2	0.00000	1.732051	0.00000	1.0000	0.05943
24	5	0.00000	1.732051	0.00000	1.0000	-0.00888
96	4	0.00000	1.732051	0.00000	1.0000	-0.15674
5	4	-3.75000	1.732051	-2.16506	0.0304*	-1.06761
24	4	-3.75000	1.732051	-2.16506	0.0304*	-1.07649

C

Nonparametric Comparisons For Each Pair Using Wilcoxon Method

q*	Alpha
1.95996	0.05

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann
5	3	5.50000	2.078024	2.64675	0.0081*	0.174466
5	2	3.16667	2.078024	1.52388	0.1275	0.137024
5	4	2.50000	2.078024	1.20307	0.2290	0.140730
24	3	1.16667	2.081666	0.56045	0.5752	0.032943
4	3	1.00000	2.078024	0.48123	0.6304	0.030565
24	2	0.00000	2.081666	0.00000	1.0000	-0.000857
24	4	0.00000	2.081666	0.00000	1.0000	-0.008708
4	2	-0.50000	2.081666	-0.24019	0.8102	-0.050942
3	2	-0.83333	2.081666	-0.40032	0.6889	-0.088300
5	1	-1.83333	2.074375	-0.88380	0.3768	-0.081782
96	2	-1.83333	2.081666	-0.88070	0.3785	-0.576755
96	3	-1.83333	2.081666	-0.88070	0.3785	-0.562085
96	4	-1.83333	2.081666	-0.88070	0.3785	-0.598830
96	5	-1.83333	2.078024	-0.88225	0.3776	-0.743963
96	24	-1.83333	2.081666	-0.88070	0.3785	-0.562855
96	1	-2.50000	2.078024	-1.20307	0.2290	-0.825745
2	1	-3.16667	2.078024	-1.52388	0.1275	-0.305303
4	1	-3.50000	2.078024	-1.68429	0.0921	-0.301725
24	1	-3.83333	2.078024	-1.84470	0.0651	-0.273268
3	1	-4.16667	2.078024	-2.00511	0.0450*	-0.288670
24	5	-4.16667	2.078024	-2.00511	0.0450*	-0.181108

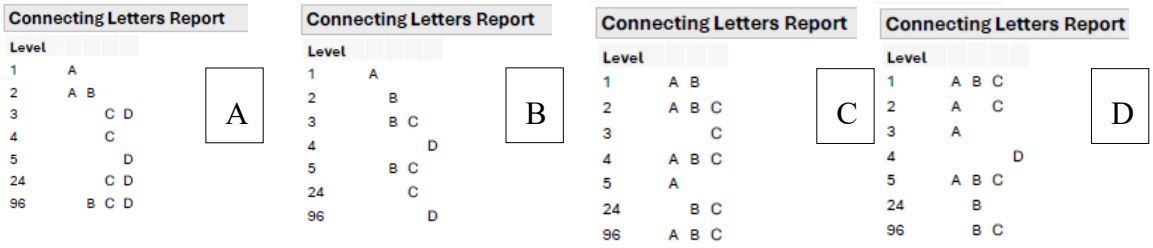
D

Nonparametric Comparisons For Each Pair Using Wilcoxon Method

q*	Alpha
1.95996	0.05

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann
4	1	5.83333	2.081666	2.80224	0.0051*	2.19295
4	2	5.83333	2.081666	2.80224	0.0051*	2.27373
4	3	5.83333	2.081666	2.80224	0.0051*	2.36033
24	2	5.83333	2.081666	2.80224	0.0051*	0.46387
24	3	5.83333	2.081666	2.80224	0.0051*	0.62874
96	3	4.16667	2.081666	2.00160	0.0453*	0.83662
96	2	3.83333	2.081666	1.84147	0.0656	0.78075
5	3	3.16667	2.081666	1.52122	0.1282	0.44408
96	1	3.16667	2.081666	1.52122	0.1282	0.69471
96	5	3.16667	2.081666	1.52122	0.1282	0.35866
24	1	2.50000	2.081666	1.20096	0.2298	0.28690
5	2	1.83333	2.081666	0.88070	0.3785	0.37776
96	24	1.83333	2.081666	0.88070	0.3785	0.32138
5	1	1.50000	2.081666	0.72058	0.4712	0.31061
24	5	0.83333	2.081666	0.40032	0.6889	0.17933
2	1	-0.50000	2.081666	-0.24019	0.8102	-0.12752
3	1	-1.16667	2.081666	-0.56045	0.5752	-0.17167
3	2	-1.16667	2.081666	-0.56045	0.5752	-0.10019
5	4	-5.83333	2.081666	-2.80224	0.0051*	-1.89936
24	4	-5.83333	2.081666	-2.80224	0.0051*	-1.83862
96	4	-5.83333	2.081666	-2.80224	0.0051*	-1.47430

Supplementary Figure 41 The values representing the differences in log cell counts (CFU/mL) measured by the PMA-qPCR method among the (A) CC strain of *E. coli* in the control medium, (B) FI strain of *E. coli* in the control medium, (C) CC strain of *E. coli* in the dried fruit-mimicking medium, and (D) in the FI strain of *E. coli* in the dried fruit-mimicking medium



Supplementary Figure 42 Connecting letters report on the significant differences in log cell counts (CFU/mL) measured by the PMA-qPCR method among the (A) CC strain of *E. coli* in the control medium, (B) FI strain of *E. coli* in the control medium, (C) CC strain of *E. coli* in the dried fruit-mimicking medium, and (D) in the FI strain of *E. coli* in the dried fruit-mimicking medium. Levels not connected by the same letter are significantly different.

A

onparametric Comparisons For Each Pair Using Wilcoxon Method

q* 1.95996 Alpha 0.05

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann
3	1	5.83333	2.081666	2.80224	0.0051*	1.82000
4	1	5.83333	2.081666	2.80224	0.0051*	1.91500
5	1	5.83333	2.081666	2.80224	0.0051*	2.11500
5	2	5.83333	2.081666	2.80224	0.0051*	1.61500
4	2	5.16667	2.081666	2.48199	0.0131*	1.41500
24	1	5.16667	2.081666	2.48199	0.0131*	1.75500
3	2	4.83333	2.081666	2.32186	0.0202*	1.32000
5	3	4.50000	2.081666	2.16173	0.0306*	0.51000
5	4	3.66667	2.078024	1.76450	0.0776	0.33000
24	2	3.16667	2.081666	1.52122	0.1282	1.27000
2	1	2.66667	2.078024	1.28327	0.1994	0.56500
4	3	1.66667	2.078024	0.80204	0.4225	0.10500
24	3	-0.50000	2.081666	-0.24019	0.8102	-0.11000
96	1	-1.16667	2.081666	-0.56045	0.5752	-0.58000
24	4	-1.50000	2.081666	-0.72058	0.4712	-0.23500
24	5	-3.83333	2.081666	-1.84147	0.0656	-0.56000
96	2	-4.16667	2.081666	-2.00160	0.0453*	-1.08000
96	3	-5.83333	2.081666	-2.80224	0.0051*	-2.27000
96	4	-5.83333	2.081666	-2.80224	0.0051*	-2.49000
96	5	-5.83333	2.081666	-2.80224	0.0051*	-2.90000
96	24	-5.83333	2.081666	-2.80224	0.0051*	-2.27500

B

Nonparametric Comparisons For Each Pair Using Wilcoxon Method

q* 1.95996 Alpha 0.05

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann
4	1	5.50000	2.081666	2.64211	0.0082*	0.95000
3	1	4.83333	2.081666	2.32186	0.0202*	0.73500
2	1	4.50000	2.081666	2.16173	0.0306*	0.88000
24	1	4.33333	2.078024	2.08531	0.0370*	0.64500
5	1	4.16667	2.081666	2.00160	0.0453*	0.87000
4	3	2.00000	2.078024	0.96245	0.3358	0.16000
4	2	0.83333	2.081666	0.40032	0.6889	0.07000
5	3	0.66667	2.078024	0.32082	0.7483	0.14500
5	2	-0.16667	2.081666	-0.08006	0.9362	-0.05000
5	4	-0.33333	2.078024	-0.16041	0.8726	-0.06500
3	2	-1.16667	2.081666	-0.56045	0.5752	-0.10500
24	5	-1.16667	2.081666	-0.56045	0.5752	-0.30000
24	3	-1.50000	2.081666	-0.72058	0.4712	-0.09000
24	2	-1.66667	2.078024	-0.80204	0.4225	-0.32000
96	1	-2.83333	2.081666	-1.36109	0.1735	-0.51500
24	4	-3.33333	2.078024	-1.60409	0.1087	-0.34000
96	2	-5.83333	2.081666	-2.80224	0.0051*	-1.51000
96	3	-5.83333	2.081666	-2.80224	0.0051*	-1.30500
96	4	-5.83333	2.081666	-2.80224	0.0051*	-1.55000
96	5	-5.83333	2.081666	-2.80224	0.0051*	-1.42500
96	24	-5.83333	2.081666	-2.80224	0.0051*	-1.13000

C

onparametric Comparisons For Each Pair Using Wilcoxon Method

q* 1.95996 Alpha 0.05

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann
3	2	1.33333	2.516611	0.52981	0.5962	0.06000
4	2	0.66667	2.516611	0.26491	0.7911	0.03000
5	2	0.66667	2.516611	0.26491	0.7911	0.04000
5	4	0.33333	2.515313	0.13252	0.8946	0.01000
5	3	-0.22222	2.514013	-0.08839	0.9296	-0.01000
4	3	-0.33333	2.512713	-0.13266	0.8945	-0.01000
24	2	-3.44444	2.515313	-1.36939	0.1709	-0.18000
24	3	-4.66667	2.511412	-1.85818	0.0631	-0.22000
24	5	-5.11111	2.514013	-2.03305	0.0420*	-0.31000
24	4	-5.22222	2.515313	-2.07617	0.0379*	-0.17000
3	1	-5.33333	2.514013	-2.12144	0.0339*	-0.46000
2	1	-5.77778	2.516611	-2.29586	0.0217*	-0.55000
4	1	-6.00000	2.514013	-2.38662	0.0170*	-0.57000
5	1	-6.22222	2.514013	-2.47502	0.0133*	-0.51000
24	1	-7.44444	2.515313	-2.95965	0.0031*	-0.90000
96	1	-8.88889	2.489192	-3.57099	0.0004*	-8.03000
96	2	-8.88889	2.489192	-3.57099	0.0004*	-7.83000
96	3	-8.88889	2.489192	-3.57099	0.0004*	-7.79000
96	4	-8.88889	2.489192	-3.57099	0.0004*	-7.86000
96	5	-8.88889	2.489192	-3.57099	0.0004*	-7.84000
96	24	-8.88889	2.489192	-3.57099	0.0004*	-7.56000

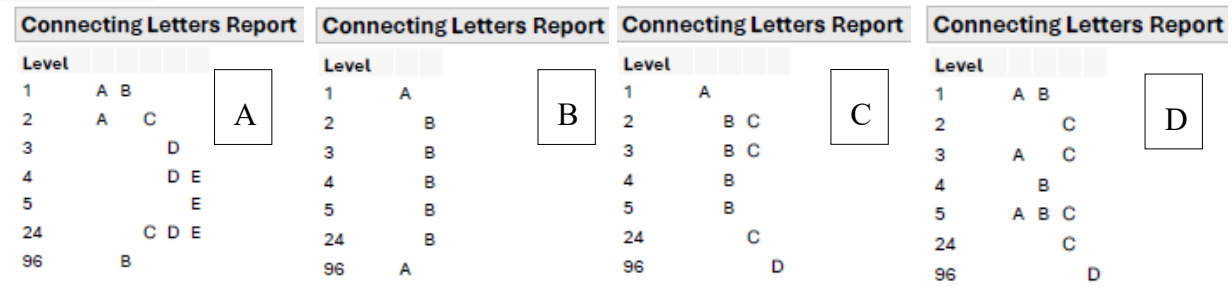
D

nparametric Comparisons For Each Pair Using Wilcoxon Method

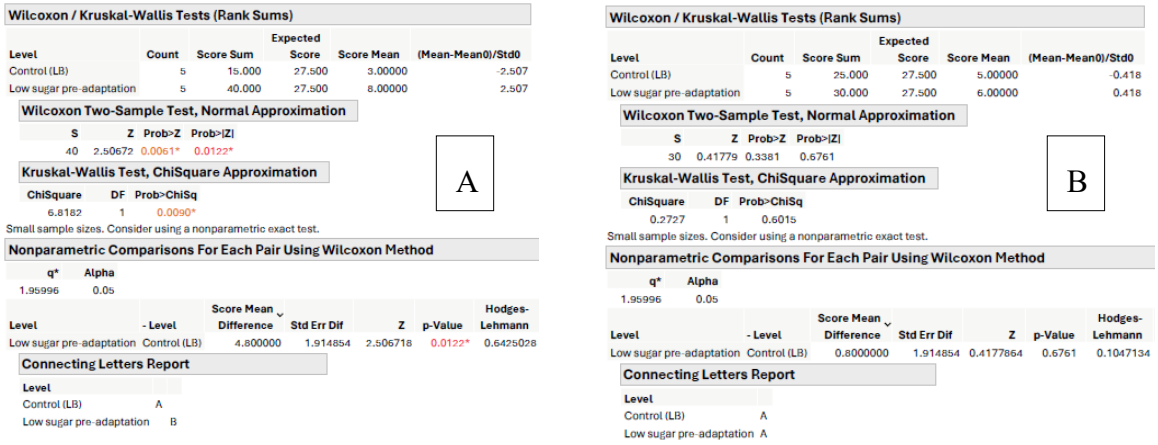
q* 1.95996 Alpha 0.05

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann
4	2	7.33333	2.514013	2.91698	0.0035*	0.61000
4	3	6.22222	2.516611	2.47246	0.0134*	0.45000
5	2	4.33333	2.515313	1.72278	0.0849	0.35000
5	3	3.11111	2.516611	1.23623	0.2164	0.34000
4	1	2.44444	2.514013	0.97233	0.3309	0.10000
3	2	1.33333	2.516611	0.52981	0.5962	0.10000
24	2	0.77778	2.512713	0.30954	0.7569	0.04000
24	3	-0.55556	2.515313	-0.22087	0.8252	-0.02000
5	1	-1.22222	2.512713	-0.48642	0.6267	-0.08000
5	4	-2.88889	2.514013	-1.14911	0.2505	-0.18000
24	5	-3.55556	2.514013	-1.41429	0.1573	-0.27000
3	1	-4.88889	2.516611	-1.94265	0.0521	-0.34000
24	1	-5.44444	2.515313	-2.16452	0.0304*	-0.43000
2	1	-6.22222	2.514013	-2.47502	0.0133*	-0.50000
24	4	-6.33333	2.515313	-2.51791	0.0118*	-0.61000
96	1	-8.88889	2.498365	-3.55788	0.0004*	-7.60000
96	2	-8.88889	2.498365	-3.55788	0.0004*	-6.98000
96	3	-8.88889	2.498365	-3.55788	0.0004*	-7.26000
96	4	-8.88889	2.498365	-3.55788	0.0004*	-7.70000
96	5	-8.88889	2.498365	-3.55788	0.0004*	-7.57000
96	24	-8.88889	2.498365	-3.55788	0.0004*	-6.98000

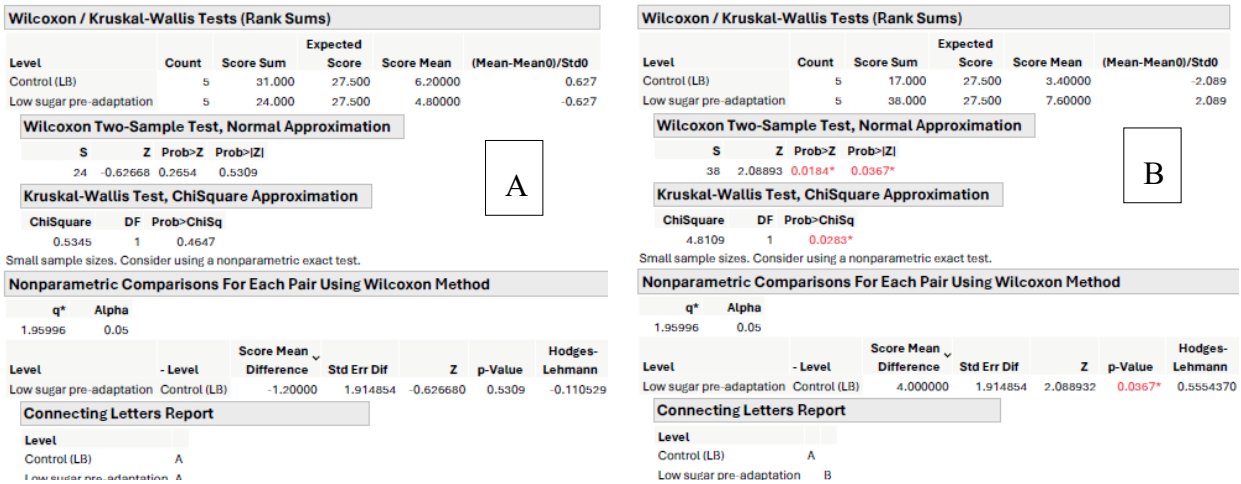
Supplementary Figure 43 The values representing the differences in log cell counts (CFU/mL) measured by the plating method among the (A) CC strain of *E. coli* in the control medium, (B) FI strain of *E. coli* in the control medium, (C) CC strain of *E. coli* in the dried fruit-mimicking medium, and (D) in the FI strain of *E. coli* in the dried fruit-mimicking medium.



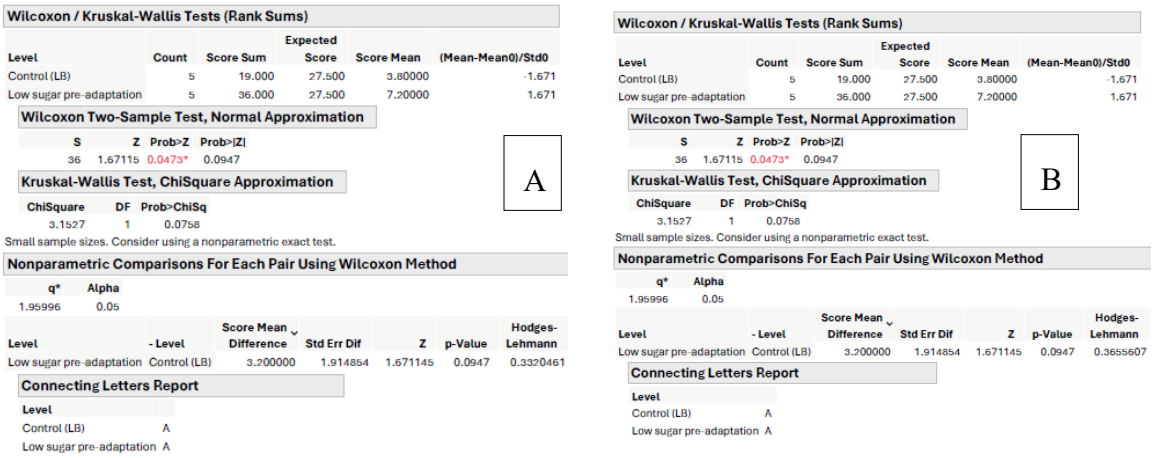
Supplementary Figure 44 Connecting letters report on the significant differences in log cell counts (CFU/mL) measured by the plating method among the (A) CC strain of *E. coli* in the control medium, (B) FI strain of *E. coli* in the control medium, (C) CC strain of *E. coli* in the dried fruit-mimicking medium, and (D) in the FI strain of *E. coli* in the dried fruit-mimicking medium. Levels not connected by the same letter are significantly different.



Supplementary Figure 45 The values representing the differences in the log cell count of pre-adapted cells following a transfer to prune-mimicking medium at (A) 4 hours, and (B) 24 hours.



Supplementary Figure 46 The values representing the differences in the log cell count of pre-adapted cells following a transfer to raisin-mimicking medium at (A) 4 hours, and (B) 24 hours.



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
Control (LB)	5	19.000	27.500	3.80000	-1.671
Low sugar pre-adaptation	5	36.000	27.500	7.20000	1.671

Wilcoxon Two-Sample Test, Normal Approximation

S	Z	Prob>Z	Prob>|Z|
36	1.67115	0.0473*	0.0947

Kruskal-Wallis Test, ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
3.1527	1	0.0758

Small sample sizes. Consider using a nonparametric exact test.

Nonparametric Comparisons For Each Pair Using Wilcoxon Method

q*	Alpha
1.95996	0.05

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann
Low sugar pre-adaptation	Control (LB)	3.200000	1.914854	1.671145	0.0947	0.3320461

Connecting Letters Report

Level	
Control (LB)	A
Low sugar pre-adaptation	A

A

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
Control (LB)	5	19.000	27.500	3.80000	-1.671
Low sugar pre-adaptation	5	36.000	27.500	7.20000	1.671

Wilcoxon Two-Sample Test, Normal Approximation

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Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann
Low sugar pre-adaptation	Control (LB)	3.200000	1.914854	1.671145	0.0947	0.3655607

Connecting Letters Report

Level	
Control (LB)	A
Low sugar pre-adaptation	A

B

Supplementary Figure 47 The values representing the differences in the log cell count of pre-adapted cells following a transfer to dried apricot-mimicking medium at (A) 4 hours, and (B) 24 hours.

Declaration of Students and Doctoral Candidates on the Use of Artificial Intelligence (AI)"

1. general information:

Name of the student:	**Viktória Dobó**
Neptun ID:	**IMXFSV**
Level of program (mark with X):	☐ BSc/BA ☐ MSc/MA ☒ Doctoral School (PhD) ☐ Other: ……
Name and code of the subject*:	
Title of the work:	**Microbiota analysis of dried fruits and stress modelling in *Escherichia coli***

* Not required to be completed in the case of a doctoral dissertation.

2. Declaration on the Use of AI

I, the undersigned, fully aware of my ethical responsibility, make the following declaration:

(Please choose one of the options below!)

☐ A) I have not used any artificial intelligence system or service.

(If you selected this option, completing the subsequent tables is not required.)

☒ B) I have used an artificial intelligence system or service.

(Please fill in the relevant tables!)

3. Details of Artificial Intelligence Usage

TABLE I: Assistant or Minor Usage (e.g., translation, language proofreading, brainstorming, etc.)

(For these uses, attaching the specific prompts and responses is not required.)

Purpose of Use	**Name and Version of the AI Tool Used**	**Affected Section (if not applicable to the entire text)**
Translation, language proofreading, and grammar and spelling check.	Grammarly; DeepL; ProWritingAid	

TABLE II: Significant Content Contribution (e.g., generating an entire figure or a longer text section)

(In these cases, documenting the key prompts used and the raw responses provided by the AI, and attaching them as an appendix to the work, is required.)

Purpose of Use	**Name, Version, and Access Information of the AI Tool Used**	**Exact Number of the Affected Chapter / Figure / Table**	**Entry Number of the Appendix Containing the Prompt Log**

3/A. Additional Rules Prescribed by the Lecturer (if any)

If the instructor or supervisor of the course has established specific rules or expectations regarding the use of AI tools, please summarize them in the field below:

For example: prohibition of AI use for certain types of tasks; only specific tools are permitted; different citation requirements; documentation format, etc.

Rules Prescribed by the Lecturer or Supervisor

……

……

……

……

4. Declaration Applicable to All Students:

I declare that I have critically reviewed, edited, and incorporated any content potentially generated by AI in all cases. I take full responsibility for every element of the submitted work, including its originality and scientific validity. I acknowledge that the Hungarian University of Agriculture and Life Sciences may check the submitted work with an artificial intelligence detector and may initiate proceedings if my declaration is found to be false or incomplete.

Place and Date: Budapest, 2025. November 19.

....

Signature of the Student **Signature of the Advisor/Supervisor**

.....

Signature of the Advisor/Supervisor

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