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SOKFÉLESEGÉNEK JELLEMZÉSE**

Characterization of the microbial diversity of dried fruits

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1. Introduction

Dried fruits are good sources of antioxidants (like phenols) and micronutrients (vitamin A, calcium, kalium, iron or folate), therefore their consumption is highly beneficial. Furthermore, dried fruits are an adequate source of insoluble fibers (Gyurova et al., 2014). According to the Centre for the Promotion of Imports from developing countries, Europe is the world's largest consumer of dried fruit, with a 26% consumption share in 2017. In terms of dried apricots, Sweden represents the largest consumer market in Europe. With regard to prunes, France is the dominant consumer, while the Dutch are the most avid consumers of raisins in the region. To ensure that any kind of commercially available dried fruit is safe, it is essential to ascertain the microbiota of these products and identify their micro- and pathobiota.

Determination of the microbiota from dried fruits could be challenging because of their complex characteristics. Moreover, the elevated sugar content of the dried fruits can influence the metabolic activity of the microorganisms present in them. The high sugar concentration results in elevated osmotic pressure, which is regarded as an environmental stress factor. This can impede the reproduction of the microorganisms or even have a lethal effect. Therefore, this study is focusing on the determination of sugar concentration of different dried fruits and establish a correlation between the microbiota and the sugar concentration.

A further objective of this study was to monitor the microbial growth rate in media with different sugar concentrations. The media were prepared to demonstrate the real sugar concentration of three dried fruits: raisins, prune, and apricot. The growth rate of the test microorganism, *E. coli*, in the mimicking media was compared with that of the bacterium in low-sugar medium and in LB broth as well. Two *E. coli* strains were investigated in this study, a laboratory strain and an *E. coli* isolated from a food with low-water activity.

My work represents a continuation of the research initiated by Viktória Dobó. In her PhD thesis, an initial description of the microbiota has already been provided. My objective was to undertake a comparative analysis of the results and to employ alternative methodologies (culture dependent and culture independent) for the determination of the microbiota.

2. Objective

This study was part of a collaboration between the Hungarian University of Agriculture and Life Sciences, Budapest, Hungary, and the University of Natural Resources and Life Sciences, Vienna, Austria. This study is also a continuation of one of the previous works, the PhD thesis of Viktória Dobó.

The objective of this study is to determine the microbial diversity of dried fruits with different methods. MALDI-TOF MS is used for identification of colonies of culturable microorganisms in the case of all tested dried fruit samples. A more advanced technology, the Nanopore sequencing will also be used for nucleotide order determination of the 16S rDNA gene. Growth rate of two *E. coli* strains is going to be investigated (to compare a laboratory and an isolated strain), as certain strains of this species can be either human pathogenic or hygiene indicator. It is relatively easy for *E. coli* bacteria to contaminate the dried fruits if the hygienic conditions are poor during the production chain. One of the test strains is a laboratory strain originating from the National Collection of Agricultural and Industrial Microorganisms, Institute of Food Science and Technology, Hungarian University of Agriculture and Life Sciences, Budapest, while the other strain is an isolated *E. coli* from a food with low water activity. The two strains will be compared to each other in different sugar containing media. The goal of the study is to see whether there is any statistical difference between the sugar concentration and the growth rate of different strains of *E. coli*.

Another aim of this study is to determinate how the microbiota is depending on the sugar concentration of the dried fruits, and is there any collapse between the number of the microorganisms and the sugar concentration, more specifically is there any inhibitory effect of the high sugar concentration on the microbiome of the samples.

3. Literature review

3.1. Dried fruits and health

A lot of study shows that the dried fruits have a great health benefit for the human's body. In the study of Sadler et al. (2019) the impact of the dried fruits for the human health was investigated. In their study they described that dried fruits are nutritionally equivalent to fresh fruits in smaller serving sizes. Also, from other studies (He et al., 2007, Lichtenstein et al., 2006, Slavin & Lloyd, 2012) it is attributable that 3-5 servings of dried fruit a day can protect against non-communicable diseases (NCDs), such as cardiovascular diseases, diabetes, chronic kidney diseases or neurological disorders. Other benefits of the dried fruits are that they are easy to store all around the year and easy to distribute. In the study of Chang et al. (2016) the popularity of dried fruits was described. In Table 1. the propensity to consume in order is listed.

Table 1: Consumed amount of dried fruits in a consumption order (Source: Chang et al., 2016)

Dried fruits	Consumed amount (in metric tonnes)
Raisins and currants	1,394,566
Dates	756,000
Prunes	216,853
Cranberries	152,000
Figs	135,744
Apricots	88,129

We know that the dried fruits are beneficial for the human health, but what does make them so healthy? The answer can be attributed to the phytochemicals.

3.1.1. Phytochemicals

"Phytochemicals are non-nutritive, naturally occurring, and biologically active compounds found in the plant kingdom. However, a large percentage of phytochemicals is still unknown and remains to be identified by the scientific community" (Quideau et al., 2011, Tomás-Barberán & Andrés-Lacueva, 2012).

Phytochemicals are able to prevent or reduce oxidative stress (based on the Health Benefits of Dietary Phytochemicals in Whole Foods chapter of the Nutritional Health). Moreover, phytochemicals can improve the digestive health as well. In the study of Baek et al. (2016) the scientists made a clinical trial to demonstrate the beneficial effect of the flavonoid rich diet. The trial was randomized and double-blinded. The patients who were suffering from constipation got either *F. carica* paste (n=40) or placebo (n=40) for 8 weeks. They found that transit time in the colon was significantly reduced, the stool quality improved, and abdominal discomfort was reduced. In some studies from 2013, it was described that dried fruits provide a wide range of phytochemicals, such as phenolic acids, flavonoids, phytoestrogens, and carotenoids (Alasalvar & Shahidi, 2013a, Alasalvar & Shahidi, 2013b). Figure 1. shows the most important phenolic compounds described in the case of dried fruits.

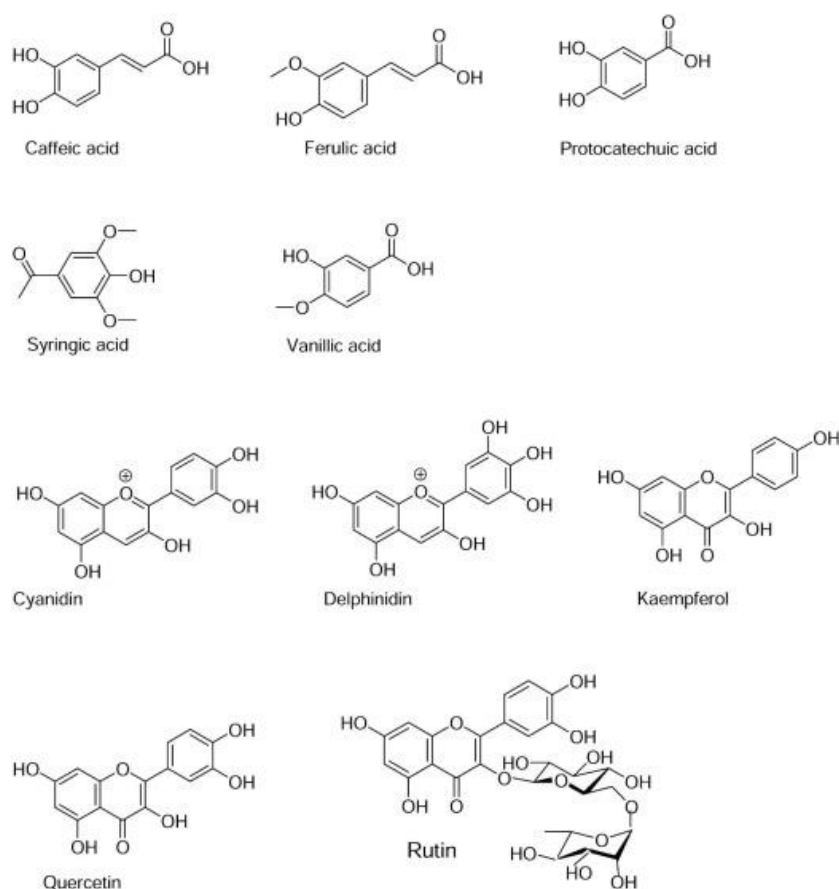


Figure 1: Chemical structures of representative phenolic compounds (phenolic acids, flavonoids, and anthocyanidins) reported in dried fruits (Source: Alasalvar et al., 2013)

Stacewicz-Sapuntzakis and coworkers (2010) showed that prunes contain large amounts of phenolic compounds (184 mg/100 g), which can delay glucose absorption. One of the findings of the study was that the "*Phenolic compounds in prunes had been found to inhibit human LDL oxidation in vitro, and thus might serve as preventive agents against chronic diseases, such as heart disease and cancer.*" They also researched the potassium content of prunes. It was found that the high potassium content of prunes (745 mg/100 g) might be beneficial for cardiovascular health. Dried prunes are an important source of boron, which can be able to prevent osteoporosis. The research showed that the serving of prunes (100 g) fulfils the daily requirement for boron (2 to 3 mg).

3.1.2. Fibers

Dried fruits are also a good source of fiber. In 2014, the European Food Safety Authority (EFSA) authorized a health claim for dried plums/prunes and gastrointestinal health. This claim states that "*Dried plums/prunes can contribute to normal bowel function*". To obtain the claimed effect, about 100 g/day (~8–12 prunes, depending on their size) of prunes should be consumed. Based on the study of Alasalvar (2023) dried fruits can contain 3.7–9.8 g/100 g of dietary fiber. The recommended consumption of dietary intake is 14 g/day, therefore 20–30 g

dried fruit per/day provides 10–16% of the daily dietary fiber intake depending on the fruit. Still, the biggest benefit of dried fruits is that the bioactive compounds and antioxidant activities are higher than those of their corresponding fresh counterparts. Some studies suggest that fruits rich in fibers and oligosaccharides might promote the growth of the healthy microorganism and act as prebiotics (El-Gawad et al., 2005; Scheid et al., 2013). Figure 2. shows the effect of dried fruits on the gut microbiota.

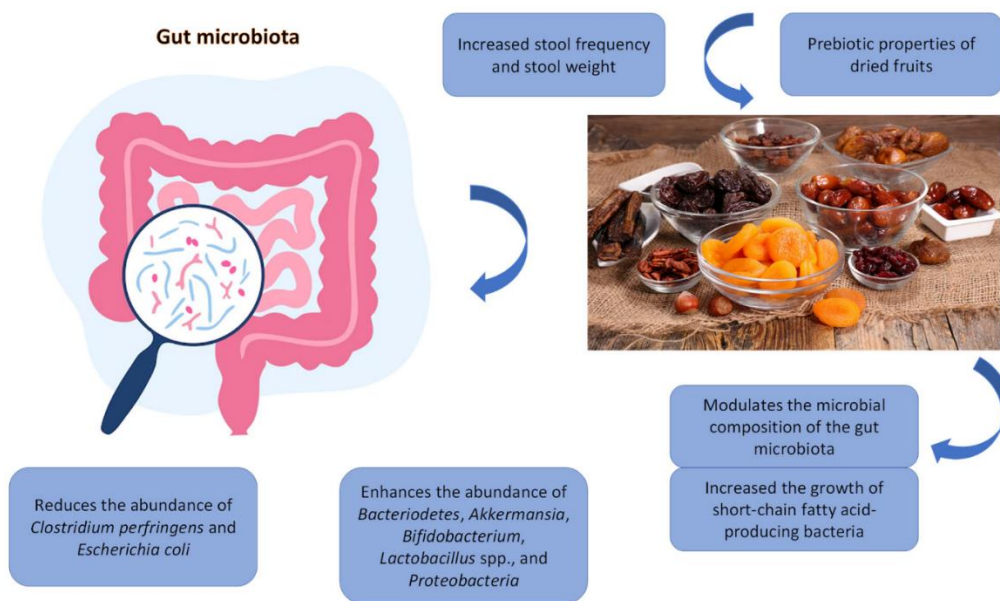


Figure 2: Effects of dried fruit consumption on the gut microbiota (Source: Alasalvar et al., 2023)

3.1.3. Glycaemic index and type II diabetes

In the case of dried fruits, the high sugar content may raise question marks for the consumer. Is it healthy to eat dried fruits if they contain a lot of sugar? Many studies show even if the sugar content is high in dried fruits, they do not mediate a rapid rise in blood sugar concentration, possibly because of high fiber, fructose, and sorbitol content. In dried fruits, glycaemic index ranges go from low (e.g., dried prunes) to medium (e.g., dried figs) or high (e.g., dates). Another benefit is that dried fruits with a low glycaemic index could help to reduce the risk of diabetes (Alasalvar & Shahidi, 2013a, Alasalvar & Shahidi, 2013b). Other studies (Al-Farsi & Lee, 2008, Anderson & Waters, 2013, Kundu & Surh, 2013, Lever et al., 2014, Vayalil, 2012) show that individuals who consume dried fruits regularly have a lower risk of obesity, certain types of cancer, type II diabetes or inflammatory bowel disease. The result of the study of Muraki et al. (2013) showed that three servings of grapes and raisins per week can lower the risk of type II diabetes with 12%. Kanellos et al. (2013) provided a randomized study with healthy subjects and 15 type II diabetic patients. One group received 74 g of raisins, while the another received 50 g of glucose as a reference food. Blood samples were collected at 0, 30, 60, 90, 120, 150 and 180 minutes after the consumption. Figure 3. shows that the peak of the serum glucose was at 30 minutes in healthy subjects and at 60 minutes in T2D, for both treatments (no difference between the raisins or the sugar consumption, people with type II diabetes produced a bigger

glucose peak but later than the healthy persons). On the other hand, the difference of the glucose levels was statistically different between the raisins (triangle on the figure) and the glucose consumption (square on the figure) for both groups.

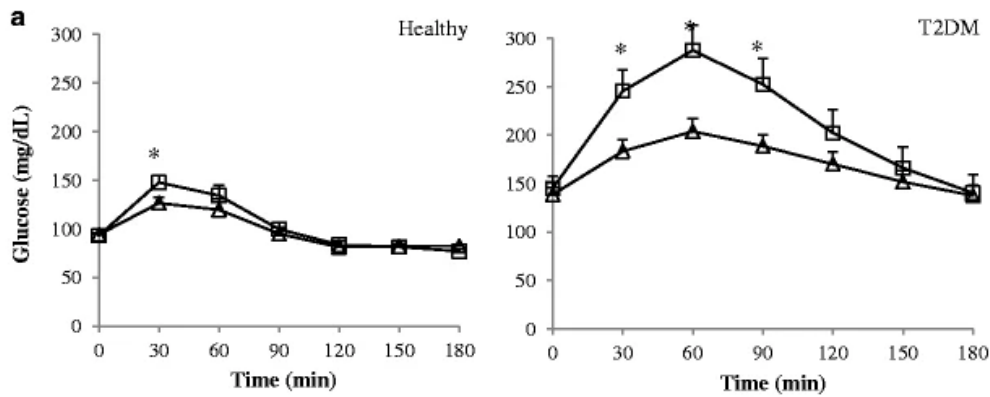


Figure 3: Glucose peaks in healthy and T2D group of persons after consumption of raisins or glucose

The same pattern could be observed in the case of the insulin peaks (Figure 4).

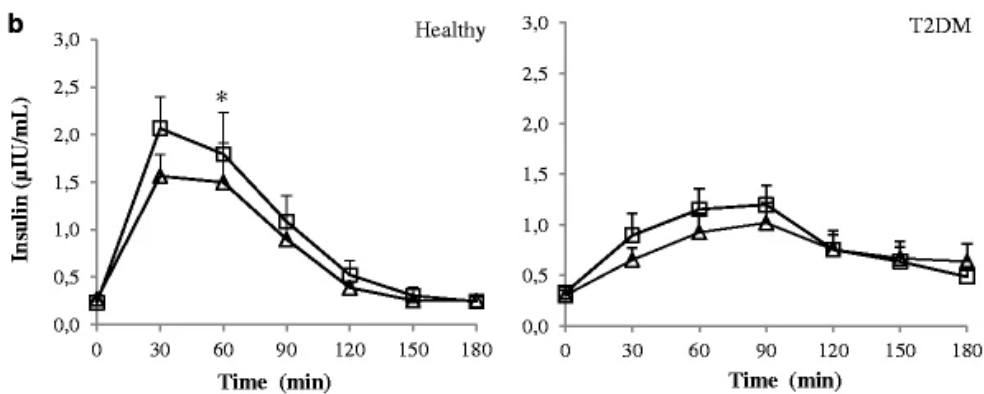


Figure 4: Insulin peaks in healthy and T2D group of persons after consumption of raisins or glucose

The explanation of this phenomenon could be the phenolics and dietary fiber content of the dried fruits. Based on the study of Wursch et al. (1997) the dietary fiber intake could improve glycaemic control which also can lead to lower plasma lipid concentrations in patients with type II diabetes.

3.1.4. Vitamin content

In addition to the numerous other health benefits, dried fruits frequently contain a high concentration of vitamins. In Table 2. nutrient content of prune is described.

Table 2: Nutrient content in prune in RDA (based on Stacewicz-Sapuntzakis et al., 2013)

Nutrient in prune	Concentration in 100 g serving (RDA*)
Magnesium	10 %
Niacin (Vitamin B ₃)	12 %
Vitamin B ₆	12%
Riboflavin (Vitamin B ₂)	14 %
Copper	31 %

*RDA: recommended dietary allowance

The Vitamin K₁ content of the prune is higher than in any other fruits (fresh or dried), and comparable to cashews and hazelnuts (Dismore et al., 2003). The nutrient content shows that consuming dried fruits is highly beneficial if someone would like to increase a vitamin intake, not only during the sunny months but even in winter. Figure 5. summarizes the health benefits of dry fruit consumption.

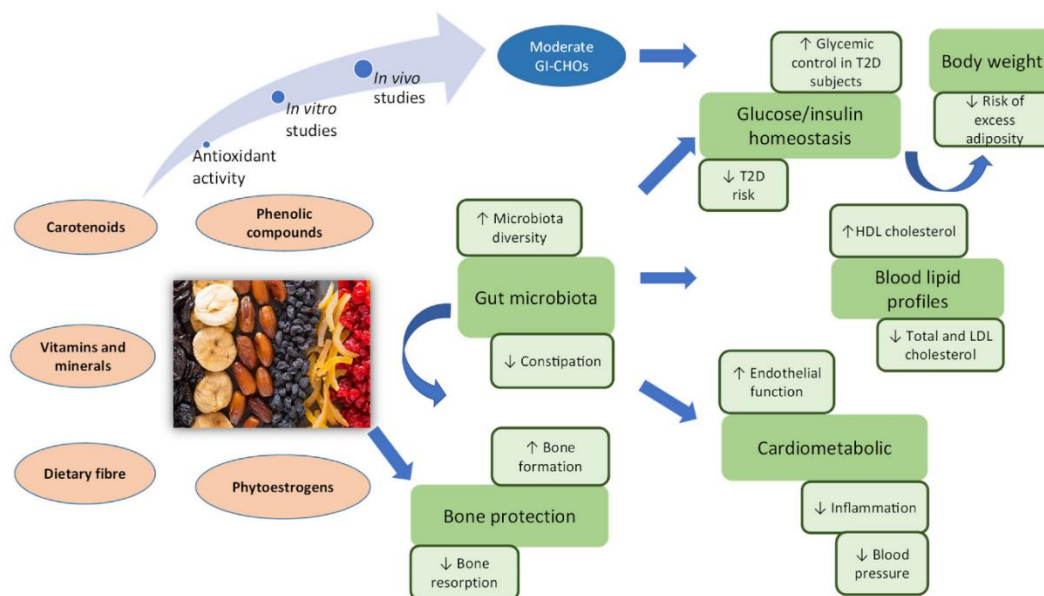


Figure 5: Health benefits of dry fruit consumption (Dargenio et al., 2023)

3.2. Microbial characteristics of dry fruits and the relevance of pathogenic bacteria

The complexity of the dried fruit matrix (low water activity, high sugar concentration) limits the growth of microorganisms. Wehner et al. described the microbiota of dried fruits in 1970. Table 3. shows the results of their study. The researchers found that *Aspergillus niger*, *Aspergillus flavous*, *Bacillus spp.*, *Mucor spp.*, *Rhizopus spp.*, and *Micrococcus* were the dominant microorganisms in different samples (such as raisins, apple, apricot, figs and prunes).

Table 3: Average type of microorganisms after incubation at 25°C and 37°C (based on Wehner et al., 1970)

Sample name	Microorganisms name	
	25°C	37°C
Raisins	<i>Aspergillus niger</i> , <i>Bacillus</i> spp., <i>Micrococcus</i> spp.	<i>Aspergillus niger</i> , <i>Aspergillus flavus</i> , <i>Bacillus</i> spp.
Dried aricot	<i>Aspergillus niger</i> , <i>Bacillus</i> spp., <i>Micrococcus</i> spp.	<i>Aspergillus niger</i> , <i>Bacillus</i> spp., <i>Micrococcus</i> spp.
Prunes	<i>Aspergillus niger</i> , <i>Bacillus</i> spp., <i>Micrococcus</i> spp.	<i>Aspergillus niger</i> , <i>Aspergillus flavus</i> , <i>Bacillus</i> spp., <i>Micrococcus</i> spp.

Nevertheless, the low water activity can inhibit the growth of microorganisms, and some studies have demonstrated that the dominant foodborne pathogens are capable of surviving under these condition. A study by Sheng et al. (2023) revealed that *Salmonella* spp., *E. coli* (STEC) and *Listeria monocytogenes* can survive on the surface of dried fruits, including apricots, cranberries, dates, plums, raisins, strawberries, and tomatoes. Furthermore, Bourdoux et al. (2016) identified *Salmonella* spp. and *Bacillus cereus* as the primary causes of outbreaks associated with dried fruits.

3.3. Outbreaks associated with dried fruits

To ensure the safety of dried fruits, the pathogen microorganisms' ability to survive and reproduce must be investigated. The water activity (a_w) of the dried fruits is approximately 0.85, which is below the minimum a_w required for bacterial growth, estimated to be around 0.87 (Beuchat et al., 2013). Consequently, dried fruits are regarded as generally safe food products. On the other hand, it is important to note that even food with low water activity could be contaminated with fungi, so the dried fruits are not sterile. This could be a reason why food borne outbreaks can occur. The most recent outbreak associated with dried fruits occurred in March 2019. Thirty-nine people were confirmed with salmonellosis caused by *Salmonella* Agbeni because of consumption of a contaminated dried fruit mix in Norway (Norwegian Institute of Public Health, 2019). Several studies show that the microbial contamination can occur during the harvesting, drying, storage, transportation, and handling of dried products. Furthermore, a couple of previous study shows that food borne pathogens can survive on the surface of the dried fruits. According to the World Health Organization (WHO) unsafe food containing harmful bacteria, viruses, parasites or chemical substances causes more than 200 different types of diseases (Internet 1). In the annual announcement of WHO from 2007 to 2015 each year more than one million people are infected with foodborne pathogens. The risk of foodborne diseases has increased during the last decades, despite the improvements in the production of food stuffs. It is estimated that almost one-quarter of the world population is at risk of foodborne illnesses (Silk et al., 2013). The study of Dobó et al. (2023) reviewed the food-borne outbreaks related to low water activity food products (Table 4).

Table 4: Association of foodborne pathogens with outbreaks of low water activity foods (Source: Dobó et al., 2023)

Low water-activity products	Pathogenic microorganism	Disease	Reference
Raw cake batter	<i>E. coli</i>	<i>E. coli</i> infection	CDC 2021
Flour	Shiga toxin-producing <i>E. coli</i> (STEC)	<i>E. coli</i> infection	CDC 2016
Flour	<i>E. coli</i>	<i>E. coli</i> infection	CDC 2019
Flour (wheat, spelt, and rye)	Shiga toxin-producing <i>Escherichia coli</i> (STEC)	<i>E. coli</i> infection	Food Safety News 2020
In-shell hazelnuts	<i>E. coli</i> O157:H7	<i>E. coli</i> infection	CDC 2011
Raw refrigerated, pre-packaged cookie dough	<i>E. coli</i> O157:H7	<i>E. coli</i> infection	CDC 2009
Exotic dried fruits	<i>Salmonella</i> Agbeni	Salmonellosis	Johansen et al. 2021
Dried fruit or ice cream served in open containers	Hepatitis A virus (HAV)	Hepatitis	Howitz et al. 2005
Powdered milk formula	<i>Enterobacter sakazakii</i>	Necrotising enterocolitis	Van Acker et al. 2001
Chocolate products	<i>Salmonella</i> Typhimurium	Salmonellosis	WHO 2022

According to the review of Dobó et al. (2023) *E.coli* (mainly STEC and O157:H7) and *Salmonella* cause the highest number of outbreaks related to low water activity food products.

3.3.1. *Salmonella* genus

Salmonella genus belongs to the Enterobacteriaceae family of Gram-negative, rod-shaped bacteria. The three species of *Salmonella* are *S. enterica*, *S. bongori*, and *S. subterranea*. *S. enterica* has more than 2300 serotypes (USDA, Internet 2) but *S. Typhimurium* and *S. Enteritidis* are the most common pathogens related to food (Robinson, 2013). *S. Enteritidis* is one of the most frequently isolated foodborne pathogens, mainly eggs and poultry meat can be contaminated with *S. Enteritidis*. *S. Typhimurium* is one of the most prevalent serotypes globally, often found in beef, poultry and milk products. The most typical characteristics of *Salmonella* genus are listed in Table 5.

Table 5: Basic characteristics of *Salmonella* spp. (based on O’Beirne and Francis, 2003)

Basic characteristics of <i>Salmonella</i> species	
Classification	Bacteria
Spore forming ability	Non-spore formers
Shape, motility	Rod-shaped
Temperature preference	Mesophilic (T _{opt.} : 35-43 °C)
Gram staining	Gram-negative
Lactose fermentation	Non-fermenter
Catalase test	Catalase positive
Oxidase test	Oxidase negative

The study by Ehuwa et al. (2021) shows that salmonellosis is the second most reported gastrointestinal disorder in the EU resulting from the consumption of *Salmonella*-contaminated foods. Figure 6. shows all the symptoms which can occur if someone is infected with a pathogenic serotype of *Salmonella*.



Figure 6: Symptoms of salmonellosis

Figure 6. shows the food types which have the biggest risk to be contaminated with *Salmonella*. The *Salmonella* food related outbreaks are a common problem even in the developed countries. According to the European Food Safety Authority (EFSA) the reported salmonellosis cases are over 91,000 in the EU per year (Internet 3). Figure 7. shows data about the most common food types associated with *Salmonella* outbreaks.

Foods associated with *Salmonella* outbreaks

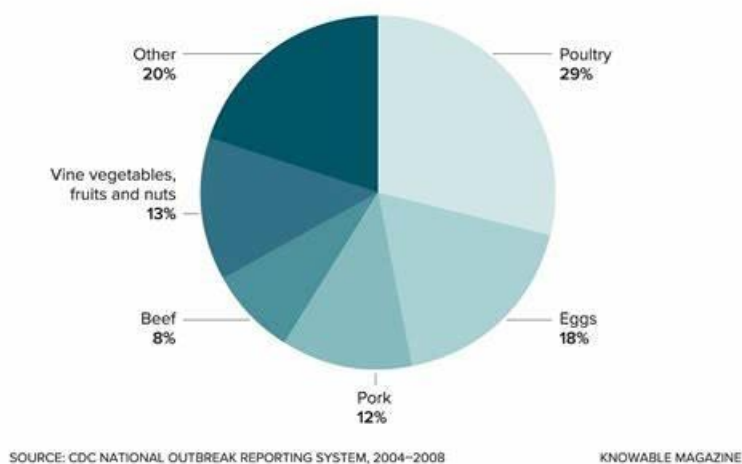


Figure 7: Food types associated with *Salmonella* outbreaks (Source: Internet 4)

The biggest problem related to *Salmonella* is its survival for extended periods in low moisture food products. In the case of spices and herbs, this ability of survival is particularly a big problem, because the worldwide trade of these products provides a great way for *Salmonella* to spread. Table 6. shows the duration of survival of different *Salmonella* serotypes in dry food products.

Table 6: *Salmonella* survival in different food types (Source: Ehuwa et al., 2021)

Food	<i>Salmonella</i> Serotypes	Survival Times	Reference
Dried milk products	<i>S. Infantis</i> , <i>S. Typhimurium</i> , <i>S. Eastbourne</i>	≤10 months	Ray et al. (1972)
Desiccated plastic surface Pasta	<i>S. Typhimurium SL 1344</i> , <i>S. Infantis</i> , <i>S. Typhimurium</i> , <i>S. Eastbourne</i>	<100 weeks ≤12 months	Gruzdev et al. (2012) Rayman et al. (1979)
Milk chocolate	<i>S. Infantis</i> , <i>S. Typhimurium</i> , <i>S. Eastbourne</i>	>9 months at 20 °C	Tamminga et al. (1976)
Bitter chocolate	<i>S. Eastbourne</i>	≤9 months at 20 °C	
Peanut butter	<i>S. Agona</i> , <i>S. Enteritidis</i> , <i>S. Michigan</i> , <i>S. Montevideo</i> , <i>S. Typhimurium</i>	≤24 weeks at 5 °C ≤6 weeks at 21 °C	Kozak et al. (2013) Burnett et al. (2000)
Paprika powder	multiple serotypes	>8 months	Ray et al. (1971)

3.3.2. *Escherichia coli*

According to the World Health Organization *E. coli* is a bacterium that is commonly found in warm-blooded animals, mainly in the gut of humans. Table 7. shows some important characteristics relevant to *E. coli*.

Table 7: Basic characteristics of *E. coli* (based on Mueller Mcand Tainter CR. *Escherichia coli* Infection, 2013, NCBI)

Basic characteristics of <i>Escherichia coli</i>	
Classification	Bacillus
Spore forming ability	Non-spore former
Shape, motility	Flagellated
Temperature preference	Mesophilic (T _{opt.} : 37 °C)
Gram staining	Gram-negative
Lactose fermentation	Ferment lactose (grows on MacConkey agar)
Catalase test	Catalase positive
Oxidase test	Oxidase negative

Most of the strains of *E. coli* are harmless for humans. Some strains, however, such as Shiga toxin-producing *E. coli* (STEC), can cause severe foodborne disease. It is transmitted with the consumption of contaminated food, such as raw or undercooked ground meat products, raw milk, and contaminated raw vegetables and sprouts.

"STEC can grow in temperatures ranging from 7 °C to 50 °C, with an optimum temperature of 37 °C. Some STEC can grow in acidic foods, down to a pH of 4.4, and in foods with a minimum water activity (a_w) of 0.95. STEC is destroyed by thorough cooking of foods

until all parts reach a temperature of 70 °C or higher. E. coli O157:H7 is the most important STEC serotype in relation to public health; however, other serotypes have frequently been involved in sporadic cases and outbreaks." (WHO, 2018)

Symptoms of the diseases caused by STEC could include diarrhoea (in some cases bloody diarrhoea) and abdominal cramps. Vomiting and fever may also occur. The incubation period can range from 3 to 8 days and most patients recover within 10 days. However, in a small proportion of patients (particularly young children and the elderly), the infection may lead to a life-threatening disease such as haemolytic uraemic syndrome (HUS). Antibiotics are not part of the treatment of patients with STEC disease and may possibly increase the risk of subsequent HUS. WHO handled a guide how to prevent the infection in the case of fruits and vegetables. The "*Five keys to growing safer fruits and vegetables*" provides rural workers who grow fresh fruits and vegetables for themselves, their families and for sale in local markets. It contains the key practices to prevent microbial contamination during planting, growing, harvesting and storing.

Table 8. shows how grape extract can inhibit the activity of *E. coli* and how the effect is observed.

Table 8: Fruit extracts to control *E. coli* (based on Heliyon et al., 2020)

Nr .	Fruit extract	Extraction/ Concentration	Active molecule(s) identified	Effect observed	Reference
1	Grape seed and pomace extract	N/A	?	Reduction of Shiga toxin expression (gene transcription level)	Quinones et al., 2009
2	Grape	Commercial extract	?	Block toxin binding to cell surface, prevent unfolding and translocation into cytosol of CTA1, abolish catalytic activity	Reddy et al., 2013
3	Grape	Fruit extract	Epigallocatechin gallate and procyanidin B2	Inhibit CT binding to cell surface	Cherubin et al., 2016
4	Grape seed	10% ethanol extract, 0.5 mg ml ⁻¹	?	Effect on quorum-sensing and virulence factors of non-O157:H7	Sheng et al., 2016

The results show that both inhibition of gene expression and blocking of binding and penetration are effective as well. With grape seed extract even the inhibition of quorum-sensing is possible.

3.4. Pathogen survival on dry fruits surface

Beuchat and Mann (2014) reported in their study that *Salmonella* survived for 182 days on dried strawberries and 242 days on dried cranberries and raisins when inoculated products were stored at 4 °C. When stored at 25 °C, *Salmonella* could survive on dried apples for more than 28 days (DiPersio et al., 2003) and *L. monocytogenes* could survive in dried peaches for at least 14 days (Dipersio et al., 2004). In the study of S.S. Canakapalli (2021) *Salmonella* spp., *Escherichia coli* O157:H7, and *Listeria monocytogenes* survival was monitored for 6 months on the surface of dried peaches, dried peaches processed with sulfur, dried pluots processed with sulfur, sundried tomatoes, high-moisture Medjool dates, and low-moisture Medjool dates. Figure 8. illustrates that the *E. coli* growth reached the limit of detection after 150 and 180 days in the case of dates, less than 30 days in the surface of plums and after 60 days on sundried tomatoes in all media. Figure 9. shows the findings of the *Salmonella* survival study. The results are comparable for plums and sundried tomatoes, however, *Salmonella* was still detectable on the surface of dates after 180 days.

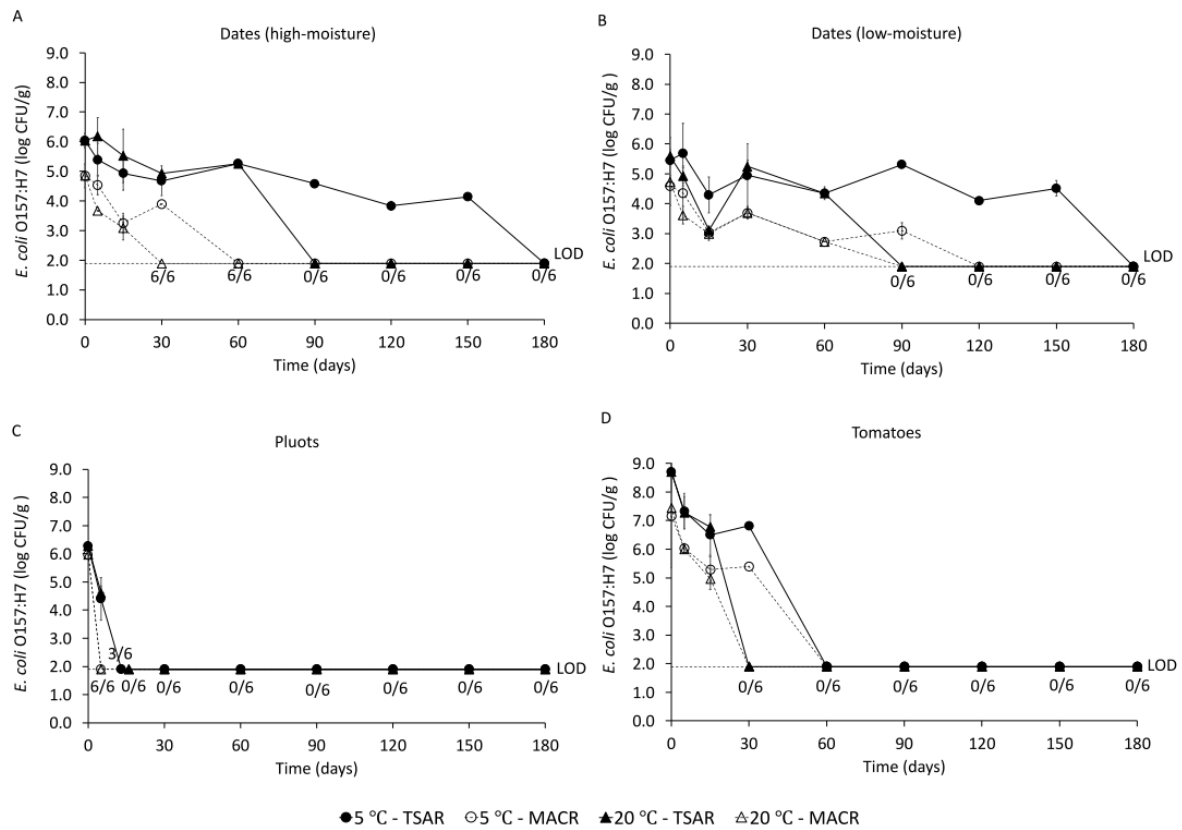


Figure 8: *E. coli* O157:H7 survival on dates, pluots and sundried tomatoes

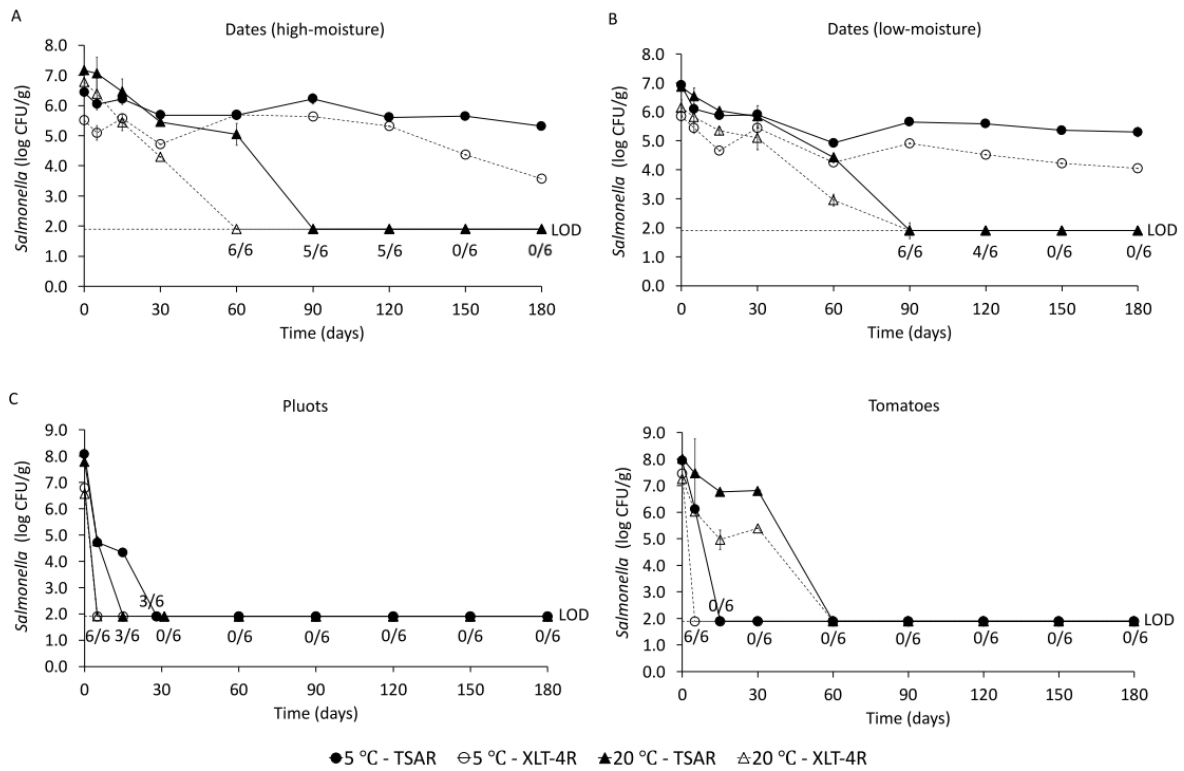


Figure 9: *Salmonella* survival on dates, pluots and sundried tomatoes

The study of Zhuosheng et al. from 2021 described the survival of the most common foodborne pathogens (*Salmonella*, *Listeria monocytogenes* and Shiga-toxin producing *Escherichia coli*, known as STEC) on dried apricots with and without SO₂ treatment. Their study shows when dried apricots were inoculated with the wet carrier, all three pathogens survived the 48- hour drying period. Among all three pathogens, STEC survived with higher culturable numbers for a longer period of time than the other two pathogens with no SO₂ treatment over the 90-day storage period. When pathogens were inoculated via the dry carrier, *Salmonella* survived with higher culturable numbers among three pathogens with no SO₂ treatment, indicating its enhanced survivability after being dried on sand and exposed to stress conditions before inoculation. In addition, regardless of the inoculation carriers, pathogens survived for longer time or with higher levels with no SO₂ treatment, indicating the antimicrobial properties of free SO₂. In the summary of their study, it was reported that the pathogens can survive on dried fruits; factors affecting their survival include the types of the pathogens, inoculation methods, and the use of sulphur dioxide.

3.5. Sugar content of dry fruits and the osmotic stress respond

A substantial amount of research has been conducted to investigate the impact of high sugar concentration on the osmotic stress response in *E. coli*. In a study conducted by Karlheinz et al. (2009) it was discovered that the presence of sugars can trigger the synthesis of trehalose in *E. coli*. This acts as an osmoprotectant, stabilising proteins and cellular structures under stress. The results demonstrated that the transport systems ProP, ProU, BetT, and BetU are involved in the uptake of osmoprotectants, which assist *E. coli* in managing osmotic stress in high sugar environments.

4. Materials and methods

4.1. Media:

1. Buffered pepton water: 25.5 g of buffered pepton water powder was dissolved in 1 liter of deionized water. It was homogenized with a magnetic stirrer and autoclaved for 15 minutes at 121 °C.
2. LB broth: 25 g of LB broth powder was dissolved in 1 liter of deionized water. It was homogenized with a magnetic stirrer and autoclaved for 15 minutes at 121 °C.
3. LB agar: 25 g of LB broth powder and 15 g of agar were dissolved in 1 liter of deionized water. After homogenizing in a stirring water bath (at 99 °C) for 20 minutes, it was autoclaved for 15 minutes at 121 °C, cooled down to 50 °C and poured into plates.
4. Low sugar broth, apricot mimicking LB broth, prune mimicking LB broth and raisin mimicking LB broth contained different sugars in diverse ratios as indicated in Table 9.

Table 9. Sugar content of low sugar broth and dried fruit-mimicking LB broth media used in this study.

Name of the mimicking broth	Glucose concentration (g/100 ml)	Fructose concentration (g/100 ml)	Saccharose concentration (g/100 ml)
Low sugar broth	5,4	-	-
Apricot broth	33,1	12,5	7,89
Prunes broth	25,5	12,4	0,15
Raisin broth	31	34,7	-

5. TSA agar: 40 g TSA was dissolved in 1 liter of deionized water. After Homogenization in a stirring water bath (99 °C) for 20 minutes, it was autoclaved for 15 minutes at 121 °C, cooled down to 50 °C, and poured into plates.
6. TSA agar with actidion: 40 mg actidion was dissolved in 1 ml of ethanol and added to the cooled TSA agar (1 liter). The agar was poured into Petri dishes.
7. DG18 agar: 31.6 g DG18 agar powder was dissolved in 1 liter of deionized water. After homogenization in a stirring water bath (at 99 °C) for 20 minutes, 175 ml of glycerol was added to it. The agar was poured in plates after autoclaving for 15 minutes at 121 °C, and cooling down to 50 °C.

4.2. Methods

4.2.1. Isolation of microorganisms from dried fruit samples (Screening round 3)

During the previous experiments two rounds of the screening was already completed. The aim of the first round was to ascertain the *E. coli* concentration in the dried fruits samples. The second run was about not only to determine the *E. coli* concentration, but to determinate the total bacterial, yeast and mould counts as well. Moreover, the aim was to identify the isolated microorganisms using of the Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry (MALDI-TOF MS) device. In the frame of this study, in the third round of the experiment, the main goal was to identify the bacteria, yeasts and moulds not only by MALDI-TOF MS, but with 16S rDNA sequencing as well.

The samples were weighted into Stomacher bags with buffered peptone water in 1:5 dilution ratio (10 g of sample and 50 ml of sterile buffered peptone water).

For homogenization a Stomacher device (Seward Stomacher 400 circulator) was used for 2 minutes at 230 rpm. Two types of agar were used for the plating: DG18 agar was used for yeast and mould determination, while for the bacterial count estimation Triptone Soya (TS) or Plate Count (PC) Agar supplemented with actidion (for inhibition of the growth of fungi) was applied. On Figure 10. the layout of the isolation is summarized.

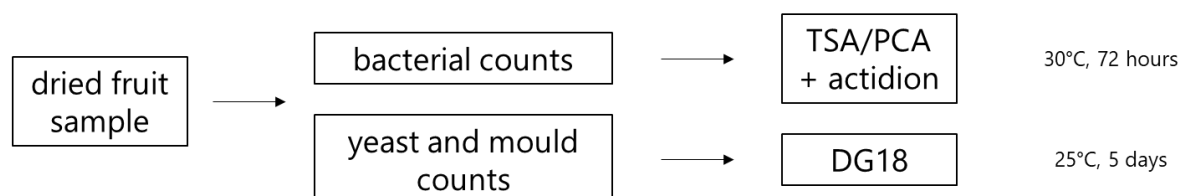


Figure 10: Structure of the Screening round 3

4.2.2. Matrix-Assisted Laser Desorption/Ionization Time of Flight - Mass Spectrometry (MALDI-TOF MS)

In order to determine the microbiota of dried fruit samples, MALDI-TOF MS was used. Colonies grown on the plates from the isolations were used to identify the culturable microorganisms.

With a sterile toothpick one colony from the agar was picked and transferred to the MALDI-TOF target plate. The spots were overlayed with 1 µl of 70% formic acid and airdried at room temperature. After drying 1 µl of HCCA matrix were added.

The HCCA matrix in an undissolved form was provided. The matrix was dissolved in 250 µl OS-solution and vortexed at room temperature until the solution was clear.

4.2.3. Identification of the bacterial isolates by 16S rDNA sequencing

1. DNA isolation:

For the isolation of the DNA from the food sample, the DNeasy PowerFood Microbial DNA Isolation Kit (Quiagen) was used as described by the manufacturer.

2. Polymerase chain reaction (PCR):

In order to amplify the 16S rDNA genes of the bacteria isolated from the dried fruit samples, the polymerase chain reaction (PCR) method was used. First the II/17 (forward) and II/18 (reverse) primers were diluted to 10 pmol/µl concentration, then the master mix was prepared according to Table 10.

Table 10: Master mix for amplifying the 16S rDNA gene of bacteria by PCR

	Mastermix for one sample	Mastermix for "n" sample
AccuStart II	12.5 μ l	12.5 μ l $\times$ (n + 3)*
UHQ	9.5 μ l	9.5 μ l $\times$ (n + 3)*
Forward primer	1 μ l	1 μ l $\times$ (n + 3)*
Reverse primer	1 μ l	1 μ l $\times$ (n + 3)*
Total amount	24 μ l	24 μ l $\times$ (n + 3)*

*: n plus negative control, positive control and pipetting loss (altogether 3 more samples)

Into the PCR tubes 24 μ l master mix and 1 μ l of the isolated DNA was added. The tubes were vortexed and spined. The 16S rDNA gene was amplified according to the protocol in Figure 11.

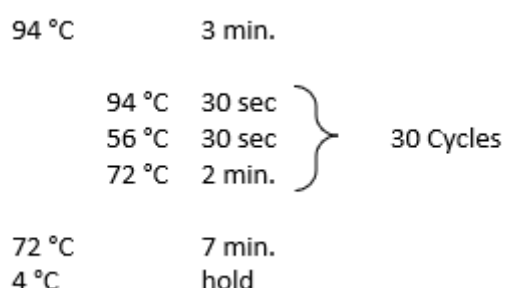


Figure 11: Parameters of PCR used for amplification of 16S rDNA genes

3. Determination of DNA concentration using Qubit 2.0 fluorometer:

In order to determine the DNA concentrations of the samples, the Qubit (Invitrogen) device was used. All of the reagents were used at room temperature. For the working solution (WS) 199 $\times$ n μ l of the Qubit Buffer and 1 $\times$ n μ l Qubit reagent was added. The “n” means the number of the samples which was measured at one time. In the case of the standard solutions, 190 μ l was added from the WS to the Qubit assay tubes and 10 μ l of the provided standards. In the case of the samples, 199 μ l was added from the WS to the Qubit assay tubes and 1 μ l of the samples to measure the DNA concentration.

After the tubes were loaded all of them was vortexed 2-3 seconds and incubated at room temperature for 2 minutes. After the incubation period, the tubes were read with the Qubit 2.0 Fluorometer in the dsDNA – High sensitivity assay. In every measurement the stock concentration was calculated.

4. Gel electrophoresis:

After the polymerase chain reaction, agarose gel electrophoresis was used for detection of the amplified DNAs. For the electrophoresis an agarose gel was prepared according to the following steps:

- Heat water bath to 50°C
- Prepare 0,75x TAE buffer by diluting 50x TAE buffer (Art.Nr.: A4686, Applichem) with deionized water
→ 15 ml 50x TAE/1 l water

- Weigh the necessary amount of LE-agarose in a sterile flask with a flamed spatula and fill it up with 0.75x TAE buffer as described in Table 11.

Table 11: Gel weighting for gel-electrophoresis

Concentration	Amount of agarose		
	1%	1.5%	2%
Small gel: 40ml 0.75x TAE buffer	0.4 g	0.6 g	0.8 g
Large gel: 70ml 0.75x TAE buffer	0.7 g	1.05 g	1.4 g

- Dissolve by heating in a microwave
- Cool it to 50°C in the water bath
- Prepare gel tray by sealing the open ends with tape
- Align the pouring plate perfectly horizontally
- Place gel tray on pouring plate and pour the solution in the tray, avoid bubbles
- Insert comb
- Let gel polymerize for 30 minutes.

Before loading the electrophoresis chamber has to be prepared as well. For this the cooling system has to be turned on and the chamber has to be filled with approximately 1.9 liter of 0.75X TAE-buffer. Afterwards the pump has to be turned on for a short period of time to make sure the pump system is filled with buffer, then it has to be turn off again. After preparing the chamber, the tape from the gel tray was removed and the gel was put into the chamber. The comb was removed carefully. On parafilm 1.5 µl loading dye and 5 µl sample was mixed by slowly pipetting up and down. 5 µl of the mixture was loaded into the wells and 4 µl of marker was loaded into the first and last wells (100 bp (A5216_AppliChem); 100 bp ext. (T835.2_CarlRoth, 100-5.000 bp)). After the sample loading, the program was chosen with 80 V, 2000 mA and 300 W settings. The electrodes were connected to the power supply unit and the electrophoresis was started. When the voltage reached the set 80 V value, the pump was set to the “pump delay 4.5 min” setting.

5. Gel staining for gel electrophoresis:

In order to make the position of DNAs visible, the Gel Red dye solution was used. To prepare the Gel Red solution, 250 ml sterile, deionized water and 70 µl Gel Red was mixed in a staining tray. The gel was put into the solution and the rocker was turned on for 30 minutes. After the incubation period the gel was put into the Gel Doc device (Bio-Rad) with avoiding the bubbles underneath the gel and positioning into the middle of the drawer of the Gel Doc device. A photo of the gel was taken and saved for evaluation.

4.2.4. Sequencing

Oxford Nanopore device is a pocket-size, portable, real-time sequencing machine. The device does the sequencing based on the change of the electric current. The main parts of the device are the nanopores (nanoscale protein pores) which are the biosensors. The other important part is the flow cell which contains an electrolyte solution where a constant voltage is applied. As the DNA flows thorough the nanopore, it changes its position from the negatively charged ‘cis’ side to the positively charged ‘trans’ side (translocation). The changes in the ionic current are correspond to the nucleotide sequence which is interpreted by the MinKNOW software. The motor protein of the translocation could control the speed of the procedure but also has helicase

activity to unwind the double-stranded DNA, because the Nanopore device can handle only single-stranded DNA (Wang et al, 2021). Most important steps of sequencing (like the sample preparation and the flow cell loading) were done based on the manufacturers' protocol.

4.2.5. 4.2.4. Sugar and sulfur-dioxide determination with Rida Cube device

In order to measure the sugar and the SO₂ concentrations of the dried apricot, prune and raisins samples, the Rida Cube device was used. For the first step the samples were soaked in sterile deionized water in 6 ± 1 °C for overnight in 1:2 dilution ratio (5 g sample and 10 g sterile deionized water). For the homogenization of the samples Ultraturrax device was used for 2 minutes at 8000 m⁻¹ efficacy. After the homogenization step, the samples were centrifuged in 4000 rpm for 10 minutes. For the sugar concentration measurement, the Rida Cube Sucrose / D-Glucose / D-Fructose RCS4190 kit was used. According to the kit description, 20 µl supernatant was added to the provided Rida Cube tube. For the SO₂ concentration measurement, the Rida Cube SO₂-Total RCS4600 kit was used with 100 µl of the supernatant.

4.2.6. 4.2.5. Determination of growth curves by Bioscreen

In order to determine the growing potential of *E.coli* and to compare the laboratory strain with the isolated one, an experiment was done using a Bioscreen device (Growth Curves Bioscreen C Pro) for 7 days at 37 °C. First an overnight culture was made from both strains in three parallel. That the optical density of the overnight cultures was measured in 600 nm and adjusted to 0.1 applying fresh culture media either of LB or the mimicking ones. The following formula was used for the calculations:

$$OD_1 \times V_1 = OD_2 \times V_2$$

OD₁: the measured OD of the overnight culture

V₁: amount of overnight culture used to set the OD to 0.1

OD₂: the adjusted OD; 0,1

V₂: the final volume of the solutions; 5 ml

After the calculation, the amount of V₁ was discarded from the fresh broth and same amount of overnight culture was added. Figure 11. shows the structure of the well-plate loading.

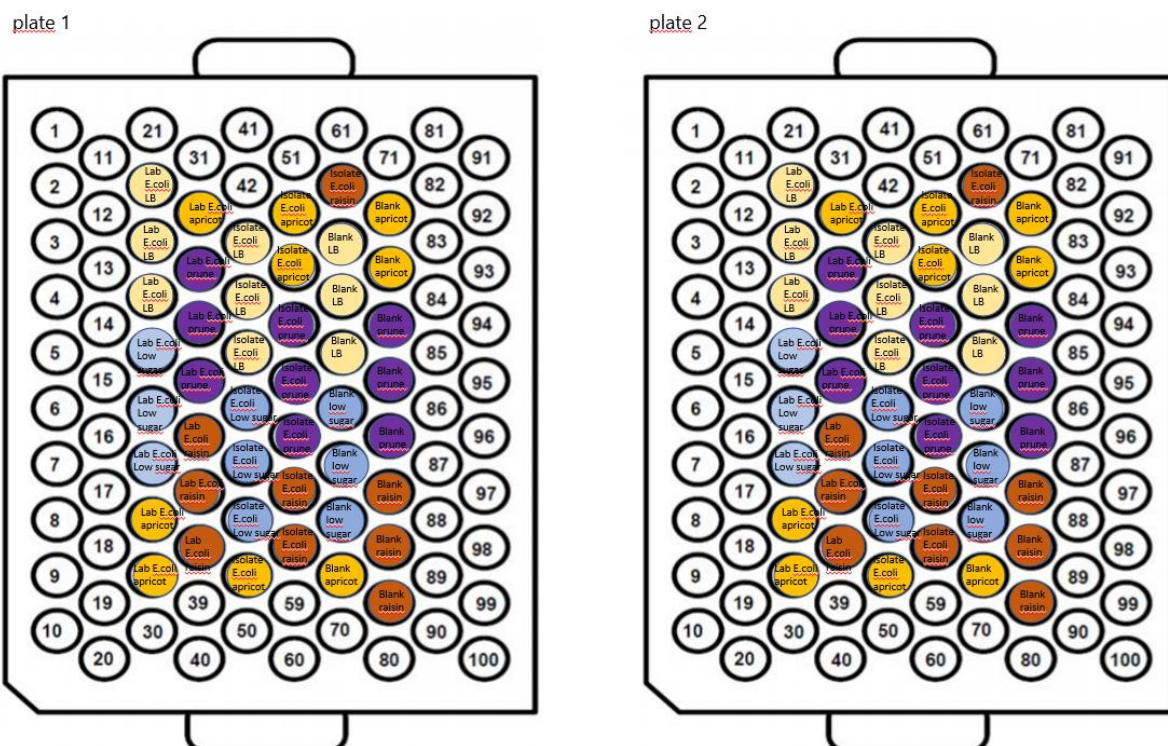


Figure 12.: The location of samples in the Honeycomb plate.

Figure 12. shows the loading positions of the Honeycomb plate. All the samples were loaded in three parallels with all the media. The protocol in Table 12. was used in the Bioscreen.

Table 12: Bioscreen device working parameters

Temperature	37 °C
Filters	600 nm
Shaking	before the measurement, linear pattern, low amplitude
Shaking duration	15 seconds
Measurement interval	every 30 minutes
Experiment duration	7 days

The results were evaluated with the JMP software.

5. Results

5.1. Results of MALDI TOF-MS analysis of bacteria derived from different dried fruit samples

One of the aims of the research was to determine the dominant microorganism of dried fruits. It is a fundamental right and a basic consumer demand that food products should be safe for human consumption. This applies as much to dried apricots, raisins and prunes as to any other foodstuff. It is therefore vital to investigate the microbiota of these products. The first research field of this study was the determination of typical microbiota of dried fruits. The samples were taken from different markets, where the storage conditions were different as well. In the frame of the research, altogether 31 samples (eleven dried apricot, 11 raisin and nine prune samples) were studied. In tables 13-15. the results of MALDI TOF-MS analyses are shown.

Table 13: Results of identification by MALDI TOF-MS in the case of bacteria isolated from raisins

Sample number	Sample origin	Isolated bacteria	Nr. of isolated bacteria types	MALDI score value
6	Hungary	<i>Bacillus cereus</i>	9	1,91
		<i>Bacillus velezensis</i>		1,95
		<i>Ureibacillus sinduriensis</i>		1,98
		<i>Priestia flexa</i>		2,34
		<i>Bacillus licheniformis</i>		2,01
		<i>Micrococcus luteus</i>		2,23
		<i>Peribacillus simplex</i>		1,83
		<i>Alkalihalobacillus clausii</i>		1,76
		<i>Niallia circulans</i>		2,05
7		<i>Bacillus velezensis</i>	3	1,72
		<i>Niallia circulans</i>		1,7
		<i>Cytobacillus horneckiae</i>		1,7
10		<i>Peribacillus muralis</i>	1	1,71
12		<i>Bacillus mycoides</i>	4	2,09
		<i>Bacillus velezensis</i>		2,05
	<i>Peribacillus simplex</i>	2,1		
	<i>Bacillus cereus</i>	2,03		
15	<i>Metabacillus idriensis</i>	1	1,81	
18	<i>Bacillus mycoides</i>	2	2	
	<i>Bacillus cereus</i>		2,29	
23	Austria	<i>No organism identification possible</i>	1	N/A
24		<i>Priestia megaterium</i>	4	2,25
		<i>Kocuria rosea</i>		2,47
		<i>Micrococcus luteus</i>		2,36
		<i>Bacillus pumilus</i>		1,92
26		<i>Bacillus pumilus</i>	2	1,76
		<i>Bacillus subtilis</i>		2,05

Sample number	Sample origin	Isolated bacteria	Nr. of isolated bacteria types	MALDI score value
27		<i>Priestia megaterium</i>	1	2,31
31		<i>Bacillus mycoides</i>	4	2,02
		<i>Bacillus cereus</i>		2,02
		<i>Micrococcus luteus</i>		2,35
		<i>Bacillus thuringiensis</i>		1,9

Table 13. shows that in the case of raisins altogether 32 microbes were isolated, and the dominant microorganisms belonged to Gram-positive group of bacteria. Most of them naturally occur in the human body or in the nature. For example, *Bacillus licheniformis* and *Ureibacillus sinduriensis* can often be found in the soil. Others, like *Bacillus cereus* (which was represented by four isolates) can cause food poisoning, or some strains can even cause more serious health issues. Another possibly harmful bacterium is *Kocuria rosea*, which can infect the human body and is able to cause bacteraemia (bacteria in the bloodstream) or endocarditis (infection of heart membrane or heart valves) (Purty et al 2013). *Priestia flexa* is a relative new microorganism, but some recent studies have found that certain strains can be harmful for the human body. In 2024, a new study came out with strains 21LM07, 21LM367, and 21LM1136, which are described as possible causative agents of urinary tract infections (Gbégbé et al 2024). On the other hand, *Priestia flexa* strains can be useful as well. In a study from 2023 (Deswal et al., 2023), the researchers got the result that *Priestia flexa* strain KS1 can degrade the mucin in the faeces, as they said; "*P. flexa can produce glycan hydrolysis enzymes that convert complex mucin oligosaccharide chains into simple glycans*". Some species have been demonstrated to exert beneficial effects on human health. For instance, *Bacillus velezensis* has been shown to possess probiotic properties.

Table 14: MALDI TOF-MS - identified bacteria isolated from dried apricot samples

Sample number	Sample origin	Isolated bacteria	Nr. of isolated bacteria types	MALDI score value	
1	Hungary	<i>Paenibacillus glucanolyticus</i>	1	1,8	
5		<i>Bacillus licheniformis</i>	3	2,05	
		<i>Bacillus mojavensis</i>		1,93	
		<i>Bacillus subtilis</i>		2,27	
9		<i>Bacillus mojavensis</i>	2	1,88	
		<i>Solibacillus silvestris</i>		1,7	
13		<i>Bacillus cereus</i>	2	2,05	
		<i>Micrococcus luteus</i>		2,28	
17		<i>No organism identification possible</i>	1	N/A	
20		<i>Bacillus cereus</i>	1	2,14	
22	Austria	<i>Paenibacillus lautus</i>	2	1,91	
		<i>Priestia megaterium</i>		2,1	
		25	<i>Bacillus cereus</i>	2	2,21
			<i>Enterococcus mundtii</i>		2,64
		28	<i>Bacillus mycoides</i>	1	1,91

Sample number	Sample origin	Isolated bacteria	Nr. of isolated bacteria types	MALDI score value
29		<i>Peribacillus simplex</i>	2	1,81
		<i>Cytobacillus firmus</i>		2,13
30		<i>Paenibacillus tylopili</i>	1	1,77

Out of the 18 isolates in one case identification was not possible, while the other isolates belonged to the Gram-positive group of bacteria. Fifteen isolates were rod shape, spore forming bacteria, and only two of them (*Enterococcus mundtii* and *Micrococcus luteus*) were morphologically distinct bacteria. *Enterococcus mundtii* was investigated in the case study of Higashide et al. (2005). The results show that *E. mundtii* causes endophthalmitis (inflammation of inner eye), so it can be considered as illness causing bacterium. The Gram-positive bacteria found in dried apricot were similar to bacteria of the raisins. The third group of samples consisted of prune samples, and Table 15. shows the results of the identification of bacteria by MALDI TOF MS.

Table 15: Bacteria from prune samples identified with MALDI TOF-MS

Sample number	Sample origin	Sample name	Isolated bacteria	Nr. of isolated bacteria types	MALDI score value
2	Hungary	Prunes	N/A	1	N/A
3			No organism identification possible	1	N/A
4			<i>Micrococcus luteus</i>	1	2,17
8		Mix of dried banana, prunes, blueberry, raisin and other	<i>Bacillus subtilis</i>	1	2,26
11		Prunes	<i>Bacillus mycoides</i>	1	1,94
14			<i>Bacillus licheniformis</i>	1	2,3
16			<i>Cytobacillus horneckiae</i>	1	1,89
19			N/A	1	N/A
21	Austria		N/A	1	N/A

In the case of prune samples, only five isolates could be identified, of which four were determined to be spore forming bacteria and one was identified as *Micrococcus luteus*, which is a common contaminant. Not only the diversity of bacterial species was described, but fungi were investigated with the MALDI-TOF MS device as well. There were three types of fungi which were detectable: *Zygosaccharomyces rouxii* (in Sample 1 and Sample 22), *Aspergillus*

niger (in Sample 24) and *Rhizopus stolonifer* (in Sample 26). It was a reasonable result as the samples were in open containers.

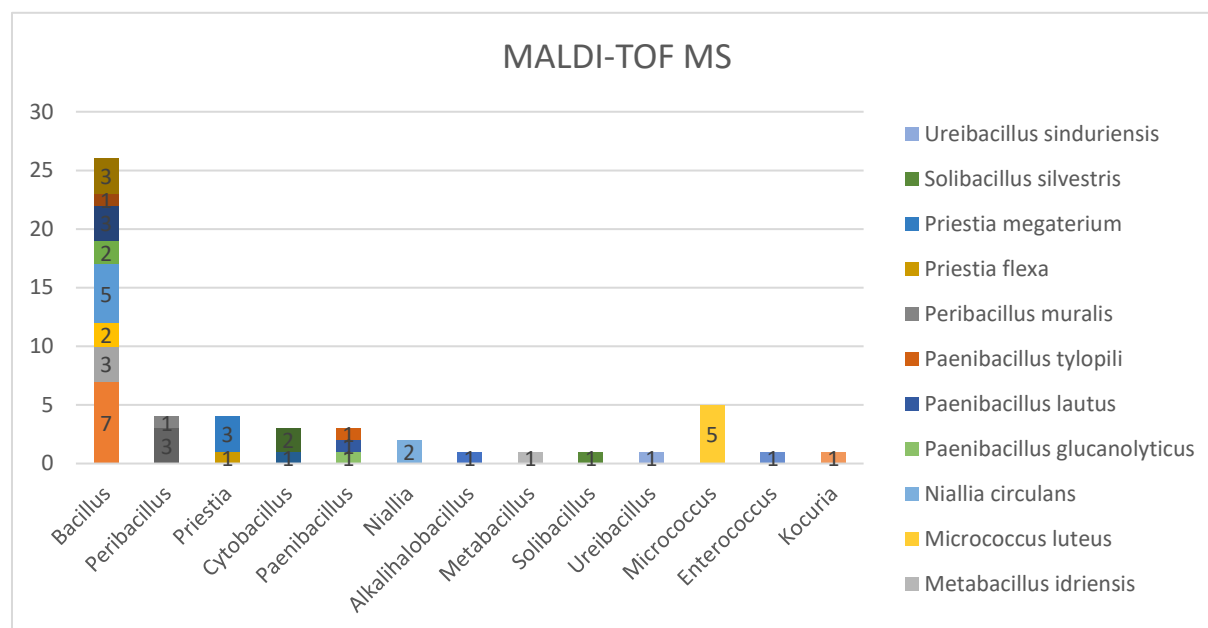


Figure 13 Frequency of bacteria isolated from dry fruit (raisins, apricot and prune) samples

In conclusion 25 types of bacteria and 3 types of fungi were identified with the MALDI-TOF MS device. Many bacteria proved to be *Bacillus* species (29 out of 53, which is 54.7%), which could be transferred from soil, environment, or even from human skin to the fruit, and are usually harmless. Moreover, majority of the isolates (46 out of 53 identified ones, which is 86.8% of the strains) are spore forming bacteria (Fig. 13). The presence of them in products with low water activity is not considered a noteworthy phenomenon, as endospores, as a particularly resilient formula, are capable of enduring adverse environmental conditions. Consequently, spore-forming bacteria can become dominant even in dried fruits characterised by low water activity.

5.2. Sequencing results

Annex 1. shows the result of the 16S rDNA sequencing. In total, it can be said that 124 types of bacteria were detected 616 times. In the case of twelve samples (samples no. 5, 6, 7, 8, 12, 13, 20, 24, 25, 26, 27, and 31) MALDI TOF-MS results yielded comparable outcomes. The highest number of matches was in the case of sample no. 6, but samples no. 8, 20, 25, and 27 fully matched with those of the MALDI-TOF MS results. Obviously, the sequencing gave more accurate overview of the microbiota. Bacterial identification of sample no. 13 by MALDI-TOF MS revealed *Bacillus cereus* and *Micrococcus luteus*. It is well known that *Micrococcus luteus* is the most common microorganism found in the laboratory environment and on human skin. It is interesting to note that the spore-forming microorganisms make up the majority of the microbiota detected by 16S rDNA sequencing, which is similar to the MALDI-TOF MS results. On the other hand, foodborne pathogens such as *Bacillus cereus* occur. Figure 14 shows the detailed results of the 16S rDNA sequencing. The figure shows only those microorganisms that had at least two species. It is easy to see that the *Bacillus spp.* had the highest number of

identification, which is similar to the results of MALDI-TOF MS. *Bacillus cereus* was detectable 7 times with the MALDI-TOF MS and 13 times with the 16S sequencing.

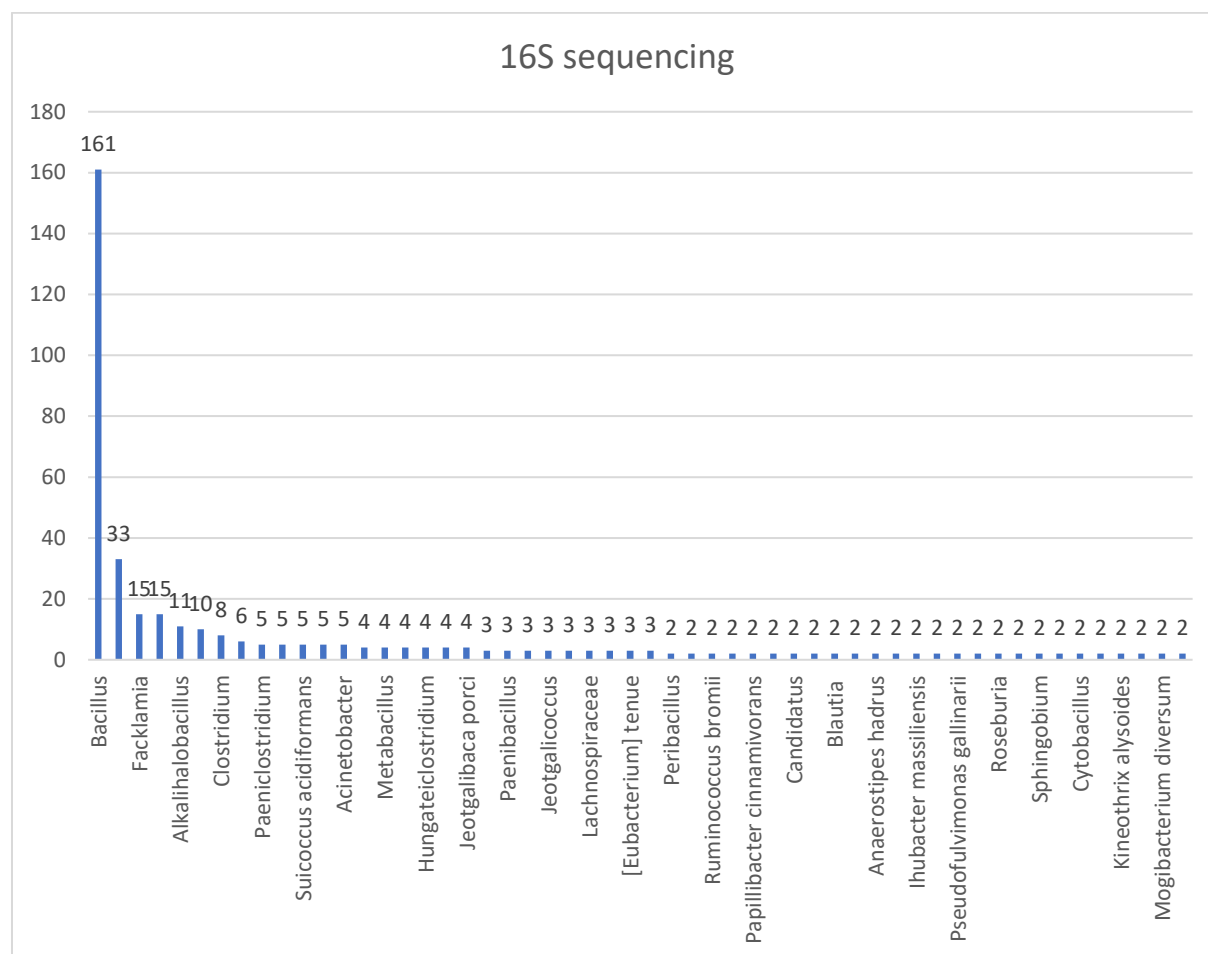


Figure 14: Results of microbiota determination by 16S rDNA sequencing

5.3. Comparison of the microbial diversity of Hungarian and Austrian dried fruit samples

The origin of the samples is Hungary for the first twenty samples (64.5%) and Austria for the remaining eleven samples (35.5%). The aim was to determine any differences between the microbial content of the two groups of samples, focusing mainly on bacteria. *Bacillus spp.* were the most isolated species in both the Hungarian and the Austrian samples. *Priestia spp.*, *Peribacillus spp.* and *Micrococcococcus spp.* were detectable from both origin.

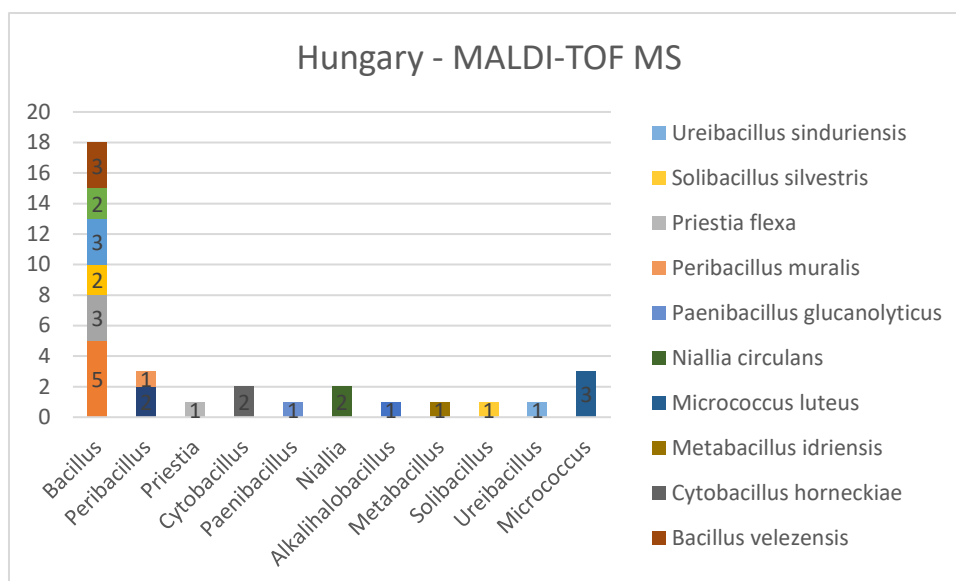


Figure 15: Bacteria of Hungarian dried fruit samples identified by MALDI-TOF MS

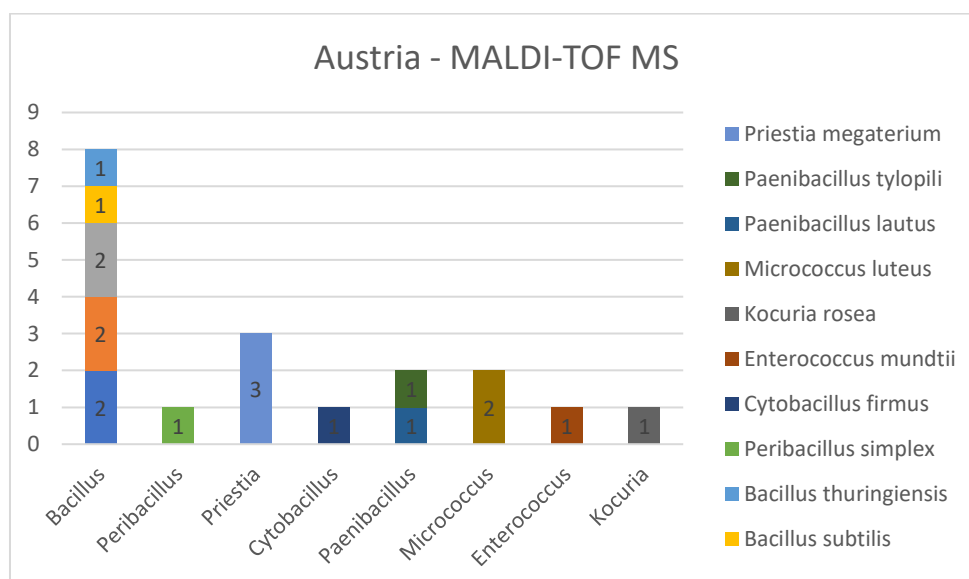


Figure 16: Bacteria of Austrian dried fruit samples identified by MALDI-TOF MS

The MALDI-TOF MS (culture-dependent) method revealed the presence of eleven different genera in the Hungarian samples and eight genera in the Austrian samples. This discrepancy is not deemed to be substantial, despite the variability observed in the sample sizes under examination.

The 16S rDNA sequencing comparison is illustrated in Figures 17 and 18. It can be observed that there is a discrepancy between the results of the MALDI-TOF MS analysis. In the case of sequencing, the second most common microorganisms identified were *Staphylococcus spp.* in both the Hungarian and Austrian samples.

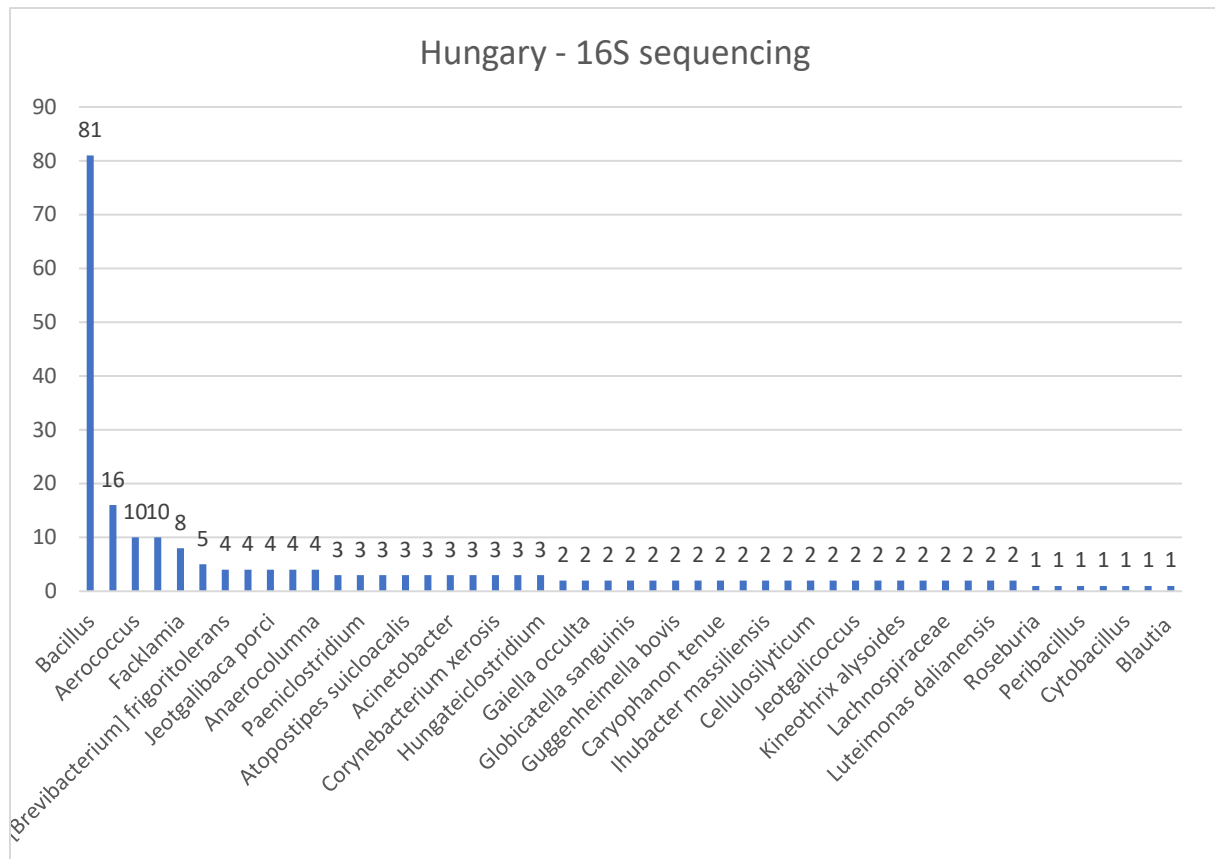


Figure 17: Bacterium biota of Hungarian dried fruit samples determination by 16S rDNA sequencing

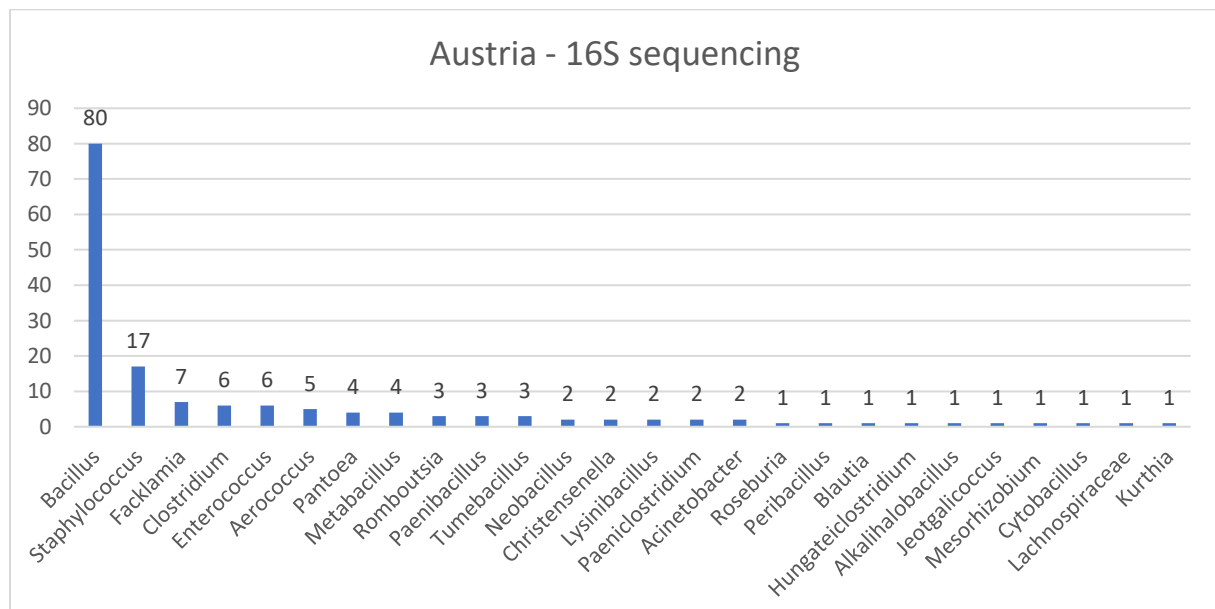


Figure 18: Bacterium biota of Austrian dried fruit samples determination by 16S rDNA sequencing

In case of Hungarian samples 42 genus was detectable with 16S sequencing (for samples where at least two microorganisms were present). Austrian samples contained 16 genus (for samples

where at least two microorganisms were present). With MALDI-TOF MS this number is lower, 11 genus was detectable (even one microorganisms is present) from Hungarian sample and 8 genus from Austrian sample. It is important to note that this difference can occur from the different number of samples. There was 20 Hungarian and 11 Austrian samples measured.

5.4. Sugar content of the dried fruits, and correlations between the sugar content and the number of detected bacteria

In the scientific literature contains data regarding the sugar content of dried fruit samples that were the subject of this study. Table 16. shows the summarised result based on literature survey.

Table 16: Sugar content of raisins, dried apricot and prune (based on the Food Data central data, Internet 5, 6, 7)

Name of the product	Sugar concentration (g/l)
Raisins	657
Dried apricot	534.9
Prune	380.5

As evidenced in Table 16, the mean sugar content is highest in raisins, while prunes exhibit the lowest concentration of sugar. To measure the sugar content of the investigated dried fruit samples, a RIDA CUBE device was used. Table 17. shows the results of sugar contents typical to the analysed samples, while the averages of measured sugar concentrations of each sample category are listed in Table 18.

Table 17: The sugar concentration of the test samples presented in an ascending order

Sample number	Name of the fruits	Calculated sugar concentration (g/l)	Nr. of isolated bacteria types MALDI-TOF MS	Nr. of isolated bacteria types SEQUENCING
25	Dried apricots	13,63	2	8
9	Dried apricots	14,63	2	8
19	Prunes	18,50	1	71
29	Dried apricots	36,00	2	44
11	Prunes	42,13	1	22
5	Dried apricots	44,08	3	2
6	Raisins	48,63	9	132
20	Dried apricots	53,25	1	5
17	Dried apricots	60,92	1	6
30	Dried apricots	68,75	1	2
3	Prunes	76,00	1	2
16	Prunes	78,00	1	1
14	Prunes	81,00	1	4
13	Dried apricots	81,75	2	8
1	Dried apricots	93,67	1	5

Sample number	Name of the fruits	Calculated sugar concentration (g/l)	Nr. of isolated bacteria types MALDI-TOF MS	Nr. of isolated bacteria types SEQUENCING
22	Dried apricots	114,00	2	8
23	Raisins	124,63	1	4
2	Prunes	124,75	1	5
18	Raisins	144,00	2	3
15	Raisins	156,13	1	7
28	Dried apricots	170,00	1	2
26	Raisins	171,25	2	27
24	Raisins	187,25	4	6
21	Prunes	192,88	1	100
27	Raisins	193,63	1	9
31	Raisins	202,63	4	7
12	Raisins	208,50	4	71
7	Raisins	231,13	3	6
4	Prunes	231,75	1	4
8	Mix of dried banana, prunes, blueberry, raisin and other	266,00	1	32
10	Raisins	285,00	1	5

Table 18: Average measured sugar concentration from the samples

Sample name	Average sugar concentration (g/L)	Standard deviation
Apricot	68,24	43,73
Prune	105,63	73,44
Raisins	177,52	61,16

A comparison of the measured sugar concentration with the data from the scientific literature data (and the applied media in this study) (Table 15.) revealed that the sugar concentrations measured in this study were consistently lower than those reported in the literature. However, the standard deviation is also considerable, indicating that the sugar concentration is markedly influenced by the product. In general, the sugar concentration of the samples did not reach that of the prepared media (which is based on literature data). Table 17. shows that the number of microorganisms that could be identified by MALDI-TOF MS was not affected by the sugar concentration, as no correlation was identified between the sugar concentration and the culturable microorganism types. The results demonstrate that the number of detectable microorganisms amenable to sequencing is also independent of the sugar content.

MALDI TOF-MS identified 25 microbial types 59 times, whereas sequencing identified 124 bacterium types 616 times. The results of the experiment demonstrate that sequencing is a more effective method for detecting a wider range of microorganisms. The relatively low concentration of sugar in the environment was insufficient to support bacterial survival due to

the lack of essential nutrients, which is corroborated by the low number of bacteria detected through culturing.

5.5. The growth rate of *E. coli* strains in presence of diverse sugar contents

One of the objectives of this study was to examine the growth rate of *E. coli* in order to ascertain its capacity to survive in dried fruits. In establishing the rate of growth, the Bioscreen device was employed using LB broth, low sugar content LB broth, and three mimicking sample media (dried apricot, prune and raisins) for cell propagation. Table 19. summarises the sugar contents of the prepared media.

Table 19: Sugar concentrations of the culture media used in Bioscreen tests

Name of the broth	Glucose concentration (g/100 ml)	Fructose concentration (g/100 ml)	Saccharose concentration (g/100 ml)	Total sugar (g/100 ml)
LB medium	0	0	0	0
Low sugar broth	5.4	0	0	5.4
Apricot broth	33.1	12.5	7.89	53.49
Prunes broth	25.5	12.4	0.15	38.05
Raisin broth	31	34.7	0	65.7

The bioscreen measurement was performed in 4 parallels. The diagrams present a comparison of the mean values of the four parallel samples in the case of the laboratory and the isolated strains. The first medium which effect was tested on growth of *E. coli* strains was the LB broth. The mean value of the non-sugar containing LB was around 0.7 in the case of the laboratory strain, and around 1.0 in the case of the isolated strain. As illustrated in Figure 19., the μ is of equivalent value. However, in the case of the laboratory strain, the reproduction rate declines over time and does not attain the same cell concentration as the other strain.

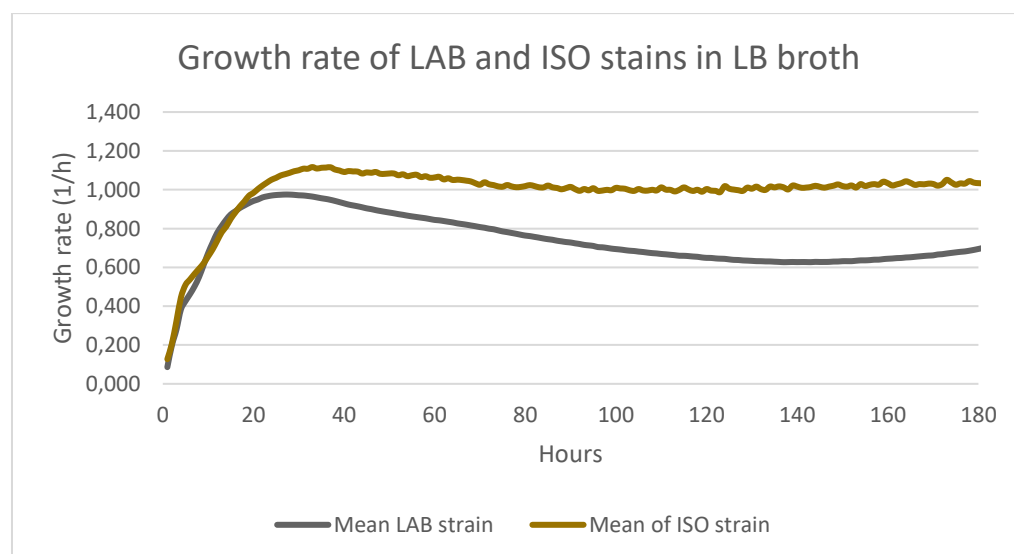


Figure 19: Growth curves of the laboratory and isolated *E. coli* strains in LB broth

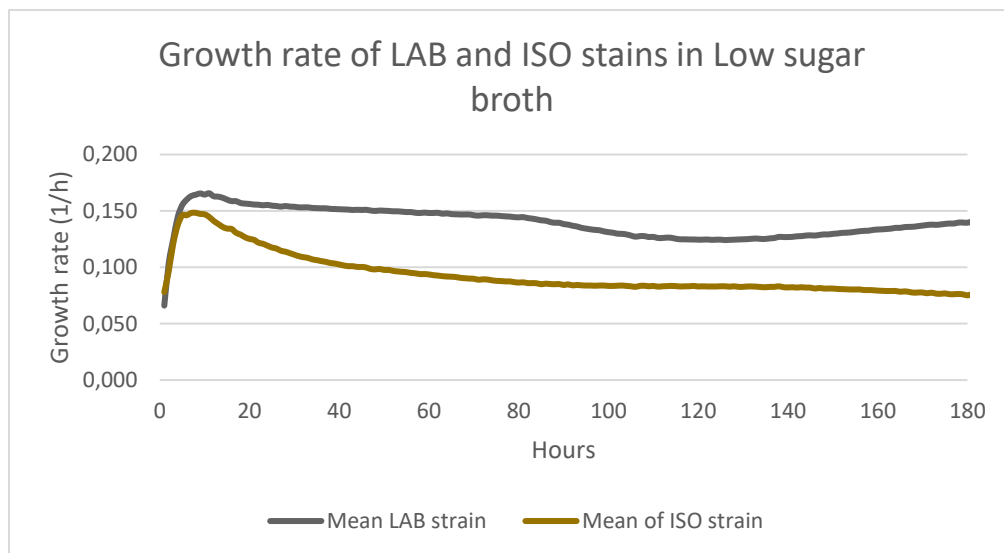


Figure 20: Growth curves of the laboratory and isolated *E. coli* strains in low sugar broth

The first comparison of the LB broth was with the low sugar broth, which contained 5.4 g glucose/100 mL of medium. It was expected that the low concentration of sugar would increase the growth of the strains because the increased carbohydrate level, but the results showed different tendency. In the case of the laboratory strain the mean value was around 0.15, while the mean value of the isolated strain was around 0.075. The results of the mimicking broth media could give an answer why this low concentration of sugar did not lead to higher mean values in the case of the growth rate for the tested strains.

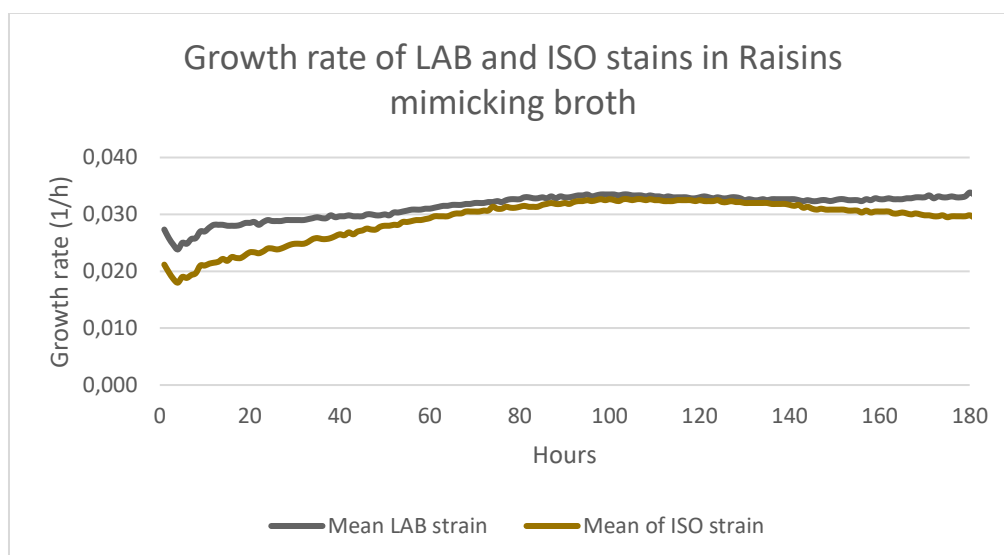


Figure 21: Growth curves of the laboratory and isolated *E. coli* strains in raisins mimicking broth

It is known from the literature (see Table 15.) that raisins have generally the highest sugar concentration out of the tested samples, and in this study the mimicking broth also contained the highest sugar concentration (more than 30% of glucose and 30% of fructose). As it can be seen from Fig. 21. the mean values were around 0.03-0.05 in the case of raisins mimicking

broth. Moreover, an interesting phenomenon happened in the first 0.5-1 hour. The optical density started to decrease, and after a short time the OD started to increase. This phenomenon could be explained as an adaptation period for higher sugar concentration, or osmotic stress.

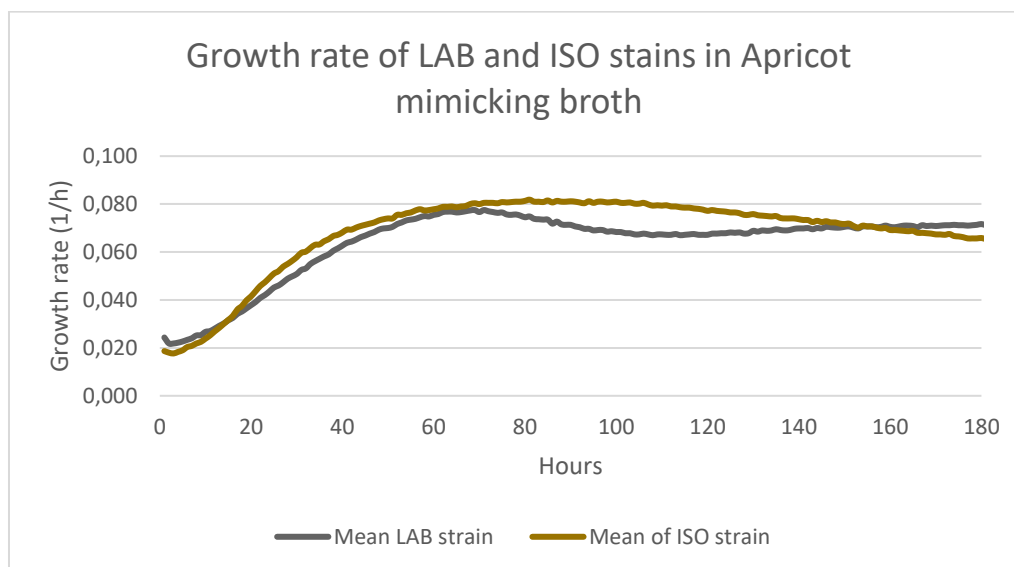


Figure 22: Growth curves of the laboratory and isolated *E. coli* strains in dried apricot mimicking broth

Adaptation was also observed in the case of peach-mimicking medium, although this was not as significant as in the case of raisins. Furthermore, it can be observed that the growth was more pronounced in the case of peaches, which can be attributed to the lower fructose concentration (instead of 34.7% it was only 12.5 %.), as glucose was present in almost the same concentration.

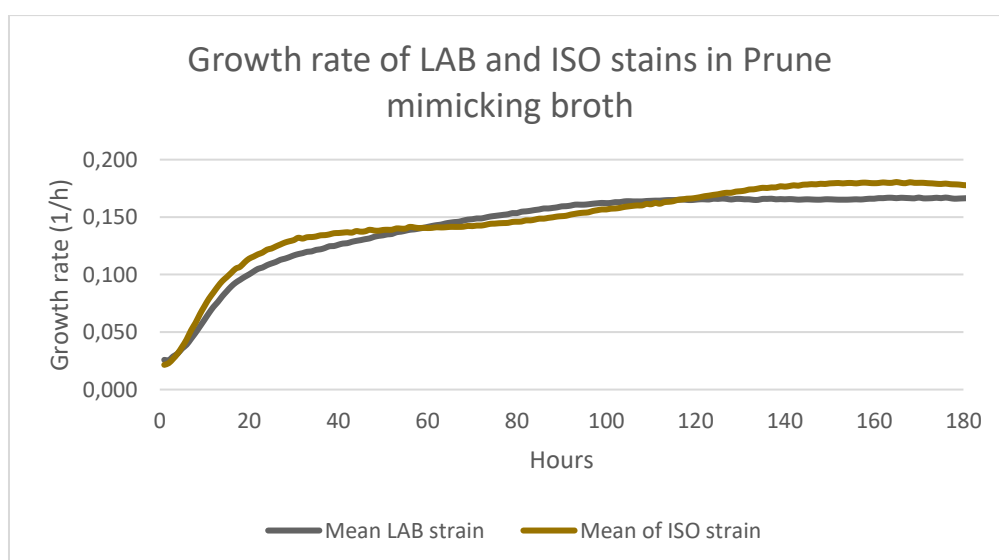


Figure 23: Growth curves of the laboratory and isolated *E. coli* strains in prune mimicking broth media

The highest growth rate was observed in the case of prune mimicking medium. The prune medium was set to have the lowest sugar concentration, which could be the reason for this outcome. Moreover, the lower glucose (25.5%) and much lower sucrose (0.15%) concentrations may have the positive effect on higher growth rate of the tested *E. coli* strains. On the other hand comparing to the Bioscreen method to the MALDI TOF MS method, prune media results are controversial. The Bioscreen the growth rate results shows that not even the higher sugar concentration can inhibit the bacteria growth. Table 20 shows the sugar concentration and the related isolated bacteria. As illustrated even with the highest sugar concentration (sample 10, 281 g/L) *Peribacillus muralis* was culturable!

Table 20: Sugar concentration and MALDI-TOF MS isolated bacteria

Sample number	Sample	Isolated bacteria	Sugar concentration (g/L)
25	Dried apricot	<i>Bacillus cereus</i>	13,63
		<i>Enterococcus mundtii</i>	
9	Dried apricot	<i>Bacillus mojavensis</i>	14,63
		<i>Solibacillus silvestris</i>	
19	Prunes	No peak	18,50
29	Dried apricot	<i>Peribacillus simplex</i>	36,00
		<i>Cytobacillus firmus</i>	
11	Prunes	<i>Bacillus mycoides</i>	42,13
5	Dried apricot	<i>Bacillus licheniformis</i>	44,08
		<i>Bacillus mojavensis</i>	
		<i>Bacillus subtilis</i>	
6	Raisins	<i>Bacillus cereus</i>	48,63
		<i>Bacillus velezensis</i>	
		<i>Ureibacillus sinduriensis</i>	
		<i>Priestia flexa</i>	
		<i>Bacillus licheniformis</i>	
		<i>Micrococcus luteus</i>	
		<i>Peribacillus simplex</i>	
		<i>Alkalihalobacillus clausii</i>	
		<i>Niallia circulans</i>	
20	Dried apricot	<i>Bacillus cereus</i>	53,25
17	Dried apricot	No organism identification possible	60,92
30	Dried apricot	<i>Paenibacillus tylopili</i>	68,75
3	Prunes	No organism identification possible	76
16	Prunes	<i>Cytobacillus horneckiae</i>	78,00
14	Prunes	<i>Bacillus licheniformis</i>	81,00
13	Dried apricot	<i>Bacillus cereus</i>	81,75
		<i>Micrococcus luteus</i>	

Sample number	Sample	Isolated bacteria	Sugar concentration (g/L)
1	Dried apricot	<i>Paenibacillus glucanolyticus</i>	93,67
22	Dried apricot	<i>Paenibacillus lautus</i>	114,00
		<i>Priestia megaterium</i>	114,00
23	Raisins	No organism identification possible	124,63
2	Prunes	No peak	124,75
18	Raisins	<i>Bacillus mycoides</i>	144,00
		<i>Bacillus cereus</i>	
15	Raisins	<i>Metabacillus idriensis</i>	156,13
28	Dried apricot	<i>Bacillus mycoides</i>	170,00
26	Raisins	<i>Bacillus pumilus</i>	171,25
		<i>Bacillus subtilis</i>	
24	Raisins	<i>Priestia megaterium</i>	187,25
		<i>Kocuria rosea</i>	
		<i>Micrococcus luteus</i>	
		<i>Bacillus pumilus</i>	
21	Prunes	No peak	192,88
27	Raisins	<i>Priestia megaterium</i>	193,63
31	Raisins	<i>Bacillus mycoides</i>	202,63
		<i>Bacillus cereus</i>	
		<i>Micrococcus luteus</i>	
		<i>Bacillus thuringiensis</i>	
12	Raisins	<i>Bacillus mycoides</i>	208,50
		<i>Bacillus velezensis</i>	
		<i>Peribacillus simplex</i>	
		<i>Bacillus cereus</i>	
7	Raisins	<i>Bacillus velezensis</i>	231,13
		<i>Niallia circulans</i>	
		<i>Cytobacillus horneckiae</i>	
4	Prunes	<i>Micrococcus luteus</i>	231,75
8	Mix of dired banana, prunes, blueberry, raisin and other	<i>Bacillus subtilis</i>	266,00
10	Raisins	<i>Peribacillus muralis</i>	285,00

Generally, if either a pathogenic or an indicator *E. coli* would present in a dried fruit, they will be able to grow (the sugar concentration would not inhibit the growth) or even survive. The Bioscreen results were statistically evaluated with the JMP software.

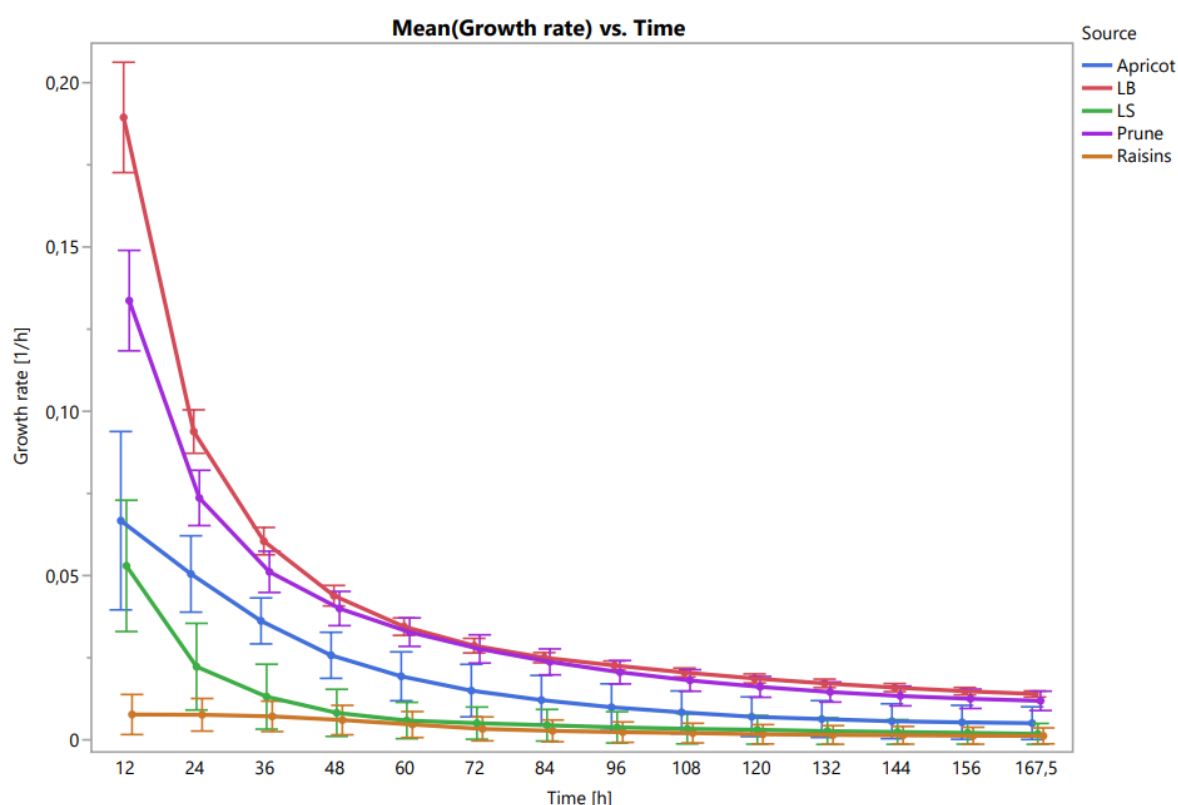


Figure 24: Growth rate of the laboratory and isolated *E. coli* strains over the time in all types of media

Figure 24. shows that the highest growth rate of *E. coli* over time was in the LB broth, which was expected as this medium was originally designed to promote the growth of *E. coli*. It contains nutrients, e.g. peptone, yeast extract and sodium chloride, that can support the cell propagation, however, it does not contain any sugar. Low sugar medium contains only 5.4 g/100 mL of glucose, which could be a lower concentration than the optimal for *E.coli* to grow. Prune mimicking broth, on the other hand, contains higher concentration (38.05 g/100 mL) of sugars, but this concentration covers the optimal range. The dried apricot mimicking broth reaches a higher concentration than the prune broth (53.49 g/100 mL), this could be the reason why the growth rate is lower in case of the dried apricot broth. On the other hand, the 53.49 g/100 mL sugar concentration showed higher growth rate than the 5.4 g/100 mL glucose concentration. This is reasonable because the apricot mimicking broth contained 33.1 g/100 mL glucose and the rest of the concentration is added up by fructose and saccharose, but the low sugar broth contained only 5.4 g/100 mL of glucose. For the more precise statistical evaluation the JMP software was used in 12 hours time blocks. Table 21. shows the first 12 hours of growth rate to compare all media.

Table 21: Results of the first 12 hours of *E. coli* growth rate's statistics

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	Lower CL	Upper CL
Prune	Apricot	11,9167	2,886124	4,12895	<,0001*	0,062722	0,043990	0,087289
Prune	LS	11,9167	2,886124	4,12895	<,0001*	0,081961	0,061842	0,097786
LB	Apricot	10,9083	2,780388	3,92331	<,0001*	0,120977	0,097193	0,150011
LS	Apricot	-4,2500	2,886751	-1,47224	0,1410	-0,015933	-0,039085	0,006675
LS	LB	-10,9083	2,780388	-3,92331	<,0001*	-0,138980	-0,149554	-0,110925
Prune	LB	-10,9083	2,779603	-3,92442	<,0001*	-0,056621	-0,068766	-0,038608
Raisins	LB	-10,9083	2,778032	-3,92664	<,0001*	-0,175454	-0,199185	-0,167847
Raisins	Apricot	-11,7500	2,884868	-4,07298	<,0001*	-0,066983	-0,080291	-0,039538
Raisins	LS	-11,9167	2,884868	-4,13075	<,0001*	-0,043558	-0,063601	-0,027753
Raisins	Prune	-11,9167	2,884240	-4,13165	<,0001*	-0,125159	-0,137824	-0,114441

The p-values shows that all the growth rate in the media were significantly different (in a 99.9% of significance level) from each other except the LS medium and the dried apricot mimicking broth. After one day, all media showed significant differences compared with each other. (Table 22).

Table 22: Statistical analysis of results after one day of incubation for *E. coli* growth rate

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	Lower CL	Upper CL
Prune	Apricot	11,9167	2,886751	4,12805	<,0001*	0,020460	0,013066	0,030901
Prune	LS	11,9167	2,886751	4,12805	<,0001*	0,052368	0,038645	0,062650
LB	Apricot	10,9083	2,780388	3,92331	<,0001*	0,040158	0,033510	0,051487
Raisins	LS	-7,7500	2,886751	-2,68468	0,0073*	-0,015257	-0,026999	-0,001487
LS	Apricot	-10,0833	2,886751	-3,49297	0,0005*	-0,025664	-0,043928	-0,017603
Prune	LB	-10,7250	2,780388	-3,85738	0,0001*	-0,021061	-0,026874	-0,012766
LS	LB	-10,9083	2,780388	-3,92331	<,0001*	-0,075366	-0,083241	-0,057232
Raisins	LB	-10,9083	2,780388	-3,92331	<,0001*	-0,084576	-0,091495	-0,080100
Raisins	Apricot	-11,9167	2,886751	-4,12805	<,0001*	-0,045585	-0,050846	-0,036815
Raisins	Prune	-11,9167	2,886751	-4,12805	<,0001*	-0,065184	-0,073094	-0,059311

After 36 hours (Table 23) the too low (prune) and the too high sugar containing media (raisins) showed a non-significant difference, but the LB, prune and apricot media were still significantly different from each other.

Table 23: Statistical analysis of results after 36 hours of incubation for *E.coli* growth rate

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	Lower CL	Upper CL
Prune	LS	11,9167	2,886751	4,12805	<,0001*	0,038892	0,027834	0,045672
Prune	Apricot	11,5833	2,886751	4,01258	<,0001*	0,013533	0,008297	0,020788
LB	Apricot	10,9083	2,780388	3,92331	<,0001*	0,022894	0,018549	0,028461
Raisins	LS	-3,5833	2,886124	-1,24157	0,2144	-0,005434	-0,014933	0,002892
Prune	LB	-7,7917	2,780388	-2,80237	0,0051*	-0,009858	-0,014639	-0,003585
LS	LB	-10,9083	2,780388	-3,92331	<,0001*	-0,049841	-0,055900	-0,037250
Raisins	LB	-10,9083	2,779603	-3,92442	<,0001*	-0,052870	-0,057411	-0,048418
LS	Apricot	-11,2500	2,886751	-3,89711	<,0001*	-0,021469	-0,033444	-0,014908
Raisins	Apricot	-11,9167	2,886124	-4,12895	<,0001*	-0,029798	-0,034799	-0,024236
Raisins	Prune	-11,9167	2,886124	-4,12895	<,0001*	-0,043228	-0,048797	-0,038862

After 2 days and further (Tables 24-26.) the same phenomenon appeared. The raisins-LS and the prune-LB media were no longer statistically different from each other.

Table 24: Statistical analysis of results after 48 hours of incubation for *E. coli* growth rate

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	Lower CL	Upper CL
Prune	LS	11,9167	2,886751	4,12805	<,0001*	0,032603	0,024743	0,036895
Prune	Apricot	11,2500	2,886751	3,89711	<,0001*	0,013377	0,008337	0,019986
LB	Apricot	10,9083	2,780388	3,92331	<,0001*	0,017137	0,012684	0,023862
Raisins	LS	-1,7500	2,886124	-0,60635	0,5443	-0,001241	-0,009491	0,003854
Prune	LB	-5,2250	2,780388	-1,87923	0,0602	-0,004306	-0,008064	0,000548
LS	LB	-10,9083	2,780388	-3,92331	<,0001*	-0,036846	-0,042248	-0,028573
Raisins	LB	-10,9083	2,779603	-3,92442	<,0001*	-0,038382	-0,041773	-0,033178
LS	Apricot	-10,9167	2,886751	-3,78164	0,0002*	-0,017163	-0,025112	-0,011666
Raisins	Apricot	-11,9167	2,886124	-4,12895	<,0001*	-0,019683	-0,025779	-0,014767
Raisins	Prune	-11,9167	2,886124	-4,12895	<,0001*	-0,034254	-0,037919	-0,028946

Table 25: Statistical analysis of results after 60 hours of incubation for *E. coli* growth rate

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	Lower CL	Upper CL
Prune	LS	11,9167	2,886124	4,12895	<,0001*	0,026954	0,022281	0,031732
Prune	Apricot	11,0833	2,886124	3,84021	0,0001*	0,012802	0,007364	0,018494
LB	Apricot	10,7250	2,780388	3,85738	0,0001*	0,014264	0,009239	0,020501
Raisins	LS	-1,0833	2,886751	-0,37528	0,7075	-0,000977	-0,005715	0,003240
Prune	LB	-3,0250	2,779603	-1,08829	0,2765	-0,001966	-0,005554	0,002007
LS	Apricot	-10,2500	2,886751	-3,55070	0,0004*	-0,013881	-0,019702	-0,007660
LS	LB	-10,9083	2,780388	-3,92331	<,0001*	-0,029108	-0,033071	-0,023780
Raisins	LB	-10,9083	2,780388	-3,92331	<,0001*	-0,029926	-0,033340	-0,026231
Raisins	Apricot	-11,0000	2,886124	-3,81134	0,0001*	-0,014781	-0,020974	-0,009558
Raisins	Prune	-11,9167	2,886124	-4,12895	<,0001*	-0,027991	-0,031609	-0,023943

Table 26: Statistical analysis of results after 72 hours of incubation for *E. coli* growth rate

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	Lower CL	Upper CL
Prune	LS	11,9167	2,886751	4,12805	<,0001*	0,022714	0,018386	0,026822
LB	Apricot	10,3583	2,780388	3,72550	0,0002*	0,012854	0,007691	0,018791
Prune	Apricot	10,2500	2,886751	3,55070	0,0004*	0,011743	0,006357	0,018241
Raisins	LS	-1,9167	2,886751	-0,66395	0,5067	-0,001682	-0,005545	0,001976
Prune	LB	-3,2083	2,780388	-1,15392	0,2485	-0,001400	-0,004228	0,002004
LS	Apricot	-8,2500	2,886751	-2,85788	0,0043*	-0,010751	-0,016690	-0,004044
Raisins	Apricot	-9,5833	2,886751	-3,31976	0,0009*	-0,012578	-0,018216	-0,006344
LS	LB	-10,9083	2,780388	-3,92331	<,0001*	-0,024219	-0,028096	-0,019581
Raisins	LB	-10,9083	2,780388	-3,92331	<,0001*	-0,025453	-0,028282	-0,022544
Raisins	Prune	-11,9167	2,886751	-4,12805	<,0001*	-0,023861	-0,027604	-0,020623

By the end of the experiment almost all media were non-significantly different (Table 27), except the corner points of the Figure 24.

Table 27: Statistical analysis of results after 167.5 hours of incubation for *E. coli* growth rate

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	Lower CL	Upper CL
Prune	LS	11,9167	2,886751	4,12805	<,0001*	0,010214	0,007218	0,013064
LB	Apricot	10,9083	2,780388	3,92331	<,0001*	0,009164	0,003645	0,013466
Prune	Apricot	8,9167	2,886751	3,08882	0,0020*	0,006938	0,002162	0,011232
Raisins	LS	-1,5833	2,886124	-0,54860	0,5833	-0,000356	-0,003757	0,001442
LS	Apricot	-4,5833	2,886751	-1,58771	0,1124	-0,003826	-0,006799	0,000735
Raisins	Apricot	-4,9167	2,886124	-1,70355	0,0885	-0,004782	-0,006918	0,000306
Prune	LB	-6,1417	2,780388	-2,20892	0,0272*	-0,002258	-0,004041	-0,000254
LS	LB	-10,9083	2,780388	-3,92331	<,0001*	-0,012612	-0,015038	-0,009184
Raisins	LB	-10,9083	2,779603	-3,92442	<,0001*	-0,013631	-0,014829	-0,010207
Raisins	Prune	-11,9167	2,886124	-4,12895	<,0001*	-0,010898	-0,013044	-0,007899

In summary, it is not only visible but statistically proven that the growth rate was the best in the case of LB medium (as it is designed to be beneficial for growth of *E. coli*), and the prune and dried apricot mimicking broth (due to the fact that their sugar concentrations are at the optimal level).

Following the examination of the media, an additional comparison could be made between the laboratory and the isolated strains of *E. coli*. The isolate was derived from a low water activity food, while the laboratory strain originates from the National Collection of Agricultural and Industrial Microorganisms (NCAIM), Institute of Food Science and Technology, Hungarian University of Agriculture and Life Sciences, Budapest, Hungary. The statistical evaluation showed that at 95% significance level in the case of LB, apricot, and prune mimicking broths there were no differences.

Table 28: Comparison of the growth rate for isolated and laboratory *E. coli* strain in dried apricot mimicking broth

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	Lower CL	Upper CL
Lab strain	Isolate	-7,48810	7,505553	-0,997674	0,3184	-0,002178	-0,006710	0,0018788

Table 29: Comparison of the growth rate for isolated and laboratory *E. coli* strain in LB broth

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	Lower CL	Upper CL
Lab strain	Isolate	1,900000	6,855655	0,2771435	0,7817	0,0004997	-0,003315	0,0042811

Table 30: Comparison of the growth rate for isolated and laboratory *E. coli* strain in dried prune mimicking broth

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	Lower CL	Upper CL
Lab strain	Isolate	-7,50000	7,505544	-0,999261	0,3177	-0,002040	-0,006034	0,0020176

The statistical proof of the difference between the two strains is evident in the case of the broth with an insufficiently low sugar concentration (LS broth) and the broth with an excessively high sugar concentration (raisins mimicking broth).

Table 31: Comparison of the growth rate for isolated and laboratory *E. coli* strain in raisins mimicking broth

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	Lower CL	Upper CL
Lab strain	Isolate	-25,9286	7,505359	-3,45467	0,0006*	-0,001804	-0,003296	-0,000688

Table 32: Comparison of the growth rate for isolated and laboratory *E. coli* strain in low sugar (LS) broth

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	Lower CL	Upper CL
Lab strain	Isolate	65,25000	7,504499	8,694784	<,0001*	0,0077794	0,0067571	0,0090255

In conclusion, the individual results for the two strains demonstrated that, with the exception of the excessively high sugar concentration (raisin media) and the insufficiently low sugar concentration (low sugar media), optimal growth was observed when the media contained an appropriate, yet not excessive, concentration of sugar (as seen in the prune and apricot broths).

6. Conclusion

The microbiota of three types of dried fruit samples was determined made by two methods: MALDI-TOF MS and 16S rDNA sequencing. Although the sequencing method yielded more precise results regarding the microbiota, the majority of the samples also exhibited culturable microorganisms, which were identified through MALDI-TOF MS. This method identified 25 microbial types 59 times. A total of 124 microbial species were identified through sequencing, with 616 instances of detection. Twelve samples exhibited concordance between the two analytical methods, while four samples demonstrated complete concordance between the two identification methods. The majority of the identified microorganisms were spore-forming and non-culturable. Nevertheless, the foodborne pathogen *Bacillus cereus* was identified in both the culturable and non-culturable detection methods.

The growth rate of the *E. coli* which was measured by the Bioscreen device, which showed that even in the lowest sugar concentration environment, *E. coli* is present and able to grow. Nevertheless, a statistical difference was observed between the various sugar concentration media. The highest growth rate of the *E. coli* was observed in prune broth, while the lowest was noted in raisin broth. The discrepancy in of the growth rates of these two broths is notable.

In order to build upon the findings of this study, it would be beneficial to measure the effects of other dried fruits or to investigate additional stress factors, such as UV irradiation or disinfections.

7. References

1. Gyurova, Desislava Krasteva, Enikova, Rositsa Kirilova (2014): Dried Fruits – Brief Characteristics of their Nutritional Values. Journal of Food and Nutrition Sciences. Vol. 2, No. 4, 2014, pp. 105-109. DOI: [Dried Fruits – Brief Characteristics of their Nutritional Values. Author's Own Data for Dietary Fibers Content, Journal of Food and Nutrition Sciences, Science Publishing Group](#)
2. Sadler, M. J., Gibson, S., Whelan, K., Ha, M. A., Lovegrove, J., & Higgs, J. (2019). Dried fruit and public health – what does the evidence tell us? International Journal of Food Sciences and Nutrition, 70(6), 675–687. DOI: [Full article: Dried fruit and public health – what does the evidence tell us?](#)
3. S. K. Chang, C. Alasalvar, F. Shahidi (2016): Review of dried fruits: Phytochemicals, antioxidant efficacies, and health benefits, Journal of Functional Foods Volume 21 , March 2016, Pages 113-132. DOI: [Review of dried fruits: Phytochemicals, antioxidant efficacies, and health benefits - ScienceDirect](#)
4. He F.J., C.A. Nowson, M. Lucas, G.A. MacGregor (2007): Increased consumption of fruit and vegetables is related to a reduced risk of coronary heart disease, Meta-analysis of cohort studies Journal of Human Hypertension, 21, pp. 717-728. DOI: [1002212.pdf \(nature.com\)](#)
5. A.H. Lichtenstein, L.J. Appel, M. Brands, M. Carnethon, S. Daniels, H.A. Franch, B. Franklin, P. Kris-Etherton, W.S. Harris, B. Howard, N. Karanja, M. Lefevre, L. Rudel, F. Sacks, H.L. Van, M. Winston, J. Wylie-Rosett (2006): Diet and lifestyle recommendations revision 2006, A scientific statement from the American Heart Association Nutrition Committee Circulation, 114, pp. 82-96. DOI: [Scopus - Document details - Diet and lifestyle recommendations revision 2006: A scientific statement from the American heart association nutrition committee](#)
6. J.L. Slavin, B. Lloyd (2012): Health benefits of fruits and vegetables, Advances in Nutrition, 3, pp. 506-516. DOI: [Health Benefits of Fruits and Vegetables - ScienceDirect](#)
7. S. Quideau, D. Deffieux, C. Douat-Casassus, L. Pouységu (2011): Plant polyphenols: Chemical properties, biological activities, and synthesis, Angewandte Chemie International Edition, 50, pp. 586-621. DOI: [Plant Polyphenols: Chemical Properties, Biological Activities, and Synthesis \(wiley.com\)](#)
8. F.A. Tomás-Barberán, C. Andrés-Lacueva (2012) Polyphenols and health: Current state and progress, Journal of Agricultural and Food Chemistry, 60, pp. 8773-8775. DOI: [Plant Polyphenols: Chemical Properties, Biological Activities, and Synthesis \(wiley.com\)](#)
9. Liu, R.H. (2023). Health Benefits of Dietary Phytochemicals in Whole Foods. In: Temple, N.J., Wilson, T., Jacobs, Jr., D.R., Bray, G.A. (eds) Nutritional Health. Nutrition and Health. Humana, Cham. https://doi.org/10.1007/978-3-031-24663-0_14
10. Baek HI, Ha KC, Kim HM, Choi EK, Park EO, Park BH, Yang HJ, Kim MJ, Kang HJ, Chae SW. (2016): Randomized, double-blind, placebo-controlled trial of Ficus carica paste for the management of functional constipation, Asia Pac J Clin Nutr. 2016;25(3):487-96. doi: 10.6133/apjcn.092015.06.
11. C. Alasalvar, F. Shahidi (2013): Composition, phytochemicals and beneficial health effects of dried fruits: an overview C. Alasalvar, F. Shahidi (Eds.), Dried fruits: Phytochemicals and health effects, Wiley-Blackwell, Oxford, pp. 1-18. DOI: [Composition, Phytochemicals, and Beneficial Health Effects of Dried Fruits: An Overview - Dried Fruits - Wiley Online Library](#)
12. C. Alasalvar, F. Shahidi (2013): Nutritional composition, phytochemicals, and health benefits of dates C. Alasalvar, F. Shahidi (Eds.), Dried fruits: Phytochemicals and health effects, Wiley-Blackwell, Oxford, pp. 428-443. DOI: [Composition, Phytochemicals, and Beneficial Health Effects of Dried Fruits: An Overview - Dried Fruits - Wiley Online Library](#)

13. European Food Safety Authority (EFSA). Scientific Opinion on the Substantiation of a Health Claim Related to Prunes and Contribution to Normal Bowel Function Pursuant to Article 14 of Regulation (EC) No 1924/2006. *EFSA J.* 2014, 12, 3892.
14. U.S. Department of Agriculture (USDA). National Nutrient Database for Standard Reference Legacy Release. 2018. Available online: <https://ndb.nal.usda.gov/ndb/search/list> (accessed on 1 March 2023).
15. U.S. Department of Agriculture (USDA). Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids; The National Academies Press: Washington, DC, USA, 2005.
16. Ishiwata, K.; Yamaguchi, T.; Takamura, H.; Matoba, T. (2004): DPPH Radical-Scavenging Activity and Polyphenol Content in Dried Fruits. *Food Sci. Technol. Res.* 2004, 10, 152–156. DOI: [DPPH Radical-Scavenging Activity and Polyphenol Content in Dried Fruits \(jst.go.jp\)](https://doi.org/10.3177/JST.04.10.152)
17. Vinson, J.A.; Zubik, L.; Bose, P.; Samman, N.; Proch, J. (2005) Dried Fruits: Excellent In Vitro and In Vivo antioxidants. *J. Am. Coll. Nutr.* 2005, 24, 44–50. DOI: [Dried Fruits: Excellent in Vitro and in Vivo Antioxidants: Journal of the American College of Nutrition: Vol 24, No 1 \(tandfonline.com\)](https://doi.org/10.1080/07315724.2005.10718681)
18. Rababah, T.M.; Ereifej, K.; Howard, L. (2005): Effect of Ascorbic Acid and Dehydration on Concentrations of Total Phenolics, Antioxidant Capacity, Anthocyanins, and Color in Fruits. *J. Agric. Food Chem.* 2005, 53, 4444–4447. DOI: [Effect of Ascorbic Acid and Dehydration on Concentrations of Total Phenolics, Antioxidant Capacity, Anthocyanins, and Color in Fruits | Journal of Agricultural and Food Chemistry \(acs.org\)](https://doi.org/10.1021/jf050840a)
19. Threlfall, R.; Morris, J.; Meullenet, J.F. (2007): Product Development and Nutraceutical Analysis to Enhance the Value of Dried Fruit. *J. Food Qual.* 2007, 30, 552–566. DOI: [PRODUCT DEVELOPMENT AND NUTRACEUTICAL ANALYSIS TO ENHANCE THE VALUE OF DRIED FRUIT - THRELFALL - 2007 - Journal of Food Quality - Wiley Online Library](https://doi.org/10.1111/j.1365-3030.2007.01911.x)
20. M. Al-Farsi, C.Y. Lee (2008) Nutritional and functional properties of dates: A review *Critical Reviews in Food Science and Nutrition*, 48 (2008), pp. 877-887. DOI: [Nutritional and Functional Properties of Dates: A Review: Critical Reviews in Food Science and Nutrition: Vol 48, No 10 \(tandfonline.com\)](https://doi.org/10.1080/10407170701488881)
21. J.W. Anderson, A.R. Waters (2013): Raisin consumption by humans: Effects on glycemia and insulinemia and cardiovascular risk factors *Journal of Food Science*, 78 (2013), pp. A11-A17. DOI: [Raisin Consumption by Humans: Effects on Glycemia and Insulinemia and Cardiovascular Risk Factors - Anderson - 2013 - Journal of Food Science - Wiley Online Library](https://doi.org/10.1111/1365-3030.12111)
22. J.K. Kundu, Y.-J. Surh (2013): Cancer chemopreventive effects of selected dried fruits C. Alasalvar, F. Shahidi (Eds.), *Dried fruits: Phytochemicals and health effects*, Wiley-Blackwell, Oxford (2013), pp. 19-51. DOI: [Cancer Chemopreventive Effects of Selected Dried Fruits - Dried Fruits - Wiley Online Library](https://doi.org/10.1111/1365-3030.12111)
23. E. Lever, J. Cole, S.M. Scott, P.W. Emery, K. Whelan (2014) Systematic review: The effect of prunes on gastrointestinal function *Alimentary Pharmacology and Therapeutics*, 40 (7) (2014), pp. 750-758. DOI: [Alimentary Pharmacology & Therapeutics | Pharmacology Journal | Wiley Online Library](https://doi.org/10.1111/apt.12681)
24. P.K. Vayalil (2012) Date fruits (*Phoenix dactylifera* Linn): An emerging medicinal food *Critical Reviews in Food Science and Nutrition*, 52 (2012), pp. 249-271. DOI: [Date Fruits \(Phoenix dactylifera Linn\): An Emerging Medicinal Food: Critical Reviews in Food Science and Nutrition: Vol 52, No 3 \(tandfonline.com\)](https://doi.org/10.1080/10407170.2012.688881)
25. Muraki I, Imamura F, Manson J E, Hu F B, Willett W C, van Dam R M et al. (2013): Fruit consumption and risk of type 2 diabetes: results from three prospective longitudinal cohort studies *BMJ* 2013; 347. DOI: [Fruit consumption and risk of type 2 diabetes: results from three prospective longitudinal cohort studies | The BMJ](https://doi.org/10.1136/bmj.f688)

26. Stacewicz-Sapuntzakis M. (2013): Dried plums and their products: composition and health effects--an updated review. *Crit Rev Food Sci Nutr.* 2013;53(12):1277-302. DOI: [Dried plums and their products: composition and health effects--an updated review - PubMed \(nih.gov\)](#)
27. MacKenzie L. Dismore, David B. Haytowitz, Susan E. Gebhardt, James W. Peterson, Sarah L. Booth (2003): Vitamin K content of nuts and fruits in the US diet, *Journal of the American Dietetic Association*, Volume 103, Issue 12, Pages 1650-1652. DOI: [Vitamin K content of nuts and fruits in the US diet - ScienceDirect](#)
28. F.C. Wehner, C.J. Rabie (1970): Micro-organisms in nuts and dried fruits, *Phytophylactica* 2, 165-170, DOI: [The micro-organisms in nuts and dried fruits](#)
29. Lina Sheng, Luxin Wang (2023): Approaches for a more microbiologically and chemically safe dried fruit supply chain, *Current Opinion in Biotechnology*, Volume 80, 102912. DOI: [Approaches for a more microbiologically and chemically safe dried fruit supply chain - ScienceDirect](#)
30. S. Bourdoux, D. Li, A. Rajkovic, F. Devlieghere, M. Uyttendaele (2016): Performance of drying technologies to ensure microbial safety of dried fruits and vegetables, *Compr Rev Food Sci Food Saf*, 15 (2016), pp. 1056-1066. DOI: [Performance of Drying Technologies to Ensure Microbial Safety of Dried Fruits and Vegetables - Bourdoux - 2016 - Comprehensive Reviews in Food Science and Food Safety - Wiley Online Library](#)
31. Internet 1: [Food safety](#) (WHO)
32. Dobó V, Homlok R, Mohácsi-Farkas C, Belák Á. Effect of gamma irradiation, high sugar content and antimicrobials on survival of *Escherichia coli*: A review. *Czech J. Food Sci.* 2023;41(4):231-247. doi: [Czech Journal of Food Sciences: Effect of gamma irradiation, high sugar content and antimicrobials on survival of Escherichia coli: A review](#)
33. Internet 2: [Salmonella and Salmonellosis | Food Safety and Inspection Service](#) (USDA)
34. S. Robinson (2013): The Big Five: Most Common Salmonella Strains in Foodborne Illness Outbreaks, *Food Safety News*. DOI: [The Big Five: Most Common Salmonella Strains in Foodborne Illness Outbreaks | Food Safety News](#)
35. Internet 3: [Foodborne diseases | EFSA](#)
36. Internet 4: [Salmonella: Why it's a chicken and egg thing | The Counter](#)
37. Ehuwa O, Jaiswal AK, Jaiswal S. Salmonella, Food Safety and Food Handling Practices. *Foods*. 2021 Apr 21;10(5):907. DOI: [Salmonella, Food Safety and Food Handling Practices - PubMed](#)
38. Ray, B.; Jezeski, J.; Busta, F. Isolation of *Salmonellae* from naturally contaminated dried milk products: Iii. Influence of pre-enrichment conditions. *J. Milk Food Technol.* **1972**, 35, 607–614. DOI: [ISOLATION OF SALMONELLAE FROM NATURALLY CONTAMINATED DRIED MILK PRODUCTS: III. INFLUENCE OF PRE-ENRICHMENT CONDITIONS - ScienceDirect](#)
39. Gruzdev, N.; Pinto, R.; Sela, S. Persistence of *Salmonella enterica* during dehydration and subsequent cold storage. *Food Microbiol.* **2012**, 32, 415–422. DOI: [Persistence of Salmonella enterica during dehydration and subsequent cold storage - PubMed](#)
40. Rayman, M.; D'AOUST, J.-Y.; Aris, B.; Maishment, C.; Wasik, R. Survival of microorganisms in stored pasta. *J. Food Prot.* **1979**, 42, 330–334. DOI: [Survival of Microorganisms in Stored Pasta. | Semantic Scholar](#)
41. Tamminga, S.; Beumer, R.; Kampelmacher, E.; Van Leusden, F. Survival of *Salmonella eastbourne* and *Salmonella typhimurium* in chocolate. *Epidemiol. Infect.* **1976**, 76, 41–47. DOI: [Survival of Salmonella eastbourne and Salmonella typhimurium in milk chocolate prepared with artificially contaminated milk powder - PubMed](#)
42. Kozak, G.; MacDonald, D.; Landry, L.; Farber, J. Foodborne outbreaks in Canada linked to produce: 2001 through 2009. *J. Food Prot.* **2013**, 76, 173–183. DOI: [Foodborne outbreaks in Canada linked to produce: 2001 through 2009. - Abstract - Europe PMC](#)

43. Quinones B., Massey S., Friedman M., Swimley M.S., Teter K. Novel cell-based method to detect Shiga toxin 2 from *Escherichia coli* O157:H7 and inhibitors of toxin activity. *Appl. Environ. Microbiol.* 2009;75:1410–1416. DOI: [Novel cell-based method to detect Shiga toxin 2 from Escherichia coli O157:H7 and inhibitors of toxin activity - PubMed](#)
44. Reddy S., Taylor M., Zhao M., Cherubin P., Geden S., Ray S., Francis D., Teter K. Grape extracts inhibit multiple events in the cell biology of cholera intoxication. *PloS One.* 2013;8 DOI: [Grape extracts inhibit multiple events in the cell biology of cholera intoxication - PubMed](#)
45. Cherubin P., Garcia M.C., Curtis D., Britt C.B., Craft J.W., Jr., Burrell H., Berndt C., Reddy S., Guyette J., Zheng T., Huo Q., Quinones B., Briggs J.M., Teter K. Inhibition of cholera toxin and other AB toxins by polyphenolic compounds. *PloS One.* 2016;11 DOI: [Inhibition of Cholera Toxin and Other AB Toxins by Polyphenolic Compounds - PubMed](#)
46. Sheng L., Olsen S.A., Hu J., Yue W., Means W.J., Zhu M.J. Inhibitory effects of grape seed extract on growth, quorum sensing, and virulence factors of CDC “top-six” non-O157 Shiga toxin-producing *E. coli*. *Int. J. Food Microbiol.* 2016;229:24–32. DOI: [Inhibitory effects of grape seed extract on growth, quorum sensing, and virulence factors of CDC "top-six" non-O157 Shiga toxin producing E. coli - PubMed](#)
47. Burnett, S.; Gehm, E.; Weissinger, W.; Beuchat, L. Survival of *Salmonella* in peanut butter and peanut butter spread. *J. Appl. Microbiol.* **2000**, *89*, 472–477. DOI: [Survival of Salmonella in peanut butter and peanut butter spread. - Abstract - Europe PMC](#)
48. Ray, B.; Jezeski, J.; Busta, F. Isolation of *Salmonellae* from naturally contaminated dried milk products: II. Influence of storage time on the isolation of *Salmonellae*. *J. Milk Food Technol.* **1971**, *34*, 423–427. DOI: [ISOLATION OF SALMONELLAE FROM NATURALLY CONTAMINATED DRIED MILK PRODUCTS: II. INFLUENCE OF STORAGE TIME ON THE ISOLATION OF SALMONELLAE - ScienceDirect](#)
49. Davarynejad, D. G., Hamed, K., Nyéki J., Szabó Z. & Nagy P. T. (2012): Levels of some micronutrient in dried and fresh fruit samples of apricot cultivars, *International Journal of Horticultural Science* 2012, 18 (1): 25–30. DOI: [161872441.pdf \(core.ac.uk\)](#)
50. Bennett, L. E., Singh, D. P., & Clingeffer, P. R. (2010). Micronutrient Mineral and Folate Content of Australian and Imported Dried Fruit Products. *Critical Reviews in Food Science and Nutrition*, 51(1), 38–49. DOI: [Micronutrient Mineral and Folate Content of Australian and Imported Dried Fruit Products: Critical Reviews in Food Science and Nutrition: Vol 51 , No 1 - Get Access \(tandfonline.com\)](#)
51. Stacewicz-Sapuntzakis, M., Bowen, P. E., Hussain, E. A., Damayanti-Wood, B. I., & Farnsworth, N. R. (2001). Chemical Composition and Potential Health Effects of Prunes: A Functional Food? *Critical Reviews in Food Science and Nutrition*, 41(4), 251–286. DOI: [Chemical Composition and Potential Health Effects of Prunes: A Functional Food?: Critical Reviews in Food Science and Nutrition: Vol 41, No 4 \(tandfonline.com\)](#)
52. Kanellos, P.T., Kaliora, A.C., Liaskos, C. et al. (2013): A Study of Glycemic Response to Corinthian Raisins in Healthy Subjects and in Type 2 Diabetes Mellitus Patients. *Plant Foods Hum Nutr* 68, 145–148. DOI: [A Study of Glycemic Response to Corinthian Raisins in Healthy Subjects and in Type 2 Diabetes Mellitus Patients | Plant Foods for Human Nutrition \(springer.com\)](#)
53. Wursch P, Pi-Sunyer FX (2002) The role of viscous soluble fiber in the metabolic control of diabetes: a review with special emphasis on cereals rich in beta-glucan. *Diabetes Care* 20:1774–1780. DOI: [The Role of Viscous Soluble Fiber in the Metabolic Control of Diabetes: A review with special emphasis on cereals rich in β-glucan | Diabetes Care | American Diabetes Association \(diabetesjournals.org\)](#)
54. Chiu, H. F., Huang, Y. C., Lu, Y. Y., Han, Y. C., Shen, Y. C., Golovinskaia, O., ... Wang, C. K. (2017). Regulatory/modulatory effect of prune essence concentrate on intestinal function and blood lipids. *Pharmaceutical Biology*, 55(1), 974–979. DOI: [Full article:](#)

[Regulatory/modulatory effect of prune essence concentrate on intestinal function and blood lipids \(tandfonline.com\)](https://doi.org/10.1080/00087041.2019.1644444)

55. Ibrahim A. Abd El-Gawad, E.M. El-Sayed, S.A. Hafez, H.M. El-Zeini, F.A. Saleh (2005): The hypocholesterolaemic effect of milk yoghurt and soy-yoghurt containing bifidobacteria in rats fed on a cholesterol-enriched diet. *International Dairy Journal*, Volume 15, Issue 1, Pages 37-44. DOI: [The hypocholesterolaemic effect of milk yoghurt and soy-yoghurt containing bifidobacteria in rats fed on a cholesterol-enriched diet - ScienceDirect](https://doi.org/10.1016/j.idj.2004.12.001)
56. Marlene Maria Amaral Scheid, Yara Maria Franco Moreno, Mário Roberto Maróstica Junior, Glaucia Maria Pastore (2013): Effect of prebiotics on the health of the elderly. *Food Research International*, Volume 53, Issue 1, Pages 426-432. DOI: [Effect of prebiotics on the health of the elderly - ScienceDirect](https://doi.org/10.1016/j.foodres.2012.12.011)
57. World Health Organization (WHO) (2007-2015) Estimates of the Global Burden of Food Borne Diseases; Foodborne Disease Burden Epidemiology Reference Group. http://apps.who.int/iris/bitstream/10665/199350/1/9789241565165_eng.pdf?ua=1
58. Shamloo E, Hosseini H, Abdi Moghadam Z, Halberg Larsen M, Haslberger A, Alebouyeh M. (2019): Importance of *Listeria monocytogenes* in food safety: a review of its prevalence, detection, and antibiotic resistance. *Iran J Vet Res.*;20(4):241-254. DOI: [Importance of Listeria monocytogenes in food safety: a review of its prevalence, detection, and antibiotic resistance - PMC \(nih.gov\)](https://doi.org/10.30453/ijvr.2019.204241)
59. Silk, BJ , Mahon, BE , Griffin, PM , Gould, LH , Tauxe, RV , Crim, SM , Jackson, KA , Gerner-Smidt, P , Herman, KM , Henao, OL (2013): Vital signs: *Listeria* illnesses, deaths, and outbreaks - United States, 2009-2011. *MMWR.*;62:448–452. DOI: [Vital signs: Listeria illnesses, deaths, and outbreaks--United States, 2009-2011 - PubMed \(nih.gov\)](https://doi.org/10.15585/mmwr.mm6204a1)
60. D. O’Beirne, G.A. Francis, (2003): 12 - Reducing pathogen risks in MAP-prepared produce. *Woodhead Publishing Series in Food Science, Technology and Nutrition, Novel Food Packaging Techniques*, 231-275. DOI: [Reducing pathogen risks in MAP-prepared produce - ScienceDirect](https://doi.org/10.1533/9780857099444.ch12)
61. Zhuosheng Liu, Chao Liao, Kayla Golson, Shelley Phillips, Luxin Wang (2021): Survival of common foodborne pathogens on dried apricots made with and without sulfur dioxide treatment. *Food Control*, Volume 121, 107569. DOI: [Survival of common foodborne pathogens on dried apricots made with and without sulfur dioxide treatment - ScienceDirect](https://doi.org/10.1016/j.foodcon.2021.107569)
62. L. R. Beuchat, E. Komitopoulou, H. Beckers, R. P. Betts, F. Bourdichon, S. Fanning, H. M. Joosten, B. H. Ter Kuile (2013): Low Water Activity Foods: Increased Concern as Vehicles of Foodborne Pathogens. *Journal of Food Protection*, Volume 76, Issue 1, Pages 150-172. DOI: [Low--Water Activity Foods: Increased Concern as Vehicles of Foodborne Pathogens - ScienceDirect](https://doi.org/10.4315/0391-3059-76.1.150)
63. Beuchat LR, Mann DA. (2014): Survival of salmonella on dried fruits and in aqueous dried fruit homogenates as affected by temperature. *J Food Prot.* 77(7):1102-9. DOI: [Survival of salmonella on dried fruits and in aqueous dried fruit homogenates as affected by temperature - PubMed \(nih.gov\)](https://doi.org/10.4315/0391-3059-77.7.1102)
64. P. A. DiPersio, P. A. Kendall, J. N. Sofos, M. Calicioglu (2003): Inactivation of *Salmonella* during drying and storage of apple slices treated with acidic or sodium metabisulfite solutions. *J Food Prot.* 66(12):2245-51. DOI: [Inactivation of Salmonella during drying and storage of apple slices treated with acidic or sodium metabisulfite solutions - PubMed \(nih.gov\)](https://doi.org/10.4315/0391-3059-66.12.2245)
65. P. A. DiPersio, P. A. Kendall, J. N. Sofos, (2004): Inactivation of *Listeria monocytogenes* during drying and storage of peach slices treated with acidic or sodium metabisulfite solutions. *Food Microbiology*, Volume 21, Issue 6, Pages 641-648. DOI: [Inactivation of Listeria monocytogenes during drying and storage of peach slices treated with acidic or sodium metabisulfite solutions - ScienceDirect](https://doi.org/10.1016/j.fm.2004.05.001)
66. Canakapalli, Sushumna Sri (2021): The Microbial Safety of Dried Fruits, UC Davis. DOI: [The Microbial Safety of Dried Fruits](https://doi.org/10.1016/j.fm.2021.107569)

67. Altendorf K, Booth IR, Gralla J, Greie J, Rosenthal AZ, Wood JM. 2009. Osmotic Stress. 3:10.1128/ecosalplus.5.4.5. DOI: [Osmotic Stress | EcoSal Plus](#)
68. Wang, Y., Zhao, Y., Bollas, A. et al. Nanopore sequencing technology, bioinformatics and applications. Nat Biotechnol 39, 1348–1365 (2021). DOI: [Nanopore sequencing technology and its applications - PubMed](#)
69. Gbégbé DA, Kacou OBC, N'zi NP, Angaman DM (2024): *Priestia flexa* as a Novel Urinary Tract Pathogen in Daloa, Côte d'Ivoire: Insights From Genomic Sequencing. Int J Genomics. 2024 Aug 3;2024:6239250. DOI: [Priestia flexa as a Novel Urinary Tract Pathogen in Daloa, Côte d'Ivoire: Insights From Genomic Sequencing - PubMed \(nih.gov\)](#)
70. Deswal G, Selwal MK, Nirvan H, Selwal KK (2023): *Priestia flexa* KS1: A new bacterial strain isolated from human faeces implicated in mucin degradation. Int Microbiol. 2023 Aug;26(3):475-486. DOI: [Priestia flexa KS1: A new bacterial strain isolated from human faeces implicated in mucin degradation - PubMed \(nih.gov\)](#)
71. Purty S, Saranathan R, Prashanth K, Narayanan K, Asir J, Sheela Devi C, Kumar Amarnath S. The expanding spectrum of human infections caused by Kocuria species: a case report and literature review. Emerg Microbes Infect. 2013 Oct;2(10):e71. DOI: [The expanding spectrum of human infections caused by Kocuria species: a case report and literature review - PMC](#)
72. Dého Aristide Gbégbé, Okran Beyosse Christophe Kacou, N'goran Parfait N'zi, Djédoux Maxime Angaman. *Priestia flexa* as a Novel Urinary Tract Pathogen in Daloa, Côte d'Ivoire: Insights From Genomic Sequencing. International Journal of Genomics. 2024. DOI: [Priestia flexa as a Novel Urinary Tract Pathogen in Daloa, Côte d'Ivoire: Insights From Genomic Sequencing - Gbégbé - 2024 - International Journal of Genomics - Wiley Online Library](#)
73. Deswal G, Selwal MK, Nirvan H, Selwal KK. *Priestia flexa* KS1: A new bacterial strain isolated from human faeces implicated in mucin degradation. Int Microbiol. 2023 Aug;26(3):475-486. DOI: [Priestia flexa KS1: A new bacterial strain isolated from human faeces implicated in mucin degradation - PubMed](#)
74. K. Smaluch, B. Wollenhaupt, H. Steinhoff, D. Kohlheyer, A. Grünberger, C. Dusny (2022): Assessing the growth kinetics and stoichiometry of *Escherichia coli* at the single-cell level, Engineering in Life Sciences, vol.3, no. 1. DOI: [Assessing the growth kinetics and stoichiometry of Escherichia coli at the single-cell level - Smaluch - 2023 - Engineering in Life Sciences - Wiley Online Library](#)
75. Internet 5: Food Data Central - Raisins. [FoodData Central](#)
76. Internet 6: Food Data Central - Prune. [FoodData Central](#)
77. Internet 7: Food Data Central - Dried apricot. [FoodData Central](#)