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Microbiological and sensory analysis of cultivated mushrooms from retail

Diplomarbeit

zur Erlangung der Würde einer Magistra medicinae veterinariae

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Content

Abbreviations

%	Percent
°C	Degree Celsius
acc.	According
ALOA	Listeria Agar nach Ottaviani und Agosti
AMC	Aerobic mesophilic bacteria
BPW	Buffered Pepton Water
BR	Rose Bengal Chloramphenicol Agar
CFU	Colony forming unit
e.g.	Exempli gratia
ECVPH	European college of veterinary public health
EB	Enterobacteriaceae
etc.	et cetera
FF	Full-Fraser
g	Gram
GSP	Pseudomonas/Aeromonas Selective Agar acc. to Kielwein
HF	Half-Fraser
ISO	International Organization for Standardization
KOH	Potassium hydroxide
LAB	lactic acid bacteria
MKTT	Muller-Kauffmann Tetrathionate-Novobiocin Broth
ml	Milliliter
MRS	Lactobacillus Agar acc. to De Man, Rogosa and Sharpe
MYP	Mannitol Egg Yolk Polymyxin Agar
neg.	Negative
pH	pH-level
pos.	Positive
PS	Pseudomonadaceae
RVS	Rappaport Vassiliadis soya peptone broth
spp.	Species pluralis

TSA	Tryptic Soy Agar plus 0,6 % Yeast
VRBG	Violet Red Bile Glucose Agar
XLD	Xylose-Lysin-Desoxycholat-Agar
µl	Microliter

1. Introduction

1.1. Background information

1.1.1. Mushrooms

Like plants and animals, mushrooms are eukaryotic organisms. In the past, they were mistakenly assigned to the plants, but now it is known that they form an independent group and have more in common with animals. Along with that group, they belong to the heterotrophic organisms and feed on organic nutrients that they externally break down using enzymes and then absorb. Furthermore, like animals, they produce the storage product glycogen. However, unlike animals, they have vacuoles and cell walls as they occur in plants and thus differ from that group. They can be differentiated from plants mainly by the lack of plastids and consequently by the absence of photosynthesis. Another difference is the presence of chitin, which does not occur in plants. In contrast, they lack the typical plant substance cellulose (Webster and Weber, 2007; Stephenson, 2010; Watkinson et al., 2015).

The taxonomic classification of these complicated organisms is not easy, and although there has been significant improvement in recent decades, there are still many left that need to be correctly classified. Today, the kingdom of fungi comprises the following phyla: *Chytridiomycota, Zygomycota, Glomeromycota, Ascomycota,* and *Basidiomycota.* The latter includes *Pleurotus ostreatus* (Oyster mushroom/ *P. ostreatus*), *Pleurotus eryngii* (King oyster mushroom/ *P. eryngii*), and *Lentinula edodes* (Shiitake mushroom/ *L. edodes*) among others (Lutzoni et al., 2004).

Mushrooms always require a substrate such as soil, wood, or other living or dead tissue to grow. The little differentiated body of the fungus, also called Thallus, consists of microscopically fine, filamentous hyphae. These form a widely branched network-like structure called mycelium, which propagates into or onto said substrate. Depending on the type of fungus, hyphae can be subdivided by septa into separate compartments or occur undivided and continuously. These septa are permeable to smaller cell components and cytoplasm. Each compartment contains one or more nuclei. In hyphae without septa, the nuclei are scattered in the cytoplasm. Many types of fungi produce fruiting bodies, which are also composed of hyphae and stand out from the substrate. These are used for propagation, persistence, and spread by the formation of spores and represent only a small part of the entire mushroom. In addition, unicellular fungi such as yeasts exist (Webster and Weber, 2007; Stephenson, 2010; Watkinson et al., 2015).

Fungi have the potential for sexual and asexual reproduction, with asexual reproduction being predominant in most species. Asexual reproduction can occur through fragmentation of

the thallus, simple cell division, or budding, resulting in a simple genetic copy of an individual. Sexual reproduction, like in other organisms, involves the fusion of two gametes, the fusion of nuclei, and thereby the emergence of new genetic material. Both ways of reproduction take place predominantly through the formation and distribution of spores. Ultimately, these spores are released, spread, and subsequently start to form a new mycelium (Webster and Weber, 2007; Stephenson, 2010; Watkinson et al., 2015).

1.1.2. Consumption and market development

Mushrooms are becoming increasingly popular not only for their exceptional taste and high nutritional value (Heleno et al., 2010; Mattila et al., 2010; Kalač, 2013; Valverde et al., 2015), but also for their medicinal properties. Not only do they have antitumor, antiviral, antioxidant, and antibacterial properties, but they also have positive and protective effects on the cardiovascular system and the liver (Lindequist et al., 2005; Guillamón et al., 2010; Chang and Wasser, 2012; Patel and Goyal, 2012; Valverde et al., 2015; Ferreira et al., 2017).

In total, the value of the mushroom industry in 2021 was around $ 50.3 billion (US Dollars), with an expected annual growth of 9.7 % from 2022 to 2030 (Grand view research, 2022). In 2021, the global mushroom market size was 15.3 million tons. With an estimated annual growth rate of 6.74 %, the worldwide market is expected to increase to 24.1 million tons by 2028. The increasing demand for a low-calorie, high-nutritional, and protein-rich diet is expected to be a key driver for the mushroom market in the upcoming years (Fortune business insights, 2022). From 2009 to 2019, an increase in worldwide mushroom and truffle production from 7.5 to 11.8 million tons was already observed (FAO, 2021).

However, from 2006 to 2022, the consumption of mushrooms in Austria did not increase and remained steady at approximately 18 thousand tons (Statista, 2023). With only 15 %, Austria shows rather low self-sufficiency when it comes to cultivated mushrooms (Bundesministerium für Land- und Forstwirtschaft, Regionen und Wasserwirtschaft, 2023).

Due to their short shelf life, fresh mushrooms raise challenges for manufacturers in terms of production and distribution. Nevertheless, as of 2021, with an 89.5 % sales share, the fresh form made up the bulk of the global mushroom market (Grand view research, 2022).

The most commonly produced and popular mushrooms are button-type mushrooms, followed by shiitake and oyster-type mushrooms (Fortune business insights, 2022). In 2021, with a volume share of 61.8 %, the button-type mushroom category dominated the global mushroom market. An expected revenue-based growth of 9.8 % by 2030 is predicted for the button-type mushroom. Their lower cost, compared to their competitors like shiitake and

oyster-type mushrooms, is expected to be the driving force of the button type mushroom market. However, it is estimated that oyster type mushrooms will exhibit the highest annual growth rate with 11.3 % by the year 2030 (Grand view research, 2022).

Time and again, the Asia Pacific region has been the biggest producer and consumer of mushrooms. As of 2021, it held a market share of 78.6 %. China in particular proved to be the largest producer and consumer, with an average annual consumption of up to 10 kilograms (kg) per person. Europe, on the other hand, relies mostly on imports. In 2019, mushroom imports in Europe were valued at $ 183 million. The COVID-19 pandemic and the implemented lockdowns and restrictions on hotels, restaurants, and food chains, caused a drop in imports and throttled the European mushroom market (Grand view research, 2022).

1.1.3. General quality requirement / Codex Austriacus

The Codex Alimentarius Austriacus (Austrian Food Book, Österreichisches Lebensmittelbuch, ÖLMB) is used for the announcement of product names, definitions, methods of examination and assessment principles, as well as for directives for the marketing of goods (Wirtschaftskammer Österreich, 2018).

Chapter B 27 is devoted to the topic of 'mushrooms and mushroom products' and includes general guidelines, requirements for mushrooms and mushroom products, and assessment principles. The two tables in the appendix of said chapter contain a list of German and scientific names of mushrooms and their classification, as well as a description of standards for mushrooms and mushroom products, such as the content of NaCl (sodium chloride) and other substances, water content, mineral and organic contaminants, and percentage of maggot-damaged mushrooms. Edible mushrooms are defined as the edible fruit bodies of the mushroom species mentioned in the appendix, which are directly placed on the market or processed. Mushroom products are defined as processed and/or preserved mushrooms (Codex Alimentarius Austriacus, Codexkapitel B 27 - Pilze und Pilzerzeugnisse).

Fresh edible mushrooms are required to be marketed as whole fruiting bodies, which have been carefully tested for their identity. Fresh mushrooms must be sensorily immaculate, not overripe, overly watery, or visibly mouldy. They must be largely free of soil, leaves, and coniferous needles and not significantly altered by maggot infestation. Mushroom products are required to be made from fresh edible mushrooms, cleaned, and free from parts that are not commonly used, especially those that are infested with maggots.

The assessment is made in accordance with the general principles of assessment of Codex Chapter A3 (Codex Alimentarius Austriacus, Codexkapitel A3 - Allgemeine

Beurteilungsgrundsätze. Codex Standard for Edible Fungi and Fungus Products, CODEX STAN 38-1981)

1.2. Microbiology and spoilage

1.2.1. Microbiology load of spoilage bacteria and potential mushroom pathogens

As with other crops, freshly cultivated mushrooms are not spared from spoilage and disease. Their production, shelf life, and overall value can be negatively affected by a variety of microorganisms such as bacteria, viruses, and fungi (Fermor, 1987; Eppo, 2010; Eastwood et al., 2015). Due to their high water content ranging from about 80 % to 90 %, neutral pH of around 6.9 and high respiration rate mushrooms offer an optimal environment for a wide variety of pre- and post-harvest existing bacteria and microorganisms (Reis et al., 2012; Jiang et al, 2018; Cliffe-Byrnes and O' Beirne, 2007; Manzi et al., 2004; Ares et al., 2006). This existing microflora, which may stem from any stage of production, can promote the spoilage of fresh mushrooms.

Previous studies have found the total aerobic mesophilic microbiological load (AMC) of fresh mushrooms, like *Pleurotus eryngii, Pleurotus ostreatus*, and *Lentinula edodes* ranging from 4.9–7.0 log CFU/g; 4.3–6.0 log CFU/g and 3.3–8.4 log CFU/g, respectively. These numbers include values that were measured up to 16 days post harvest (Venturini et al., 2011; Reyes et al., 2004; Kim et al., 2013; Jiang et al., 2013). In 1987, Doores et al. described a connection between declining mushroom quality post-harvest and bacterial growth. The total bacterial count found on *Agaricus bisporus* pre- and post-harvest ranged from 6.3 log CFU/g to 7.2 log CFU/g pre-harvest and 7.0 log CFU/g to 11.0 log CFU/g up to 10 days post-harvest at 13 °C. Cap opening and browning were used as a quality index. The vast majority of the bacteria found were ascribed to the family of *Pseudomonadaceae* (PS) (Doores et al., 1987; Venturini et al. 2011). Time and again, it has been confirmed that a high microbiological contamination coincides with the decline of sensory quality, and thus shelf life of edible mushrooms. If one compares different shelf life-prolonging methods, especially when examining treated and untreated mushrooms, the connection between the degree of bacterial contamination and the sensory quality becomes apparent. Indicators used to determine the quality of mushrooms may include color for the determination of the mushroom's quality may be color, firmness, off-odor, soluble solid content, protein content and enzyme activity (Jiang et al., 2013; Xu et al., 2016; Wang et at., 2017)

Over the past decades, PS have repeatedly been one of the most represented group of bacteria found on fresh cultivated and wild mushrooms (Doores et al., 1987; Danell et al., 1993; Reyes et. al., 2004; Venturini et al., 2011; Kim et al., 2013; Jiang et al., 2013). For *Pleurotus eryngii, Pleurotus ostreatus*, and *Lentinula edodes* average values ranging from 4.4–7.7 CFU/g were described (Venturini et al., 2011; Jiang et. al., 2013; Reyes et. al., 2004; Manthou et. al., 2019). In 2002, Eastwood and Burton described that PS, favoring moist conditions, are the main reason for the spoilage of mushrooms by causing mottled brown lesions on the surface, similar to a bruise (Eastwood and Burton, 2002). The most notable and important causative agent for the classical bacterial blotch disease of mushrooms is *Pseudomonas tolaasii* and its destructive lipopeptide toxin tolaasin. First described by Tolaas in 1915, it leads to cap discoloration, often wet and sunken in lesions, pin death, and is responsible for a high loss of yield (Tolaas, 1915; Fermor, 1987; Savoie et al., 2016). Bacterial blotch disease has been found to affect and spoil a variety of edible mushrooms, with *Lentinula edodes, Pleurotus eryngii, Pleurotus ostreatus, Agaricus bitorquis, Agaricus campestris*, and *Flammulina velutipes* just being a small subset that can be affected (Fletcher, 1979; Lee and Cha, 1998; Rainey et al., 1992; Tsuneda et al., 1995). A number of other *Pseudomonas* species spoiling edible mushrooms by causing similar blotch diseases have been described. *Pseudomonas agarici, Pseudomonas costantinii* and *Pseudomonas reactans*, together with *Pseudomonas gingeri* are said to be the main cause of drippy gill or yellow blotch, brown blotch, and ginger blotch, respectively (Young, 1970; Munsch et al., 2002; Iacobellis and Lo Cantore, 2003; Wong et al., 1982).

Although PS make up the vast majority of bacteria found on fresh mushrooms, they are not the only cause leading to their spoilage. *Enterobacteriaceae* (EB) have been found to a lower, but non-negligible proportion on fresh mushrooms, with average values ranging from 2.7–3.7 log CFU/g for *Pleurotus eryngii, Pleurotus ostreatus* and *Lentinula edodes* (Reyes et al., 2004; Venturini et al., 2011). However, higher mean values of up to 6.5 log CFU/g for other mushroom species were described (Venturini et al., 2011; González et al., 2012; Donzellini et al., 2018). In 1996, Ingils et al. described a link between *Ewignella americana* and the browning disorder called ‘internal stipe necrosis’ (Inglis et al., 1996). Later on, Reyes et al. examined the occurrence and mycopathogenic potency of EB on *Lentinula edodes*, *Pleurotus ostreatus*, and *Agaricus bisporus*. Again, the majority of the EB isolated were identified as *Ewignella americana*, which was ascribed to be responsible for browning lesions and necrosis in the center of the *Agaricus bisporus* stalk. However, no necrosis or browning

disorder could be induced in *Lentinula edodes* and *Pleurotus ostreatus* (Reyes et al., 2004). Further studies also identified *Ewignella americana* as the causative agent for internal stem necrosis in cultivated mushrooms (Lee et al., 2009; Madbouly et al., 2014; Hamidizade et al., 2022).

Other microorganisms, such as lactic acid bacteria (LAB), yeasts, and mold, may be found on fresh mushrooms like *Pleurotus eryngii, Pleurotus ostreatus*, and *Lentinula edodes* but usually in fewer numbers with average values ranging from 1.7–2.1 log CFU/g for LAB and 2.6–3.4 log CFU/g for yeasts and mold (Venturini et al., 2011; Siyoum et al., 2015; Dril et al., 2021). A lot of important fungal contaminants are known to be limiting factors for worldwide mushroom production by causing diseases like green mold, dry bubble, wet bubble, cobweb, and others (Biswas and Kuiry, 2013; Fletcher and Gaze, 2007).

But not all contaminants are harmful. Mushrooms are grown in different soils or certain selective substrates that provide them with the nutrients they need for fructification. The existing microflora in said soil has a strong influence on growth and fructification and is, in some cases, supportive or even required. They can be helpful by suppressing competing fungi with secreted antibiotics, protecting against plant parasites or improving nutrition by breaking down complex polycarbohydrates (Carrasco and Preston, 2020). *Pseudomonas putida*, for example, has a positive and stimulating effect on hyphal growth and colony morphology of *Agaricus bisporus* (Rainey, 1991). Even fluorescent PS can be beneficial by promoting the fructification of *Pleurotus ostreatus* (Cho et al., 2003).

1.2.2. Occurrence of potential human pathogens

The highest priorities for the food industry are the safety of their products and ensuring that certain quality standards throughout the production chain are met. Even though a multitude of safety measures are in place to avoid contaminations with foodborne pathogens, they still occur, and tainted products, such as fresh mushrooms, can end up in retail. An important instrument in ensuring food safety is the Rapid Alert System for Food and Feed (RASFF) of the European Union. Implemented for the exchange of information between member countries, it supports a rapid response by food safety authorities in case of public health risks arising from the food chain. The system provides a service whereby vital and urgent notifications are sent, received, and responded to around the clock collaboratively and efficiently. To make information available to non-member countries, such as consumers,

business operators, and authorities worldwide, an interactive, searchable online database, called “RASFF Window” was created. It grants access to summarized information about the most recently submitted RASFF notifications and allows searching for information on all notifications issued in the past, without revealing commercial details such as brands or company names. For non-member countries, “RASFF Window” represents a major source of information concerning products that have been exported or imported, by a member country.

With their potency to cause serious diseases in humans, *Bacillus (B.) cereus, Listeria (L.) monocytogenes*, and *Salmonella* spp. are among the best-monitored human pathogens found in food. In the years from 1999 to 2022, the European Union's Rapid Alert System for Food and Feed recorded 68 instances of contamination with foodborne pathogens. Among those reported cases, *Salmonella* spp. (n = 24), *B. cereus* (n = 15), and *L. monocytogenes* (n = 15) were the most common foodborne agents in fresh, dried, brined and canned mushrooms (RASFF Window, 2023; EU open data portal, 2023).

However, although several regulations are in place to ensure food safety, there are no specific legal regulations regarding the occurrence of *B. cereus, L. monocytogenes*, and *Salmonella* spp. in fresh mushrooms.

1.2.2.1. Salmonella spp.

Several studies have investigated the prevalence of *Salmonella* spp. among other different foodborne pathogens on mushrooms. Venturini et al. tested 402 mushroom samples comprising 22 different species for their microbiological quality and safety. No *Salmonella* spp. could be detected in any of the examined *Pleurotus eryngii, Pleurotus ostreatus*, and *Lentinula edodes* samples (Venturini, et al., 2011). In 2003, 202 samples of mushrooms from US-retail were examined, but no *Salmonella* spp. was found (Strapp et al., 2003). In 2006, among 100 (uncategorized) examined mushroom samples from US-retail, only five (5.0 %) tested positive for *Salmonella* spp. (Samadpour et al., 2006). Lastly in 2020, *Salmonella* spp. was not detected in any of the tested mushroom samples originating from the Canadian retail market (Zhang et al., 2020). However, again in 2020, the CDC investigated an outbreak of *Salmonella* spp. infections linked to dried wood ear mushrooms, and 55 people were infected through mushrooms used only in restaurants (CDC, 2020).

1.2.2.2. Bacillus cereus

In 2021, a study investigated the microbiological safety of grey oyster mushrooms. The occurrence of *B. cereus* was tested and values ranging from 0.5 ± 0.7 log CFU/g at day zero

to 1.7 ± 2.4 log CFU/g at day twelve after storage at 4 °C were found. A similar increase from 0.5 ± 0.7 to 2.7 ± 3.8 log CFU/g after six days for samples stored at 25 °C was detected (Suhaili et al., 2021) Previously, Shiitake mushrooms in whole and sliced fresh form, were found to contain 1.0 log CFU/g of *B. cereus* (Kim et al., 2016). Messelhäusser et al., found 81.5 % of its analyzed dry mushroom samples to be contaminated with enterotoxigenic *B. cereus* (Messelhäusser et al., 2014). *B. cereus* was found in 28.3 % of Chinese mushroom samples in 2020, with average contamination levels varying from 0.5 to 3.0 log CFU/g (Liu et al., 2020). In most clinically relevant cases, 5 to 8 log cells/spores per g of contaminated and ingested food were linked to *B. cereus*-caused foodborne illnesses. Lower food contamination levels (3–4 log CFU/g) were found in certain cases, nevertheless (European Food Safety Authority, 2005). In 2011, *B. cereus* related foodborne intoxications and toxicoinfections increased by 122.2 %, according to the European Food Safety Authority (EFSA) (European Food Safety Authority and European Centre for Disease Prevention and Control, 2013). *B. cereus* is easily found in the environment, notably in the digestive tracts of invertebrates and in organic matter such as rotting or spoiled vegetables (Bottone, 2010). Both diarrheal and emetic symptoms are two forms of food poisoning that this spore-forming bacterium can inflict upon people. Of particular concern is the potential for cereulide toxin synthesis by *B. cereus* and its emesis-inducing potency. Cereulide, in contrast to diarrheal toxins, is pre-formed in food and can result in foodborne intoxications immediately after tainted food is consumed. Cereulide is frequently created in favorable environments such as those found in foods rich in starches, carbohydrates, vitamins, and trace minerals, as well as in physiological settings like pH neutrality and water activity ranging from intermediate to high (Messelhäusser et al., 2014).

1.2.2.3. Listeria monocytogenes

According to Regulation VO (EG) 2073/2005, *L. monocytogenes* must not be detected in five samples of 25 g each in ready-to-eat foods. In addition, the microbiological count of 100 CFU/g must not be exceeded during the entire shelf life (VO (EG) 2073/2005). With fatality rates of up to 20–30 %, *L. monocytogenes* continues to be a serious public health issue. The intracellular foodborne pathogen, which is particularly dangerous for those with impaired immune systems, pregnant women, the elderly, and newborns, causes listeriosis, a deadly condition linked to meningitis, meningoencephalitis, and abortion (Lomonaco et al., 2015). *L. monocytogenes* can survive in a variety of unfavorable conditions, including a broad range of pH levels, low temperatures, and high salinities (Gandhi and Chikindas, 2007). As a result of

L. monocytogenes biofilm's great resistance to disinfectants, food plants continue to be tainted (Nakamura et al., 2013; Piercey et al., 2017). It has been discovered in both manufacturing and processing facilities in the surroundings of a mushroom production site (Viswanath et al., 2013; Murugesan et al., 2015). *L. monocytogenes* contamination of edible mushroom products has been documented in several nations in the past (Cordano and Jacquet, 2009; Venturini et al., 2011; Viswanath et al., 2013; Wu et al., 2015). In 2006, Samadpour et al. investigated the incidence of *L. monocytogenes* in mushrooms from retail in the USA and found only 1 % out of 100 samples to be positive (Samadpour et al., 2006). In Norway, only 0.6 % (n = 1/156) of fresh mushroom samples from retail tested positive for *L. monocytogenes* (Johannessen et al., 2002), and in Canada, only 0.8 % (n = 8/1008) were tainted with the pathogen (Zhang et al., 2020). In Spain, not one sample of cultivated mushrooms tested positive for *L. monocytogenes* (Venturini et al., 2011). In a study describing the occurrence of *L. monocytogenes* on edible mushrooms in Chinese markets, approximately 21.2 % of mushroom samples tested positive. However, contamination rates for *P. ostreatus*, *P. eryngii*, and *L. edodes* in comparison ranged rather low with 6.7 %, 4.4 %, and 2.9 %, respectively (Chen et al., 2018). A similar study investigating the prevalence of *L. monocytogenes* on *Flammulina velutipes* described higher values and found that as much as 18.6 % out of 295 samples were contaminated (Chen et al., 2014). The CDC reported an outbreak of *L. monocytogenes* infections, resulting in four deaths in 2020. Source of the tainted Enoki mushrooms was most likely South Korea (CDC, 2020). In 2022 an RASFF alert was issued for Enoki mushrooms contaminated with *Listeria* in Slovenia and the Netherlands. The contaminated mushrooms originated in China and South Korea (RASFF Window, 2023).

1.3. Aim of this study

The primary idea of this study was the consideration that fresh mushrooms belong to highly perishable products, lose their sensory quality easily, and therefore only have a short shelf life. Main reasons are their high water content, neutral pH, and high respiration rate, which provide a suitable environment for the pre- and post-harvest existing bacteria, including food-relevant pathogenic organisms (Reis et al., 2012; Jiang et al, 2018; Cliffe-Byrnes and O'Beirne, 2007; Manzi et al., 2004; Ares et al., 2006).

The main hypothesis was that the total bacterial load increases fast and to a high degree during storage, and that at the same time the sensory quality, influenced by the increasing microbiological load, decreases accordingly. In order to confirm or even refute this theory

and to estimate the shelf life of *Pleurotus eryngii, Pleurotus ostreatus*, and *Lentinula edodes* available in Austrian trade, the microbiological and sensory quality of a total of 40 samples were examined on the day of purchase and after storage.

2. Material and methods

2.1. Study design and sampling

Over a twelve-week period from April to June 2018 a total of 40 packs of mushrooms, consisting of *Pleurotus eryngii* (from Austria and Korea), *Pleurotus ostreatus* (from Austria and Germany), and *Lentinula edodes* (from Austria and Poland) were analyzed for their microbiological load and sensory quality. Furthermore, the study was focused on potential food-associated human pathogens. In order to exclude cross-contamination no loose goods but only already packed mushrooms were bought and used in this study. To ensure accurate analysis, always two packs of mushrooms with the same Lot-number were purchased: one to be analyzed on the day of purchase and the other after storage. When selecting the samples, care was taken to buy the freshest packs available to ensure a maximum interval between analyses. Since there is no legal requirement for mushrooms to have an expiration date, the Lot-number, consisting of the calendar week and day of packaging, was used to identify the most recently packed and therefore freshest mushrooms. Only mushrooms offered by one Austrian retail market were labeled with an expiration date. For *Pleurotus eryngii* (King oyster mushroom), it was set at 17 days, and for *Pleurotus ostreatus* (Oyster mushroom) and *Lentinula edodes* (Shiitake mushroom) at seven days after packaging. This storing time was taken over for all samples in the study. All samples were analyzed for their AMC, the count of EB, PS, LAB, yeasts, molds, and *B. cereus*, as well as the presence of *L. monocytogenes* and *Salmonella* spp.

In addition, the sensory quality of all 40 samples on the day of purchase and again after storage was examined. Attributes investigated were appearance, texture, aroma and taste after cooking.

2.2. Material

2.2.1. Obtained samples

All samples were purchased in different Austrian supermarkets. The majority of examined samples were mushrooms produced in Austria, some were imports from South Korea, Germany and Poland (Table 1). In store, all mushrooms were refrigerated, and apart from transport, they were continuously stored in our laboratory refrigerator at a constant temperature of 4 °C. All examined mushrooms were already packaged using a variety of different packaging methods.

Table 1: Mushroom samples (n = 40), analyzed in this study focusing on origin.

Country of Origin	Producer	Number of samples
Pleurotus ostreatus (Oyster mushroom)		
Austria	C	8
Poland	E	2
Pleurotus eryngii (King oyster mushroom)		
Austria	A	10
South Korea	B	10
Lentinula edodes (Shiitake mushroom)		
Austria	C	8
Germany	D	2
Sum	4 different producers	40

2.2.2. Materials for microbiological and sensory examination

An overview of the materials used for the microbiological and sensory analysis can be found in the appendix (supplementary Table 1).

2.3. Method

2.3.1. Microbiological analysis

Microbiological tests were performed according to ISO standards. 25 grams (g) of mushroom, cut into small pieces by sterilized scissors, were weight into a sterile stomacher filter bag (Seward Ltd., Worthing, United Kingdom), mixed with 225 ml of room tempered buffered peptone water (BPW, Oxoid Ltd., Basingstoke, United Kingdom), and homogenized with a Laboratory Blender Stomacher 400 (Seward Ltd., Worthing, United Kingdom). This solution was serially diluted with a ratio of 1:10 in Ringer's solution (B. Braun Melsungen AG, Melsungen, Germany) to a maximal dilution level of 10^{-8}. 100 µl of each dilution stage were evenly spread on duplicate plates of Tryptic Soy Agar (TSA, Merck KGaA, Darmstadt, Germany) and single plates of Violet Red Bile Dextrose agar (VRBD, Biokar Diagnostics, Beauvais Cedex, France) for the analysis of total bacterial count (ISO 4833-2:2013) and the detection of EB (ISO 21528-2:2004). For the detection of PS species (ISO 13720:2010), Glutamate starch phenol red agar (GSP, Merck KGaA, Darmstadt, Germany) was used, while for LAB (ISO 15214:1998) a selective Agar according to De Man, Rogosa and Sharpe (MRS, Biokar Diagnostics, Beauvais Cedex, France) and for yeasts and molds (ISO 21527-

1:2008) Rose-Bengal-Chloramphenicol-Agar (BR, Oxoid Ltd., Basingstoke, United Kingdom) was used. The detection limit was 10^2 colony-forming units per gram (CFU/g). In addition to that, 1 ml from the first dilution level was equally divided into 333 µl each and evenly spread on three plates of Mannitol Egg Yolk Polymyxin Agar (MYP, Oxoid Ltd., Basingstoke, United Kingdom) for the detection of *B. cereus* (ISO 7932:2004). Figure 1 shows the scheme of the dilution series.

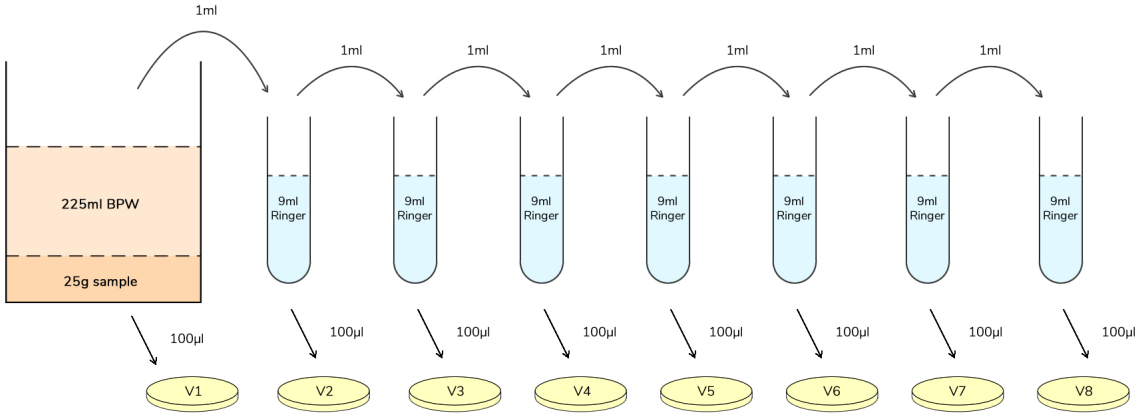

Figure 1: Scheme of dilution series.

The TSA, VRBD, MYP, and MRS plates were all incubated at 30 °C for 24 to 48 hours. If necessary, the plates were left at room temperature for another 24 hours or more to ensure optimal growth of the bacterial colonies. GSP and BR agar plates were incubated at 25 °C for 24 hours to five days, depending on the growth rate of the present bacteria, yeasts, and molds.

All colonies were counted on plates with less than 300 colonies and on plates of the next higher dilution level. Each colony was differentiated and sorted by optical features such as color, size, luster, elevation, and the presence of a halo. Three or more colonies with clean margins of each group were picked to be subcultured on TSA plates. Those were incubated for 24 hours at 37 °C.

The different types of bacteria in question were subsequently identified by a combination of their typical appearance on their selective agar and by analyzing distinctive properties such as the response to hydrogen peroxide (H_2O_2) (VWR, laboratory equipment, Pennsylvania, USA) which determines the presence or absence of the enzyme catalase, the response to potassium hydroxide (KOH) (VWR, laboratory equipment, Pennsylvania, USA) which identifies them as gram-positive (gram+) or gram-negative (gram-), and the presence of the enzyme cytochrome-c-oxidase, which was determined using the Kovacs reagent (VWR,

laboratory equipment, Pennsylvania, USA). EB, for example, form red colonies surrounded by reddish precipitation zones on their selective agar VRBD (Merck, 2005). They are gram- and possess both the catalase and cytochrome-c-oxidase enzymes **(**Mossel et al., 1962; Octavia and Lan, 2014; Donnenberg, 2015). PS colonies are blue-violet surrounded by a red-violet zone on their selective agar GSP (Merck, 2005). They are gram- and possess the catalase enzyme but not cytochrome-c-oxidase (Kielwein, 1969; Burns, 2018). *B. cereus* shows with his dry colonies, the bright pink background surrounded by an egg yolk precipitate a very typical growth on MYP agar (Merck, 2005). It's a gram+ bacterium with both the catalase and cytochrome-c-oxidase enzymes (Mossel et al., 1967; Coia and Cubie, 1995). LAB show compact or feathery, small, opaque, and white colonies on MRS agar (Merck, 2005). They are gram+ and have neither the catalase nor cytochrome-c-oxidase enzyme (Bratcher, 2018).

Yeasts and molds were identified by their characteristic outer appearance. *Penicilium* spp. show a very distinct growth of white mycelia and green spores. Yeasts form colonies with a size of 1–4 millimeters (mm) and are typically pink in color. Suspicious yeast colonies were further examined by microscopy and identified by their round-oval cells of approximately 4 micrometers (µm) in size (Barnett et al.,1983).

The colony-forming units per gram (CFU/g) were calculated using the following formula. The quantitative results were calculated according to ISO 7218:2007/Amd.1:2013. For this purpose, colony counts of at least ten up to a maximum of 300 on one plate were used.

$$\frac{\sum C}{V * \{n1 + (n2 * 0{,}1)\} * d}$$

$\sum$ C = Sum of all colonies of all counted plates.

V = Volume of dilutions pipetted onto the plates in ml.

n1 = Number of plates of lower dilution.

n2 = Number of plates of higher dilution.

d = Dilution factor of the lower dilution.

2.3.1.1. Salmonella spp.

For this study, a horizontal method for the detection of *Salmonella* spp. adapted from ISO 6579-1:2017 was used. The homogenate from the sample preparation, consisting of 25 g of

mushroom and 225 ml of buffered peptone water, was incubated for 18–24 hours at 37 °C. For selective enrichment, 100 µl were transferred into Rappaport-Vassiliadis soya peptone broth (RVS, Oxoid Ltd., Basingstoke, United Kingdom), and incubated for 24 hours at 42 °C. Additionally, 1 ml was transferred into Muller-Kauffmann Tetrathionate-Novobiocin Broth (MKTT, Oxoid Ltd., Basingstoke, United Kingdom) and incubated for 24 hours at 37 °C. Afterwards, 3 µl of each enrichment broth were streaked on Xylose-Lysin-Desoxycholat Agar plates (XLD, Oxoid Ltd., Basingstoke, United Kingdom) and incubated at 37 °C for 24 hours.

2.3.1.2. Listeria monocytogenes

A horizontal method for the detection of *Listeria* spp., adapted from ISO 11290-1:2017 was used. For pre-enrichment in a selective liquid medium, 25 g of cut-up sample were weighed into a sterile stomacher filter bag and homogenized with 225 ml of semi-fraser broth (DF, Biokar Diagnostics, Beauvais Cedex, France). This mixture was then incubated at 30 °C for 24 hours. After incubation, for enrichment in a second selective medium, 100 µl were carried over into 10 ml of full-fraser broth (FF, Merck KGaA, Darmstadt, Germany), which was then incubated at 37 °C for 48 hours. Afterwards, 3 µl were streaked evenly on Listeria Agar plates according to Ottaviani and Agosti (ALOA, Merck KGaA, Darmstadt, Germany) and incubated at 37 °C for 48 hours.

Table 2: Colony morphology on selective agar plates used for enumeration and differentiation of hygiene indicators and foodborne pathogens.

Selective plates	Colony Morphology	Company
Violet Red Bile Dextrose Agar (VRBD)	EB: red colonies surrounded by reddish precipitation zones.	Biokar Diagnostics, Beauvais Cedex, France
Glutamat-Stärke-Phenolrot-Agar (GSP)	PS: blue-violet colonies surrounded by a red-violet zone.	Merck, KGaA, Darmstadt, Germany
Rose-Bengal Chloramphenicol Agar (BR)	Yeasts: matt or shiny round colonies, typically pink in color. Molds: flat or fluffy spreading colonies with white mycelia and green spores.	Oxoid Ltd., Basingstoke, United Kingdom
Lactobacilli selective Agar acc. to De Man, Rogosa and Sharpe (MRS)	LAB: compact or feathery, small, opaque, and white colonies.	Biokar Diagnostics, Beauvais Cedex, France
Mannitol Egg Yolk Polymyxin Agar (MYP)	Presumptive BC: dry colonies, bright pink background surrounded by an egg yolk precipitate.	Oxoid Ltd., Basingstoke, United Kingdom
Listeria Agar acc. to Ottaviani and Agosti (ALOA)	LM: blue green colonies with opaque halo.	Merck, KGaA, Darmstadt, Germany
Xylose-Lysin-Desoxycholat-Agar (XLD)	S: black center and a lightly transparent zone of reddish color.	Oxoid Ltd., Basingstoke, United Kingdom
Abbreviations: EB–*Enterobacteriaceae*, PS–*Pseudomonaceae*; AE–*Aeromonadaceae*; LAB–lactic acid bacteria; BC–*Bacillus cereus*; LM–*Listeria monocytogenes*; S–*Salmonella* spp.		

2.3.2. Sensory analysis

The sensory analysis of all mushrooms was performed as a descriptive test with following quality evaluation according to DIN 10969:2018. Sensory characteristics, such as appearance, texture and consistency, aroma, and taste were assessed by three panelists trained by our laboratory. The published quality standards, including the Codex Alimentarius

Standard, were used for the sensory evaluation of the King oyster, Oyster and Shiitake mushrooms. The following worksheet considering the mushroom specific characters described by Zawirska-Wojtasiak et al. (2009), Boin et al., (2016), Zheng et al., (2017), Politowicz et al. (2017), Aisala et al. (2018) and Codex Standard for Edible Fungi and Fungus Products (CODEX STAN 38-1981) was used for this purpose (Table3).

Table 3: Quality standards for sensory evaluation of the King oyster, Oyster, and Shiitake mushroom.

	King oyster mushroom	***Oyster mushroom***	***Shiitake mushroom***
Appearance	Cap: brown-grey, dry-velvety Stem: white-beige, dry-velvety Lamellae: light brown	Cap: Smooth, gray-beige (bluish/dark gray possible) Stem: white-beige, dry-velvety Lamellae: white light, in older mushrooms light beige → White naturally occurring weave on hat and stem is not objectionable.	Cap: very dry, chapped skin or with a colorless veil, light to reddish brown, brownish spots possible Stem: whitish-brownish, dry-velvety Lamellae: white, yellow-reddish brown (depending on age)
Consistency	Cap: firm Stem: firm elastic Lamellae: firm	Cap: firm Stem: firm elastic Lamellae: firm	Cap: firm Stem: firm elastic Lamellae: firm
Aroma/Smell	Cap: pure, typical aroma, mild umami Stem: pure, typical aroma, mild umami Lamellae: pure	Cap: pure, typical aroma, mild umami Stem: pure, typical aroma, mild umami Lamellae: pure	Cap: pure, typical aroma, mild umami, mild garlic Stem: pure, typical aroma, mild umami Lamellae: pure
Taste	Cap and Stem: typical aroma, slightly spicy-peppery	Cap and Stem: typical aroma, slightly spicy	Cap and Stem: strong spicy, umami

To analyze the taste, 10 g freshly sliced mushroom heads and stems were sautéed in rapeseed oil (1:10, oil:mushrooms) for 3–5 minutes.

Specific product defects for mushrooms were defined for each characteristic. A five-point-scale was used to describe the quality deviations (deviation from the quality: 5 - no deviation, 4 - slight deviations, 3 - moderate deviations, 2 - significant deviations, 1 - strong deviations, 0 - not rateable) and a mean value was calculated from this for each sample. The used test scheme can be found in the appendix (supplementary Figure 1).

3. Statistics

For statistical processing, the bacterial counts were logarithmized and tested for normal distribution using the Komolgorov-Smirnov and the Shapiro-Wilk test. Statistical analysis was performed with IBM SPSS version 29 (SPSS Inc., Chicago, IL, USA). T-test was applied to compare groups and an error level of $p < 0.05$ was fixed. The Pearson correlation coefficient (r) was calculated to determine the correlation between the AMC and the sensory quality score.

4. Results

4.1. Results of microbiological and sensory analysis

No mushroom sample, either on the day of purchase or after storage, tested positive for *Salmonella* spp., *L. monocytogenes, Listeria* spp., or presumptive *B. cereus.*

The highest average and total amounts of AMC (7.7 log CFU/g before; 8.6 log CFU/g after storage), EB (7.6 log CFU/g before; 8.0 log CFU/g after storage), and PS before and after storage (7.0 log CFU/g before, 8.2 log CFU/g after storage) were found on King oyster mushrooms originating in South Korea. King oyster mushrooms coming from Austria showed the lowest average and total counts of AMC (mean 2.1 log CFU/g, max 2.9 CFU/g before; mean 2.2 CFU/g, max 4.5 CFU/g after storage), EB (mean 1.6 log CFU/g, max 2.7 CFU/g before; mean 1.6 CFU/g, max 3.9 CFU/g after storage), and PS (mean 1.6 log CFU/g, max 2.8 CFU/g before; mean 1.0 CFU/g, max 1.0 CFU/g after storage) before and after storage. The highest mean amounts of molds were found on Austrian King oyster mushrooms before and on Korean King oyster mushrooms after storage (3.5±0.2 log CFU/g before; 3.7±1.5 log CFU/g after storage). The highest mean amounts of yeasts were found on Shiitake mushrooms before and on Korean King oyster mushrooms after storage (2.4±1.8 log CFU/g before; 2.9±1.9 log CFU/g after storage). Only two samples, one Shiitake and one King oyster, tested positive for LAB, both samples were analyzed before storage (4.0 log CFU/g and 5.4 log CFU/g).

After storage the AMC increased significantly by 0.9 log CFU/g for all examined samples ($p < 0.05$). However, no significant increase of EB, PS, yeasts, or molds after storage for all samples was observed ($p > 0.05$). The average EB, PS, yeasts or molds showed an increase of 0.1 log CFU/g, 0.5 log CFU/g, 0.4 log CFU/g and 0.3 log CFU/g, respectively, after storage. With a rise of 1.1 log CFU/g after storage, King oyster mushrooms in general showed a significant increase of AMC ($p < 0.05$). King oyster mushrooms from Korea exhibited the highest mean and significant increase of AMC and PS with 2.2 log CFU/g and 2.0 log CFU/g, respectively ($p < 0.05$ each).

The mean sensory quality score of all examined mushrooms decreased not significantly by 0.4 points, with Korean King oyster mushrooms showing the highest, but still not significant decrease by 0.9 points ($p > 0.05$ each). King oyster mushrooms, especially King oyster mushrooms originating in Austria, exhibited a significant decrease of sensory quality with

minus 0.7 points and minus 0.5 points, respectively ($p < 0.05$ each).

Table 4: Microbial counts and sensory quality scores of all investigated samples before and after storage**.**

Mushroom		AMC	EB	PS	Molds	Yeasts	QS
Pleurotus eryngii	before storage	4.0 ± 2.0 [a] (1.0–7.7) [b]	3.6 ± 2.2 (1.0–7.6)	3.2 ± 2.0 (1.0–7.0)	3.4 ± 0.3 (2.9–3.8)	1.1 ± 0.4 (1.0–2.4)	4.2 ± 1.1 (1.4–5.0)
	after storage	5.1 ± 3.1 [c] (1.0–8.6)	4.4 ± 3.0 (1.0–8.0)	3.9 ± 3.0 (1.0–8.2)	3.5 ± 1.1 (1.0–5.0)	1.9 ± 1.6 (1.0–5.5)	3.5 ± 1.1 [c] (1.6–4.8)
Pleurotus ostreatus	before storage	3.4 ± 2.4 (1.0–6.9)	2.2 ± 1.7 (1.0–5.4)	3.0 ± 2.5 (1.0–6.7)	2.7 ± 1.2 (1.0–4.0)	2.1 ± 1.4 (1.0–4.0)	3.9 ± 1.4 (1.3–5.0)
	after storage	5.3 ± 1.5 (3.8–7.9)	2.2 ± 1.5 (1.0–4.5)	4.3 ± 2.2 (1.0–7.8)	3.4 ± 1.2 (1.0–4.3)	2.3 ± 1.6 (1.0–4.9)	4.0 ± 1.0 (2.0–5.0)
Lentinula edodes	before storage	3.7 ± 1.8 (1.0–6.3)	3.0 ± 2.1 (1.0–6.3)	2.7 ± 2.1 (1.0–5.8)	2.2 ± 1.2 (1.0–4.4)	2.4 ± 1.8 (1.0–5.5)	4.1 ± 0.3 (3.7–4.3)
	after storage	3.3 ± 1.8 (1.0–6.6)	2.0 ± 2.0 (1.0–6.1)	2.1 ± 2.2 (1.0–6.6)	2.8 ± 1.5 (1.0–5.2)	1.8 ± 1.5 (1.0–4.8)	3.8 ± 0.4 (3.3–4.6)
All samples	before storage	3.8 ± 2.1 (1.0–7.7)	3.1 ± 2.1 (1.0–7.6)	3.0 ± 2.2 (1.0–7.0)	2.9 ± 1.0 (1.0–4.4)	1.7 ± 1.3 (1.0–5.5)	4.1 ± 1.0 (1.3–5.0)
	after storage	4.7 ± 2.6 [c] (1.0–8.6)	3.2 ± 2.7 (1.0–8.0)	3.6 ± 2.8 (1.0–8.2)	3.3 ± 1.3 (1.0–5.2)	2.0 ± 1.6 (1.0–5.5)	3.7 ± 1.0 (1.6–5.0)

Microbial counts are calculated in log CFU/g. before storage = day of purchase; after storage at 4 °C for seven days (Oyster and Shiitake) or 12 days (King oyster); AMC–aerobic mesophilic count; EB–*Enterobacteriaceae*; PS–*Pseudomonadaceae*; QS–sensory quality score.

a Mean ± SD. b Minimum–Maximum. c significant difference after storage ($p < 0.05$).

Both times, before and after storage, a strong negative correlation between the AMC and the sensory quality score was found (before r = -0.787; after storage r = -0.621).

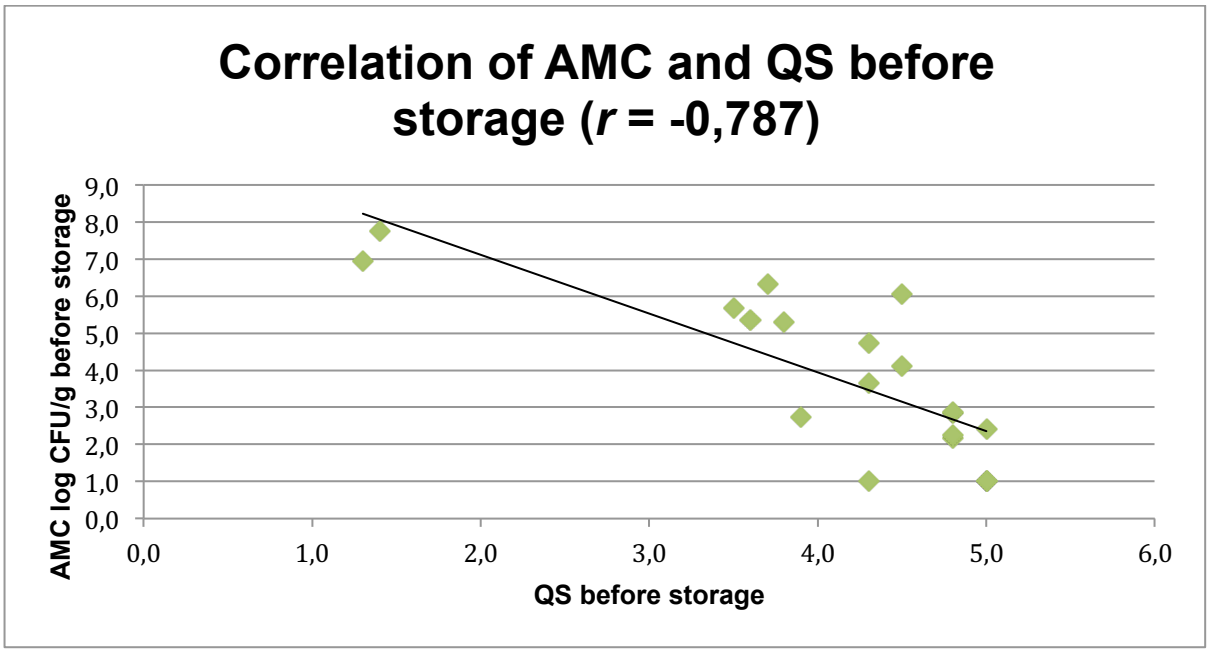

Figure 2: Correlation between the total aerobic mesophilic bacteria (AMC) in log CFU/g and the sensory quality score (QS) before storage. Pearsons correlation coefficient: r = -0.787.

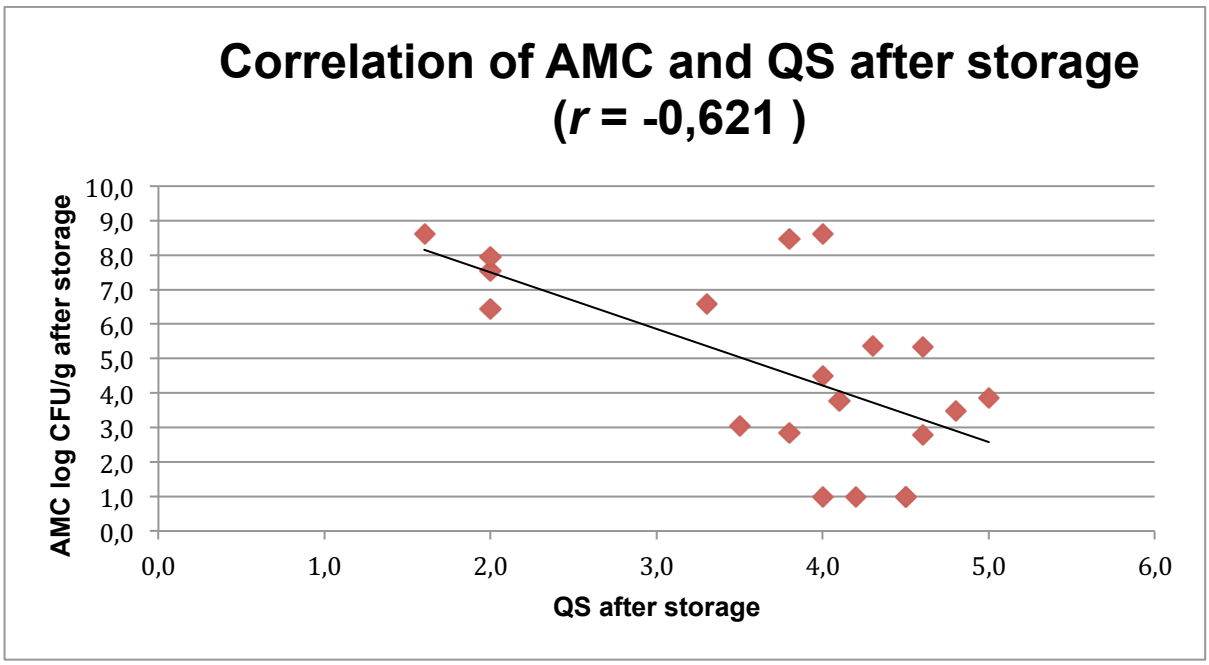

Figure 3: Correlation between the total aerobic mesophilic bacteria (AMC) in log CFU/g and the sensory quality score (QS) after storage. Pearsons correlation coefficient: r = -0.621.

4.2. Microbiological contamination and sensory quality categorization

All samples' microbial quality scores both before and after storage were divided into five degrees of contamination based on the AMC load: “low”: < 5.0 log CFU/g; “medium”: 5.1–6.5 log CFU/g; “high”: 6.6–8.0 log CFU/g; and “very high”: > 8.1 log CFU/g (in accordance with the fresh product standard of our own laboratory).

On the day of purchase, none of the samples showed contamination levels categorized as “very high”. After storage, only 15 % (n = 3/20) of investigated samples evidenced a “very high” contamination level (AMC >8,1 log CFU/g), al of which were king oyster mushrooms from South Korea. All King oyster mushrooms from Austria were ascribed to the category of “low” contamination (AMC <5.0 log CFU/g) before and after storage. In general 96.2 % (n = 25/26) of all mushrooms originating in Austria showed a “low” or “medium” contamination level, where as only 50 % (n = 7/14) of mushrooms imported to Austria exhibited the same levels of microbiological quality. A significant difference between Austrian and imported mushrooms concerning their AMC was found ($p < 0.05$). 80 % (n = 4/5) before; n = 4/5 after storage) of Shiitake mushrooms were ascribed to the contamination category “low” before and even after storage. The remaining 20 % (n = 1/5) of Shiitake mushrooms showed an increase in their contamination level from “medium” (AMC 5.1–6.5 log CFU/g) to “high” (AMC 6.6–8.0 log CFU/g). Initially 60 % (n = 3/5) of Oyster mushrooms evidenced a “low”, and 20 % (n = 1/5) a “medium” contamination level. After storage, 40 % showed a “low” (n = 2/5) and 40 % (n = 2/5) a ”medium” contamination level. There was no change in the contamination levels “high” (20 % before, 20 % after storage) or “very high” (0 % before, 0 % after storage). Figure 4 presents the individual mushroom species before and after storage and their microbiological contamination.

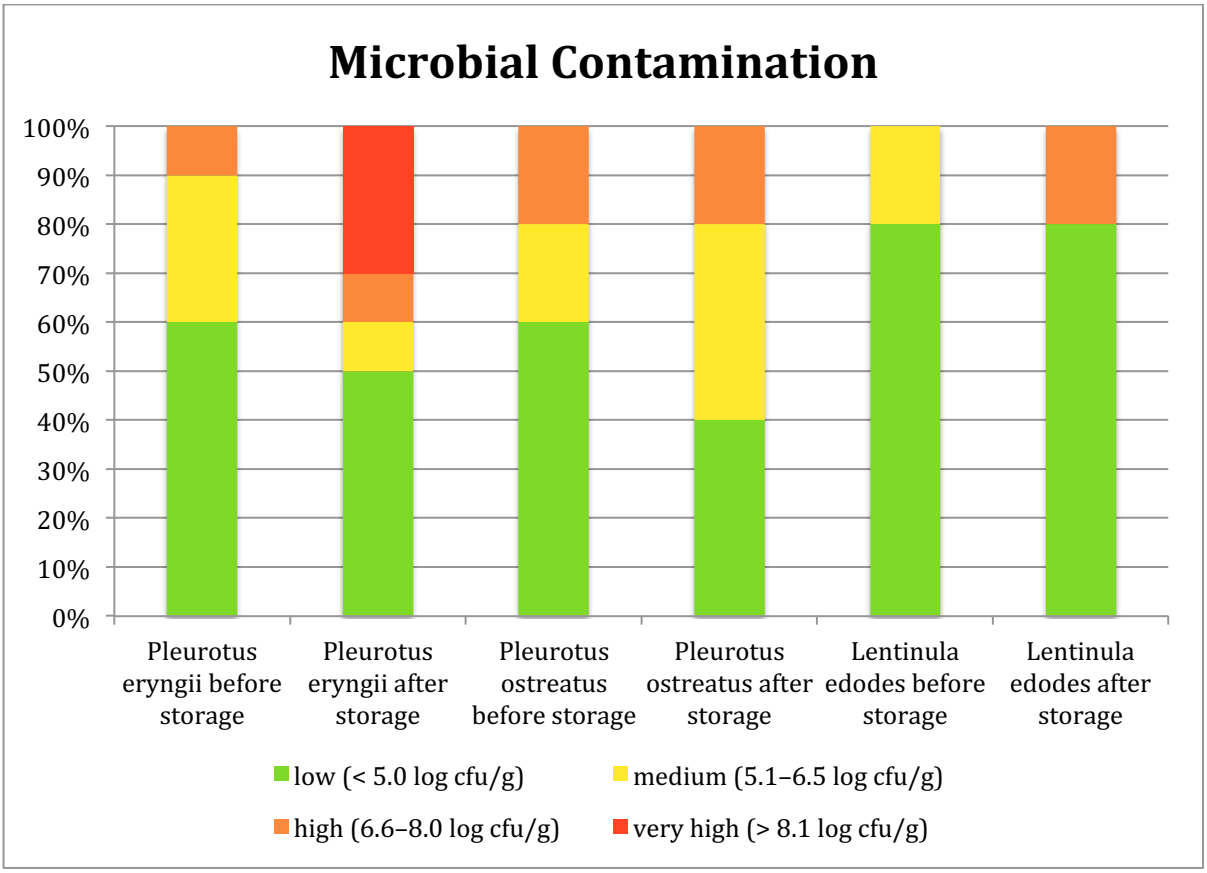

Figure 4: Individual mushroom species before and after storage and their microbiological contamination.

The results of the sensory quality analysis for each sample were graded as follows: 5.0 to 4.0 “very good or good”; 3.9 to 3.0 “satisfactory”; 2.9 to 2.0 “still acceptable” and below 1.9 “not acceptable” and/or inadequate for human consumption.

Initially, only 10 % (n = 2/20) of tested mushroom samples showed a “not acceptable” (QS < 1.9) quality score and were deemed unsuitable for consumption. After storage, the amount doubled and 20 % (n = 4/20) were “not acceptable”, with the majority (n = 4/6) being King oyster mushrooms originating in South Korea before and after storage. All of those six “not acceptable” samples showed a visible overgrowth of foreign mold, and therefore did not meet the Codex Alimentarius standard. In comparison, all King oyster mushrooms originating in Austria, before and after storage, showed a “very good or good” (QS: 5.0 to 4.0) sensory quality. Mushrooms originating in Austria displayed a quite good sensory quality with 96.2 % (n = 25/26) being ascribed to the category “very good or good” or “satisfactory”. Only 64.3% (n = 9/14) of imported samples were categorized as “very good or good” or “satisfactory”.

Concerning their QS, a significant difference was found between Austrian and imported mushrooms ($p < 0.05$). During storage, a slight but general decline in sensory quality was observed, with the percentage of “very good or good” categorized samples decreasing from 65 % to 60 % (n = 13/20 before storage; n = 12/20 after storage), and a decrease in the category “satisfactory” (QS: 3.9 to 3.0) from 25 % to 20 % (n = 5/20 before storage; n = 4/20 after storage). No sample, neither before nor after storage, displayed a "still acceptable" (QS: 2.9 to 2.0) sensory quality score. Only 16.7 % (n = 2/12) of all examined samples were classified as "very good or good" when the bacterial count reached 5.5 log CFU/g or higher. Conversely, 50 % (n = 6/12) of the samples were categorized as "not acceptable". The most frequent observed sensory deviations and deficits were a softening cap, fibrous stem, brown lesions, loss of aroma and taste, and an overall rubbery consistency. Figure 5 provides an overview of the individual mushroom species and their sensory quality before and after storage.

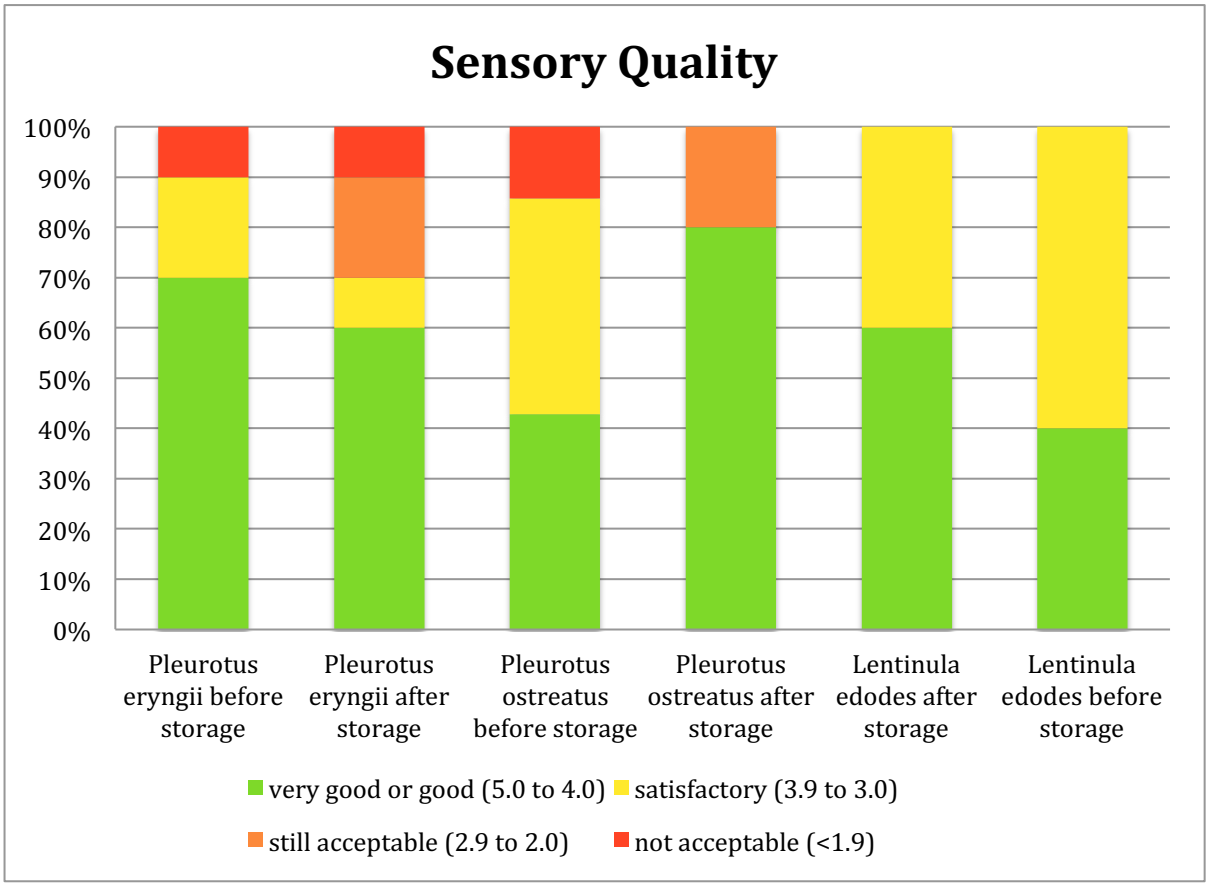

Figure 5: Overview of the individual mushroom species and their sensory quality before and after storage.

5. Discussion and conclusion

Within the scope of this work, the microbiological load and quality, and the sensory quality of a total of 40 edible mushrooms offered in Austrian supermarkets were examined on the day of purchase and after storage.

The AMC counts varied widely, ranging from 1.0–7.7 log CFU/g before and 1.0–8.6 log CFU/g after storage. These values are comparable to those from previous studies finding AMC values pre- and post-storage to range from 3.3–8.4 log CFU/g (Venturini et al., 2011; Reyes et al., 2004; Kim et al., 2013; Jiang et al., 2013).

Mean PS values were ranging from 2.7 to 3.2 log CFU/g before and 2.1 to 4.3 log CFU/g after storage. For *Pleurotus eryngii, Pleurotus ostreatus*, and *Lentinula* edodes slightly higher average levels ranging from 4.4–7.7 CFU/g were described (Venturini et al., 2011; Jiang et. al., 2013; Reyes et. al., 2004; Manthou et. al., 2019).

Mean EB levels found on the examined mushroom samples ranged from 2.2 to 3.6 log CFU/g before and 2.0 to 4.4 log CFU/g after storage. Previous studies found the average amount of EB for *Pleurotus eryngii, Pleurotus ostreatus* and *Lentinula edodes* to be ranging at similar levels going from 2.7 to 3.7 log CFU/g (Reyes et al., 2004; Venturini et al., 2011). However, higher mean values of up to 6.5 log CFU/g for other mushroom species were also described (Venturini et al., 2011; González et al., 2012; Donzellini et al., 2018).

Only two out of all 40 samples, one Shiitake and one King oyster, tested positive for LAB. Both samples were analyzed before storage (4.0 log CFU/g and 5.4 log CFU/g). Previous studies found lower values ranging from 1.7–2.1 log CFU/g (Venturini et al., 2011). Due to the small number of positive cases, quantitative comparison with the literature was not possible.

Counts of yeasts and molds were rather low also, with mean values ranging from 2.9–3.3 log CFU/g and 1.7–2.0 log CFU/g before and after storage, which is in accordance with already published studies (2.6–3.4 log CFU/g for yeasts and molds) (Venturini et al., 2011; Siyoum et al., 2015; Dril et al., 2021).

Neither *Salmonella* spp., *L. monocytogenes, Listeria* spp. or presumptive *B. cereus* were detected in any sample, demonstrating a high degree of safety of fresh mushrooms on the Austrian market. Concerning *Salmonella* spp., this is comparable with previous studies, which found no to very low contamination (Venturini et al., 2011; Strapp et al., 2003; Samadpour et al., 2006). *B. cereus* was recovered from 28.3% of Chinese mushroom samples in previous studies, with contamination levels varying from 0.5 to 3.0 log CFU/g (Liu et al., 2020; Suhaili et al., 2021). Previous studies conducted in the USA, Norway, Canada, and Spain reported no or very low prevalence of *L. monocytogenes* (0 % to 1 %) (Samadpour et al., 2006; Johannessen et al., 2002; Zhang et al., 2020; Venturini et al., 2011). A Chinese study, on the other hand, found slightly higher contamination rates for *P. ostreatus, P. eryngii*, and *L. edodes* with values ranging from 6.7 %, 4.4 % and 2.9 %, respectively (Chen et al., 2018). In 2020, the CDC reported an outbreak of *L. monocytogenes* infections, resulting in four deaths. South Korea was most likely the source of the contaminated Enoki mushrooms (CDC, 2020). Recently, a RASFF alert was issued for Enoki mushrooms originating in China and South Korea contaminated with *Listeria* in Slovenia and the Netherlands (RASFF Window, 2023). Due to *L. monocytogenes* high survivability a general high standard of hygiene during all steps of production is recommended (Nakamura et al., 2013; Piercey et al., 2017; Pennone et al., 2020).

The microbiological load (AMC) was divided into four contamination grades. (“low”: < 5.0 log CFU/g; “medium”: 5.1–6.5 log CFU/g; “high”: 6.6–8.0 log CFU/g; and “very high”: > 8.1 log CFU/g). Only 7.5 % (n = 3/40) of all examined *Pleurotus eryngii*, *Pleurotus ostreatus*, and *Lentinula edodes* samples showed a “very high” contamination level and with 80 % (n = 32/40) of all tested specimens showing a “low” or “medium” contamination level, the mushrooms found in Austrian retail markets can be considered to be of rather good microbiological quality. All three samples with a “very high” contamination level were King oyster mushrooms originating in South Korea. In comparison, all King oyster mushrooms from Austria were ascribed to the category of “low” contamination before and after storage. 96.2 % (n = 25/26) of mushrooms of Austrian origin and only 50 % (n = 7/14) of mushrooms imported to Austria showed a “low” or “medium” contamination level. A significant difference between Austrian and imported mushrooms concerning their AMC/microbiological quality was found ($p < 0.05$). A probable prolonged transporting time as cause needs to be taken into consideration. Other factors that can influence the change in sensory and microbiological quality, next to prolonged storage and transporting times, may include the usage of different

types of packaging, different storage temperatures, different hygiene and processing standards during all stages of production, and other shelf life prolonging treatments (Jiang et al., 2013; Wang et at., 2017; Santana et al., 2007; Manthou et al., 2019; Parentelli et al., 2007; González-Fandos et al., 2001; Pennone et al., 2020)

For sensory quality testing, a five-point scale for different characteristics was used. The sensory quality number QS calculated from this was graded and categorized from “very good or good”, “satisfactory”, “still acceptable” to “not acceptable”. Overall, the examined mushrooms available in Austrian retail showed a rather good sensory quality. 85 % (n = 34/40) of examined mushrooms exhibited a “very good or good” or “satisfactory” quality. The remaining 15 % (n = 6/40) showed a “not acceptable” sensory quality, with the majority (n = 5/6) originating form South Korea or Poland. All of these six "not acceptable" samples had a visible overgrowth of foreign mold and therefore did not correspond to the Codex Alimentarius standard. 96.2 % (n = 25/26) of Austrian mushrooms displayed a “very good or good” or “satisfactory” sensory quality. In comparison only 64.3% (n = 9/14) of imported samples were to be ascribed to the same categories. There was a significant difference between imported and Austrian mushrooms in terms of their QS ($p < 0.05$).

Although no significant increase for all samples concerning their QS after storage could be detected ($p > 0.05$ each), a significant increase in AMC was observed ($p < 0.05$), thus it is recommended that consumers reach for the freshest and, if possible, local products to keep the risk of a high microbiological contamination as low as possible. Even though no legal requirement in Austria for a best-before date for fresh mushrooms exists, due to the significant increase in AMC after storage it is still advised that retailers set one, in order to be able to offer customers a safe and high-quality product. Furthermore, due to the partially high microbiological load especially after storage, it is not recommended to consume mushrooms in raw form.

Both times, before and after storage, a strong negative correlation between the AMC and the sensory quality score was found (before $r = -0.787$; after storage $r = -0.621$). This shows and confirms that a high bacterial count has a negative effect on the sensory quality and thus the shelf life. It has been repeatedly confirmed that a high microbial contamination causes edible mushrooms to lose sensory quality and, consequently, shelf life. The connection between the level of bacterial contamination and the sensory quality can be observed when analyzing

various shelf life-prolonging techniques, particularly when comparing treated versus untreated mushrooms. For the purpose of determining the quality of mushrooms, indicators such as color, hardness, off-odor, soluble solid content, protein content, and enzyme activity may be utilized (Jiang et al., 2013; Xu et al., 2016; Wang et at., 2017).

6. Summary

In this study, a total of 40 mushroom samples, consisting of *Pleurotus eryngii*, *Pleurotus ostreatus*, and *Lentinula edodes* available at Austrian retail markets, were analyzed for their sensory and microbiological quality before and after storage at 4 °C.

All samples were tested for their total aerobic mesophilic microbiological load (AMC), the count of *Enterobacteriaceae* (EB), *Pseudomonas* spp. (PS), Lactic acid bacteria (LAB), yeasts, molds, and the presence of the food-borne human pathogens *Bacillus cereus*, *Listeria monocytogenes* and *Salmonella* spp.

The microbiological quality was based on the total AMC load and graded from “low”, “medium”, “high” to “very high”. The AMC counts varied widely, ranging from 1.0 log CFU/g to 7.7 log CFU/g before and 1.0 log CFU/g to 8.6 log CFU/g after storage. Only 7.5 % (n = 3/40) of all samples showed a “very high” contamination level, while 80 % (n = 32/40) of all tested specimens showed a “low” or “medium” contamination level.

EB and PS levels before and after storage also varied widely (EB: 1.0–7.6 log CFU/g before and 1.0–8.0 log CFU/g after storage, PS: 1.0–7.0 log CFU/g before and 1.0–8.2 log CFU/g after storage). Yeasts and molds were found in lower numbers, with average values before and after storage ranging from 2.9 to 3.3 log CFU/g and 1.7 to 2.0 log CFU/g, respectively. LAB was only found on two samples, both being analyzed before storage (4.0 log CFU/g and 5.4 log CFU/g).

Salmonella spp., *Listeria monocytogenes, Listeria* spp., or presumptive *Bacillus cereus* could not be found in any sample.

For quality testing, a five-point scale for different sensory quality characteristics was used. The sensory quality number QS calculated from this was graded and categorized from “very good or good”, “satisfactory”, “still acceptable”, to “not acceptable”. Before storage, 90 % (n = 18/20) of tested mushrooms showed a “very good or good” or “satisfactory” sensory quality. After storage, 80 % still (n = 16/20) exhibited a “very good or good” or “satisfactory” quality. Combined, 85 % (n = 34/40) of all samples were assigned to those two sensory quality categories. The remaining 15 % (n = 6/40) were graded as “not acceptable” and inadequate for human consumption.

Even though high values of AMC were found and low quality scores were determined, the overall microbiological and sensory quality of mushrooms in Austrian retail markets, before and even after storage, can be considered good. Customers are still advised to choose the freshest and local products to keep the risk of a high microbiological contamination as low as possible. Austrian retailers should set a best-before date out of their own responsibility in

order to be able to offer their customers the best possible and safest goods. In the scope of this study, contrary to the initially assumption that mushrooms spoil quickly and badly, and despite a significant increase in AMC after storage was found, no excessive spoilage or deterioration in sensory quality was detected for mushrooms offered in Austrian trade.

7. Zusammenfassung

In dieser Studie wurden insgesamt 40 im österreichischen Einzelhandel erhältliche Pilzproben bestehend aus *Pleurotus eryngii, Pleurotus ostreatus* und *Lentinula edodes*, vor und nach Lagerung bei 4 °C auf ihre sensorische und mikrobiologische Qualität untersucht.

Alle Proben wurden auf ihre aerobe mesophile Gesamtkeimzahl (AMC), die Anzahl von *Enterobacteriaceae* (EB), *Pseudomonadaceae* (PS), Milchsäurebakterien (LAB), Hefen und Schimmelpilze sowie auf das Vorkommen der potentiell humanpathogenen Bakterien *Bacillus cereus*, *Listeria monocytogenes* und *Salmonella* spp. getestet.

Die mikrobiologische Qualität wurde auf Basis der AMC nach den folgende Kategorien bewertet: „gering“, „mittel“, „hoch“ bis „sehr hoch“. Die AMC-Werte variierten stark und reichten von 1.0 log KBE/g bis 7.7 log KBE/g vor und 1.0 log KBE/g bis 8.6 log KBE/g nach der Lagerung. 7.5 % (n = 3/40) aller Proben wiesen einen „sehr hohen“ Kontaminationsgrad auf. 80 % (n = 32/40) aller getesteten Proben wiesen einen „niedrigen“ oder „mittleren“ Kontaminationsgrad auf.

Die EB- und PS-Werte vor und nach der Lagerung schwankten ebenfalls stark (EB: 1.0–7.6 log KBE/g vor und 1.0–8.0 log KBE/g nach der Lagerung, PS: 1.0–7.0 log KBE/g vor und 1.0–8.2 log KBE/g nach Lagerung).

Hefen und Schimmelpilze wurden in geringerer Zahl gefunden, mit Mittelwerten zwischen 2.9–3.3 log KBE/g und 1.7–2.0 log KBE/g vor und nach der Lagerung.

LAB wurde nur bei zwei Proben gefunden, die beide vor der Lagerung analysiert wurden (4.0 log KBE/g und 5.4 log KBE/g).

Salmonella spp. *Listeria monocytogenes, Listeria* spp. oder *Bacillus cereus* wurden in keiner Probe gefunden. Zur Qualitätsprüfung wurde eine Fünf-Punkte-Skala für verschiedene sensorische Qualitätsmerkmale verwendet. Die daraus errechnete sensorische Qualitätszahl QS wurde benotet und von „sehr gut oder gut“, „befriedigend“, „noch akzeptabel“ bis „nicht akzeptabel“ kategorisiert.

Vor der Lagerung zeigten 90 % (n = 18/20) der getesteten Pilze eine „sehr gute oder gute“ oder „befriedigende“ sensorische Qualität. Nach der Lagerung zeigten noch 80 % (n = 16/20) eine „sehr gute oder gute“ oder „befriedigende“ Qualität. Zusammen waren 85 % (n = 34/40) aller Proben diesen beiden sensorischen Qualitätskategorien zuzuordnen. Die restlichen 15 % (n = 6/40) wurden als „nicht akzeptabel“ und nicht für den menschlichen Verzehr geeignet eingestuft.

Obwohl teilweise hohe Gesamtkeimzahl-Werte und niedrige Qualitätswerte festgestellt wurden, kann die mikrobiologische und sensorische Qualität der im österreichischen Handel

angebotenen Pilze vor und auch nach Lagerung als gut angesehen werden. Den Kunden wird dennoch empfohlen möglichst frische und regionale Produkte zu wählen, um das Risiko einer hohen mikrobiologischen Kontamination so gering wie möglich zu halten. Österreichische Einzelhändler sollten aus eigener Verantwortung ein Mindesthaltbarkeitsdatum festlegen, um ihren Kunden die bestmögliche und sicherste Ware anbieten zu können. Im Rahmen dieser Studie konnte, entgegen der ursprünglichen Annahme dass Pilze schnell und stark verderben und trotz eines signifikanten Anstiegs der Gesamtkeimzahl nach der Lagerung, kein übermäßiger Verderb oder Verschlechterungen der sensorischen Qualität der im österreichischen Handel angebotenen Pilze festgestellt werden.

8. Literature

AISALA, H., LAAKSONEN, O., MANNINEN, H., RAITTOLA, A., HOPIA, A., SANDELL, M. (2018): Sensory properties of nordic edible mushrooms. *Food Research International, 109*, 526–536. https://doi.org/10.1016/j.foodres.2018.04.059

ARES, G., PARENTELLI, C., Ga´MBARO, A., LAREO, C., LEMA, P. (2006): Sensory shelf life of shiitake mushrooms stored under passive modified atmosphere. *Postharvest Biology and Technology, 41*(2), 191–197. https://doi.org/10.1016/j.postharvbio.2006.03.013

BARNETT, J.A., PAYNE, R.W., YARROW, D. (1983): Yeasts: characteristics and identification, 3rd edn. Cambridge University Press. Cambridge, UK. https://doi.org/10.1046/j.1525-1470.2001.1862020a.x

BISWAS, M., KUIRY, S. (2013): Yield performance of different species of oyster mushroom (*Pleurotus* spp.) under the agroecological condition of lateritic zone of West Bengal, India. *International Journal of Bio-resource and Stress Management, 4*(1), 43–46.

BOIN, E., AZEVEDO, C., NUNES, J., GUERRA, M.M. (2016): Consumer acceptability and descriptive characterization of fresh and dried king oyster (*Pleurotus eryngii*) and hedgehog (*Hydnum repandum*) mushrooms. *Journal of Food Research, 5*(4), 55. https://doi.org/10.5539/jfr.v5n4p55

BOTTONE, E.J. (2010): *Bacillus cereus*, a volatile human pathogen. *Clinical Microbiology Reviews, 23*(2), 382–398. https://doi.org/10.1128/CMR.00073-09

BRATCHER, D.F. (2018): Other gram-positive *Bacilli*, In LONG, S.S., PROBER, C.G., FISCHER, M., *Principles and practice of pediatric infectious diseases* (Fifth Edition). Elsevier. Philadelphia, USA. https://doi.org/10.1016/C2013-0-19020-4

Bundesministerium für Arbeit, Soziales, Gesundheit und Konsumentenschutz. Codex Alimentarius Austriacus, Codexkapitel B27 - Pilze und Pilzerzeugnisse, http://www.lebensmittelbuch.at/pilze-und-pilzerzeugnisse/ (accessed 12.11.2019)

Bundesministerium für Arbeit, Soziales, Gesundheit und Konsumentenschutz. Codex Alimentarius Austriacus, Codexkapitel A3 - Allgemeine Beurteilungsgrundsätze, http://www.lebensmittelbuch.at/allgemeine-beurteilungsgrundsaetze/(accessed 12.11.2019)

Bundesministerium für Land- und Forstwirtschaft, Regionen und Wasserwirtschaft. (2023): Selbstversorgungsgrad bei Lebensmitteln. https://info.bml.gv.at/themen/lebensmittel/lebensmittel-in-oesterreich/selbstversorgungsgrad.html (accessed 18.05.2023)

BURNS, J.L., (2018): *Pseudomonas* species and related organisms. In LONG, S.S., PROBER, C.G., FISCHER, M., *Principles and Practice of Pediatric Infectious Diseases* (Fifth Edition). Elsevier. Philadelphia, USA. https://doi.org/10.1016/C2013-0-19020-4

CARRASCO, J., PRESTON, G.M. (2020): Growing edible mushrooms: a conversation between bacteria and fungi. *Environmental Microbiology, 22*(3), 858–872. https://doi.org/10.1111/1462-2920.14765

CDC, Center for Disease Control and Prevention (2020): https://www.cdc.gov/salmonella/stanley-09-20/index.html. (accessed 18.05.2023)

CHANG, S.T., WASSER, S.P. (2012): The role of culinary-medicinal mushrooms on human welfare with a pyramid model for human health. *International Journal of Medicinal Mushrooms, 14*(2), 95–134. https://doi.org/10.1615/intjmedmushr.v14.i2.10

CHEN, M., WU, Q., ZHANG, J., GUO, W., WU, S., YANG, X. (2014): Prevalence and contamination patterns of *Listeria monocytogenes* in *Flammulina velutipes* plants. *Foodborne Pathogens and Disease, 11*(8), 620–627.

https://doi.org/10.1089/fpd.2013.1727

CHEN, M., CHENG, J., WU, Q., ZHANG, J., CHEN, Y., ZENG, H., YE, Q., WU, S., CAI, S., WANG, J., DING, Y. (2018): Prevalence, potential virulence, and genetic diversity of *Listeria monocytogenes* isolates from edible mushrooms in Chinese markets. *Frontiers in Microbiology, 9*, 1711.
https://doi.org/10.3389/fmicb.2018.01711

CHO, Y.S., KIM, J.S., CROWLEY, D.E., & CHO, B.G. (2003): Growth promotion of the edible fungus *Pleurotus ostreatus* by fluorescent pseudomonads. *FEMS Microbiological Letter, 218*(2), 271–276.
https://doi.org/10.1016/S0378-1097(02)01144-8

CLIFFE-BYRNES, V., O'BEIRNE, D. (2007): Effects of gas atmosphere and temperature on the respiration rates of whole and sliced mushrooms (*Agaricus bisporus*) - implications for film permeability in modified atmosphere packages. *Journal of Food Science, 72*(4), E197–E204.
https://doi.org/10.1111/j.1750-3841.2007.00321.x

Codex Standard for Edible Fungi and Fungus Products, CODEX STAN 38-1981,
http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B38-1981%252FCXS_038e.pdf, (accessed 13.08.2020)

COIA J., CUBIE H. (1995): *Bacillus cereus*. In COIA J., CUBIE H., *The Immunoassay Kit Directory* (Series B: Infectious Diseases), vol 2/1/3. 648–649. Springer. Dordrecht, Netherlands.
https://doi.org/10.1007/978-94-009-0359-3

CORDANO, A.M., JACQUET, C. (2009): *Listeria monocytogenes* isolated from vegetable salads sold at supermarkets in Santiago, Chile: prevalence and strain characterization. *International Journal of Food Microbiology, 132*(2–3), 176–179.
https://doi.org/10.1016/j.ijfoodmicro.2009.04.008

DANELL, E., ALSTRÖM, A., TERNSTRÖM, A., (1993): *Pseudomonas fluorescens* in association with fruit bodies of the ectomycorrhizal mushroom *Cantharellus cibarius. Mycology Research, 97*(9), 1148–1152. https://doi.org/10.1016/S0953-7562(09)80519-4

DE MAN, J.D., ROGOSA, M., SHARPE, M.E. (1960): A medium for the cultivation of Lactobacilli. *Journal of Applied Bacteriology, 23*, 130–135. https://doi.org/10.1111/j.1365-2672.1960.tb00188.x

DIN 10969:2018: Sensory analysis - Descriptive analysis with following quality evaluation, Deutsches Institut für Normung e.V. (DIN), Beuth Verlag, Germany. https://dx.doi.org/10.31030/2833424

DONNENBERG M.S., (2015): *Enterobacteriaceae*. In BENNETT, J.E., DOLIN, R., BLASER, M.J. *Principles and practice of infectious diseases* (Eighth Edition). Elsevier Saunders. Philadelphia, USA. https://doi.org/10.1016/C2012-1-00075-6

DONZELLINI, M., LORINI, C., PALAZZONI, S., BECCIOLINI, M., CIBECCHINI, A., TALINI, M., BONACCORSI, G. (2018): Research on mesophilic aerobic microorganisms and *Enterobacteriaceae* in cultivated and commercialized *Agaricus bisporus*. *Annali di Higiene: medicina Preventiva e di Comunita, 30*(2), 104–111. https://doi.org/10.7416/ai.2018.2201

DOORES, S., KRAMER, M., BEELMAN, R.B., (1987): Evaluation and bacterial populations associated with fresh mushrooms (*Agaricus bisporus*). In WUEST, P.J., ROYSE, D.J., BEELMAN, R.B., *Developments in Crop Science*, Vol 10, 283–294. https://doi.org/10.1016/B978-0-444-42747-2.50035-X

DRIL, A., MAYURNIKOVA, L., ROZHDESTVENSKAYA, L. (2021): The influence of ionizing radiation on free protein content and microorganisms' growth in oyster mushrooms. *IOP Conference Series: Earth and Environmental Science, 640*(3), 032060. https://doi.org/10.1088/1755-1315/640/3/032060

EASTWOOD, D., BURTON, K. (2002): Mushrooms – A matter of choice and spoiling oneself. *Microbiology Today, 29*, 18–19.

EASTWOOD, D., GREEN, J., GROGAN, H., BURTON, K. (2015): Viral Agents Causing Brown Cap Mushroom Disease of *Agaricus bisporus*. *Applied and Environmental Microbiology, 81*(20), 7125–7134.
https://doi.org/10.1128/AEM.01093-15

EPPO (2010): Fungal diseases on cultivated mushrooms of *Agaricus* spp. *EPPO Bulletin, 40,* 270–273.
https://doi.org/10.1111/j.1365-2338.2010.02384.x

European Commission, EU open data portal (2023):
https://data.europa.eu/data/datasets/restored_rasff?locale=en (accessed 10.01.2023)

European Food Safety Authority (EFSA) (2005): Opinion of the scientific panel on biological hazards on *Bacillus cereus* and other *Bacillus* spp. in foodstuffs. *The EFSA Journal, 175*, 1–48.
https://doi.org/10.2903/j.efsa.2005.175

European Food Safety Authority (EFSA) and European Center of Disease Control (ECDC) (2013): The European Union Summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2011. *The EFSA Journal, 11*(4), 3129, 250.
https://doi.org/10.2903/j.efsa.2013.3129

FERMOR, T.R. (1987): Bacterial diseases of edible mushrooms and their control. *Developments in Crop Science, 10*, 361–370.
https://doi.org/10.1016/B978-0-444-42747-2.50043-9

ISABEL C.F.R. FERREIRA, Â.F., HELENO, S.A. (2017): Chemical, nutritional, and bioactive potential of mushrooms. In DIEGO, C.Z., PARDO-GIMÉNEZ, A., *Edible and Medicinal Mushrooms: Technology and Applications.* Wiley-Blackwell
https://doi.org/10.1002/9781119149446.ch21

Food and Agriculture Organization of the United Nations (FAO). FAOSTAT Database (2021): http://www.fao.org/ faostat/en/#data/QC/, (accessed 07.02.2021).

FLETCHER, J.T. (1979): Bacteria and mushrooms. *Mushroom Journal, 82*, 451–457.

FLETCHER, J.T., GAZE, R.H. (2007): Mushroom pest and disease control: A colour handbook. 1st ed. CRC Press.
https://doi.org/10.1201/b15139

Fortune Business Insights, (2022): Mushroom market size, share & COVID-19 impact analysis, by type (button, shiitake, oyster, and others), by form (fresh, frozen, dried, and canned), and regional forecast, 2021–2028.
https://www.fortunebusinessinsights.com/industry-reports/mushroom-market-100197 (accessed 18.05.2023)

GANDHI, M., CHIKINDAS, M.L. (2007): *Listeria*: a foodborne pathogen that knows how to survive. *International Journal of Food Microbiology, 113*, 1–15.
https://doi.org/10.1016/j.ijfoodmicro.2006.07.008

GONZÁLEZ, A.J., GEA, F.J., NAVARRO, M.J., FERNÁNDEZ, A. M. (2012): Identification and RAPD-typing of *Ewingella americana* on cultivated mushrooms in Castilla-La Mancha, Spain. *European Journal of Plant Pathology, 133*(3), 517–522.
https://doi.org/10.1007/s10658-012-9952-1

GONZÁLEZ-FANDOS, E., OLARTE, C., GIMÉNEZ, M., SANZ, S., SIMÓN, A. (2001): Behaviour of *Listeria monocytogenes* in packaged fresh mushrooms (*Agaricus bisporus*). *Journal of Applied Microbiology*, *91*(5), 795–805.
https://doi.org/10.1046/j.1365-2672.2001.01452.x

Grand view research, (2022): Mushroom market size, share & trends analysis report by product (button, shiitake, oyster), by form, by distribution channel, by application (food, pharmaceuticals, cosmetics), by region, and segment forecasts, 2022–2030.
https://www.grandviewresearch.com/industry-analysis/mushroom-market (accessed 18.05.2023)

GUILLAMÓN, E., GARCÍA-LAFUENTE, A., LOZANO, M., D'ARRIGO, M., ROSTAGNO, M., VILLARES, A., ALFREDO, M. (2010): Edible mushrooms: role in the prevention of cardiovascular diseases. *Fitoterapia, 81*(7), 715–723. https://doi.org/10.1016/j.fitote.2010.06.005

HAMIDIZADE, M., TAGHAVI, S.M., MOALLEM, M., AEINI, M., FAZLIARAB, A., ABACHI, H., HERSCHLAG, R.A., HOCKETT, K.L., BULL, C.T., OSDAGHI, E. (2022). *Ewingella americana*: an emerging multifaceted pathogen of edible mushrooms. *Phytopathology, 113*(2), 150–159. https://doi.org/10.1094/PHYTO-08-22-0299-R

HELENO, S.A., BARROS, L., SOUSA, M.J., MARTINS, A., FERREIRA I.C.F.R. (2010): Tocopherols composition of Portuguese wild mushrooms with antioxidant capacity. *Food Chemistry, 119*(4), 1443–1450. https://doi.org/10.1016/j.foodchem.2009.09.025

IACOBELLIS, N.S., LO CANTORE, P. (2003): *Pseudomonas 'reactans'* a new pathogen of cultivated mushrooms. In IACOBELLIS, N.S., COLLMER, A., HUTCHESON, S.W., MANSFIELD, J.W., MORRIS, C.E., MURILLO, J., SCHAAD, N.W., STEAD, D.E., SURICO, G., ULLRICH, M.S., *Pseudomonas syringae and related pathogens*. Springer, Dordrecht, Netherlands. https://doi.org/10.1007/978-94-017-0133-4_65

INGLIS, P.W., PEBERDY, J. F. (1996): Isolation of *Ewingella americana* from the cultivated mushroom, *Agaricus bisporus*. *Current Microbiology, 33*(5), 334–337. https://doi.org/10.1007/s002849900124

ISO 11290-1:2017: Microbiology of the food chain - Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. - Part 1: Detection method, Beuth-Verlag, Germany.

ISO 13720:2010: Meat and meat products - Enumeration of presumptive *Pseudomonas* spp. Beuth-Verlag, Germany.

ISO 15214:1998: Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of mesophilic lactic acid bacteria. Colony-count technique at 30 degrees Celsius. Beuth-Verlag, Germany.

ISO 21527-1:2008: Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of yeasts and moulds - Part 1: colony count technique in products with water activity greater than 0,95. Beuth-Verlag, Germany.

ISO 21528-2:2004: Microbiology of the food chain - Horizontal method for the detection and enumeration of *Enterobacteriaceae* - Part 2: Colony-count technique, Beuth-Verlag, Germany.

ISO 4833-2:2013: Microbiology of the food chain - Horizontal method for the enumeration of microorganisms - Part 2: Colony count at 30 degrees C by the surface plating technique, Beuth-Verlag, Germany.

ISO 6579-1:2017: Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella – Part 1: Detection of Salmonella spp., Beuth-Verlag, Germany.

ISO 7218:2007/Amd.1:2013: Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations - Amendment 1, Beuth-Verlag, Germany.

ISO 7932:2004: Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of presumptive *Bacillus cereus* - Colony-count technique at 30 °C. Beuth-Verlag, Germany.

JIANG, T., FENG, L., ZHENG, X., LI, J. (2013): Physicochemical responses and microbial characteristics of shiitake mushroom (*Lentinus edodes*) to gum arabic coating enriched with Natamycin during storage. *Food Chemistry*, *138*(2-3), 1992–1997. https://doi.org/10.1016/j.foodchem.2012.11.043

JIANG, H., MIRAGLIA, D., RANUCCI, D., DONNINI, D., ROILA, R., BRANCIARI, R., LI, C. (2018): High microbial loads found in minimally-processed sliced mushrooms from Italian market. *Italian Journal of Food Safety, 7*(1), 7000. https://doi.org/10.4081/ijfs.2018.7000

JOHANNESSEN, G., LONCAREVIC, S., KRUSE, H. (2002): Bacteriological analysis of fresh produce in Norway. *International Journal of Food Microbiology, 77*(3), 199–204. https://doi.org/10.1016/s0168-1605(02)00051-x

KALAČ, P. (2013): A review of chemical composition and nutritional value of wild growing and cultivated mushrooms. *Journal of the Science of Food and Agriculture, 93*(2), 209–218. https://doi.org/10.1002/jsfa.5960

KIELWEIN, G. (1969): Ein Nährboden zur selektiven Züchtung von *Pseudomonaden* und *Aeromonaden*. *Archiv für Lebensmittelhygiene, 20*, 131–133.

KIM, S.W., KIM, S., LEE, H.J., PARK, J.W., RO, H.S. (2013): Isolation of fungal pathogens to an edible mushroom, *Pleurotus eryngii*, and development of specific ITS primers. *Mycobiology, 41*(4), 252–255. https://doi.org/10.5941/MYCO.2013.41.4.252

KIM, C., NARTEA, T.J., PAO, S., LI, H., JORDAN, K.L., XU, Y.X., STEIN, R.A., SISMOUR, E.N. (2016): Evaluation of microbial loads on dried and fresh shiitake mushrooms (*Lentinula edodes*) as obtained from internet and local retail markets, respectively. *Food Safety, 4*(2), 45–51. https://doi.org/10.14252/foodsafetyfscj.2016005

KRAUSE, R., (2012): Pilzleitfaden zum Frischegrad verschiedener Pilzsorten. Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit. https://www.lgl.bayern.de/lebensmittel/warengruppen/wc_27_pilze/frischegrad_pilze.htm (accessed 18.11.2019)

LEE, H.I., CHA, J.S. (1998): Cloning of a DNA fragment specific to *Pseudomonas tolaasii* causing bacterial brown blotch disease of oyster mushroom (*Pleurotus ostreatus*). *Korean Journal Plant Pathology, 14*, 177–183.

LEE. C.J., JHUNE C.S., CHEONG J.C., YUN H. S., CHO W.D. (2009): Occurrence of internal stipe necrosis of cultivated mushrooms (*Agaricus bisporus*) caused by *Ewingella americana* in Korea. *Mycobiology, 37*(1), 62–66. https://doi.org/10.4489/MYCO.2009.37.1.062

LI, D., QIN, X., TIAN, P., WANG, J. (2016): Toughening and its association with the postharvest quality of king oyster mushroom (*Pleurotus eryngii*) stored at low temperature. *Food Chemistry, 196*, 1092–1100. https://doi.org/10.1016/j.foodchem.2015.10.060

LINDEQUIST, U., NIEDERMEYER, T.H.J., JÜLICH, W.D. (2005): The pharmacological potential of mushrooms. *Evidence-based Complementary and Alternative Medicine, 2*(3), 285–299. https://doi.org/10.1093/ecam/neh107

LIU, C., YU, P., YU, S., WANG, J., GUO, H., ZHANG, Y., ZHANG, J., LIAO, X., LI, C., WU, S., GU, Q., ZENG, H., ZHANG, Y., WEI, X., ZHANG, J., WU, Q., DING, Y. (2020): Assessment and molecular characterization of *Bacillus cereus* isolated from edible fungi in China. *BMC microbiology*, *20*(1), 310. https://doi.org/10.1186/s12866-020-01996-0

LOMONACO, S., NUCERA, D., FILIPELLO, V. (2015): The evolution and epidemiology of *Listeria monocytogenes* in Europe and the United States. *Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases, 35*, 172–183. https://doi.org/10.1016/j.meegid.2015.08.008

LUTZONI, F., KAUFF, F., COX, C.J., MCLAUGHLIN, D., CELIO, G., DENTINGER, B., PADAMSEE, M., HIBBETT, D., JAMES, T.Y., BALOCH, E., GRUBE, M., REEB, V., HOFSTETTER, V., SCHOCH, C., ARNOLD, A.E., MIADLIKOWSKA, J., SPATAFORA, J., JOHNSON, D., HAMBLETON, S., CROCKETT, M., SHOEMAKER,

R., SUNG, G.H., LÜCKING, R., LUMBSCH, T., O'DONNELL, K., BINDER, M., DIEDERICH, P., ERTZ, D., GUEIDAN, C., HANSEN, K., HARRIS, R.C., HOSAKA, K., LIM, Y.W., MATHENY, B., NISHIDA, H., PFISTER, D., ROGERS, J., ROSSMAN, A., SCHMITT, I., SIPMAN, H., STONE, J., SUGIYAMA, J., YAHR, R., VILGALYS, R. (2004): Assembling the fungal tree of life: progress, classification, and evolution of subcellular traits. *American Journal of Botany, 91*(10), 1446–80. https://doi.org/10.3732/ajb.91.10.1446

MADBOULY, A., EL-SHATOURY, E., ABOUZEID, M. (2014): Etiology of stipe necrosis of cultivated mushrooms (*Agaricus bisporus*) in Egypt. *Phytopathologia Mediterranea, 53*, 124–129. https://doi.org/10.14601/Phytopathol_Mediterr-13224

MANTHOU, E., TARLAK, F., LIANOU, A., OZDEMIR, M., ZERVAKIS, G.I., PANAGOU, E.Z., NYCHAS, G.J.E. (2019): Prediction of indigenous *Pseudomonas* spp. growth on oyster mushrooms (*Pleurotus ostreatus*) as a function of storage temperature. *LWT, 111*, 506–512. https://doi.org/10.1016/j.lwt.2019.05.062

MANZI, P., MARCONI, S., AGUZZI, A., PIZZOFERRATO, L. (2004): Commercial mushrooms: nutritional quality and effect of cooking. *Food Chemistry, 84*(2), 201–206. https://doi.org/10.1016/S0308-8146(03)00202-4

Market research future: (2019): Edible mushroom market global research report by type (white button, portobello, shiitake, oyster, and others), category (organic and conventional), distribution channel (store-based and non-store-based), and region (North America, Europe, Asia-Pacific, and rest of the world) - forecast till 2024 (november 2019) https://www.marketresearchfuture.com/reports/edible-mushroom-market-4351 (accessed 9.11.2019)

MATTILA, P., KÖNKÖ, K., EUROLA, M., PIHLAVA, J.M., ASTOLA, J., VAHTERISTO, L., HIETANIEMI, V., KUMPULAINEN, J., VALTONEN, M., PIIRONEN, V. (2001):

Contents of vitamins, mineral elements, and some phenolic compounds in cultivated mushrooms. *Journal of Agricultural and Food Chemistry, 49*(5), 2343–2348. https://doi.org/10.1021/jf001525d

MAU, J.L., LIN, Y.P., CHEN, P.T., WU, Y.H., PENG, J.T. (1998): Flavor compounds in king oyster mushrooms *Pleurotus eryngii*. *Journal of Agricultural and Food Chemistry, 46*(11), 4587–4591. https://doi.org/10.1021/jf980508+

McGLYNN, W. (2009): The importance of food pH in commercial canning operations. Food Technology Fact Sheet. Food and Agricultural Products Research and Technology Center, Oklahoma State University. http://pods.dasnr.okstate.edu/docushare/dsweb/Get/Rendition-3801/FAPC-118web.pdf (accessed 15.11.2019)

Merck KGaA. (2005): Microbiology manual 12th edition. Merck, KGaA, Darmstadt, Germany.

MESSELHÄUSSER, U., FRENZEL, E., BLÖCHINGER, C., ZUCKER, R., KÄMPF, P., EHLING-SCHULZ, M. (2014): Emetic *Bacillus cereus* are more volatile than thought: recent foodborne outbreaks and prevalence studies in Bavaria (2007–2013). *BioMed Research International, 2014*, 1–9. https://doi.org/10.1155/2014/465603

MOSSEL, D.A., MENGERINK, W.H., SCHOLTS, H.H. (1962): Use of a modified MacConkey agar medium for the selective growth and enumeration of *Enterobacteriaceae*. *Journal of Bacteriology, 84*(2), 381. https://doi.org/10.1128/jb.84.2.381-381.1962

MOSSEL, D.A.A., KOOPMANN, M.J., JONGERIUS, E. (1967): Enumeration of *Bacillus cereus* in foods. *Journal of Applied Microbiology, 15*, 650–653. https://doi.org/10.1128/am.15.3.650-653.1967

MUNSCH, P., ALATOSSAVA, T., MARTTINEN, N., MEYER, J.M., CHRISTEN, R., GARDAN, L. (2002): *Pseudomonas costantinii* sp. nov., another causal agent of

brown blotch disease, isolated from cultivated mushroom sporophores in Finland. *International Journal of Systematic and Evolutionary Microbiology, 52*(Pt 6), 1973–1983.
https://doi.org/10.1099/00207713-52-6-1973

MURUGESAN, L., KUCEROVA, Z., KNABEL, S.J., LaBORDE, L.F. (2015): Predominance and distribution of a persistent *Listeria monocytogenes* clone in a commercial fresh mushroom processing environment. *Journal of Food Protection, 78*(11), 1988–1998.
https://doi.org/10.4315/0362-028X.JFP-15-195

NAKAMURA, H., TAKAKURA, K.I., SONE, Y., ITANO, Y., NISHIKAWA, Y. (2013): Biofilm formation and resistance to benzalkonium chloride in *Listeria monocytogenes* isolated from a fish processing plant. *Journal of Food Protection, 76*(7), 1179–1186.
https://doi.org/10.4315/0362-028X.JFP-12-225

OCTAVIA, S., LAN, R. (2014): The Family *Enterobacteriaceae*. In ROSENBERG, E., DELONG, E.F., LORY, S., STACKEBRANDT, E., THOMPSON, F., *The Prokaryotes*. Springer. Berlin, Germany. https://doi.org/10.1007/978-3-642-38922-1_167

PARENTELLI, C., ARES, G., CORONA, M., LAREO, C., GÁMBARO, A., SOUBES, M., LEMA, P. (2007): Sensory and microbiological quality of shiitake mushrooms in modified-atmosphere packages. *Journal of the Science of Food and Agriculture, 87,* 1645–1652.
https://doi.org/10.1002/jsfa.2848.

PATEL, S., GOYAL, A. (2012): Recent developments in mushrooms as anticancer therapeutics: a review. *3 Biotech, 2*(1), 1–15.
https://doi.org/10.1007/s13205-011-0036-2

PENNONE, V., DYGICO, K.L., COFFEY, A., GAHAN, C.G.M., GROGAN, H., MCAULIFFE, O., BURGESS, C.M., JORDAN, K. (2020). Effectiveness of current hygiene practices on minimization of *Listeria monocytogenes* in different mushroom production-related environments. *Food Science & Nutrition*, *8*(7), 3456–3468.
https://doi.org/10.1002/fsn3.1629

PIERCEY, M.J., ELLS, T.C., MACINTOSH, A.J., HANSEN, L.T. (2017): Variations in biofilm formation, desiccation resistance and Benzalkonium chloride susceptibility among *Listeria monocytogenes* strains isolated in Canada. *International Journal of Food Microbiology, 257*, 254–261. https://doi.org/10.1016/j.ijfoodmicro.2017.06.025

POLITOWICZ, J., LECH, K., LIPAN, L., FIGIEL, A., CARBONELL-BARRACHINA, A. (2017): Volatile composition and sensory profile of shiitake mushrooms as affected by drying method: Aroma profile of fresh and dried *Lentinula edodes*. *Journal of the Science of Food and Agriculture, 98*(4), 1511–1521. https://doi.org/10.1002/jsfa.8622

RAINEY, P.B. (1991): Effect of *Pseudomonas putida* on hyphal growth of *Agaricus bisporus*. *Mycological Research, 95*(6), 699–704. https://doi.org/10.1016/S0953-7562(09)80817-4

RAINEY, P.B., BRODEY, C.L., JOHNSTONE, K. (1992): Biology of *Pseudomonas tolaasii*, cause of brown blotch disease of the cultivated mushroom. *Advanced Plant Pathology, 8*, 95–117.

Rapid Alert System for Food and Feed (RASFF). RASSF Portal. https://webgate.ec.europa.eu/rasff-window/screen/search. (accessed 11.11.2022)

REIS, F.S., BARROS, L., MARTINS, A., FERREIRA, I.C.F.R. (2012): Chemical composition and nutritional value of the most widely appreciated cultivated mushrooms: An inter-species comparative study. *Food and Chemical Toxicology, 50*(2), 191–197. https://doi.org/10.1016/j.fct.2011.10.056

REYES, J.E., VENTURINI, M.E., ORIA, R., BLANCO, D., (2004): Prevalence of *Ewingella americana* in retail fresh cultivated mushrooms (*Agaricus bisporus, Lentinula edodes,* and *Pleurotus ostreatus*) in Zaragoza (Spain). *FEMS Microbiology Ecology, 47*(3), 291–296. https://doi.org/10.1016/S0168-6496(03)00283-6

RYU, J.S., KIM, M.K., IM, C.H., SHIN, P.G. (2015): Development of cultivation media for extending the shelf-life and improving yield of king oyster mushrooms (*Pleurotus eryngii*). *Scientia Horticulturae, 193*, 121–126. https://doi.org/10.1016/j.scienta.2015.07.005

SAMADPOUR, M., BARBOUR, M.W., NGUYEN, T., CAO, T.M., BUCK, F., DEPAVIA, G.A., MAZENGIA, E., YANG, P., ALFI, D., LOPES, M., STOPFORTH, J.D. (2006): Incidence of enterohemorrhagic *Escherichia coli, Escherichia coli O157, Salmonella,* and *Listeria monocytogenes* in retail fresh ground beef, sprouts, and mushrooms. *Journal of Food Protection, 69*(2), 441–443. https://doi.org/10.4315/0362-028x-69.2.441

SANTANA, C., VANETTI, M., KASUYA, M.C. (2007): Microbial growth and colour of minimally processed shiitake mushroom stored at different temperatures. *International Journal of Food Science & Technology, 43*, 1281–1285. https://doi.org/10.1111/j.1365-2621.2007.01606.x.

SAVOIE, J.M., MATA, G., LARGETEAU, M. (2016): New prospects in pathogen control of button mushroom cultures. In PETRE, M., *Mushroom Biotechnology*. Elsevier. Philadelphia, USA. https://doi.org/10.1016/B978-0-12-802794-3.00006-0

SIYOUM, N.A., SURRIDGE, K., VAN DER LINDE, E.J., KORSTEN, L. (2015): Microbial succession in white button mushroom production systems from compost and casing to a marketable packed product. *Annals of Microbiology, 66*(1), 151–164. https://doi.org/10.1007/s13213-015-1091-4

Statista Inc. (2023): Konsum von Champignons und Pilzen in Österreich in den Jahren 2006/7 bis 2021/22. https://de.statista.com/statistik/daten/studie/436611/umfrage/konsum-von-champignons-und-pilzen-in-oesterreich/ (accessed 18.05.2023)

STEPHENSON, S.L. (2010): The kingdom fungi. Timber Press, Inc. Portland, Oregon. ISBN13: 9780881928914.

STRAPP, C.M., SHEARER, A.E.H., & JOERGER, R.D. (2003): Survey of retail alfalfa sprouts and mushrooms for the presence of *Escherichia coli O157:H7, Salmonella,* and *Listeria* with BAX, and evaluation of this polymerase chain reaction–based system with experimentally contaminated samples. *Journal of Food Protection, 66*(2), 182–187.
https://doi.org/10.4315/0362-028x-66.2.182

SUHAILI, M., NOR-KHAIZURA, M-A-R., NUR HANANI, Z.A., ISMAIL-FITRY, M.R., SAMSUDIN, N.I.P., JAMBARI. N.N. (2021): Assessment of microbiological safety and physicochemical changes of grey oyster mushroom (*Pleurotus sajor-caju*) during storage at 4 °C and 25 °C. *Sains Malaysiana, 50*, 2993–3002.
https://doi.org/10.17576/jsm-2021-5010-13

TOLAAS, A.G. (1915): A bacterial disease of cultivated mushrooms. *Phytopathology, 5*, 51-54.

TSUNEDA, A., SUYAMA, K., MURAKAMI, S., OHIRA, A. (1995): Occurrence of *Pseudomonas tolaasii* on fruiting bodies of *Lentinula edodes* formed *Quercus* logs. *Mycoscience, 36*, 283–288.
https://doi.org/10.1007/BF02268603

VALVERDE, M.E., HERNÁNDEZ-PÉREZ, T., PAREDES-LÓPEZEDIBLE, O. (2015): Edible mushrooms: improving human health and promoting quality life. *International Journal of Microbiology, 2015*(7), 14.
https://doi.org/10.1155/2015/376387

VENTURINI, M.E., REYES, J.E., RIVERA, C.S., ORIA, R., BLANCO, D. (2011): Microbiological quality and safety of fresh cultivated and wild mushrooms commercialized in Spain. *Food Microbiology, 28*(8), 1492–1498.
https://doi.org/10.1016/j.fm.2011.08.007

VERORDNUNG (EG) Nr. 2073/2005 DER KOMMISSION vom 15. November 2005 über mikrobiologische Kriterien für Lebensmittel, Pub. L. No. L338/1, 26 (2005). https://eur-lex.europa.eu/legal-content/DE/ALL/?uri=CELEX%3A32005R2073, (accessed 11.11.2022)

VISWANATH, P., MURUGESAN, L., KNABEL, S.J., VERGHESE, B., CHIKTHIMMAH, N., LaBORDE, L.F. (2013): Incidence of *Listeria monocytogenes* and *Listeria* spp. in a small-scale mushroom production facility. *Journal of Food Protection, 76*(4), 608–615. https://doi.org/10.4315/0362-028X.JFP-12-292

WANG, Q., CHU, L., KOU, L. (2017): UV-C treatment maintains quality and delays senescence of oyster mushroom (*Pleurotus ostreatus*). *Scientia Horticulturae, 225,* 380–385. https://doi:10.1016/j.scienta.2017.07.019

WATKINSON, S.C., BODDY, L., MONEY, N.P. (2015): The Fungi. Elsevier Ltd. Amsterdam, Netherlands. ISBN: 9780123820358.

WEBSTER, J., WEBER, R. (2007): Introduction to Fungi. Cambridge University Press. Cambridge, UK. ISBN 13: 9780521807395.

Wirtschaftskammer Österreich, Codex Alimentarius, Österreichisches Lebensmittelbuch, Stand: 20.02.2018, unter: https://www.wko.at/branchen/industrie/nahrungs-genussmittelindustrie/Codex_Alimentarius.html (accessed 10.11.2019)

WONG, W.C., FLETCHER, J.F., UNSWORTH, B.A., PREECE, T.F. (1982): A note on ginger blotch, a new bacterial disease of the cultivated mushroom, *Agaricus bisporus. Journal of Applied Bacteriology, 52*, 43–48. https://doi.org/10.1111/j.1365-2672.1982.tb04371.x

WU, S., WU, Q., ZHANG, J., CHEN, M., YAN, Z.A., HU, H. (2015): *Listeria monocytogenes* prevalence and characteristics in retail raw foods in China. *PLOS ONE, 10*(8), e0136682.

https://doi.org/10.1371/journal.pone.0136682

XU, Y., TIAN, Y., MA, R., LIU, Q., ZHANG, J. (2016): Effect of plasma activated water on the postharvest quality of button mushrooms, *Agaricus bisporus*. *Food Chemistry, 197,* 436–444.
https://doi:10.1016/j.foodchem.2015.10.144

YOUNG, J.M. (1970): Drippy gill: a bacterial disease of cultivated mushrooms caused by *Pseudomonas agarici* n. sp*. New Zealand Journal of Agricultural Research, 13*(4), 977–990.
https://doi.org/10.1080/00288233.1970.10430530

ZAWIRSKA-WOJTASIAK, R., SIWULSKI, M., MILDNER-SZKUDLARZ, S., WĄSOWICZ, E. (2009): Studies on the aroma of different species and strains of *Pleurotus* measured by GC/MS, sensory analysis and electronic nose. *Acta Scientiarum Polonorum Technologia Alimentaria, 8*, 47–61.

ZHANG, H., YAMAMOTO, E., MURPHY, J., LOCAS, A. (2020): Microbiological safety of ready-to-eat fresh-cut fruits and vegetables sold on the Canadian retail market. *International Journal of Food Microbiology*, *335,* 108855.
https://doi.org/10.1016/j.ijfoodmicro.2020.108855

ZHENG, H.G., CHEN, J.C., AHMAD, I. (2017): Preservation of king oyster mushroom by the use of different fermentation processes. *Journal of Food Processing and Preservation, 42*, e13396
https://doi.org/10.1111/jfpp.13396

9. Figures and tables

10. Appendix

Supplementary Table 1: Overview of the devices and consumables used for the microbiological and sensory analysis.

LABEL	PRODUCER/DISTRIBUTOR
1. Technical devices	
Stomacher filter-bags	Seward Ltd., Worthing, United Kingdom
Stomacher 400	Seward Ltd., Worthing, United Kingdom
Incubator 30 °C	Ehret, Emmendingen, Germany
Incubator 37 °C	Ehret, Emmendingen, Germany
Incubator 42 °C	Sanyo Denki K.K., Osaka, Japan
Pipette 100–1000 µl	Thermo Fisher Scientific Inc., Waltham, USA
Pipette 10–100 µl	Thermo Fisher Scientific Inc., Waltham, USA
Pipette tip 1250 µl	Peqlab, Erlangen, Germany
Pipette tip 100 µl	Peqlab, Erlangen, Germany
Vortexer	VWR, laboratory equipment, Pennsylvania, USA
Latex gloves	B. Braun Melsungen AG, Melsungen, Germany
Pasteur pipette	Karl Hecht GmbH & Co KG, Sondheim, Germany
10 ml tubes	Sarstedt AG&Co. KG, Nümbrecht, Germany
Sterilized Scissors and forceps	
Inoculation loops	VWR, laboratory equipment, Pennsylvania, USA
2. Chemicals, reagents and solutions	
Ringer's solution	B. Braun Melsungen AG, Melsungen, Germany
Hydrogen peroxide (H_2O_2)	VWR, laboratory equipment, Pennsylvania, USA
Kovacs Reagent	VWR, laboratory equipment, Pennsylvania, USA
Potassium hydroxide (KOH)	VWR, laboratory equipment, Pennsylvania, USA
Mikrozid	Schülke&Mayr, Norderstedt, Germany
3. Culture media	
Tryptic Soy Agar (TSA)	Merck, KGaA, Darmstadt, Germany
Violet Red Bile Dextrose Agar (VRBD)	Biokar Diagnostics, Beauvais Cedex, France
Glutamat-Stärke-Phenolrot-Agar (GSP)	Merck, KGaA, Darmstadt, Germany
Lactobacilli selective Agar acc. to De Man, Rogosa and Sharpe (MRS)	Biokar Diagnostics, Beauvais Cedex, France
Rose-Bengal Chloramphenicol Agar (BR)	Oxoid Ltd., Basingstoke, United Kingdom
Mannitol Egg Yolk Polymyxin Agar (MYP)	Oxoid Ltd., Basingstoke, United Kingdom

Xylose-Lysin-Desoxycholat-Agar (XLD)	Oxoid Ltd., Basingstoke, United Kingdom
Buffered Pepton Water (BPW)	Oxoid Ltd., Basingstoke, United Kingdom
Rappaport Vassiliadis soya peptone broth (RVS)	Oxoid Ltd., Basingstoke, United Kingdom
Muller-Kauffmann Tetrathionate-Novobiocin Broth (MKTT)	Oxoid Ltd., Basingstoke, United Kingdom
Demi-Fraser (DF)	Biokar Diagnostics, Beauvais Cedex, France
Full-fraser Bouillon (FF)	Merck, KGaA, Darmstadt, Deutschland
Listeria Agar acc. to Ottaviani and Agosti (ALOA)	Merck, KGaA, Darmstadt, Germany
Materials used for the sensory analysis	
Rapeseed oil (Mazola® Peter Kölln GmbH & Co. KGaA, Elmshorn, Germany)	
Non-stick pan	
Kitchen knife, stainless steel	
Plastic cutting board	
Fork, stainless steel	
Ceramics plates	
Tap water to drink in-between	

Supplementary Figure 1: Sensory test scheme for fresh mushrooms.

Sensorik-Prüfschema für frische Pilze nach DIN 10969:2018

Punkte und Bewertung

Punkte	Qualitätsbeschreibung	Abweichungen vom Standard
5	Sehr gut	Keine Abweichung vom Standard
4	Gut	Geringfügige Abweichungen
3	Zufriedenstellend	Leichte Abweichungen
2	Weniger zufriedenstellend	Deutliche Abweichungen
1	Nicht zufriedenstellend	Starke Abweichungen
0	Ungenügend	sehr starke Abweichungen, nicht bewertbar

Keine Bewertung

0. Verpackung

Gesamtbild der Probe	0 1	MHD-Angabe	0 1	
intakt	0 1	Kühlhinweis	0 1	
Folie	0 1	Kennzeichnung konform	0 1	
Spankorb	0 1			
Pappkorb	0 1			

Gewichtungs-Faktor = Gewichtete Bewertung

1. Äußeres, Aussehen, Farbe, Zusammensetzung im nicht zubereitetem Zustand — Bewertung 5 4 3 2 1 0 X 3 =

Pilze gesamt		**Hut**	**hellbraun, trocken, samtig**		**Stiel**		**Lamellen hellbraun, trocken**	
1	Sortierung ungleichmäßig 4 3 2 -	8	Farbveränderung 4 3 2 1 -		43	Farbveränderung 4 3 2 1 -	15	Farbveränderung 4 3 2 1
2	miner. Verunreinig. zu hoch* 4 3 2	9	Oberfläche feucht/klebrig 4 3 2 1 -		44	Oberfläche feucht/klebrig 4 3 2 1 -	16	klebrig/mazeriert 4 3 2 1
	*max. 0,5 %	10	braune Flecken 4 3 2 1 -		13	braune Flecken 4 3 2 1 -		
3	org.Verunreinigung zu hoch 4 3 2	11	gelbe Flecken 4 3 2 1 -		14	gelbe Flecken 4 3 2 1 -		
	max. 8,0 %	12	Schimmel - 2 1 -					
4	Anteil madengesch. Pilze 4 3 2 1							
	max. 1 %							
5	Anteil stark madengesch. Pilze 4 3 2 1							
	max. 0,5 %							
6	zu reif 4 3 2							
7	zu alt 4 3 2							

45 **Sonstige Mängel*** - 4 3 2 1 -

*** Beschreibung

Gewichtungs-Faktor = Gewichtete Bewertung

3. Konsistenz — Bewertung 5 4 3 2 1 0 X 2 =

Pilze gesamt		**Hut**	**fest bis elastisch**		**Stiel**	**fest-elastisch**		**nach Erhitzung**	
17	weich 4 3 2 1 -	19	weich 4 3 2 1 -		22	verminderte Elastizität 4 -		42	gummiartig 4 3 2 -
18	wässrig (Prüfung im Anschnitt) 4 3 2 -	20	mehlig 4 3 2 1 -		23	gummiartig 4 3 2 1 -		25	zäh 3 2 1 -
30	eingetrocknet 3 2 1	21	schwammig 4 3 2 1 -		24	faserig 4 3 2 1 -		26	faserig 3 2 1 -

45 **Sonstige Mängel*** - 4 3 2 1 -

*** Beschreibung

Gewichtungs-Faktor = Gewichtete Bewertung

4. Geruch — Bewertung 5 4 3 2 1 0 X 2 =

27	flach/neutral (Aromaverlust) 4 3 -	31	dumpf/muffig 3 2 1 -
28	alt 3 2 1 -	32	faulig 2 1 -
29	fremdartig 3 2 1	33	phenolisch 3 2 1 -

45 **Sonstige Mängel**** - 4 3 2 1 -

*** Beschreibung - - - - - 0

Gewichtungs-Faktor = Gewichtete Bewertung

5. Geschmack nach Erhitzung — Bewertung 5 4 3 2 1 0 X 3 =

Pilze gesamt			
34	fade (Aromaverlust Umami) 4 3 2 1 -	38	phenolisch - 3 2 1 -
35	bitter 4 3 2 1 -	39	dumpf, muffig - 3 2 1 -
36	adstringierend 4 3 2 1 -	40	faulig - - - 1 -
37	süßlich 4 3 2 1 -	41	charakt. Eigengeschmack fehlt 4 3 2 -

45 **Sonstige Mängel**** - 4 3 2 1 -

*** Beschreibung - - - - - 0

*** unbedingt erläutern
Verkehrsfähigkeit am Untersuchungstag:

Gewichtete Gesamtbewertung
Summe der Gewichtungsfaktoren = ____ / 10 Qualitätszahl

Datum: Prüfer:

11. Disclaimer

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