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Quality and Safety of Dried Mushrooms Available at Retail Level

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Abstract: Pathogenic microorganisms surviving in dry products have regularly led to recalls and foodborne disease outbreaks. Therefore, the microbiological quality of 61 dried mushrooms samples purchased online and in supermarkets were analyzed. Counts of aerobic mesophiles (AMCs), *Enterobacteriaceae* (EB), yeasts and molds, presumptive *Bacillus cereus* (pBC), the presence of *Salmonella* spp., and *L. monocytogenes* were investigated. Isolates of pBC were screened for their partial *panC* gene sequences and their toxin genes' profiles. The microbiological quality of the dried mushrooms investigated in this study was generally found to be acceptable. Average AMCs, EB, yeasts, and molds were 3.9 log, 1.1 log, 1.6 log, and 1.5 log cfu/g, respectively. All mushroom samples tested negative for *Salmonella* spp. and *L. monocytogenes.* Presumptive BC were detected in 59.0% of the samples, but the contamination level was low (1.0 to 3.4 log cfu/g). None of the isolates were positive for the *ces* gene. Incomplete labeling was found in 45.9% of the samples, mainly in the form of missing heating instructions (31.1%) and/or country of origin (16.3%). Contamination by pathogens can occur in dried mushrooms. Adequate information on home cooking practices is essential to reduce the risk of foodborne illness to the consumer and to provide a safe food product.

Keywords: microbiology contamination; *Listeria monocytogenes*; *Salmonella*; presumptive *Bacillus cereus*; *Bacillus cereus* toxin profile; adequate labeling

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1. Introduction

In Europe, the consumption of dried mushrooms has increased steadily in recent years, and accounts for around 20.0% of the global dried mushroom market. The most common dried groups of cultivated mushrooms are the genus *Agaricus*, mainly button mushrooms, shiitake mushrooms, and wood ears (syn. mu err), while wild-grown species include porcini, chanterelles, black trumpets, and morels [1]. Freshly harvested mushrooms are highly perishable due to their high water content and contamination with microorganisms in nature [2]. Drying is an important preservation technique, which reduces the moisture content to prevent microbial growth and quality loss due to chemical and biochemical reactions [3]. Bacteria, yeasts, and molds, including foodborne pathogens, are able to survive the drying process and survive for several months or years in low-moisture foods ($a_w < 0.85$) [4]. The average aerobic mesophilic count (AMC) of dried mushrooms sold on the Internet and in local markets ranges from 0.8 to 4.6 log of colony-forming units (cfu)/g. Yeasts and molds have only been found at low levels (0.4 to 2.2 log cfu/g) [5–7]. After rehydration in water, dried mushrooms can be used in various meals, and the number of total coliforms could increase by 2.0 log cfu/g in jelly mushrooms after soaking in sterile water for four hours at room temperature [7]. Dried mushrooms are regularly contaminated with foodborne pathogens, with *Salmonella* spp. and *Bacillus* (*B.*) *cereus* being the most important [6,8–11]. In 2020, an outbreak caused by *Salmonella* Stanley in dried wood ear mushrooms was published by the Centers for Disease Control and Prevention [9]. Reports

of the presence of *Listeria* (*L.*) *monocytogenes* have been associated with fresh cultivated and wild mushrooms and have led to notifications and recalls in Europe and the United States [11–14]. Recalls of sunflower seeds, several nut species, and dried fruits that were contaminated with *L. monocytogenes* in the USA and Canada indicate that this pathogen is regularly present in dried food [15,16]. Few studies have reported on the microbial status and presumptive *B. cereus* (pBC) in dried mushrooms.

However, no data are available that focus on the prevalence of food-borne pathogens (*Salmonella* spp., *L. monocytogenes*, and pBC) after the common rehydration procedure before mushrooms are used in the kitchen. Therefore, the aim of the present study was to investigate the potential risk of humans consuming dried mushrooms that are marketed online and in Asian supermarkets in Vienna, Austria, (i) focusing on the microbiological safety after soaking the mushrooms in water and (ii) reviewing the mandatory labeling requirements of the European Union (EU) [17]. Additionally, *Salmonella*, *L. monocytogenes*, and pBC isolates were confirmed by specific PCR methods. *B. cereus* were screened for their toxin gene profile.

2. Materials and Methods

2.1. Collection of Mushrooms

In total, 61 samples of packed dried mushrooms (at least 100 g/sample) were randomly purchased online (37 samples), in Asian supermarkets (18 samples), in a whole foods store, and in a supermarket (2 samples each) in Vienna, Austria. The samples originated from China (33 samples), Bosnia and Herzegovina (6 samples), Bulgaria (5 samples), Spain (3 samples), Greece (2 samples), Montenegro, Poland, and Vietnam (1 sample each). The country of origin was not provided for nine of the samples. The set of dried mushrooms consisted of 11 different mushroom species, which were mainly offered as pure species. Twenty-one different suppliers were included in the study. Details are presented in Table 1. Samples were stored in a dark and dry place until microbiological analysis (1–7 days).

Table 1. Sample description of dried mushroom species included in this study.

Classification [A–E]	Mushroom Species [F]	Samples (%) [G]
Agaricales [A]		total 25 (41.0)
	Shiitake (*Lentinula edodes*)	18 (29.5)
	Portobello (*Agaricus bisporus*)	3 (4.9)
	Oyster mushroom (*Pleurotus ostreatus*)	2 (3.3)
	Poplar fieldcap mushroom (*Cyclocybe cylindracea*)	1 (1.6)
	Mixed mushroom species with Shiitake and Oyster	1 (1.6)
Agaricomycotina species without gills [B]		total 13 (21.3)
Auriculariales	Wood ear (*Auricularia auricula-judae*)	10 (16.4)
Tremellomycetes	Jelly mushroom (*Tremella fuciformis*)	2 (3.3)
Polyporales	Maitake (*Grifola frondosa*)	1 (1.6)
Cantharellales [C]		total 10 (16.4)
	Chanterelle (*Cantharellus cibarius*)	6 (9.8)
	Horn of plenty (*Craterellus cornucopioides*)	2 (3.3)
	Mixed mushroom species with *Cantharellales*	2 (3.3)
Boletales [D]		total 10 (16.4)
	Porcini (*Boletus edules*)	9 (14.8)
	Mixed mushroom species with Porcini	1 (1.6)
Pezizomycotina [E]		total 3 (4.9)
	Morel (*Morchella* spp.)	
TOTAL		total 61 (100)

Abbreviations: Classification [A,C,D,E] according to taxonomic position or [B] common characteristics of the group; [F] common species name and scientific name in brackets; [G] number of samples and percentage of the total.

2.2. Microbiological Enumeration and Detection

Usually, dried mushrooms are rehydrated in water before further processing and being used in dishes. Therefore, 50 g of each sample was soaked in a sterile plastic bag with 100 mL of sterile distilled water at room temperature for one hour according to ISO 6887-4:2017 [18]. Subsequently, a 75 g portion of the rehydrated mushroom sample (corresponds to 25 g dry matter) was diluted in 225 mL buffered peptone water (BPW) (Biokar, Groupe Solabia, Pantin Cedex, France), homogenized for 60 s in a laboratory blender (Stomacher, Seward Limited, West Sussex, UK), and serial decimal diluted in Ringer's solution (B. Braun, Melsungen, Germany). AMC, *Enterobacteriaceae* (EB), ppBC, yeasts, and molds were enumerated according to the corresponding ISO standards or in-house methods (Supplemental Table S1). Microbiological counts were calculated according to ISO 7218:2007/Amd.1:2013 [19]. For the detection of *Listeria* spp., *L. monocytogenes,* and *Salmonella* spp., a 75 g portion of rehydrated mushrooms each was enriched and streaked on selective agar according to the ISO standards (Supplemental Table S1).

Typical colony morphologies (1–5 isolates) for EB, pBC, *Listeria spp., L. monocytogenes,* and *Salmonella* spp. were selected and sub-cultivated on Tryptic Casein Soy Agar with 0.6% yeast extract (TSAYE, Biokar) for further confirmation. Colonies that were typical for coliform bacteria on Violet Red Bile Glucose Agar (pink to red or purple colonies with and without precipitation halos) were further differentiated on Brilliance *Escherichia* (*E.*) *coli*/Coliform Agar (Thermo Fisher Scientific Inc., Oxoid, Waltham, MA, USA) according to the manufacturer's colony morphologies (presumptive *E. coli*: purple, coliform bacteria: pink). These results were used to enumerate *E. coli* and coliforms in dried mushroom samples. Presumptive BC grown on MYP were further streaked on *Bacillus* Chromo Select Agar with Polymyxin B (Sigma-Aldrich, St. Louis, MO, USA) and 10.0% egg yolk suspension (Thermo Fisher Scientific Inc., Oxoid). Blue growing colonies with an opaque halo were used for the final confirmation steps of the pBC. For further confirmation by PCR, the purified *Salmonella*, *Listeria,* and pBC isolates were stored at −80 °C in 25% glycerol stocks in the strain collection of the Unit of Food Microbiology (University of Veterinary Medicine, Vienna, Austria).

The microbiological quality of dried mushrooms was assessed as satisfactory according to the values for the AMC load $\leq$ 6.0 log cfu/g; for EB $\leq$ 4.0 log cfu/g; and for pBC $\leq$ 2.0 log cfu/g (according to the recommended limits of the German Society for Hygiene and Microbiology (DGHM) [20], as well as for yeasts $\leq$ 4.0 log cfu/g and molds $\leq$ 3.0 log cfu/g (according to our internal standard). The critical limit of pBC was defined by the recommendations of the DGHM [20] for dried mushrooms, with $\geq$3.0 log cfu/g.

2.3. Investigation of Presumptive Salmonella, Listeria, and B. cereus isolates by PCR

2.3.1. DNA Extraction

DNA was extracted by the Chelex method published by Walsh et al. [21]. A loop of the pure culture grown on TSAYE was resuspended in 0.1 M Tris-HCl buffer (Sigma-Aldrich), followed by the addition of Chelex® 100 Resin (Bio-Rad, Hercules, CA, USA). The Chelex suspension was then boiled for 10 min (100 °C) and centrifuged at 14,000 rpm for 5 sec. The supernatant was transferred to a 1.5 mL Maximum Recovery Eppendorf Tube® (Eppendorf, Hamburg, Germany) and stored at −20 °C.

2.3.2. Confirmation of Salmonella and *Listeria* spp.

Isolates with suspected *Salmonella* spp. were confirmed by PCR amplification of the invasion protein (*invA*) gene according to the method published by Rahn et al. [22]. Differentiation of *Listeria* spp. was performed by a multiplex PCR assay targeting the invasion-associated protein (*iap*) gene [23].

2.3.3. Characterization of Presumptive *B. cereus* by *panC* Typing and Screening of Toxin and Crystal Protein (*cry*) Genes

Presumptive BC isolates (n = 42) were selected from all positive samples, and for further characterization, the partial *panC* gene was sequenced by LGC, Berlin, Germany. Amplification was carried out according to Guinebretière et al., and the sequences were compared with a specific online database (https://www.tools.symprevius.org/Bcereus/english.php, accessed on 18 October 2023) to assign *B. cereus* isolates to phylogenetic groups I-VII [24,25].

Additionally, the isolates were tested for their toxin profile by conventional PCR technique according to Fuchs et al. [26]. Three pore-forming enterotoxins genes—non-hemolytic (*nheA* and *B*), hemolysin bl (*hbl A* and *D*), and cytolysin K (*cyt K-2*)—were selected. The presence of the emetic toxin cereulide gene *ces* was analyzed as previously published by Dzieciol et al. [27]. The presence of the *cry* gene encoding insecticidal crystal proteins (d-endotoxin) that are characteristic of *B. thuringiensis* strains was detected by conventional PCR technique as described by Wei et al. [28].

3. Results and Discussion

3.1. Microbiological Quality

In dried mushrooms soaked for one hour, the average AMC (3.4 log to 5.0 log cfu/g), counts of yeasts (1.2 to 2.5 log cfu/g), and molds (1.3 to 2.4 log cfu/g) were consistent with results from dried mushrooms without a soaking procedure [5,6]. The counts of EB were in the range from 3.4 log to 5.0 log cfu/g, and in nine samples (14.8%), low counts (<3.0 log cfu/g) of coliform bacteria were detected (Table 2). *E. coli* was not detected in any of the samples. In contrast, in a study presented by Ajis et al. [7], 15.0% of the samples had a load of coliforms that was above 3.0 log cfu/g. The counts of pBC (>log 2.0 cfu/g in 11 samples, 18.0%) and EB (>log 4.0 cfu/g in 6 samples, 9.8%) were most frequently above the defined microbiological criteria established in this study. Increased AMC (>6.0 cfu/g in 4 samples, 6.6%) were found only within mushrooms of the *Cantharellales* group (Table 2). Microbiological limits were exceeded in 17 samples (27.9%) for at least one parameter (AMC, EB, yeasts, molds, and pBC), with *Agaricales* mushrooms always being below the defined limits and mushrooms of the *Cantharellales* group most frequently (13.1%) exceeding these limits (Figure 1). In one *Cantharellales* sample (*Craterellus cornucopioides*), the results of all the investigated microbiological parameters were above the limits. The microbial load of different mushroom categories varied widely (AMC log 4.4–9.4 cfu/g) with fresh *Cantharellales* and *Auriculariales*, which achieved the highest of AMC (8.2–9.4 log cfu/g) [12,29]. The natural microbiome of Portobello mushrooms (*Agaricus bisporus*) preharvest and after packaging was already dominated by Gram-negative bacteria [30]. The AMC detected in dried mushrooms should be lower, due to the drying process. Hot-air-drying of Portobello mushrooms resulted in a 2.3 log reduction in the AMC [31], but unfavorable storage conditions (temperature 37 °C, real humidity 85%) can cause a rebound of the aerobic mesophilic count in dried mushrooms [32].

3.2. Detection of Foodborne Pathogens

L. monocytogenes, *Listeria* and *Salmonella* spp. were not detected in any sample of the dried mushroom samples in this study. On the other hand, the presence of *Salmonella* spp. in dried mushrooms has been regularly reported [9,11]. *L. monocytogenes* was more commonly associated with fresh mushrooms, and both cultivated and wild mushrooms were affected [11–14].

Table 2. Average microbiological counts and samples above the limit values of dried mushrooms from online shops and Austrian supermarkets. Mean microbiological counts and limit values are given in log cfu/g.

Classification (Groups)	n	Average Microbiological Counts					Number (Percentage) of Samples above the Limits				
		AMC	EB/ [C]	Yeasts	Molds	BC	AMC [D] >6.0	EB [D] >4.0	Yeasts [D] >4.0	Molds [D] >3.0	BC [D] >2.0
Agaricales	25	3.4 ± 1.0 [A] (2.0–5.6) [B]	0.5 ± 0.5/[1] (0.3–2.6)	1.2 ± 0.5 (1.0–2.8)	1.3 ± 0.6 (1.0–2.9)	0.7 ± 0.5 (0.3–1.9)	0	0	0	0	0
Agaricomycotina [E]	13	4.0 ± 1.1 (1.0–5.2)	1.8 ± 1.4/[5] (0.3–4.6)	1.6 ± 1.2 (1.0–4.6)	1.6 ± 0.6 (1.0–2.8)	1.4 ± 0.9 (0.3–2.9)	0	1 (7.7%)	1 (7.7%)	0	3 (23.1%)
Cantharellales	10	5.0 ± 1.4 (3.1–6.6)	2.0 ± 2.5/[2] (0.3–6.2)	2.5 ± 1.3 (1.0–4.5)	1.9 ± 1.1 (1.0–4.3)	1.8 ± 0.7 (0.3–2.7)	4 (40.0%)	3 (30.0%)	2 (20.0%)	1 (10.0%)	5 (50.0%)
Boletales	10	3.5 ± 0.6 (2.7–4.7)	1.6 ± 1.6/[1] (0.3–4.4)	1.6 ± 1.0 (1.0–4.0)	1.5 ± 0.7 (1.0–2.9)	1.5 ± 1.0 (0.3–3.4)	0	1 (10.0%)	0	0	2 (20.0%)
Pezizomycotina	3	4.2 ± 0.8 (3.0–5.0)	2.6 ± 1.7 (0.3–4.3)	1.6 ± 0.8 (1.0–2.8)	2.4 ± 0.1 (2.3–2.5)	0.9 ± 0.9 (0.3–2.2)	0	1 (33.3%)	0	0	1 (33.3%)

AMC—aerobic mesophilic counts; EB—*Enterobacteriaceae*; BC—presumptive *Bacillus cereus*; counts of 1.0 and 0.3 cfu/g signify values below the detection limit and are used for the calculation of the mean. [A] mean ± SD; [B] minimum–maximum; [C] number of samples positive for coliforms (<3.0 log cfu/g); [D] microbiological limits; [E] this group represented different species without gills.

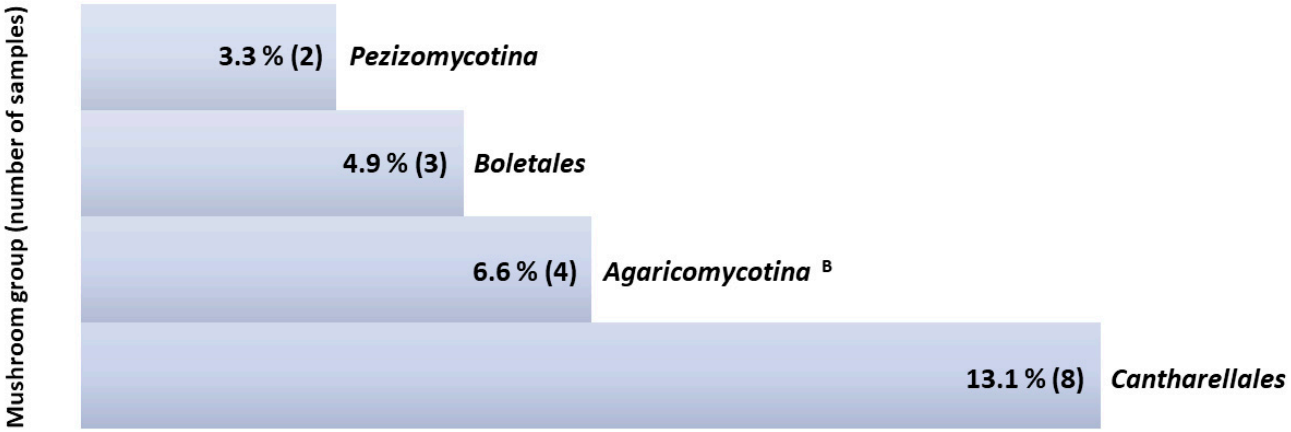

Figure 1. Samples with microbiological values above the specified limits in at least one parameter [A], purchased from online shops and Austrian supermarkets. Percentage (%) of total (n = 61) and number of samples within each group. [A] AMC, EB, yeasts, molds, and BC; [B] this group represented different species without gills.

Presumptive BC were identified in 36 samples (59.0%). In dried mushrooms analyzed in Germany, pBC were isolated from 81.5% and, recently, 64.3% [8,10]. However, in our study, the pBC counts were low to moderate (1.0–3.4 log cfu/g). These results are consistent with previous reports that found pBC between 0.5 and 3.6 log cfu/g both for dried and fresh mushrooms [33,34]. According to the *panC* gene sequencing and toxin gene typing, the 42 BC isolates were affiliated to the phylogenetic groups II, III, IV, V, and VI and toxin profiles A (*nhe*+, *hbl*+, *cytk2*+), C (*nhe*+, *hbl*+), D (*nhe*+, *cytk2*+), and F (*nhe*+) (Figure 2). Based on the PCR, none of the isolates were positive for the *ces* gene, but in previous studies, BC isolates possessing the *ces* gene were observed in both fresh (3.0%) and dried (8.1%) mushroom samples [8,34]. The *panC* IV cluster in combination with toxin gene profile A was identified most frequently (52.8%/36 BC-positive samples) in our study and was observed in every group except *Pezizomycotina*. Subsequently, the *panC* group toxin profile combinations VI/C, VI/F, II/C, and V/C were found in 19.4%, 13.9%, 11.1%, and 5.6% of the positive samples, respectively (Figure 2). This is in agreement with a study by Fuchs et al. [26], who also found a predominance of the *panC* cluster IV/A in 33.0% and VI/C in 10.4% of different food products. In fresh mushrooms, toxin gene profile A was also the most common (62%) among isolates [34]. Isolates assigned to toxin profile F in combination with *panC* groups II, III, IV, and V were present in one sample each. The *cry*

gene, which is characteristic of *B. thuringiensis*, a species that also belongs to the BC group, was detected in only four (6.5%/42) of these isolates, associated to the mushroom samples of the *Agaricales*, *Agaricomycotina* (wood ear mushroom), and *Chanterellales* (Figure 2). *B. thuringiensis* is present naturally in the environment, and defined subspecies use it as a biological insecticide to promote plant production [35]. Assessing the potential health risk posed by BC, the combination of enterotoxin profiles, the presence of the *ces* gene, and the contamination level in the food has to be taken into account. In cell bioassays, the cytotoxic activity of the enterotoxin toxin profiles (A, C, D, and F) that were also found in this study appears to be variable and ranged from low to high [36]. Outbreaks were often associated with pBC counts above 5.0 log cfu/g in the foodstuff [37]. On the other hand, cases induced by lower BC counts could be traced back to strains encoding cereulide and diarrheal toxin genes [38]. The mushrooms investigated in the present study contained low BC counts, except for one *Boletus* sample, which had a pBC count above the critical limit of 3.0 log cfu/g [20], and the *ces* gene, which induces the pre-formation of the heat-stable emetic toxin cereulide in food, was not detected in any BC isolate. Considering food safety issues, it is important to note that foodborne pathogens can survive the common drying technologies in fruits, vegetables, and mushrooms [4,31,39–41]. Current studies showed that after the drying process at 55 to 90 °C, the populations of *Salmonella* spp., *L. monocytogenes*, and *B. cereus* in mushrooms were reduced, but not inactivated completely [31,41]. During storage of Portobello mushrooms at room temperature, the three pathogens remained viable for over two months in vacuum-packaging [31]. After long-term storage for 180 days on enoki and wood ear mushroom, a residual population of *Salmonella enterica* and *L. monocytogenes* was detected [40]. An adequate heat treatment of the rehydrated mushrooms destroys vegetative cells, but spores of BC can survive the procedure, and it can lead to regrowth at improper storage temperatures (>4.0 °C cold storage, <60 °C hot storage) for several hours [37]. Further, the rehydration procedure can promote the germination of bacteria, possibly associated with the production of heat-stable toxins. The counts of *Salmonella* Typhimurium, *L. monocytogenes*, and pBC increased after soaking the dried Portobello mushrooms overnight by about 2.0 log cfu/g [31]. With a focus on this food safety issue, the soaking time for dried mushrooms should be kept as short as possible. In previous studies, soaking in water at room temperature for 20 to 30 min showed an adequate rehydration capacity [42–44]. Therefore, food suppliers should not recommend longer soaking times to increase food safety.

	II Toxin C	II Toxin F	III Toxin D	III Toxin F	IV Toxin A	IV Toxin F	V Toxin C	V Toxin F	VI Toxin C	VI Toxin F
total (%)	11.1	2.8	2.8	2.8	52.8	2.8	5.6	2.8	19.4	13.9
Agaricales					p[2]		p			
Agaricomycotina [B]					p[1]	p	p		p	p
Boletales	p	p	p		p				p	p
Cantharellales				p	p[1]				p	p
Pezizomycotina								p		

Figure 2. *PanC* groups (II–VI) and toxin gene profiles (A–F) of *B. cereus* isolates (n = 42) detected in 36 dried mushroom samples. [B] This group represented different species without gills. [1, 2] Number of samples containing *cry*-gene-positive *B. cereus*.

3.3. Labeling

Foods intended for sale to consumers in the EU must be labeled with information such as their name (common name of the mushroom species), net quantity, nutritional

value, storage recommendations (minimum durability, storage conditions), food business operator, and country of origin [17,45]. In our study, 16.3% (n = 10/61) of the samples had incomplete labeling, which occurred mainly in samples purchased from online shops (n = 9/10) (Figure 3). Most commonly, the country of origin was missing (14.7%; n = 9/10); only one sample purchased on the Internet was sold without any indication of the net quantity. In a Canadian study, the country of origin was also missing, and only 34.4% of fresh fruit samples were correctly labeled [46]. In addition to the mandatory particulars, information may be required to protect the health of consumers [17]. In order to reduce the food risks associated with the consumption of dried mushrooms, labeling regarding cooking treatment (heating instructions) allows for the use of a safe food product. In the present study, this important label information was missing on 31.1% (n = 19/61) of the mushroom packages, with most of the samples being mushrooms of the *Agaricales* group and wild mushrooms, *Boletales*, *Cantharellales*, and *Pezizomycotina* (Figure 3). Samples purchased on the Internet and in Asian supermarkets were almost equally affected. We also found that six samples (9.8%) without heating information were contaminated with pBC. This is in agreement with a report from the German Food Authority [10], where dried mushrooms containing pBC and not labeled with cooking instructions (21.4%) were considered "unfit for consumption".

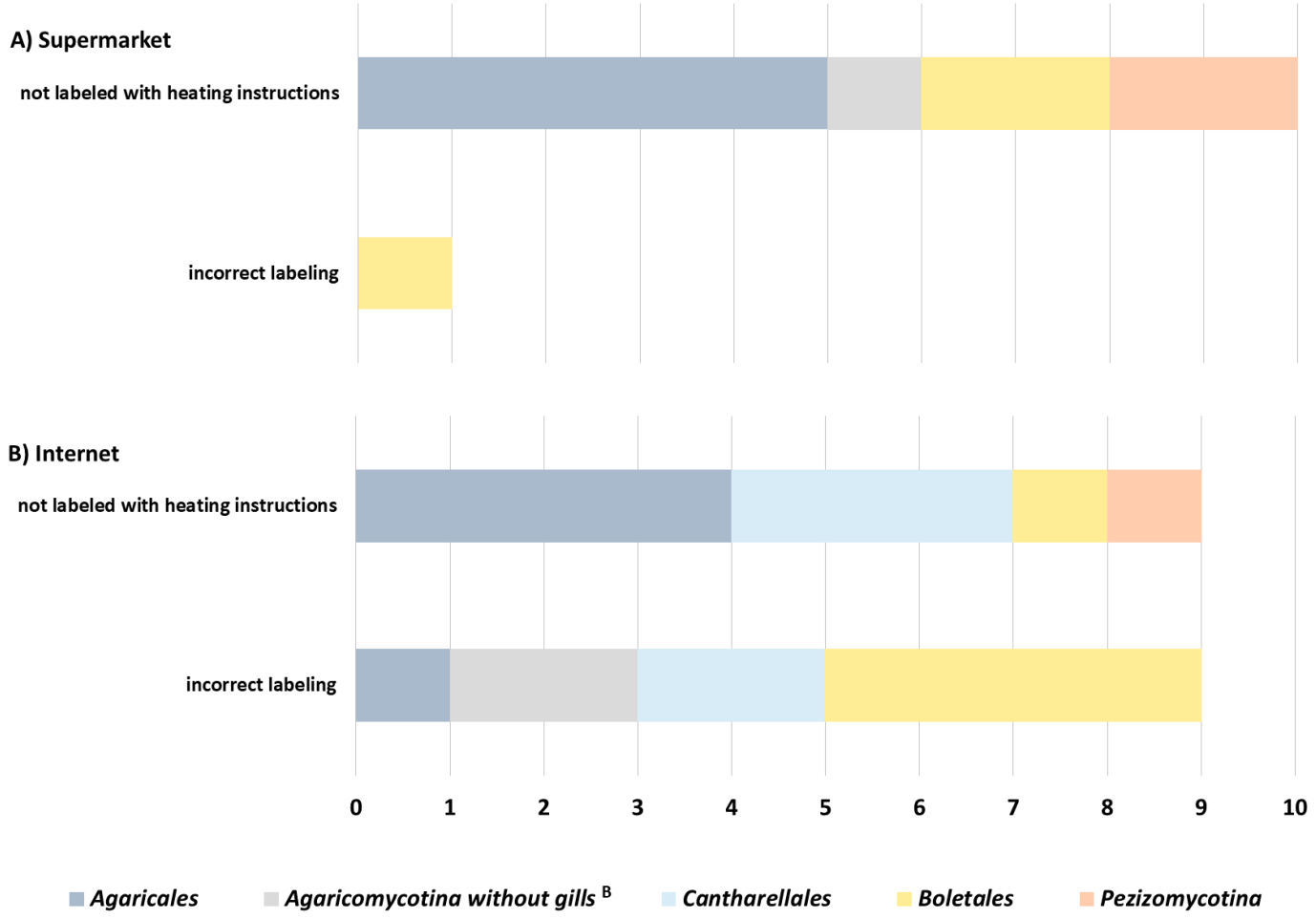

Figure 3. Number of dried mushrooms purchased from online shops and Austrian supermarkets which had incorrect labeling in terms of information required according to Regulation (EU) 1169/2011. [B] This group represented different species without no gills.

4. Conclusions

The microbiological quality of the dried mushrooms investigated in this study was generally found to be acceptable. Fresh mushrooms may have a naturally high microbial load and a more diverse microbiota. The samples did not contain the foodborne pathogens *L. monocytogenes* and *Salmonella* spp. However, the presence of pBC was high, but only one sample had counts > 3.0 log cfu/g. None of the BC isolates carried the emetic toxin gene (*ces*), but enterotoxin genes were present. BC strains representing *panC* group IV and toxin profile A were highly abundant. Almost half of the samples (45.9%) were inadequately labeled, mainly lacking heating instructions and/or country of origin. Adequate information on preparation behaviors (soaking procedure, cooking instructions, storage after cooking) at home is mandatory to reduce the risk of foodborne infections for the consumer and provide a safe food product. Country-of-origin labeling provides food traceability and information to consumers to make a purchase decision.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app14052208/s1, Table S1: Methods and media used for microbiological enumeration and detection in dried mushrooms.

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