

# Nutritional properties, microbial and sensory quality, and formation of biogenic amines in wild-grown mushrooms (*Cantharellus cibarius* & *Boletus edulis*) from Austrian local markets



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## ABSTRACT

Wild-grown mushroom species chanterelle (*Cantharellus cibarius*) and porcini mushroom (*Boletus edulis*), available at Austrian local markets, were evaluated regarding microbial and sensory quality, content of biogenic amines, and proximate chemical composition. Chemical composition varied widely with species, but showed no correlation with sensory quality and microbial load. Chanterelles were characterized by high levels of aerobic mesophilic counts, regardless of sensory quality. In both mushroom species, poor sensory quality was associated with increased counts of *Enterobacteriaceae*. In porcini mushrooms, the level of  $\beta$ -phenylethylamine, cadaverine, histamine, and tyramine correlated to the count and type of microorganism. The level of biogenic amines does not pose a risk to healthy individuals. Almost half of the samples purchased were sensorically deficient, indicating handling under poor hygienic conditions or being offered for sale for too long. To obtain wild mushrooms of excellent quality, all relevant stakeholders at harvest and retail level should be trained in hygienic handling of mushrooms.

## 1. Introduction

Wild-grown mushrooms, mainly the very popular chanterelle (*Cantharellus cibarius*) and porcini mushrooms (*Boletus edulis*), have been commercialized as seasonal products in local Austrian markets. Consumers prefer to eat mushrooms from wild gathering rather than cultivated ones, because of the more intense flavor and relevance for healthy lifestyle of the former (Schunko & Vogl, 2020). In general, mushrooms have become an important source of nutrients due to the high protein and low fat/energy content (Barros et al., 2008a, 2008b).

However, mushrooms are highly perishable due to their water content of about 90%, neutral pH, high CO<sub>2</sub> respiration rate and the presence of different populations of bacteria, yeasts, and molds. These characteristics lead to an increase of the initial microbial load and a loss of sensory quality within a few days (Barros et al., 2008; Schill et al., 2021; Venturini et al., 2011). Thus, after harvesting, the fresh mushrooms should be delivered to consumer markets as soon as possible. Furthermore, spoiled mushrooms have to be excluded from human consumption (Codex Alimentarius Standard (CXS) 38, 1981).

Wild mushrooms also have the potential to harbor foodborne pathogens derived from the natural environment and wildlife (Plaza-Rodríguez et al., 2021; Venturini et al., 2011). Additionally, formation of biogenic amines in mushrooms is a well-known issue (Dadáková et al., 2009; Kalač, 2014), since biogenic amines may also pose a risk to human health (Biohaz, 2011). Biogenic amines differ from polyamines by being formed primarily through bacterial decarboxylation of free amino acids. Hence  $\beta$ -phenylethylamine, cadaverine, histamine, tryptamine and tyramine are classified as biogenic amines, while spermidine, and spermine are biologically active polyamines and putrescine can be classified in both groups, as it is a precursor for spermidine and spermine (Dadáková et al., 2009; Kalač, 2014).

In the present study, the potential public health risk of two wild-grown mushroom species (chanterelle and porcini mushroom) available at Austrian local markets was evaluated focusing on (i) the microbiological and (ii) sensory quality, (iii) analyzing eight biogenic amines and polyamines as well as (iv) proximate chemical composition for an overall evaluation of the suitability for human consumption. Additionally, a possible relationship between microbial load, sensory quality and the content of biogenic amines was examined.

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## 2. Materials & methods

### 2.1. Mushroom sampling

Wild mushrooms of the species *Cantharellus cibarius* (chanterelle,  $n = 41$ ) and *Boletus edulis* (porcini mushroom,  $n = 40$ ) originating from Austria, Romania ( $n = 6$ ), Serbia, and Slovenia (one each) were analyzed in the period from August to September 2020. Fresh loose mushroom samples (500 g each) were randomly purchased from eight local markets (A, B, C, D, E, F, G, I,  $n = 77$ ) in and around Vienna and one Austrian internet retailer (H,  $n = 4$ ) and immediately transported to the laboratory at the University of Veterinary Medicine Vienna under refrigerated conditions (7 °C). On the same day, the microbiological load, sensory status and the chemical composition was determined. Samples for biogenic amines were stored at 2 °C (one day) or frozen at -20 °C (two to ten days) until analysis.

### 2.2. Microbiological enumeration and detection

Mushrooms (25 g of stem and cap) were randomly selected from the batch and aseptically placed in a Stomacher bag (Interscience, Saint Nom la Brétèche, France). Each sample was decimal diluted in 225 mL buffered peptone water (BPW) (Biokar, Groupe Solabia, Pantin Cedex, France), and homogenized for 2 min in a lab blender (Interscience). Subsequent serial dilutions were made up to dilution  $10^{-8}$  in sterile Ringer's solution (B. Braun, Melsungen, Germany) and plated (100 µL each) on different agar media for the enumeration of aerobic mesophilic counts (AMC) on Trypto-Casein Soy Agar with 0.6% yeast extract (TSAYE, ISO 4833-2, 2013); (Biokar), *Enterobacteriaceae* (EB) on Violet Red Bile Glucose (VRBG) Agar (ISO 21528-2, 2017); (BioMérieux, Marcy-l'Etoile, Frankreich), presumptive *Bacillus cereus* group (BCG) on Mannitol Egg Yolk Polymyxin (MYP) Agar (ISO 7932, 2004); (BioMérieux), and yeasts and molds (YM) on Yeast Glucose Chloramphenicol (YGC) Agar (incubation 25 °C, 72–96 h); (BioMérieux). The detection limit of the AMC, EB and YM was 2.0 log cfu/g, of BCG 1.0 log cfu/g (1 mL of dilution  $10^{-1}$  was divided on three MYP plates). Each colony morphology (AMC), typical colonies for EB and YM and BCG were counted ( $\geq 10$  and  $\leq 300$  colonies for each dilution) and included in the calculation of the results (expressed in cfu/g). Colonies suspicious for BCG were also confirmed by Bacillus ChromoSelect (BCS) Agar with Polymyxin B (Sigma-Aldrich, St. Louis, USA) and 10.0% egg yolk suspension (Oxoid Ltd., Hampshire, UK). The typical blue colonies with an opaque halo were used for the final calculation of the BCG count. Detection of *Salmonella* spp. was performed according to ISO 6579-1, 2017 (25 g sample in 225 mL BPW (Biokar), homogenization for 2 min). Further each 100 µL and 1 mL of the BPW enrichment was inoculated to Rappaport-Vassiliadis Soya (RVS; BioMérieux), and Muller-Kauffmann Tetrathionate-Novobiocin (MKTTn; BioMérieux) broth. *Salmonella* spp. were isolated on Xylose Lysine Desoxycholate (XLD) Agar (BioMérieux).

The *Listeria* spp. and *Listeria monocytogenes* detection was performed according to ISO 11290-1, 2017 (25 g in 225 ml half Fraser broth (HFB; Biokar), homogenized for 2 min). Subsequently, 100 µL HFB was transferred to 10 ml Fraser broth (FB; (BioMérieux). One loop (10 µL) each of the HFB and FB was streaked on selective *Listeria* Agar acc. to Ottaviani & Agosti (ALOA; BioMérieux).

The typical colonies for EB and foodborne pathogenic bacteria (*Salmonella* spp., *L. monocytogenes*, BCG) were sub-cultivated on TSAYE for further differentiation. The initial differentiation for EB included potassium hydroxide (KOH; 3%; Merck KgaA, Darmstadt, Germany), catalase (3%; Merck KgaA) and cytochrome-oxidase testing ((BioMérieux). Isolates suspicious for *Listeria* and *Salmonella* spp. were confirmed according to the specific ISO standards.

Microbial quality of fresh mushrooms was evaluated as satisfactory according to values for the AMC load  $\leq 6.5$  log cfu/g; for EB and yeasts  $\leq 4.0$  log cfu/g, for molds  $\leq 3.0$  log cfu/g, and for BCG  $\leq 2.0$  log cfu/g (according to our internal laboratory standard for fresh products).

### 2.3. Sensory evaluation

The sensory quality was analyzed on the day of purchase by a descriptive method according to DIN EN ISO 13299 (2016) 09. Fresh wild mushrooms of good quality should be clean, firm, undamaged, and largely free from maggot damage (maximum 6.0%), mineral and organic impurities (Codex Alimentarius Austriacus, 2014; Codex Alimentarius Standard (CXS) 38, 1981). The typical aroma (odor) of chanterelles is described as like “cooked carrots” with a “forest note” and that of porcini mushrooms like “mashed potatoes” and “typical mushroom” (Aisala et al., 2018).

The sensory characteristics were evaluated by five panelists trained according to DIN EN ISO 8586 (2014) and standards of descriptive analyses. The evaluation was performed on the basis of appearance (including maggot damage), status of hymenium, consistency (texture), and aroma (supplements Table 1).

Each parameter was rated separately using a five-point scale, where “5” represented a very good quality acc. to the standard and “1” the lowest (strong deviations in relation to the standard). The final sensory quality score (QS) was calculated by dividing the total score by the sum of all tested parameters and the mushroom sample was evaluated according a grading system: 5.0 to 4.0 “very good or good”; 3.9 to 3.1 “satisfactory”; 3.0 to 2.1 “still acceptable” and below 2.1 “not acceptable” and inadequate for human consumption. Mushrooms damaged more than 6.0% by maggots were also not acceptable for consumption (QS < 2.0).

### 2.4. Proximate chemical composition

For chemical analysis, each of the 81 mushroom samples were minced separately (400 g of stem and cap) by a mill (GM 200, Retsch GmbH, Haan, Germany), and were used for analysis of proximate chemical composition (water content, ash, crude protein, and crude fat), chitin, as well as biogenic amine content.

Moisture content was analyzed according to Beluhan and Ranogajec (2011) with some modifications. Ten g mushroom sample was mixed with sea sand (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) and dried for 4 h at 103 °C, which was sufficient to reach a constant weight. The dried sample was subsequently used for crude fat analysis, which done by the gravimetric method by Weibull-Stoldt where the Soxhlet extraction (Soxtherm extractors Gerhardt, Königswinter, Germany) was done with ether as a solvent (Matissek et al., 2010).

The ash analysis was performed according to the method of Beluhan and Ranogajec (2011) with some modifications. A 10 g sample of mushrooms was dry ashed in a muffle furnace (CWF1100, Carbolite Gero GmbH & Co. KG, Neuhausen, Germany) at 600 °C for five to six hours and the resultant ash was weighed.

Crude protein was calculated as total nitrogen determined by the Kjeldahl method (AOAC, 1995) (Speed Digester K-425, Scrubber B-414, and KjelFlex K-360 all from Büchi, Flawil, Switzerland) minus chitin nitrogen and then multiplied by the conversion factor 6.25. The factor 6.25 is the factor traditionally used when determining the crude protein content of a food via the Kjeldahl analysis. However, due to the chitin content of mushrooms, this leads to an overestimation of the crude protein content, and a factor of 4.38 is recommended instead (Kalač, 2013) when chitin is not determined.

Carbohydrate content of the mushrooms was estimated on the basis of the content of crude protein, lipid and ash, i.e. total carbohydrates = 100 - (g of moisture + g of protein + g of fat + g of ash).

Energy content was calculated per 100 g fresh matter (FM) as 17 kJ/g protein, 37 kJ/g fat, and 17 kJ/g carbohydrate (including fiber) according to the Atwater general factor system as described by FAO (2003).

### 2.5. Chitin determination

Chitin content was determined according to our in-house-method. Thirty g minced mushroom sample was weighed into a beaker and dried

**Table 1**  
Average microbial counts and samples above the microbial limit values of wild-grown mushroom species from Austrian local markets at the day of purchase. Mean microbial counts and limit values are given in log cfu/g.

Species	n	Average microbial counts				Number (percentage) of samples above the microbial limits					
		AMC	EB	Yeasts	Molds	BC	AMC > 6.5 <sup>d</sup>	EB > 4.0	Yeasts > 4.0	Molds > 3.0	BC > 2.0
Chanterelle ( <i>Cantharellus cibarius</i> )	41	9.0 ± 0.6 <sup>a, c</sup> (7.6 – 10.5) <sup>b</sup>	6.0 ± 1.6 <sup>c</sup> (1.0 – 8.6)	5.7 ± 1.5 <sup>c</sup> (1.0 – 8.2)	2.4 ± 1.3 (1.0 – 6.0)	0.6 ± 0.6 (0.3–2.6)	41 (100.0%) <sup>d</sup>	38 (92.7%)	34 (82.9%)	10 (24.4%)	2 (4.9%)
Porcini mushroom ( <i>Boletus edulis</i> )	40	5.2 ± 1.5 <sup>c</sup> (2.0 – 8.4)	4.1 ± 1.7 <sup>c</sup> (1.0 – 7.5)	4.2 ± 1.1 <sup>c</sup> (1.0 – 6.2)	2.4 ± 0.7 (1.0 – 4.5)	0.9 ± 0.8 (0.3–2.9)	7 (17.5%)	19 (47.5%)	21 (52.5%)	7 (17.5%)	3 (7.5%)

AMC – aerobic mesophilic counts; EB – *Enterobacteriaceae*; BC – presumptive *Bacillus cereus*; Counts of 1.0 and 0.3 cfu/g signifies value below the detection limit and used for the calculation of the mean.

<sup>a</sup> Mean ± standard Deviations;

<sup>b</sup> Minimum – Maximum;

<sup>c</sup> Mean values differ significantly ( $p < 0.05$ ) between the mushroom species;

<sup>d</sup> Microbial limits.

in a drying oven (Memmert GmbH & Co.KG, Schwabach, Germany) over night at 103 °C. The next day, 80 mL NaOH (8%) was added to the dried sample. The sample was shaken in a water bath (1083, GFL, Burgwedel, Germany) at 70 °C for three hours followed by a brief homogenization by a magnetic stirrer (RSM-02HP, Phoenix Instruments, Garbsen, Germany). The sample was then put back into the water bath overnight for digestion. The following day, the sample was filtered through a folded filter (MN 615 1/4, 150 mm, Macherey-Nagel, Düren, Germany) and washed with approximately 400 mL distilled water. The filtration residue was pH neutralized with 0.5 M HCl and dried overnight at room temperature. Neutralization step is important in order to prevent heavy expansion. This dried sample was used for nitrogen analysis by the Kjeldahl method (AOAC, 1995). The chitin content was calculated by multiplying the determined nitrogen content with a factor 14.51, which is equivalent to the molecular weight of chitin divided by the molecular weight of nitrogen.

## 2.6. Determination of biogenic amines and polyamines

Determination of eight biogenic amines and polyamines ( $\beta$ -phenylethylamine, cadaverine, histamine, putrescine, tryptamine, tyramine, spermidine, and spermine) was done by derivatization with dansyl chloride, separation by high performance liquid chromatography (HPLC) with a reversed phase (RP) C18 column and detection by a photodiode array (PDA) detector at 254 nm, according to the method by Paulsen et al. (1997) with a few modifications.

For the extraction procedure, 10 g of minced mushrooms combined with 90 mL trichloric acid (10%) and homogenized by Ultra Turrax (B52, IKA, Staufen, Germany) for 1 min. Then, the sample was filtrated through a folded filter (MN 615 1/4, 150 mm, Macherey-Nagel) and a 0.2  $\mu$ m membrane filter (Chromafil Xtra PET-20/25, Macherey-Nagel). The extraction procedure was reminiscent of that validated by Reis et al. (2020), which employed on a variety of edible mushrooms.

Subsequently, derivatization was done by pipetting 400  $\mu$ L of the filtrate, 1 mL saturated sodium hydrogen carbonate, and 1 mL dansyl chloride solution (5 mg dansyl chloride in 1 mL acetone) into a 50 mL pointed flask and heating the mixture at 70 °C for 10 min. After cooling, the samples were evaporated under vacuum at 40 °C (Rotavapor, R-100, Büchi) and dissolved in 2 mL HPLC grade acetonitrile (Chem Lab, Zedelgem, Belgium) and centrifuged at 16,000 rpm (approx. 20,000 g) (3K30, Sigma-Aldrich) for 45 min. The supernatant was used for HPLC analysis. For the calibration curve, 10 mg of the eight amines ( $\beta$ -phenylethylamine, cadaverine, histamine, putrescine, spermidine, spermine, tyramine and tryptamine, all from Sigma-Aldrich) were each dissolved in 25 mL HCl (0.1 N). The amine standards were derivatized in the same way as the samples and measured with the HPLC using different injection volumes. The calibration curve consisted of the peak area (y-axis) and the calculated concentration of the amine standard (x-axis) in mg/kg. The concentrations of the respective biogenic amines in the samples were then determined by comparing peak areas. The derivatized amines were determined by HPLC (Alliance 2695, Waters, Milford, USA) with an RP C18 column (RP 18–3  $\mu$ m 125 × 3 mm, ACE, UK), and detected by a PDA detector at 254 nm (PDA 996, Waters).

Chromatographic separation was carried out using a gradient elution of (A) acetic acid (0.02 N) / acetonitrile (9:1), (B) acetic acid (0.02 N) / acetonitrile / methanol (1:4,5:4,5) as follows: 0–2 min, A 50%, B 50%; 2–4 min, A 50–40%, B 50–60%, 4–6 min, A 40–30%, B 60–70%; 6–10 min, A 30–20%, B 70–80%; 10–12 min, A 20–10%, B 80–90%; 12–14 min, A 10–0%, B 90–100%; 14–18 min, A 0–10%, B 100–90%; 18–20 min, A 10–20%, B 90–80%; 20–22 min, A 20–30%, B 80–70%; 22–23 min, A 30–40%, B 70–60%; 23–24 min, A 40–50%, B 60–50%. The flow rate was kept constant at 0.5 mL/min.

## 2.7. Statistics

Descriptive statistics (mean, standard deviation, minimum- and maximum values) of microbial counts, sensory quality scores, chemical composition, and biogenic amines were performed using Graph Pad Prism version 8.1.1 for Windows (GraphPad Software, San Diego, California USA, [www.graphpad.com](http://www.graphpad.com)). Gaussian distribution of data was assessed by the Kolmogorov-Smirnov test. Comparisons of two categories (sensory quality level according to microbial load and content of biogenic amines) were calculated via the Mann Whitney *U* test (not normally distributed) or unpaired *t*-test (normally distributed). A *p*-value of *p* < 0.05 was considered statistically significant.

## 3. Results and discussion

### 3.1. Microbiological quality and detection of foodborne pathogens

The results of average microbial counts of chanterelle and porcini mushrooms are presented in Table 1. The average counts of AMC, EB, and yeasts of chanterelle (9.6, 6.0, and 5.7 log cfu/g) were significantly higher than those in porcini mushrooms (5.2, 4.1, and 4.2 log cfu/g) (*p* < 0.05). These findings are in accordance with data from other studies, which also observed significantly higher counts for aerobic mesophilic load, EB, and yeasts in chanterelle than in porcini mushrooms (Gaglio et al., 2019; Venturini et al., 2011). The levels of AMC and EB at markets found in those two studies were, however, lower than those observed in our study and have been reported as follows: AMC 7.4 and 8.2 log cfu/g and EB 1.0 and 5.3 log cfu/g for chanterelle and AMC 4.4 and 4.6 log cfu/g and EB 1.3 and 2.7 cfu/g for porcini mushrooms. The counts of yeasts in porcini mushrooms in the studies by Venturini et al. (2011) and Gaglio et al. (2019) were comparable to that of our investigations (4.5 and 4.7 log cfu/g), while higher loads (7.4 log cfu/g) were found in chanterelle. On the other hand, counts of molds (2.4 log cfu/g) were lower than those reported by Venturini et al. (2011) and Gaglio et al. (2019) (3.0 and 4.2 log cfu/g for porcini mushrooms; 3.7 and 4.0 log cfu/g for chanterelle).

Limits on microbial counts of freshly traded mushrooms are not regulated in the European Union legislation. For this reason, the results of AMC, EB, yeasts, molds and presumptive *Bacillus* (*B.*) *cereus* were evaluated according to our internal laboratory standard for fresh products. In our study, in all chanterelle samples (100%) and only in 17.5% of porcini mushrooms, the values of AMC exceeded the limit of 6.5 log cfu/g (Table 1). Counts about the limit value of more than 4.0 log cfu/g for EB and yeasts were detected in 97.2% and 82.5% of chanterelle samples, while only 47.5% and 52.5% of porcini mushrooms had higher counts for EB and yeasts, respectively. Microbial limits (> 3.0 log cfu/g) for molds were exceeded in 24.4% of chanterelle and 17.5% of porcini mushrooms. In total 33.3% of the samples (chanterelle 19.5%, *n* = 8; porcini 47.5%, *n* = 19) contained presumptive *B. cereus*. BC counts above the limit value (> 2.0 log cfu/g) were found in only 4.9% of chanterelle and 7.5% of porcini mushrooms. BC counts of more than 3.0 log cfu/g, which is defined as a critical limit for BC could not be observed in any sample of wild-grown mushrooms tested in our study. In mushrooms collected in Chinese markets, presumptive BC were isolated from 28.3% of the samples with contamination levels ranging from 3 to 1100 cfu/g (C. Liu et al., 2020).

*Salmonella* spp., *L. monocytogenes*, and *Listeria* spp. were not detected in any mushroom samples (*n* = 81). Venturini et al. (2011) also could not isolate *Salmonella* spp. in wild mushrooms from Spanish markets. However, 11.5% of the wild mushrooms were positive for *L. monocytogenes*, including four porcini samples.

### 3.2. Sensory quality

Average sensory quality scores were 3.4 in chanterelle and 3.2 in porcini mushrooms. Only 34.6% (*n* = 28/81) of mushroom samples were

evaluated as “very good or good” (QS: 5.0 to 4.0) and 17.3% (*n* = 14/81) as “satisfactory” (QS: 3.9 to 3.1) (Fig. 1). In total, 22.2% of both the mushroom species (*n* = 18/81) had deficiencies in sensory quality and were unfit for consumption (“not acceptable”, QS < 2.0), most frequent in porcini mushrooms (27.5%, *n* = 11/40). The majority of these samples were harvested in Austria (83.3%, *n* = 15/18), while three were from Romania. The analysis shows further, that 66.6% of the deficient samples (QS < 2.0) were sold at markets A and B. The most frequent changes observed in these mushrooms were deficits in appearance (cap and stem discoloration, gill macerated or dried), consistency (soft), and aroma (old, musty, putrid). Six porcini samples were also infested by high levels of maggots (damage more than 6%).

### 3.3. Chemical composition

The chemical composition of the two wild-grown mushroom species chanterelle and porcini mushrooms are given in Table 2. Not surprisingly, there is quite a difference in composition depending on species, with the chanterelle containing much less protein than porcini mushrooms. That chanterelle has a low protein content also stated by Danell and Eaker (1992) in their study, which was especially important for highlighting the analytical difficulties of determining the protein content of mushrooms due to inference by other *N*-containing compounds, particularly chitin.

A number of studies have investigated the chemical composition of various wild-growing mushroom species. Relatively recent reports for chanterelle and porcini mushrooms are shown in Table 3. A crude protein content of 16.7 g/100 g dry matter (DM) (Table 2) lies in the lower range of the literature values (12–69 g/100 g DM), highlighting the natural differences occurring, even within species, and also the potential overestimation of protein content if the Kjeldahl method is used with a conversion factor of 6.25, i.e. with no regard for chitin content. Nevertheless, the study, which found the highest protein content, actually used the recommended conversion factor of 4.38. Further factors influencing the determined nutritional composition are development stage of the fruiting body as well as sample location (Kalač, 2013).

Carbohydrate content (including chitin) of the wild mushrooms was estimated on the basis of the content of crude protein, lipid and ash, i.e. total carbohydrate by difference as was also the case for the values found in literature (Table 3) except for Liu et al. (2016), who used the phenol-sulfuric acid method. In the present study, the highest carbohydrate content was found in chanterelle (70.7 g/100 g DM) compared to 53.0 g/100 g for porcini mushrooms. As evident from Table 3, there is a large variety in the literature values for carbohydrate content.

Dietary fiber is defined as non-digestible carbohydrate polymers of benefit to human health (Cheung, 2013). Chitin, a straight-chain (1→4)- $\beta$ -linked polymer of *N*-acetyl-glucosamine, is the main dietary fiber in mushrooms along with  $\beta$ -glucans, both of which are water-insoluble dietary fibers found in the mushroom cell wall. Consumption of 100 g of fresh mushrooms can easily provide up to 25% of the recommended daily intake of dietary fiber (Cheung, 2013).

Nonetheless, many studies determine the chemical composition of mushrooms without examining the chitin content. Vetter (2007) investigated the chitin content of three species of cultivated mushrooms and found the content to be highly species specific, ranging from 3.3 to 7.3% of DM. The author further concluded that the chitin content of a mushroom species is heavily influenced by nutrition. The species specificity was confirmed by Nitschke et al. (2011), who detected chitin in the range from 0.4 to 9.8% of DM in six species of cultivated mushrooms. Table 2 shows that the two investigated species of wild mushrooms all have chitin contents at or above the upper range of what was found in the studies by Vetter (2007) and Nitschke et al. (2011). This may well be explained by species specificity and the influence of nutrition as both of those studies studied cultivated mushrooms, i.e. none of the same species as the wild-growing ones investigated in the present study.

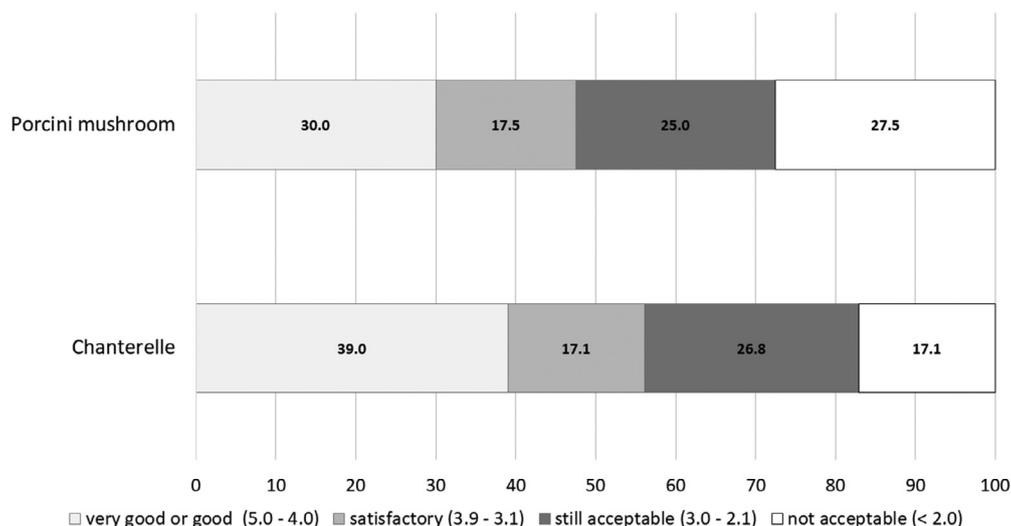


Fig. 1. Categorization of sensory quality of wild mushroom species. Percentage (%) of tested samples among several ranges of score of sensory quality (QS) analyzed on the day of purchase.

Table 2

Proximate composition (g/100 g dry matter) of two species of wild-grown mushrooms found in Austrian markets reported as means  $\pm$  standard deviation with ranges shown in parentheses.

Species	n	Chitin <sup>a</sup>	Crude protein <sup>b</sup>	Fat	Ash
Chanterelle ( <i>Cantharellus cibarius</i> )	41	10.1 $\pm$ 0.8 (7.7–11.9)	16.7 $\pm$ 1.8 (13.8–21.4)	0.5 $\pm$ 0.6 (0.0–3.0)	12.0 $\pm$ 1.4 (8.6–15.3)
Porcini mushroom ( <i>Boletus edulis</i> )	40	8.4 $\pm$ 1.7 (5.9–12.4)	39.7 $\pm$ 4.1 (31.9–50.6)	0.7 $\pm$ 0.6 (0.0–2.9)	6.6 $\pm$ 1.0 (4.4–8.6)

<sup>a</sup> Calculated as chitin nitrogen x 14.51.

<sup>b</sup> Calculated as protein nitrogen x 6.25.

Table 3

Results from literature for proximate composition (g/100 g dry matter) of wild-grown chanterelle and porcini mushrooms reported as means  $\pm$  standard deviation (if available).

Species	n	Crude protein	Fat	Total carbohydrate	Ash	Refs.
Chanterelle <i>Cantharellus cibarius</i>	3	69.14 $\pm$ 3.26	4.49 $\pm$ 0.06	14.25 $\pm$ 3.96	12.12 $\pm$ 0.26	Barros et al. (2008)
	3	53.7	2.9	32.0	11.5	Barroset al. (2008)
	3	30.91 $\pm$ 0.28	1.9 $\pm$ 0.61	52.5 $\pm$ 0.24	8.8 $\pm$ 0.05	Beluhan & Ranogajec (2011)
	3	21.03 $\pm$ 0.04	2.17 $\pm$ 0.04	65.06 $\pm$ 0.11	9.57 $\pm$ 0.08	Fogarasi et al. (2018)
	3	12.37 $\pm$ 0.01	2.82 $\pm$ 0.01	72.24 $\pm$ 0.03	12.57 $\pm$ 0.02	Jedidi et al. (2016)
Porcini mushroom <i>Boletus edulis</i>	3	17.18 $\pm$ 0.92	4.60 $\pm$ 0.13	71.15 $\pm$ 1.55	7.07 $\pm$ 0.59	Barros et al. (2008)
	3	36.91 $\pm$ 0.02	2.92 $\pm$ 0.41	64.27 $\pm$ 0.21	5.3 $\pm$ 0.87	Beluhan & Ranogajec (2011)
	3	36.24 $\pm$ 0.12	1.92 $\pm$ 0.09	46.23 $\pm$ 0.22	8.38 $\pm$ 0.07	Fogarasi et al. (2018)
	3	10.65 $\pm$ 0.47	2.23 $\pm$ 0.02	81.86 $\pm$ 0.41	5.26 $\pm$ 0.44	Heleno et al. (2015)
	3	23.91 $\pm$ 0.04	3.85 $\pm$ 0.01	57.54 $\pm$ 0.02	14.70 $\pm$ 0.01	Jedidi et al. (2016)
	3	26.84 $\pm$ 1.14	4.11 $\pm$ 0.09	58.66 $\pm$ 6.59	8.13 $\pm$ 0.79	Liu et al. (2016)
		30.59 $\pm$ 0.63	2.17 $\pm$ 0.13	58.23 $\pm$ 5.54	6.93 $\pm$ 0.53	
		29.94 $\pm$ 0.43	6.93 $\pm$ 0.21	50.82 $\pm$ 5.93	9.73 $\pm$ 1.01	
		30.17 $\pm$ 0.66	1.96 $\pm$ 0.07	59.13 $\pm$ 3.51	7.19 $\pm$ 0.38	

Regarding fat content, the 0.5 and 0.6/100 g DM found for chanterelle and porcini mushrooms, respectively (Table 2), were all below the values reported in literature, which were at or above 2 g/100 g DM (Table 3). However, Barros et al. (2008) found a fat content of 0.4 g/100 g DM for the species *Lycoperdon perlatum*, also known as the common puffball, so some mushrooms do have that low of a fat content.

Ash content was found to be 12.0 and 6.6 g/100 g DM for chanterelle and porcini mushrooms, respectively (Table 2), which were within the ranges reported in literature (Table 3).

Energy content was calculated as 140 kJ/100 g FM for chanterelle and 199 kJ/100 g FM for porcini mushrooms, which is well in line with previously published results (Barros et al., 2008; Beluhan & Ranogajec, 2011), and which confirms the nutritional value of mushrooms as having low energy contents and high contents of fiber.

### 3.4. Biogenic amines and polyamines

Mushrooms are known to contain biogenic amines (Dadáková et al., 2009; Kalač, 2014), which may pose a risk to human health (Biohaz, 2011). In the present study, seven of the eight investigated biogenic amines and polyamines were detected in one or more of the two investigated wild-grown mushroom species (Table 4) with  $\beta$ -phenylethylamine and spermine only being detected in porcini mushrooms and tryptamine being detected in neither of the two species.

A few studies have previously determined biogenic amine contents in fresh, wild-growing mushrooms (Dadáková et al., 2009; Jabłońska-Ryś et al., 2020; Kalač & Křížek, 1997), though most studies seem to focus on cultivated mushrooms as shown by Dadáková et al., (2009). The biogenic amine content in freshly harvested chanterelle mushrooms was

**Table 4**

Content of eight biogenic amines (in mg/kg fresh matter (FM) and mg/kg dry matter (DM)) of wild-grown chanterelle ( $n = 41$ ) and porcini ( $n = 40$ ) mushrooms available on Austrian local markets reported as ranges.

Species	Chanterelle ( <i>Cantharellus cibarius</i> )		Porcini mushroom ( <i>Boletus edulis</i> )	
	mg/kg FM	mg/kg DM	mg/kg FM	mg/kg DM
$\beta$ -Phenylethylamine	ND*	ND*	ND*-1295.4	ND*-10,446.8
Cadaverine	54.4–147.6	584.9–1587.1	ND*-840.7	ND*-6779.8
Histamine	ND*-95.7	ND*-1029.0	ND*-407.0	ND*-3282.3
Putrescine	ND*-71.4	ND*-767.7	32.4–2143.1	261.3–17,283.1
Spermidine	11.2–48.5	120.4–521.5	13.8–194.8	111.3–1571.0
Spermine	ND*	ND*	ND*-53.0	ND*-427.4
Tryptamine	ND*	ND*	ND*	ND*
Tyramine	ND*-12.5	ND*-134.4	ND*-68.1	ND*-549.2

\* ND signifies that the biogenic amine was not detected.

determined by Dadáková et al. (2009), who detected putrescine, spermidine, and spermine at 37.4, 74.5, and 24.3 mg/kg fresh matter (FM), respectively. In turn, Jabłońska-Ryś et al. (2020) reported values of 197.0 for spermidine and 61.3 for putrescine, calculated in mg/kg FM in frozen chanterelle. In the present study, the content of putrescine in positive samples (43.0%,  $n = 18/41$ ) and of spermidine in all samples are comparable with those published by Jabłońska-Ryś et al. (2020) (Table 4). The biogenic amine content of porcini mushrooms was analyzed in two different years, with phenylethylamine, putrescine, spermidine, and spermine in the ranges 43.3–158.0, 894.0–935.0, 110.0–324.0, and 19.6–55.9 mg/kg DM, respectively (Dadáková et al., 2009). In mushrooms of the family *Boletaceae*, putrescine was detected in all mushrooms and cadaverine only in *Boletus* (syn. *Xerocomus*) *badius* (Kalač & Křížek, 1997). The biogenic amine levels in fresh samples were in the ranges 43.2–80.4 mg/kg FM, (565.0–1010.0 mg/kg DM) for putrescine and 4.0–9.4 mg/kg FM (50.4–119.0 mg/kg DM) for cadaverine. After 48 h of storage, the authors reported even higher putrescine levels (5500.0 mg/kg DM) for sliced mushrooms. Histamine and tyramine has not been detected in any sample of fresh chanterelle and mushrooms of the family *Boletaceae* in the previous studies. Jabłońska-Ryś et al. (2020) analyzed cadaverine, histamine, putrescine, spermidine, and tyramine in processed porcini mushrooms (pickled and dried) and mushroom concentrate with the highest concentrations of biogenic amines being found in the dried samples. These samples contained 3863.9–5338.9 mg/kg cadaverine, 4152.3–21,126.9 mg/kg histamine, 3606.2–10,761.1 mg/kg putrescine, 6522.9–22,601.2 mg/kg spermidine and, 0.0–2928.1 mg/kg tyramine. Putrescine and spermidine were present in all fresh porcini samples in our study and the observed levels were in a similar range to those was found in the previous studies. Cadaverine, histamine, and tyramine were detected in 30.0% ( $n = 12$ ), 42.5% ( $n = 17$ ), and 37.5% ( $n = 15/40$ ) of porcini samples, respectively. The detected levels of cadaverine, histamine, and tyramine varies widely, and the very high contents are comparable to the results obtained in dried mushrooms (Jabłońska-Ryś et al., 2020).

According to the European Food Safety Authority (EFSA) (Biohaz, 2011) histamine and tyramine are the most relevant biogenic amines from a food safety perspective. Ingestion of < 50 mg of histamine per person per meal has been found to lead to no adverse effects for healthy individuals with no histamine intolerance. The same is the case for the intake of < 600 mg tyramine/meal for healthy individuals not taking monoamino oxidase inhibitor (MAOI) drugs and < 6–50 mg/meal if taking MAOI drugs, depending on the specific drug (Biohaz, 2011).

For putrescine and cadaverine, the currently available data do not make the determination of a level for acute adverse health effects in humans possible, and studies on  $\beta$ -phenylethylamine and tryptamine are, likewise, limited. A non-observed adverse effect level (NOEAL) of 444.75 mg/kg for putrescine and 255.45 mg/kg for cadaverine have been found to be cytotoxic on intestinal cell cultures (Del Rio et al., 2019), which were higher levels than those detected in fresh wild mush-

rooms in the present study, except of one spoiled sample (unfit for consumption) containing putrescine and cadaverine contents above the published NOEAL.

Even less information seems to be available on the potential adverse health effects of spermidine and spermine (Biohaz, 2011) apart from information on cytotoxic effects in intestinal cell cultures, which were much higher than found normally in mushrooms (Del Rio et al., 2018).

In fact, these polyamines are regularly found in mushrooms and they are important for many aspects of development and maintenance of normal cellular functions (Kalač, 2014). Based on these limits, our data suggest that consumption of mushrooms at a level of 100 g/person in a meal is unlikely to cause any adverse effects of histamine, as the highest level detected was 40.7 mg/100 g fresh mushroom in one porcini sample and the levels were even lower for chanterelles (Table 4). For tyramine, only one porcini mushroom (and none of the chanterelles) was above the 6 mg/meal limit at 6.8 mg/100 g fresh porcini mushroom, which may cause a risk for patients taking classical MAOI drugs, according to EFSA's scientific opinion (Biohaz, 2011).

However, it can be speculated that the effect of the various biogenic amines may be additive or synergistic, resulting in the combined level of biogenic amines present in some mushrooms to cause a potential risk. Nevertheless, the sum of biogenic amines is generally not used when performing exposure assessments (Biohaz, 2011).

### 3.5. Effect of sensory quality on microbial load and biogenic amines

Chanterelles purchased at local markets were always found to have a very high bacterial load ( $AMC > 6.5 \log \text{ cfu/g}$ ), regardless of their sensory quality. Further, the counts of yeasts ( $> 4.0 \log \text{ cfu/g}$ ) and molds ( $> 3.0 \log \text{ cfu/g}$ ) had no effect on the sensory scoring. In chanterelle that were classified as sensory deficient ( $QS < 3.1$ , “still acceptable” or “not acceptable”), the count of EB was above the limit ( $> 4.0 \log \text{ cfu/g}$ ) in all samples and the mean count was significantly higher than in those with a QS of 5.0 to 3.1 ( $p < 0.05$ ) (Fig. 2). Generally, for chanterelles fresh harvested or purchased at retail the reported microbial loads were very high, while results according sensory aspects were not noted (Gaglio et al., 2019; Venturini et al., 2011). In the case of porcini mushrooms, counts of aerobic mesophiles (AMC), EB, and yeasts above the limit were observed more frequently in samples with poor sensory quality ( $QS < 3.1$ ), and the mean counts were significantly higher ( $p < 0.05$ ) (Fig. 2). Similarly, in cultivated King Oyster, Oyster and Shiitake mushrooms, it was observed that higher counts of aerobic mesophiles were more often associated with losses in sensory quality (Schill et al., 2021).

The abundance of biogenic amines found in the two wild mushroom species is summarized in Fig. 2. There was no correlation between sensory quality and microbial load and mean levels of different biogenic amines in the chanterelle mushrooms analyzed in this study. However, porcini mushrooms showing sensory deficits ( $QS < 3.1$ ) and higher microbial loads (AMC, and yeasts above the limits) had higher

	Chanterelle QS		Porcini mushroom QS	
	5.0 - 3.1	< 3.1	5.0 - 3.1	< 3.1
AMC > 6.5 <sup>a</sup>	100.0	100.0	0.0	33.3 <sup>b</sup>
EB > 4.0 <sup>a</sup>	87.0	100.0 <sup>b</sup>	21.1	71.4 <sup>b</sup>
Yeasts > 4.0 <sup>a</sup>	78.3	94.4	21.1	80.9 <sup>b</sup>
Moulds > 3.0 <sup>a</sup>	21.7	22.2	15.8	19.0
β-Phenylethylamine	0.0	0.0	100.0	100.0 <sup>b</sup>
Cadaverine	100.0	100.0	10.5	47.6 <sup>b</sup>
Histamine	65.2	72.2	57.9	57.1
Putrescine	34.8	55.6	100.0	100.0
Spermidine	100.0	100.0	100.0	100.0
Spermine	0.0	0.0	5.3	4.8
Tyramine	21.7	11.1	47.4	76.2

AMC – aerobic mesophilic counts; EB – *Enterobacteriaceae*. Tryptamine were below the limit of detection (0.52 mg/kg).

**Fig. 2.** Heatmap showing the relative abundance of chanterelle and porcini samples which exceed the specified limit of microbial counts and containing seven biogenic amines relative to the sensory quality (QS). <sup>a</sup>Microbial counts are given in log cfu/g. <sup>b</sup>Mean values differ significantly ( $p < 0.05$ ) between the QS. AMC – aerobic mesophilic counts; EB – *Enterobacteriaceae*. Tryptamine were below the limit of detection (0.52 mg/kg).

mean contents of β-phenylethylamine and cadaverine ( $p < 0.05$ ). Higher mean levels of tyramine correlated with the AMC and the count of yeasts, while the β-phenylethylamine, histamine, and tyramine contents were influenced by the count of EB ( $p < 0.05$ ). A study designed by Jaworska et al. (2020) showed an increase of β-phenylethylamine in cultivated *Agaricus biosporus* mushrooms during eight days of storage at 7 and 19 °C according to the AMC and the count of EB. The authors reported that there was also a positive correlation between histamine and putrescine contents and the count of EB.

In sliced *Boletus* (syn. *Xerocomus*) species with signs of spoilage, the content of putrescine and cadaverine increased significantly during storage (Kalač & Křížek, 1997). The biogenic amines are formed primarily by bacteria containing decarboxylase activity (Dadáková et al., 2009). In many food products, bacteria of the *Enterobacteriaceae* family and different gram positive bacteria have been described as producers of the different biogenic amines (Biohaz, 2011). Contents of corresponding amino acids (arginine, histidine, lysine, methionine, phenylalanine, and tryptophan) were detected in chanterelle and porcini mushrooms as well (Beluhan & Ranogajec, 2011).

#### 4. Conclusion

Harvesting wild edible mushrooms to sell at small local markets has been a common practice in Austria. In the study presented here, the majority of chanterelle and porcini mushrooms were collected in Austria, with only 9.1% of the mushrooms purchased originating from Romania, Serbia, and Slovenia. The nutritional values of the two mushroom species in our study varied widely, but no correlation was found with sensory quality and microbial load. In general, chanterelle mushrooms were always characterized by high levels of aerobic mesophilic counts, regardless of their sensory quality. Furthermore, we can conclude that in both mushroom species, poor sensory quality was associated with increased counts of EB. Only in porcini samples was the level of β-phenylethylamine, cadaverine, histamine, and tyramine influenced by the count and type of microorganisms. The analyzed content of biogenic amines observed in our study indicated no risk for healthy consumers relating to the usual consumption quantity of mushroom per person. Almost half of the samples purchased at local markets were evaluated as

sensorially deficient (QS < 3.1). This leads to the assumption that these mushrooms have been handled under poor hygiene facility management or/and offered for sale for too long. The shelf life of fresh mushrooms is influenced by many factors, among them at harvest level: age of the fruiting body, climatic conditions, handling by the collectors (e.g. removal of mineral and organic impurities, storage conditions) and during storage at the local market: type of packaging, duration, and temperature. To obtain wild mushrooms of excellent quality, all relevant stakeholders at the harvest and at the retail stage should be trained in hygienic handling of mushrooms.

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All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in Food Chemistry Advances.

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#### CRediT authorship contribution statement

**Kathrine H. Bak:** Formal analysis, Visualization, Methodology, Writing – original draft. **Susanne Bauer:** Investigation, Methodology,

Validation. **Julia Rattner**: Investigation. **Martin Wagner**: Writing – review & editing. **Martina Ludewig**: Conceptualization, Formal analysis, Funding acquisition, Writing – original draft.

## Data Availability

Data available on request from the corresponding author.

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## Supplementary materials

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