



Microbiological Characteristics of Wild Edible Mushrooms and Effect of Temperature during Storage of *Morchella conica*

R. Gaglio¹, A. Saitta¹, M. Cruciata¹, A. La Rosa², P. Barbaccia¹, G. Moschetti¹,
L. Settanni^{1*}✉

1. Dipartimento Scienze Agrarie, Alimentari e Forestali, Università di Palermo, Viale delle Scienze 4, 90128 Palermo, Italy

2. Cooperativa Silene, Via D'Ondes Reggio 8/A, Palermo, 90100, Italy

HIGHLIGHTS

- This work highlights consistent differences in terms of microbiological properties among different mushrooms species.
- Microbial counts of wild mushrooms were generally higher than those registered for marketed samples.
- Total mesophilic populations developed at high levels very quickly at temperatures higher than 15 °C.

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Acronyms and abbreviations

TMC=Total Mesophilic Count
TPC=Total Psychrotrophic Count
LAB=Lactic Acid Bacteria
PCA=Plate Count Agar
CPS=Coagulase Positive Staphylococci
CFU=Colony Forming Unit

ABSTRACT

Background: The continuous worldwide increase of consumption of fresh mushrooms has registered in the recent years. The major goal of this study was to determine the microbiological characteristics of wild edible mushrooms and effect of temperature during storage of *Morchella conica*.

Methods: Wild mushrooms of the species *Boletus edulis*, *Cantharellus cibarius*, and *Leccinum aurantiacum* were collected in a mixed forest of *Picea abies*, *Betula pendula*, and *Pinus sylvestris* located in Tartu territory, Estonia. Faecal indicators, potential pathogens, spoilage bacteria, and microfungi (yeasts and moulds) were evaluated. *M. conica* was microbiologically investigated for 24 days under different thermal regimes, including 4, 8, 12, 15, 20, and 28 °C. The statistical analysis was conducted with SAS 9.2 software.

Results: The microbial counts of wild mushrooms, ranging from 6.81 to 7.68 log₁₀ CFU/g for total mesophilic count, were generally higher ($p<0.05$) than those registered for marketed samples ranging from 4.60 to 7.39 log₁₀ CFU/g. The dynamics of total mesophilic microorganisms on *M. conica* stored at different temperatures indicated that stationary and death phases occurred earlier with increasing temperature and that the highest levels were registered at 28 °C at the 2nd day of storage.

Conclusion: This work highlights consistent differences in terms of microbiological properties among different mushrooms species. The results clearly showed that total mesophilic populations developed at high levels quickly at temperatures more than 15 °C, but also the refrigeration did not stop the microbiological decay of mushrooms.

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Introduction

The continuous worldwide increase of consumption of fresh mushrooms registered in the recent years (Venturini et al., 2011) is mainly imputable to their supply in pro-

teins, vitamins, especially those of group B (Barros et al., 2007; Bernas et al., 2006) that are commonly provided by meat products (Khan et al., 2011), and minerals (Hong et

* Corresponding author. ✉ luca.settanni@unipa.it
ORCID ID: <https://orcid.org/0000-0001-7019-3598>

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al., 2005). Mushrooms represent an important source of precursors of vitamin D (Mattila et al., 1994). Edible mushrooms also provide bioactive compounds (Valverde et al., 2015) and might exert several positive effects on human health such as hypocholesterolemic, antioxidant, antimicrobial, and anticancer activity (Lindequist et al., 2005; Teoh et al., 2018). Different antimicrobial compounds have been isolated from mushrooms (Nelson et al., 2016; Rathee et al., 2012). Consumption of edible mushrooms showed also positive effects in the prevention of diseases of the gastrointestinal tract due to their generation of short chain fatty acids that influence the intestinal microbial populations, in particular, increasing the abundance of Bifidobacteriales and decreasing that of Fusobacteriales (Zhao et al., 2018). The low content of fat and the high fiber content of mushrooms are particularly appreciated by consumers. Hence, fresh mushrooms are strategic components of a healthy diet (Guillamón et al., 2010).

Fresh mushrooms, like in case of vegetable matrices, are naturally contaminated by eukaryotic (moulds and yeasts) and prokaryotic (bacteria) organisms (Alfonzo et al., 2017; Corsetti et al., 2007). Like in case of vegetable matrices, the environmental contamination of mushrooms might depend on several factors, mainly animal, faeces, soil, and air (Buck et al., 2003). Mushrooms are characterized by a high moisture content, high water activity (≥ 0.98), and neutral pH; these conditions favor microbial growth after harvesting and during storage of mushroom limiting strongly their shelf life (Venturini et al., 2011).

In the present work, wild mushrooms collected in woods or purchased from markets in Estonia were characterized for the main microbiological parameters. Furthermore, the mushroom *Morchella conica* collected in Sicily, that has not been object of microbiological characterization in past, was used to evaluate the effect of different thermal regimes applied during storage on the total mesophilic bacterial community.

Materials and methods

Sample collection

Wild mushrooms of the species *Boletus edulis*, *Cantharellus cibarius*, and *Leccinum aurantiacum* were collected in a mixed forest of *Picea abies*, *Betula pendula*, and *Pinus sylvestris* located in Tartu territory, Estonia during July 2017. Mushrooms of the same species were purchased in a popular retail market of Tartu, Estonia and were all collected by local mushroom hunters. Ten sporomata of each species were included in the study both for the “wild” and “market” trials. All mushrooms were transported under refrigerated conditions in a

portable fridge containing reusable ice packs to the Laboratory of Agricultural Microbiology, SAAF Department-University of Palermo for analyses.

Microbiological analyses

Mushrooms were microbiologically investigated for 10 microbial groups, including Total Mesophilic Count (TMC), Total Psychrotrophic Count (TPC), members of Enterobacteriaceae family, total and faecal coliforms, enterococci, pseudomonads, Coagulase Positive Staphylococci (CPS), Lactic Acid Bacteria (LAB), yeasts and moulds. Ten g of each mushroom were collected (in triplicate, using three sporomata randomly chosen for each species and trial) and subjected to the decimal serial dilution in Ringer’s solution (Sigma-Aldrich, Milan, Italy). Cell suspensions were plated and incubated under different conditions specific for each microbial group: TMC were spread plated on Plate Count Agar (PCA), incubated aerobically at 30 °C for 72 h; Enterobacteriaceae pour plated on double-layer Violet Red Bile Glucose Agar (VRBGA), incubated aerobically at 37 °C for 24 h; TPC was measured on PCA, incubated aerobically at 7 °C for 7 days; total and faecal coliforms on double-layer Violet Red Bile Agar (VRBA), incubated aerobically at 37 °C and 44 °C, respectively, for 24 h; enterococci on Kanamycin Aesculin Azide (KAA) agar, incubated aerobically at 37 °C for 24 h; pseudomonads on *Pseudomonas* Agar Base (PAB) supplemented with 10 mg ml⁻¹ cetrimide fucidin, incubated aerobically at 20 °C for 48 h; CPS on Baird Parker (BP) agar with rabbit plasma fibrinogen supplement, incubated aerobically at 37 °C for 48 h; mesophilic rod LAB on de Man-Rogosa-Sharpe (MRS) agar, incubated anaerobically at 30 °C for 48 h; mesophilic coccus LAB on M17 agar, incubated anaerobically at 30 °C for 48 h; yeasts on Dichloran Rose Bengal Chloramphenicol (DRBC) agar, incubated aerobically at 25 °C for 48 h; moulds on Malt Agar (MA), incubated aerobically at 25 °C for 7 days. All media and supplements were purchased from Oxoid (Milan, Italy).

Effect of temperature and time on the microbiological characteristics of mushrooms

M. conica was collected in April 2017 in the Ficuzza Forest, Palermo, Sicily, Italy in a mixed forest of *Quercus pubescens* s.l. and *Fraxinus angustifolia*. Identification of mushrooms was carried out using fresh ascomata. This species were used as model mushroom to evaluate the effect of storage time and temperature on the microbiological characteristics of wild mushrooms. *M. conica* samples were stored under different temperatures (4, 8, 12, 15, 20, and 28 °C) for 24 days. For this purpose, *M. conica* harvested in high amounts was divided into 103 samples (in duplicate for a total of 206

samples) of approximately 15 g and packed into sterile BagLight^R 400 Multilayer^R bags (Interscience, Saint Nom, France) sealed with a foil welding machine (Laica VT3112, Vicenza, Italy) in order to avoid further contaminations. One aliquot was analyzed soon after packaging and, for each of the six thermal regimes, 17 aliquots were subjected to storage.

Microbiological investigations were performed on *M. conica* from each bag stored at a given temperature at the following sampling days: 0 (at the moment of packaging), 1, 2, 3, 4, 7, 8, 9, 10, 11, 14, 15, 16, 17, 21, 22, 23, and 24. All these samples were analyzed for the levels of TMC. Preparation of samples and TMC determination performed as reported above.

Statistical analyses

Microbiological data were subjected to one-way Analysis of Variance (ANOVA). Pair comparison of treatment means was achieved by Duncan's procedure at $p < 0.05$. Differences between wild and market mushrooms were evaluated with the Generalized Linear Model (GLM) procedure. The statistical analysis was conducted with SAS 9.2 software (Statistical Analysis System Institute Inc., Cary, NC, USA).

Results

The results of the microbiological investigation of *B.*

edulis, *C. cibarius*, and *L. aurantiacum* are reported in Table 1. Faecal coliforms, enterococci, and CPS were not detected in any sample. All other microbial groups were counted at statistically different levels. The levels of each microbial group were generally significantly different ($p < 0.05$) between wild and market mushrooms with the exception of TPC and pseudomonads for *C. cibarius* and filamentous fungi for *L. aurantiacum*. The microbial counts of wild mushrooms, ranging from 6.81 to 7.68 log₁₀ CFU/g for TMC, were higher than those registered for marketed samples ranging from 4.60 to 7.39 log₁₀ CFU/g. In particular, the highest viable counts were detected on wild *C. cibarius* whose levels of TMC and yeasts were 8.18 log₁₀ CFU/g. Furthermore, for all samples, the levels of TMC were superimposable to those of yeasts, showing that the microbial community of the mushrooms analyzed was dominated by yeasts. Except rod LAB on *C. cibarius*, for all microbial groups object of the survey, the levels detected on wild mushrooms were higher than those found on the purchased ones belonging to the same species as indicated by $p < 0.001$ resulting from the interaction among the groups of wild and marketed mushrooms for several microbial populations (TMC, members of Enterobacteriaceae family, yeasts, LAB rods, and cocci). The better microbiological quality of the purchased mushrooms in the present investigation was also highlighted by the total absence of total coliforms and the very low levels of members of the Enterobacteriaceae family.

Table 1: Levels (log₁₀ CFU/g) of microorganisms on wild and market mushrooms

Mushrooms	Media											
	PCA 28 °C	PCA 7 °C	MA	VRBGA	VRBA 37 °C	VRBA 44 °C	PAB	DRBC	CAA	M17	MRS	BP
Wild:												
<i>B. edulis</i>	6.81±0.33 ^b	6.85±0.40 ^b	4.60±0.26 ^b	5.16±0.18 ^b	2.59±0.21 ^b	<1 ^a	6.40±0.60 ^b	6.48±0.41 ^b	<2 ^a	5.40±0.24 ^b	4.40±0.54 ^b	<2 ^a
<i>C. cibarius</i>	8.18±0.48 ^b	7.66±0.18 ^a	4.48±0.19 ^b	1.70±0.09 ^b	<1 ^a	<1 ^a	7.51±0.24 ^a	8.18±0.29 ^b	<2 ^a	3.98±0.25 ^a	<1 ^a	<2 ^a
<i>L. aurantiacum</i>	7.68±0.30 ^b	7.86±0.29 ^b	3.78±0.36 ^a	4.63±0.42 ^b	2.78±0.45 ^b	<1 ^a	7.48±0.12 ^b	7.26±0.33 ^b	<2 ^a	7.60±0.55 ^b	3.74±0.44 ^b	<2 ^a
Market:												
<i>B. edulis</i>	4.60±0.24 ^a	5.48±0.46 ^a	4.18±0.31 ^a	1.30±0.15 ^a	<1 ^a	<1 ^a	3.70±0.27 ^a	4.70±0.45 ^a	<2 ^a	4.65±0.14 ^a	<1 ^a	<2 ^a
<i>C. cibarius</i>	7.39±0.31 ^a	7.63±0.35 ^a	3.70±0.15 ^a	1.00±0 ^a	<1 ^a	<1 ^a	7.35±0.14 ^a	7.46±0.51 ^a	<2 ^a	6.48±0.34 ^b	2.54±0.20 ^b	<2 ^a
<i>L. aurantiacum</i>	5.72±0.16 ^a	5.64±0.18 ^a	4.02±0.30 ^a	<1 ^a	<1 ^a	<1 ^a	4.48±0.32 ^a	4.93±0.44 ^a	<2 ^a	5.70±0.11 ^a	3.26±0.17 ^a	<2 ^a
Statistical significance	***	**	**	***	**	N.S.	**	***	N.S.	***	***	N.S.

Results indicate mean values±S.D of three plate counts (carried out in triplicate). Data within column followed by the same letter between the species of wild and market mushrooms are not significantly different according to Duncan's test. p value: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; N.S., not significant. Abbreviations: *B.*, *Boletus*; *C.*, *Cantharellus*; *L.*, *Leccinum*; PCA 30 °C, plate count agar incubated at 30 °C for total mesophilic counts; PCA 7 °C, plate count agar incubated at 7 °C for total psychrotrophic counts; MA, for moulds; VRBGA, violet red bile glucose agar for members of Enterobacteriaceae family; VRBA 37 °C, violet red bile agar incubated at 37 °C for total coliforms; VRBA 44 °C, violet red bile agar incubated at 44 °C for faecal coliforms; PAB, *Pseudomonas* agar base for pseudomonads; DRBC, dichloran rose bengal chloramphenicol agar for yeasts; CAA, kanamycin aesculin azide agar for enterococci; M17, medium 17 agar for coccus lactic acid bacteria; MRS, de Man-Rogosa-Sharpe agar for rod lactic acid bacteria; BP, Baird Parker for coagulase positive staphylococci

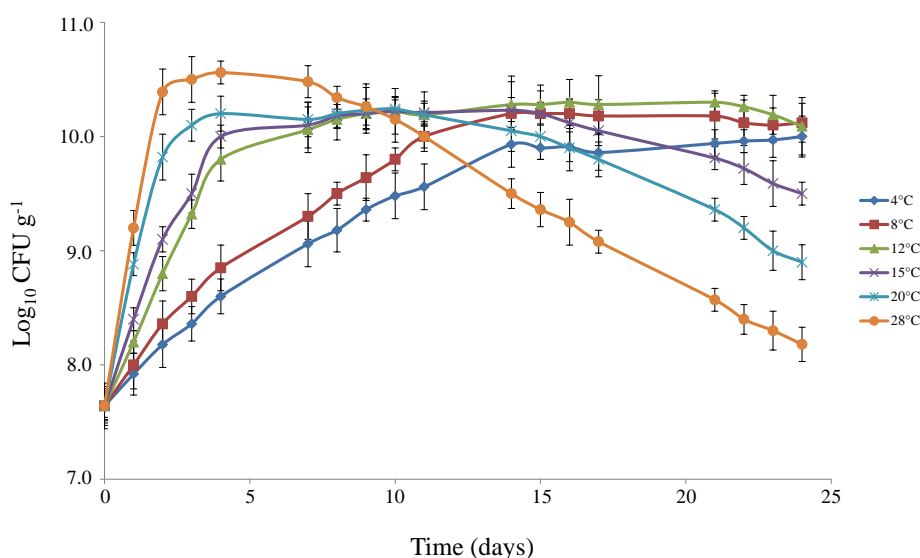


Figure 1: Evolution of TMC on *Morchella conica* stored at different temperatures for 24 days. Results indicate mean values \pm S.D. of three plate counts (carried out in triplicate). Vertical bars represent S.D. of the mean

The effect of the temperature and the storage time was thoroughly evaluated on wild *M. conica*. To this purpose, TMC were followed by plate count, since they were found to represent the dominant microbial group of wild and purchased mushrooms. The dynamics of TMC is reported in Figure 1; the graphic clearly showed the differences induced by the storage temperature. Except for the stationary phase in the tract 7-11 days for the trials carried out at 12, 15, and 20 °C (with cell densities in the range 10.15–10.19 log₁₀ CFU/g) and in the tract 14 days–end of observation for the trials carried out at 8 and 12 °C (with cell densities in the range 10.15–10.19 log₁₀ CFU/g) for which the data were not statistically different ($p > 0.05$), the majority of the differences registered during the experimentation were significant ($p < 0.05$).

Considering the general trend of the effect of temperature on TMC, it was clear that as the temperature increased a more inclined slope of the exponential growth phase, the plateau of living cells was reached earlier and its duration was shorter, while the decline phase occurred faster. As a matter of fact, the death phase began at days 8, 14, 17, and 21 from storage at temperature 28 to 12 °C, respectively. At 4 and 8 °C, the stationary phase started at day 14 and no death phase could be observed. The highest levels of TMC were registered for the trial at 28 °C that reached 10.39 log₁₀ CFU/g at the 2nd day of storage.

Discussion

The first part of this work was forwarded to the microbiological analyses of collected wild and also purchased marketed mushrooms in Tartu, Estonia. The results indicated the presence of TMC and yeasts at dominant levels until 8.18 log₁₀ CFU/g, followed by the presence of psychrotrophic microorganisms, including pseudomonads, moulds, members of Enterobacteriaceae family, and LAB. In a similar work, Venturini et al. (2011) analyzed the microbial properties of fresh cultivated and wild mushrooms sampled from Zaragoza, Spain belonging to several species, including *B. edulis* and *C. cibarius*, and commercialized in Spain. *B. edulis* and *C. cibarius* were only sampled for the wild trial. The levels of mesophilic aerobes, *Pseudomonas* genus, Enterobacteriaceae family, LAB, yeasts, and moulds reported by Venturini et al. (2011) were almost superimposable to those showed by our investigation, even though our levels of yeasts for *C. cibarius* were higher and those of Enterobacteriaceae members were lower both on *C. cibarius* and *B. edulis*. Ezekiel et al. (2013) analyzed various mushrooms, including *C. cibarius*, marketed in Nigeria and reported levels of moulds slightly lower than 3.5 log₁₀ CFU/g which are comparable to those of *C. cibarius* analyzed in this study. Low levels of coliforms (below the detection limit) have been reported also for other species of mushrooms (González-Fandos et

al., 2000). To our knowledge, *L. aurantiacum* has been object of study for the content of trace elements (Gorbunov et al., 2013; Orita et al., 2018), but this is the first report on its microbiological characteristics. The differences registered for the microbiological levels of *B. edulis*, *C. cibarius*, and *L. aurantiacum* ($p < 0.05$) might be imputable to the diverse surface/volume ratios of the mushroom species considered in this study, as well as to their different nutrient availability. Regarding the significant differences found among wild and market mushrooms, the reason for the lower counts registered for all marketed mushrooms could be due to the conditions applied during conservation that better preserve their microbiological quality characteristics.

A few works had been carried out on the evolution of the microorganisms responsible for the microbiological decay of mushrooms. To this purpose, considering that *M. conica* has not been object of microbiological characterization in past, our study focused to deepen the knowledge on the microbiological characteristics of this niche edible mushroom. The choice of this species was also due to the fact that, at the season of monitoring, it was easily available in Sicily. Thus, it represented an optimal model to perform a study on the effect of the temperature on the microbiological characteristics of mushrooms minimizing the influence of the transport that might accelerate microbial decay, even if it occurs under refrigeration (La Scalia et al., 2016, 2017). Our work evidenced a clear effect of the temperature for TMC. With the increasing temperature, they developed until high levels very rapidly. This is due to the fact that mesophilic microorganisms develop at the highest rates between 20 and 30 °C. At lower temperatures, the growth was particularly slow and no death phase was registered for the refrigeration temperatures 4 and 8 °C.

A previous investigation on this topic was previously carried out by Parentelli et al. (2007) who focused on *Lentinula edodes* packaged under several different conditions and stored at 5 °C for 20 days. In that work, the aerobic mesophilic bacteria count decreased soon after the monitoring period and those authors concluded that the shelf-life of *L. edodes* during storage does not depend on mesophilic bacterial growth. In a study conducted on *Agaricus bisporus* stored at 17 and 25 °C, the decrease of aerobic bacteria in non-perforated films was observed after 5 days (González-Fandos et al., 2000). Our study confirmed the trend found by Simón et al. (2005) who designed an investigation about the microbiological quality of *A. bisporus* in fresh sliced form and packaged in modified atmospheres. These researchers stated that aerobic bacteria counts were at very high level (7.5 log₁₀ CFU/g) since the beginning of the monitoring and increased, depending on the atmosphere composi-

tion, until 8–10 log₁₀ CFU/g at day 12 (Simón et al., 2005).

Conclusion

Although several information are available in literature on the microbiological characteristics of fresh mushrooms marketed or collected in woods/forests, this is one of the first reports on *B. edulis*, *C. cibarius*, and *L. aurantiacum* sampled in Estonia. Furthermore, the edible mushroom *M. conica* was microbiologically characterized for the first time. This work highlights consistent differences, in terms of microbiological levels and composition of the microbial groups among mushrooms belonging to different species and sampled from different sources such as directly from forests or purchased in retail markets. Since all mushrooms were, indeed, wild and collected by hunters, their microbiological quality, besides environmental factors, could also depend on the post-harvest contamination due to transport and, especially storage conditions. The results clearly showed that total mesophilic populations developed at high levels very quickly at temperatures higher than 15 °C, but also the refrigeration did not stop the microbiological decay for this high perishable product.

Author contributions

L.S. and A.S. designed the project of study; R.G., M.C., P.B., and A.L.R. conducted the experiments; R.G. and G.M. analyzed the data; L.S. wrote the manuscript. All authors read and approved the revised manuscript.

Conflicts of interest

There was no conflict of interest in this study.

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