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MICROBIOTA QUALITY OF FRESH AND DRIED EDIBLE MUSHROOMS CONSUMED IN THE HO MUNICIPALITY, GHANA

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Abstract

Mushrooms, despite their nutritional and medicinal attributes, are prone to microbial contamination, probably due to poor handling and preservation methods. The microbiological quality of fresh and dried edible mushrooms in the Ho Municipality was examined in this study. Per conventional protocols, bacteria tests were conducted on various media to quantify and identify pathogenic microbial species, including Salmonella, Escherichia coli, total coliforms, Staphylococcus aureus, and heterotrophic bacteria. Fresh mushrooms had a bacterial count ranging from 6.01–6.78 log_{10} CFU/g, whereas dry mushrooms had a count ranging from 5.68–6.64 log_{10} CFU/g. Fungal studies were performed on Dichloran Rose Bengal Chloramphenicol (DRBC) and Oxytetracycline Glucose Yeast Extract (OGYE) media at three different points per location. Nine different fungal species from seven different genera were isolated on both fresh and dried media. These species included Aspergillus (A. niger, A. fumigatus, A. alutaceaus, A. ochraceous), Rhizopus (R. stolonifer), Mucor (M. racemosus), Fusarium (F. oxysporum), Penicillium (P. digitatum), Trichoderma harzianum, and Rhodotorula sp. For fresh mushrooms, the range of fungal counts on both media was 3.56±1.1–4.35±0.92 and 3.32±0.93 log_{10} CFU/g, respectively. In terms of dry, 3.31±0.7–4.3±0.81 and 3.75±1.16–4.229±0.85 log_{10} CFU/g, respectively. The samples from various sites showed statistically significant ($p < 0.05$) differences. The pH readings for both fresh and dried samples fell between 6.62±0.09 and 6.83±0.08, and between 5.95±0.09 and 6.3±0.08. It can be concluded that the majority of mushrooms were acceptable for consumption according to the International Commission for Microbiological Specifications of Foods (ICMSF). This raises some public health concerns, as consuming those mushrooms increases the food safety risk in the Ho municipality.

Keywords
Fresh mushrooms, Dried mushrooms, Bacteria, Fungi, Contaminants, Ghana

Introduction

Mushrooms have gained widespread recognition in recent times, as they are used to prepare a variety of delectable dishes, such as soups, stews, and pizza (Lyle, 2016). They are also used as special food ingredients for prominent people (Halling, 2006) owing to their unique culinary attributes. Mushrooms are naturally endowed with numerous vital nutrients, such as protein, fibre, vitamins, and minerals (Kortei et al., 2017a,b; Mattila et al., 2001). Over time, mushroom consumption has dramatically expanded worldwide (Chang and Miles, 2004; Ezekiel et al., 2013; Mattila et al., 2001; Venturini et al., 2011), probably because they are used as alternative sources of proteins to combat protein malnutrition (Dunkwal et al., 2007; Singh et al., 1995). In developing countries, especially, animal sources of protein are not accessible and so mushrooms have been used as a promising alternative to meat. Mushrooms are also well endowed with innumerable bioactive compounds (Valverde et al., 2015) such as hypocholesterolemic, antioxidant (Kortei et al., 2014b; Obodai et al., 2017), antimicrobial (Nelson et al., 2019; Rathee et al., 2012; Sánchez, 2017), and anticancer activity (Lindequist et al., 2005; Pandya et al., 2019) which may be responsible for their medicinal potentials to human health. Eating edible mushrooms has also been shown to be beneficial in preventing gastrointestinal disorders because they produce short-chain fatty acids that affect the populations of microorganisms in the intestines, specifically increasing the abundance of Bifidobacteriales and decreasing that of Fusobacteriales (Zhao et al., 2018).

Among the common types of mushrooms consumed globally are button, oyster, and shiitake mushrooms (Gebretsadkan, 2015). The *Pleurotus* species, or oyster mushrooms, are, without a doubt, the most widely consumed type of mushrooms in Ghana (Adjapong et al., 2015; Kortei and Wiafe-Kwagyan, 2014; Kortei et al., 2018a; Obodai and Apetorgbor, 2008). This is likely because oyster mushrooms are delicious when used as a recipe in both local and foreign cuisines. Also, this particular species is readily available on supermarket shelves, neighborhood shops, markets, farm gates, and street vendors (Kortei et al., 2018a).

Fresh mushrooms are ideal for microbial proliferation due to their high moisture content, neutral pH, and water activity of 0.98 or higher (Martínez-Carrera et al., 2000). They are physiologically classified as high-respiring products (Kader and Saltveit, 2002), making the wilting from water loss occur rapidly. Additionally, microbial contamination reduces

their post-harvest shelf life to a few days. Harvested by bare hands, fresh mushrooms are transported to a processing facility where they are put into retail containers without any cleaning. However, when compared to other fresh foods, little is known about the microbial makeup of fresh mushrooms. ImathIu (2017) said that despite significant advancements in lowering the prevalence of specific pathogens in foods through improved farming techniques and food regulation, the sanitary quality of mushrooms has become a global source of great concern.

Globally, eukaryotic organisms (molds and yeasts) and prokaryotic (bacteria) organisms have been found in fresh mushrooms, just like some components of vegetables (Deng et al., 2020; Pepper and Gentry, 2015). Contaminated mushrooms can cause vomiting, diarrhea, headaches, chills, dizziness, impaired vision, and eventually death, which are signs of a foodborne infection.

Some predisposing factors of fresh mushroom contamination with microorganisms to cause foodborne infection include high water activity and high moisture content (Venturini et al., 2011).

Some previous studies in Ghana by Kortei et al. (2014a) have reported many foodborne bacteria species, such as *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, and coliform species. Likewise, some fungal species, including yeasts such as *Saccharomyces cerevisiae* and *Rhodotorula* sp., and molds such as *Aspergillus* (*A. niger*, *A. flavus*, *A. fumigatus*, *A. tamarii*), *Rhizopus* (*R. oligosporus*), *Mucor* (*M. racemosus*), *Fusarium* (*F. oxysporum*), *Penicillium* (*Penicillium* sp.), and *Trichoderma* (*T. viride*), have been isolated from dried mushrooms (Kortei et al., 2018b). However, this study examined the microbiological composition of both fresh and dried edible mushrooms in the Ho Municipality to update the microorganisms that contaminate mushrooms consumed in the Volta region of Ghana.

Materials and Methods

Study Site Description

The Volta Region of Ghana has Ho Municipal as its administrative capital. It is one of the twenty-five Municipalities and districts of the region. This Municipality is also the viable nucleus of the region. The municipality consists of seven hundred and seventy-two communities and a land size of 573.2 km^2 (221.3 sq. mi) per records of the Ghana Statistical Service (Ghana Statistical Service, 2014) (Figure 1). Ho spans 11.65 square kilometers and is located between latitudes 6° 20′ N and 6° 55′ N and longitudes 0° 12′ E and 0° 53′ E (Ghana Statistical Service, 2014).

Collection of mushrooms

Thirty-six samples (n=36) in all, consisting of eighteen fresh and eighteen dried mushroom samples were collected. For fresh mushrooms, 3 different sets of replicates were obtained from each of the 6 sellers in the open markets in Ho, Volta Region, Ghana. The same technique was employed for the dried mushrooms. The samples were collected at random over the course of two weeks in November and December of 2020. About 200 g of each sample was taken and stored in sterile specimen containers (Nasco, USA) in accordance with the description given by Kortei et al. (2020). After that, they were transported in an ice chest freezer (Thermos 7750, China). Within two hours after being collected, cold packs were utilized aseptically at 10 °C and brought to the University of Health and Allied Sciences' (UHAS) Microbiology Laboratory for microbiological investigation.

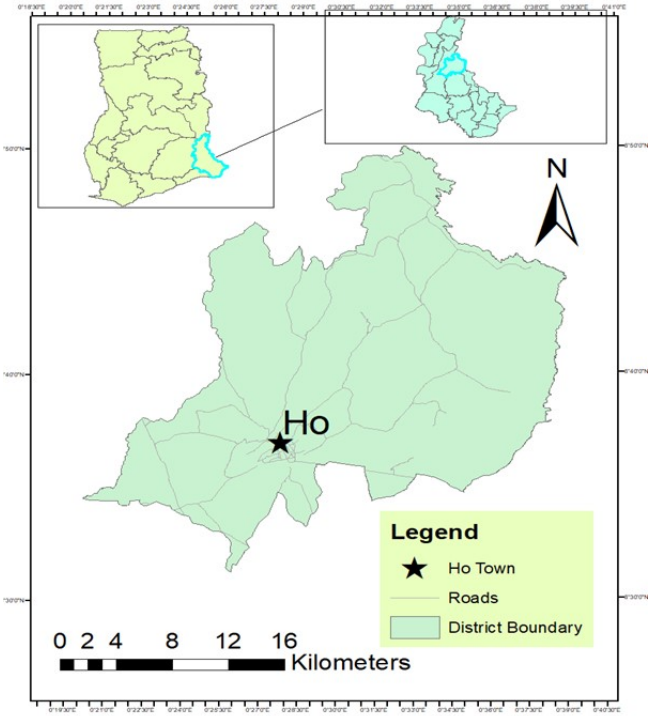

Figure 1. Map of Ghana showing Ho in the Volta Region where sampling of samples was done. Adapted from Kortei et al. (2022)

Mushroom Sample Preparation

One gram (1 g) of the sample was weighed into ten milliliters (10 mL) of distilled water for fungi under aseptic circumstances. Ten milliliters (10 mL) of distilled water were measured into sterile sample containers. The samples of mushrooms were ground into a powder and left in distilled water in a sterile container for two hours. Using an orbital shaker (Gallenkamp, England), one milliliter (1 mL) of the mushroom sample solution was pipetted into sterile sample bottles together with nine milliliters (mL) of distilled water. The bottles were then agitated at 120 rev/min for five to ten minutes. For the mushrooms, three serial dilutions (10^{-1} – 10^{-3}) were made, and aliquots containing one milliliter of each dilution were employed in the analysis.

Forty-five milliliters (45 mL) of distilled water were measured in sterile sample containers for bacteria under aseptic circumstances. Five grams (5 g) of the sample were weighed into the

45 mL of distilled water. Each milliliter (1 mL) of fresh and dried mushroom sample was pipetted into sterile sample vials with 9 mL of distilled water. The samples were then shaken on an orbital shaker (Gallenkamp, England) at 120 rev/min for five to ten minutes. For the mushrooms, six serial dilutions (10^{-1} through 10^{-6}) were made, and aliquots containing one milliliter of each dilution were employed in the analysis.

Bacteria Analysis

Using conventional techniques for the number of heterotrophic bacteria, total coliforms, *Staphylococcus aureus*, *Salmonella*, and *Escherichia coli* species, studies of bacteria were performed to quantify and identify harmful bacteria. The bacteriological analyses were conducted, and the samples were plated on Nutrient agar, MacConkey agar, Mueller-Hinton agar, and Salmonella-Shigella agar. The samples were then incubated at 37 °C for 48 hours using the 4^{th} and 5^{th} dilutions.

For the bacteriological testing, mesophilic (loving moderate temperatures) and microorganism colonies (such as bacteria developed in 48 hours on an agar plate) were cultured at a regulated temperature of 37 °C. The total plate count (TPC) of these organisms was measured. The physical traits of the bacteria's colonies allowed for the identification of the newly emerging species after 48 hours. Colony Forming Units per Gram (CFU/g) were used to compute the duplicate mean counts.

Gram Staining and Confirmatory Tests

The Gram's staining method and other related confirmatory tests were done according to procedures prescribed by Moyes et al. (2009).

Suspected colonies of *E. coli* were subcultured into EC Broth (Oxoid CM853), pH 6.9, followed by Tryptone Water (Oxoid CM87), pH 7.5, for the indole test. All were incubated at 44°C for 24 h, according to NMKL No.125 (2005) (Kortei et al., 2020).

Suspected colonies of *Staphylococcus* sp. were confirmed as coagulase-positive on rabbit coagulase plasma (C14389) according to NMKL Method No. 66 (2009) (Kortei et al., 2020).

The suspected *Bacillus cereus* was confirmed on Blood Agar Base (Oxoid CM0055) for the presence of hemolysis as described in NMKL No. 67, 2010 (Kortei et al., 2020).

Suspected *Salmonella* species were confirmed by a biochemical test on Triple Sugar Iron Agar (Vm381715 214, Merck KGaA Darmstadt, Germany) and a serological test using Salmonella Polyvalent Agglutinating Sera (30858501ZD01, UK) (Kortei et al., 2020).

Suspected colonies of *Listeria monocytogenes* were confirmed for catalase, gram, motility test, and Blood Agar Base to determine the presence of hemolysis.

Fungal Analysis

Fungal Plating

One gram (1 g) of each sample was transferred into 9 ml of sterile distilled water. The samples were soaked overnight. All samples were weighed using an electronic balance (OHAUS®, Germany) with a readability of 0.01 g. Each stock solution was serially diluted in 9 ml of peptone (0.1%) water in ten-fold increments from stock 10^{0} to 10^{-3}. One milliliter (1 ml) of each serial dilution was plated in either Dichloran Rose Bengal Chloramphenicol (DRBC) (Oxoid CM727, Basingstoke, United Kingdom) or Oxytetracycline Glucose Yeast Extract (OGYE) (Oxoid CM727, Basingstoke, United Kingdom). Agar media plates were prepared according to the manufacturer's instructions and incubated at 25°C for 5–7 days as outlined by Odamtten et al. (2018).

Enumeration and Identification of Fungi

The number of molds and yeast that emerged after 4 days was recorded. Using a colony counter (STAR 8500, Funke Gerber, Germany), the enumerations were conducted. The formula according to Kortei et al. (2021) and Odamtten et al. (2018), was used to compute colony-forming units per gram:

$$\text{CFU/g} = \frac{\text{no. of colonies} \times \text{reciprocal of dilution factor}}{\text{volume of culture plate}} \quad (1)$$

The percentage occurrence of fungal species was calculated using the formula:

$$\text{Percentage (\%) occurrence of fungal species} = \frac{\text{number of fungal species}}{\text{total number of fungi isolated}} \times 100 \quad (2)$$

Identification

Using standard identification guidelines (Moss, 1989; Samson et al., 1995), molds and yeast that appeared were recognized based on their morphological and cultural characteristics. Their microscopic characteristics were also observed with the microscope (Leica DM 750, Wetzlar, Germany) with a magnification × 400.

Determination of pH

The pH of the samples was determined by soaking the pulverized mushroom samples in distilled water for at least 3 h and measuring directly with a bench pH meter (Jenway 3510, United Kingdom) after calibration using standard buffer parameters 4.0 and 7.0 pH.

Data Analysis

Standard deviations and means, two types of descriptive statistics, were employed. A one-way analysis of variance (ANOVA) was performed on the data. Duncan's Multiple Range Test was used to separate the means, and significances were accepted at a 5% level ($p<0.05$). Version 22 of the Statistical Package for the Social Sciences (SPSS) program was utilized for the investigation. Logarithmic values were generated from the standard forms for the numbers of bacteria and fungi.

Table 1. pH of fresh and dried mushrooms

	S1	S2	S3	S4	S5	S6
Fresh mushroom	6.71±0.08	6.72±0.08	6.83±0.08	6.62±0.09	6.69±0.08	6.70±0.08
Dried mushroom	5.95±0.09	6.00±0.08	6.10±0.07	6.30±0.08	6.20±0.07	6.00±0.10

Note: n=3, values are Mean±SD

Table 2. Mean fungal counts (Log_{10} CFU/g) of fresh mushroom samples isolated on two different growth media

Media	S1	S2	S3	S4	S5	S6
DRBC	4.20±1.01	4.35±0.92	3.56±1.10	3.79±0.90	3.93±0.74	4.11±1.19
OGYE	3.93±0.99	3.32±0.93	4.22±0.99	3.89±0.72	4.04±0.85	4.07±0.82

Note: DRBC = Dichloran Rose Bengal Chloramphenicol; OGYE = Oxytetracycline Glucose Yeast Extract. Values are Mean±SD

Table 3. Mean fungal counts (Log_{10} CFU/g) of dried mushroom samples isolated on two different growth media

Media	S1	S2	S3	S4	S5	S6
DRBC	3.53±0.80	3.31±0.70	4.30±0.81	4.03±0.73	4.13±0.65	4.11±0.93
OGYE	3.80±1.21	3.81±1.18	4.18±0.79	3.75±1.16	3.87±1.19	4.29±0.85

Note: DRBC = Dichloran Rose Bengal Chloramphenicol; OGYE = Oxytetracycline Glucose Yeast Extract. Values are Mean±SD

Results

pH Determination for both fresh and dried mushrooms

The pH of both fresh and dried mushroom samples was measured and calculated. The average and standard deviation pH values for fresh and dry mushroom samples were also recorded. The pH value ranges for fresh and dried mushroom samples were 6.62–6.83 and 5.95–6.3, respectively (Table 1). The average pH value for fresh and dry mushroom samples was 6.71±0.07 and 6.09±0.14, respectively (Table 1). The standard deviation value for fresh and dried mushroom samples recorded was 0.14, respectively. There was no statistical difference ($p<0.05$) among the samples.

Fungal Counts

The results of the fungal counts and profile of the mushroom sample are presented in Tables 2 and 3. The mean fungal count and standard deviation of fresh mushrooms recorded on Dichloran Rose Bengal Chloramphenicol (DRBC) and Oxytetracycline Glucose Yeast Extract (OGYE) for samples 1, 2, 3, 4, 5, and 6 were 4.2 and 3.93, 4.35 and 3.32, 3.56 and 4.22, 3.79 and 3.89, 3.93 and 4.04, 4.11 and 4.07, respectively. And, the mean fungal count for DRBC and OGYE of dried mushrooms recorded for samples 1, 2, 3, 4, 5, and 6 were 3.53 and 3.8, 3.31 and 3.81, 4.3 and 4.18, 4.03 and 3.75, 4.13 and 3.87, 4.11 and 4.29, respectively (Table 3). For DRBC, the mean fungal count for fresh mushrooms of the first samples was lower, but in the subsequent sample was marginally higher, while for OGYE, the first sample was higher and marginally higher in the subsequent samples as well. Again, for the first media, DRBC, the mean fungal count for dried mushrooms of the first samples was marginally higher in the subsequent samples while for OGYE the first sample was lower but marginally higher in the subsequent samples.

Generally, comparing the mean fungal counts of DRBC and OGYE for fresh and dried samples, there was no statistically significant difference ($p<0.05$) between fresh and dried mushroom samples.

According to the International Commission for Microbiology Specifications for Food (International Commission of Microbiology Specifications of Foods (ICMSF), 1996), counts of $<10^2$ are satisfactory, 10^2 – $<10^4$ are marginal/borderline, and $>10^4$ could be injurious to the consumer.

Percentage (%) Occurrence of Fungal Species

A total of nine (9) fungal species belonging to seven (7) genera were identified for fresh mushrooms; *Aspergillus fumigatus*, *Aspergillus niger*, *Fusarium oxysporum*, *Rhodotorula* species, *Penicillium digitatum*, *Rhizopus stolonifer*, *Mucor racemosus*, *Aspergillus ochraceous*, *Trichoderma harzianum*, and yeasts belonging to the genera *Aspergillus*, *Fusarium*, *Penicillium*, *Rhodotorula*, *Mucor*, *Rhizopus* and *Trichoderma*.

For the dried samples, a similar set of fungal species were isolated and included *Fusarium verticillioides*. Additionally, the most frequently occurring species was *Rhizopus stolonifer*, while the least occurring was *Trichoderma harzianum*.

Some potentially toxigenic fungi species, *A. niger*, *A. flavus*, *A. fumigatus*, *P. digitatum* and *Rhodotorula* were isolated in the various samples (Tables 4 and 5).

Tables 4 and 5 show the fungal species that were isolated from the fresh and dried mushroom samples.

Table 4. Percentage occurrence (%) of fungi on fresh mushrooms

S1	S2	S3	S4	S5	S6
A. fumigatus (20%) *A. niger* (25%) *F. oxysporum* (30%) *Rhodotorula* sp. (7%) *P. digitatum* (18%)	*R. stolonifer* (90%) *A. niger* (10%)	*R. stolonifer* (30%) *M. racemosus* (30%) *A. niger* (25%) *A. ochraceous* (15%)	*F. oxysporum* (25%) *T. harzianum* (10%) Yeast spp. (20%) *A. niger* (45%)	*R. stolonifer* (100%)	*R. stolonifer* (40%) *M. racemosus* (20%) *A. niger* (40%)

Note: Values in parentheses represent percentage occurrence of each fungal species

Table 5. Percentage occurrence (%) of fungi on dried mushrooms

S1	S2	S3	S4	S5	S6
R. stolonifer (45%) *A. niger* (35%) *M. racemosus* (20%)	*A. fumigatus* (30%) *A. niger* (25%) *F. oxysporum* (10%) *F. verticillioides* (25%) *Rhodotorula* sp. (10%)	*T. harzianum* (10%) *F. verticillioides* (35%) *R. stolonifer* (20%) *A. niger* (15%) *M. racemosus* (20%)	*A. niger* (15%) *A. fumigatus* (20%) *A. terreus* (8%) *F. verticillioides* (12%) *R. stolonifer* (45%)	*A. niger* (10%) *F. verticillioides* (15%) *M. racemosus* (20%) *R. stolonifer* (55%)	*T. harzianum* (20%) *A. niger* (45%) *F. verticillioides* (35%)

Note: Values in parentheses represent percentage occurrence of each fungal species

Table 6. Mean $\log_{10}$ CFU/g of fresh mushroom samples isolated on different growth media

Fresh samples	NA	SSA	MCA	MHA
S1	6.43	6.64	6.51	6.77
S2	6.78	6.37	6.63	6.01
S3	6.10	6.28	6.08	6.02
S4	6.77	6.61	6.72	6.59
S5	6.49	6.01	6.57	6.06
S6	6.75	6.10	6.02	6.18

Note: NA = Nutrient Agar; SSA = Salmonella-Shigella Agar; MCA = MacConkey Agar; MHA = Mueller-Hinton Agar

Table 7. Mean $\log_{10}$ CFU/g of dried mushroom samples isolated on different growth media

Dried samples	NA	SSA	MCA	MHA
S1	6.33	6.07	5.96	6.64
S2	6.46	6.10	6.40	6.70
S3	6.46	6.16	6.33	6.29
S4	5.97	6.04	5.97	6.62
S5	6.24	6.10	6.04	6.27
S6	5.94	5.80	5.78	5.68

Note: NA = Nutrient Agar; SSA = Salmonella-Shigella Agar; MCA = MacConkey Agar; MHA = Mueller-Hinton Agar

Bacterial Counts

The result obtained from the bacteriological analysis is presented in Tables 6 and 7. The mean $\log_{10}$ CFU/g for the 4^{th} and 5^{th} dilutions isolated on different media of 6 fresh mushrooms and 6 dried mushrooms are presented. For the fresh mushroom samples from samples 1 to 6, ranges of 6.1–6.78, 6.01–6.64, 6.02–6.72, and 6.01–6.77 mean $\log_{10}$ CFU/g were recorded for NA, SSA, MCA, and MHA, respectively. For the dried mushroom samples from sample 1 to sample 6, a range of 5.94–6.46, 5.8–6.16, 5.78–6.4, and 5.68–6.7 mean $\log_{10}$ CFU/g were recorded for NA, SSA, MCA, and MHA, respectively.

Discussion

Eating edible mushrooms can improve metabolism and alter the composition of the microbiota since they are a natural source of beneficial substances (Mattila et al., 2001). Finding novel compounds with antibacterial and anti-inflammatory properties led to the use of mushroom species in microbiota regulation. Due to the increased use of antibiotics and subsequent growth of antibiotic resistance, the present biopharmaceutical industry needs these solutions (Lopez-Santamarina et al., 2020; Shimizu et al., 2020).

A variety of bioactive components found in mushrooms, such as polysaccharides, proteins, or secondary metabolites, including polyphenols, alkaloids, steroids, and terpenes, contribute to the health benefits of intake (Tung et al., 2020). The two types of polysaccharides found in fungi, homopolysaccharides and heteropolysaccharides, may affect the microbiota based on their structure and, indirectly, on their degree of solubility. Fungal biological activity is mostly attributed to soluble chemicals, of which only a tiny part is D-glucans (e.g., lentinan from *Lentinus edodes*, schizophyllan from *Schizophyllum commune*, or ganoderan from *Ganoderma lucidum*). Fungal polysaccharides are mostly composed of insoluble fibers, such as cellulose and lignin (Tu et al., 2021; Vamanu et al., 2021).

pH

Worthy of note, a critical condition that facilitates the growth of micro-organisms in fresh and dried mushrooms is pH content (Adams et al., 2000; Bamforth and Cook, 2019). The pH values obtained for fresh mushrooms in this study agreed with the study conducted by Jiang et al. (2018), who also reported the pH of ready-to-cook mushrooms ranging from 6.73 to 7.12. Again, the pH value obtained for dried mushrooms in this study agrees with the results of Yang et al. (2019), who also reported that the pH of shiitake mushrooms processed by hot air drying ranged from 5.95 to 6.36. These pH

ranges are most likely to support the growth of *Salmonella* species, *Escherichia coli*, *Listeria monocytogenes*, and many fungal species in foods. *Salmonella* species will grow at a wide pH range from 4.05–9.5 (Fatica and Schneider, 2011) and the mushroom samples had pH values within this range (4.05–9.5) which supported the growth of *Salmonella* sp. *Escherichia coli* was found in the mushroom samples in this study because *Escherichia coli* survives in a wide pH range of 4.4–10 (Gullian-Klanian and Sánchez-Solis, 2018) and the mushroom samples had a pH range within 4.4–10 that supported *Escherichia coli*. According to Lado and Yousef (2007), *Listeria monocytogenes* can thrive in a wide pH range of 4.0–9.6. The pH ranges of the mushroom samples were within this range (4–9), which allowed *Listeria monocytogenes* to grow. Nevarez et al. (2009) found that the mushroom samples had pH values between 5 and 9, which acted as a factor that favored the growth of fungal species. Some fungal species are known to thrive over a broad pH range of 5 to 9.

Fungal Counts

Fungal contamination of foodstuffs is a common occurrence but a serious issue in sub-Saharan Africa (Ezekiel et al., 2013). Several studies that have been conducted over these years have revealed that there has been contamination of molds especially *Aspergillus*, *Fusarium*, and *Penicillium* species, and their toxic secondary metabolites in foodstuffs, which are harmful to humans (Bankole and Adebanjo, 2003; Makun et al., 2013). Results obtained in this work suggest a greater number was in line with the range of values obtained by other researchers from other parts of the world. Suhaili et al. (2021) reported a yeast and mold count of 4.73–7.37 $\log_{10}$ CFU/g in grey oyster mushrooms (*Pleurotus sajor-caju*) in Malaysia. In a related study conducted by Kim et al. (2016), yeast and mold counts of 6.0 $\pm$ 0.3 log CFU/g were recorded in *Lentinula edodes*. Venturini et al. (2011) in Spain reported high levels of yeasts (5.8 $\pm$ 0.6 and 3.9 $\pm$ 0.7 log CFU/g) and molds (4.7 $\pm$ 1.3 and 3.9 $\pm$ 0.8 log CFU/g) in fresh wood ear mushrooms (*Auricularia auricula-judae*) and shiitake (*Lentinula edodes*), which is quite similar to the result of the yeast and mold obtained in this study. From Italy, Gaglio et al. (2019) recorded a range of 3.70$\pm$0.15–8.18$\pm$0.29 $\log_{10}$ CFU/g in wild edible mushrooms and found that TMC levels were lower in all samples than yeast levels, indicating that yeasts predominated in the microbial community of the studied mushrooms. Kortei et al. (2018b) also reported fungal counts of a range 2.4–4.6 $\log_{10}$ CFU/g in dried oyster mushrooms (*Pleurotus ostreatus*) stored in different packaging materials in Ghana.

In disagreement with the results obtained in this study, Schill et al. (2021) reported comparatively low fungal counts of 2.2–2.9 $\log_{10}$ CFU/g in *Pleurotus eryngii*, *Pleurotus ostreatus* and *Lentinula edodes* in Austria. Furthermore, Ergönül et al. (2018) also reported a yeast and mold count of 1.9$\pm$0.2–3.3$\pm$0.6 $\log_{10}$ CFU/g on eight (8) different species of wild edible mushrooms in Turkey.

Some of the mean yeast and mold counts of samples in both fresh and dried mushrooms were within the acceptable limit of yeast and mold count based on European Union Commission Recommendation (directive 2004/24/EC), which is <5 $\times$ 10^3 (<3.7 $\log_{10}$) CFU/g, while the others were above the acceptable limit. The count of yeast and molds that were above the acceptable limits points to poor hygienic practices at the production and handling levels, and this calls for stricter enforcement of GAP and GHP.

Percentage Occurrence (%) of Fungi in Fresh Mushrooms

The fungal species that were isolated from both fresh and dried mushrooms include *Aspergillus fumigatus*, *Aspergillus niger*, *Fusarium oxysporum*, *Rhodotorula* sp., *Penicillium digitatum*, *Rhizopus stolonifer*, *Mucor racemosus*, *Trichoderma harzianum*, Yeast species, *Fusarium verticillioides*, *Aspergillus terreus*, *Aspergillus ochraceous*, and these results are in agreement with the results obtained by Kortei et al. (2018b), who also isolated *A. niger*, *A. flavus*, *A. fumigatus*, *M. racemosus*, *Rhodotorula* species and *R. oligosporus* in their study. *Rhizopus stolonifer* was the most dominant species in both fresh and dried mushrooms in this study. Fungal species and yeast are mainly responsible for the contamination and spoilage of foods in Ghana, and these species can cause serious health problems when they grow on foods and are consumed by humans (Kortei et al., 2018b, 2017b).

Ezekiel et al. (2013) identified several molds in dried mushroom samples in Nigeria and they included: *Aspergillus* (*A. flavus*, *A. niger*-clade, *A. parvisclerotigenus*, *A. tamarii* and other *Aspergilli*); *Fusarium* spp.; *Penicillium* spp.; *Mucor* spp. and other *Mucorales*; *Trichoderma* spp.

Choi et al. (2010) noted various pathogens such as *Aspergillus* spp., *Mucor* spp., *Penicillium* spp., and *Trichoderma* spp. damage mushrooms by inhibiting growth. *Penicillium* competes for preoccupancy with green spores and inhibits the formation of fruiting bodies, resulting in the spores spreading out in the middle and top portions of the mushrooms. It is interesting to note that the green mold, or *Penicillium digitatum*, that was found in this study is likely to be a strict wound pathogen that may infect fresh fruit in the field, packing house, distribution, and marketing. It is known to damage most fresh food, including mushrooms.

Generally, food products that are not well handled and stored are of primary concern to humans, as these food products can contain harmful microorganisms when consumed and cause a variety of illnesses in humans. A considerable number of fungal spores thrive in the atmosphere, and these fungal spores, when dry, float in the atmosphere and find suitable conditions where they can survive and start their growth cycle again (Money, 2016).

Fungi may produce potent mycotoxins, which can cause severe diseases; among them are cancers, and it can also damage vital organs of humans, such as the liver, kidney, and brain (Patial et al., 2018; Zain, 2011). Different kinds of fungi found in foods, including *Fusarium*, *Penicillium*, and *Aspergillus*, are

of potential threat to humans because of their toxicogenic potential or ability to contaminate foods and cause serious illness to humans with various symptoms, including blurred vision, dizziness, chills, headaches, diarrhea, and vomiting (Kortei et al., 2018b). A previous study conducted by Ezekiel et al. (2013) also isolated *Fusarium*, *Penicillium*, *Trichoderma*, and aflatoxigenic *Aspergillus* from dried mushrooms. The findings of this study corroborate with that of Ezekiel et al. (2013), as they investigated the fungal and mycotoxins of dried edible mushrooms in Nigeria. Sun drying is one of the best methods to preserve mushrooms, as it prolongs their shelf life. The low fungal count in the dried mushroom may be due to the impact of sun drying, which reduces the water activity and moisture content of the mushroom, thereby hampering biological and microbial activities (Kumar et al., 2013).

Toxicogenic species

Although almost all fungal species are capable of producing mycotoxins, many are natural venomous compounds (Hathout et al., 2020; Hathout and Aly, 2014; Reddy et al., 2010). When present in high concentrations in food, they can lead to a variety of health issues, including mortality, in both people and animals. It is interesting to know that no level of mycotoxin above zero is thought to be safe when it comes to species that produce deadly mycotoxins. The JECFA supports "Reduction to As Low As Reasonably Achievable" as the safe level in foods once certain toxins have a significant chance of being genotoxic, carcinogenic, etc. (Hathout et al., 2020).

Bacteria

The range of bacterial counts recorded in this study was in the same range of values recorded in a previous study by Venturini et al. (2011), who recorded values of 5.30 $\pm$ 0.6 log CFU/g for the microbial count of mesophilic microorganisms in the same species of oyster mushroom (*P. ostreatus*). Recent studies by Suhaili et al. (2021) reported ranges of 3.87–7.45 and 3.37–5.74 log CFU/g, respectively, for Total Plate Count and *E. coli* in a related mushroom species, grey oyster mushrooms (*Pleurotus sajor-caju*).

Salmonella spp., *E. coli*, and coliforms were discovered in some of the fresh oyster mushrooms contaminated in Dhaka, Bangladesh, according to a study (Kamal et al., 2010). The highest level recorded on a normal plate count was 8.9 log CFU/g. According to Kim et al. (2016), shiitake mushrooms grown in Virginia, USA, were likewise found to have *Listeria* spp. and had an aerobic mesophilic count of 7.5 $\pm$ 1.1 log CFU/g and a coliform count of 1.9 $\pm$ 1.1 log MPN/g. Because of cross-contamination from its growth substrate and during postharvest processing, fresh grey oyster mushrooms may include rotting and harmful microbes.

A comparable pattern was documented when *P. ostreatus* was stored in different polymeric packaging; from the first day of storage to the last, the coliform count rose from 5.53 to 8.52 log CFU/g (Sapata et al., 2009). There is currently no restriction on the presence of *E. coli* on raw fresh fruits and vegetables, even though the bacteria do not survive for very long on plant surfaces and is solely linked to recent water pollution.

The average counts of mesophilic aerobic microorganisms in *B. edulis* and *A. auricula-judae* were found to range from 4.4 to 9.4 log CFU/g. With counts ranging from 4–6 log CFU/g, *L. edodes*, *P. ostreatus*, *Pleurotus eryngii*, and *B. edulis* were the four species with the lowest microbial total load. Eleven out of the species had levels ranging from 6 to 8 log CFU/g. *Agrocybe cylindracea*, *Amanita cecilae*, *Cantharellus cibarius*, *Lactarius deliciosus*, *Suillus collinitus*, and *Tricholoma myomyces* were the sources of the AMB counts of 8.4 log CFU/g, 7.4 log CFU/g, 7.6 log CFU/g, 8.2 log CFU/g, 8.8 log CFU/g, and 6.9 log CFU/g, according to Ergönül et al. (2018). *Boletus reticulatus* had a statistically significantly higher TMAB count ($P<0.05$) than the other mushroom samples.

According to Schill et al. (2021)'s recent investigation on the impact of storage on the microbiological quality of *Pleurotus eryngii*, *Pleurotus ostreatus*, and *Lentinula edodes* in Austria, AMCs at the retail level recently ranged from log 1.7 to 7.8 on the day of purchase and from 1.7 to 9.4 log CFU/g. The following AMCs were recorded at the retail level by Venturini et al. (2011), Kim et al. (2016), and Reyes et al. (2004): 7.7 to 8.4 log CFU/g for champignon, 5.0 to 5.3 log CFU/g for oyster, 4.9 to 6.9 log CFU/g for shiitake, and 5.9 log CFU/g for king oyster.

Although little evidence exists about the occurrence of *Salmonella* species in fresh mushrooms, *Salmonella* was isolated from fresh mushroom samples in this study which agreed with the findings of Venturini et al. (2011). Again, with results of Samadpour et al. (2006), who isolated *Salmonella* in fresh mushrooms as well as *Salmonella* in 5% of fresh mushrooms commercialized in Seattle (USA) respectively. In recent times, Kragh et al. (2022) reported the occurrence of *Salmonella typhimurium* on sliced mushrooms during drying in a household dehydrator in Denmark.

Listeria monocytogenes was also isolated from mushroom samples in this study, which corroborates the findings of Kim et al. (2016), who also reported the presence of *Listeria monocytogenes* in 11% of the 28 local mushroom samples. In their study, Messelhäusser et al. (2014) found that the most common zoonotic agents in dried and brined mushrooms were *Bacillus cereus*, *Clostridium* sulfite reducer/*C. perfringens*, *Salmonella* spp., and *Listeria monocytogenes* in fresh mushrooms. Interestingly, they found that 81.5% of the dried mushroom samples they tested had enterotoxigenic *B. cereus* (>5.0 log CFU/g).

It appears that *L. monocytogenes* is a foodborne pathogen of relatively less significance in dried mushrooms compared to *Salmonella* spp. and *B. cereus*, given the frequency with which it has been reported by some previous researchers (Chen et al., 2018; Murugesan et al., 2015; Venturini et al., 2011) and its ability to grow in mushrooms as observed in the present study. However, it should not be disregarded, as *L. monocytogenes* can survive in a dehydrated state on stainless steel surfaces

for months (Vogel et al., 2010). They are also known to be isolated from a variety of low-moisture foods (LMFs) after storage for up to a year (Taylor et al., 2018), representative of how manufacturers of LMFs still need to pay attention to *L. monocytogenes*.

Because they are highly perishable, it is essential to dehydrate them in order to lower the water activity and extend their shelf life. According to Villaescusa and Gil (2003), the low shelf life is caused by both having a fast respiration rate and the cuticle's nonappearance, which protects the plant from microbial attack and physical harm.

Processing methods such as solar drying, sun drying, oven drying, etc., of mushrooms result in the lowering of the moisture content or water activity, thereby discouraging bacterial growth. Additionally, the differences in bacterial counts could be due to the different locations where the samples were purchased. In the African setting, it is common to see them displayed in the open retail markets, which are most often exposed to contamination and have no control over temperature storage. Although there have been food regulations and better farm practices in the world to reduce the incidence of various foodborne pathogens in foods, the hygienic quality of mushrooms is of great public concern in the world (Balali et al., 2020; Kortei et al., 2014a). Gastroenteritis is one of the complications that arises as a result of the contamination of microbial agents in foods (Adu-Gyamfi and Nketsia-Tabiri, 2007; Meng and Doyle, 2002; Mor-Mur and Yuste, 2010).

Public Health implications

It is worthy of note that the consumption of microbial-contaminated mushrooms can have significant health effects, such as foodborne illnesses caused by pathogens like bacteria, fungi, parasites etc. Similarly, on a large scale, their consumption may cause outbreaks and epidemics especially in communal settings such as restaurants, eateries, festivals, markets etc. Again, some problems associated with gastrointestinal symptoms like vomiting, nausea, diarrhea and abdominal pains. Also, some individuals may experience allergic reactions to certain microorganisms or toxins produced by them. Lastly, toxicity issues may arise from certain toxins produced that can cause severe health issues, including liver and kidney damage. To curtail these challenges to a large extent, it is vital to ensure proper handling, storage, and cooking of mushrooms to minimize the risk of microbial contamination.

In the surveillance and regulation, authorities need stricter monitoring, sourcing, processing and labelling to protect populations. Also, the enforcement of guidelines to minimize contamination.

Conclusion

The study provided a general overview of the microbial quality of both fresh and dried mushroom samples. The study found that the microbial load of fresh mushrooms was higher compared to the microbial load of dried mushroom samples. It may be concluded that some fresh mushroom samples are okay, while those that are beyond the permitted level are a public health concern, as their consumption raises the danger of food contamination in the Ho municipality. To lessen food safety issues, the general population must be educated about the health risks of consuming low-quality microbes. The findings of these studies have provided baseline knowledge and research-based guidelines on microbial loads of both fresh and dried mushrooms to the general public and mushroom producers. Consumers are advised to take safe hygiene precautions and thoroughly wash mushrooms before eating them. Additionally, the findings of this work satisfy SDG 3: Ensure healthy lives and promote well-being for all at all ages, and SDG 17: Strengthen the means of implementation and revitalize the Global Partnership for Sustainable Development.

Author Contribution

FN, NKK, PCAA, and GA: conceived and designed the experiments; performed the experiments; NKK and GA analyzed and interpreted the data; contributed reagents, materials, analysis tools, or data; NKK, PCAA, JN, TA, and PCAA, wrote the paper. FN and NKK: performed experiments. FN, NKK, JN, TA and GA: Analyzed and interpreted data.

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Availability of data and materials

Data shall be made available by the corresponding author upon request.

Declarations

Ethics approval and consent to participate

The data generated for this article did not involve both animal and human subjects, which would have warranted the need for ethical clearance. All data obtained and used in the study were on fungal material (mushrooms).

Consent for publication

The data presented in this article does not contain any individual data.

Competing interests

The authors declare that they have no competing interest.

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