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Research Article

THE IMPACT OF FUNGAL SPOILAGE ON MUFFINS WITH CHESTNUT, COCONUT OR WHEAT FLOUR

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ABSTRACT

In this study, muffins made with various flour types (chestnut, coconut, and wheat) and sweeteners (sugar and stevia) are examined for fungal spoilage. Fungal isolation and identification were carried out using Potato Dextrose Agar (PDA) incubation and microscopic analysis. *Penicillium* spp. was found to be the most common spoilage genus in all muffins. Food spoilage fungi like *Penicillium*, *Nigrospora*, and *Rhizopus* were highly tolerant of the muffin made with wheat flour and stevia. Muffins containing chestnut flour, with either sugar or stevia, also showed high acceptability to food spoilage fungi such as *Penicillium*, *Alternaria*, *Aspergillus*, and *Fusarium*. Conversely, muffins made with a blend of chestnut-coconut flour and stevia showed lower fungal growth. Fungal contamination was not solely influenced by moisture content, suggesting that flour type and sweetener, as well as their antimicrobial qualities, also contribute to fungal growth.

1.Introduction

Food spoilage, particularly due to fungi, is a major concern in the food industry, especially with bakery products like muffins. Research indicates that muffins are primarily affected by fungi such as *Aspergillus, Penicillium*, and *Rhizopus* species (Morassi et al., 2018). These fungi thrive in the nutrient-rich environments created by flour and other ingredients. Several factors influence the growth of spoilage fungi, including water activity, pH, temperature, and the presence of preservatives (Snyder et al., 2019). For example, lower water activity and pH levels can inhibit fungal growth, whereas higher moisture content may encourage it (Arroyo et al., 2008).

The composition of flour, particularly its gluten content, can affect the growth of fungi in muffins. Research showed that gluten, the main protein in wheat flour, can be associated with celiac disease (Gojković Cvjetković et al., 2024). Moreover, to prevent fungal spoilage in muffins during production and storage, it is essential to consider the sources of fungal contamination, including raw materials, processing environments, and air (Copetti et al., 2025).

Fungal spoilage in food products can pose health risks due to the production of mycotoxins by certain mold species, such as *Penicillium* and *Aspergillus*. These mycotoxins can have various toxic effects on humans, including carcinogenic, immunotoxic, and neurotoxic effects (Ribes et al., 2018). To combat fungal spoilage, a variety of natural compounds have been studied for their effectiveness in inhibiting mold growth in fruits and vegetables. These include essential oils, preservatives, natural products, chemical fungicides, nanoparticles, and chitosan (Souza et al., 2024). While synthetic chemicals like sorbic acid and potassium sorbate are commonly used as preservatives to prevent fungal spoilage in food products (Bento de Carvalho et al., 2024), there is a growing demand for “natural” and “chemical-free” foods. This has led to increased research into clean-label preservative alternatives. Coconut flour has demonstrated antimicrobial properties. The high phenolic content of coconut shell pyrolytic oil has been shown to inhibit the growth of wood-decay fungi, suggesting its potential as an antimicrobial agent (Shiny et al., 2014). Furthermore, studies have shown that the nutritional composition of coconut flour is comparable to that of wheat flour, and it is gluten-free, making it a healthy option for individuals with celiac disease (Karandeep et al., 2019).

While coconut flour has shown antimicrobial properties, its effectiveness in preventing fungal spoilage in food products is not widely covered in existing literature. Therefore, we presented experimental data to provide insights into the antimicrobial properties of coconut flour and its potential applications in food products, such as muffins. Further, in this research, we compared coconut flour with chestnut flour, which is gluten-free and rich in starch, minerals, vitamins, fiber, and antioxidants (Paciulli et al., 2019).

2. Materials and Methods

2.1.Cake samples

The ingredients used for muffin preparation included wheat flour, coconut flour, chestnut flour, butter, baking powder, and eggs, as presented by Raczyk et al. (2021). In addition to sugar, stevia was used to create muffins with a bio-functional profile that highlighted the quality characteristics of coconut and chestnut flour (Difonzo et al., 2020). Vanilla and orange extracts, along with beaten yolks and milk, were added sequentially, and meringue was incorporated at the final stage. The muffins were baked in an air fryer at 165°C for 20 minutes (Baranda et al., 2025).

The composition of the muffins was:
Muffin 1: Made with wheat flour and stevia;
Muffin 2: Made with chestnut flour and stevia;
Muffin 3: Made with a blend of chestnut and coconut flour (80:20) and stevia;
Muffin 4: Made with chestnut flour and sugar

2.2.Determination of moisture content

The moisture content of the muffins was determined according to AOAC methods with some modifications (AOAC, 2005). Briefly, 5g of sample was weighed and placed in a pre-weighed aluminum pan, then placed in the thermo balance (Kern DBS, Balingen, Germany) to remove the moisture content at 120°C until constant weight. Once the thermobalance stopped, the final weight of the sample was recorded, and the percentage of moisture content was calculated. The moisture content of all samples was measured on days 1,2,4 and 8, with day 1 referring to the day the muffins were prepared.

2.3.Detection of Fungus in Muffins Products

To isolate fungi from muffins, small samples of the muffin products were placed onto a Petri dish containing potato dextrose agar (PDA, Biolab) using sterile forceps. The PDA plates were sealed and incubated at 25°C for 5 days and 10 days. Fungal growth for each type of muffin treatment (e.g., muffins made with wheat flour) was observed both visually and macroscopically at the 5-day and 10-day intervals.

2.4.Pathogen Isolation and Culture Conditions

We utilized the book "Fungi and Food Spoilage" (Pitt and Hocking, 2022) as a manual for identifying fungal species, focusing primarily on their cultural and physiological characteristics. We also examined microscopic features of the fungi, such as the type of conidia, the structure of conidiophores (e.g., penicilli conidiophores), the types of phialides etc. For microscopic observation, we used a CETI compound microscope (Medline Scientific) equipped with a HAYEAR 5MP 1/2.5" live-color image camera. Furthermore, we employed the microscope scale to measure the length of the conidia, aiding in the distinction of fungal species e.g. *Aspergillus nidulans*.

2.5.Statistical Analysis

IBM SPSS Statistics v.28.0 was used for statistical analysis. Every experiment was carried out in triplicate, and the results are presented as the mean ± standard deviation. To identify statistically significant differences, a One-way ANOVA test followed by Duncan's post hoc test was performed, at a significance level of 0.05.

3. Results and Discussion

3.1.Moisture content

Moisture is a physicochemical factor attribute of bakery products, closely linked to product freshness. It plays a main role in the staling process, which can negatively impact on the sensory qualities of the products (Marciniak-Lukasiak et al., 2022). The moisture content of the muffins is shown in Table 1

Table 1. Moisture content of muffins.

	% Moisture content			
Days	**Muffin 1**	**Muffin 2**	**Muffin 3**	**Muffin 4**
Day 1	31.30 ± 0.28 ^{bD}	34.94 ± 0.09 ^{cA}	36.74 ± 0.77 ^{dD}	28.22 ± 0.18 ^{aC}
Day 2	31.19 ± 0.07 ^{bD}	34.90 ± 0.02 ^{cA}	36.66 ± 0.04 ^{dD}	28.14^{a4} ± 0.028 ^{aC}
Day 4	29.01 ± 0.29 ^{bC}	34.79 ± 0.03 ^{cA}	35.95 ± 0.09 ^{dC}	27.43^{a3} ± 0.20 ^{aB}
Day 6	27.91 ± 0.19 ^{bB}	34.69 ± 0.04 ^{cA}	35.07 ± 0.08 ^{dB}	25.44^{a3} ± 0.17 ^{aB}
Day 8	25.44 ± 0.09 ^{bA}	34.65 ±0.54 ^{cA}	33.51 ± 0.73 ^{cA}	24.10^{a1} ± 0.04 ^{aA}

Table values are means ± standard deviations (*n*=3). Different uppercase superscript letters (a, b, c, d) in the same row represent statistical differences (p ≤ 0.05). Different uppercase superscript letters (A, B, C, D) in the same column represent statistical differences (p ≤ 0.05).

The moisture content of the muffins was monitored on days 1,2,4,6 and 8, and the findings indicated that all muffins' moisture levels stayed consistent during the first two days. Muffins 1, 3 and 4 showed a decrease in moisture content over storage, whereas Muffin 2 remained comparatively stable in moisture content. Soumya et al. reported muffins losing moisture content over time (Soumya et al., 2016).

Muffin 4 with chestnut flour and sugar had the lowest moisture content of all samples, likely due to the presence of sugar that serves as a preservative. Sugar impacts on baked goods, and not just because it gives cakes and cookies their texture and softness (Indrani and Rao, 2008). Additionally, it delays spoiling and prevents dehydration by serving as a water-holding agent (Sivasankar, 2002; Indrani and Rao, 2008). Muffin 1, 3, and 3 followed, ranked by increasing moisture levels. Interestingly, Muffin 3 had the highest moisture content until day 6, but by day 8, there were no discernible differences between Muffins 2 and 3 in terms of moisture levels.

According to the literature, coconut flour typically has a lower moisture content than flour blends like wheat-coconut flour (50:50). Cakes made entirely of wheat flour may have less moisture than cakes made with coconut flour or coconut-wheat blends (Afoakwah et al., 2019). Furthermore, compared to wheat flour, chestnut flour is known to exhibit more consistent moisture behavior, exhibiting less variation between adsorption and desorption at low moisture levels, suggesting more predictable moisture retention during processing or storage in dry conditions (Zhu, 2017)

3.2.Detection of Fungus in Muffins Products

Penicillium was the most common species found in all muffin products. This fungus was observed by the 10th day of incubation. In muffins made with wheat flour, the observed fungal species included *Penicillium* (*P. expansum*), *Nigrospora*, and *Rhizopus* (*R. oligosporus*). In muffins made with chestnut flour, the fungal species present were *Penicillium*, *Alternaria*, *Aspergillus* (*A. nidulans*), and *Fusarium* spp (*F. oxysporum*).

Microscopic distinctive features of Fungal isolates

According to the book "Fungi and Food Spoilage" by Pitt and Hocking (2022), several fungal species were identified as listed below.

Alternaria spp.

In our research *Alternaria* isolates (Figure 1), are characterized by the production of large brown conidia that have transverse septa and usually thin longitudinal septa. These conidia develop from inconspicuous conidiophores and feature a distinct conical narrowing, referred to as a "beak," at the apex (Pitt and Hocking, 2022). They often arrange themselves in chains (Figure 1d). Microscopic examination reveals that secondary conidia are produced at the tips of short hyphal segments (Figure 1a), and these conidia are distinctly clavate, or club-shaped. The primary microscopic differences between *A. alternata* and *A. infectoria* lie in their conidial formation. In *A. alternata*, conidia (Figure 1d and e), measure between 20–40 × 8–12 μm and form continuous chains without narrow hyphal elements between spores. In contrast, *A. infectoria* produces secondary conidia (Figure 1b and c), that measure 20–50 × 7–14 μm, often separated by short hyphal segments (Silva et al., 2014). Additionally, *Alternaria* is a significant mycotoxigenic fungal genus, responsible for producing over 70 identified metabolites. These toxins can exhibit considerable toxicity, including mutagenic and carcinogenic effects, DNA strand breakage, disruption of sphingolipid metabolism, and inhibition of enzyme activity and photophosphorylation (Barkai-Golan & Paster. 2008; Lee et al., 2015; Escrivá et al., 2017).

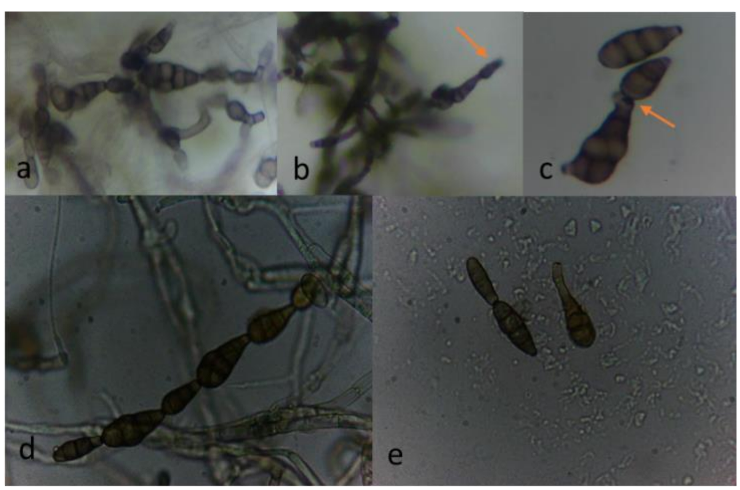

Figure 1. (a) Conidia clavate (club shaped), *A. infectoria*: Primary conidia producing secondary conidia (b, arrow) and conidia often separated by short hyphal lengths (c, arrow), *A. alternata*: the conidia in continuous chains, "conidia *in situ"* (d) and conidia (e).

Nigrospora spp.

Nigrospora is distinguished by its production of relatively large, solitary, jet-black conidia (16-18μm) with smooth walls and an oblate shape (Figure 2). It primarily acts as a plant and seed pathogen but can also be found in air, soil, and water (Zhang et al., 2024). Species of this genus function as pathogens, endophytes, and saprobes across various hosts, with *N. oryzae* and *N. sphaerica* being as the most frequently reported *Nigrospora* pathogens (Hao et al., 2020). However, there have been no reports of mycotoxin production in *Nigrospora* species (Pitt and Hocking, 2022). The genus *Nigrospora* is characterized by its distinctive morphological feature of producing black conidia on white mycelium (Figure 2b and c). This feature is a significant identifying trait for the genus and is frequently mentioned in studies focusing on the taxonomy and identification of *Nigrospora* species.

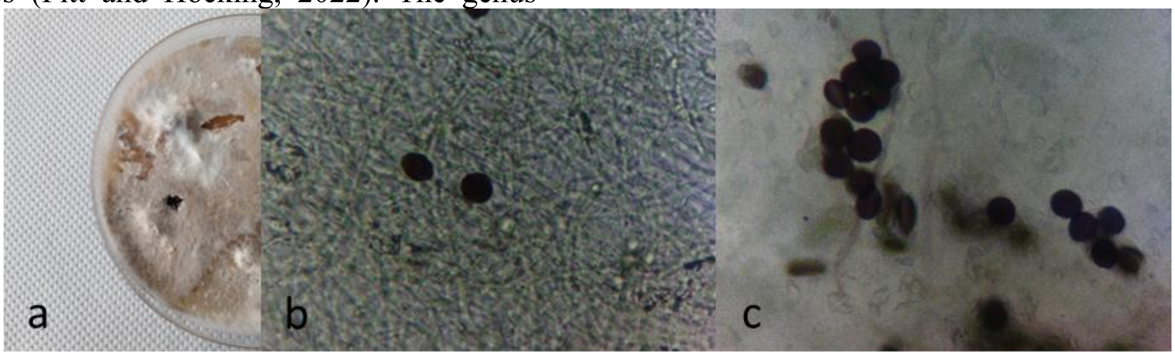

Figure 2. Morphological description of *Nigrospora* spp. growth on PDA (a), mycelial features and black conidia (b-c)

Penicillium spp.

Penicillium spp. are filamentous fungi with branched conidiospores and round, unicellular conidia (2.5-5µm in diameter), (Figure 2b,c,d,e and f). Species are classified by their method of conidia production: in some, conidia form directly on phialides, while in others, conidiophores bear metulae that support the phialides, or may branch before producing metulae (Torelli et al., 2006). The species commonly occurring in foods are similar in color and general colony appearance (Pitt and Hocking, 2022). According to all the above, it is clear that *Penicillium* taxonomy is not easy for the inexperienced researchers.

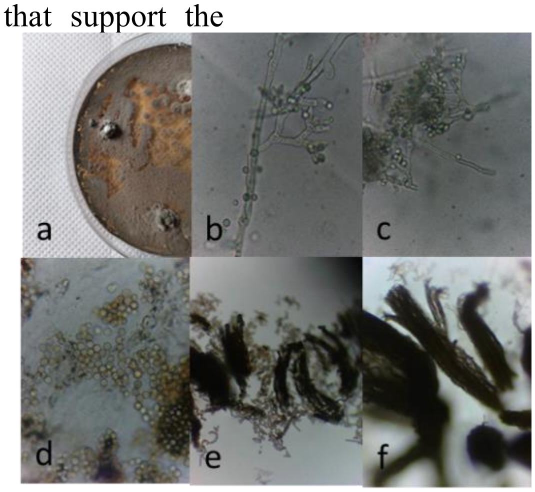

Figure 3. Morphological description of *Penicillium* spp., growth on PDA (a). Microscopic characteristics: phialides and simple conidiophore showing conidiophores showing two-stage branching (b-c), conidia (d), long chains of single-celled phialoconidia (e-f)

The identified *Penicillium* species are among the most significant producers of mycotoxins (Rundberget et al., 2004); while they are rarely linked to human infections due to limited growth at 37°C, the main health risk arises from consuming food contaminated with mycotoxins from these species (Perrone and Susca, 2017). For Instance, in *P. expansum*, the formation of patulin and citrinin are probably not coupled to each other but may formed in different periods (Max Rubner-Institut, 2024).

Penicillium expansum

Penicillium expansum typically exhibits a floccose (woolly) colony texture, which can help differentiate it from other *Penicillium* species that may have a velutinous (velvety) texture (Figure 3a). The conidiophores of P. expansum are smooth-walled and can be monoverticillate or biverticillate, producing chains of conidia (Figure 3e and f). The conidia are globose to subglobose, smooth-walled, and can vary in color from blue-green to olive-green (Figure 3d).

Aspergillus spp.

Aspergillus species are generally characterized by conidiophores with large, often thick-walled stipes and swollen tips, known as vesicles (Figure 4). These vesicles are typically roughly spherical (Figure 4b and c) but may be elongated or less prominently swollen in certain species. Vesicles bear densely packed phialides, or both metulae and phialides, which are typically produced simultaneously. Moreover, *Aspergillus* spp. are highly prevalent fungi that contaminate a variety of food sources and produce mycotoxins, a group of toxic biochemical (Aflatoxins, ochratoxin A, patulin, citrinin, aflatrem, secalonic acids, cyclopiazonic acid, terrein, sterigmatocystin, and gliotoxin) that not only are playing a critical role in agriculture but these mycotoxins are harmful to human health (Frisvad et al., 2004; Pfliegler et al., 2020, Navale et al., 2021; Max Rubner-Institut, 2024).

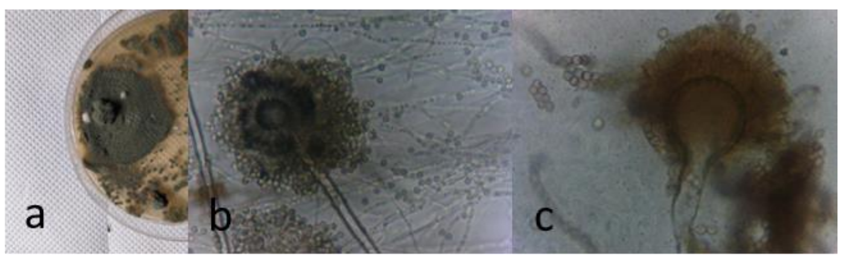

Figure 4. Morphological description of *Aspergillus* spp., mycellial fungal colonies growth on PDA (a) Microscopic characteristics including conidiophore, vesicle, matulae, phialides and conidia (b-c).

Aspergillus nidulans (*Emericella* spp.)

Conidia form in dry chains, creating dark yellow-green columns from solitary, upright, aseptate, brown, and smooth conidiophores (Figure 5). Conidiogenous cells (phialides) are situated on supporting cells at the swollen tips of conidiophores, featuring short necks. Conidia (12–18 μm long × 8–10 μm) are globose with rough walls (Figure 5a, b, c and d).

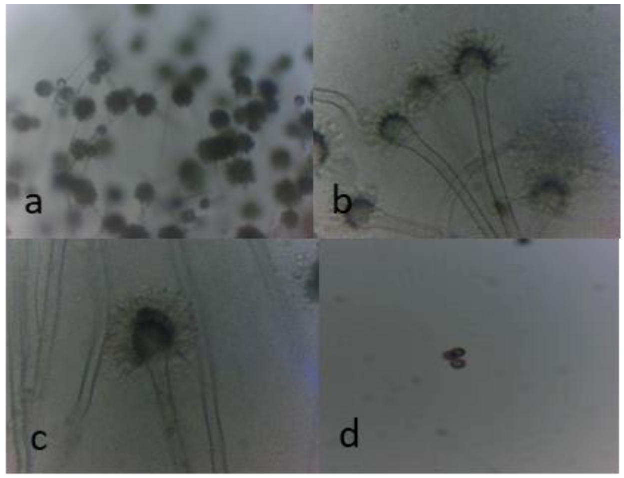

Figure 5. Morphological description of *Emericella* spp., conidiophores and phialides (a-c), conidia (d).

Fusarium spp. (*Fusarium oxysporum*)

Fusarium spp. typically exhibits white to cream colonies with sparse mycelium on PDA (Figure 6a). Conidiophores in *Fusarium* spp. are often long and monophialidic, producing microconidia (Figure 6b, and c). Macroconidia of *Fusarium* spp. are typically cylindrical and slightly curved, with blunt and rounded apical cell (Figure 6d). *Fusarium oxysporum*, this species produces macroconidia on sporodochia, which are dense masses of fungal hyphae. These macroconidia are typically airborne and play a significant role in the spread of the pathogen (Figure 7).

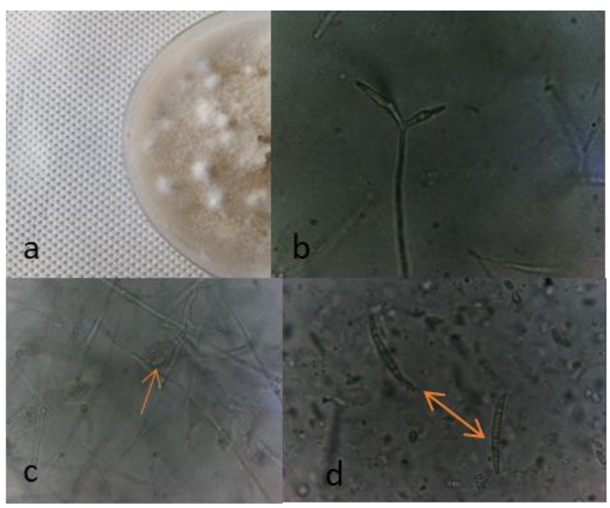

Figure 6. Morphological description of *Fusarium* spp., mycelial fungal growths on PDA (a), conidiophores (b), false heads (c, arrow) and macroconidia (d, arrow).

Fusarium species are a diverse and resilient group of fungi that significantly impact agriculture and the economy (Arumugam et al., 2021). Their growth in the field is highly influenced by environmental conditions, particularly the presence of high moisture and warm temperatures, which promote *Fusarium* development and subsequently reduce product quality (Chandravarnan et al., 2022). Compounding this issue, several *Fusarium* species produce secondary metabolites, including the mycotoxins fumonisin B1 and fusaric acid, which are toxic or even carcinogenic to both humans and animals. These harmful mycotoxins are commonly found in cereal-based foods, animal feed, and various other products consumed daily, potentially contributing to plant disease and posing ongoing risks within the food supply chain (Escrivá et al., 2015).

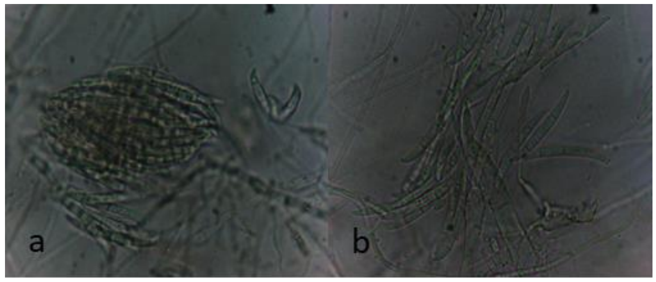

Figure 7. Morphological description of *Fusarium oxysporum*, macroconidia produced on sporodochia (a) macroconidia developed from sporodochia and microconidia developed from monophialides.

Rhizopus oligosporus

Rhizopus oligosporus spores develop within a sporangium that contains fluid, which dries at maturity. This process is crucial for the survival and dispersal of the fungus (Figure 8c and d). Hyphae may have specialized structures that aid in identification, such as septa with clamp connections and bottle-shaped cystidia with bent neck (Figure 8b).

The classification of *Rhizopus* species has seen substantial advancement, particularly over the past 40 years, as taxonomy has shifted from traditional methods to molecular approaches (Sjamsuridzal et al., 2021). Distinguishing between *Rhizopus* species relies on various morphological features, such as the length of rhizoids and sporangiophores, sporangia diameter, columella shape, and the size, shape, and surface texture of sporangiospores. These specific characteristics provide critical insights into species identification within the genus, complementing modern molecular techniques and enhancing the accuracy of *Rhizopus* taxonomy.

After the microscopic analysis, the sample was *R. oligosporus* which is part of the *R. microsporus* group, a collection of morphologically similar species linked to food fermentation, pathogenicity, or the production of undesirable metabolites such as rhizonins and rhizoxins (Jennessen et al., 2008).

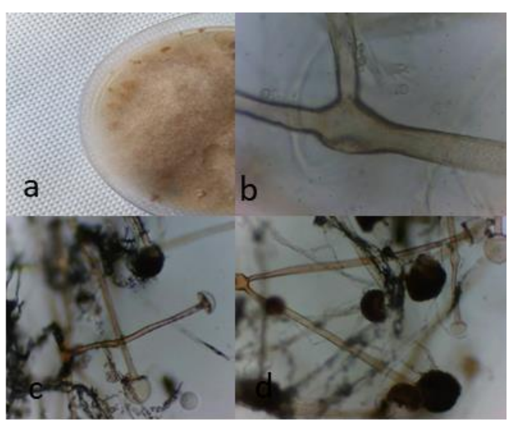

Figure 8. Morphological and Macroscopic description of *Rhizopus oligosporus*: stolons and typically unbranched sporangiophores with black, rounded sporangia at the sporangiophore tips without rhizoids (a-c).

4. Conclusions

This study examined the fungal spoilage of muffins prepared using various flour types (chestnut, coconut, and wheat) and sweeteners (sugar and stevia). On the 10th day of incubation, all muffins showed fungal spoilage with *Penicillium* as a predominant species. The muffin made with wheat flour and stevia was prone to food spoilage fungi, while the muffins made with chestnut flour, either with stevia or sugar, also showed a high level of fungal contamination such as *Penicillium* and *Aspergillus*. In contrast, the muffin with a blend of chestnut-coconut flour and stevia was less vulnerable to food spoilage by fungi.

Despite the expectation that fungal growth would be encouraged by higher moisture levels, this was not a consistent predictor across all samples. Compared to the muffin made with wheat flour and stevia, the muffin made with a blend of chestnut and coconut flour and stevia had a higher moisture content but exhibited noticeably less fungal growth. The type of flour, the initial microbial load, and the presence of bioactive compounds are some of the variables that affect fungal spoilage in addition to moisture levels.

The biochemical composition of each flour affects not only the physicochemical and sensory properties of the products, but also their antioxidant and antibacterial properties (Durazzo et al., 2013; Smith et al., 2016). In fact, chestnut flour, which is rich in plant lignans and phenolics, has antibacterial and antioxidant properties (Durazzo et al., 2013). Furthermore, when coconut flour is added to rice noodles, not only improved the product but also may have extended its shelf life (Sundaresan et al., 2023). According to the literature, coconut flour when combined with other flour, increased dietary fiber and antioxidant levels (Adelove et al., 2020). Furthermore, microbial growth may be inhibited by the low water activity of coconut flour (Jiamjariyatam et al., 2021).

Literature supports our findings because fungal counts were found in wheat flour, and coconut flour. In particular, other researchers found that there was microbial contamination in wheat flour (Berghofer et al., 2003; Morassi et al., 2018; Lesniewska et al., 2021).

Aspergillus and Fusarium spp. are common in wheat flour (Tournas & Niazi, 2018). Even more, bakery products, including muffins, are susceptible to spoilage by fungi such as *Aspergillus, Penicillium,* and *Fusarium* species (Tournas & Niazi, 2018; Abdelhameed & Khalifa, 2024). Further, mycotoxins like deoxynivalenol (DON) are frequently found in wheat and its derivatives, posing significant health risks (Arrúa Alvarenga et al., 2018). Gluten-free bread with the addition of chestnut flour also showed microbial contamination such as yeast, mold and *Bacillus* spp (Lesniewska et al., 2021). The replacement of sugar in muffins or in bakery products by stevia has been investigated in several studies. Stevia is a source of bioactive substances and provides anti-microbial, antioxidant activities and other health benefits (Ahmad et al., 2020) and stevia extracts can be used as natural preservatives in food applications (Ortiz-Viedma et al., 2017, Ahmad et al., 2020).

Stevia's antimicrobial properties seem to be dose-dependent (Abou-Arab et al., 2010; Puri and Sharma, 2011; Singh et al., 2012; Ahmad et al., 2020). Research has shown that while full replacement tends to lower product quality, partial substitution of stevia for sucrose in muffins and other bakery good has no distinct effect on the sensory qualities of the final product (Kulthe et al., 2014; Karp et al., 2016; 2017; Vatankhah et al., 2017; Ahmad et al., 2020). Natural preservatives, such as lactic acid bacteria (LAB) and plant essential oils, can prolong the shelf life of baked goods by preventing the growth of fungi (Ranjith et al., 2021). It is known that both coconut and chestnut flour increase food product's antioxidant activity (Smith et al., 2016; Choi et al., 2024). Other researchers found that adding more chestnut powder to muffins increased their total polyphenol and flavonoid contents which improved their antioxidant activity (Choi et al., 2024).

Because coconut flour has a low moisture content (Jiamjariyatam et al., 2021), it may result in products that have a drier texture and less water activity, both of which can affect the growth of spoilage fungi. Its antioxidant potential has been demonstrated through various assays, such as Trolox Equivalent Antioxidant Capacity (TEAC), DPPH radical scavenging, and Ferric Reducing Antioxidant Potential (FRAP). These experiments imply that coconut flour muffins might have better functional qualities (Smith et al., 2016).

Although coconut flour has antimicrobial, anticancer and cardiovascular protective effects (Shiny et al., 2014; Karandeep et al., 2019), its anti-fungal activity in food applications has not been well documented. Consistent with our results, a study on coconut flour-based bio-yogurt beverages demonstrated antifungal activity as no distinct growth of yeast or mold was shown (Salama et al., 2019). Based on these findings, we propose that coconut flour, like the other plant-based extracts and essential oils, should be investigated further because of its antifungal qualities (Copetti et al., 2025).

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