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Original Article

A comparative study on the use of white light, black light 365 nm and 395 nm for screening mold on food surfaces

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Abstract

Mold contamination on food presents significant threats to food safety and public health, necessitating the detection and prevention of mold presence in food products to ensure their quality and safety. The study investigates the effectiveness of white light, black light at 365 nm, and black light at 395 nm for early mold detection on food surfaces. Analyzing 100 food samples through potassium hydroxide preparation and microscopic examination confirmed mold presence. Black lights at both 365 nm and 395 nm demonstrated superior performance over white light in detecting mold. ROC curve analysis supported the increased sensitivity and specificity of black lights in identifying both contaminated and uncontaminated mold on food. Integrating black light into mold detection methods holds promise for enhancing accuracy and reducing health risks associated with mold-contaminated food. While these results are encouraging, further research is required to validate findings and optimize mold detection effectiveness on food surfaces.

Keywords: white light, black light, ultraviolet light 365 and 395 nm, fungi, mold, food screening

1. Introduction

Extended storage of food often leads to the growth of mold, primarily due to the presence of mold spores in the surrounding environment, which can contaminate the food (Stobnicka-Kupiec, Gołofit-Szymczak, & Górny, 2019). Initial stages of mold growth, starting from spores and progressing to hyphae, are not easily visible to the naked eye. To detect these stages, a commonly employed method is the use of a potassium hydroxide (KOH) preparation (Ponka & Baddar, 2014). Under microscopic examination, mold can be identified by its elongated and branch-like hyphae. Additionally, it can be recognized by spore bulbs known as arthroconidia that emerge from the hyphae tips, or by circular budding yeasts that undergo division (Moore & Robson, 2013). The growth time of molds can vary significantly. Some molds can develop within as little as 12-24 hours, while others may take weeks before becoming visible. As the hyphae grow and form a network, they can change color and appear as fine, fluffy fibers known as mycelia. Molds can exhibit a wide range of colors, including black, brown, gray, white, yellow, green, and others under normal visible light or white light, mycelium is typically visible, while hyphae are not easily seen. Consequently, food contaminated with mold in the hyphae stage is often consumed unknowingly, leading to potential health issues.

Several methods have been utilized to identify molds on food surfaces, including rapid testing kits (Deák, 1995), immunochemical techniques (Li, Marquardt, & Abramson, 2000), molecular techniques (Boysen, Eriksson, &

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Schnürer, 2001; Rai & Bai, 2014), tomography (Zhou, Wu, & Chen, 2022), and culture-based techniques (Erkmen, 2022). However, these methods often require considerable time, and are costly and impractical for routine food screening (Rico-Munoz, Samson, & Houbraken, 2019).

Yabe *et al.* demonstrated the effectiveness of using ultraviolet light type A (UVA) with a wavelength range of 365-395 nm, commonly referred to as black light, in detecting mold in laboratory settings (Yabe, Ando, Ito, & Terakado, 1987). UVA light, with a wavelength range of 315-400 nm, can detect fungal hyphae. Certain substances, including mold, absorb UVA light and emit light at different wavelengths, resulting in a fluorescent glow. Mold typically exhibits fluorescence in shades of blue and green (Häder & Tevini, 1987).

Black light detection has been employed across various fields. In household purposes, black light is used to detect pet urine stains (Coats & Ferguson, 1989). In medicine, Wood's lamp or UV lamps aid in diagnosing a range of skin diseases by examining the fluorescence of skin lesions, including those caused by fungal infections (Veasey, Miguel, & Bedrikow, 2017). Furthermore, black light detection is utilized for identifying mold on building walls to assess water leakage (Suchorab *et al.*, 2019), and analyzing trace substances in serum (Kearse, 2020).

Despite its various applications, there have been limited studies exploring the use of black light for detecting mold hyphae on food surfaces. However, researchers have proposed the hypothesis that black light could serve as an effective method for quickly identifying mold on food surfaces and facilitating the detection of suspicious mold features. The ultimate objective is to provide consumers with information that empowers them to make informed decisions about food consumption, promoting food safety and reducing potential health risks. Further research in this area will be crucial to assess the feasibility and effectiveness of utilizing black light for mold detection on food surfaces.

2. Materials and Methods

2.1 Sample preparation

Altogether 100 fresh food items based on their commonality in households and their varying textures were prepared. Bakery products, fruits, and meat represent a range of food types commonly consumed by people and are known to be susceptible to fungal contamination. The absence of fungal contamination was ensured by testing each item with a 20% KOH preparation. Aseptic techniques were used to divide the food into two groups. The first group was designated as the control group. Each food item was transferred individually into separate sterilized petri dishes using sterilized forceps. Each dish was securely covered with its lid to prevent any external contamination and maintain sterility and consistency throughout the experiment. To simulate fungal spore contamination for the experimental group, wipe various types of food on a table surface exposed to environmental factors, such as a table with dust or soil residues. Subsequently, the contaminated food was transferred into petri dishes. Each petri dish was labeled with the food sample, date, and time. The control and experimental groups were paired side by side for observation.

2.2 Screening for mold

To identify the early stages of mold growth on food surfaces, each food sample was screened every 12 hours using three different light sources: white light, UVA light with a wavelength of 365 nm, and UVA light with a wavelength of 395 nm. Photos were taken at each screening time for each light wavelength for comparison. Samples were carefully examined by an inspector, who used a regular flashlight and two different black light flashlights, which were emitting wavelengths of 365 nm and 395 nm, respectively. During the inspection, the presence or absence of mold was recorded for each sample. Any suspicious findings, such as changes in color, darkening, fading, glowing, or alterations in the texture of the food observed under each wavelength compared to the previous record and photographs, were documented.

2.3 Laboratory analysis of mold contamination

All samples, whether showing suspected or non-suspected findings during the screening, were subjected to laboratory analysis for mold contamination. Microscopic examination was conducted using a 20% KOH preparation. Positive mold contamination was indicated by the presence of any form of mold in the samples. Mold can be identified through three different findings: 1) Hyphae: branch-like tubular structures. 2) Arthroconidia: spore bulbs emerging from the tip of the hyphae, and 3) Budding yeast: round or oval structures with budding. Negative mold contamination indicated the absence of any mold growth.

2.3.1 20% KOH preparation technique

In the microscopic examination using a 20% KOH preparation, a small portion of each food sample displaying suspected or non-suspected findings was placed on a glass slide using sterilized forceps. A drop of 20% KOH solution was then applied to cover the food sample entirely, followed by the gentle placement of a cover slip to create a flat viewing surface. The prepared slide was subsequently observed under a microscope at various magnifications to determine the presence or absence of mold structures, including hyphae, arthroconidia, and budding yeast. All identified mold structures and their abundance were meticulously documented, and photographs of the microscopic images were taken for further analysis and record keeping.

2.4 Statistical analysis

Data were divided into suspected and non-suspected fungi. Fungal examinations yielded positive and negative results, which were then used to calculate parameter values, including true positive (TP), false positive (FP), true negative (TN), and false negative (FN). The TP group was correctly identified as containing mold by the screening method and confirmed through laboratory analysis. The FP group was incorrectly identified as containing mold by the screening method, but confirmed through laboratory analysis to be free of mold. The TN group was correctly identified as not containing mold by the screening method and confirmed through laboratory analysis, while the FN group was incorrectly identified as not containing mold by the screening

method but confirmed through laboratory analysis to have mold contamination.

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of white light and black light at each wavelength were calculated and reported with a 95% confidence interval (CI) using Pearson's chi-square test and Fisher's exact test. For sensitivity, specificity, PPV, and NPV, the following formulas were applied:

- Sensitivity = true positive / (true positive + false negative)
- Specificity = true negative / (true negative + false positive)
- PPV = true positive / (true positive + false positive)
- NPV = true negative / (true negative + false negative)

Categorical data of larger sample sizes in bakery and fruit items were analyzed using Pearson's chi-square test. Smaller sample sizes of meat and vegetable items were analyzed using Fisher's exact test for diagnostic analysis.

To evaluate the performance of different light sources, we utilized the area under the curve (AUC) of the receiver operating characteristic (ROC) curve. A higher AUC value indicates better discrimination between positive and negative cases. The statistical significance of differences in AUC values between different light sources was determined using a p-value. A p-value of less than 0.05 was considered statistically significant, suggesting a significant difference in the performance of the evaluated light wavelengths.

3. Results and Discussion

During white light examination, suspected mold contamination on food surfaces can be identified by the presence of thin, fine fibers and fluffy areas, typically appearing white or black. However, these characteristics can sometimes blend with the food's natural color and texture, making them less distinct. When examined under black light at wavelengths of 365 nm and 395 nm, potential mold contamination becomes more readily identifiable through fluorescence or color changes. Colors can vary, ranging from purple, blue, and green to orange, yellow, or even paler or darker shades of the food item's original color (Figure 1). This fluorescence or color change makes suspected mold contamination stand out as bright spots or patches against the darker background of the food surface. The intensity and pattern of fluorescence may vary depending on the type of food and the severity of contamination.

This study explored the characteristics of mold growth on the surface of various foods when examined using both white light and black light at wavelengths of 365 nm and 395 nm. Observations revealed no distinctive changes during white light screening. However, variations in the appearance of mold contamination were observed under both wavelengths of black light, depending on the type of food. For example, an éclair exhibited a pale green color under 365 nm and a pale white color under 395 nm (Figure 1, item e). Toddy palm displayed a blue-white fluorescence under 365 nm, whereas under 395 nm, it showed a pale purple fluorescence (Figure 1, item l). Yellow-orange spots were detected on jackfruit under both 365 nm and 395 nm black light examinations (Figure 1, item j).

Some food items did not display any characteristics of mold contamination when examined under black light at one specific wavelength but became apparent when examined with the other wavelength. For instance, strawberries showed

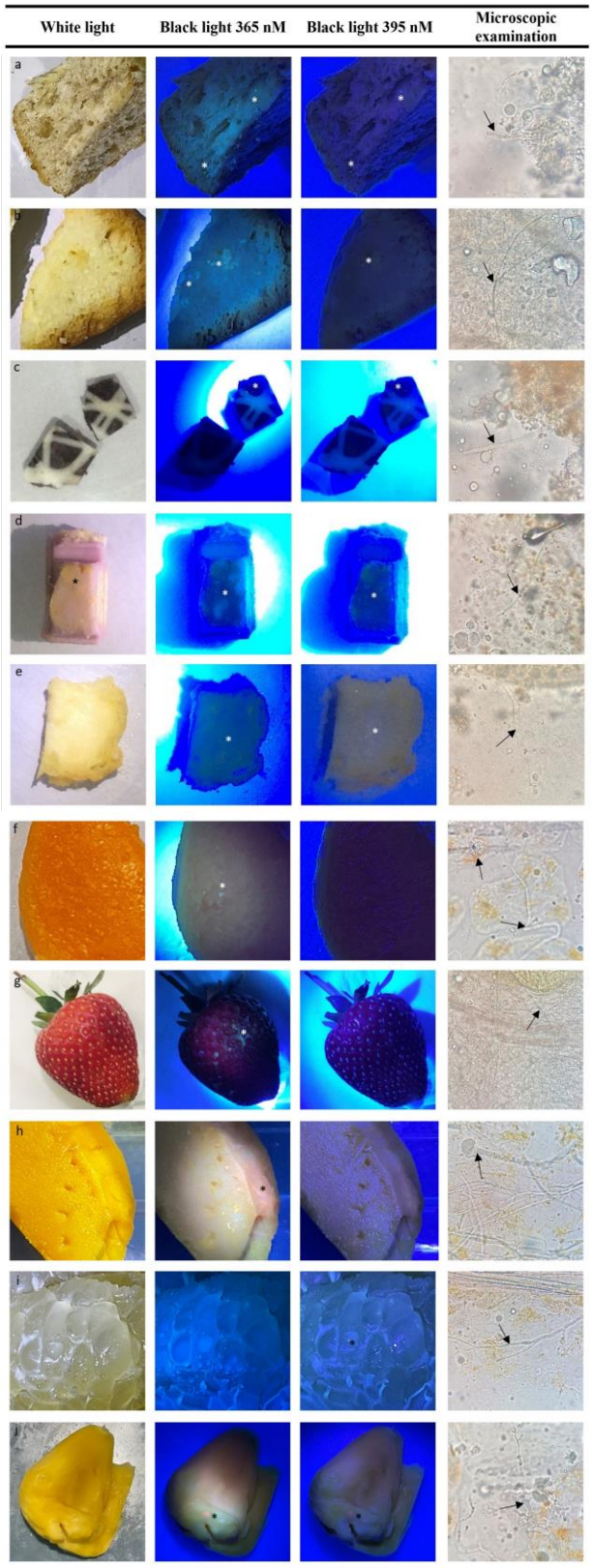
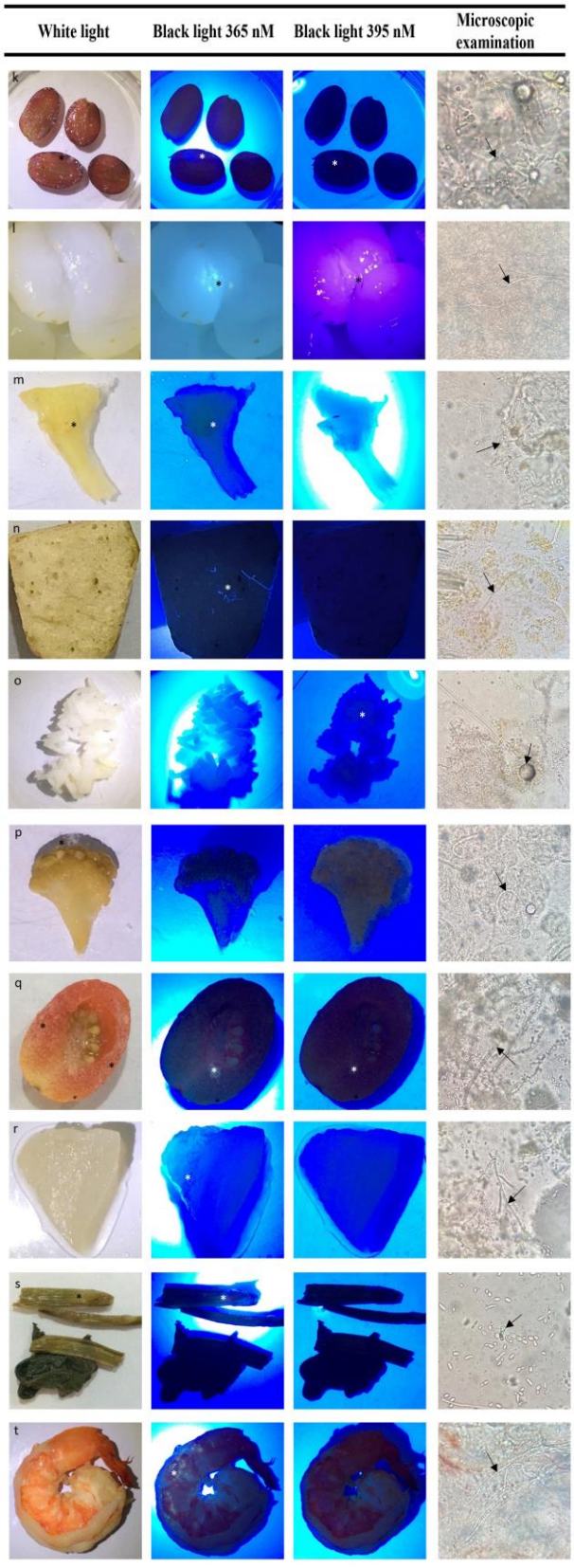


Figure 1. Detection of mold contamination through white light, black light (365 nm and 395 nm), and microscopic examination



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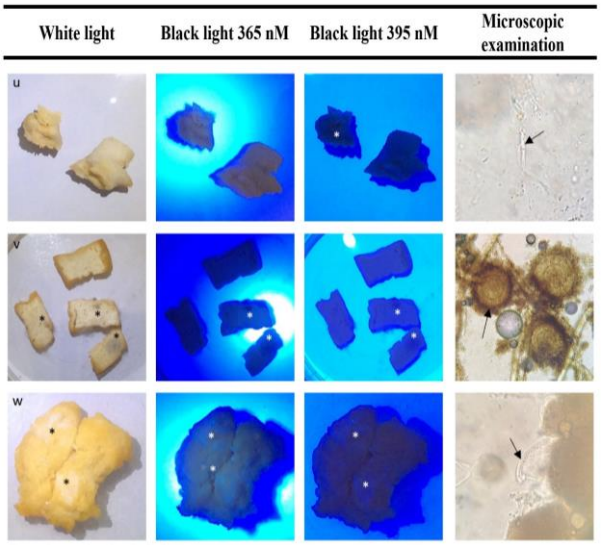


*Areas of suspected mold contamination. (arrow) Identification of mold (hyphae:long tubular structure; spore:round structure) under microscope. Food items: a=bread, b=pie, c= chocolate, d=wafer, e= éclair, f= cream filling, g=strawberry, h=mango, i=pomelo pulp, j=jackfruit, k=grape, l=toddy palm, m=pineapple, n=guava, o=rice, p=cauliflower, q=baby tomato, r=winter melon, s=morning glory, t= shrimp, u=chicken meat, v=tofu, w=fried egg

Figure 1. Continued.

a distinct white-green appearance under 365 nm black light, while no noticeable difference was observed under 395 nm black light (Figure 1, item g). Ripe mangoes displayed a red-orange appearance under 365 nm black light, whereas 395 nm black light did not show fluorescence (Figure 1, item h). Pomelo pulp reflected a purple hue under 395 nm black light but showed no color change under 365 nm (Figure 1, item i). Similarly, shrimp appeared normal under 395 nm black light but exhibited a pale-yellow glow under 365 nm black light, suggesting potential mold contamination (Figure 1, item t).

It is important to note that the appearance of mold on food surfaces when examined under black light at wavelengths of 365 nm and 395 nm can vary. Several factors, including the specific type of mold, the quantity of mold, and the inherent characteristics of each food type, can contribute to these observed variations. Contamination is generally easier to detect on foods with smooth and monochromatic textures, such as fruits, compared to foods with rough surfaces or a combination of colors, like baked goods, vegetables, and meat products. Further research is needed to explore these factors and gain a more comprehensive understanding of how mold appears on food surfaces under different black light wavelengths.

Altogether 100 food samples were analyzed, comprising 52 positive mold samples and 48 negative mold samples identified through a 20% KOH preparation under microscopic examination. The white light screening identified 17 items of mold contamination on food surfaces, while the black light at 365 nm and 395 nm detected 51 and 50 items of mold, respectively (Table 1). These findings highlight the superior performance of the black light in identifying a higher number of potential contamination items compared to white light.

Table 1. Data of suspected mold contamination on food surface by white light and black light at wavelengths of 365 nm and 395 nm.

Methods	Items marked as mold contamination on food surface (N=100)		
	Positive	Negative	%Positive (95%CI) ¥
Mold presence	52	48	52 (42.2-61.8)
White light	17	83	17 (9.6-24.4)
Black light 365 nm	51	49	51 (41.2-60.8)
Black light 395 nm	50	50	50 (40.2-59.8)

N, number of total food items in study; %, percentage; CI, confidence interval. ¥ Calculated via normal approximation to the binomial methods.

Table 2. Performance comparison of different light sources for screening mold contamination on food.

Mold contamination	White light (95%CI) ¥			Black light 365 nm (95%CI) ¥			Black light 395 nm (95%CI) ¥		
	Detected	Not detected	Total	Detected	Not detected	Total	Detected	Not detected	Total
Positive	TP 16	FP 1	17	TP 42	FP 9	51	TP 42	FP 8	50
Negative	FN 36	TN 47	83	FN 10	TN 39	49	FN 10	TN 40	50
total	52	48	100	52	48	100	52	48	100
Sensitivity	30.8 (21.7 - 39.8)			80.8 (73 - 88.5)			80.8 (73 - 88.5)		
Specificity	97.9 (95.1 - 100.7)			81.3 (73.6 - 88.9)			83.3(76 - 90.6)		
PPV	94.1 (89.5 - 98.7)			82.4 (74.9 - 89.8)			84 (76.8 - 91.2)		
NPV	56.6 (46.9 - 66.3)			79.6 (71.7 - 87.5)			80 (2.2 - 87.8)		
Prevalence (%Positive)	17 (9.6-24.4)			51 (41.2-60.8)			50 (40.2-59.8)		

Note: TP = true positive; FP = false positive; TN = true negative; FN = false negative; PPV = positive predictive value; NPV = negative predictive value, CI = confidence interval. ¥ Calculated via normal approximation to the binomial methods.

Sensitivity represents the ability of each light wavelength to accurately detect genuine mold contaminations. In this study, the black light exhibited a sensitivity of 80.8%, indicating that it correctly identified mold in 80.8% of samples that were genuinely contaminated. Conversely, white light had a substantially lower sensitivity of 30.8%. This discrepancy suggests that black light is significantly more effective than white light in identifying mold on food surfaces.

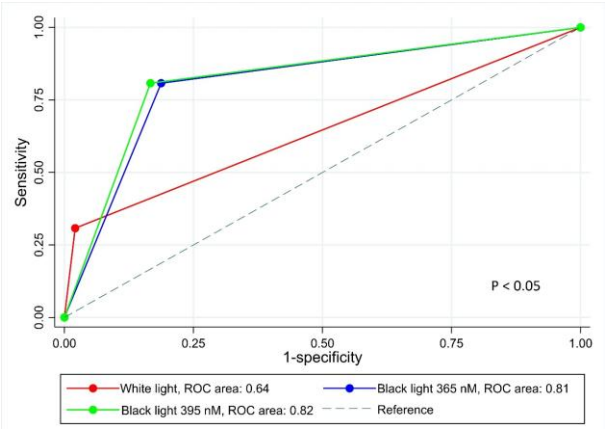
Specificity measures the ability of the different light sources to correctly identify food samples without mold contamination. All three wavelengths displayed high specificity, with white light recording the highest at 97.9%, and black light at 365 nm and 395 nm registering values of 81.3% and 83.3%, respectively. Thus, all light wavelengths effectively and accurately identified food samples that were free from mold contamination.

PPV indicates the probability that a food item identified as positive genuinely contains mold. White light demonstrated the highest PPV at 94.1%, suggesting its precision in mold detection. This heightened accuracy may stem from the fact that mold is typically detected on food surfaces under white light when the mold colony has grown larger, becoming more conspicuous. Thus, observing suspected mold on food under white light strongly suggests the presence of actual mold. In comparison, black light at both 365 nm and 395 nm had PPVs of 82.4% and 84%, respectively. This suggests that black light, while slightly less accurate than white light, remains a reliable method for identifying mold contamination on food surfaces.

NPV assesses the likelihood that a sample identified as negative truly does not contain mold. White light exhibited an NPV of only 56.6%, pointing to a higher chance of false negatives. Conversely, black light at both 365 nm and 395 nm demonstrated NPVs of 79.6% and 80%, respectively. This highlights the superior capability of black light to accurately confirm the absence of mold, minimizing the potential for false reassurances.

The statistical data strongly supports the assertion that black light demonstrates higher sensitivity, underscoring its proficiency in detecting mold contamination even at lower concentrations. Conversely, elevated PPV values of white light can be attributed to its effectiveness in identifying intense and larger mold colonies. While this characteristic of white light is beneficial for detecting well-established mold growths, it may inadvertently overlook early-stage or less pronounced contamination, posing potential health risks. Thus, solely relying on white light may not offer a comprehensive assessment of mold contamination. To ensure a more thorough evaluation, incorporating black light, especially at wavelengths of 365 nm and 395 nm, in conjunction with white light can significantly bolster mold detection capabilities, thereby enhancing food safety and safeguarding consumer health.

When comparing the area under curve (AUC) of the receiver operating characteristic (ROC) curves for all three types of light sources (Figure 2), the AUC values for white light and black light at wavelengths of 365 nm and 395 nm were found to be 0.64, 0.81, and 0.82, respectively.



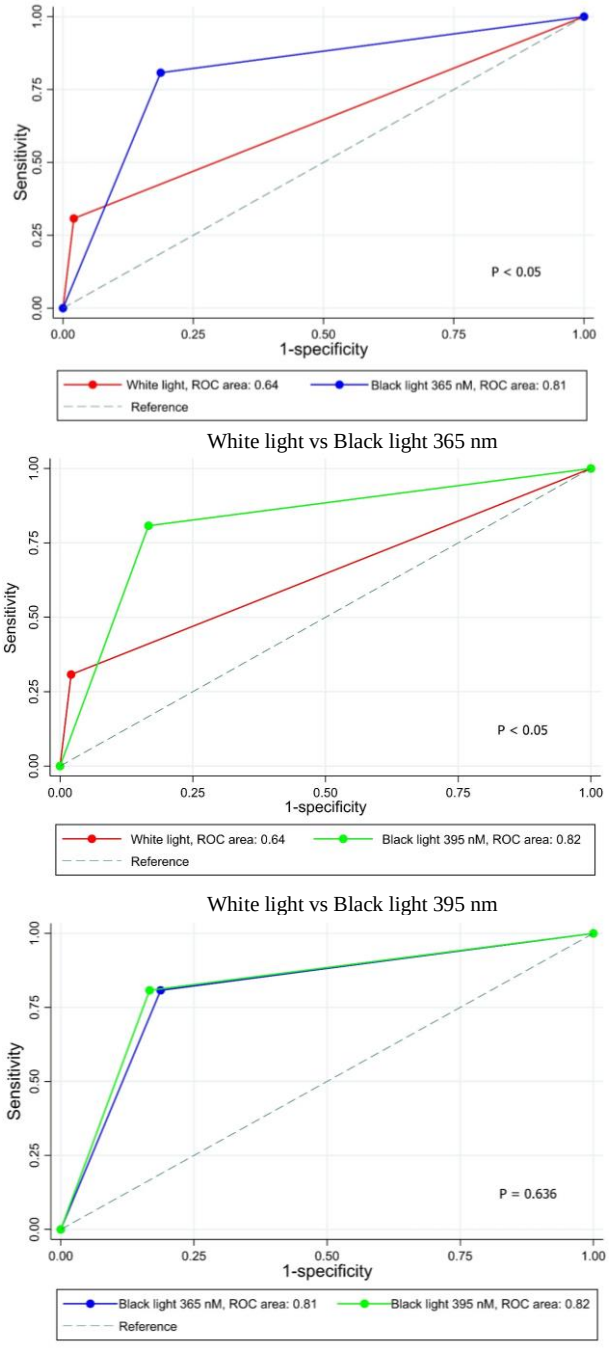
P, p-values for difference in Area under curve (AUC) of receiver operating characteristic (ROC) curve between two techniques using Pearson's chi-square test.

Figure 2. Receiver operating characteristic (ROC) curve for diagnostic sensitivity and specificity of mold contamination detection on food surfaces, categorized by light wavelengths

Differences were statistically significant ($P < 0.05$) when pairwise comparisons of the AUC values were made using the ROC curve data (Figure 3). Specifically, the performance of black light at both wavelengths was significantly superior to that of white light ($P < 0.05$). However, there was no significant difference between the AUC values of black light at the two wavelengths ($P = 0.636$).

The AUC analysis of the ROC curves underscores that black light at both 365 nm and 395 nm outperformed white light, as evidenced by their higher AUC values. This suggests better overall performance in distinguishing between contaminated and non-contaminated food samples. Notably, both wavelengths of black light demonstrated comparable performance in detecting mold contamination, with no significant difference observed in their respective AUC values.

The study results provide comprehensive insights into the efficacy of different light sources for mold detection on food surfaces. Sensitivity values indicate that black light, especially at wavelengths of 365 nm and 395 nm, excels in detecting even subtle mold contamination, underscoring its proficiency at lower concentrations compared to white light. Conversely, white light, with its higher true positive and PPV values, demonstrates a strength in identifying more pronounced mold colonies but may overlook early-stage or less visible contamination, posing potential health risks. Specificity across all methods remains high, suggesting reliable identification of mold-free food samples. The PPV values highlight white light's accuracy in confirming genuine mold when detected, while black light's superior NPV values imply a reduced likelihood of false negatives. Furthermore, the AUC analysis solidifies black light's superiority over white light, with both 365 nm and 395 nm wavelengths demonstrating enhanced performance in distinguishing between contaminated and uncontaminated samples. In conclusion, while white light offers precision in detecting visible mold colonies, incorporating black light, particularly at wavelengths of 365 nm and 395 nm, enhances sensitivity and overall mold detection capabilities, ensuring food safety and consumer protection.



P, p-values for difference in Area under curve (AUC) of receiver operating characteristic (ROC) curve between two techniques using Pearson's chi-square test.

Figure 3. Pairwise receiver operating characteristic (ROC) curve for diagnostic sensitivity and specificity of mold contamination detection on food surfaces

Findings of this study hold significant implications for food safety and public health. Given that mold

contamination can compromise the safety and quality of food products, early and accurate detection is crucial to prevent potential health risks to consumers. The study's demonstration of black light's superior sensitivity in detecting mold, especially at wavelengths of 365 nm and 395 nm, highlights the importance of utilizing advanced and sensitive screening methods in food inspection processes.

By incorporating black light alongside traditional methods like white light, food safety regulators and industry professionals can enhance their mold detection capabilities, ensuring that contaminated products are identified and removed from the supply chain before reaching consumers. This proactive approach not only safeguards public health by minimizing the ingestion of mold-contaminated food but also upholds the integrity and trustworthiness of the food industry. Thus, investing in and adopting sensitive screening tools like black light is paramount in maintaining high standards of food safety and protecting public health.

While the study provides valuable insights into mold detection on food surfaces using various light sources, its limitations must be acknowledged. The sample size used may not fully capture the diversity of mold contamination across different food types and conditions, potentially limiting the generalizability of the findings. Additionally, the study's focus on specific food types could overlook variations in mold growth and detection methods across a broader range of food products. Moreover, while the efficacy of black light in mold detection is highlighted, practical considerations such as equipment availability, cost-effectiveness, and required expertise for device operation may impact its widespread adoption in real-world settings. Future research should address these limitations to ensure the comprehensive and practical application of black light for enhancing mold detection in food safety protocols.

Future research endeavors could delve deeper into understanding the influence of environmental factors, such as temperature, humidity, and food storage conditions, on mold appearance and visibility under various light wavelengths. This exploration could provide valuable insights into optimizing mold detection techniques under different environmental settings, thereby enhancing the accuracy and reliability of detection methods. Additionally, investigating the feasibility of integrating black light technology into existing food safety protocols would be beneficial. This would entail assessing the practicality, cost-effectiveness, and ease of implementation of black light devices in routine food inspection processes. Understanding these aspects can pave the way for developing standardized protocols that incorporate black light technology, ultimately bolstering food safety measures and protecting public health.

5. Conclusions

Black light at wavelengths of 365 nm and 395 nm outperforms white light in detecting early mold contamination on food surfaces. Its higher sensitivity and comparable specificity make it a valuable tool for early mold screening. Findings emphasize the importance of further research to better understand the variations in mold growth appearance among different types of food. Incorporating black light sources can enhance the accuracy of mold detection methods and mitigate potential health risks associated with mold-contaminated food. Further research is needed to validate these findings and improve mold detection techniques on food surfaces.

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