Erciyes Üniversitesi
Enginoscope Dergisi
Cilt 1, Sayı 1, 2025

Erciyes University
Enginoscope Journal
Volume 1, Issue 1, 2025

AI-Assisted Fungal Infection Detection Using Impedimetric Odour Biosensors

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(Alınış / Received: 11.12.2024, Kabul / Accepted: 02.01.2025, Online Yayınlanma / Published Online: 31.01.2025)

Keywords
Fungal Infection,
Impedimetric Biosensor,
Electronic Nose,
Artificial Intelligence

Abstract: Yeast infections have been widely recognized and if no quick and accurate treatment method is applied, they can be very dangerous and might even turn into death. In comparison with old-fashioned diagnostic solutions such as Culturing, which takes around one to three days to reveal yeast infections, rapid and effective treatment is often not initiated. In the current study a novel method is offered involving the extraction of yeast fungal strain identification in a rapid, cost-effective, and accurate way. Through the application of a gelatin-based hydrogel coating that represents the way in which odor receptors attach to cells a sensing concept for impedimetric odor was constructed. The hydrogel was further improved by adding glycerol for its structural stability and graphite powder for its better conductivity. The process of making a sensor involved applying the modified hydrogel to wires made of copper. The sensor was then exposed to the odor molecules from culture tests of *Candida albicans, Candida glabrata, and Candida tropicalis*, which were placed in a controlled environment. Changes in impedance took place, and these measurements were analyzed using a Random Forest machine learning algorithm that helped to get 94% classification success. This new testing process may lead to a revolution in the era of clinical diagnostics. It will enable speediness, simplicity, as well as precision in the detection of yeast fungal infections, which, in turn, will decrease health risks leading to unnecessary treatment costs by approved drug companies.

İmpedimetrik Koku Biyosensörü ile Yapay Zekâ Destekli Mantar Enfeksiyonu Tespiti

Anahtar Kelimeler
Mantar Enfeksiyonu,
İmpedimetrik Biyosensör,
Elektronik Burun,
Yapay Zeka

Öz: Mantar enfeksiyonları oldukça yaygın olmakla birlikte, hızlı ve doğru bir tedavi yöntemi uygulanmazsa çok tehlikeli sonuçlara ve hatta ölüme bile yol açabilmektedir. Mantar enfeksiyonunu teşhis etmek için günümüzde kullanılan, yaklaşık bir ila üç gün sürebilen kültürleme gibi eski moda teşhis çözümleri hızlı ve etkili tedavi imkanını kısıtlamaktadır. Çalışmamızda hızlı, uygun maliyetli ve doğru bir şekilde mantar suşlarının tespit ve sınıflandırılmasını içeren yeni bir yöntem sunulmaktadır. Koku reseptörlerinin, koku molekülleri ile bağlanmasını taklit eden jelatin bazlı bir hidrojel kaplamanın iletken bir bakır tele uygulanmasıyla, koku tespiti için impedimetrik bir algılama konsepti oluşturulmuştur. Hidrojel elektrolit, yapısal kararlılığı için gliserol ve daha iyi iletkenliği için grafit tozu eklenerek daha da geliştirilmiştir. Sensör fabrikasyonu ise modifiye edilmiş hidrojelin, bakırdan yapılmış tellere uygulanması ile yapılmıştır. Sensör yapısı kontrollü bir ortama yerleştirilen *Candida albicans, Candida glabrata ve Candida tropicalis* kültür testlerinden gelen koku moleküllerine maruz bırakılmıştır. Hidrojel yapısında meydana gelen empedans değişikliklerinin Random Forest makine öğrenme algoritmasıyla sınıflandırılmasıyla %94 sınıflandırma başarısı ile enfeksiyonlar tespit edildi. Bu yeni koku biyosensörü, yerinde teşhis çağında bir devrime yol açabilecek potansiyele sahiptir. Mantar enfeksiyonlarının hızlı, basit ve hassas şekilde tespiti ve sınıflandırılması ile gereksiz tedavi maliyetleri düşürülecek ve sağlık riskleri azaltılacaktır.

1. Introduction

A fungal infection is a health problem caused by fungal species such as Candida and Aspergillus. In individuals with weakened immune systems, these infections can be more serious and have a systemic effect. For instance, HIV/AIDS patients, cancer patients receiving chemotherapy and individuals exposed to long-term antibiotic use are particularly at risk [8].Furthermore, Fungal infections have become a major health problem worldwide [11]. with a significant prevalence reported in immunocompromised individuals [11]. reported that HIV/AIDS patients, organ transplant recipients and individuals exposed to long-term steroid use are at a higher risk of fungal infections. A study from developing countries shows that fungal infections due to Candida species are particularly high [1]. Candida is a common fungus that can cause infections of the skin, mouth, nails and genitals [1]. Such infections are more common in individuals with weakened immune systems and can lead to serious complications [11].

A number of methods are used in the diagnosis of fungal infections and this process involves several challenges. Diagnosis Typically includes an accurate assessment of symptoms, examination of the clinical picture and the use of laboratory tests [15] . Especially in immunocompromised patients, symptoms can often be vague, making diagnosis difficult [15]. Clinical assessments include taking the patient's history and physical examination, as well as identifying symptoms, which vary depending on the specific type of fungus [22] . However, these methods alone are not sufficient and laboratory tests are often added to the diagnostic process.

Laboratory diagnosis of fungal infections usually involves microscopy, culture and molecular methods [22]. In some cases, extra methods such as microscopy and culture are time-consuming and the results are sometimes uncertain [23]. Furthermore, the specific diagnosis can be further complicated by the more serious nature of the infection, especially in immunocompromised patients [23]. Despite these challenges, accurate and rapid diagnosis of fungal infections is important for effective treatment and disease control. Research is focusing on improving diagnostic methods and developing new diagnostic tools, which could lead to the development of more effective control strategies against fungal infections.

In recent years, there have been significant developments in the methods used in the diagnosis of fungal infections and innovative approaches in this field have come to the forefront [5]. In particular, molecular biology and genetics-based diagnostic methods have emerged as an alternative to traditional microbiological methods [7]. Molecular diagnostic methods are usually based on the identification of fungal DNA and allow for a faster and more accurate recognition of the disease[5]. PCR (Polymerase Chain Reaction) based methods provide high sensitivity in identifying specific causes of disease [17]However, these methods often have challenges, such as being expensive and requiring expertise[7]. Furthermore, rapid diagnostic tests developed as an alternative to culture-based methods have also made significant progress[18]. These tests allow for faster diagnosis of the infection, but may have some limitations in identifying specific fungal species [27]. Challenges of new diagnostic methods include factors such as lack of standardization, cost, and widespread applicability [25]. Therefore, further research and development is needed before these techniques can be used effectively in clinical practice.

In addition to traditional methods for diagnosing fungal infections, electronic nose technologies have gained attention for their potential to provide rapid and accurate diagnoses [28]. These artificial sensory systems detect specific odor profiles of biological materials. A major challenge in this field is the cost of applying a bio-sensing surface specific to odor molecules to simulate the olfactory mechanism. Gelatin, an amino acid-rich protein, can simulate the amino acid content of olfactory receptors, making it a suitable artificial odor receptor [12]. Studies indicate that odor molecules bind to odor receptors via ionic and hydrogen bonds, mediated by functional groups on amino acids at the binding site [2].

Electronic noses can identify specific markers of fungal infections by detecting metabolic products or volatile organic compounds, offering faster results than traditional microbiological culture techniques [24, 20]. However, these methods face limitations in detecting a broad spectrum of fungi, lack standardization, and are costly, hindering widespread adoption [20, 16]. Further research is needed to improve standards, specificity, and cost-effectiveness.

Electronic nose (eNose) technologies offer non-invasive, rapid, and real-time diagnostics by analyzing complex odor profiles, making them ideal for disease detection and monitoring. Unlike traditional tools, eNoses utilize sensor arrays and pattern recognition algorithms to identify a wide range of volatile organic compounds (VOCs) and physiological indicators. Their applications span food quality, environmental monitoring, and medical diagnostics. While challenges like selectivity, humidity sensitivity, and achieving human-nose-level sensitivity remain, advancements in sensor technology and intelligent algorithms promise broader clinical integration and enhanced diagnostic precision[30].

AI integration has advanced electronic nose methods for fungal infection diagnosis, addressing specificity issues in distinguishing similar odors [29]. AI algorithms enhance the ability of electronic noses to identify characteristic odors of specific fungal infections, increasing diagnostic sensitivity [26, 11]. For instance, machine learning algorithms have demonstrated improved discrimination of *Candida* species compared to other microorganisms [3]. Although promising, this field requires further development to address challenges such as standardization, scalability, and differentiating between fungal types.

Building on the potential of AI in enhancing electronic nose specificity, recent research highlights the efficiency of classification algorithms like Random Forest (RFC) in handling imbalanced datasets. RFC has demonstrated superior performance in classifying binary and multi-class datasets, particularly in mushroom classification tasks. Studies reveal that RFC outperforms other decision tree-based algorithms, such as Reduced Error Pruning (REP) Tree, in terms of accuracy, precision, and sensitivity [19, 22]. Ensemble methods, including Bagging, Boosting, and RFC, also show that RFC consistently delivers the highest accuracy in determining mushroom edibility and toxicity [23].

The focus of this project is to classify these different environments with machine learning algorithms (Random Forest and K-Nearest Neighbor), using as data the impedance differences caused by three different yeast fungi and air environments in the impedimetric odor sensor. This classification focuses on the detailed analysis of the features of the samples and the accurate discrimination between different classes. It is also aimed to compare the performance of these two algorithms to determine which one performs better in classification[4].

The aim of the project is to diagnose fungal infection by measuring the sensor impedance change caused by the interaction of odor molecules, which occur as a result of fungal infection and exhibit different molecular structure according to the type of fungus causing the infection, with the impedimetric odor sensor created with copper wires coated with gelatin-based coating, and classifying these data with artificial intelligence.

Since different odor molecules bind to the gelatin-based layer in different ways, the electrical properties and therefore the impedance of the copper wire and the gelatin-based layer on it were different. Taking advantage of these differences, the impedance of the copper wire with the gelatin layer was measured with an impedance analyzer capable of applying oscillatory signals with different frequencies in the range of 0.1–1 MHz, and the data were classified by artificial intelligence to classify the type of mushrooms with different odors.

2. Material and Methods

This study aims to develop an impedance-based method for the classification of yeast species (*Candida albicans, Candida tropicalis, Candida glabrata*, and air) By using AI Tools and machine learning models. The methodology involves the preparation of gelatin-based hydrogel coatings, impedance measurement using an AIM4300 impedance analyzer, feature extraction and selection, and the setup of machine learning models (k-NN and Random forest) for classification.

2.1. Gelatin-Based Hydrogel BioSensor Preparation

The gelatin-based hydrogel coating was prepared using a simple method. 1 g gelatin (Dr. Oetker, 100% Bovine Gelatin), 1 ml glycerol (Merck, Vegetable origin) and 0.5 g graphite powder were mixed in 10 ml distilled water at 40°C and 70 wires cut from copper wires with a copper diameter of 0.25 mm and a length of 10 cm were soaked in the gelatin-based mixture for 2 minutes. After this process, the gelatin-based coated wires were suspended to dry at room temperature and dried for 1 day.

2.2. Yeast Fungus Samples

The study utilized yeast samples from the Candida genus, including *Candida albicans (CA), Candida tropicalis (CT) and Candida glabrata* (CG) strains. The samples were obtained from Erciyes University Medical Faculty Medical Microbiology Laboratory in culture medium in petri dishes. The relevance of these strains lies in their clinical significance as pathogens, making them suitable for developing diagnostic techniques through impedance spectroscopy.

2.3. Impedance Measurement:

We used the AIM4300 impedance analyzer for impedance measurements. In each petri dish, copper wires with gelatin-based coating suspended in air were exposed to this environment for 5 minutes, provided that they did not touch the yeast fungi culture and the petri dish was closed. A total of 67 impedance measurements were then made, 26 for CA, 13 for CT, 13 for CG and 15 measurements in the empty petri dish. Impedance measurements were performed with an impedance analyzer generating a 1 V oscillatory AC signal with a frequency increment of 1 kHz in the frequency range of 100 kHz - 1 MHz. In each impedance measurement, resistance, reactance and phase angle values were recorded for 1000 different frequency values. In total, 67 different impedance measurements were made with 67 different wires. Figure 1 shows the measurement system.

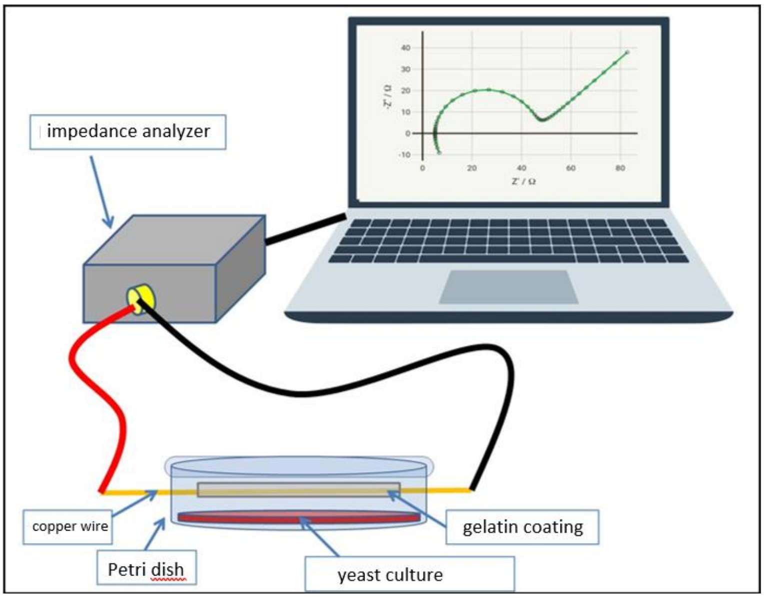

Figure 1. Impedance measurement system of copper wire with gelatin-based coating

Environmental conditions such as temperature and humidity are critical factors that can interfere with impedance measurements by altering the sensor's electrical properties and the behavior of odor molecules. In our study, since it was conducted in a controlled laboratory setting, temperature and humidity were maintained at stable levels to minimize any potential interference. This controlled environment ensures that the results primarily reflect the sensor's interaction with the target molecules rather than environmental variability.

However, for clinical or field applications, where environmental factors cannot always be controlled, it is essential to characterize and understand their impact on impedance measurements. Before transitioning to clinical applications, further studies should be conducted to evaluate the sensor's performance under varying environmental conditions and to develop methods to compensate for or mitigate such interferences. For this study, the focus remained on demonstrating the sensor's performance under ideal, controlled conditions rather than addressing these environmental challenges.

2.4. Feature Extraction and Selection

The Impedance data collected from yeast fungus samples and air was processed to find important features needed for classification. Each .csv file held 1000 data points. These data points matched different frequency values. They included parameters like phase angle, resistance and reactance. These features represent the basic resistance properties of the fungus types and the air samples.

The raw data files were read sequently using Python's pandas library. The data then converted into DataFrames for preprocessing. to ensure consistency, numbers with commas as decimal separators were standardized to dots. They converted into floating-point numbers. Subsequently, the data from each species (*Candida albicans, Candida glabrata, Candida tropicalis, and air*) were merged into distinct NumPy arrays for efficient processing. These arrays were combined into a single dataset (X) to enable comparative analysis of impedance features across classes.

Feature selection was implicitly applied by focusing on the most relevant impedance parameters: phase angle, resistance, and reactance. This approach ensured that only the critical attributes required for machine learning were retained while reducing redundancy. Additionally, a label vector (Y) was generated, categorizing the samples into four classes: *Candida albicans, Candida glabrata, Candida tropicalis, and air*. This step organized the dataset for subsequent classification and analysis, facilitating the effective application of machine learning algorithms.

This streamlined approach to feature extraction and selection enabled the creation of a structured dataset that encapsulated the distinguishing characteristics of the fungal species and air, forming the foundation for the classification task.

SMOTE (Synthetic Minority Oversampling Technique): The data augmentation method creates synthetic samples to increase the number of samples in the minority class. Instead of randomly copying minority instances, this technique generates new instances using the neighborhoods found between minority class instances. SMOTE is a frequently used balancing method with different studies and modifications. Traditional SMOTE creates synthetic samples by generating random samples between minority class instances and their nearest neighbors (Liu et al., 2024). As can be seen in Figure 2, the dataset is balanced with the SMOTE technique. [23].

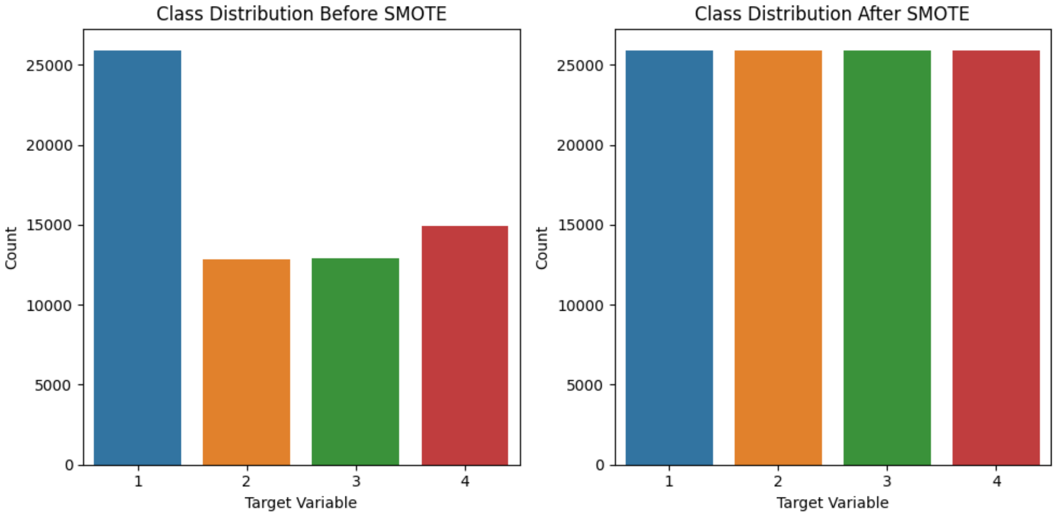

Figure 2. Equalizing the data distribution of classes, before and after SMOTE

2.5. Proposed Methods:

The classification model selection focused on using Random Forest and K-Nearest Neighbor (K-NN) classifiers because of their effectiveness with high-dimensional data and their robust handling of noise and overfitting. Here's a detailed explanation of each of the used models:

2.5.1. Random Forest Classifier:

Random Forest is a learning method that builds many decision trees during training. It then chooses the class that appears most often for classification or the average prediction for regression from these trees. Each tree uses different parts of the data and different features are picked randomly at each decision point.[4]

Experts selected the Random Forest model for its strong ability to work with large and complex datasets. Our impedance data fits this description. This model works well with high-dimensional data. It reliably classifies information even when there is noise or many unnecessary features. The model reduces overfitting by averaging predictions from various trees. Overfitting becomes less of an issue through this process[14].

2.5.2. K-Nearest Neighbor (K-NN) Classifier:

K-NN uses simple rules to learn by examples. It guesses the group of a new item by checking nearby data points. The algorithm finds its k nearest neighbors, where k is a number set by users. The new item gets the group that most of these neighbors belong to[10].

K-NN is often picked because of its simplicity and strength in recognizing patterns. It does not need any special modeling rules. This is very helpful with data that have tricky boundaries that are hard to separate using straight lines. In our study, the dataset is small. K-NN probably is a straightforward choice for grouping samples by likeness[10].

2.6. Set-up the Models:

Two classification models, Random Forest and K-Nearest Neighbor (K-NN), were used for the task. The dataset split into training and testing sets with a stratified 80-20 ratio. The class proportions stayed consistent across both sets.

Random Forest Model:
The Random Forest classifier used 100 estimators. Hyperparameter tuning happened with Grid Search Cross-Validation to find the best settings like the number of estimators and the maximum depth of decision trees. This method was chosen for its ability to work with high-dimensional data and very effectively deal with noise. It really helps stop overfitting with its ensemble method.

K-NN Model:

The K-NN algorithm checked how well it worked by testing different values of K, which is the number of neighbors it looks at for sorting. First, k=1 was used. Cross-validation then helped find the best K value. Confusion matrices showed how many correct and incorrect guesses the model made for each type. To improve the KNN model's performance, hyperparameter tuning was implemented by testing a range of k values, to determine the optimal number of neighbors for the model. This process involved iteratively training the KNN model with different k values, predicting outcomes on the test set, and calculating the accuracy for each configuration. The k value that produced the highest accuracy was selected as the optimal parameter, ensuring the model's performance was maximized. [32].

Training Process:
The models went through training and checking many times to improve their performance and get better accuracy on the test group. Metrics like accuracy, precision, recall, F1-score and confusion matrix used to judge

how well the model told different fungal species apart using the impedance data. It really gave a full look at how the classifiers worked.

3. Results

3.1. Random Forest Classification Results

The classification report shows the accuracy, precision, recall and F1-score of the model for each class. In this case, the weighted average accuracy was 0.94 (94%), indicating a very high accuracy of classification. Precision indicates how accurate the prediction of a particular class is in the cases where it is predicted. For example, the precision for class 0, CA, is 97%. 97% of the instances predicted by the model in class 0 actually belong to class 0 (CA). This report shows that our model performs well overall, with high precision, sensitivity and F1-score, especially in classes 0 (CA) and 2 (CT). However, there is some accuracy degradation in the 1st (CG) and 3rd (Air) classes, and these classes show lower performance.

Table 1 shows the classification report of the Random Forest model and Figure 3 shows the confusion matrix.

Table 1. Classification report obtained from random forest model

	Precision	**recall**	**F1-score**	**support**
Class 0	**0.971445**	**0.969549**	**0.970496**	**5123**
Class 1	**0.863402**	**0.907157**	**0.884739**	**2585**
Class 2	**0.975126**	**0.971727**	**0.973424**	**2582**
Class 3	**0.926511**	**0.892196**	**0.909030**	**3024**
Accuracy			**0.94**	**13314**
Macro avg	**0.93**	**0.94**	**0.93**	**13314**
Macro avg	**0.94**	**0.94**	**0.94**	**13314**

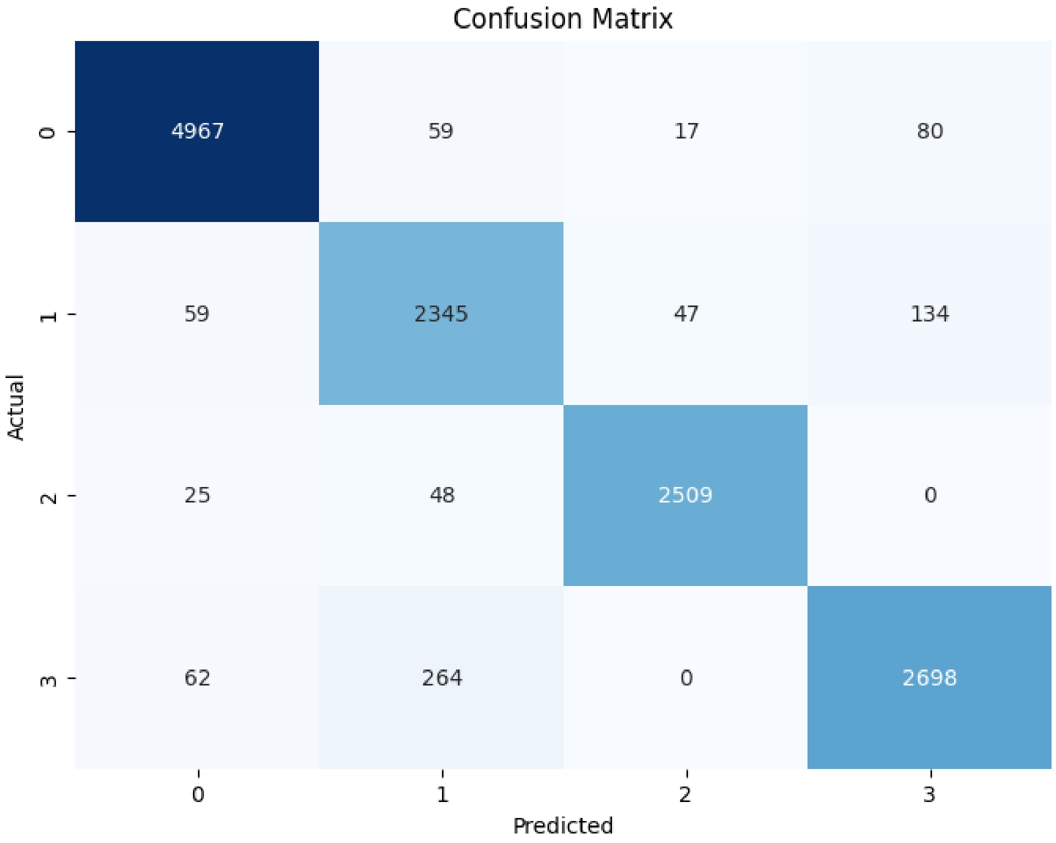

Figure 3. Confusion matrix in the random forest classification model

After hyperparameter tuning with the Grid research method, we also observed some small improvements. In particular, we can see slight changes in the precision and recall values for some classes. For example, for class 1 (CG), we observe an increase in recall and a decrease in precision (Table 2).

Table 2. Random Forest classification report after Grid search method

	Precision	**recall**	**F1-score**	**support**
Class 0	**0.97**	**0.97**	**0.97**	**5123**
Class 1	**0.85**	**0.93**	**0.89**	**2585**
Class 2	**0.98**	**0.97**	**0.97**	**2582**
Class 3	**0.94**	**0.88**	**0.91**	**3024**
Accuracy			**0.94**	**13314**
Macro avg	**0.94**	**0.94**	**0.94**	**13314**
Macro avg	**0.94**	**0.94**	**0.94**	**13314**

From these findings, we conclude that the base case of our Random Forest classification model performs reasonably well and can be slightly improved by hyperparameter tuning, but that these improvements do not make a significant difference.

3.2. K-NN Classification Results:

Table 3. Classification report obtained from the K-NN model

	Precision	**recall**	**F1-score**	**support**
Class 0	**0.89**	**0.90**	**0.90**	**5123**
Class 1	**0.75**	**0.79**	**0.77**	**2585**
Class 2	**0.94**	**0.97**	**0.97**	**2582**
Class 3	**0.83**	**0.79**	**0.81**	**3024**
Accuracy			**0.86**	**13314**
Macro avg	**0.85**	**0.85**	**0.85**	**13314**
Macro avg	**0.86**	**0.86**	**0.86**	**13314**

Considering the weighted average accuracy in Table 3, 0.86 (or 86%) seems to be a high accuracy, but it is low compared to Random Forest. Although it performs particularly well for class 0 (CA) and class 2 (CT), it performs relatively poorly for class 1 (CG) and class 3 (Air).

When the confusion matrix in Figure 4 is examined, it is seen that misclassifications increase.

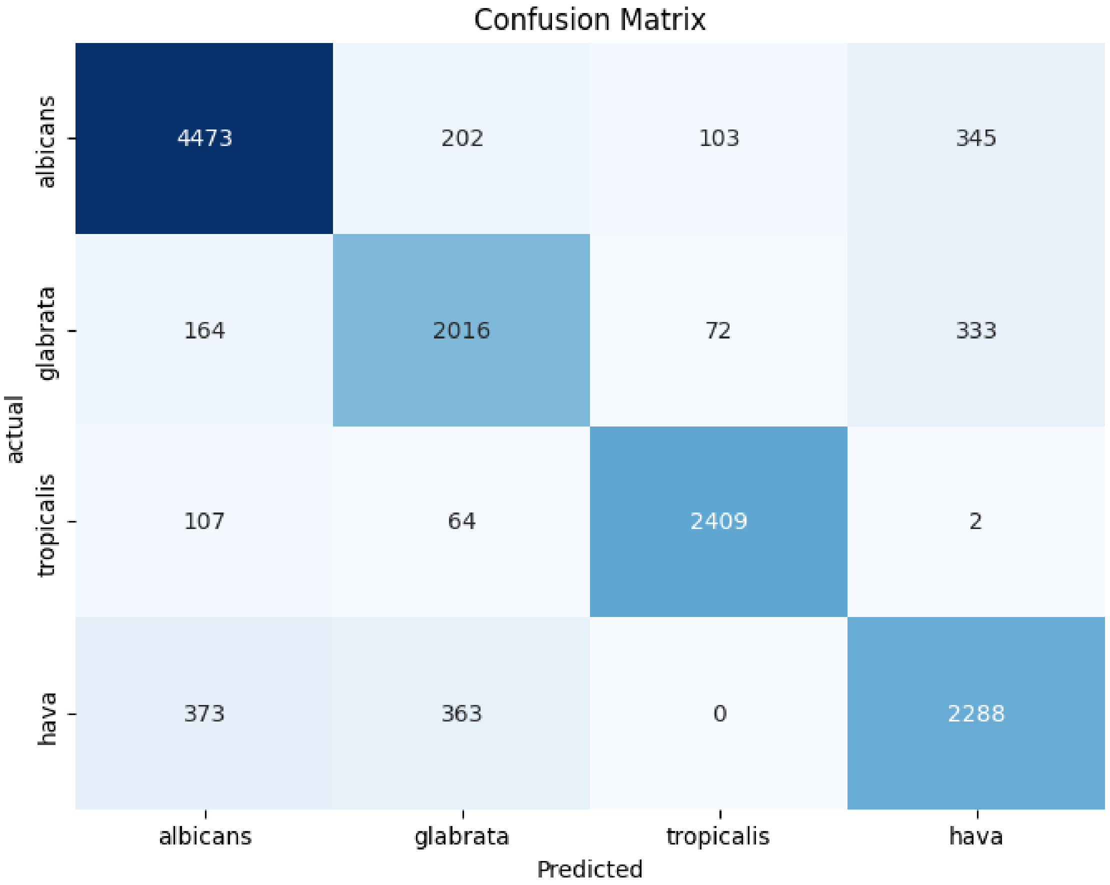

Figure4. Confusion matrix of the KNN classification model

Again, after hyperparameter tuning with the Grid Search method, we couldn't observe any significant improvements beyond small and statistically insignificant differences. Here, we can conclude that the k-nearest neighbor (KNN) algorithm struggled to enhance classification performance due to its inherent limitations, particularly with imbalanced or complex datasets [31].

4. Discussion and Conclusion

The results obtained show promise for predicting which strain of yeast fungus is responsible for infections caused by yeast fungi and for making accurate diagnoses. Considering the simplicity, cheapness, implementation time and speed of the sensor, as well as the accuracy of the classification system, it is thought to have the potential to be used in determining the type of yeast fungal infections and even in other applications where similar electronic nose applications would be advantageous[14,30]. The results obtained for the performance of Random Forest and KNN models reflect the different advantages and disadvantages of each model.

Random Forest:

- Random Forest is a powerful model that generally gives very good results when configured correctly and is resistant to overfitting in many cases.
- Our results show that the Random Forest model has high accuracy and good performance in general. In particular, the high precision and sensitivity values for class 0 (CA) and class 2 (CT) demonstrate the model's ability to accurately predict these classes.
- However, it is also observed that the performance for class 1 (CG) and class 3 (Air) is relatively poor compared to the other classes. However, given the limited number of training data sets, the model can be improved.

KNN:

- KNN is a model characterized by its simple structure and easy comprehension. However, these advantages apply to large data sets.
- The performance of KNN is lower than that of Random Forest. In particular, the low precision and sensitivity values for class 1 (CG) and class 3 (Air) indicate that the model tends to mispredict these classes.

A complex model like Random Forest performed quite well. This is because Random Forest is generally a model that can give good results in large datasets by taking into account the interactions of different features and is resistant to overfitting. In general, our results emphasize that the Random Forest model has high accuracy rates and good performance and show that with this sensor system and classification model, fungal strains that cause yeast infection in humans can be diagnosed by odor[04].

Optimization of the sensor system was not performed within the scope of this project. With optimization, electronic nose biosensors can be developed to obtain more accurate and sensitive results by increasing the type and size of the conductive material as well as the quality and specificity of the gelatin-based coating.

By increasing and diversifying the volume of the data set, the accuracy and precision in the classification phase can be increased. In addition, testing with different classification models will be beneficial in determining the most appropriate result.

To adapt this sensor and modeling system for clinical use, several enhancements are essential. First, the sensor requires optimization to achieve clinical-grade performance by improving the specificity and sensitivity of the gelatin-based hydrogel coating, potentially through advanced conductive materials or hybrid biocompatible coatings tailored for fungal detection. The system must also be miniaturized and integrated into portable devices for point-of-care diagnostics[34]. Adherence to clinical regulatory standards, such as ISO 13485, is crucial to ensure safety, efficacy, and reproducibility[33]. Expanding the dataset to include diverse patient samples and fungal strain variants will enhance the model's robustness and generalizability. Furthermore, the classification models should undergo rigorous validation on large, independent clinical datasets to ensure reliable performance. The system interface should be user-friendly, with automated reporting for clinicians, and it should integrate seamlessly with hospital electronic health records (EHR) for efficient data logging and management. Finally, ensuring cost-effectiveness in manufacturing and operation is vital for widespread adoption in healthcare settings. Addressing these aspects will enable this biosensor and modeling system to become a viable clinical tool for diagnosing yeast fungal infections and other applications.

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