Ferdowsi University of Mashhad

Iranian Food Science and Technology Research Journal

Iranian food science and technology Association

Vol.21 No.3

2025

ISSN:1735-4161

Contents

Iranian Food Science and Technology Research Journal

Vol. 21 No. 3 2025

Published by: Ferdowsi University of Mashhad, (College of Agriculture), Iran

Executive Manager: N. Shahnoushi, Department of Agricultural Economics, Ferdowsi University of Mashhad, Iran

Editor-in-Chief: M. Yavarmanesh, Department of Food Science and Technology, Ferdowsi University of Mashhad, Iran

Publisher Ferdowsi University of Mashhad

Address: College of Agriculture, Ferdowsi University of Mashhad, Iran

P.O.BOX: 91775- 1163

Fax: (98)051-38787430

E-Mail: ifstrj@um.ac.ir

Web Site: https://ifstrj.um.ac.ir

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Iranian Food Science and Technology Research Journal
Homepage: https://ifstrj.um.ac.ir

Research Article
Vol. 21, No. 3, Aug.-Sep. 2025, p. 261-269

Classification of Iranian Wheat Flour by FT-MIR Spectroscopy based on Max-Relevance Min-Redundancy Wavelength Selection Coupled with SVM

A. Kazemi[1*], A. Mahmoudi[1], S.H. Fattahi[2]

1- Department of Biosystems Engineering, University of Tabriz, Tabriz, Iran
(*- Corresponding Author Email: amirkazemi422@gmail.com)
2- Department of Biosystems Engineering, University of Maragheh, Maragheh, Iran

Received: 22.06.2024 Revised: 01.11.2024 Accepted: 20.11.2024 Available Online: 17.06.2025	**How to cite this article:** Kazemi A., Mahmoudi, A., & Fattahi, S.H. (2025). Classification of Iranian wheat flour by FT-MIR spectroscopy based on max-relevance min-redundancy wavelength selection coupled with SVM. *Iranian Food Science and Technology Research Journal, 21*(3), 261-269. https://doi.org/10.22067/ifstrj. 2024.88624.1342

Abstract

Different varieties of wheat as one of the strategic crops are cultivated in Iran based on the specific geographical and climatic conditions of each area. Classification of wheat varieties is important in order to guarantee the final products acquired from wheat flour. Fourier Transform-Mid Infrared (FT-MIR) spectroscopy as a nondestructive approach combined with chemometrics was employed to classify four varieties of Iranian wheat. 160 samples were analyzed and various preprocessing algorithms were used to correct unwanted information. Then, Principal Component Analysis (PCA) as unsupervised and Support Vector Machine (SVM) as supervised models with Max-Relevance Min-Redundancy (MRMR) feature selection algorithm were applied to investigate the classification of these varieties. The best result of SVM model without feature selection was with S-G+D2+MSC preprocessing with 99.4% of accuracy. The output of 100% with SVM model and MRMR feature selection algorithm confirmed the capability of FT-MIR spectroscopy method for classification of Iranian wheat flour varieties.

Keywords: Classification, FT-MIR spectroscopy, PCA, Preprocessing, Wheat flour

Introduction

Wheat stands out as the most commonly used cereal due to its high carbohydrate content, providing enough calories to satisfy both human and animal needs on a daily basis. Several factors contribute to the differences in bread wheat flour, such as the type of wheat variety, the growing conditions, and the environmental conditions. These factors vary between regions and from year to year, impacting the quality of flour. Therefore, if various types of wheat are intentionally combined, variations and inconsistencies can lead to variations in the quality of the resulting wheat flour and it is important to assess these factors before and during the trading process to ensure the quality of the wheat flour. Unfortunately, mixing varieties from different wheat classes can occasionally occur during storage, which cause potentially lower quality and is the primary concern for both buyers and sellers due to the fact that the product may not meet the specifications and expectations when delivered. Therefore, it is important to evaluate the uniformity and consistency of the wheat varieties before they are utilized in food processing facilities. Achieving the correct wheat quality will minimize the low crop output and boost production, that is the primary

https://doi.org/10.22067/ifstrj. 2024.88624.1342

objective for profitable wheat production. In Iran, the authentication of various wheat flour varieties, such as Baran, Homa, Sardari, Hashtrood, and Sadra, is a significant concern due to their popularity and productivity. The yield of such varieties ranging from 1200-1600 kg per hectare in the northwest region of the country (Khojastehnazhand & Roostaei, 2022).

Currently, various traditional techniques, including chemical and mechanical methods, were utilized for classification. However, these approaches had several drawbacks, such as being destructive, expensive, and time-consuming. As a result, there is a need for alternative methods that are non-destructive, cost-effective, and efficient. Vibrational spectroscopies such as Fourier Transform-Infrared (FT-IR) spectroscopy are commonly chosen for authenticating flour varieties due to their non-destructive nature, affordability, and user-friendly application. The FTIR method is a reliable tool for identifying biochemical fingerprints, allowing for the detection of trace compounds in intricate food compositions with subtle differences between closely related samples. Additionally, this technique provides precise measurement capabilities and efficient screening processes (Deniz *et al*., 2018; Ellis, Muhamadali, Haughey, Elliott, & Goodacre, 2015). In FTIR spectra, the positions of bands are associated with structural information, whereas the areas of the bands indicate the concentration of specific molecules. This information can be valuable for examining food samples based on their contents of proteins, carbohydrates, lipids, and nucleic acids (Deniz *et al*., 2018).

After data acquisition, it is essential to establish a link between the targeted sample characteristics and the IR absorption or transmittance measurements. Chemometrics is the keystone that utilizes spectral data alongside pattern recognition methods in order to efficiently tackle authentication challenges in products. The combination of FT-IR and chemometrics has been applied in numerous studies. For example, Wadood et al. (2019) utilized NIR spectroscopy combined with chemometric methods to classify wheat based on its production year, geographical origin, and genotypes. The results of LDA model indicated classification accuracies of 69% for production year, between 72.2% and 100% for geographical origin, and 69% for genotypes (Wadood, Guo, Zhang, & Wei, 2019).

In another study, De Girolma et al. used FT-NIR and FT-MIR techniques to evaluate identification of durum wheat pasta adulterated with common wheat. The results of LDA and PLS-DA models to classify samples had acceptable classification accuracy ranged from 80% to 95% for three groups, and from 91% to 97% for two groups (De Girolamo *et al*., 2020).

In another research, FT-IR spectroscopy combined with chemometrics were applied to detect the adulteration of blankit in wheat flour. By evaluating various preprocessing techniques with different models, the SVM model achieved the highest classification accuracy, attaining 100% for two classes (pure and adulterated) and 79.62% for five classes (pure and adulterated at various adulteration levels). This demonstrates the effectiveness of FT-MIR spectroscopy combined with chemometric methods for identifying fraud in wheat (Kazemi, Mahmoudi, & Khojastehnazhand, 2023).

The aim of this research is to classify four major varieties of Iranian wheat flour through Fourier Transform-Mid Infrared (FT-MIR) spectroscopy combined with chemometric analysis. In this study, the Max-Relevance Min-Redundancy (MRMR) algorithm was used to assess the performance of feature selection with SVM model to develop a robust classification method for Iranian wheat flour varieties. This methodology can enhance quality control, prevent mislabeling, and address food fraud, thus supporting the Iranian wheat industry in meeting stringent quality standards and reinforcing consumer confidence.

Materials and Methods

After preparing the sample classes and getting spectral data from each sample, various data preprocessing methods were applied to remove unwanted effects on acquired spectral data. Then firstly, PCA analysis was performed for clustering and SVM model without application of feature selection method was created. MRMR feature selection algorithms were then applied to compare the outcomes. The flowchart of steps for classification of Iranian wheat flour is shown in Fig. 1.

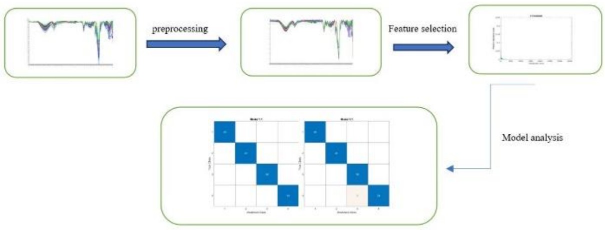

Fig. 1. The flowchart of wheat flour classification using FT-MIR spectroscopy approach

Data acquisition

After getting wheat seed varieties including (Baran, Hashtrood, Homa, Sadra) from dryland agricultural research institute in Maragheh, Iran, some initial processes including removing impurities from samples, milling the seeds with benchtop mill, and passing from sieve (420µm) were done to acquire pure flour samples of wheat. Then 40 samples of each variety were transferred to the laboratory in microtubes. All flour samples were then scanned by an FT-MIR spectrometer (Perkin Elmer, USA) equipped with an Attenuated Total Reflectance (ATR). Approximately 50mg of each sample was loaded on the ATR crystal and pressed until the signal intensity could be measured. The acquired spectra were in transmission mode and in the range of 400-4000 cm^{-1} with a resolution of 0.5 cm^{-1}.

Preprocessing of Spectral Data

Data were preprocessed before being explored or classified. Dealing with uninformative spectra due to light scattering or system noise is a key aspect of spectral data analysis (López-Maestresalas *et al*., 2019). There are 2 primary categories of preprocessing methods in NIR spectroscopy: spectral normalization and spectral derivatives. Multiplicative Scattering Correction (MSC) and Standard Normal Variate (SNV) are the most common used algorithms used to correct the scatter effects from spectral data. Savitzky-Golay derivative is one of the most common algorithms which is used to smooth the spectra and eliminate the useless changes in signals (Holden, Wolfe, Ogejo, & Cummins, 2021; Sacré *et al*., 2014). In this study, the combination of S-G smoothing (window size of 15 points), first and second derivatives, and MSC algorithms were applied. All of preprocessing methods were applied by Unscrambler X10.4 (Camo software, Oslo, Norway).

MRMR Feature Selection

Using all the different characteristics of a spectrum to create the model can lead to overfitting, which can make it harder for the model to apply to new data. Furthermore, in datasets with many variables, some of the features may be duplicate or provide redundant information. This indicates that some characteristics are closely related to other characteristics, leading to redundant information being supplied. This significantly increases the complexity of training the model and can lead to overfitting problems. To enhance the model's effectiveness, it is crucial to decrease the dimensionality of the data. Feature selection is one of the techniques that involves selecting a specific subset of unique and independent features to improve the modeling process by removing irrelevant elements from the original set. Maximum-Relevance Minimum-Redundancy (MRMR) is an algorithm that selects features by minimizing the correlation among variables to reduce redundancy in information. The main goal of MRMR is to find the most fitting group of attributes through maximizing correlation with target values and minimizing redundancy among variables (Ma, Chen, & Liu, 2024).

The MRMR feature selection method takes into account both the relationship between the input variable features and the target variable, as well as the redundancy between features, in order to select the most optimal subset of representative features. Before applying the learning algorithm, this method employs a filtering algorithm, like the MRMR algorithm, to choose a subset of features (Ma *et al*., 2024). By minimizing overlap between the new feature and the chosen subset, this method enhances the relationship between the new feature and the target variable (Ramírez-Gallego *et al*., 2017). The ultimate target is to screen and determine the most appropriate feature subset.

Mutual information (MI) is a core concept in information theory, that measures the dependency level between two random variables. After picking a specific subset S of features, we can measure the correlation between these features and the target value y using MI as:

$$I(S,y) = \frac{1}{|s|} \sum_{x_i \in S} I\ (x_i\ ; y)$$

In the same way, within the unique subset S, the redundancy concept is defined as R by employing MI.

$$R(S) = \frac{1}{|s|^2} \sum_{x_i, x_j \in S} I(x_i\ , x_j)$$

The i_{th} feature variable within the feature subset S is represented by the variable x_i.

In selecting wavelengths for NIR spectral characteristics, the chosen wavelengths must adhere to two fundamental principles simultaneously. The goal is to increase the mutual information between the chosen feature subset S and the target value y, making sure that each feature in S has a meaningful impact on the target. Additionally, the aim is to decrease redundancy among the features in subset S in order to decrease correlation between model variables and improve the model's efficiency and performance. In order to simultaneously follow the constraints noted, it is possible to enhance the difference in importance between MI and redundancy R (Ma *et al*., 2024).

Modelling

The output data obtained from different preprocessing methods were applied to build classification models firstly without application of feature selection and then with application of feature selection algorithm. The applied classification models were unsupervised (PCA) and supervised (SVM). Among the unsupervised clustering methods available, including PCA, hierarchical cluster analysis (HCA), and K-means, PCA is often preferred for detecting potential data patterns through examination of variable similarities and differences (Granato, Santos, Escher, Ferreira, & Maggio, 2018). In the present investigation, PCA model was utilized on the FTIR-S-G-D2-MSC spectra to enhance data visualization within a transformed space. By utilizing PCA, the fundamental data is summarized into a score plot and loading plot derived from the original dataset. The score plot is considered as a useful tool for identifying patterns in highly correlated data, allowing us to observe how samples from similar groups tend to cluster together. Furthermore, the loading plot can uncover valuable insights about the original dataset by identifying which wavenumbers show the highest data variance and contribute most significantly to group clustering and separation. Therefore, by implementing a PCA model to reduce the original number of variables, the decision-making process for selecting classification algorithms in machine learning tests is streamlined and made more intuitive.

Support vector machine as one of the best techniques for classification, unlike clustering algorithms, is considered in the category of supervised learning and has two phases of training and testing. The result of this model is a representation of data in a multi-dimensional space where the data is divided in classes and the ranges between the data are determined by a hyperplane. The SVM utilizes a method known as kernel trick, including Linear, Polynomial, Radial Basis function, and Sigmoid for data conversion. In simpler terms, SVM uses intricate calculations to figure out how to differentiate the data according to specified labels or results (Khojastehnazhand & Roostaei, 2022). In the present research, all of modelling algorithms were applied by MATLAB R2017b (The MathWorks, MA, Natick, USA).

Results and Discussion

PCA

The acquired spectra of flour samples is shown in Fig. 2.

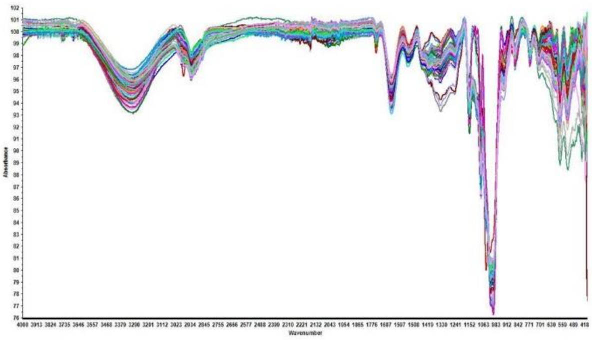

Fig. 2.The raw acquired spectra for flour varieties

PCA model was applied on the spectral data to observe any possible similarities and differences among four samples. PCA categorizes vast datasets with common identifying features in to clusters using an unsupervised approach. In PCA interpretation, it is typical for each separate cluster to contain samples with the highest spectral similarities that mirror composition similarities (Keshavarzi, Barzegari Banadkoki, Faizi, Zolghadri, & Shirazi, 2020). Fig.3.a, illustrates the score plot of flour samples. PC1, PC2, and PC3 account for 53%, 21%, and 7% of the overall variances, respectively. The explained-variance plot Fig. 3.b, gives an indication of how much of the variation in the data is described by the different components. According to Fig. 3.b, the optimal value of the factor for the model is 7, and factors number first and second had more ability to describe independence variables, among which the first factor had the largest contribution in describing the data. From the fourth factor onwards, the increase in the number of main components was ineffective and may decrease the validity of the model. The classification of flour classes was acceptable according to the obtained score plot. Various classes were identified separately and clustered together. However, some samples from Homa and Baran varieties showed overlap most likely due to the similarities of their composition. In addition, the loading plot of NIR data were obtained to determine optimal wavelengths. Fig. 3.c, shows the obtained loading plot for the first three PCs. The regression coefficients of each wavelength at each PC in PCA loadings provide insight in to which wavelengths have the greatest impact on the discrimination (Barbin, Badaro, Honorato, Ida, & Shimokomaki, 2020). When there is a larger absolute loading value for a certain wavelength region, the connection to PC becomes stronger. The peaks at 3350, 950, and 530 cm^{-1} related to the fats, unsaturated bonds C=C connected to the oxygen atoms (Coţovanu & Mironeasa, 2022).

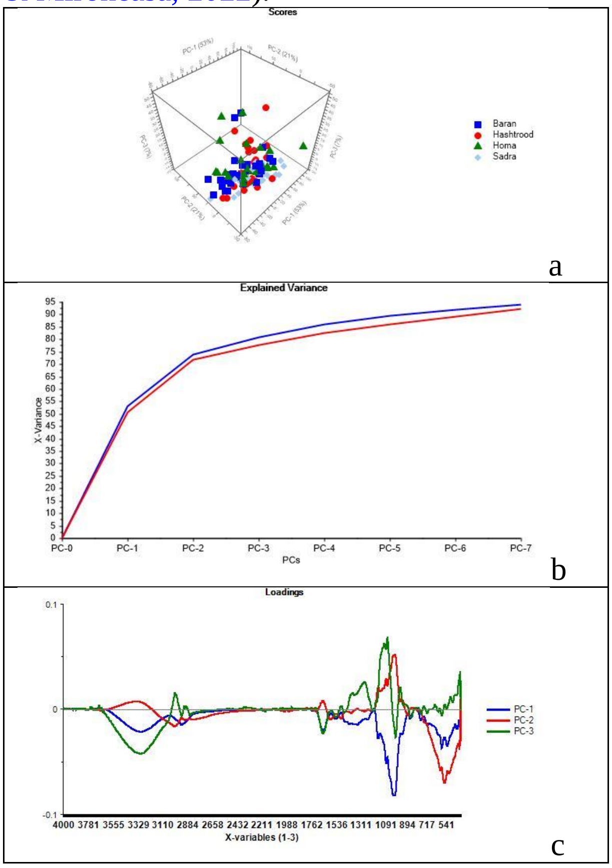

Fig. 3. a) The score plot of PCA model b) The explained-variance plot of PCA model c) The loading plot of PCA model

SVM Model

SVM, an algorithm rooted in statistical learning theory, is recognized for its ability to classify samples with its nonlinear computational approach. SVM model with 70% of data for training and 30% for test was employed for the obtained data with various preprocessing methods. The application of SVM model on the spectral data for flour variety classification yielded promising results, particularly after the preprocessed data with S-G + D2 + MSC method. The combination of pretreatment algorithms played a significant role in enhancing the discriminative capabilities of SVM model, S-G filtering helps in reducing noise and smoothing the spectral data, thereby improving the signal-to-noise ratio. The application of the second or first derivative enhances the spectral features by highlighting variations in the spectral curve, making subtle differences between flour varieties more pronounced. Additionally, MSC preprocessing addresses multiplicative effects such as baseline shifts and scaling differences, thereby normalizing the spectral data and mitigating variability related to instrumental differences or sample presentation. As it is observable from Table.1, the result of MSC preprocessing was weaker than D1 or D2. The choice of preprocessing method can affect the performance of the model trained on the preprocessed data. If the model is better suited to the features generated by derivative preprocessing, it might perform better compared to when trained on features generated by MSC preprocessing. Furthermore, certain preprocessing techniques may be better suited to handling specific types of data. In the spectral data of present research, the multiplicative effect was weaker than noises and the peaks of data had to be clearer by derivative algorithms. According to Table.1, the application of SVM model on the spectral data for classification of flour varieties had yielded acceptable results, particularly with the application of various kernel functions. In this study, we explored the performance of SVM model applying linear, quadratic, and cubic kernels. The outcomes indicated that the linear kernel outperformed other kernels, achieving the accuracy of 99.4%. Linear kernels establish decision boundaries that are linear hyperplanes in the feature space. The reason for better performance of linear kernel might be the less complexity of dataset. Furthermore, quadratic and cubic kernels introduce higher degrees of non-linearity in to the decision boundary, which can lead to overfitting, particularly when dealing with high-dimensional data and the risk of overfitting is less with a linear kernel, as it imposes a simpler, more constrained decision boundary among classes. In another study, Mohamed *et al.* used combined handheld spectrometer and SVM model to classify three different types of flour (whole wheat, organic wheat, and rice flour). The outcome of 100% for accuracy was acquired with the proper preprocessing algorithm (Mohamed, Solihin, Astuti, Ang, & Zailah, 2019). Therefore, the result of present study was similar to the result of that study, however, the classification in our study was between 4 flour varieties and a little more difficult than 3 classes. In another research, Sampaio *et al.* applied near-infrared (NIR) spectroscopy associated to SVM model created after MSC preprocessing showed the result of 91% accuracy for prediction dataset which was weaker than the outcomes of present study (Sampaio, Castanho, Almeida, Oliveira, & Brites, 2020). In order to decrease and select important features in the dataset, MRMR feature selection algorithm was also applied to illustrate the effects of each feature on the classification results. Removing of irrelevant features can lead to improved classification results. Furthermore, with utilizing just a fraction of the available features will greatly enhance the systems speed (Karimi, Kondrood, & Alizadeh, 2017; Khojastehnazhand & Roostaei, 2022). The acquired plot of MRMR feature selection algorithm is shown in fig.4, the threshold of important feature was selected 250 cm^{-1} by trial and error.

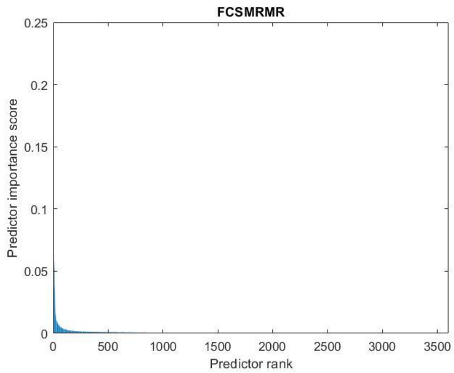

Fig. 4. The plot of MRMR feature selection algorithm

Table 1, shows the result of SVM model without application of feature selection and with feature selection algorithm selecting 250 important features. As shown in the table, SVM model was improved in S-G and S-G+MSC preprocessing methods. The accuracy of S-G+D2+MSC was improved from 99.4% to 100% and was selected as the best model. However, the feature selection algorithm did not have especial effect of S-G+D1, S-G+D2 models. In a similar research Khojastehnezhand and Roostaei applied this algorithm to the dataset of wheat seeds classification by computer vision technique. The SVM model had not improved in any of the number of features used (Khojastehnazhand & Roostaei, 2022). For further studies it is suggested to explore the effects of other feature selection algorithms on the machine learning algorithms like SVM.

Conclusion

The classification of wheat flour was explored with the combination of Ft-MIR spectroscopy and chemometrics techniques. PCA model as unsupervised method was used to reduce dimensionality of data and explore similarities of samples of classes. SVM algorithm as supervised model was applied to investigate classification. The best outcome was with dataset with S-G+D2+MSC preprocessing with 99.4% of accuracy. Then with the application of MRMR feature selection method the accuracy improved to 100%. Therefore, the applicability of FT-MIR spectroscopy and machine learning algorithms for classification of Iranian wheat flour as one of the strategic crops was approved.

Table 1- The results of SVM model for different preprocessing methods

Model	SVM			MRMR + SVM		
Kernel function	Linear	Quadratic	Cubic	Linear	Quadratic	Cubic
S-G	78.6	78	74.8	83	81.1	80.5
S-G+MSC	75.5	73.6	73	78.6	76.7	76.7
S-G+D1	99.4	99.4	99.4	99.4	99.4	99.4
S-G+D2	99.4	99.4	99.4	99.4	99.4	99.4
S-G+D1+MSC	99.4	99.4	99.4	99.4	99.4	99.4
S-G+D2+MSC	99.4	99.4	99.4	100	100	100

Author Contributions

Amir Kazemi: Conceptualization, Methodology, Software, Formal analysis, Investigation, Resources, Data curation, Writing–review & editing, Project administration, Writing-Original Draf, **Asghar Mahmoudi:** Software, supervision, validation, writing-reviewing, and editing.

Founding Source

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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مقاله پژوهشی
جلد ۲۱، شماره ۳، مرداد- شهریور ۱٤۰٤، ص. ۲٦۹-۲٦۱

طبقه‌بندی آرد گندم ایرانی با استفاده از طیف‌سنجی FT-MIR بر پایه‌ی انتخاب طول موج با بیشینه‌ی ارتباط و کمینه‌ی افزونگی، همراه با ماشین بردار پشتیبان SVM

امیر کاظمی[1]*- اصغر محمودی[1]- سید حسین فتاحی[2]

تاریخ دریافت: ۱۴۰۳/۰۴/۰۲
تاریخ پذیرش: ۱۴۰۳/۰۸/۳۰

چکیده

انواع واریته گندم، به‌عنوان یکی از محصولات راهبردی، در ایران بر اساس شرایط خاص جغرافیایی و اقلیمی هر منطقه کشت می‌شوند. طبقه‌بندی این واریته‌های گندم برای تضمین کیفیت محصولات نهایی حاصل از آرد گندم اهمیت دارد. در این پژوهش، از طیف‌سنجی مادون قرمز میان‌ناحیه با تبدیل فوریه (FT-MIR) به‌عنوان روشی غیرمخرب، همراه با شیمی‌سنجی، برای طبقه‌بندی چهار رقم از گندم ایرانی استفاده شد. در مجموع ۱۶۰ نمونه مورد تحلیل قرار گرفت و از الگوریتم‌های مختلف پیش‌پردازش برای حذف اطلاعات ناخواسته بهره گرفته شد. سپس، از تحلیل مؤلفه‌های اصلی (PCA) به‌عنوان مدل بدون ناظر و ماشین بردار پشتیبان (SVM) به‌عنوان مدل با ناظر، همراه با الگوریتم انتخاب ویژگی با بیشینه‌ی ارتباط و کمینه‌ی افزونگی (MRMR)، برای بررسی رده‌بندی این گونه‌ها استفاده شد. بهترین نتیجه مدل SVM بدون انتخاب ویژگی، با پیش‌پردازش ترکیبیS-G+D2+MSC ، دقتی برابر با ۹۹/۴ درصد به‌دست آورد. خروجی ۱۰۰ درصد حاصل از مدل SVM همراه با الگوریتم انتخاب ویژگی MRMR، توانمندی روش طیف‌سنجی FT-MIRرا در رده‌بندی گونه‌های آرد گندم ایرانی تأیید کرد.

واژه‌های کلیدی: آرد گندم، پیش‌پردازش، طبقه بندی، طیف‌سنجی تبدیل فوری

۱- گروه مهندسی بیوسیستم، دانشگاه تبریز، تبریز، ایران
(نویسنده مسئول: Email: a.kazemi@tabrizu.ac.ir)
۲- گروه مهندسی بیوسیستم، دانشکده کشاورزی، دانشگاه مراغه، مراغه، ایران

https://doi.org/10.22067/ifstrj. 2024.88624.1342

Iranian Food Science and Technology
Research Journal
Homepage: https://ifstrj.um.ac.ir

Research Article
Vol. 21, No. 3, Aug.-Sep. 2025, p. 271-286

Implementation of Several Data Mining Strategies on Electronic Nose Data for Identifying Gluten in Cheese

M. Nasiri-Galeh[1*], M. Ghasemi-Varnamkhasti[2]

1- Department of Information Technology Management, Faculty of Management and Economics, Tarbiat Modares University, Tehran, Iran
(*- Corresponding Author Email: m_nasiri@modares.ac.ir)
2- Department of Biosystems Mechanical Engineering, Faculty of Agriculture, Shahrekord University, Shahrekord, Iran

Received: 27.08.2024 Revised: 28.12.2024 Accepted: 29.12.2024 Available Online: 17.06.2025	**How to cite this article:** Nasiri-Galeh, M., & Ghasemi-Varnamkhasti, M. (2025). Implementation of several data mining strategies on electronic nose data for Identifying gluten in cheese. *Iranian Food Science and Technology Research Journal, 21*(3), 271-286. https://doi.org/10.22067/ifstrj.2024.89599.1360

Abstract

Electronic nose is an electronic device for smell detection. The data obtained from this device are stored in the form of numbers in different columns, which are related to the data of two types of cheese namely gluten-free cheese and cheese with gluten. It is not enough to make decisions and judge the data unless discovering the relationships and patterns between the data obtained to determine the relation of new data recorded by the device to the type of cheese, for this purpose, data mining and machine learning methods have been used in this research. Data mining includes various algorithms such as classification, clustering, and obtaining association rules. To get a better result from the data, a data mining process was performed on 105 different permutations of the models, and 13 models with the highest accuracy in understanding the relationships between the data were chosen. In this research, with data mining methods, cheese with gluten and gluten-free cheese data were classified into separate categories, and a model was created to predict the type of new input data in terms of the nature of cheese (gluten-free and with gluten). With analyzing 105 Permutations, Finally, the best suitable model to be used for data classification using the Random Forest algorithm and MinMaxScaler for scaling was selected with a prediction accuracy of 99.8% for both test and training datasets.

Keywords: Data classification, Data mining, Decision tree, Electronic nose, Machine learning

Introduction

Celiac disease is one of the most common diseases related to nutrition (Gh. Shekari, 2024). People suffered by this disease are allergic to gluten in food, so it is necessary to design a method to detect gluten in food with high accuracy, and one of these methods is the application of electronic nose. Smell collection technology was first created in 1982 with the invention of an array of sensors (Persaud & Dodd, 1982).

In recent years, electronic nose (E-nose) technology has been widely utilized in the food industry to assess quality, authenticity, and safety of products (Wilson, 2009). These devices, by mimicking the human olfactory system, can detect and differentiate volatile compounds present in food products. E-nose consists of an array of chemical sensors that respond to volatile organic compounds, generating unique olfactory patterns. These patterns are analyzed using data mining techniques and multivariate analysis methods, such as Principal Component Analysis (PCA),

https://doi.org/10.22067/ifstrj.2024.89599.1360

Linear Discriminant Analysis (LDA), and Artificial Neural Networks (ANN), enabling precise differentiation and identification of various samples (Zhang, 2023).

The applications of E-nose technology in the food industry are vast, including the evaluation of meat quality, detection of spoilage, determination of shelf life, and identification of food fraud (Zhao, 2024). Recently, there has been increasing attention on employing this technology to evaluate the chemical and sensory properties of dairy products, particularly cheese. Studies have demonstrated that E-nose systems can identify different types of cheese based on their olfactory patterns and even predict the intensity of aroma with high accuracy (Fernandez, 2023).

On the other hand, the increasing prevalence of gluten-related diseases, particularly celiac disease, has brought significant attention to the production and monitoring of gluten-free food products. Celiac disease is a chronic autoimmune disorder triggered by the consumption of gluten, a protein found in wheat, barley, and rye, which damages the small intestine's villi. In individuals with celiac disease, even trace amounts of gluten can cause severe complications, including malabsorption of nutrients, weight loss, gastrointestinal problems, and long-term risks such as osteoporosis and intestinal cancers (Thompson, 2023).

Given the global rise in the number of celiac patients and the growing demand for accurate and rapid detection of gluten in food products, advanced technologies like the electronic nose, combined with data mining methods, offer a promising non-destructive solution for quality control and the identification of potential gluten contamination, especially in dairy products. Moreover, utilizing advanced data analysis techniques, such as machine learning algorithms and sophisticated modeling, can significantly enhance the accuracy and efficiency of E-nose systems for gluten detection (Yu, 2024).

One of the key innovations of this study is that the employed methods can be integrated into the design and development of next-generation electronic nose devices. Incorporating advanced algorithms and precise modeling will improve the performance of these systems, increase detection accuracy, and reduce potential errors. Such improvements will contribute to the development of smarter and more efficient devices that can better meet the demands of the food industry.

This paper investigates gluten detection in cheese samples using data generated by an electronic nose and several data mining methods. The study is structured to include the following sections: an Introduction, providing the background and motivation for the research; Materials and Methods, detailing the data collection and preprocessing steps; Algorithms Used, describing the machine learning techniques applied; Modeling, outlining the process of building and validating the models; and Results and Discussion, presenting the findings and their implications.

Justification for the Study

The use of electronic noses (E-noses) in food quality control has become increasingly significant due to their ability to provide rapid and non-invasive detection of various food components. In the context of gluten detection in dairy products such as cheese, this technology offers a promising alternative to conventional methods. Traditional gluten detection methods, such as ELISA (Enzyme-Linked Immunosorbent Assay) and PCR (Polymerase Chain Reaction), although accurate, are often labor-intensive, time-consuming, and require specialized laboratory conditions. Given the growing concerns about gluten contamination in foods and the rising prevalence of gluten-related disorders, there is a critical need for more efficient, accurate, and user-friendly detection methods. This study aims to apply advanced data mining strategies to E-nose data, providing a novel approach for the reliable identification of gluten in cheese.

Research Gaps

1. **Insufficient Application of E-noses in Gluten Detection**: While E-noses have been widely applied in various fields of food analysis, their specific use for gluten detection in

dairy products remains underexplored. Existing studies have primarily focused on other food matrices, leaving a significant gap in the research related to dairy products, particularly cheese (Bhattacharya, 2008).

2. **Limited Integration of Data Mining Techniques**: Previous research utilizing E-noses has often relied on basic statistical analysis rather than employing advanced data mining techniques. This has limited the potential of E-noses in detecting complex food contaminants like gluten. There is a need for studies that explore a broader range of data mining algorithms to improve the accuracy and robustness of gluten detection (Wilson, 2013)
3. **Challenges in Ensuring Cross-Product Applicability**: Most existing studies on gluten detection are focused on specific food products or use limited datasets, which hampers the generalizability of the findings. There is a lack of comprehensive studies that validate the effectiveness of these techniques across different dairy products, making it difficult to apply these findings in a broader context (Karoui, 2011). This study aims to address this gap by using diverse data sets and evaluating multiple data mining techniques to develop a more generalizable model.

Materials and Methods

In this study, all data mining processes were performed by manual coding in the Python programming language (Python Software Foundation), which are explained in the following algorithms used in this research. The tested samples are gluten-free cheese and gluten-containing cheese, prepared from reputable stores. Data collection was done by the electronic nose device made by the authors, which will be briefly explained below.

Data Collection Electronic Nose Device

In this study, both gluten-free and gluten-containing cheese products were examined. For the gluten-free samples, commercially available products specifically designed for individuals with celiac disease were utilized. These products were verified to be gluten-free through reference methods prior to their inclusion in the analysis, ensuring their suitability for the study.

For the gluten-containing samples, regular cheese products available in the market, known to contain gluten, were used. These products were selected to represent typical gluten-containing cheeses and provided a reliable basis for comparison in the analysis.

The data collection process was completely carried out by the electronic nose device made by the authors, which is briefly explained below. Fig. 1 shows a schematic diagram of an electronic nose.

Data Processing and Preprocessing

Data obtained from the electronic nose was initially in raw form and stored in JSON format. To prepare the data for analysis, visualization, and modeling, it was first necessary to convert it into formats compatible with data modeling and visualization tools. This conversion ensured that the data could be effectively interpreted and utilized for subsequent processes.

Following this, a comprehensive data preprocessing phase was conducted to enhance data quality and ensure accuracy. This process included:

1. Data Cleaning: Outlier values were identified and removed, and missing or invalid data points were appropriately handled to prevent them from compromising the results.
2. Normalization: To ensure consistency across features, scaling methods such as MinMaxScaler were applied to normalize the data. This step allowed all features to have comparable ranges, which is crucial for the optimal performance of many machine learning algorithms.

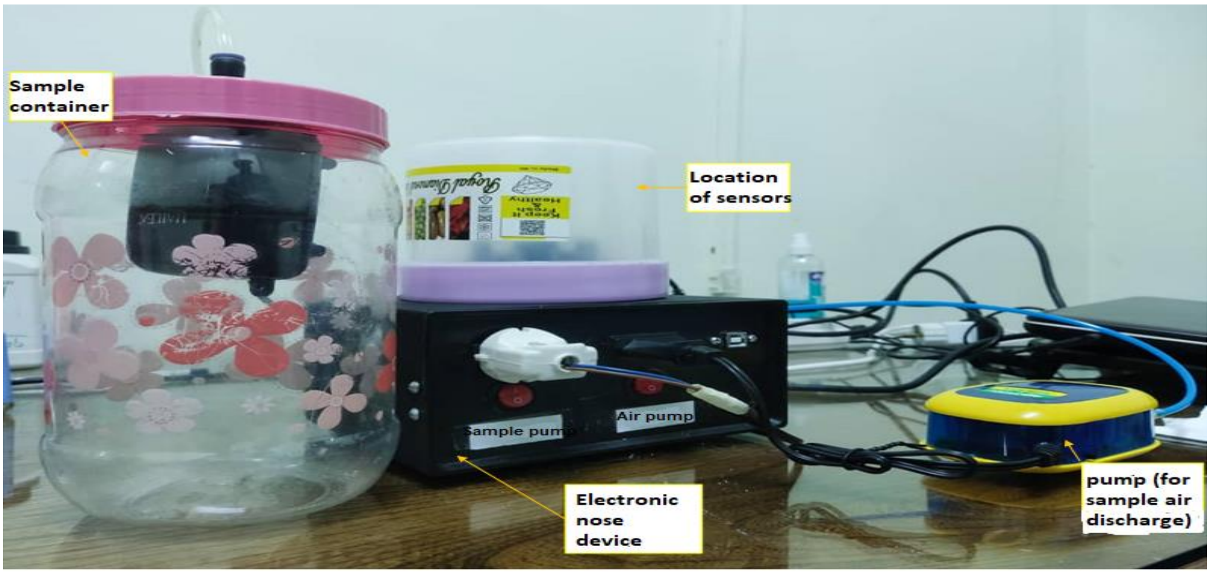

Fig. 1. The electronic nose device used in the research

The sample to be tested is placed in the sample container, then by the sample air discharge pump, the sample air, which is accompanied by the smell emitted from the sample, is directed to the location of sensors, and the sensors then store the desired data.

In addition, specific preprocessing strategies were tailored to meet the requirements of different machine learning models. For certain models, dimensionality reduction techniques were applied to simplify the dataset and enhance performance without sacrificing accuracy. This was done when it was determined that reducing the number of features would lead to better outcomes. Conversely, for models where dimensionality reduction was unnecessary or unsuitable, alternative methods of data preparation were employed.

After completing these preprocessing steps, the data was fully processed, cleaned, normalized, and formatted. The resulting output, as shown in Fig. 2, served as the foundation for the machine learning processes described in subsequent sections.

Furthermore, due to the high accuracy achieved with the applied machine learning methods, additional techniques, such as the area under the curve (AUC) or integration-based methods, were deemed unnecessary. The robustness and precision of the employed algorithms ensured reliable results, eliminating the need for supplementary approaches and streamlining the analysis.

Fig. 2 shows an example of a graph obtained from a sampling device.

Used Algorithms

AdaBoost

Boosting is an approach to machine learning based on the idea of creating a strong rule from by combining of relatively weak rules (Schapire, 2013) which was introduced in 1990 by Freund and Schapire (Schapire, 197-227). Booting algorithms for classification and regression issues provide a solution for easier comparison of algorithms (Hu, 2008) The AdaBoost algorithm was the first Boosting algorithm developed as an application algorithm and used in a variety of applications such as classification issues (Freund, 1997).

The AdaBoost algorithm is a boosting classification method designed to enhance weak classifiers and transform them into strong ones. It typically begins with a basic classification algorithm, which is used as a template to train an initial classifier on the training data. The algorithm then adjusts the sample weights based on the performance of this classifier. These re-weighted samples are subsequently used to train the next-level learner. An iterative design of this process was conducted and the algorithm assigns optimized weights to each learner was achieved, ultimately forming the final, robust classification model (Wang, 2019).

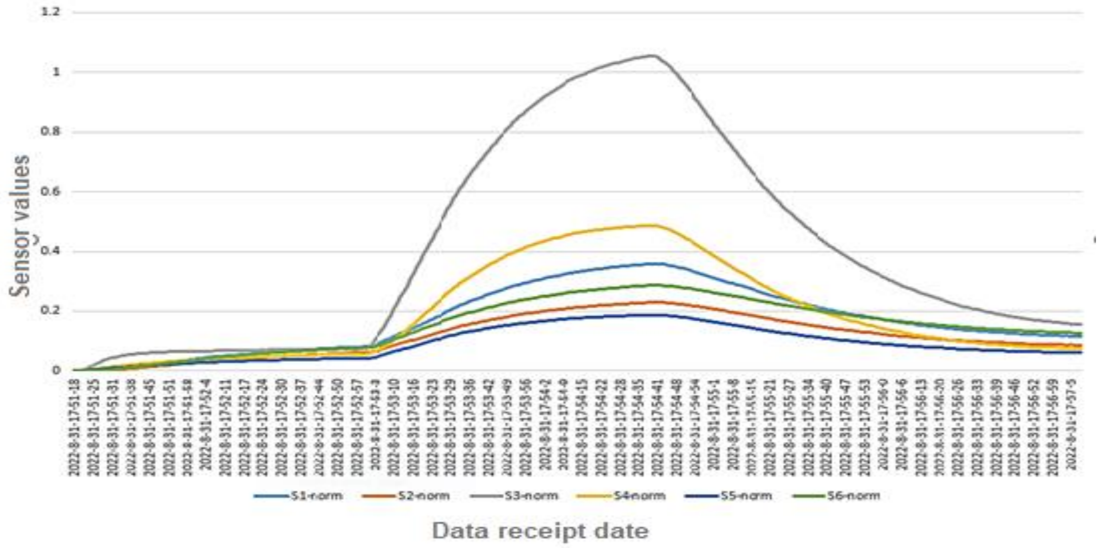

Fig. 2. A diagram drawn with the data collected by the 6 sensors of the device
This chart displays data collected by six sensors, with each sensor's data represented in a different color. Data were plotted based on date and categorized by the type of sensor.

Decision Tree

Decision Tree classification is one of the most well-known machine-learning techniques presented by Quinlan (Quinlan, 1993). The Decision Tree in classification is one of the multi-stage decision-making methods. The general method in the Decision Tree is that a complex decision is divided into a set of simple decisions and finally, by solving a set of these simple decisions, the desired output for the main complex decision is reached.

After the Decision Tree is created, it can be used to classify the test data that have the same characteristics as the training data (Stein, 2005). The general method of the Decision Tree can be displayed in Fig. 3. As shown in this figure, the complex decision, which is a set of pixels on the left side, is transformed into simple decisions on the right side at each stage, so that by solving those simple decisions, the complex decision is finally solved.

Decision Tree models are suitable for data mining due to their acceptable accuracy and low computational cost (Du, 2002). Most Decision Tree classifiers (such as C4.5) perform the classification in two steps: tree construction and tree pruning.

In tree construction, the decision tree model is built by recursive partitioning. Tree pruning is used to improve the generalization of a decision in a Decision Tree, as well as to prune the leaves and branches responsible for classifying single or very small vector data (Du, 2002).

Random Forest

The Random Forest was invented in the early 2000s by L. Breiman (Breiman, 2001). Random Forest is a collection of trees that are taught independently (Breiman, 2001). For the final prediction, the Random Forest combines the predictions of all trees with the average, which is a generalization property (Criminisi, 2011) .

By random sampling, a subset of educational data is used to learn a separate tree (Ren, 2015).

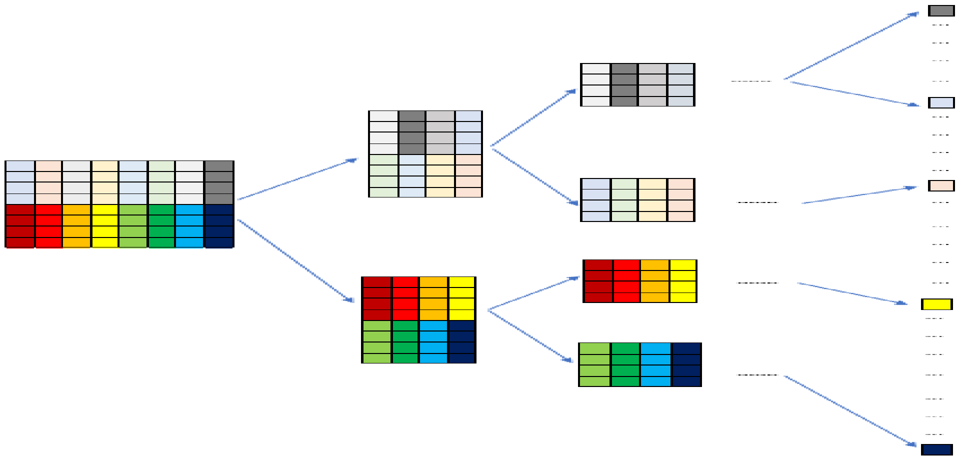

Fig. 3. A general image of the division of decision making in the Decision Tree

Deep trees are the main source of the power of a Random Forest; although reduction randomness can also improve the robustness of an individual tree, it is preferred to increase the depth of trees because a special randomization procedure is required to ensure complementarity with other trees (Ren, 2015).

Among machine learning algorithms, the Random Forest works very well in terms of the accuracy of forecasting and interpretation of the model (Qi, 2012). The Random Forest algorithm for classification and regression is based on the accumulation of a large number of decision trees.

Random Forest focuses on three features (Breiman, 2001):

1. Provides accurate predictions for many applications.
2. It can measure the importance of each variable in the model made by teaching the model and ranking the variables according to their ability to predict the response.
3. The pairwise closeness between samples can be measured by the training model.

The Random Forest method can be used for a set of forecasting issues and receives quantitative parameters as input. It can also deal with really large systems (Biau, 2016).

Support Vector Machine (SVM)

Support vector machines (SVM) were presented by Vladimir Vapnik (Vapnik, 1998) in the field of statistical learning theory and minimizing structural risk. It works successfully on various classification and forecasting problems.

In machine learning, the Support Vector Machine (SVM) method is a supervised learning approach used for both classification and regression tasks. An SVM classifier works by distinguishing between different data sets, often through the creation of a non-linear decision boundary.

The algorithm takes a labeled training dataset as input, where each data point belongs to a specific category. During the training process, the SVM constructs a model that can determine the category of new examples, enabling accurate classification of unseen data.

Modeling

Data classification was utilized in this research because the dataset comprises two distinct types of data, and the objective is to define separate regions that can classify new data accurately. The Python programming language was used for modeling, along with various machine learning algorithms. Specifically, the sklearn library was employed for implementing the algorithms and defining the search space, while the matplotlib library was used to generate graphical outputs. Additionally, the NumPy and pandas libraries facilitated numerical operations on the data. Various algorithms were applied for classification, scaling, and dimensionality reduction, resulting in 105 permutations of different algorithm combinations to identify the optimal output.

Results and Discussion

Explanation of the Data Entry into the Analysis

One way to ensure the accuracy of the output data and verify the results during testing is to repeat the test. In this data collection, both gluten-containing and gluten-free cheeses were tested, and the data was recorded. For each type of cheese, the test was repeated 7 times, resulting in a total of 14 tests. The outputs from these tests were stored in separate files across 10 columns for each test, which included data on time, temperature, humidity, device voltage, and readings from 6 gas sensors.

For the machine learning analysis, there are two types of data: training data and testing data. To improve the algorithm's accuracy and ensure precise results, one of the 7 experiments for each type of cheese was used as the test data, while all 14 experiments were used as the training data.

The Results of Machine Learning

Almost all well-known models and algorithms were used interchangeably for three tasks: classification, scaling, and dimensionality reduction. To select the optimal model, it is crucial that the accuracy percentages of both the test and training data are high and similar. A significant discrepancy between the accuracy of the test and training data indicates that the model is unsuitable for prediction and may be overfitting, that means it is not a good candidate for final predictions. To determine the best model, 105 permutations were tested, of which 13 permutations with the highest prediction accuracy are described below, along with their corresponding outputs.

Explanation of the Table

The output table generated from the implementation of machine learning models contains several key performance metrics that provide insights into the efficacy of the models. Below is an explanation of the metrics included in the table:

- **Precision**: This metric indicates the accuracy of the model in predicting correct outputs. It represents the probability that a positive prediction by the model is actually correct. A higher precision value reflects fewer false positives, emphasizing the reliability of the predictions.
- **Recall**: Also known as sensitivity or coverage, this metric shows the percentage of actual positive instances in the data that were correctly identified by the model. It measures the extent to which the model has successfully captured relevant data points during the modeling process.
- **F1-Score**: This metric is the harmonic mean of Precision and Recall. It provides a single performance score that balances both metrics, particularly useful when there is an uneven distribution of classes or when both false positives and false negatives need to be considered equally.

These metrics collectively offer a comprehensive evaluation of the model's performance, ensuring that both accuracy and coverage are accounted for. The detailed results from the table guide the selection of the most effective model for further analysis or application.

1-<Classifier: AdaBoost> <Scaler: MinMaxScaler>

This model uses the AdaBoost algorithm to classify data and MinMaxScaler to scale.

Table 1- Prediction accuracy of data classification model with AdaBoost and MinMaxScaler scaling

Type of cheese	Precision	Recall	F1-score
Gluten	0.996	0.993	0.995
Gluten-free	0.993	0.996	0.995

The prediction accuracy of the model is 99.6% for gluten-containing cheese and 99.3% for gluten-free cheese.

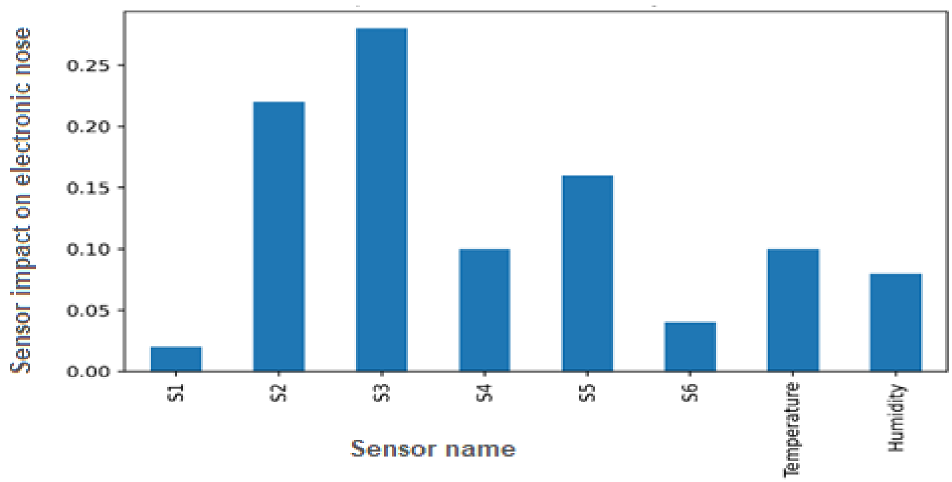

Fig. 4. The effect of each of the electronic nose sensors on the data classification model with AdaBoost and MinMaxScaler scaling

In model 1, the S3 and S2 sensors have the most impact, respectively.

2-<Classifier: AdaBoost> <Scaler:StandardScaler>

In this model, the AdaBoost algorithm is used for data classification and StandardScaler is used for scaling.

In model 2, the S3 and S2 sensors have the most impact, respectively.

3-<Classifier: AdaBoost>

In this model, the AdaBoost algorithm is used to classify data.

In model 3, sensors S3 and S2 have had the greatest impact, respectively.

4-<Classifier: DecisionTree-entropy > <Scaler: StandardScaler>

In this model, DecisionTree-entropy algorithm is used for data classification and StandardScaler is used for scaling.

Table 2- Prediction accuracy of data classification model with AdaBoost and StandardScaler scaling

Type of cheese	Precision	Recall	F1-score
Gluten	0.986	0.991	0.988
Gluten-free	0.991	0.986	0.988

The prediction accuracy of the model is 98.6% for gluten-containing cheese and 99.1% for gluten-free cheese.

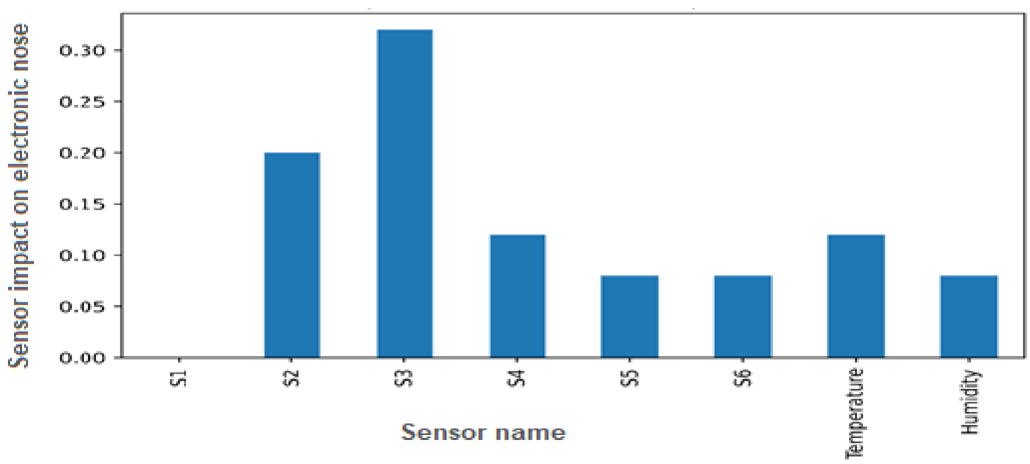

Fig. 5. The effect of each of the electronic nose sensors on the data classification model with AdaBoost and StandardScaler scaling

Table 3- Accuracy of data classification model with AdaBoost

Type of cheese	Precision	Recall	F1-score
Gluten	0.996	0.987	0.992
Gluten-free	0.988	0.997	0.992

The prediction accuracy of the model is 99.6 % for gluten-containing cheese and 98.8 % for gluten-free cheese.

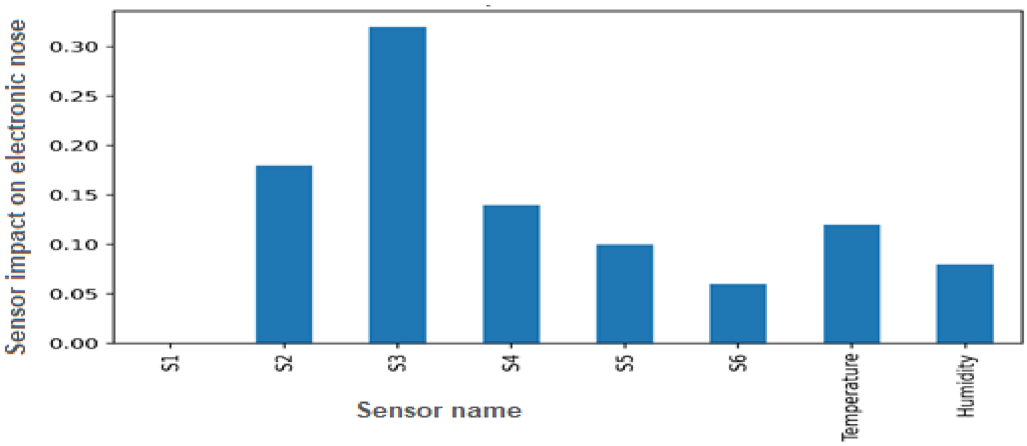

Fig. 6. The effect of each of the electronic nose sensors on the data classification model with AdaBoost

Table 4- Prediction accuracy of classification model with DecisionTree-entropy and scaling with StandardScaler

Type of cheese	Precision	Recall	F1-score
Gluten	0.995	0.995	0.995
Gluten-free	0.995	0.995	0.995

The prediction accuracy of the model is 99.5% for gluten-containing cheese and 99.5% for gluten-free cheese.

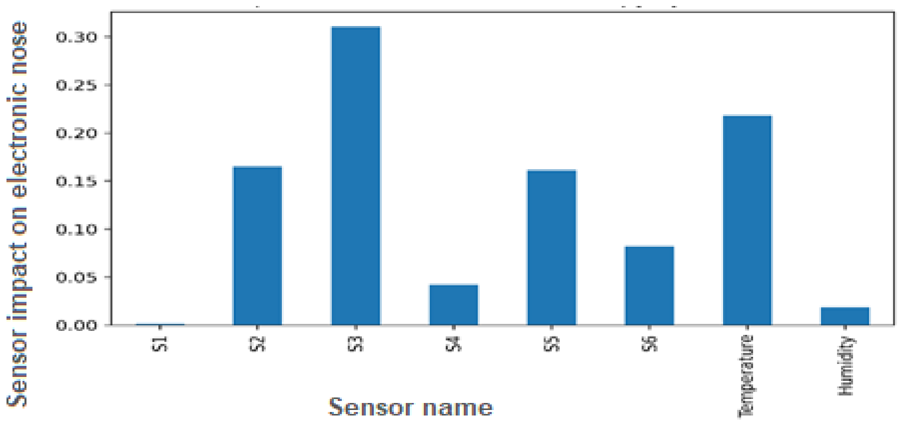

Fig. 7. The effect of each of the electronic nose sensors in the classification model with DecisionTree-entropy and scaling with StandardScaler

In model 4, S3 and Temperature sensors have had the greatest impact, respectively.

5-<Classifier: DecisionTree-entropy >

In this model, DecisionTree-entropy algorithm is used for data classification.

Table 5- Prediction accuracy of classification model with DecisionTree-entropy

Type of cheese	Precision	Recall	F1-score
Gluten	0.997	0.995	0.996
Gluten-free	0.995	0.996	0.995

The prediction accuracy of the model is 99.7% for gluten-containing cheese and 99.5% for gluten-free cheese.

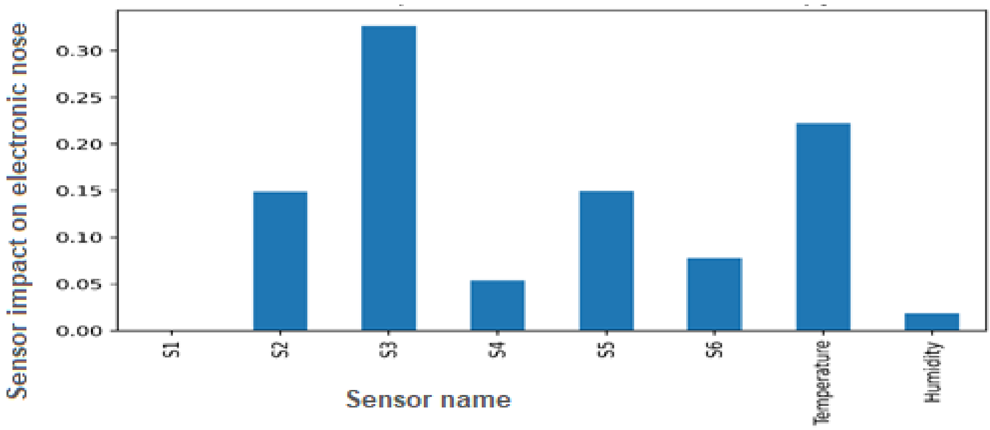

Fig. 8. The effect of each of the electronic nose sensors in the classification model with DecisionTree-entropy

In Model 5, the S3 and Temperature sensors had the most impact, respectively.

6-<Classifier: DecisionTree-gini> <Scaler: MinMaxScaler>

In this model, DecisionTree-gini algorithm is used for data classification and MinMaxScaler is used for scaling.

In Model 6, S3 and Temperature sensors have had the greatest impact, respectively.

7-<Classifier: DecisionTree-gini> <Scaler: StandardScaler>

In this model, DecisionTree-gini algorithm is used for data classification and StandardScaler is used for scaling.

The prediction accuracy of the model is 99.3% for gluten-containing cheese and 99.1% for gluten-free cheese.

8-<Classifier: DecisionTree-gini>

In this model, DecisionTree-gini algorithm is used for data classification.

Table 6- Prediction accuracy of classification model with DecisionTree-gini and scaling with MinMaxScaler

Type of cheese	Precision	Recall	F1-score
Gluten	0.996	0.996	0.996
Gluten-free	0.996	0.996	0.996

The prediction accuracy of the model is 99.6% for gluten-containing cheese and 99.6% for gluten-free cheese.

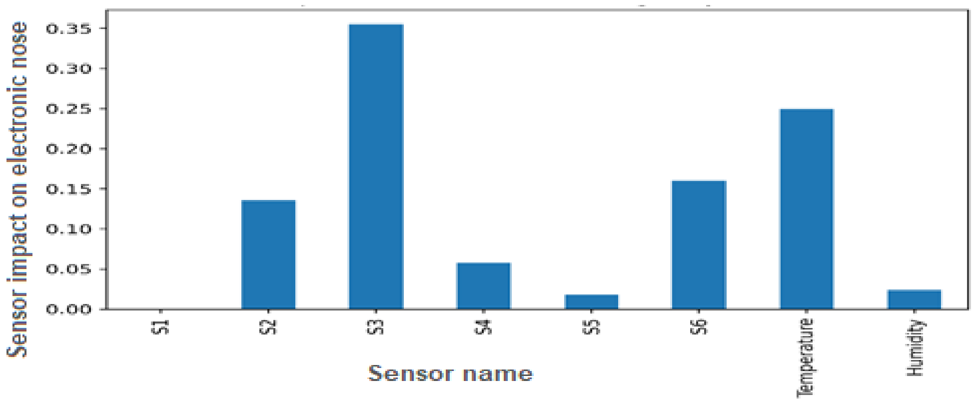

Fig. 9. The effect of each of the electronic nose sensors in the classification model with DecisionTree-gini and scaling with MinMaxScaler

Table 7- Prediction accuracy of classification model with DecisionTree-gini and scaling with StandardScaler

Type of cheese	Precision	Recall	F1-score
Gluten	0.993	0.991	0.992
Gluten-free	0.991	0.993	0.992

Table 8- Prediction accuracy of classification model with DecisionTree-gini

Type of cheese	Precision	Recall	F1-score
Gluten	0.995	0.993	0.994
Gluten-free	0.993	0.995	0.994

The prediction accuracy of the model is 99.5% for gluten-containing cheese and 99.3% for gluten-free cheese.

9-<Classifier: RandomForest> <Scaler: MinMaxScaler> <DimReducer: KernelPCA-poly>

In this model, RandomForest algorithm is used for data classification, MinMaxScaler is used for scaling, and KernelPCA-poly is used for dimensionality reduction.

Table 9- Prediction accuracy of classification model with RandomForest, scaling with MinMaxScaler and dimensionality reduction with KernelPCA-poly

Type of cheese	Precision	Recall	F1-score
Gluten	0.985	0.969	0.977
Gluten-free	0.971	0.986	0.978

The prediction accuracy of the model is 98.5% for gluten-containing cheese and 97.1% for gluten-free cheese.

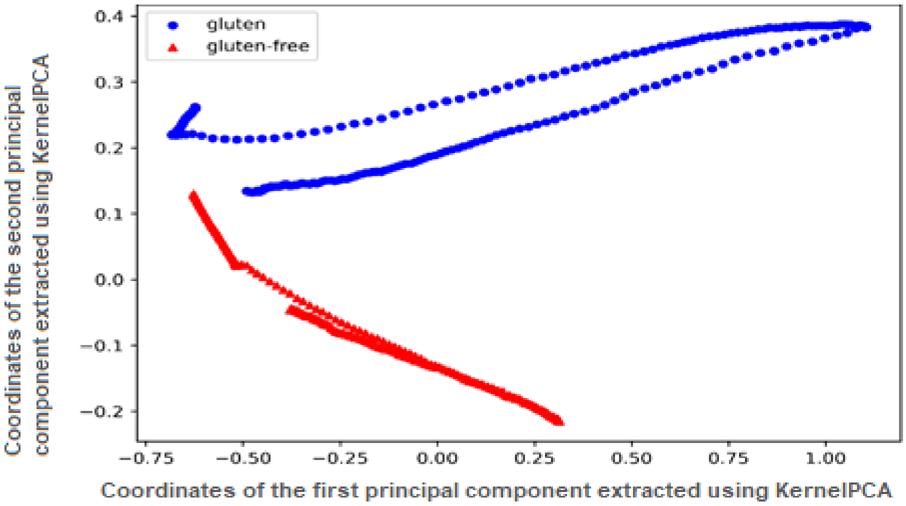

Fig. 10. Classification of data and drawing of the separating area in the classification model with RandomForest, MinMaxScaler scaling and dimensionality reduction with KernelPCA-poly

Gluten-containing and gluten-free cheeses are separated by delineating the area and with different colors with classification by RandomForest model, MinMaxScaler scaling, and KernelPCA-poly dimensionality reduction. In the classification method, as shown in Fig. 10, with the entry of new data, its area and, as a result, the type of data division that belongs to cheese with gluten or gluten-free is determined.

10-<Classifier: RandomForest> <Scaler: MinMaxScaler>

In this model, RandomForest algorithm is used for data classification and MinMaxScaler is used for scaling.

Table 10- Prediction accuracy of classification model with RandomForest and scaling with MinMaxScaler

Type of cheese	Precision	Recall	F1-score
Gluten	0.998	0.998	0.998
Gluten-free	0.998	0.998	0.998

The prediction accuracy of the model is 99.8% for gluten-containing cheese and 99.8% for gluten-free cheese.

11-<Classifier: RandomForest> <Scaler: StandardScaler>

In this model, RandomForest algorithm is used for data classification and StandardScaler is used for scaling.

Table 11- Prediction accuracy of classification model with RandomForest and scaling with StandardScaler

Type of cheese	Precision	Recall	F1-score
Gluten	0.998	0.997	0.997
Gluten-free	0.998	0.998	0.997

The prediction accuracy of the model is 99.8% for gluten-containing cheese and 99.6% for gluten-free cheese.

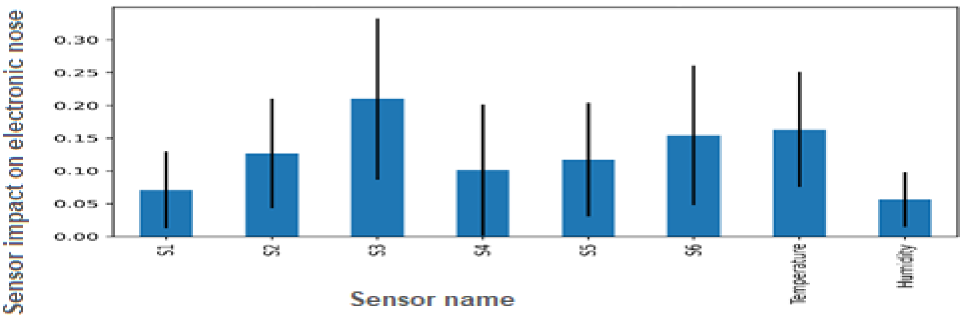

Fig. 11. The effect of each of the electronic nose sensors in the classification model with RandomForest and scaling with StandardScaler

In Model 11, S3 and Temperature sensors have had the most impact, respectively. The black line in each bar shows the influence interval of each sensor on the model, which is finally calculated as the average of this interval and is drawn as a bar graph.

12-<Classifier: RandomForest>

In this model, RandomForest algorithm is used for data classification.

Table 12- Prediction accuracy of classification model with RandomForest

Type of cheese	Precision	Recall	F1-score
Gluten	1.0	0.996	0.998
Gluten-free	0.997	1.0	0.998

The prediction accuracy of the model is 100% for gluten-containing material and 99.7% for gluten-free material.

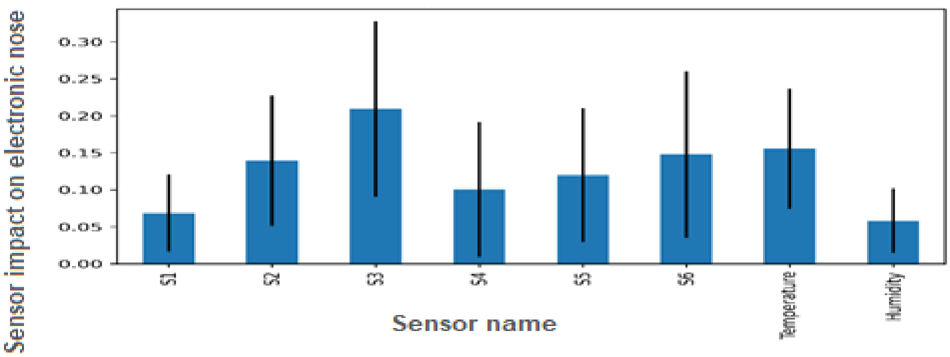

Fig. 12. The effect of each of the electronic nose sensors in the classification model with RandomForest

In Model 12, S3 and Temperature sensors have had the most impact, respectively. The black line in each bar shows the influence interval of each sensor on the model, which is finally calculated as the average of this interval and is drawn as a bar graph.

13-<Classifier: SVC > <Scaler: MinMaxScaler>

In this model, SVC algorithm is used for data classification and MinMaxScaler is used for scaling.

Table 13- Prediction accuracy of classification model with SVC and scaling with MinMaxScaler

Type of cheese	Precision	Recall	F1-score
Gluten	0.993	0.968	0.98
Gluten-free	0.969	0.993	0.981

The prediction accuracy of the model is 99.3% for gluten-containing cheese and 96.9% for gluten-free cheese.

By observing the set of conditions, one of the models with 99.8% prediction accuracy for two datasets, was selected as the best model. In this model, the Random Forest algorithm was used for data classification, and MinMaxScaler was applied for scaling.

Conclusion

This study explored the application of electronic nose technology combined with advanced data mining and machine learning techniques for the detection of gluten in cheese samples. The primary focus was on distinguishing between gluten-free and gluten-containing cheeses using data collected from electronic nose sensors. The raw data, stored in structured tagged tables, was preprocessed to ensure quality and accuracy. Given the large volume of data and the necessity of repeated experiments for reliability, traditional methods of analysis were deemed inefficient due to their high time and cost requirements, as well as their limited accuracy.

In contrast, this research demonstrated that modern data mining and machine learning techniques provide a more effective solution. These methods not only reduce time and cost but also enhance the accuracy and reliability of gluten detection. By analyzing and modeling the sensor data using these innovative approaches, it was possible to accurately classify the cheese samples into gluten-free and gluten-containing categories.

The findings of this research highlight the potential of leveraging advanced computational techniques to improve food safety and quality assessment processes. Specifically, this study offers a cost-effective and efficient method for identifying gluten in food products, which could significantly benefit individuals with celiac disease or gluten sensitivity. The ability to distinguish gluten content in cheese with high accuracy using electronic nose technology is a step forward in addressing the dietary needs of this population.

In conclusion, the methodology developed in this research can be adapted for broader applications in food safety, potentially contributing to the development of new, efficient devices for gluten detection. These advancements could pave the way for more precise and accessible gluten-testing technologies, thereby improving the quality of life for people who need to adhere to a strict gluten-free diet.

Author Contributions

Mohammad Nasiri-Galeh: was responsible for data curation, formal analysis, investigation, methodology, resources, visualization, and writing – original draft. **Mehdi Ghasemi-Varnamkhasti:** contributed to conceptualization, supervision, and writing – review and editing.

Funding Source

The sampling device used in this study was developed under the Shahid Ahmadi Roshan project with financial support from the Iranian Elite Foundation. The device was designed and constructed by a dedicated team. In this study, samples were collected using this device, and data analysis was performed on the collected data. All costs related to sampling and data analysis were covered by the authors.

Acknowledgments

The authors would like to express their sincere appreciation to Mohammad Hossein Shams and Dariush Valipour, members of the electronic nose development team, for their valuable assistance in the construction of the device.

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مقاله پژوهشی
جلد ۲۱، شماره ۳، مرداد- شهریور ۱٤۰٤، ص. ۲۸٦-۲۷۱

پیاده‌سازی چندین استراتژی داده‌کاوی روی داده‌های بینی الکترونیکی برای شناسایی گلوتن در پنیر

محمد نصیری گله[۱*]- مهدی قاسمی ورنامخواستی[۲]

تاریخ دریافت: ۱۴۰۳/۰۶/۰۶
تاریخ پذیرش: ۱۴۰۳/۱۰/۰۹

چکیده

بینی الکترونیکی یک دستگاه الکترونیکی برای تشخیص بو است. داده‌های به‌دست‌آمده از این دستگاه به‌صورت عددی و در ستون‌های مختلف ذخیره می‌شوند که مربوط به داده‌های دو نوع پنیر بدون گلوتن و پنیر حاوی گلوتن هستند. این داده‌ها به‌تنهایی برای تصمیم‌گیری و قضاوت کافی نیستند و لازم است روابط و الگوهای میان آن‌ها کشف شود تا مشخص شود داده‌های جدید ثبت‌شده توسط دستگاه به کدام دسته از پنیرهای دارای گلوتن یا بدون گلوتن تعلق دارند. به همین منظور، در این تحقیق از روش‌های داده‌کاوی و یادگیری ماشین استفاده شده است. داده‌کاوی شامل الگوریتم‌های متنوعی مانند طبقه‌بندی، خوشه‌بندی و استخراج قوانین وابستگی است. برای دستیابی به نتایج بهتر، فرآیند داده‌کاوی بر روی ۱۰۵ ترکیب مختلف از مدل‌ها انجام شد و ۱۳ مدلی که بالاترین دقت را در درک روابط میان داده‌ها داشتند، در تحقیق ذکر شده‌اند. در این پژوهش، با استفاده از روش‌های داده‌کاوی، داده‌های مربوط به پنیرهای دارای گلوتن و بدون گلوتن در دسته‌های جداگانه طبقه‌بندی شدند و مدلی جهت پیش‌بینی نوع داده‌های جدید از نظر ماهیت پنیر (دارای گلوتن یا بدون گلوتن) ایجاد شد. پس از تحلیل ۱۰۵ ترکیب مختلف، در نهایت مدلی که از الگوریتم Random Forest برای طبقه‌بندی و از MinMaxScaler برای مقیاس‌بندی داده‌ها استفاده می‌کرد، به‌عنوان بهترین مدل با دقت پیش‌بینی ۹۹.۸ درصد برای هر دو مجموعه داده‌های آموزش و آزمون انتخاب شد.

واژه‌های کلیدی: بینی الکترونیکی، داده کاوی، درخت تصمیم، طبقه‌بندی داده‌ها، یادگیری ماشین

۱- گروه مدیریت فناوری اطلاعات، دانشکده مدیریت و اقتصاد، دانشگاه تربیت مدرس، تهران، ایران
(*- نویسنده مسئول: Email: m_nasiri@modares.ac.ir)
۲- گروه مهندسی مکانیک بیوسیستم ، دانشکده کشاورزی، دانشگاه شهرکرد، شهرکرد، ایران

https://doi.org/10.22067/ifstrj.2024.89599.1360

Iranian Food Science and Technology Research Journal
Homepage: https://ifstrj.um.ac.ir

Research Article
Vol. 21, No. 3, Aug.-Sep. 2025, p. 287-301

The Effect of Soy Protein Concentrate/Whey Protein Edible Coatings on the Quality of Semi-dried Potato Slices

Z. Moslehi[1], M. Bolandi[1], S.H. Ziaolhagh[2*], S. Bani[1]
1- Department of Food Science and Technology, Damghan branch, Islamic azad University, Damghan, Iran
2- Agricultural Engineering Research Department, Agricultural and Natural Resources Research and Education Center of Semnan Province (Shahrood), AREEO, Shahrood, Iran
(*- Corresponding Author Email: h.ziaolhagh@areeo.ac.ir)

Received:	02.10.2024	**How to cite this article:**
Revised:	12.02.2025	Moslehi, Z., Bolandi, M., Ziaolhagh, S.H., & Bani, S. (2025). The effect of soy protein
Accepted:	15.02.2025	concentrate/whey protein edible coatings on the quality of semi-dried potato slices.
Available Online:	17.06.2025	*Iranian Food Science and Technology Research Journal, 21*(3), 287-301. https://doi.org/10.22067/ifstrj.2025.90051.1373

Abstract

Edible coatings can be an effective and environmentally friendly method for preserving food quality during storage. This concept sets the research stage that explores how coatings made from soy protein concentrate and whey protein can enhance the chemical stability of potato slices, thus improving their preservation and overall quality during storage. The study lays the groundwork for investigating the effects of these coatings on various physicochemical properties of semi-dried potatoes, ultimately highlighting their potential benefits in food preservation. In this research, the impact of different concentrations (2.5, 4, and 5 w/w %) of soy protein concentrate and whey protein on some physicochemical properties of semi-dried potatoes (color, rehydration of dried slices, reducing sugars, starch, ascorbic acid, moisture, oil absorption, texture crispness, and sensory properties) during 60 days of storage were investigated. The results showed that semi-dried potatoes coated with soy protein concentrate and whey protein had the highest moisture content and the lowest oil absorption and crispiness compared to the control sample. The sensory properties of coated samples were different from those of uncoated samples. Panelists also accepted the taste of coated semi-dried potatoes. The applied edible coatings significantly affected the ascorbic acid and reducing sugar content. The lowest and highest amount of starch was observed in the control and coated samples, respectively. These characteristics show that coatings based on soy protein concentrate and whey protein considered to be an excellent choice to reduce oil absorption and increase shelf life of potato slices.

Keywords: Dried potato, Edible coating, Shelf life, Soy protein, Whey protein

Introduction

Research in the field of food packaging is mostly focused on environmentally friendly or biodegradable films made of polysaccharides, fats, proteins, or a mixture of these compounds. Protein coatings are well-connected to the hydrophilic surface and prevent oxygen and carbon dioxide, but are not resistant to water penetration (Moslehi *et al*., 2023). These films are prepared from edible proteins of plant and animal origins, like zein, wheat gluten, soy, peanut, albumin, gelatin, collagen, casein, and whey proteins (Ananey-Obiri *et al*., 2018). Edible coatings act as good moisture insulation and reduce these problems. When packing, the water leak in the package creates an unfavorable appearance for the consumer.

https://doi.org/10.22067/ifstrj.2025.90051.1373

Edible coatings preserve the product's nutrients and eliminate using moisture-absorbing pads in the packages. The speed of rancidity and browning reactions in foods is reduced when surrounded by coatings with low permeability to oxygen. In addition, the loss of volatile aromatic substances and the absorption of external odors from the environment are limited by edible coatings. Some researchers have used food coatings containing antioxidants or antimicrobial substances as active packaging for the direct treatment of food, and the results reported by these researchers indicated that this action delayed pungency, unfavorable color changes, and reduced microbial load (Moslehi *et al*., 2015). Sarfraz *et al*. (2024) discussed the benefits of edible coatings, particularly their contribution to improving food safety and quality. They discussed chitosan-based films, which provide antimicrobial and antioxidant properties, thermal stability, and improved barrier capacity, making them suitable for sustainable food packaging (Sarfraz *et al*., 2024). Kandasamy *et al.* (2021) discussed the development and potential of whey protein-based edible films and coatings as sustainable food packaging solutions (Kandasamy *et al*., 2021). These films, made from whey protein isolate or concentrate, offer excellent mechanical and barrier properties, flexibility, and transparency while carrying active ingredients like antimicrobials and antioxidants. Di Pierro *et al*. (2018) highlighted the diverse proteins found in whey, which can be utilized in active food packaging (Di Pierro *et al*., 2018). These proteins offer the potential to develop bioactive packaging materials with health benefits, extend shelf life, and enhance food safety. In addition, the environmental problems caused by food packaging waste will be reduced. Water constitutes a high percentage of the fresh weight of fruits and vegetables. Therefore, compared to other plant products such as seeds, they show high metabolic activity. This metabolic activity continues after harvest, so it causes many fruits to be perishable goods. One of the ways to improve the durability of fruits is to reduce their moisture so that microorganisms cannot grow. Drying is one of the significant processes that can increase the shelf life of food after harvesting (Martínez-Pineda *et al*., 2021).

The drying process is widely used to preserve fruits and vegetables, the primary purpose of which is to bring water to a level that minimizes microbial spoilage, chemical reactions (Such as color and taste), and enhances the mechanical properties (such as fracture resistance) (Giancaterino *et al*., 2024). In addition to play a protective effect on the product, drying significantly reduces the weight and volume and thus reduces the cost of transportation and storage of the product (Wang *et al*., 2023). One of the most fundamental goals of drying agricultural products is to remove water to a certain level to slow down microbial spoilage and chemical interactions (Rashid *et al*., 2022). In this research, we aimed to enhance the chemical stability of semi-dried potato sticks during storage by applying appropriate edible coatings. Since the semi-dried potato slices available in frozen conditions are kept in the freezer, it is possible to keep them for a longer time in the refrigerator by semi-drying them. By using the coating of soy protein and whey protein, which improves the quality characteristics and increases the shelf life of potato slices, it is possible to provide a product that can be made in easier conditions and offered with better quality.

Materials and Methods

Materials

Whey protein (85%) was bought from Arla Denmark, and soy protein was collected from the Somiah Company in Behshahr. N-hexane (with a purity of 95%), 2,6-dichlorophenol, indophenol, calcium chloride, sodium hydroxide, Fehling A and B, and glycerol were obtained from Merck, Germany, and frying oil (a mixture of sunflower, soybean, and cottonseed) was purchased from Behshahr Industrial Company.

Preparation of Potato Sticks

The Agria potatoes were obtained from farmers in Golestan province. The potatoes were peeled with a sharp knife and sliced with a homemade slicer into sticks with dimensions of 6×1×1 cm. They were then blanched for 4 minutes in boiling water and immediately washed with cold water (Shakouri *et al*., 2018).

Preparation of Coatings from Whey Protein and Soy Protein Concentrate

The concentrations of whey protein and soy protein concentrates were chosen based on findings from a literature review and initial laboratory tests. Whey protein concentrate with concentrations of 2.5%, 4%, and 5% (w/w dry matter) was prepared in distilled water and stirred on an Erlenmeyer shaker (Mrhei-Standard/Heidolph, Japan) at 180 rpm for 1 hour. The solution was then heated in a water bath (Shimaz co. SHVB15) at 90°C for 1 hour and immediately placed in ice water to prevent protein denaturation. As a plasticizer, 2.5% (w/w dry matter) of glycerol was added to the solution and stirred for 10 minutes in an Erlenmeyer shaker at 140 rpm. The solution was then filtered (Sheerzad *et al*., 2024). Soy protein concentrate was dissolved in distilled water at three concentrations of 2.5%, 4%, and 5% (w/w dry matter), then glycerol was added as a plasticizer (50% of the weight of soy protein concentrate). The pH of the solution was adjusted to 10 with 0.1N sodium hydroxide. The solution was stirred on a magnetic hot plate for 20 minutes (Erdem & Kaya, 2021). Potato sticks were immersed in protein coating solutions for 2 minutes, after which the coated and uncoated samples were placed in the oven (model D91126, Memert, Germany). For the heat to reach all sample surfaces uniformly, the samples were continuously turned upside down during drying. The drying process continued until the moisture content of the samples reached 30%. The control (Semi-dried sticks without coating treatment) and treatment (coated) samples were then packed in polyethylene bags and stored at 4°C. The qualitative properties of control and treatment samples were determined after 20, 40, and 60 days of storage (Karacabey *et al*., 2023).

Methods

The tests carried out in this research include: colorimetry, rehydration of dried slices, measuring the amounts of reducing sugars, starch, ascorbic acid, moisture, oil absorption, texture crispness, and sensory evaluation.

Colorimetry

Image processing was used for colorimetric measurement of coated and uncoated samples. An HP Scanjet scanner was used to prepare images of samples with a resolution of 300 dpi. The images were converted from RGB to LAB color space using the software's color-space converter plug-in. Then the b*, a*, and L* parameters of the potato images were obtained by Image J software version 1.5 (Ziaolhagh *et al*., 2017)

Rehydration

To check the samples' water absorption ability, they were immersed in distilled water (approximately 100 ml of water per 5 grams of the sample) at ambient temperature. The rehydration was continued until the potato slices attained constant weight. The maximum ability of the samples to reabsorb water was calculated from Equation (1), where M_1 and M_2 are the sample weights before and after rehydration, respectively (Jafari *et al*., 2019).

$$\%rehydration = \frac{M_2 - M_1}{M_2} \times 100 \qquad (1)$$

Starch and Reducing Sugars

The polarimetry method was used to determine starch. The semi-dried potatoes were ground by a Nicer Dicer model chopper for 3 minutes at high speed. Ten milliliters of distilled water and 65 ml of calcium chloride were added to 2.5 g of powdered potato in a 250 ml Erlenmeyer flask. The solution was heated for 25 minutes at 120ºC, cooled to room temperature, poured into a 100 ml flask, and washed with calcium chloride. Then 2 ml of sucrose was added to a balloon and was made up to 100 ml with calcium chloride. The content of the balloon was filtered and poured into the WXG-4 Disk polarimeter-chin cell, and the percentage of starch was calculated from Equation (2) (Parvaneh, 2022).

$$C = \frac{100\,A}{L[\![\alpha]\!]} \times \frac{100}{W} \qquad (2)$$

Where C is the starch percent, A is the optical rotation in degrees, L is the length of the polarimeter cell (dm), α is the specific rotation degree (195.2) and W, is the sample weight. Fehling method was used to determine reducing sugar (Parvaneh, 2022).

Ascorbic Acid

Ascorbic acid was measured according to the method described by the National Standard of Iran, No. 5609 (Iran Institute of Standards and Industrial Research, 1994). Five grams of powdered potato samples were dissolved in 5% (w/v) metaphosphoric acid and titrated against 2,6-dichloroindophenol solution. The ascorbic acid content was expressed as milligrams per 100 grams of dried potato.

Moisture

The moisture content was measured using the weight difference method following the approach of Ziaolhagh and Kanani, with some modifications (Ziaolhagh & Kanani, 2021). Approximately 5 grams of each sample were placed in designated containers and dried in an oven set at 105ºC for about 5 hours until a stable weight was reached. The moisture content was calculated by determining the weight difference, as outlined in Equation (3).

$$\%Moisture = \frac{M_1 - M_2}{M_1} \times 100 \quad (3)$$

M_1 and M_2 are the sample weights before and after drying, respectively.

Oil Absorption

Frying was done using a home fryer and a mixture of soybean and sunflower oil (Bahar brand) at 175°C. The semi-dried potato samples were fried in oil until a light color and crispy texture were obtained (2.5 minutes). Then, the samples were cooled to room temperature. Soxhlet extractor (Jerhardt, Germany) with n-hexane was used to determine oil content. The oil absorption ratio was reported as the percentage difference between the amount of oil in the sample before and after frying (Shakouri *et al*., 2018).

Crispness

The crispiness of potato slices was evaluated using Kramer's test with a texture tester (HOUNSFIELD H5KS). Five vertical blades were employed, and four potato sticks were tested in each trial. The blades moved at a speed of 96 mm per minute, with a penetration depth set to 13 mm. In each test, the amount of force required for cutting was recorded (Grau *et al*., 2024).

Sensory Evaluation

Ten trained panelists, aged 20 to 40 years, evaluated the sensory characteristics of the potatoes, including color, texture, taste, and overall acceptance, based on a five-point hedonic scale. The potato samples were fried before being given to the panelists for sensory evaluation. In addition, the panelists were asked to drink cold water after testing each sample.

Statistical Analysis

All measurements were done in three repetitions using "Repeated Measure" and "Univariate" tests. Muchly's Test of Sphericity was performed before the repeated-measure test. Analysis was performed using SPSS 0/17 (SPSS Inc., Chicago, IL).

Result and Discussion

Colorimetric

Fig. 1A-C show the lightness (L*), red-green (a*), and yellow-blue (b*) of semi-dried potatoes. The results showed that the brightness increased significantly as the concentration of both coatings increased (p>0.05). In all concentrations and for both types of coatings, the lowest level of lightness was on day 60 (Fig. 1A). The intensity of the green color of the samples increased with increasing concentration (decrease of a*). Moreover, the a* increased in the control sample. In addition, the a* increased during storage. The results of the statistical analysis also showed that the interaction effect of time and concentration was significant (p>0.05) (Fig. 1B). The yellow-blue index (b*) in the semi-dried potato samples coated with soy protein concentrate and whey protein increased over time. This index decreased significantly as the concentration of the coating proteins increased (p<0.05). In addition, the b* value of uncoated samples was more significant than that of coated samples (Fig. 1C).

Laksana *et al*. (2024) showed that fresh-cut apples covered by whey protein had the highest value in lightness/darkness (L*) and yellow/blue index (b*) (p<0.05) (Laksana *et al*., 2024). The authors attributed the obtained results to the oxygen barrier and the antioxidant property of whey protein, which was attributed to the anti-browning amino acid cysteine. The green-red index (a*) of the potatoes coated with soy protein concentrate and whey protein was estimated as -2.26 and -2.99, respectively, which were significant (p<0.05). This agreed with the findings of other authors who worked on potatoes, apples, and pears (Aayush *et al*., 2022).

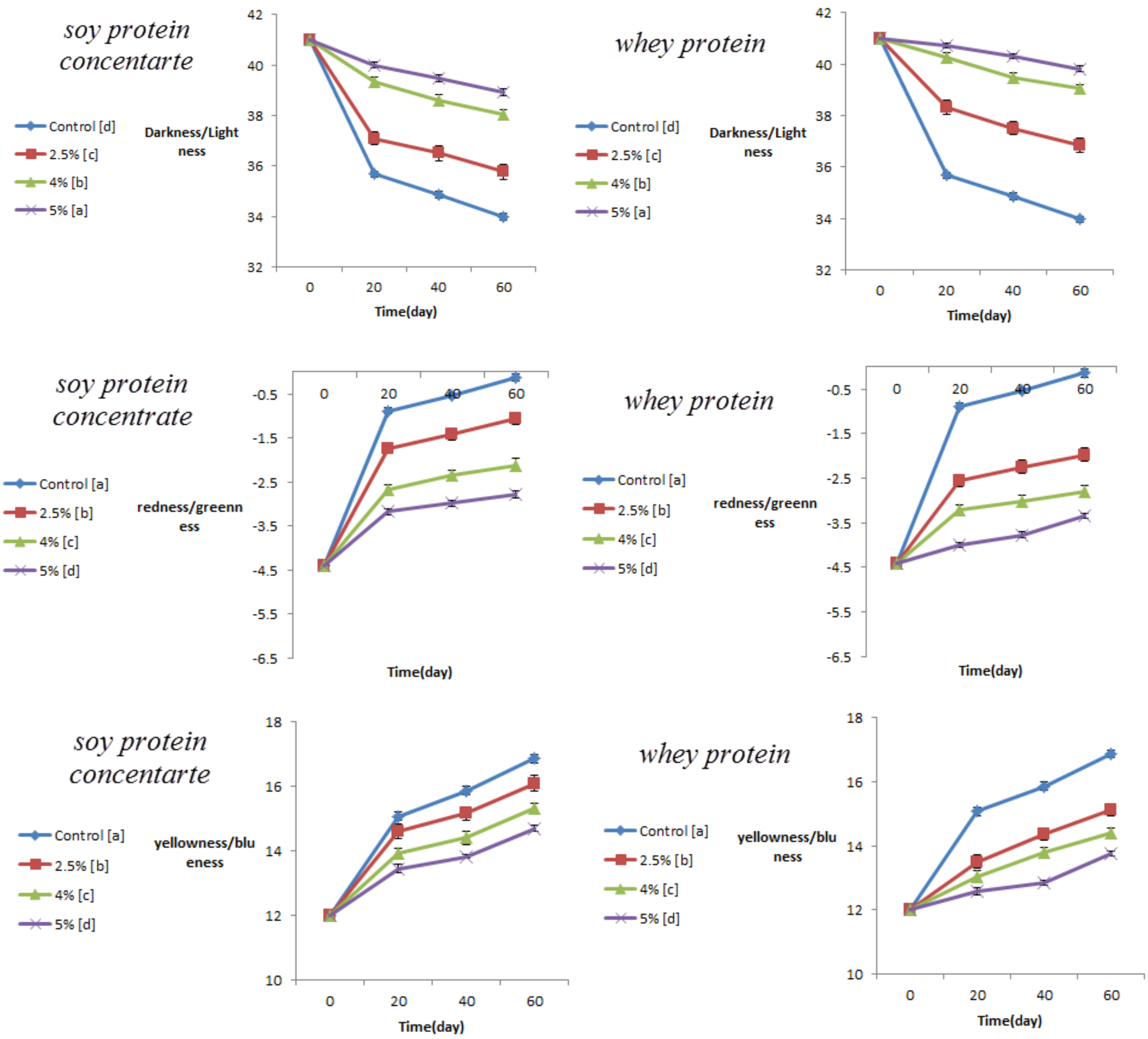

Fig. 1. The effect of different concentrations of soy and whey proteins on (A) the light-darkness index (L*), (B) the red/green index (a*), and (C) the yellow-blue index (b*) of semi-dried potatoes

Rehydration

Fig. 2 shows the effect of whey and soy protein coatings on the rehydration characteristics of semi-dried potatoes during 60 days of storage. Samples with 5% soy or whey proteins absorbed the most water. This observation could be related to the constant storage capacity of dry matter. With the increase in coating concentration, the amount of water absorption increased due to the change in water absorption capacity (WAC). Rehydration decreased significantly by storage time ($p<0.05$). Most potatoes and their products are made up of starch and sugars, and the starch changes over time, which leads to the so-called staleness or retrogradation. Retrogradation is a phenomenon that occurs as a result of the staling of starch materials, and generally, the connection of amylose chains to each other is responsible for its occurrence. As the retention time increases, the connection of long amylopectin branches to each other causes aggravation. These structural changes are responsible for phenomena such as the decrease in water absorption power of the samples and the decrease in their transparency (Rolandelli *et al.*, 2024).

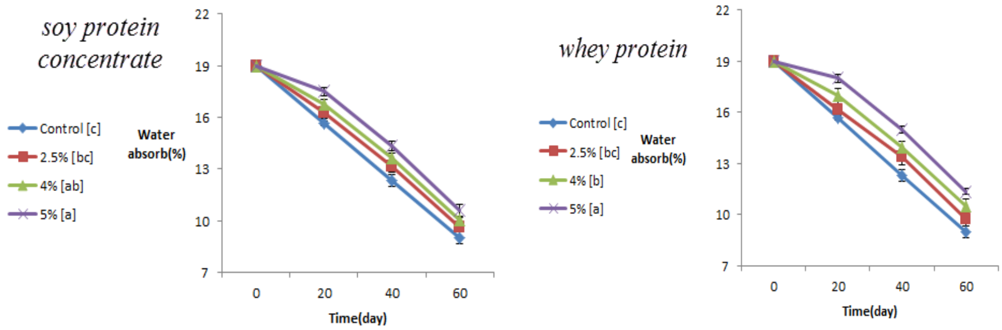

Fig. 2. The effect of soy protein concentrates and whey coating on the Water reabsorption of semi-dried potatoes

Starch and Reducing Sugar

Fig. 3 shows the amount of starch in semi-dried potatoes coated with soy protein concentrate and whey protein. Soy protein coating at a concentration of 2.5% showed no significant difference with the uncoated potato samples ($p< 0.05$). Semi-dried potatoes in both coatings had the lowest amount of starch on day 60 because the breakdown of starch with the help of amylases during drying by hot air causes a further increase in water binding capacity and a decrease in viscosity, and its digestibility increases. Therefore, starch is easily removed from dried products (Flores-Silva *et al.*, 2023).

This research found that the starch content in potatoes coated with whey protein was significantly higher than in those coated with soy protein concentrate ($p < 0.05$). Potatoes coated with 2.5, 4, and 5% of whey protein and the control sample showed no significant difference ($p>0.05$).

Fig. 4 indicates that the reducing sugar in potatoes coated with both types of protein increased with time ($p<0.05$). However, the interaction effect of time and concentration was not statistically significant ($p>0.05$). The highest amount of reduced sugar was seen in the control sample, which can be due to the rapid conversion of starch to sugar due to the reduction of moisture and acidity through physiological changes during the storage period.

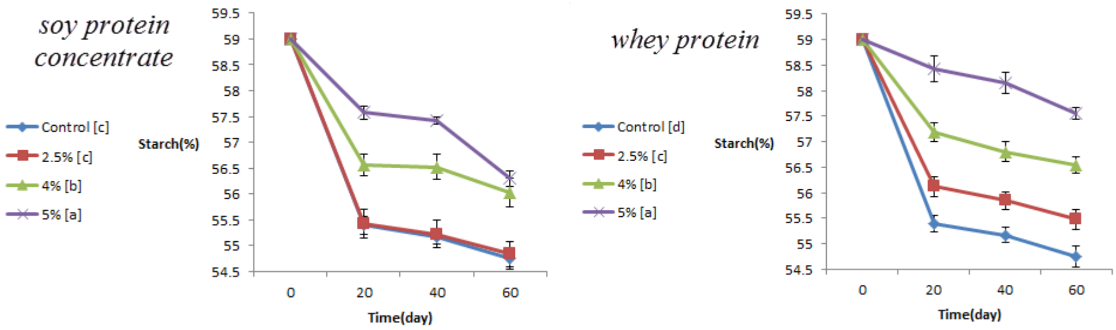

Fig. 3. The effect of soy protein concentrates and whey coating on starch in semi-dried potatoes

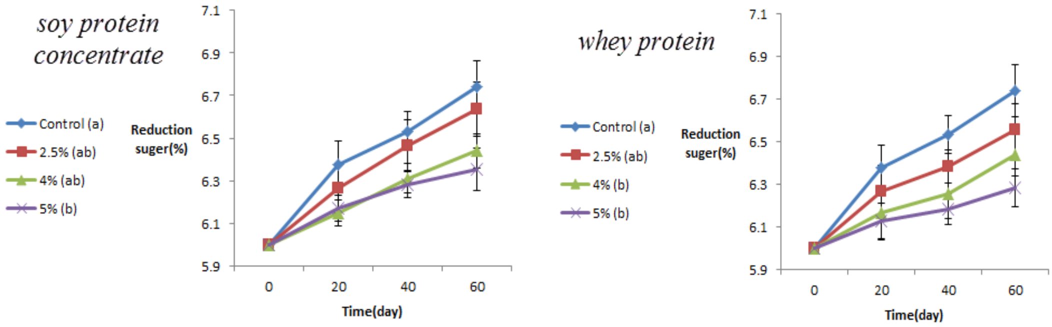

Fig. 4. The effect of soy protein concentrates and whey coating on reducing sugar in semi-dried potatoes

Ascorbic Acid

Fig. 5 shows the effect of different concentrations of soy protein concentrate and whey protein coatings on ascorbic acid. In both coatings, the ascorbic acid content of semi-dried potatoes significantly increased with the concentration increase ($p < 0.05$). The higher ascorbic acid content of the coated samples was due to the low oxygen permeability of the coating. The oxygen had less contact with the coated potatoes, which reduced enzyme activity and prevented the oxidation of ascorbic acid (Etxabide *et al*., 2023). In addition, whey protein has antioxidant properties due to its amino acid cysteine, which reduces the loss of ascorbic acid due to oxidation (Chen *et al*., 2023). During the storage time, the amount of ascorbic acid decreased ($p < 0.05$). As the storage time increased, the samples were exposed to light and air for a longer time, and as a result, the amount of ascorbic acid decreased due to its sensitivity to light and oxygen. The type of coating used showed no significant effect on the ascorbic acid content of the potato sticks during the storage period ($p>0.05$).

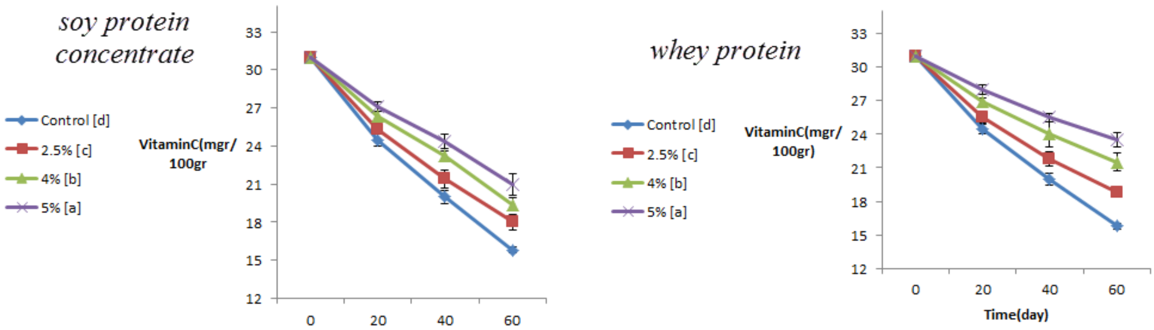

Fig. 5. The effect of soy protein concentrates and whey coating on the ascorbic acid of the semi-dried potato

Moisture

Fig. 6 shows the effect of soy concentrate and whey protein coatings on the moisture content of semi-dried potatoes during storage. Potatoes coated with 5% concentration of both types of coatings showed the highest amount of moisture. A significant decrease in water content was observed during storage time ($p<0.05$). The most important feature of food film or coating is its hindrance to moisture. Changes in the moisture level of food can occur inside or between the food and the air around it. The moisture transfer rate between the food and the surrounding atmosphere is reduced by completely covering the food with a film or coating (Sakooei-Vayghan *et al.*, 2021). These results may be attributed to the excellent barrier properties of whey proteins due to their disulfide bonds and hydrophobic effect. Also, whey protein contains calcium, which increases the hydrophobicity of the protein. The increase in hydrophobicity can be attributed to divalent calcium cations, which cause the creation of cross-links between hydroxyl groups in peptides, and as a result, the bonds with water decrease, which causes the creation of a moisture barrier structure of films (Dedebas, 2024). The amount of moisture in potatoes coated with soy protein concentrate was significantly higher than that coated with whey protein ($p < 0.05$). The soy film is a homogenized oil-in-water emulsion, and the emulsification property of proteins has a linear relationship with its hydrophobic surface, so it can be considered that soy protein is a barrier against moisture, which is due to its inherent hydrophobic properties.

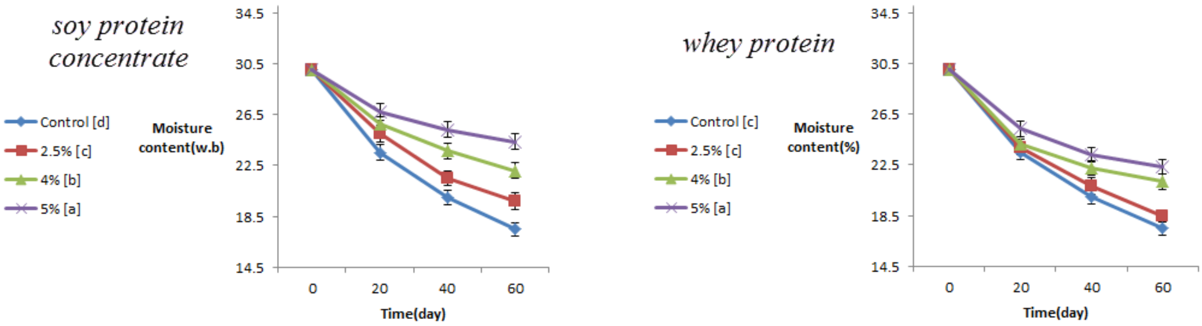

Fig. 6. The effect of soy protein concentrates and whey coating on the moisture of the semi-dried potato during a time

Oil Absorption

Fig. 7 shows the amount of oil absorption of semi-dried potatoes during 60 days of storage. Application of edible coatings on the product caused a significant decrease in oil absorption ($p<0.05$). This decrease can be attributed to the barrier properties against water vapor in different concentrations, which ultimately causes an effect on moisture and oil absorption (Abbasi *et al.*, 2015). The highest amount of oil absorption in both types of coating was estimated on day 60. On this day, the lowest moisture in the product was seen in both types of coating, which indicates the inverse relationship between moisture and oil absorption. On the other hand, the control sample had the lowest amount of moisture, which showed the highest oil absorption due to the inverse relationship between moisture and oil absorption. The interaction effect of time and concentration was also significant ($p<0.05$). The results showed that the amount of oil absorption in potato product covered by whey concentrate was less than that of the other coating. There was no significant difference between potato products covered by these two types of proteins ($p>0.05$).

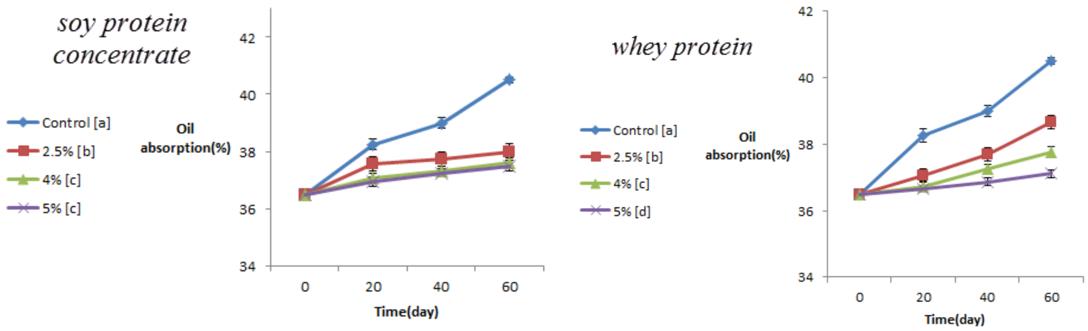

Fig. 7. The effect of soy protein concentrates and whey coating on oil absorption of the semi-dried potato during a time

Texture Crispness

Fig. 8 shows the effect of different concentrations of soy protein concentrate and whey protein on potato crispiness for 60 days. The use of protein coatings caused a significant decrease in the crispiness of the products ($p<0.05$). This may happen because a soft crust forms when the coating solution is applied. This top crust hardens during frying at high temperatures (Deo *et al.*, 2023). On the other hand, as mentioned previously, the control sample had less moisture. As the moisture content of the sample decreases, it becomes more brittle (Zielinska *et al.*, 2020). The interaction effect of time and concentration was also significant ($p<0.05$). The results showed that potatoes coated with soy protein concentrate had less crispness than samples coated with whey protein. The differences in surface properties of two coating proteins may explain this phenomenon (Jeon *et al.*, 2023).

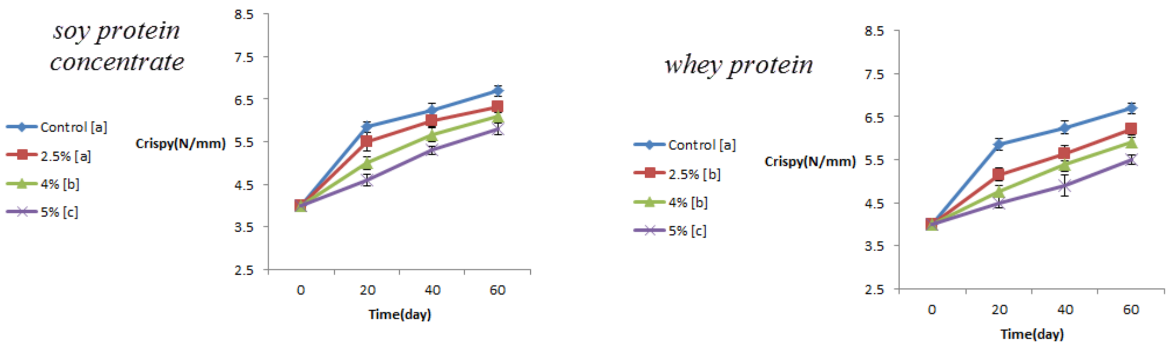

Fig. 8. The effect of soy protein concentrates and whey coating on the crispiness of the semi-dried potato during a time

Sensory Test

The sensory properties of coated semi-dried potato, including texture, taste, color, and overall acceptance, were evaluated. In both types of coatings, the concentration of 5% received the lowest score for texture (Fig. 9), which can be related to the crispiness of the product. The results regarding crispiness also indicated that using 5% concentration reduced the crispiness of the final product. The texture scores of the product on the 20th day and the first day were the lowest and the highest, respectively. Also, there was no significant difference between the samples covered with 2.5% whey protein and the control sample ($p>0.05$). The interaction effect of concentration and time was also significant ($p<0.05$).

Sensory judges evaluated the taste of the semi-dried potatoes over a storage period of 60 days. The application of the coating in the potato product at a concentration of 5% received the highest score from the sensory judges, and no significant difference was seen in the samples coated with soy protein concentrate at concentrations of 4 and 5% (Fig. 9) ($p > 0.05$). Potatoes coated with 2.5% soy protein concentrate scored lowest on the 20th day. The interaction effect of time and concentration was significant ($p<0.05$).

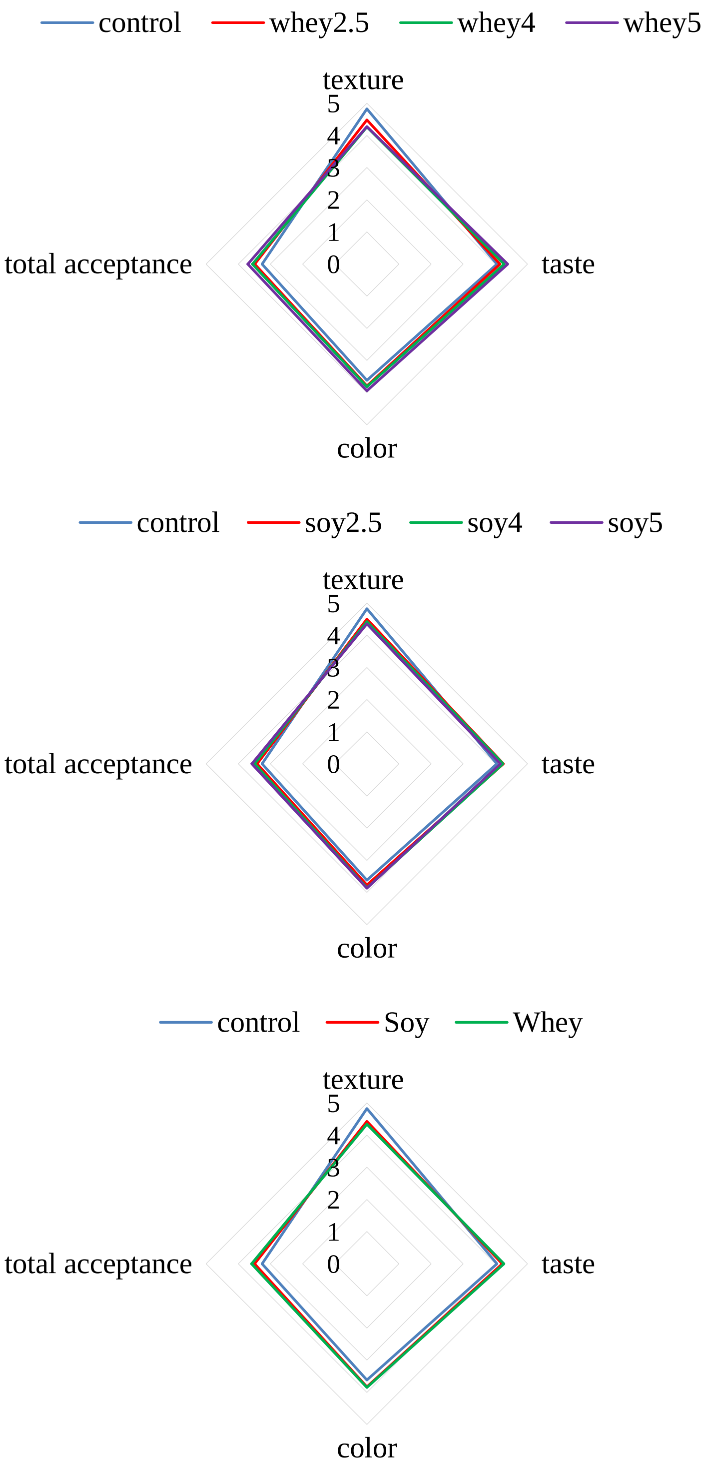

Fig. 9. The effect of soy protein concentrates and whey coating on sensory properties of the semi-dried potato during 60 days of storage

Color was another index investigated in the sensory test. The color scores showed that concentration was a compelling factor in this index, and the control sample got the lowest score. However, there was no significant difference between the control samples and potatoes with 2.5% whey protein coating. Potatoes coated with 4 and 5% soy protein also showed no significant difference ($p < 0.05$). The lowest color score in both coatings was estimated for the control sample on day 40.

The highest overall acceptance score in potato products in both types of coatings was related to the concentration of 5%; however, no significant difference was seen in the case of soy protein concentrate in concentrations of 2.5% and 4% (Fig. 9). The overall acceptance scores of potato samples decreased significantly during storage time ($p<0.05$). The interaction effect of storage time and concentration was significant ($p<0.05$).

Conclusion

As consumer awareness grows regarding environmental issues, there will be a shift towards more sustainable food preservation methods. Edible coatings, being biodegradable and derived from natural sources, are well-positioned to meet this demand. The research highlights the potential of plant-based coatings, which aligns with the global trend toward reducing plastic waste in food packaging. This study used soy protein concentrate and whey protein to coat semi-dried potatoes. The study evaluated the chemical, physical, and oil absorption properties of the coated potatoes during a 60-day storage period. Protein coatings caused the potato product to have higher moisture content than the control sample, and as a result, these coatings caused less oil absorption in the potato samples. The moisture content of semi-dried potatoes coated with soy protein concentrate was higher than that of samples coated with whey protein. The oil absorption of potato samples coated with soy protein concentrate was lower than the oil absorption of those coated with whey protein. The results proved the maintenance of ascorbic acid by coating the potatoes with proteins. In addition, coatings prevented the formation of reducing sugars in potatoes. Semi-dried potato sticks coated with soy protein concentrate and whey protein had more starch than the control samples. Soy protein concentrate and whey protein as coatings for semi-dried potatoes improved water reabsorption of potatoes during 60 days of storage. The rehydration of potatoes coated with whey protein was higher than that of soy protein concentrate. Both coatings reduced the crispness of the final product. According to the sensory evaluation, the overall acceptance score of the coated potatoes was higher than the control samples, although there was no significant difference between the two coatings. The results showed no significant difference between potatoes coated with soy protein and those coated with whey protein. However, all the investigated properties except the degree of crispness improved with increasing concentrations of both proteins. Therefore, it is recommended to use soy protein or whey protein at a concentration of 5% for coating potato slices. Future studies may focus on incorporating additional functional ingredients into edible coatings, such as antimicrobial agents, antioxidants, or nutraceuticals. This could enhance the coatings' effectiveness in preserving food quality while providing health benefits. Research may focus on optimizing coating formulations tailored to specific produce or storage conditions. This could include using different concentrations of soy and whey proteins or combining them with other biopolymers to achieve the desired physicochemical properties. Researchers may explore advancements in coating application methods, including nano-coating technologies and ultrasound-assisted techniques to enhance film properties.

Author Contribution

Z. Moslehi, S. Bani: Data curation, investigation, methodology, writing the original draft. **M. Bolandi, S.H. Ziaolhagh:** Conceptualization, data curation, software, project administration, supervision, writing review, and editing.

Acknowledgment

The authors thank the Department of Food Science and Technology, Damghan Branch, Islamic Azad University (Damghan, Iran) and Agricultural Engineering Research Department of Agricultural and Natural Resources Research and Education Center of Semnan Province (Shahrood), AREEO, (Shahrood, Iran) for their advisory support in implementing this project.

Funding Sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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مقاله پژوهشی

جلد ۲۱، شماره ۳، مرداد- شهریور ۱٤۰٤، ص. ۲۸۷-۳۰۱

تأثیر پوشش‌های خوراکی حاوی کنسانتره پروتئین سویا/ پروتئین آب پنیر بر کیفیت خلال‌های نیمه‌خشک سیب‌زمینی

زینب مصلحی[۱]- مرضیه بلندی[۱]- سیدحمیدرضا ضیاءالحق[۲*]- سیما بانی[۱]

تاریخ دریافت: ۱۴۰۳/۰۷/۱۱
تاریخ پذیرش: ۱۴۰۳/۱۱/۲۷

چکیده

استفاده از پوشش‌های خوراکی یک رویکرد پایدار و سازگار با محیطزیست برای حفظ کیفیت غذا در طول ذخیره‌سازی می‌باشد. هدف از این تحقیق بررسی تأثیر پوشش خوراکی تهیه شده از کنسانتره پروتئین سویا و پروتئین آب پنیر در افزایش پایداری شیمیایی خلال‌های سیب‌زمینی در طول دوره نگهداری می باشد. از آنجایی که معمولا خلال‌های نیمه آماده سیب‌زمینی منجمد و در فریزر نگهداری می‌شوند، می‌توان با نیمه‌خشک کردن و کاهش رطوبت آنها، این محصول را در دمای یخچال نگهداری کرد. در این تحقیق تأثیر غلظت‌های مختلف (۲/۵، ۴ و ۵ درصد وزنی) کنسانتره پروتئین سویا و پروتئین آب پنیر بر برخی ویژگی‌های فیزیکوشیمیایی سیب‌زمینی نیمه‌خشک (رنگ، جذب آب خلال‌های خشک، قندهای احیاکننده، نشاسته، اسید اسکوربیک، رطوبت، جذب روغن، تردی بافت و خواص حسی) طی ۶۰ روز نگهداری مورد بررسی قرار گرفت. نتایج نشان داد که سیب‌زمینی نیمه‌خشک پوشیده شده با کنسانتره پروتئین سویا و آب پنیر دارای بیشترین رطوبت و کمترین جذب روغن و ترد بودن نسبت به نمونه شاهد بود. تست حسی نمونه‌های پوشش داده شده با کنسانتره پروتئین سویا و آب پنیر با نمونه‌های بدون پوشش متفاوت بود و طعم سیب‌زمینی نیمه خشک با پوشش پروتئینی مورد قبول مصرف‌کنندگان قرار گرفت. پوشش‌های خوراکی اعمال‌شده تأثیر معنی‌داری بر اسید اسکوربیک و کاهش قند داشتند. کمترین و بیشترین مقدار نشاسته در نمونه‌های شاهد و پوشش داده شده مشاهده شد. این ویژگی‌ها نشان می‌دهد که پوشش‌های مبتنی بر کنسانتره پروتئین سویا و آب پنیر انتخابی عالی برای کاهش جذب روغن و افزایش ماندگاری خلال‌های نیمه‌خشک سیب‌زمینی هستند.

واژه‌های کلیدی: پروتئین آب پنیر، پروتئین سویا، پوشش‌های خوراکی، سیب‌زمینی خشک، عمر انباری

۱- گروه صنایع غذایی، دانشگاه آزاد اسلامی واحد دامغان، دامغان، ایران
۲- بخش تحقیقات فنی و مهندسی کشاورزی، مرکز تحقیقات و آموزش کشاورزی و منابع طبیعی استان سمنان (شاهرود)، سازمان تحقیقات، آموزش و ترویج کشاورزی، شاهرود، ایران
(*- نویسنده مسئول: Email: h.ziaolhagh@areeo.ac.ir)

https://doi.org/10.22067/ifstrj.2025.90051.1373

Iranian Food Science and Technology
Research Journal
Homepage: https://ifstrj.um.ac.ir

Research Article
Vol. 21, No. 3, Aug.-Sep. 2025, p. 303-316

Physicochemical, Functional and Rheological Properties of Soy Protein Isolates Prepared with Various Iranian Soybean Cultivars

B. Shokrollahi Yancheshmeh[1], M. Varidi [1*], S.M.A. Razavi [1,2*], F. Sohbatzadeh [3]

1- Department of Food Science and Technology, Ferdowsi University of Mashhad, Mashhad, Iran
(*- Corresponding Authors Email: m.varidi@um.ac.ir, s.razavi@um.ac.ir)
2- Center of Excellence in Native Natural Hydrocolloids of Iran, Ferdowsi University of Mashhad, PO Box: 91775- 1163, Mashhad, Iran
3- Department of Atomic and Molecular Physics, Faculty of Science, University of Mazandaran, Babolsar, Iran

		How to cite this article
Received:	25.12.2024	**How to cite this article:** Shokrollahi Yancheshmeh, B., Varidi, M., Razavi, S.M.A., & Sohbatzadeh, F. (2025). Physicochemical, functional and rheological properties of soy protein isolates prepared with various Iranian soybean cultivars. *Iranian Food Science and Technology Research Journal*, *21*(3), 303-316. https://doi.org/10.22067/ifstrj.2025.91259.1393
Revised:	19.01.2025	
Accepted:	02.02.2025	
Available Online:	17.06.2025	

Abstract

Soybeans, a prominent legume, offer substantial health benefits due to their rich and beneficial nutritional profile. However, the food sector requires improved protein functions. The functional and physicochemical characteristics of isolates from four widely grown soybean cultivars in Iran, namely Katul, Sahar, Tellar, and Sari, were examined in this research. The proximate analysis revealed significant differences ($p<0.05$) among the cultivars in moisture, ash, protein, and fat contents, with Katul isolates showing the highest protein (90.75%) and lowest fat (3.67%) content. Color analysis indicated significant variations in brightness (L^*), with Katul isolates being the brightest due to lower fat and ash content. Surface hydrophobicity varied significantly among cultivars, with Sahar showing the highest value (360.30 a.u.). Protein solubility was highest for Katul protein isolate (69.43%), influencing functional properties like emulsification and foaming. Cultivar-specific differences were observed in both water absorption capacity (WAC) and oil absorption capacity (OAC), with Tellar exhibiting the highest OAC (2.42 g/mL). Emulsifying properties, evaluated through emulsion stability (ES) and emulsion capacity (EC), were highest for Sari and Katul protein isolates. Foaming properties varied significantly among the samples, so that Katul protein isolate exhibiting the highest foaming capacity (180.50%) and foaming stability (66.3%), likely attributed to its high protein content. Rheological analyses revealed that Katul had the highest consistency index (K) and shear-thinning properties, while Sahar exhibited a more Newtonian-like flow behavior. Gelation studies identified Katul as the most efficient, with the lowest gelling concentration (10%), compared to Sahar's highest value (14%). These findings demonstrate the effect of soybean cultivar on the compositional and functional characteristics of protein isolates, suggesting potential applications in various food products depending on desired functional characteristics.

Keywords: Cultivar, Functionality, Plant protein, Soybean

Introduction

In recent years, global protein consumption has increased significantly. Future increases in protein consumption may be attributed to two factors: the expanding population and shifting

https://doi.org/10.22067/ifstrj.2025.91259.1393

dietary preferences, particularly the rising desire for healthful foods. It is estimated that by 2050, the world's protein consumption will have grown by 50%. In this sense, increasing food production and introducing valuable protein resources can guarantee food security (Westhoek *et al*., 2011; Henchion *et al*., 2017; Fasolin *et al*., 2019). Plant proteins have drawn a lot of interest lately due to their availability, affordability, and physicochemical characteristics (Abbou *et al*., 2019). Because of their beneficial qualities and ability to promote health, legumes are an important part of human nutrition. Furthermore, they are regarded as abundant providers of fiber, proteins, carbohydrates, and some minerals, as well as vitamins (B-vit.) (Boye *et al*., 2010). As a prominent member of the legume family, soybeans are significant because of their composition, which makes them necessary for a healthy diet. They have a significantly higher protein content (38–44%) than grains (8–15%) and other legumes (20–30%). This increases its value as a food and is one of the factors contributing to soybean's economic significance, along with its favorable amino acid profile. The physicochemical and functional characteristics of soy proteins, such as their ability to absorb water and oil and to emulsify, froth, and gel, make them valuable in food applications (Sui, Zhang, & Jiang, 2021; Yada, 2017). Soy protein isolate (SPI) is a high-quality plant-based protein with a protein content exceeding 90%, making it a suitable alternative to animal protein (Zheng *et al*., 2022).

Scientific evidence demonstrates that the molecular and chemical structures of soy proteins predominantly determine their physicochemical properties (Liu *et al*., 2015; Sui *et al*., 2021; Yada, 2017). Pulses cultivars have a major impact on the structure and composition, which in turn affects their functional qualities. Cui *et al*. (2020) examined the functional characteristics of pea protein isolate (PPI), under the influence of four different cultivars (Agasis, Spider, Trapeze and ND Trial). The findings demonstrated that cultivars significantly influenced on solubility, emulsifying capacity and stability, and foaming capacity and stability of PPI (Cui *et al*., 2020). The physicochemical and functional characteristics of the flour of six navy bean cultivars grown in two regions of Manitoba, Canada, were also studied by (Guldiken *et al*., 2021). The findings demonstrated that genotype had a significant impact on the total starch content, lipid content, and total phenolic content of raw navy bean flour. Additionally, the genotype had a significant effect on the oil holding capacity (OHC) and water hydration capacity (WHC) of navy bean flours.

Therefore, in order to manufacture quality functional ingredients, it is necessary to understand the way in which raw materials can influence the functionality of soy protein isolate (SPI). The impact of soy cultivars on the physicochemical and functional properties of SPI has not been investigated and different cultivars may have various functionalities. This study aims to investigate the physicochemical and functional properties of soy protein isolate (SPI) extracted from four prominent soybean cultivars cultivated in Iran. The research focuses on determining the physicochemical characteristics of SPI, including protein, fat, ash, moisture content, and color. Furthermore, the study evaluates the functional properties of SPI, encompassing surface hydrophobicity, water and oil absorption capacity, solubility, foaming, and emulsifying properties. This research endeavors to provide valuable insights for the development and optimization of protein-based products utilizing Iranian soybean cultivars.

Material and methods

Sample Preparation

Four soybean varieties (Katul, Sahar, Tellar, and Sari) were obtained from the Oilseeds Research Institute (Gorgan, Iran). The soybeans were manually cleaned to remove broken seeds and foreign objects. Seeds were then crushed in an electrical miller (type M20IKA) to produce full-fat flour. To obtain defatted soybean flour (DFSF), full-fat soybean flour (FFSF) was defatted with hexane at a 1:5 (w/v) mixing ratio with constant stirring for six hours at room temperature. The defatted flour was air-dried in a fume hood at room temperature, ground in a blender to ensure homogeneity, sieved through a 40-mesh screen, and stored in polyethylene bags at -20°C before further analysis.

SPI Preparation

The SPI was prepared based on the method previously described by Shokrollahi Yancheshmeh *et al*. (2022) with some modifications (Yancheshmeh *et al*., 2022). To summarize, 1M NaOH was used to adjust the pH to 9.5, 50 g of defatted flour was agitated for 1 hour at 25°C (1:20 w/v), and the mixture was centrifuged for 20 minutes at 5000×g. After collecting the supernatant, the pH was adjusted to 4.5, which is the isoelectric point for soy protein. In order to precipitate the protein, the mixture was centrifuged at 5000×g for 20 minutes. The protein was centrifuged at 5000×g for 10 minutes after being cleaned with deionized water and 1M NaOH was used to bring the pH to 7. The extraction procedure was performed at 25°C. The SPI was then freeze-dried and kept for further examination at 4°C.

Physicochemical Properties

Proximate Composition

The protein content of the SPI was determined through the Kjeldahl method (N×6.25). Its fat content was measured based on AOAC 922.06 via the Soxhlet method using the extraction apparatus of B- 811 (Buchi, Switzerland). The moisture and ash contents were quantified through AOAC 925.1 and 923.03, respectively (AOAC, 1990). All results have been expressed on dry weight basis (d.b.).

Color Measurement

In order to obtain the color indices of the samples, a Hunter Lab digital colorimeter (Color Slex, 45Reston VA, and the USA) was employed. The instrument was calibrated using a white plate that was considered as standard color (L*, a*, and b* were 98.84, − 0.73, 1.27 respectively) (Yancheshmeh *et al*., 2022).

Functional Properties

Surface Hydrophobicity Measurement

The surface hydrophobicity was determined based on the method explained by Ding *et al*. (2019) using a fluorometer (Agilent Technologies Inc., Santa Clara, CA, the USA) at λ_{ex} = 390 nm and λ_{em} = 480 nm with a slit width of 2.5 nm. Six concentrations (1-5 mg/mL) were prepared for each SPI sample. Next, 100 µL of anilino-8-naphthalenesulfonate fluorescence (ANS) solution was incorporated into 4 mL of the SPI solution. After the solution was incubated in darkness for 15 min, the absorbance value was measured. The surface hydrophobicity (H_0) was quantified as the slope of the linear regressions of the relative fluorescence intensity against protein concentration (Ding *et al*., 2019).

Protein Solubility (PS)

To determine the protein solubility (mg/ml), at first 1% (w/v) protein dispersion was made in deionized water and stirred (30 min) at ambient temperature. The solution was then centrifuged at 5000 g for 15 min. The protein content of the supernatant was measured through the Biuret method using the UV-2601 spectrophotometer (RayLeight, China) at 540 nm (Feyzi *et al*., 2015). Bovine serum albumin (BSA) was used as an external standard.

Water and Oil Absorption Capacity

The oil absorption capacity (OAC) and water absorption capacity (WAC) of the SPI samples were measured based on (Chandi & Sogi, 2007) with some modifications. For this purpose, 0.5 g (W) of the SPI was dissolved in 5 g of sunflower oil (or 5 g of distilled water for measuring WAC) in 15-mL centrifugal tubes. The tubes were vortexed 30 min at 5-min intervals for 10 s. They were subsequently centrifuged at 2000g for 20 min. Afterwards, the supernatant was removed, and finally, the sediment was weighed (W_1). The values of OAC and WAC were expressed as g/g using Eq. 1:

$$\text{WAC (or OAC)} = (W_1 - w)/w \times 100 \quad (1)$$

Least Gelling Concentration

Determination of LGC was carried out following the method described by Boye *et al.* (2010). Protein isolate solutions with specific concentrations (6, 8, 10, 12, 14, 16, 18, and 20% w/v) were prepared. After stirring for 1 hour, 8 mL of each suspension was transferred into test tubes. These samples were heated in a boiling water bath, then immediately cooled to 4°C and refrigerated overnight. Determination of LGC was performed visually by observing the gel behavior during the inversion test.

Emulsifying Properties

The emulsifying activity (EA) and emulsion stability (ES) of SPI were determined using the method described by Zhu *et al.* (2020), with some modifications. In brief, 45 ml protein suspensions (0.5% w/v or 5 mg/mL) were prepared, and pH was set at 7.0 with 0.1M NaOH or HCl. Then 15 ml of sunflower oil was added, and the mixture was gently stirred using a magnetic stirred for 5 min, followed by homogenization using an Ultratorax (IKA T25, Staufen, Germany) at 20000 rpm for 2 min. Afterwards, 50 µL of the emulsions were diluted 100 times using 0.1% SDS. Eventually, the absorbance value was immediately read at 500 nm (A_0) and after 10 min (A_{10}) using a spectrophotometer (UV–2601, RayLeight, China)

The EAI and ESI were calculated based on the following equations:

$$\text{EA (m}^2\text{/g)} = \frac{2\times 2.303\times A_0\times DF}{10000\times\phi\times L\times C} \quad (2)$$

$$\text{ES (min)} = \frac{A_0\times 10}{A_0-A_{10}} \quad (3)$$

where D stands for the dilution factor (100); C represents the concentration of the SPI (g/mL); L denotes the cuvette optical path length (1 cm); ϕ shows the emulsion oil phase fraction (0.25); and A_0 and A_{10} respectively indicate the emulsion's absorbance values at the times 0 and 10 min.

Foaming Capacity and Stability

The foaming properties of SPI were determined according to Shokrollahi Yancheshmeh *et al.* (2022) with some modifications. For this urpose, the SPI suspensions were prepared at 0.5% (w/v), and their pH values were set at 7.0 with 0.1M NaOH or HCl before the homogenization. Then suspensions were poured into a 50-mL graduated cylinder to measure the volume (V_0). The suspensions were homogenized with Ultratorax homogenizer (25 digital Model, IKEA Company) at 10,000 rpm for 2 minutes and immediately recorded foam volume (V_1) (Yancheshmeh *et al.*, 2022). The foam capacity (FC) was calculated as follows:

$$\text{FC (\%)} = (V_1/V_0)\times 100 \quad (4)$$

Foam stability (FS) was calculated after 60 min based on Eq.3:

$$\text{FS (\%)} = (V_t/V_1)\times 100 \quad (5)$$

Where V_1 represent the volume of the foam after whipping at time 0 min; and V_t denotes the volume of the foam after 60 min.

Time-independent Steady Shear Rheological Measurements

Samples (10% protein content; pH 7) were subjected to shear rates ranging from 1 to 200 s^{-1} and the resulting shear stress was recorded (at 25°C). To ascertain the samples' shear-dependent rheological properties, the Power law model (Eq.6) and Herschel-Bulkley model were (Eq.7) fitted to the experimental shear stress-shear rate data:

$$\tau = K(\gamma^{\cdot})^n \quad (6)$$

$$\tau = \tau_0 + K(\gamma^{\cdot})^n \quad (7)$$

Where, τ is the shear stress (Pa); τ_0 is the yield stress (Pa); k indicates the consistency index (Pa s^n); γ represents the shear rate (s^{-1}); and n is the flow behaviour index (dimensionless) (Steffe, 1996).

Statistical Analyses

All the measurements were at least triplicated, and the data have been expressed as mean ± standard deviation. In order to analyze the obtained data, one-way analysis of variance (ANOVA) and Duncan's multiple-range test were performed at $p<0.05$ using SPSS version 22.

Result and Discussion

Proximate Composition

The compositions of soybean seeds and protein isolates of four soybean cultivars (Katul, Sahar, Tellar, and Sari) are presented in Table 1. The moisture and ash contents of soybean seeds lay in the range of 7.56- 8.40% and 4.87-6.10% respectively. Fat and protein contents varied from 20.89-21.78% and 37.15-41.59% respectively. Moreover, the ash and moisture contents of soybean isolates were in the range of 7.94-9.04% and 2.32-3.91%, respectively. The fat and protein contents of soybeen isolates varied between 3.67-4.61% and 84.83-90.75% respectively. There were significant ($p<0.05$) differences between the moisture, ash, fat, and protein contents of the four soybean cultivars. Katul protein isolates had more protein (90.75%) and less fat (3.67%), moisture (7.94%) and ash (2.32%) than others. An analysis of variance found that cultivars played a significant role in the determination of the SPI yield ($p < 0.05$), which was maximized in the case of Katul at 28.12%, followed by Sari, Sahar, and Tellar gave lower yields at 27.12, 26.89 and 26.40 g/100 g, respectively (Table 1).

Table 1- Chemical compositions and color parameters of soybean seeds and soy protein isolates determined for different cultivars

Physicochemical properties	Cultivar			
	Katul	Sahar	Tellar	Sari
Protein content of raw seed (%)	38.50±0.22 [c]	41.59±0.41 [a]	37.15±0.35 [d]	39.16±0.14 [b]
Fat content of raw seed (%)	21.35±0.11 [b]	21.65±0.09[a]	20.89±0.18 [c]	21.78±0.16 [a]
Moisture content of raw seed (%)	8.26±0.20 [b]	7.56±0.15 [d]	8.02±0.17 [c]	8.40±0.12 [a]
Ash content of raw seed (%)	5.12±0.15 [b]	5.02±0.19 [c]	6.10±0.23 [a]	4.87±0.17 [d]
Protein content of isolate (%)	90.75±0.54 [a]	89.14±0.48 [b]	84.83±0.35 [d]	85.70±0.42 [c]
Fat content (%)	3.67±0.12 [d]	3.86±0.10 [c]	4.61±0.18 [a]	4.32±0.14 [b]
Moisture content (%)	7.94±0.10 [d]	8.49±0.18 [b]	9.04±0.20 [a]	8.14±0.19 [c]
Ash content (%)	2.32±0.32 [d]	3.91±0.19 [a]	2.84±0.20 [c]	3.12±0.15 [b]
Yield (g/100 g)	28.12±0.23 [a]	26.89±0.36 [c]	26.40±0.24 [d]	27.12±0.36 [b]
Color				
*L**	84.34±0.17 [a]	81.86±0.31 [b]	68.57±0.45 [d]	78.05±0.21 [c]
a*	-2.07±0.07 [c]	-2.12±0.10 [c]	-0.92±0.05 [a]	-1.71±0.14 [b]
b*	25.31±0.21 [c]	26.53±0.37 [a]	16.39±0.16 [d]	26.27±0.45 [b]

a–d: Means sharing the same letter in the same row do not differ significantly ($p > 0.05$).

Color

An important parameter about protein isolates is their color. The *L** parameter indicates the degree of brightness and can take values from 0 to 100. The higher the *L** value the brighter the color (Nielsen, Wrolstad, & Smith, 2010). As seen in Table 1, there was a statistically significant difference between the *L** parameter of protein isolates. The highest *L** parameter was related to Katul, probably due to the lower amount of fat and ash in Katul protein isolate. Parameter *a** ranges from negative values (indicating green color) to positive values (indicating red color) and parameter *b** also ranges from negative values (blue color) to positive values (yellow color) (Nielsen *et al*., 2010). According to Table 1, there was a significant difference between the parameters *a** and *b** of the isolates ($p < 0.05$). The highest absolute value of a* and the amount of *b** was related to Sahar protein isolate. In general, protein isolates that partially cause a brown color are desirable for use in breads and cakes, and isolates that help make the product colorless can be used in another group of light-colored breads (Singh *et al*., 2008). Based on this, it is possible to use Katul, Sahar and Sari protein isolates in some bakery products in which a brighter color is desired, and Tellar isolate to create a brown color for the bread crust in colored bread or pasta.

Surface Hydrophobicity

Cultivar had a substantial impact (p>0.05) on surface hydrophobicity values, according to the analysis of variance (Table 2). Tellar, Katul, and Sari produced isolates with lower surface hydrophobicities (337.85, 252.60, and 240 a.u., respectively), whereas Sahar produced an isolate with a greater surface hydrophobicity (360.30 a.u.). The differences in surface hydrophobicity values among the cultivars could be attributed to variations in their protein composition, structure, or amino acid profiles, which influence the exposure of hydrophobic groups. These intrinsic differences affect how the proteins interact with their environment, leading to the observed variations in surface hydrophobicity. According to Cserhalmi *et al*. (1998), surface hydrophobicity can vary depending on the variety of pea. The surface hydrophobicity values of the mixed globulin fractions obtained from five distinct pea varieties lay in the range of 21.81-43.11 a.u (Cserhalmi *et al*., 1998).

Protein Solubility

Table 2 displays the solubility values of the four SPI samples at pH 7.0. Solubility plays a key role in the functional properties of a protein, including emulsification, gelation, and foaming (Kinsella & Melachouris, 1976). Tellar had the lowest solubility, with an average value of 57.82%, based on the data. Compared to Sari (61.52%) and Sahar (64.11%), Katul (69.43%) had a higher solubility. The higher solubility observed in Katul compared to the other cultivars could be explained by differences in protein structure and composition. Proteins from Katul may have a higher proportion of hydrophilic groups exposed on their surface, facilitating better interaction with water. Additionally, variations in amino acid composition and protein folding may enhance the ability of Katul proteins to remain soluble, as solubility is closely linked to the balance between hydrophilic and hydrophobic regions. Different pea genotypes display varying solubility values at pH 7, according to Barac *et al*. 2010. Of the three experimental lines studied, two showed both the lowest (L2=70%) and highest (L1=85%) solubility values.

Table 2- Functional properties of soy protein isolates determined for different soybean cultivars

Functional properties	Cultivar			
	Katul	Sahar	Tellar	Sari
WAC (g/mL)	3.48±0.11 ^{a}	3.13±0.21 ^{c}	3.33±0.23 ^{ab}	3.22±0.30 ^{bc}
OAC (g/mL)	2.13±0.22 ^{b}	1.89±0.15 ^{c}	2.42±0.10 ^{a}	2.12±0.09 ^{b}
Solubility (%)	69.43±0.54 ^{a}	64.11±0.36 ^{b}	57.82±0.41 ^{d}	61.52±0.27 ^{c}
H0	337.85±1.01 ^{b}	360.30±2.05 ^{a}	240±0.94 ^{d}	252.60±0.97 ^{c}
LGC (%)	10.00±1.41 ^{c}	14.00±1.74 ^{a}	12.00±1.22^{b}	12.00±1.45 ^{b}

a–d: Means sharing the same letter in the same row do not differ significantly ($p > 0.05$).

Water and Oil Absorption Capacity

SPI's water absorption capacity (WAC) ranged from 3.13 to 3.48 g/mL of isolates, with a statistically negligible difference (p > 0.05) (Table 2). WAC is essential for certain product qualities, including moisture content and staling. Proteins and carbohydrates, due to their hydrophilic elements such as polar or charged side chains, are the primary chemical components that enhance the WAC of SPI. The range of values for the WAC of kidney bean flour reported by Siddiq *et al*. (2010) and Aguilera *et al*. (2011) were 2.2 to 2.7 kg/kg and 2.2 to 2.7 L/kg, respectively. Variations in a protein's ability to absorb water are connected to changes in its structure. Proteins with a higher surface concentration of hydrophilic groups typically exhibit enhanced water-binding capacity (Feyzi *et al*., 2015). Oil absorption capacity (OAC), which is essential for enhancing mouthfeel and maintaining flavor, is another important functional feature of flours (Kinsella & Melachouris, 1976). OAC varied from 1.89 to 2.42 g/mL for SPI (Table 2). The OAC of Tellar was substantially (p<0.05) higher than those of the other isolates.

The higher OAC of Tellar (2.42 g/mL) compared to other isolates may be attributed to its higher hydrophobic amino acid content and protein structure. Hydrophobic amino acids enhance the ability of proteins to bind non-polar oil molecules. This factor allows Tellar to interact more effectively with oil, resulting in a significantly higher OAC ($p<0.05$). OAC is primarily influenced by protein comprising both hydrophobic and hydrophilic moieties. Lipid hydrocarbon chains and the side chains of non-polar amino acids may have hydrophobic interactions (Wani *et al*., 2013).

Least Gelling Concentration

Significant variations were observed in the least gelling concentration (LGC) among soy protein isolates (SPI) derived from different soybean cultivars (Katul, Sahar, Tellar, and Sari). As presented in Table 2, Katul exhibited the lowest gelling concentration (LGC) (10%), suggesting superior gelation efficiency compared to the other cultivars .

The lower LGC of Katul can be attributed to its higher protein solubility (69.43%) and better water absorption capacity (WAC, 3.48 g/mL), as shown in Table 2. Higher protein solubility ensures a greater availability of protein molecules in the solution, which facilitates the formation of intermolecular interactions, such as hydrogen bonds and hydrophobic interactions, during gelation. Additionally, a higher WAC allows for better hydration of protein molecules, which is crucial for unfolding and aligning the proteins to create a stable and cohesive gel network. These combined factors enable Katul to form a stronger gel network at lower protein concentrations, resulting in its lower LGC compared to other cultivars. In contrast, Sahar exhibited the highest LGC (14%), suggesting weaker gelation properties potentially due to lower levels of hydrogen bonding and hydrophobic interactions compared to Tellar and Sari, which both demonstrated intermediate gelling concentrations (12%).

These differences could be attributed to the varying ratios of glycinin and β-conglycinin, which are the major protein components of SPI and strongly influence gelling behavior. Higher glycinin content is often associated with enhanced gelation properties due to its stronger intermolecular interactions during heat-induced gelation. Additionally, environmental factors during soybean cultivation and post-harvest processing can affect the protein composition and, consequently, functional properties like gelation. The results highlight the significance of cultivar selection for specific applications, especially in food systems that demand precise textural and structural properties. For example, Katul's lower gelling concentration makes it ideal for formulations where strong gel formation is required with minimal protein content.

Emulsifying Properties

Fig. 1A shows the emulsion capacity (EC) results for the four isolates. Sari (74%), Sahar (71.5%), and Katul (70%) exhibited similar EC values, while Tellar (53%) had the lowest. This variation in EC can be attributed to differences in protein structure, which influence the ability to stabilize oil-water interfaces. Sari, Sahar, and Katul likely have more favorable surface properties, such as a higher proportion of hydrophilic groups or better protein conformations, which enhance their ability to form stable emulsions. In contrast, Tellar’s lower EC may be due to less effective interaction between the proteins and the oil phase, possibly due to a higher presence of hydrophobic regions that hinder emulsion formation.

Barac *et al*. (2010) demonstrated that emulsifying stability (ES) does not always correlate directly with emulsifying activity (EA) (Barac *et al*., 2010). Fig. 1B presents the findings of the cultivar’s effect on ES. All isolates showed ES values greater than 35%, and cultivar selection had a significant impact on these values ($p<0.05$). Katul and Sahar cultivars exhibited significantly higher ES compared to the others, which differed markedly from each other ($p > 0.05$). These differences suggest that Katul and Sahar cultivars possess proteins that, due to their molecular structure, provide better stabilization of emulsions. The enhanced ES is likely due to sufficient steric hindrance and/or charge repulsion between oil droplets, which prevents coalescence and maintains the stability of the emulsion. This stability is crucial for applications in food systems, as it ensures uniform dispersion of oil in the aqueous phase.

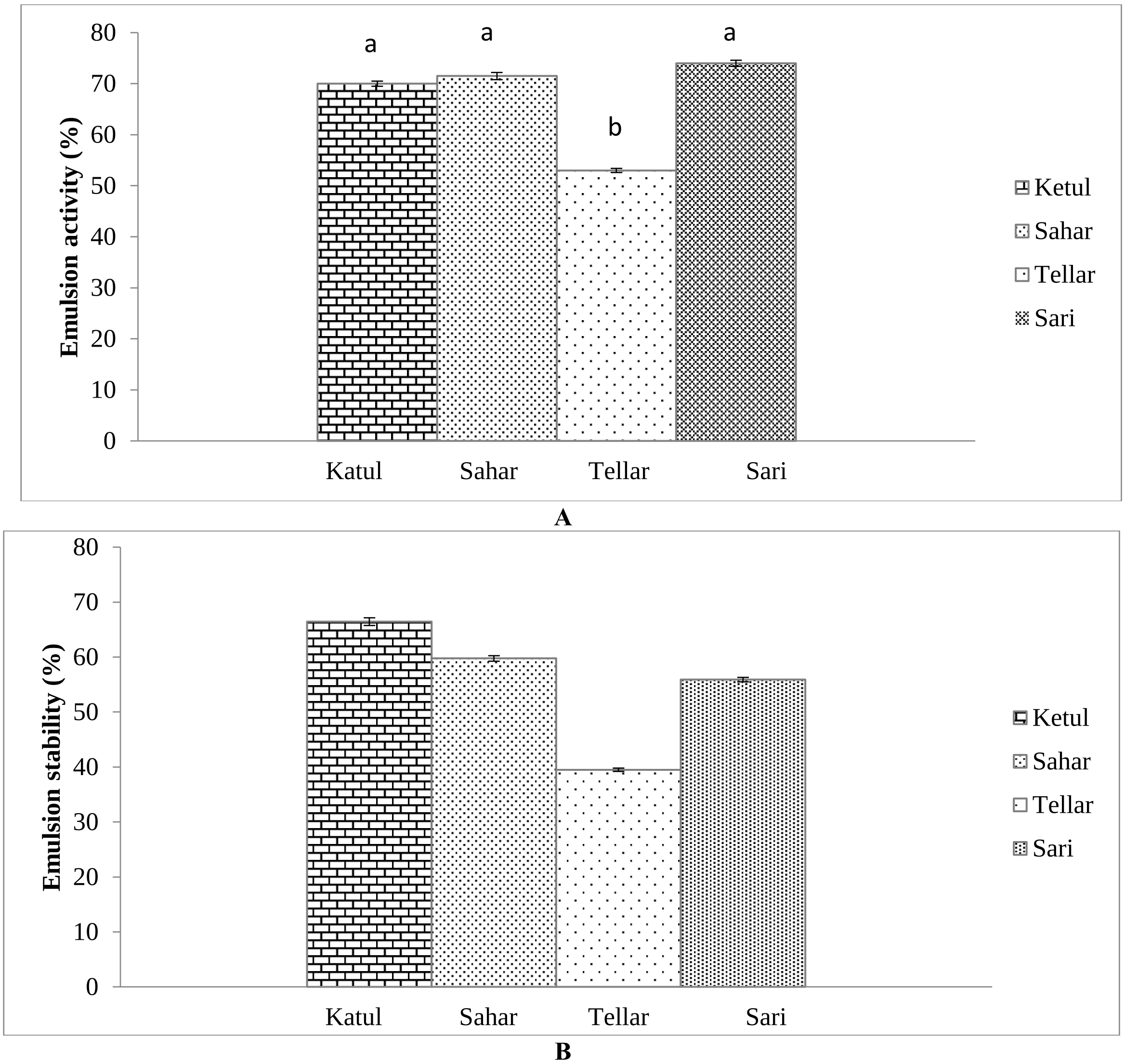

Fig. 1. Emulsifying capacity (A) and emulsion stability (B) of SPI at different cultivars

Different letters show significant differences between the cultivars ($p < 0.05$).

Foaming Properties

Foaming capacity (FC) is the ability of a protein to create foam; on the other hand, foam stability (FS) is the protein's ability to retain the foam volume for a certain duration. Flours can produce foam because of surface active proteins (Adebowale & Lawal, 2003). An overview of the effect of cultivar on FC is shown in Fig. 2A. Tellar (120%) had the lowest amount of FC, while Katul (180.50%) had the highest one, followed by Sari (171.5%) and Sahar (140.5%). Based on pea genotype, Barac *et al.* (2010) found that there are variations in FC, with Calvedon having the lowest FC (235%) and Maja having the highest (325%) (Barac *et al.*, 2010). The assessed foaming capacities of the research were higher than those of two reported commercial pea protein isolates (104% and 96%) (Soral-Smietana *et al.*, 1998). A significant association was found between solubility and FC (p<0.05), suggesting that a higher concentration of protein will move to the air-water interface and generate more foam. In the investigation of foam volume stabilities after 60 minutes conducted in this study, Sari and Tellar had the lowest values (45.75% and 34.80%) (Fig. 2B). The Katul and Sahar cultivars have higher protein contents, more initial protein molecules were presumably added to the foaming mechanism, explaining their superior foaming qualities (Feyzi *et al*., 2015).

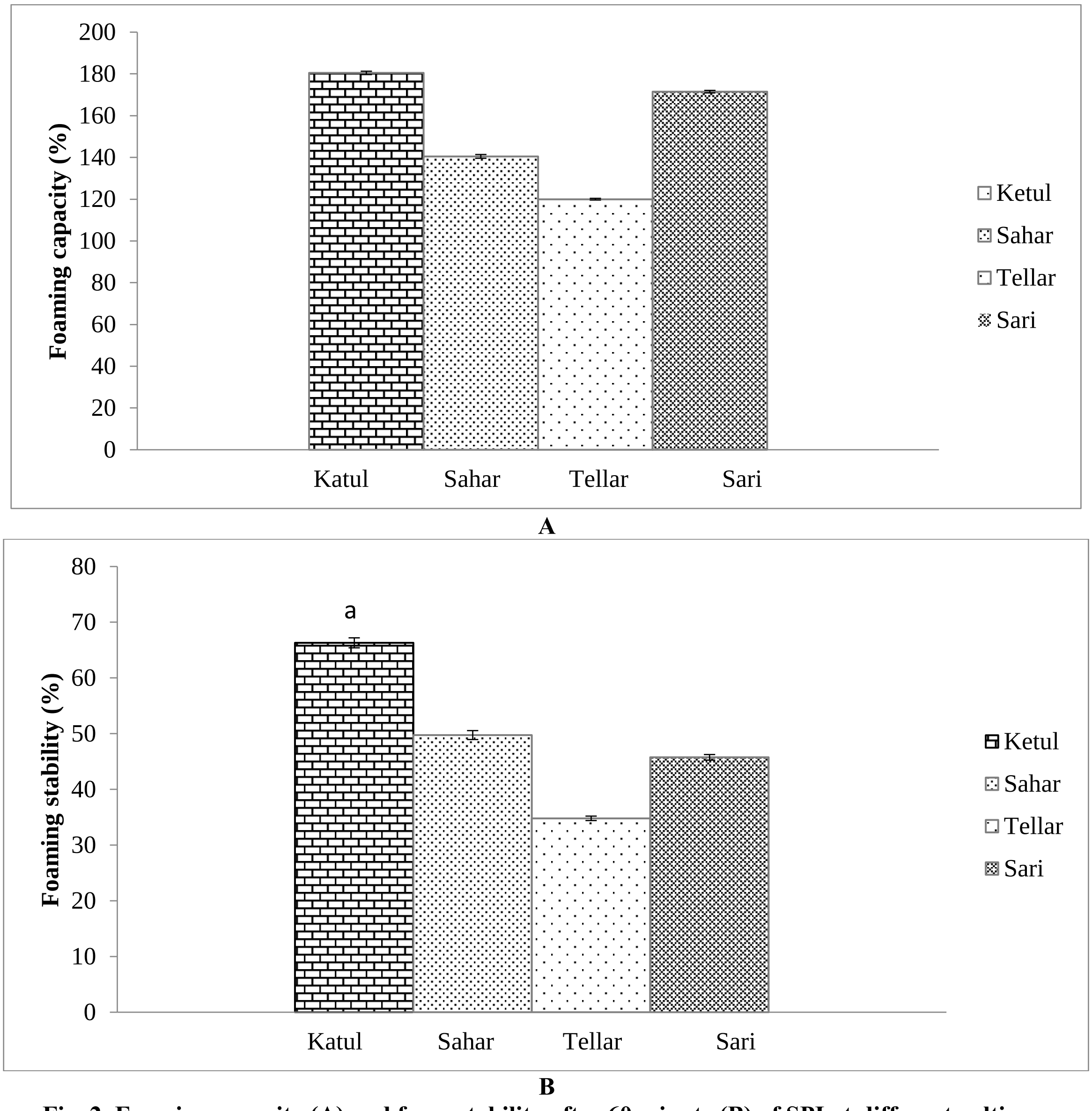

Fig. 2. Foaming capacity (A) and foam stability after 60 minute (B) of SPI at different cultivars
Different letters show significant differences between the cultivars (p < 0.05).

Steady Shear Flow Behavior

Table 3 illustrates the rheological parameters of soy protein isolates (SPI) extracted from four soybean cultivars (Katul, Sahar, Tellar, and Sari) at a 10% protein concentration. The coefficients of determination (R2) were consistently high across all cultivars, ranging from 0.954 to 0.997, signifying an excellent fit of the Power law model to the experimental data. Furthermore, the root mean square error (RMSE) values were minimal for all cultivars, with Sari displaying the lowest error (0.001), confirming the model's precision. The consistency index (K) varied significantly among cultivars, with Katul exhibiting the highest value (0.297 $Pa.s^n$), indicating the greatest viscosity, while Sahar showed the lowest value (0.133 $Pa.s^n$), representing the least viscous

behavior. The flow behavior index (n), which reflects the fluid's non-Newtonian characteristics, was highest for Sahar (0.957), suggesting its flow behavior was closest to Newtonian, whereas Katul and Sari had the lowest n values (0.887 and 0.888, respectively), indicative of a more pronounced shear-thinning behavior.

These findings reveal significant differences in the rheological properties of SPI among soybean cultivars ($p<0.05$). Katul demonstrated the highest viscosity and model reliability, whereas Sahar's SPI exhibited lower viscosity and a flow behavior closer to Newtonian fluids. These variations underscore the influence of soybean cultivar on the functional and rheological characteristics of SPI, which may have implications for their application in food systems.

Table 3- Rheological parameters of soy protein isolates determined for different soybean cultivars

Samples	Power law model			Herschel-Bulkley model			
	K ($Pa\ s^n$)	n (-)	R^2	K ($Pa\ s^n$)	n (-)	τ_0 (Pa)	R^2
Katul	0.297±0.01 ^{a}	0.887±0.00 ^{c}	0.997	0.285±0.07^a	0.897±0.00^c	0.086±0.00^a	0.999
Sahar	0.133±0.02 ^{d}	0.957±0.01 ^{a}	0.954	0.113±0.06^d	0.931±0.00^b	0.053±0.00^b	0.998
Tellar	0.183±0.00 ^{c}	0.917±0.00 ^{b}	0.987	0.145±0.09^c	0.960±0.00^a	0.031±0.00^c	0.997
Sari	0.198±0.01 ^{b}	0.888±0.00^c	0.974	0.156±0.003^b	0.927±0.00^b	0.048±0.00^b	0.989

a–d: Means sharing the same letter in the same row do not differ significantly ($p > 0.05$).

The rheological parameters of the Herschel-Bulkley model for SPI extracted from four soybean cultivars (Katul, Sahar, Tellar, and Sari) are also presented in Table 3. The coefficients of determination (R^2) for this model were consistently high, ranging from 0.989 to 0.999, indicating a superior fit of the Herschel-Bulkley model to the experimental data compared to the Power law model. This highlights the model's capability in accurately describing the rheological behavior of SPI. The yield stress (τ_0) values, which represent the minimum stress required to initiate flow, varied among cultivars. Katul exhibited the highest yield stress, indicating stronger structural resistance to flow, while Tellar showed the lowest yield stress, reflecting a weaker internal structure. The consistency index (K) values were consistent with those observed in the Power law model, with Katul demonstrating the highest viscosity and Sahar the lowest. The flow behavior index (n) closely aligned with those observed under the Power law model, with values ranging from 0.897 to 0.931. Sahar exhibited the highest -value (0.931), indicating flow behavior closest to Newtonian, whereas Katul showed the lowest-value (0.897), emphasizing its pronounced shear-thinning nature. These results reinforce the significant influence of soybean cultivar on SPI rheological properties ($p<0.05$). Katul's SPI demonstrated the highest structural integrity and viscosity, making it more suitable for applications requiring higher resistance to deformation. In contrast, Sahar's SPI showed a lower viscosity and a flow behavior closer to Newtonian fluids, making it more applicable in systems requiring easier flow.

Conclusion

The effects of four soybean cultivars (Katul, Sahar, Tellar, and Sari) on various physicochemical, functional, and rheological properties were examined in this study. The findings demonstrate that soybean cultivar significantly influences the quality and functionality of soy protein isolates (SPI). Katul and Sahar exhibited superior solubility, emulsifying, and foaming capacities compared to Tellar and Sari. These differences are consistent with their physicochemical profiles, particularly their higher protein content and lower residual fat levels, despite the removal of oil during the production of the protein isolate. In terms of rheological behavior, Katul displayed the highest consistency index (K) and significant shear-thinning properties, highlighting its potential for thickening applications. Similarly, Sahar showed the highest flow behavior index (n), indicative of a more Newtonian-like flow suitable for beverage formulations. Regarding gelation properties, Katul required the lowest gelling concentration (10%), making it the most efficient in forming gels, while Sahar needed the highest concentration (14%). Overall, this study emphasizes the importance of cultivar selection in optimizing SPI functionality for specific food industry applications. The combination of physicochemical, functional, and rheological insights provides a comprehensive understanding of how different soybean cultivars influence the final SPI product, enabling tailored applications based on desired properties.

Author Contributions

Behdad Shokrollahi Yancheshmeh: Done the experiments, analyzed and interpenetrated the data, prepared the manuscript, and revised the paper. **Mehdi Varidi:** As the corresponding author, designed the research, provided the fund and laboratory facilities, checked the data, controlled the analysis, edited the paper, revised the paper, and submitted the paper**. Seyed Mohammad Ali Razavi:** Writing review, supervision, provided the fund and laboratory facilities, checked the data, controlled the analysis, edited the paper, and revised the paper. **Farshad Sohbatzadeh:** As the advisor, reviewed the research and edited the paper.

Founding Source

This project was funded by Ferdowsi University of Mashhad, Iran (Grant No. 3/41123). The financial support is gratefully acknowledged.

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مقاله پژوهشی
جلد ۲۱، شماره ۳، مرداد- شهریور ۱٤۰٤، ص. ۳۱٦-۳۰۳

بررسی خواص فیزیکوشیمیایی، عملکردی و رئولوژیکی ایزوله‌های پروتئین سویا تهیه شده از واریته‌های مختلف سویای ایرانی

بهداد شکرالهی یانچشمه[۱]- مهدی وریدی[۱*]- سید محمدعلی رضوی[۱و۲*]- فرشاد صحبت زاده[۳]

تاریخ دریافت: ۱۴۰۳/۱۰/۰۵
تاریخ پذیرش: ۱۴۰۳/۱۱/۱۴

چکیده

علی‌رغم کشت گسترده واریته‌های سویا در مناطق مختلف ایران، تاکنون ویژگی‌های عملکردی این واریته‌ها مورد بررسی قرار نگرفته‌اند. در این پژوهش، ویژگی‌های فیزیکوشیمیایی، عملکردی و رئولوژیکی ایزوله‌های پروتئینی چهار واریته سویا شامل کتول، سحر، تِلار و ساری، که بیشترین سطح زیر کشت در ایران را دارند، مورد بررسی قرار گرفت. آنالیز آماری نتایج نشان داد بین واریته‌های مختلف تفاوت معنی‌داری ($p<0.05$) از نظر رطوبت، خاکستر، پروتئین و چربی وجود دارد، به‌طوری‌که ایزوله حاصل از واریته کتول بالاترین میزان پروتئین (۹۰/۷۵٪) و کمترین میزان چربی (۳/۶۷٪) را دارا بودند. آب‌گریزی سطحی به‌طور قابل توجهی بین واریته‌های مختلف متفاوت بود و سحر بیشترین مقدار (۳۶۰/۳۰a.u.) را نشان داد. حلالیت پروتئین در ایزوله‌های کتول (۶۹/۴۳٪) بیشترین مقدار را داشت که بر ویژگی‌های عملکردی مانند امولسیون‌کنندگی و کف‌کنندگی تأثیر می‌گذارد. ظرفیت جذب آب (WAC) و ظرفیت جذب روغن (OAC) تفاوت‌های قابل توجهی داشتند، به‌طوری‌که تِلار بالاترین ظرفیت جذب روغن ۲/۴۲ g/mL را نشان داد. خواص امولسیون‌سازی، از جمله پایداری امولسیون (ES) و ظرفیت امولسیون‌کنندگی (EC)، در ایزوله‌های پروتئینی ساری و کتول بیشترین بود. خواص کف‌کنندگی نیز تفاوت‌های قابل توجهی داشتند و کتول بالاترین ظرفیت کف‌کنندگی (۱۸۰/۵۰٪) و پایداری کف را به‌دلیل محتوای بالای پروتئین خود نشان داد. آنالیزهای رئولوژیکی نشان داد که واریته کتول دارای بالاترین شاخص قوام (K) و خواص شل شوندگی با برش است، در حالی‌که واریته سحر رفتار جریانی نزدیک به سیال نیوتنی را نشان می‌دهد. مطالعات ژل‌سازی نیز نشان داد که کتول با کمترین غلظت ژله‌ای شدن (۱۰٪) به‌عنوان کارآمدترین واریته ظاهر شد. این یافته‌ها تأثیر واریته سویا را بر ویژگی‌های عملکردی ایزوله‌های پروتئینی نشان می‌دهند و کاربردهای بالقوه‌ای را در محصولات غذایی مختلف، بسته به ویژگی‌های عملکردی موردنظر، پیشنهاد می‌کنند.

واژه‌های کلیدی: پروتئین گیاهی، سویا، واریته، ویژگی‌های عملکردی

۱- گروه علوم و صنایع غذایی، دانشکده کشاورزی، دانشگاه فردوسی مشهد، مشهد، ایران
(*- نویسندگان مسئول: Email: m.varidi@um.ac.ir, s.razavi@um.ac.ir)
۲- قطب علمی هیدروکلوئیدهای طبیعی بومی ایران، دانشگاه فردوسی مشهد، مشهد، ایران
۳- گروه فیزیک اتمی و مولکولی، دانشکده علوم پایه، دانشگاه مازندران، مازندران، بابلسر، ایران

https://doi.org/10.22067/ifstrj.2025.91259.1393

Iranian Food Science and Technology
Research Journal
Homepage: https://ifstrj.um.ac.ir

Research Article
Vol. 21, No. 3, Aug.-Sep. 2025, p. 317-335

Exogenous Melatonin Application Prolongs Citrus Fruits (*Citrus sinensis*) Shelf-life Quality by Enhancing Some Phytochemical Traits

A. Ansari[1], M. Saadatian[2*], R. Haji-Taghilou[1], K.S. Mohammad[2], R.A. Abdollah[3], A. Majid Taha[2]

1- Horticulture Department, Faculty of Agriculture, Urmia University, Urmia, Iran
2- General Science Department, Faculty of Education, Soran University, Soran, Iraq
(*- Corresponding Author Email: mohammad.saadatian@soran.edu.iq)
3- Department of Pharmacy, Rwandz Private Technical Instiute, Soran, Iraq

Received: 12.01.2025 Revised: 30.04.2025 Accepted: 07.05.2025 Available Online: 17.06.2025	**How to cite this article:** Ansari, A., Saadatian, M., Haji-Taghilou, R., Mohammad, K.S., Abdollah, R.A., & Majid Taha, A. (2025). Exogenous melatonin application prolongs citrus fruits (*Citrus sinensis*) shelf-life quality by enhancing some phytochemical traits. *Iranian Food Science and Technology Research Journal, 21*(3), 317-335. https://doi.org/10.22067/ifstrj.2025.91596.1399

Abstract

This study investigated the impact of melatonin treatments (1 mM and 2 mM) on the post-harvest quality of orange fruit during 30 and 60 days of cold storage. Parameters such as titratable acidity (TA), total soluble solids (TSS), vitamin C, antioxidant capacity, total phenolic compounds (TPC), total flavonoids compounds (TFC), enzymatic activities (PAL, CAT), and color were evaluated. Melatonin significantly improved fruit quality by maintaining higher levels of total soluble solids, vitamin C, and antioxidant capacity. Both treatments effectively reduced weight loss and enhanced the activity of antioxidant enzymes. While 2 mM melatonin showed greater efficacy in the initial stages of storage, 1 mM demonstrated better stability in maintaining quality over extended periods. Melatonin treatments also influenced color parameters, suggesting potential improvements in visual appeal. These findings highlight the potential of melatonin as a natural preservative for enhancing the post-harvest quality and extending the shelf life of orange fruit. Further research is needed to optimize melatonin concentrations and explore its integration with other preservation techniques for sustainable and efficient fruit management.

Keywords: Antioxidant, Fruit quality, Post-harvest, PAL enzyme, Weight loss

Introduction

Extending the shelf life of fruits is essential to reducing food waste, maintaining nutritional value, and ensuring the availability of fresh produce in distant markets (Bhosale & Sundaram, 2011). Longer shelf life minimizes economic losses for producers and retailers while providing consumers with consistent access to quality fruits. Effective preservation techniques, such as refrigeration, freezing, and controlled atmosphere storage, play a critical role in maintaining fruit quality during storage and transport. These methods significantly contribute to the sustainability of food supply chains by reducing spoilage and waste (Singh *et al.*, 2024; Tadapaneni *et al.*, 2014).

Oranges, as one of the most widely consumed citrus fruits globally, hold significant economic and nutritional importance. According to official statistics, the annual orange production in Iran is approximately 3 million tons, placing the country ninth in the world in terms of orange production (Sidana *et al.*, 2013). They are valued for their richness in vitamins, antioxidants, and dietary fiber, which

https://doi.org/10.22067/ifstrj.2025.91596.1399

make them a staple in many diets. However, the high perishability of oranges poses challenges in their post-harvest handling (Tütem *et al.*, 2020). Factors like weight loss, microbial spoilage, and nutrient degradation can limit their shelf life, leading to substantial losses during storage and distribution. Thus, there is a pressing need for effective strategies to enhance their post-harvest quality and extend their shelf life (Khathir *et al.*, 2019; Sicari *et al.*, 2017).

Melatonin has emerged as a promising natural compound in improving the post-harvest management of fruits (Saud *et al.*, 2023). Traditionally recognized for its role in regulating sleep and circadian rhythms in animals, melatonin is now known to be synthesized in plants as well. In plants, it performs multiple physiological functions, including stress regulation, antioxidant activity, and growth modulation (Saroj *et al.*, 2023). Its potential as a post-harvest treatment lies in its ability to mitigate oxidative stress, delay ripening, and maintain fruit quality during storage (Xue *et al.*, 2021). Melatonin enhances the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), which help in reducing ROS levels in fruits, thereby mitigating oxidative damage (Qu *et al.*, 2022). Melatonin treatment also upregulates enzymes involved in the phenylpropanoid pathway, such as phenylalanine ammonia lyase (PAL), cinnamate 4-hydroxylase (C4H), and polyphenol oxidase (PPO), leading to increased accumulation of phenolic compounds, flavonoids, and lignin, which enhance disease resistance and delay senescence (Michailidis *et al.*, 2021).

Melatonin is particularly appealing because of its compatibility with sustainable agricultural practices. Unlike synthetic chemicals that may have adverse effects on health and the environment, melatonin is a naturally occurring, non-toxic compound (Sharma *et al.*, 2024). It aligns with growing consumer demand for organic and environmentally friendly produce. Additionally, melatonin can be integrated with other preservation methods, such as cold storage or edible coatings, to create a multifaceted approach for fruit preservation.

Oxidative stress, caused by the accumulation of reactive oxygen species (ROS), is a major factor contributing to post-harvest quality loss in fruits (Neog & Saikia, 2010). ROS accelerate cellular damage, leading to faster ripening, senescence, and spoilage (Hailu *et al.*, 2008). Melatonin acts as a potent antioxidant by scavenging ROS and enhancing the activity of antioxidant enzymes, thereby reducing oxidative damage. Furthermore, it can regulate the production of ethylene -a hormone central to fruit ripening- by inhibiting its biosynthesis, slowing down ripening processes, and prolonging the storage life of fruits (Berra & Rizzo, 2009).

Studies have shown that melatonin application can effectively maintain the quality of various fruits during storage. For instance, melatonin treatment has been found to delay weight loss, reduce microbial decay, and retain firmness in fruits like strawberries, bananas, and tomatoes (Arshad & Haghshenas, 2025; El-Mogy *et al.*, 2019; Zang *et al.*, 2022). Melatonin has demonstrated the ability to slow respiration rates, inhibit fungal growth, and preserve vital nutrients, such as ascorbic acid, during extended storage. These findings highlight its potential as a natural and eco-friendly alternative to synthetic preservatives (El-Mogy *et al.*, 2019).

Despite its promise, challenges remain in the large-scale adoption of melatonin in post-harvest management. Key limitations include the need for standardized application protocols and the lack of extensive field-level research under practical conditions. Moreover, economic feasibility and scalability must be addressed to make melatonin treatments viable for widespread commercial use.

In conclusion, melatonin represents an innovative and sustainable solution to the challenges of post-harvest fruit management. Its ability to delay ripening, maintain quality, and extend shelf life offers significant benefits for reducing waste and improving the availability of high-quality fruits. As research continues to optimize its application, melatonin could play a pivotal role in creating more efficient and sustainable post-harvest systems for oranges and other perishable fruits.

Materials and Methods

Plant Materials and Experimental Design

Orange fruits (*Citrus sinensis* or *Citrus×sinensis*) were harvested at the mature green stage. To ensure consistency, the fruits were selected based on uniformity in shape, color, and size, with a preference for medium-size specimens. The selected fruits were initially weighed, and only those of medium size were selected for further analysis. Fruits displaying any signs of blemishes or disease were excluded from the study. Fruits were selected and separated, then subjected to immersion treatment in melatonin solutions at concentrations of 1 and 2 mM, as well as a control treatment, for one minute. After the treatment, the fruits were exposed to open air to allow their surfaces to dry completely. Fruits were stored in a cold room for 2 months at 0±1°C and a humidity of 90-95%. Measuring the desired parameters was carried out on days 30, and 60 after applying the treatments.

Qualitative Analysis of Fruit

To assess the qualitative changes in oranges during storage, sampling was conducted on the 14th day of cold storage. The qualitative analyses included evaluations of color, weight loss measurement, total soluble solids (TSS), titratable acidity (TA), vitamin C content, and various antioxidant properties. These properties encompassed total and enzymatic antioxidant activities, including catalase activity and phenylalanine ammonia-lyase activity, as well as total phenol and total flavonoid contents.

Color Parameters Measurements

The color of orange fruits was non-destructively measured using a colorimeter (CR-400, Konica Minolta Inc., Tokyo, Japan) following the method described by Giglio *et al*. (2023). The color parameters, including color difference index (ΔE), chroma (C), and hue angle (H), were calculated based on the *L* value (lightness), *a* value (green to red), and *b* value (blue to yellow).

Weight Loss Measurement

The weight of the fruits was measured during each experimental period using a precision scale.

Total Soluble Solids (TSS) Measurement

The total soluble solids (TSS) content was measured using a handheld refractometer (ATAGO model). A few drops of orange juice were placed on the refractometer, and the corresponding value was read from the graduated scale. Before starting the measurements, the refractometer was calibrated. After each reading, the device was rinsed with distilled water and dried for the next measurement (Marandi *et al*., 2010).

Titratable Acidity (TA) Measurement

The titration was performed using 10 mL of orange juice with 0.1 N sodium hydroxide (NaOH) solution (4 g/L) until the pH reached 8.2. The amount of acid in the juice was then expressed as a percentage based on the volume of NaOH consumed during the titration, according to the method described by Selcuk and Erkan (2015). The titratable acidity was calculated in terms of citric acid equivalent (the predominant acid in strawberries) using Equation (1).

$$TA = \frac{S \times N \times F \times E}{\mathrm{C}} \times 100$$

Total Phenol Content (TPC)

The total phenolic content was determined by combining 2 mL of a 2% sodium carbonate solution, 2.8 mL of distilled water, and 100 µL of a 50% Folin-Ciocalteu reagent with 100 µL of the fruit juice. The absorbance was recorded at 720 nm after a portion of the incubation time, using a control sample for reference. Gallic acid was utilized as the standard for constructing the calibration curve. The phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per gram of fresh plant weight, following the procedure described by Meda *et al*. (2005).

Total Flavonoid Content (TFC)

The total flavonoid content was assessed using a colorimetric assay at 380 nm, in accordance with the procedure outlined by

Pirogov *et al*. (2016) Specifically, A 30 µL aliquot of fruit juice was mixed with 150 µL of 5% sodium nitrite, 300 µL of 10% aluminum chloride, and 1000 µL of 1 mol/L sodium hydroxide. After incubating the mixture in the dark for 30 minutes, the reaction was diluted to a final volume of 5 mL with double-distilled water, and the absorbance was measured at 380 nm. The flavonoid content was calculated using a quercetin standard curve and expressed as milligrams of quercetin equivalent per milliliter of fruit juice (mg QE/mL).

Antioxidant Activity

The antioxidant activity was measured using the DPPH free radical scavenging assay. A specified volume of methanolic extract was mixed with DPPH solution and incubated in the dark for 15 to 30 minutes. Absorbance was then measured at 517 nm using a spectrophotometer. The activity was calculated using a formula, with a control sample (80% methanol) to calibrate the spectrophotometer and measure the absorbance of the DPPH solution without the extract (Chiou *et al*., 2007).

Vitamin C

The ascorbic acid (AsA) content was estimated using the method described by Vithana *et al*. (2018). The total ascorbic acid content in fruit pulp was determined by homogenizing the sample in an extraction solution, centrifuging the mixture, and measuring the absorbance of the supernatant after reaction with Dichloroindophenol reagent. The results were quantified using L-ascorbic acid as a standard and expressed as mg/kg of fresh weight.

Measurement of Catalase

Catalase (CAT) enzyme activity in the fruit was measured using the method described by Boominathan and Doran (2002). The reaction mixture consisted of 900 µL of 10 mM hydrogen peroxide (H_2O_2) prepared in phosphate-buffered saline (without PVP) and 100 µL of fruit juice placed in a glass cuvette. The decomposition of H_2O_2, catalyzed by catalase, was monitored spectrophotometrically by measuring the decrease in absorbance at 240 nm within 1 minute using a Uvi Light XS 5 SECOMAM spectrophotometer. The catalase activity was then calculated based on the rate of H_2O_2 decomposition.

$$\text{Units}\left(\frac{\text{mM}}{\text{min}}\right) = \frac{\Delta\text{oD}/\min(\text{slope}) \times \text{Vol. of assay}\ (0.0003)}{\text{Extinction Coefficient}\ (43.6)}$$

Phenylalanine Ammonia-Lyase (PAL) Activity Assay

Phenylalanine ammonia-lyase (PAL) enzyme activity was measured following the procedure outlined by D'Cunha (2005). PAL enzyme activity was measured by incubating a reaction mixture of potassium phosphate buffer, phenylalanine, distilled water, and fruit juice. After incubation, the reaction was stopped, and absorbance was measured. The activity was calculated using a cinnamic acid standard curve and expressed in mg of cinnamic acid per 100 g of fresh weight.

Experimental Design

The experiment was conducted using a completely randomized design with four replications. The treatments applied included 1 and 2 millimolar melatonin. Data were organized in tables and graphs were generated using Excel software. Statistical analysis of the data was performed using SAS 9.2 software, and mean comparisons were conducted using Duncan's multiple range test at the 5% probability levels.

Result and Discussion

The analysis of variance (ANOVA) (Table 1) shows that melatonin concentration (C) had a significant effect on most of the measured traits, including antioxidant activity, phenolic content, and weight loss, indicating its strong influence on postharvest fruit quality. Storage time (T) also significantly affected key parameters such as vitamin C and antioxidant percentage. The interaction between concentration and time (C × T) was significant for several traits, suggesting that the effect of melatonin varies depending on the duration of storage.

Table 1- ANOVA analysis for the Effects of Melatonin Concentration and Storage Time on Postharvest Orange fruits

Variable	DF	TA	TSS	Vit C	L*	a*	b*	Chroma	Hue	Loss Weight	Antioxidant %	Flavonoids	Phenols	PAL	CAT
Concentration (C)	2	11.9^{*}	0.12^{*}	62.3^{**}	10.7^{*}	7.17^{**}	13.9^{*}	0.95^{*}	101.5^{**}	122.1^{**}	102.9^{**}	15.95^{**}	332.9^{**}	36.1^{*}	7.59^{*}
Time (T)	1	1.5^{ns}	0.1^{ns}	71.7^{**}	2^{ns}	42^{*}	4.68^{*}	0.25^{ns}	20.9^{**}	115.9^{**}	18.9^{*}	12.8^{**}	49.2^{**}	209.1^{*}	1.11^{ns}
C × T	2	0.15^{*}	0.15^{ns}	8.47^{*}	0.02^{ns}	12.8^{*}	0.86^{ns}	1^{ns}	4.97^{**}	3.11^{**}	372.1^{**}	27.41^{*}	33.14^{**}	0.43^{**}	2.93^{*}
Error	17	0.9	0.34	2.5	1.5	1.05	0.94	0.54	1.32	1.53	3.5	1.65	3.5	1.34	1.22
CV%		7.6	14.3	3	2.1	3.61	2.93	5.5	4.6	7.29	3.02	4.76	2.77	7.9	21.31

* and ** significant at 0.05 and 0.01, ns: Not-significant

Titrable Acidity (TA)

The titratable acidity (TA) of orange juice exhibited notable variations during storage among different treatment concentrations (Fig. 1). On Day 30, there were no significant differences in TA levels between the control and 2 mM treatments, while the 1 mM treatment showed a slightly higher value, though this difference was not statistically significant. By Day 60, a distinct pattern emerged, with the 2 mM treatment displaying a significant increase in TA compared to both the control and 1 mM treatments. The 2 mM treatment recorded the highest TA value (1.5 g/100 ml), which was significantly greater ($p < 0.05$) than the other treatments. In contrast, the control and 1 mM treatments maintained relatively stable TA levels over time, with no significant differences observed between them. These results indicate that higher concentrations (2 mM) may lead to increased acidity during extended storage, potentially influencing the sensory characteristics of the juice.

Based on our findings, the application of melatonin can improve the content of fruit acidity. This effect was statistically significant at a concentration of 2 mM, whereas at 1 mM. Although the increase was not significant, but effectively prevented a decline in acidity (Fig. 1). Melatonin applications have been shown to maintain fruit quality characteristics during cold storage. This includes maintaining firmness, delaying changes in titrable acidity, and preserving organic acid concentrations (Carrión-Antolí *et al.*, 2022; Kucuker *et al.*, 2024). In a study, melatonin application on peach fruits was effective in maintaining the concentration of organic acids, including titratable acidity, although the effect varied depending on the concentration and compound (Kucuker *et al.*, 2024). A study on 'Newhall' navel oranges showed that melatonin treatment increased titratable acidity, suggesting an inhibition of fruit quality deterioration and delayed senescence (Ma *et al.*, 2021).

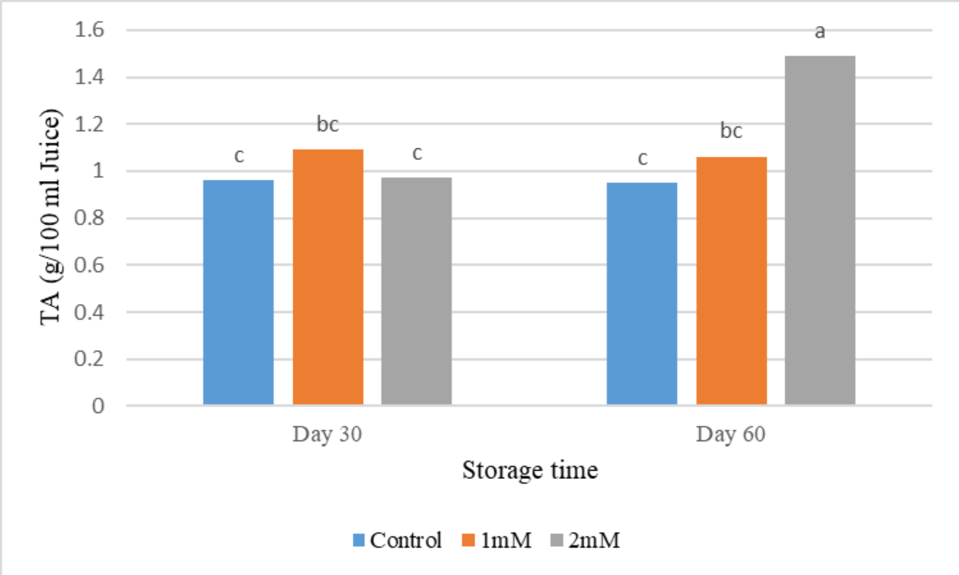

Fig. 1. Effect of different melatonin concentrations on the total acidity (TA) of orange juice over 30 and 60 days of storage

Total Soluble Solids (TSS)

The total soluble solids (TSS) content in orange juice showed significant variations among treatments and storage durations (Fig. 2). On Day 30, TSS level in the control group was significantly lower ($p < 0.05$) than those in 1 and 2 mM treatments. Among the treatments, the 2 mM group exhibited the highest TSS value, though this was not significantly different from 1 mM treatment. By Day 60, a similar trend persisted, with the 2 mM treatment maintaining the highest TSS level (approximately 14 °Brix), significantly surpassing the control and 1 mM treatments. Over time, slight reductions in TSS was observed in the control and 1 mM treatments, with the control consistently showing the lowest values at both storage intervals. These findings suggest that higher concentrations (2 mM) contribute to enhanced TSS retention during storage, potentially improving the sweetness and overall quality of the juice over extended periods.

The total soluble solids (TSS) content in oranges treated with 2 mM melatonin significantly increased compared to the control during 30 and 60 days of storage (Fig. 2). Melatonin treatments generally lead to an increase in TSS in various fruits during post-harvest storage. This is observed in pitaya, navel oranges, nectarines, peaches, jujube fruits, pomegranates, sweet cherries, passion fruits, strawberries, and kiwi berries (Ba *et al.*, 2022; Bal, 2021; Kucuker *et al.*, 2024; Ma *et al.*, 2021; Wu *et al.*, 2023). Melatonin treatments have effectively slowed the process of senescence, reduced fruit softening, and maintained the total soluble solids content in nectarines and strawberries (Bal, 2021; Liu *et al.*, 2018). Melatonin treatments have been shown to delay the decline in fruit firmness, reduce the loss of soluble solids and titratable acids, and regulate the formation of soluble pectin by inhibiting the activities of key enzymes, including pectin methylesterase (PME), polygalacturonase (PG), cellulase (Cx), and β-glucosidase (β-Glu) (Qu *et al.*, 2022).

Vitamin C Content

The vitamin C content in orange juice varied significantly among treatments and storage durations. On Day 30, the 2 mM treatment recorded the highest vitamin C concentration (50 mg/kg), though this was not significantly different from the 1 mM treatment. In contrast, the control treatment displayed a significantly lower vitamin C level ($p < 0.05$) compared to the other treatments. By Day 60, a noticeable decline in vitamin C content was observed in all groups. However, the 1 mM treatment retained a significantly higher concentration of vitamin C compared to the control and 2 mM treatments, with the control group showing the lowest value (30 mg/kg). These findings suggest that while both 1 mM and 2 mM treatments initially improve vitamin C retention, prolonged storage results in an overall decline, with the 1 mM treatment exhibiting greater stability over time. This highlights the potential of moderate concentrations (1 mM) for preserving the nutritional quality of orange juice during extended storage (Fig. 3).

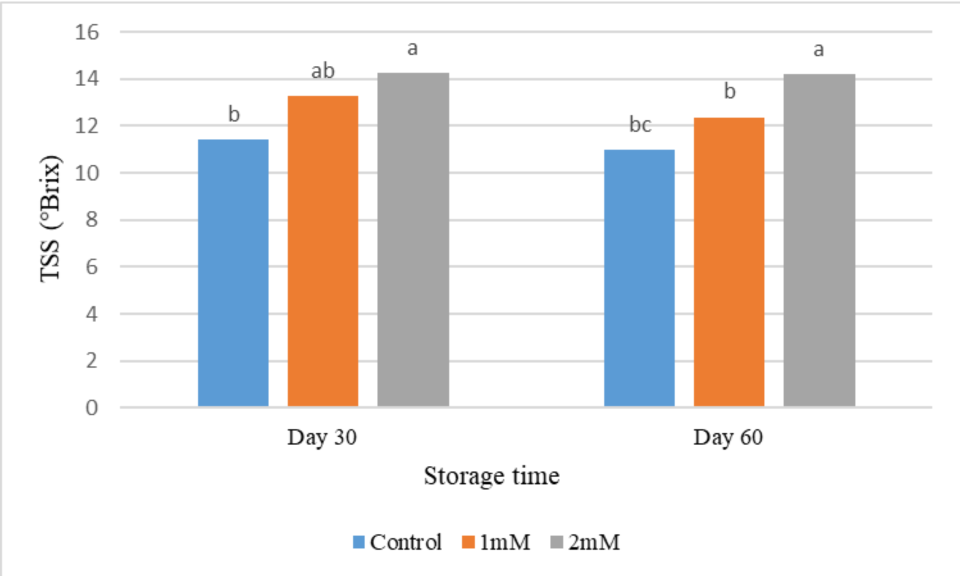

Fig. 2. Effect of different melatonin concentrations on the TSS of orange juice over 30 and 60 days of storage

In this study, vitamin C was influenced by melatonin treatment, with the application of 1 mM and 2 mM melatonin significantly enhancing its content compared to the control during both 30- and 60-day storage periods. Melatonin treatments significantly maintained higher contents of vitamin C in fresh-cut pitaya fruits during storage compared to control treatments. This suggests that melatonin can help preserve vitamin C levels in fruits post-harvest (Ba *et al.*, 2022). Also, Melatonin enhances the activity of antioxidant enzymes such as, catalase (CAT), which helps in reducing oxidative stress and preserving vitamin C (Ma *et al.*, 2021; Wang *et al.*, 2022). Furthermore, by decreasing the accumulation of reactive oxygen species (ROS) and enhancing the antioxidant defense system, melatonin helps in maintaining the nutritional quality of fruits, including vitamin C content (Song *et al.*, 2022).

Weight Loss

The graph illustrates the percentage of weight loss in samples over two storage periods (30 and 60 days) under three treatment conditions: control (untreated), 1 mM, and 2 mM. On Day 30, the control group exhibited the highest weight loss (15.25%), while the 2 mM treatment group demonstrated the lowest weight loss (5.28%). The 1 mM treatment resulted in intermediate weight loss (12.98%), highlighting the efficacy of higher concentrations in minimizing weight loss during short-term storage. By Day 60, weight loss increased among all groups, with the control group again recording the highest loss (19.05%) and the 2 mM treatment maintaining the lowest loss (11.91%). The 1 mM treatment group showed moderate weight loss (17.74%). Statistically significant differences between treatments, as indicated by the letters in the graph, underscore the effectiveness of higher treatment concentrations in reducing weight loss over extended storage periods (Fig. 4).

Melatonin treatments have consistently demonstrated a reduction in weight loss among different fruit types, including pitaya, citrus, okra, peaches, sweet cherries, and strawberries (Ba *et al.*, 2022; Ma *et al.*, 2021). Melatonin helped maintain fruit firmness and reduced weight loss by promoting stomatal closure, which minimizes water loss (Shi *et al.*, 2024). On the other hand, Melatonin is effective in reducing weight loss in post-harvest fruits by enhancing antioxidant defenses, promoting stomatal closure, and influencing gene expression related to cell wall integrity. These effects collectively contribute to maintaining fruit quality and extending shelf life during storage (Shi *et al.*, 2024).

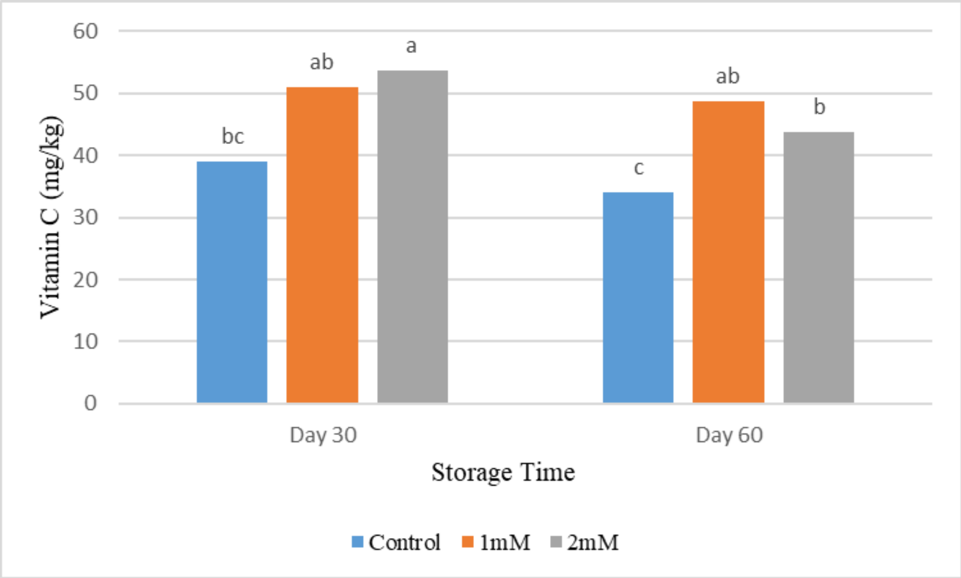

Fig. 3. Effect of different melatonin concentrations on the Vitamin C of orange juice over 30 and 60 days of storage

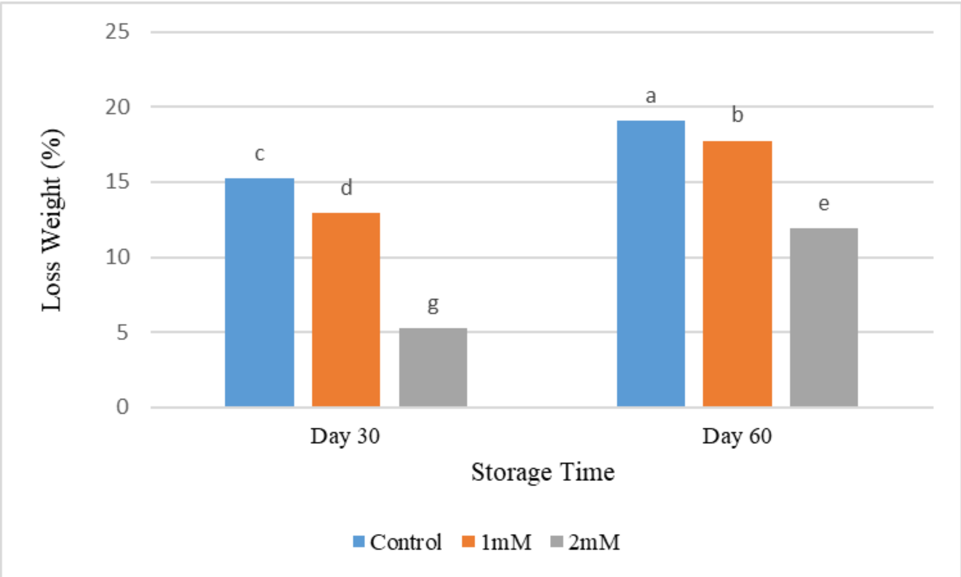

Fig. 4. Effect of different melatonin concentrations on the Vitamin C of orange juice over 30 and 60 days of storage

Antioxidant Capacity

The antioxidant capacity (%) exhibited significant variations among treatments and storage periods. On Day 30, the 2 mM treatment showed the highest antioxidant capacity (63.38%), which was significantly greater than that of the control (56%) and 1 mM (53.19%) treatments. These results highlight the superior effectiveness of the 2 mM treatment in preserving antioxidant levels during the early stages of storage. By Day 60, a notable shift in antioxidant capacity was observed among the treatments. The 1 mM treatment demonstrated the highest antioxidant capacity (72.3%), significantly surpassing the control (45%) and 2 mM (53%) treatments ($p < 0.05$). These findings suggest that while the 2 mM treatment is more effective in maintaining antioxidant capacity in the short term, the 1 mM treatment provides better retention over extended storage periods. This indicates the potential advantage of moderate concentrations (1 mM) for preserving antioxidant capacity, thereby enhancing product quality and nutritional value during long-term storage (Fig. 5).

Melatonin has been demonstrated to significantly enhance antioxidant activity in a variety of post-harvest fruits, thereby improving quality and extending shelf life (Fig. 5). Treatments with melatonin increase the activities of key antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), in fruits such as pitaya, peaches, and blueberries (Shang *et al.*, 2021). Melatonin also reduced the levels of reactive oxygen species (ROS) and lipid peroxidation, while promoting the activity of antioxidant enzymes such as ascorbate peroxidase (APX), glutathione S-transferase (GST), and phenylalanine ammonia-lyase (PAL) (Wu *et al.*, 2023). Melatonin treatments increased the levels of bioactive compounds, including total phenolics and anthocyanins, while preserving higher antioxidant activity throughout the storage period (Lorente-Mento *et al.*, 2021).

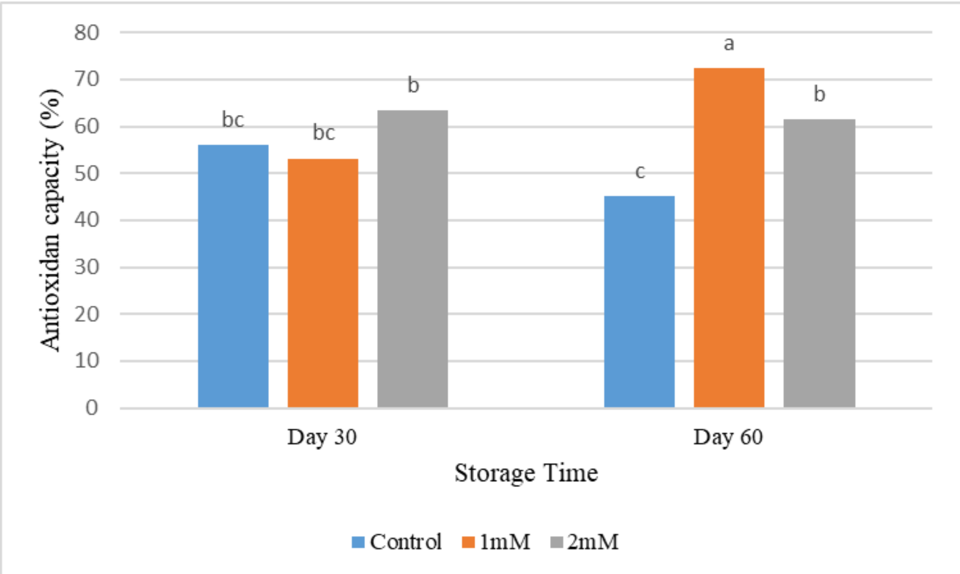

Fig. 5. Effect of different melatonin concentrations on the Vitamin C of orange juice over 30 and 60 days of storage

Total Flavonoid Content (TFC)

The total flavonoid content (TFC), expressed in mg QE/ml juice, showed significant variations among treatments and storage periods. On Day 30, the 1 mM treatment recorded the highest TFC (21.82 mg QE/ml), which was significantly higher than the control (15.83 mg QE/ml) but not significantly different from the 2 mM treatment (18.82 mg

QE/ml). The control group had the lowest TFC value, highlighting the beneficial effect of treatments in enhancing flavonoid levels during short-term storage. By Day 60, notable change in TFC level was observed among all groups. The 2 mM treatment displayed the highest TFC (28.47 mg QE/ml), significantly exceeding the 1 mM treatment (24.8 mg QE/ml) and the control (19.26 mg QE/ml). These findings underscore the superior efficacy of the 2 mM treatment in maintaining flavonoid content over extended storage periods. Overall, the results suggest that higher concentrations, such as 2 mM, are more effective in preserving TFC during prolonged storage, thereby contributing to the improved nutritional and functional quality of the product (Fig. 6).

Melatonin treatment significantly increased the total flavonoid content in orange fruits during 60 day of shelf life (Fig. 6). In a study, Melatonin treatment delayed the decrease in flavonoid content in blueberries during storage (Cao *et al*., 2024). Also, Melatonin treatment at 0.5 mM effectively preserved the flavonoid content in litchi fruits, reducing oxidative browning and maintaining overall fruit quality during storage (Marak *et al*., 2023). Exogenous melatonin application can increase non-enzymatic antioxidants, including flavonoids, thereby improving the fruit's antioxidant capacity and maintaining quality during storage (Zhang *et al*., 2024).

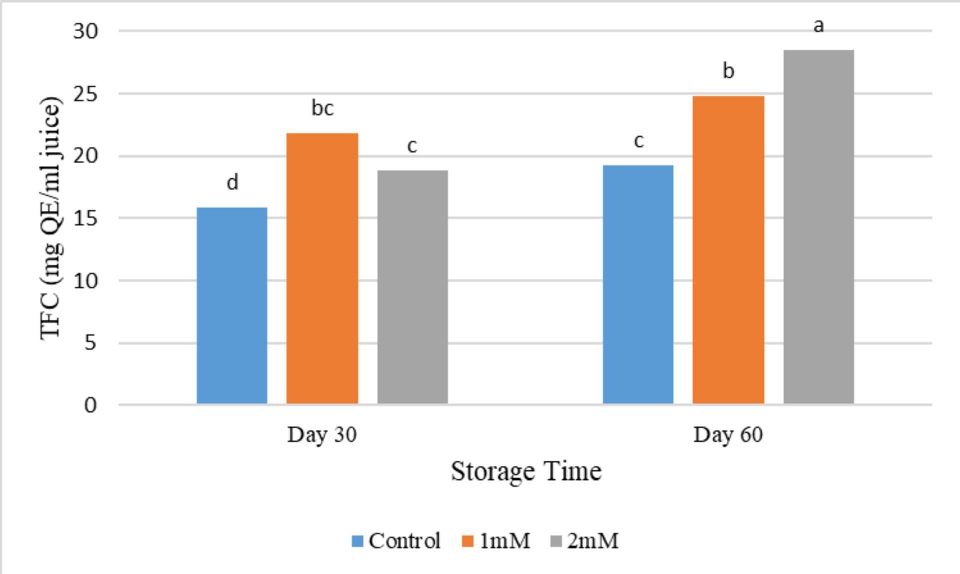

Fig. 6. Effect of different melatonin concentrations on the Vitamin C of orange juice over 30 and 60 days of storage

Total Phenolic Content (TPC)

The total phenolic content (TPC), expressed in mg GAE/ml juice, showed significant differences among treatments and storage periods. On Day 30, the 2 mM treatment recorded the highest TPC (84.68 mg GAE/ml), significantly surpassing the control (54.35 mg GAE/ml) and the 1 mM treatment (68.48 mg GAE/ml), indicating the positive impact of higher concentrations on phenolic retention during short-term storage. By Day 60, TPC decreased among all groups, with the 2 mM treatment maintaining the highest value (76.78 mg GAE/ml), significantly higher than the 1 mM (62.77 mg GAE/ml) and control (58 mg GAE/ml) groups. The control exhibited the steepest decline in TPC, underscoring the protective effect of treatments in reducing phenolic degradation over time. These results demonstrate that higher concentrations, such as 2 mM, are more effective in preserving TPC during extended storage, supporting improved antioxidant capacity and product quality (Fig. 7).

Melatonin has demonstrated a beneficial effect on enhancing the total phenolic content in various treatments during orange fruits post-harvest storage (Fig. 7). Melatonin treatments (0.1 mM) applied pre-harvest significantly increased the total phenolic content at harvest and maintained higher levels during 60 days of storage compared to control (Lorente-Mento *et al.*, 2021). Melatonin treatment (1000 µmol L^{-1}) effectively maintained higher levels of total phenolics and antioxidant activity during 40 days of storage (Bal, 2021). Application melatonin can lead to maintain the total phenolic content and enhanced antioxidant enzyme activities, which helped delay senescence and maintain fruit quality during storage (Shang *et al.*, 2021). Melatonin treatments among various fruits consistently show an increase in total phenolic content and enhanced antioxidant activity during post-harvest storage. This results in delayed senescence, improved fruit quality, and extended shelf-life.

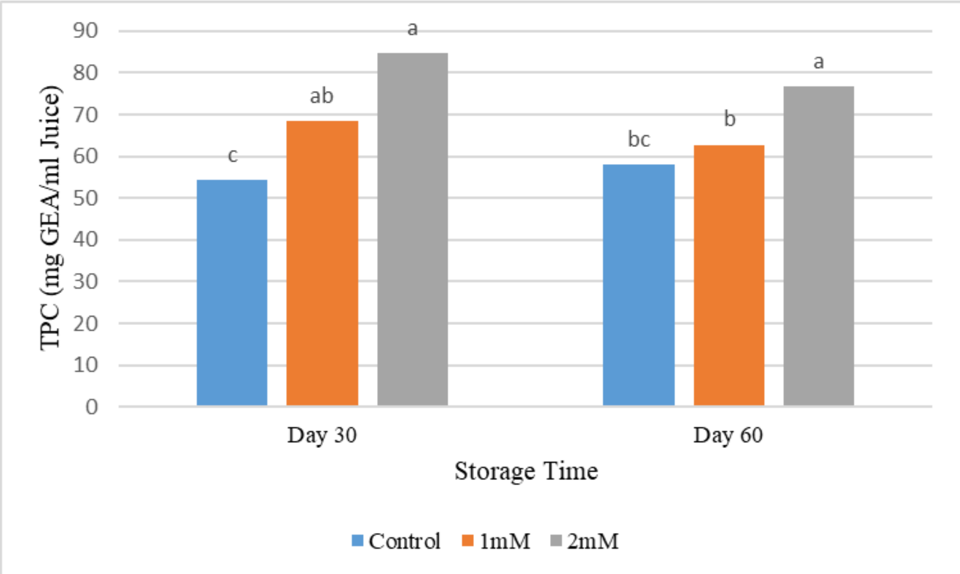

Fig. 7. Effect of different melatonin concentrations on the Vitamin C of orange juice over 30 and 60 days of storage

Phenylalanine Ammonia-Lyase (PAL)

The activity of PAL, measured in mg/g FW/min, varied significantly among treatments and storage periods. On Day 30, the 2 mM treatment showed the highest PAL activity (18.3 mg/g FW/min), significantly exceeding the 1 mM treatment (16.4 mg/g FW/min) and the control (13.45 mg/g FW/min), highlighting the role of higher concentrations in enhancing PAL activity during short-term storage. By Day 60, PAL activity declined in all treatments, with the 2 mM and 1 mM treatments maintaining similar levels (11.1 and 10.2 mg/g FW/min, respectively), both significantly higher than the control (6.4 mg/g FW/min). The control group experienced the steepest reduction in PAL activity, emphasizing the protective effect of treatments in sustaining enzyme function during extended storage. These findings underscore the effectiveness of higher concentrations (1 mM and 2 mM) in preserving PAL activity, which is crucial for secondary metabolite production and stress response, thereby enhancing product quality and stability during storage (Fig. 8).

Melatonin treatment significantly enhances PAL activity in several fruits, which is associated with improved disease resistance and delayed senescence. For instance, in peaches, melatonin treatment increased PAL activity, contributing to enhanced disease resistance and maintenance of fruit quality (Dong *et al.*, 2024; Lei *et al.*, 2022). In apricots, melatonin treatment increased PAL activity (Zhang *et al.*, 2024). Similarly, in litchis, melatonin enhanced PAL activity, which was associated with increased resistance to *Peronophythora litchii* and improved post-harvest quality (Zhang *et al*., 2021).

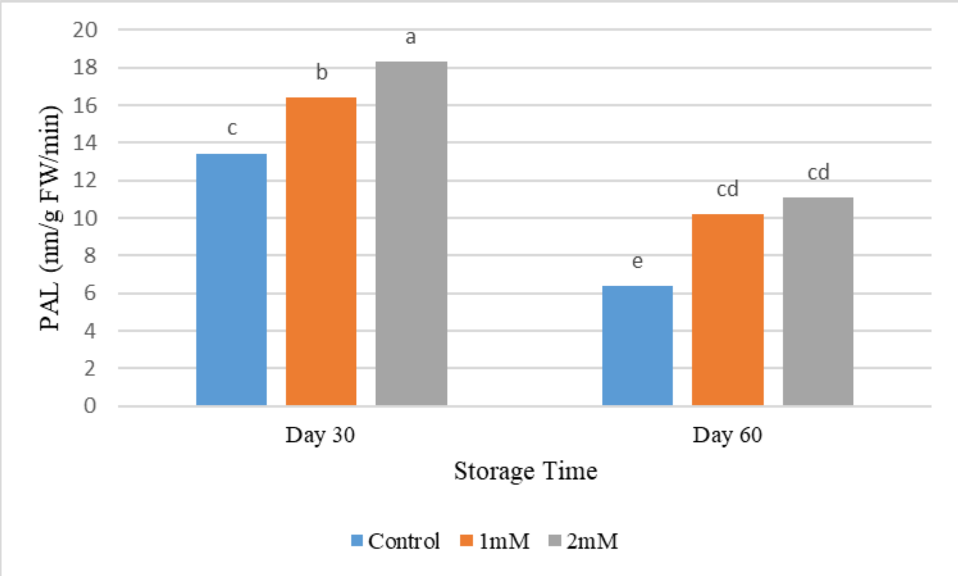

Fig. 8. Effect of different melatonin concentrations on the Vitamin C of orange juice over 30 and 60 days of storage

Catalase (CAT)

The activity of catalase (CAT) enzyme was evaluated during two storage periods (30 and 60 days) under three treatment conditions: Control, 1 mM, and 2 mM. On day 30, the Control group exhibited the lowest CAT activity compared to the treatments, although the difference was not statistically significant compared to the 1 mM treatment. Both the 1 mM and 2 mM treatments resulted in a higher CAT activity, with no significant difference between them. By day 60, a distinct trend was observed, where the Control group showed the lowest enzyme activity, while the 1 mM treatment moderately increased CAT activity. However, the 2 mM treatment significantly enhanced CAT activity, achieving the highest value among all groups. These findings indicate that the 2 mM treatment effectively boosts catalase activity during prolonged storage, suggesting its potential role in enhancing oxidative stress resistance and maintaining product quality over time (Fig. 9).

Melatonin treatments have been consistently reported to increase catalase activity in post-harvest fruits such as citrus, pitaya, apricots, raspberries, cassava, and litchi (Ba *et al*., 2022; Guo *et al*., 2021; Rahmanzadeh-Ishkeh *et al*., 2024; Zhang *et al*., 2021). This increase in catalase activity helps in scavenging reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2), thereby reducing oxidative stress and delaying senescence. For instance, in fresh-cut pitaya fruits, melatonin at 100 µmol L^{-1} significantly increased catalase activity, which contributed to lower H_2O_2 levels and delayed ripening (Ba *et al*., 2022). Similarly, in apricots, melatonin treatments enhanced catalase activity, reducing active oxygen content and delaying fruit senescence (Guo *et al*., 2021). The effectiveness of melatonin in increasing catalase activity and improving post-harvest quality appears to be dose-dependent. For example, in raspberries, the highest catalase activity was observed at a melatonin concentration of 0.1 mM (Rahmanzadeh-Ishkeh *et al*., 2024). Similarly, in eggplants, 100 µmol L^{-1} melatonin was effective in maintaining higher catalase activity and reducing chilling injury (Song *et al*., 2022).

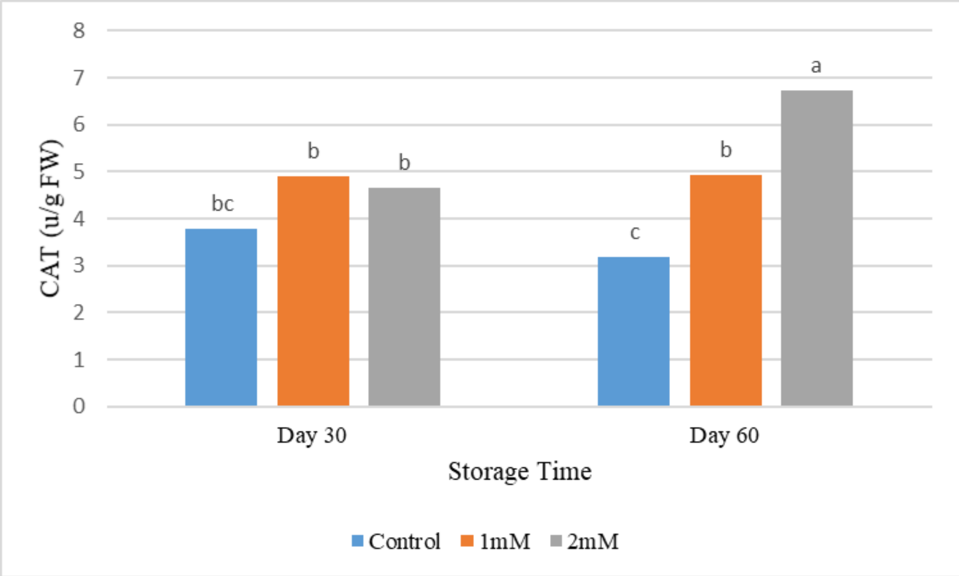

Fig. 9. Effect of different melatonin concentrations on the Vitamin C of orange juice over 30 and 60 days of storage

Color Changes

The color parameters of the samples, including a*, b*, L*, Chroma, and Hue, were measured at two storage periods (30 and 60 days) under three treatment conditions: Control, 1 mM and 2 mM melatonin (Table 2).

On Day 30, the a* and b* values were generally lower in the Control and 1 mM treatments compared to the 2 mM treatment. Specifically, a* showed the lowest value in 2 mM treatment (24.77), which was significantly lower than the Control and 1 mM treatment. The b* value followed a similar pattern, with 2 mM exhibiting a slightly lower value than the other treatments. The L* value (lightness) did not show a significant difference between the Control and 1 mM treatment, but the 2 mM treatment slightly reduced L* value. For Chroma, the 1 mM treatment had the highest value (18.95), while the 2 mM treatment exhibited the lowest (16.13). The Hue angle increased with the melatonin concentration, with 2 mM showing the highest value (27.82), indicating a noticeable shift in the color attributes. On Day 60, the a* value was significantly higher in 2 mM treatment (31.78), followed by the Control (29.03) and 1 mM treatment (26.68). The b* value was highest in the Control and 1 mM treatment, with no significant difference between them, whereas the 2 mM treatment exhibited a lower b* value (32.44). The L* value remained consistent among all treatments, with only minor variations. Chroma was highest in 1 mM treatment (19.13), while the Control and 2 mM treatment showed lower values. The Hue angle followed a similar trend as Day 30, with the 2 mM treatment showing the highest Hue angle (24.34), indicating a shift toward a more yellowish tone. These results suggest that melatonin treatments, particularly at 2 mM, significantly influence the color parameters of samples during storage, potentially enhancing visual quality attributes such as color stability and hue angle over time. The application of melatonin can enhance and promote the accumulation of fruit pigments during the storage period. Previous research findings on pear (Sun *et al.*, 2021), apple (Verde *et al.*, 2022), tomato (Sun *et al.*, 2015), and grape (Xia *et al.*, 2021) support these results.

Table 2- Effect of Melatonin Treatments on Color Parameters (a*, b*, L*, Chroma, and Hue) During Storage Periods

Storage Period	Melatonin (mM)	a*	b*	L*	Chroma	Hue
Day 30	Control	26.04b	33.69b	57.33a	17.91b	17.93f
	1mM	26.51b	33.72b	56.49a	18.95a	22.77c
	2mM	24.77bc	32.17b	54.61ab	16.13c	27.82a
Day 60	Control	29.03a	35.69a	57.95a	17.59ab	17.85f
	1mM	26.68b	35.51a	57.08a	19.13a	19.86e
	2mM	31.78a	32.44b	55.43ab	17.99b	24.34b

Conclusion

This study demonstrates the potential of melatonin treatments in maintaining the post-harvest quality of orange fruit during storage. Both 1 mM and 2 mM melatonin concentrations effectively improved critical parameters such as total soluble solids, vitamin C content, antioxidant capacity, and enzymatic activities while reducing weight loss. The 2 mM treatment exhibited greater efficacy in enhancing nutritional and sensory attributes during early storage, while 1 mM melatonin provided better stability over prolonged storage. These findings highlight melatonin's role in extending shelf life and preserving quality, emphasizing its value as a natural preservative. Further research should explore its broader applications and mechanisms.

Authors Contribution

Ansari A.: Conceptualization, Methodology, writing and revision of the manuscript. **Saadatian M.**: Formal analysis, Software, writing – original draft. **Haji-Taghilou R.**: Investigation, writing – review and editing. **Mohammad K.S.:** Visualization, Validation. **Abdollah R.A.:** Methodology, Data curation. **Majid Taha A.:** Validation, writing – review and editing.

Funding Source

This research received no external funding.

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مقاله پژوهشی
جلد ۲۱، شماره ۳، مرداد- شهریور ۱٤۰٤، ص. ۳۳۵-۳۱۷

افزایش ماندگاری کیفیت میوه پرتقال (*Citrus sinensis*) با کاربرد ملاتونین خارجی از طریق بهبود برخی ویژگی‌های فیتوشیمیایی

افسانه انصاری[۱]- محمد سعادتیان[۲*]- رامین حاجی تقی لو[۱]- کاظم صدیق محمد[۲]- راوین عبدالهادی عبدالله[۳]- عبدالسمیع مجید طاها[۲]

تاریخ دریافت: ۱۴۰۳/۱۰/۲۳
تاریخ پذیرش: ۱۴۰۴/۰۲/۱۷

چکیده

این مطالعه تأثیر تیمارهای ملاتونین (۱ و ۲ میلی‌مولار) را بر کیفیت پس از برداشت میوه پرتقال در طی ۳۰ و ۶۰ روز نگهداری در دمای سرد بررسی کرد. در این پژوهش، پارامترهایی مانند اسیدیته قابل تیتر (TA)، مواد جامد محلول کل (TSS)، ویتامین C، ظرفیت آنتی‌اکسیدانی، ترکیبات فنولی کل (TPC)، فلاونوئیدهای کل (TFC)، فعالیت آنزیم‌های پال (PAL) و کاتالاز (CAT)، و رنگ مورد ارزیابی قرار گرفت. ملاتونین به‌طور معنی‌داری کیفیت میوه را با حفظ سطوح بالاتر TSS، ویتامین C و ظرفیت آنتی‌اکسیدانی بهبود بخشید. هر دو تیمار به‌طور مؤثری از کاهش وزن میوه جلوگیری کرده و فعالیت آنزیم‌های آنتی‌اکسیدانی را افزایش دادند. در حالی‌که تیمار ۲ میلی‌مولار ملاتونین در مراحل ابتدایی نگهداری اثربخشی بیشتری داشت، تیمار ۱ میلی‌مولار در حفظ کیفیت در بازه‌های طولانی‌تر عملکرد پایدارتری نشان داد. تیمارهای ملاتونین همچنین بر ویژگی‌های رنگ میوه تأثیر گذاشتند که نشان‌دهنده بهبود احتمالی جذابیت ظاهری میوه است. این یافته‌ها پتانسیل ملاتونین را به‌عنوان نگهدارنده طبیعی برای بهبود کیفیت پس از برداشت و افزایش ماندگاری میوه پرتقال نشان می‌دهند. برای دستیابی به مدیریت مؤثر و پایدار میوه، تحقیقات بیشتری برای بهینه‌سازی غلظت‌های ملاتونین و بررسی ترکیب آن با سایر روش‌های نگهداری مورد نیاز است.

واژه‌های کلیدی: آنتی‌اکسیدان، آنزیم PAL، پس از برداشت، کاهش وزن، کیفیت میوه

۱- گروه باغبانی، دانشکده کشاورزی، دانشگاه ارومیه، ارومیه، ایران
۲- گروه علوم پایه، دانشکده آموزش، دانشگاه سوران، سوران، عراق
(*- نویسنده مسئول: Email: mohammad.saadatian@soran.edu.iq)
۳- گروه داروسازی، مؤسسه خصوصی پلی تکنیک رواندز، سوران، عراق

https://doi.org/10.22067/ifstrj.2025.91596.1399

Iranian Food Science and Technology Research Journal
Homepage: https://ifstrj.um.ac.ir

Review Article
Vol. 21, No. 3, Aug.-Sep. 2025, p. 337-357

Edible Biodegradable Films Incorporating Essential Oil-based Pickering Emulsions: A Review of Antioxidant and Antimicrobial Properties

H. Mirzaee Moghaddam[1*], A. Nahalkar[1], A. Rajaei[1*]

1- School of Agricultural Engineering, Shahrood University of Technology, Shahrood, Iran
(*- Corresponding Authors Emails: H_Mirzaee@sharoodut.ac.ir; ahmadrajaee@shahroodut.ac.ir)

Received: 15.03.2025 Revised: 21.05.2025 Accepted: 21.05.2025 Available Online: 17.06.2025	**How to cite this article:** Mirzaee Moghaddam, H., Nahalkar, A., & Rajaei, A. (2025). Edible biodegradable films incorporating essential oil-based Pickering emulsions: A review of antioxidant and antimicrobial properties. *Iranian Food Science and Technology Research Journal, 21*(3), 337-357. https://doi.org/10.22067/ifstrj.2025.92672.1416

Abstract

This article reviews the antioxidant and antimicrobial properties of biodegradable edible films based on Pickering emulsions containing essential oils. Edible biodegradable films incorporating essential oil-loaded Pickering emulsions are increasingly recognized as a promising option for sustainable food packaging. By incorporating essential oils into the emulsion matrix, the antioxidant and antimicrobial properties of these films significantly improved. Therefore, the key properties discussed in this review include antioxidant activity, antimicrobial effectiveness, and the role of these films in extending the shelf life of food products. The results showed that the incorporation of Pickering emulsions containing essential oils significantly increased the antioxidant capacity of the films, leading to a notable reduction in oxidative degradation of food. Additionally, these films exhibited effective antimicrobial activity against various foodborne pathogens such as *Escherichia coli and Staphylococcus aureus*, which is attributed to the bioactive properties of the incorporated essential oils. The films effectively inhibited microbial growth, directly contributing to enhanced food safety. The findings highlight the great potential of Pickering emulsion-based biodegradable films as a sustainable solution for food packaging with antioxidant and antimicrobial properties, ensuring longer shelf life and higher safety of packaged food products.

Keywords: Antioxidant activity, Antimicrobial, Essential oils, Edible films, Pickering emulsions

Introduction

In recent years, growing concerns over the environmental impact of synthetic plastic packaging have led researchers to explore sustainable alternatives (Bangar, Whiteside, Dunno, Cavender, & Dawson, 2023). Among these, edible biodegradable films have emerged as promising candidates for food packaging applications. These films are typically made from natural biopolymers such as proteins, polysaccharides, and lipids, offering the dual benefits of environmental friendliness and direct edibility without the need for removal before consumption (Majdzadeh, Rajaei, Mirzaee Moghaddam, & Movahednejad, 2018). However, the inherent limitations of pure edible films—such as low mechanical strength, poor barrier properties, and limited bioactivity—have encouraged the incorporation of functional agents to enhance their performance(Muñoz-Tebar, Pérez-Álvarez, Fernández-López, & Viuda-Martos, 2023).

To address these challenges, the concept of active packaging has been introduced. Active packaging not only provides a physical barrier

https://doi.org/10.22067/ifstrj.2025.92672.1416

but also interacts with the food or its environment to extend shelf life and improve safety. One widely studied approach involves the integration of bioactive compounds, particularly essential oils, known for their potent antioxidant and antimicrobial properties. These natural substances can inhibit the growth of foodborne pathogens and delay lipid oxidation, thereby maintaining food quality (Friedman, Henika, & Mandrell, 2002). However, the direct incorporation of essential oils into film matrices presents several challenges, such as high volatility, light and heat sensitivity, and strong odor, which can adversely affect both stability and consumer acceptance. These issues have driven the search for effective delivery systems that can improve the controlled release and protect the functional properties of essential oils (Shahidi & Hossain, 2022).

One innovative strategy for stabilizing essential oils is their encapsulation within emulsion systems. Among the various techniques, Pickering emulsions have garnered significant attention as a surfactant-free and biocompatible alternative to conventional emulsions (Roy & Rhim, 2021b; Visan, Popescu-Pelin, & Socol, 2021; Wardana, Wigati, Van, Tanaka, & Tanaka, 2023). These emulsions are stabilized by solid particles that irreversibly adsorb at the oil–water interface, preventing coalescence of the dispersed droplets. Solid particles such as cellulose nanocrystals, protein nanoparticles, and biopolymer-based particles have been used to create stable Pickering emulsions (Priyadarshi & Rhim, 2020; Sharkawy, Barreiro, & Rodrigues, 2020; Sun *et al*., 2020; Tavakoli-Rouzbehani *et al*., 2021). These systems offer enhanced physical stability, protection against environmental stressors, and improved retention and the controlled release of encapsulated essential oils, which are crucial for preserving their functional efficacy. (Fan *et al*., 2023).

Incorporating essential oil-loaded Pickering emulsions into edible biodegradable films offers a novel approach to developing high-performance active packaging systems (Pandita *et al*., 2024). These hybrid structures enable the controlled release of essential oils while simultaneously enhancing the mechanical and structural integrity of the film matrix. The presence of stabilizing particles in the emulsions not only improves the dispersion of the oil phase but also facilitates better interactions with the film matrix, leading to enhanced functional and physical properties (Zhang *et al*., 2024). Recent research has explored various parameters affecting these systems, including the type of stabilizing particles, the nature of the essential oil, and the oil-to-water ratio, demonstrating the significant potential of Pickering emulsions in food packaging innovations (Cheng *et al*., 2024).

In recent years, extensive research has explored the synergistic combination of essential oils and Pickering emulsions for incorporation into edible biodegradable films to enhance their antioxidant and antimicrobial properties. For instance, (Zhang *et al*., 2022) developed konjac-based films infused with oregano essential oil encapsulated in zein–pectin nanoparticles, which exhibited significant antioxidant activity. Similarly, (Roy & Rhim, 2021a) developed films using a carrageenan–agar biopolymer matrix reinforced with tea tree essential oil stabilized by nanocellulose fibers, yielding materials with notable antioxidant performance. In another study, (Zhao *et al*., 2023) incorporated clove essential oil into a film composed of potato starch and polyvinyl alcohol, observing strong antibacterial effects, particularly against *Escherichia coli* compared to *Staphylococcus aureus*. A particularly innovative approach by (Bu *et al*., 2022) involved the use of konjac glucomannan and pullulan as a film matrix, combined with tea tree essential oil delivered via cellulose nanofibril-stabilized Pickering emulsions, resulting in a hybrid system with potent antimicrobial activity against both *Escherichia coli* and *Staphylococcus aureus*. Collectively, different studies highlight the efficacy of Pickering emulsions in stabilizing volatile essential oils, facilitating their controlled release, and maintaining their bioactive properties within biopolymer-based films.

This review critically examines the role of Pickering emulsion-based delivery systems in enhancing the functional properties of essential oil-loaded edible films, with a focus on their antioxidant and antimicrobial efficacy. The mechanisms of action of essential oils, the benefits of Pickering emulsions as delivery systems, and their influence on the bioactive performance of edible films were also explored in this review. Furthermore, this review identifies key challenges—such as scalability, sensory compatibility, and long-term stability—while proposing future research directions to advance the development of next-generation active packaging. By integrating fundamental principles with cutting-edge applications, this work aims to bridge critical knowledge gaps and inspire innovative solutions for sustainable, high-performance food preservation systems.

Pickering Emulsions: Fundamentals and Applications

Definition and Mechanism

Pickering emulsions are a type of emulsion stabilized by solid particles, rather than traditional surfactants. These emulsions consist of two immiscible liquids, such as oil and water, with solid particles adsorbed at the interface between the two phases. The solid particles act to stabilize the emulsion by preventing the coalescence of oil droplets, forming a rigid structure at the oil-water interface. In contrast, conventional emulsions, rely on surfactants to reduce the interfacial tension between oil and water, preventing droplet aggregation. In Pickering emulsions, the particles adsorb onto the droplet surface, creating a physical barrier that decreases interfacial tension and provides steric and electrostatic repulsion (Mirzaee Moghaddam, 2019). This prevents the droplets from merging. The solid particles form a more stable and robust emulsion compared to surfactant-based systems, as they are less prone to desorption or leaching under challenging conditions such as temperature fluctuations or pH changes (Yang *et al*., 2017). The stabilization of oil droplets in Pickering emulsions is influenced by factors such as the size, shape, and surface characteristics of the solid particles (Hosseini, Rajaei, Tabatabaei, Mohsenifar, & Jahanbin, 2020). These particles need to be small enough to effectively stabilize the droplets, but also large enough to prevent excessive diffusion or aggregation. The wettability and surface charge of the particles also play a significant role in determining the overall stability and structure of the emulsion. For example, hydrophobic particles typically stabilize oil-in-water emulsions, while hydrophilic particles are more suited for water-in-oil emulsions (Zhao *et al*., 2022). These factors make Pickering emulsions highly effective for encapsulating and stabilizing sensitive compounds, including essential oils.

Benefits in Food Systems

In food systems, the use of Pickering emulsions provides several significant advantages, especially when it comes to encapsulating bioactive compounds such as essential oils. One of the key benefits is the enhanced stability of volatile and sensitive compounds. Essential oils, are volatile and hydrophobic, prone to degradation through factors like heat, light, and oxygen exposure. By encapsulating these oils within Pickering emulsions, their stability is improved significantly. The solid particles at the oil-water interface form a protective barrier, preventing the evaporation or degradation of the essential oils, thereby preserving their activity for longer periods (De Farias *et al*., 2025). In addition to stability, Pickering emulsions offer controlled release of active compounds, which is particularly advantageous in food applications (Nazari, Rajaei, & Moghaddam, 2025). The structure of these emulsions allows for the gradual release of bioactive compounds, such as antioxidants or antimicrobial agents, over time. This controlled release helps extend the shelf life of food products by continuously delivering active compounds that inhibit microbial growth and prevent oxidative rancidity. For instance, essential oils encapsulated within Pickering emulsions can be slowly released, maintaining their antimicrobial activity over an extended period, which is beneficial for active food packaging applications (Monjazeb Marvdashti, Yavarmanesh, & Koocheki, 2016). This approach enables a long-lasting protective effect, making it an ideal solution for food products requiring enhanced preservation. Furthermore, Pickering emulsions can improve the texture and sensory attributes of food products. The formation of stable emulsions with uniform droplet size distribution ensures smooth and consistent textures, which are crucial in products such as sauces, dressings, and beverages, where uniformity is highly valued by consumers. The use of natural stabilizers in Pickering emulsions also supports the growing demand for clean-label products, as consumers increasingly seek food products with minimal synthetic additives (Cheng *et al*., 2024).

Types of Stabilizers

The success of Pickering emulsions largely depends on the choice of stabilizers, which can be categorized into natural biopolymers and inorganic nanoparticles. Both types of stabilizers offer unique advantages and can be selected based on the specific requirements of the food system in question. Natural biopolymers, such as chitosan, cellulose, and starch, are frequently used as stabilizers in Pickering emulsions because they are abundant, biodegradable, and compatible with food systems. Chitosan, derived from the shells of crustaceans, is widely utilized in food applications due to its biocompatibility, biodegradability, and antimicrobial properties. The molecular structure of chitosan facilitates effective adsorption at the oil-water interface, stabilizing emulsions and enhancing their overall stability. Additionally, chitosan can provide functional benefits, such as improving the antioxidant properties of the emulsion, which further contributes to food preservation (Hamed, Özogul, & Regenstein, 2016). Cellulose, a naturally abundant polymer, is another effective stabilizer for Pickering emulsions. Cellulose-based materials, such as cellulose nanocrystals and cellulose nanofibers, improve the stability and rheological properties of emulsions. These materials stabilize the emulsion by forming strong, durable interfacial layers that prevent droplet coalescence and enhance the mechanical strength of the emulsion. The use of cellulose is particularly beneficial in food systems where texture and stability are key considerations (Liu *et al*., 2023). Starch, a versatile biopolymer, is often used in Pickering emulsions for its cost-effectiveness and ease of production. Modified starches and starch nanoparticles have demonstrated excellent emulsifying properties, providing stable emulsions with controlled release characteristics. Starch-based Pickering emulsions can improve both the stability and texture of food products, making them ideal for a wide range of applications (Ramos, Ramírez-López, Pinho, Ditchfield, & Moraes, 2025).

Inorganic nanoparticles are also commonly used as stabilizers in Pickering emulsions due to their excellent mechanical properties, chemical stability, and biocompatibility. Silica nanoparticles are particularly effective in stabilizing oil-in-water emulsions. They have a high surface area and are hydrophilic, which makes them suitable for applications that require high stability, such as encapsulating volatile compounds like essential oils. Silica's ability to form strong interfacial layers enhances the emulsion's stability under varying environmental conditions, such as changes in temperature and pH (Jiang, Sheng, & Ngai, 2020). While titanium dioxide (TiO_2) nanoparticles have been studied for their dual role in stabilizing Pickering emulsions and providing antimicrobial properties, their use in food applications has faced regulatory restrictions in several regions, including a ban in Iran due to safety concerns. As a result, researchers are increasingly exploring alternative inorganic or organic stabilizers—such as starch-based nanoparticles, cellulose nanocrystals, or clay minerals—that offer similar functional benefits without regulatory limitations (Das, Kumar, Singh, & Kayastha, 2024; Omidian, Akhzarmehr, & Chowdhury, 2024). The choice of stabilizer depends on the specific requirements of the food system, including the type of active ingredients to be encapsulated, the desired release profile, and the sensory attributes of the final product. By optimizing Pickering emulsions with approved stabilizers, food scientists can enhance functionality, ensuring improved stability, controlled release, and extended shelf life. These advancements provide a promising pathway for developing safer, more compliant food preservation technologies while maintaining product quality (Nahalkar, Rajaei, & Mirzaee Moghaddam, 2025).

Preparation and Characterization of Essential Oil-Loaded Pickering Emulsions

Formulation Techniques

The formulation of essential oil-loaded Pickering emulsions involves the careful selection of both the essential oils to be encapsulated and the stabilizing agents, which play a pivotal role in ensuring the stability and functionality of the emulsions. Essential oils, due to their lipophilic nature and volatility, require robust encapsulation strategies to maintain their stability and ensure their controlled release. Commonly used essential oils for encapsulation include thyme, oregano, rosemary, and clove oils, which are selected based on their bioactive properties, such as antioxidant, antimicrobial, and antifungal activities. The choice of essential oil is influenced by factors such as the intended application (e.g., food preservation, active packaging), the specific bioactive compound profile, and the compatibility of the oil with the other components of the emulsion (Mirzaee Moghaddam & Rajaei, 2021; Oun, Shin, & Kim, 2022; Priyadarshi & Rhim, 2020; Roy & Rhim, 2021a).

To achieve effective emulsion stabilization, stabilizing particles must exhibit suitable surface characteristics, such as hydrophilicity or hydrophobicity, to ensure their strong adsorption at the oil-water interface. This adsorption forms a protective barrier around dispersed droplets, preventing their coalescence and enhancing the long-term stability of the emulsion. However, recent studies have revealed that particle geometry also plays a crucial role in determining the efficiency of emulsion stabilization. As a result, increasing attention has been given to the design and utilization of non-spherical particles—including rods, fibers, ellipsoids, and cubes—as effective Pickering emulsion stabilizers (Wu & Ma, 2016).

In this context (Madivala, Fransaer, & Vermant, 2009), investigated the effect of particle shape on emulsion stability by mechanically stretching spherical polystyrene particles to create ellipsoidal shapes. These anisotropic particles demonstrated markedly improved stabilizing performance. At higher concentrations, the ellipsoidal particles assembled end-to-end at the oil-water interface, forming triangular mesh-like structures that acted as a physical scaffold, impeding droplet movement and coalescence. At lower concentrations, the particles arranged into striped patterns, still reinforcing interfacial stability. The study found a direct correlation between higher aspect ratios and enhanced emulsion stability, highlighting the influence of geometry on interfacial behavior. In another study (Kalashnikova, Bizot, Cathala, & Capron, 2011), synthesized different nanorods and employed them in the preparation of Pickering emulsions. These nanorods exhibited a strong tendency to interconnect at the interface, forming bridge-like structures that contributed to the formation of super-stable emulsions. Such arrangements substantially increased the energy barrier for droplet coalescence, providing robust resistance against phase separation and other destabilizing factors.

Emulsification methods are the next crucial step in the preparation of essential oil-loaded Pickering emulsions. Two common methods used are ultrasonication and high-pressure homogenization. Ultrasonication utilizes high-frequency sound waves to generate intense shear forces, which break up the oil phase into small droplets and create a fine emulsion. This method is particularly effective for producing emulsions with a narrow droplet size distribution and is widely used for encapsulating essential oils. High-pressure homogenization, on the other hand, involves forcing the oil-water mixture through a small orifice under high pressure, which results in the formation of fine droplets. This method is also efficient for stabilizing emulsions and can be applied at larger scales. Both methods can be optimized to achieve the desired droplet size and emulsion stability, depending on the characteristics of the essential oil and stabilizer used (Barradas & de Holanda e Silva, 2021).

Encapsulation Efficiency

Encapsulation efficiency is an important parameter for evaluating the effectiveness of Pickering emulsions in preserving essential oils and delivering them in a controlled manner. Encapsulation efficiency refers to the proportion of the essential oil that is successfully incorporated into the emulsion, compared to the total amount of oil added during the preparation process. It is typically expressed as a percentage and is influenced by factors such as the type of stabilizer used, emulsification method, and the properties of the essential oil (Cahyana *et al*., 2022). To measure encapsulation efficiency, the amount of free or unencapsulated oil is determined by techniques such as centrifugation, filtration, or solvent extraction (Rajaei, Barzegar, Mobarez, Sahari, & Esfahani, 2010). The amount of oil retained within the emulsion can then be quantified, and the efficiency can be calculated based on the ratio of encapsulated oil to the total amount of oil used in the formulation. High encapsulation efficiency is desirable because it indicates that the majority of the essential oil is effectively incorporated into the emulsion, which maximizes its functional benefits in food packaging applications (Nahalkar, Rajaei, & Mirzaee Moghaddam, (in press)). The controlled release profile of essential oils from Pickering emulsions is another critical aspect of their performance. The release of encapsulated essential oils is influenced by the properties of the stabilizing agents, the droplet size, and the emulsification method. Typically, the release rate can be controlled by adjusting the particle size of the emulsion and the thickness of the interfacial layer formed by the stabilizing agents (Lammari, Louaer, Meniai, & Elaissari, 2020).

The controlled release of essential oils is essential for applications such as active food packaging, where the goal is to provide a gradual and sustained release of bioactive compounds to enhance food preservation without overwhelming the sensory properties of the food (Karimi, Bodaghi, Rajaei, & Mojerlou, 2020). The release behavior can be studied using in vitro methods, where the emulsion is exposed to conditions that simulate real-world environments, such as acidic or alkaline conditions, temperature variations, and exposure to light or oxygen (Visan *et al*., 2021). By monitoring the concentration of released essential oil over time, a release profile can be constructed, showing how the essential oil is gradually released from the emulsion matrix. A slow and sustained release is typically ideal for maximizing the shelf life and effectiveness of essential oils in food applications.

Antioxidant and Antimicrobial Evaluation of Edible Films

In Vitro Antioxidant Activity

The evaluation of antioxidant activity is crucial for assessing the potential of edible films containing bioactive compounds, such as essential oils, to prevent oxidative spoilage and enhance food preservation. Antioxidant assays typically measure the ability of a film to scavenge free radicals or reduce oxidative damage to food components. Several in vitro methods are commonly employed to evaluate the antioxidant properties of edible films, including the DPPH and ABTS radical scavenging assays and the Ferric Reducing Antioxidant Power (FRAP) test (Benbettaïeb, Debeaufort, & Karbowiak, 2019). The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay is one of the most widely used methods to assess the free radical-scavenging ability of antioxidants. In this assay, the DPPH radical reacts with an antioxidant present in the edible film, resulting in a color change from purple to yellow (Rajaei *et al*., 2021). The extent of this color change is directly proportional to the antioxidant activity, with a higher reduction in the DPPH radical indicating stronger antioxidant properties. The scavenging ability is quantified by measuring the absorbance at 517 nm and calculating the percentage inhibition of the DPPH radical. This assay provides valuable information about the film's potential to prevent oxidative damage to food during storage (Gulcin & Alwasel, 2023).

Table 1- Some recent studies on the effect of essential oil-loaded Pickering emulsions on the antioxidant properties of edible films

Edible film	Essential oil	Pickering particle	Application	Food Product Analyzed	References
Konjac glucomannan	Oregano	Zein–pectin nanoparticle	DPPH free radical scavenging	–	(Zhang *et al*., 2022)
Carrageenan/agar	tea tree	Nanocellulose fibers	Distinct antioxidant	–	(Roy & Rhim, 2021a)
Gelatin	Cinnamon	Lignocellulose nanocrystals-tannic acid	Improve antioxidant properties	–	(Dai *et al*., 2023)
Chayote tuber starch	Cinnamon	Zein-pectin nanoparticle	Increased antioxidant activity	Ground beef	(Wu *et al*., 2023)
Chitosan	Lemon Myrtle	Alkali lignin	Improve antioxidant properties	–	(Liu, Swift, Tollemache, Perera, & Kilmartin, 2022)
Anthocyanidin/chitosan	Cinnamon-perilla	Collagen	Increase antioxidant activity	Chilled fish fillet	(Zhao, Guan, Zhou, Lao, & Cai, 2022)
Gelatin/agar	Clove	Copper-modified zinc oxide nanoparticles	Improve antioxidant properties	Pork meat	(Roy, Priyadarshi, & Rhim, 2022)
Carboxymethyl cellulose /polyvinyl alcohol	Ginger	Ginger essential oil	High antioxidant activity	Bread	(Fasihi, Noshirvani, & Hashemi, 2023)
Konjac glucomannan	Corn germ oil-oregano essential oil	Zein-pectin nanoparticle	Antioxidant activity	–	(Du *et al*., 2023)
Konjac glucomannan	Thyme	Bacterial cellulose nanofibers/soy protein isolate	Highest total phenol content and antioxidant capacities, as well as the best TEO-release property	–	(Liu, Lin, Li, & Yang, 2022)
Konjac glucomannan	Oregano	Chitin nanocrystal	Improve antioxidant properties	–	(Xu *et al*., 2023)
Chitosan	Grapefruit	Amphiphilic octenyl succinic anhydride konjac glucomannan	High efficiency of DPPH free radical scavenging	–	(Bu *et al*., 2022)
Hydroxypropyl methyl cellulose	Cinnamon	Zein/carboxymethyl tamarind gum	Improve antioxidant activity	Cherry tomatoes	(Yao *et al*., 2023)

The ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging assay is another commonly used test for evaluating antioxidant capacity

(Rajaei, Hadian, Mohsenifar, Rahmani-Cherati, & Tabatabaei, 2017). ABTS is a stable radical that, when mixed with an antioxidant, undergoes reduction, leading to a decrease in absorbance at 734 nm. The degree of inhibition of ABTS radical cation formation is used to quantify the antioxidant activity. Like the DPPH assay, the ABTS assay provides a measure of the antioxidant potential of edible films, reflecting their ability to neutralize reactive oxygen species (ROS) and prevent oxidative damage (Ilyasov, Beloborodov, Selivanova, & Terekhov, 2020).

Together, these in vitro antioxidant assays provide a comprehensive evaluation of the antioxidant capacity of edible films and can be used to compare different formulations, such as films containing essential oils or other bioactive agents. Table 1 shows some recent studies on the effect of Pickering emulsions containing essential oils on the antioxidant properties of edible films. Films with strong antioxidant properties are particularly valuable for extending the shelf life of perishable foods by reducing lipid oxidation, preserving flavor, and maintaining nutritional quality. The incorporation of Pickering emulsions containing essential oils into edible films can significantly enhance their antioxidant properties. For example in a study, a bio-based film was formulated using konjac glucomannan, thyme essential oil, and a composite of bacterial cellulose nanofibers and soy protein isolate. The resulting material exhibited the highest total phenol content and antioxidant capacity among the tested formulations. Additionally, it demonstrated the most efficient release of thyme essential oil, making it a promising candidate for active packaging applications aimed at enhancing food preservation and extending shelf life (Liu *et al*., 2022). In another study, a bioactive film composed of chitosan, lemon myrtle essential oil, and alkali lignin was developed to enhance the antioxidant properties of the material. The incorporation of lemon myrtle and alkali lignin significantly improved the film's ability to scavenge free radicals, indicating strong antioxidant activity (Liu *et al*., 2022).

Essential oils are rich in phenolic and bioactive compounds, but their high volatility and instability under environmental conditions limit their effectiveness. Pickering emulsions, stabilized by solid nanoparticles or microparticles, help reduce oxidation, control the gradual release of antioxidant compounds, and prolong their bioactivity within the edible film. This leads to increased resistance of the film against lipid oxidation and damage caused by free radicals, ultimately improving the quality and safety of packaged food products (Mirzaee Moghaddam & Rajaei, 2021; Roy & Rhim, 2021a; Roy & Rhim, 2021b; Xu *et al*., 2023).

Antimicrobial Assays

In addition to antioxidant activity, antimicrobial evaluation is an essential step in determining the efficacy of edible films for active food packaging applications. The antimicrobial properties of edible films help protect food from spoilage and contamination by inhibiting the growth of harmful microorganisms. Various in vitro methods are used to assess the antimicrobial activity of edible films, including disc diffusion, well-diffusion methods, and the determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (Benbettaïeb *et al*., 2019). The disc diffusion method is a commonly used technique for assessing the antimicrobial activity of edible films against a wide range of microorganisms. In this assay, the edible film is applied to a sterile disc, which is then placed on an agar plate inoculated with the target microorganism. The film gradually releases its antimicrobial agents, creating a zone of inhibition around the disc where bacterial growth is prevented. The size of the zone of inhibition is measured, and the larger the zone, the stronger the antimicrobial effect of the edible film. This method provides a qualitative measure of antimicrobial efficacy and is useful for screening the antimicrobial potential of films containing essential oils or other bioactive compounds (Benbettaïeb *et al*., 2019).

The well-diffusion method is another technique used to evaluate antimicrobial activity, similar to the disc diffusion method but with a slight variation. In this method, wells are punched into an agar plate, and the edible film or its extract is placed into the well. The antimicrobial agents diffuse radially outward from the well, creating a zone of inhibition. This method is often employed when higher concentrations of antimicrobial agents need to be tested, and it allows for the determination of the minimum concentration of the active compound required to inhibit microbial growth (Balouiri, Sadiki, & Ibnsouda, 2016). To further quantify the antimicrobial effectiveness of edible films, the MIC and MBC are determined. The MIC is the lowest concentration of an antimicrobial agent that prevents visible growth of the microorganism in the presence of the edible film or its extract. The MIC can be determined by preparing serial dilutions of the film or its active components and inoculating them with the target microorganism. The results are interpreted by observing the absence of microbial growth, which indicates the MIC. The MBC is the lowest concentration of the antimicrobial agent that results in the complete eradication of the microorganism, measured by the absence of growth on a subculture plate. These tests provide quantitative data on the effectiveness of edible films in inhibiting or killing microorganisms at different concentrations (Rao, Chen, & McClements, 2019).

In terms of antimicrobial activity, edible films can be tested against both gram-positive and gram-negative bacteria, as well as fungi and viruses. Gram-positive bacteria like *Staphylococcus aureus* and *Listeria monocytogenes* are often used in studies of antimicrobial edible films due to their association with foodborne illnesses. Gram-negative bacteria, such as *Escherichia coli* and *Salmonella spp.*, are also critical targets in antimicrobial food packaging, as they are responsible for a significant proportion of foodborne infections. The differences in cell wall structure between gram-positive and gram-negative bacteria may influence the effectiveness of antimicrobial agents, with gram-negative bacteria often being more resistant to certain compounds due to the presence of an outer membrane that can act as a barrier (Valencia-Chamorro, Palou, Del Río, & Pérez-Gago, 2011).

Antimicrobial tests are essential for determining the suitability of essential oil-loaded Pickering emulsions in edible films. Films containing essential oils such as thyme, oregano, or clove oil are known for their potent antimicrobial properties and have shown significant efficacy against common foodborne pathogens. For example in a recent study, a combination of polyvinyl alcohol, oregano essential oil, cinnamon essential oil, and cellulose nanocrystals (CNCs) was used to enhance the antibacterial properties. The results demonstrated significant antimicrobial activity, with *E. coli* showing greater sensitivity to cinnamon essential oil, while *Staphylococcus aureus* was more sensitive to oregano essential oil. These findings highlight the strong potential of this formulation for use in bio-based packaging materials with effective antibacterial properties (Oun *et al*., 2022). In another study, a composite film based on starch, ginger extract, and TEMPO-oxidized cellulose nanocrystals was developed to enhance antibacterial activity, particularly for food packaging applications. The formulation showed improved antimicrobial effectiveness, making it suitable for preserving perishable items such as tomatoes (Chen *et al*., 2023). Table 2 shows some recent studies on the effect of Pickering emulsions containing essential oils on the antimicrobial properties of edible films. The results from these antimicrobial assays guide the optimization of edible films for food packaging applications, ensuring that they offer both preservation and safety benefits by reducing microbial contamination and extending the shelf life of food products.

Table 2- Some recent studies on the effect of essential oil-loaded Pickering emulsions on the antimicrobial properties of edible films

Edible film	Essential oil	Pickering particle	Application	Food Product Analyzed	References
Chitosan / gelatin	Cinnamon	Zein nanoparticles	High antimicrobial against *Pseudomonad paralactis* MN10 and *Lactobacillus sakei* VMR17	–	(Fan *et al.*, 2023)
Hydroxypropyl methyl cellulose	Cinnamon	Zein/carboxymethyl tamarind gum	Improved antibacterial activity	Cherry tomatoes	(Yao *et al.*, 2023)
Chitosan	Cinnamon	Cellulose nanocrystal	Improve antibacterial properties	Pork meat	(Liu *et al.*, 2022)
Konjac glucomannan	Oregano	Zein–pectin nanoparticle	Antibacterial effect	–	(Zhang *et al.*, 2022)
Carrageenan/agar	Tea tree	Nanocellulose fibers	Antibacterial activity	–	(Roy & Rhim, 2021a)
Gelatin	Cinnamon	Lignocellulose nanocrystals-tannic acid	Improve antibacterial properties	–	(Dai *et al.*, 2023)
Chayote tuber starch	Cinnamon	Zein-pectin nanoparticle	Increased antimicrobial activity	Ground beef	(Wu *et al.*, 2023)
Chitosan	Lemon Myrtle	Alkali lignin	Improve antibacterial properties	–	(Liu *et al.*, 2022)
Potato starch and polyvinyl alcohol	Clove	Clove essential oil	Antibacterial property (showed more potent inhibition of *Escherichia coli* than *Staphylococcus aureus*).	Pork meat	(Zhao *et al.*, 2023)
Konjac glucomannan and Pullulan	Tea tree	Cellulose nanofibrils	Exhibited antimicrobial activity against *E. coli* and *Staphylococcus aureus*	–	(Bu *et al.*, 2022)
Gelatin/agar	Clove	Copper-modified zinc oxide nanoparticles	100% eradication of *L. monocytogenes* and 50% decrease in the E. coli	Pork meat	(Roy *et al.*, 2022)
Carboxymethyl cellulose /polyvinyl alcohol	Ginger	Ginger essential oil	High antimicrobial activity	Bread	(Fasihi *et al.*, 2023)
Konjac glucomannan	Corn germ oil-oregano essential oil	Zein-pectin nanoparticle	Antibacterial activity	–	(Du *et al.*, 2023)
Sodium alginate	Lemongrass	Cellulose nanofibers	Antifungal properties (*Penicillium digitatum* and *P. italicum)*	–	(Wardana *et al.*, 2023)
Chitosan	Clove	Zein and sodium caseinate	Improved antibacterial *(Escherichia coli and Staphylococcus aureus)*	–	(Hua *et al.*, 2021)
Konjac glucomannan	Oregano	Chitin nanocrystal	Improve antibacterial properties	–	(Xu *et al.*, 2023)
Polyvinyl alcohol	Oregano and cinnamon	Cellulose nanocrystals	Improved antibacterial (*E. coli* was more sensitive to CEO, while *S. aureus* was sensitive to OEO)	–	(Oun *et al.*, 2022)
Chitosan	Grapefruit	Amphiphilic octenyl succinic anhydride konjac glucomannan	Antibacterial activity	–	(Bu *et al.*, 2022)
Tapioca strach/polyvinyl alcohol	*Thymus vulgaris*	Cellulose nanocrystals	Prevent the growth of microorganisms	Fish fillets	(Guo *et al.*, 2024)
Starch	Ginger	tempo-oxidized cellulose nanocrystals	Improved antibacterial activity	tomato	(Chen *et al.*, 2023)

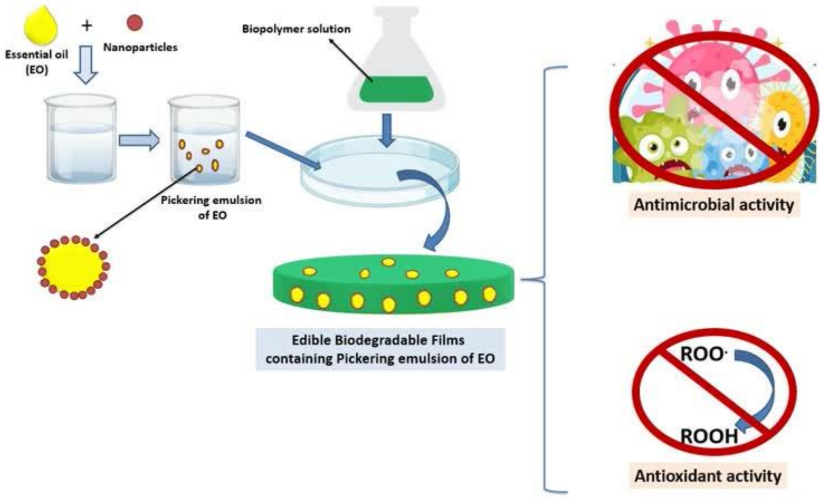

Fig. 1. Effect of Pickering emulsion containing essential oil on antioxidant and antimicrobial properties of edible film

The incorporation of Pickering emulsions containing essential oils into edible films has a significant impact on their antimicrobial properties. Essential oils contain active compounds with antimicrobial effects, but their high volatility and instability reduce their effectiveness in edible films. Pickering emulsions, by stabilizing essential oils through solid nanoparticles or microparticles, enable controlled release of antimicrobial compounds and enhance their stability. This feature helps inhibit the growth of pathogenic and spoilage microorganisms, increases food safety, and improves the shelf life of products packaged with these edible films. Fig. 1 shows the effect of Pickering emulsion containing essential oil on the antioxidant and antimicrobial properties of edible films.

Food Applications and Practical Implications

Potential Use in Food Packaging

The potential application of essential oil-loaded Pickering emulsions in food packaging represents a significant advancement in the development of active packaging systems. These systems are designed not only to provide physical protection but also to actively interact with the contents of the package to improve food preservation. Essential oils, encapsulated within Pickering emulsions, offer dual benefits in food packaging by providing antioxidant and antimicrobial properties. This dual functionality can be applied to a wide range of food products, such as meat, dairy, and fresh produce, to enhance their shelf life and ensure food safety. In the case of meat products, which are highly susceptible to spoilage due to microbial growth and oxidative rancidity, the use of edible films containing essential oils can significantly extend shelf life. Essential oils, such as oregano, thyme, or rosemary oil, have been shown to inhibit the growth of spoilage microorganisms, including Lactic acid bacteria and Enterobacteriaceae, which are commonly found in meat. Additionally, the antioxidant properties of essential oils can prevent lipid oxidation, thereby preserving the flavor and nutritional quality of the meat. By using these active films, the need for synthetic preservatives and additives can be reduced, aligning with consumer demand for cleaner, more natural products (Sánchez-Ortega *et al*., 2014). For example in a study, the use of chayote tuber starch, cinnamon, and zein-pectin nanoparticles has shown promising results in enhancing the functional properties of ground meat. Specifically, these ingredients have been found to increase antimicrobial activity against pathogens such as *Staphylococcus aureus* in ground beef. This combination of natural ingredients effectively reduced microbial growth, improved the safety and shelf life of the meat. Additionally, the incorporation of chayote tuber starch, cinnamon, and zein-pectin nanoparticles in pork meat showed a significant increase in antioxidant activity (Wu *et al.*, 2023).

Dairy products, such as cheese, milk, and yogurt, are also highly prone to microbial contamination and lipid oxidation. Essential oil-loaded Pickering emulsions offer a solution by providing antimicrobial protection against pathogens like *Listeria monocytogenes* and *Salmonella* spp., which are of particular concern in dairy processing and storage. Furthermore, the incorporation of antioxidant essential oils can help prevent the rancidity of fats in dairy products, thereby preserving their sensory properties, such as flavor and texture. The use of such packaging could also potentially extend the shelf life of dairy products, reducing food waste while maintaining product quality over time (El-Sayed, Ibrahim, & Farag, 2022).

For fresh produce, such as fruits and vegetables, the primary concerns include microbial contamination and moisture loss. Essential oil-infused edible films can prevent microbial growth, particularly mold and bacteria, which often cause spoilage in fresh produce. The antioxidant effects can also help to slow down the degradation of vitamins and other bioactive compounds in fruits and vegetables, preserving their nutritional value. Moreover, the use of such films can reduce the need for refrigeration, as they help maintain the desired humidity levels within the packaging, and further extending the freshness of the produce. By offering a biodegradable alternative to conventional plastic packaging, essential oil-loaded Pickering emulsions could also contribute to reducing the environmental impact of food packaging (Qadri, Yousuf, & Srivastava, 2015). In all these applications, the use of essential oil-based films provides an environmentally friendly solution, as they are biodegradable and derived from renewable sources, making them more sustainable than traditional petroleum-based plastics. The ability to encapsulate essential oils within Pickering emulsions further enhances the stability and controlled release of these active compounds, ensuring a prolonged protective effect throughout the shelf life of the food.

Consumer Acceptability and Regulatory Aspects

The adoption of essential oil-loaded Pickering emulsions in food packaging not only requires technical efficacy but also the acceptance of consumers and compliance with regulatory frameworks. The sensory attributes of the packaging, such as taste, aroma, and texture, play a critical role in consumer perception, especially when essential oils are involved. Sensory evaluation is, therefore, an essential part of determining the consumer acceptability of edible films used in food packaging. Sensory evaluation typically involves tests where consumers or trained panels assess the sensory characteristics of the packaged food, including any potential impact on flavor, odor, or appearance. Essential oils, while offering antimicrobial and antioxidant benefits, can impart strong aromas or flavors to the food, which might not always be desirable depending on the type of food product. For instance, essential oils like oregano or thyme may impart a noticeable flavor to meats or dairy products, which could either enhance or detract from the product's sensory appeal. As such, it is crucial to carefully select and balance the type and concentration of essential oils in the film formulations to ensure that their sensory impact is minimal or complementary to the food product (Sipos, Nyitrai, Hitka, Friedrich, & Kókai, 2021). In addition to sensory factors, consumer perception plays a significant role in determining whether these active packaging systems will be accepted in the marketplace. The growing consumer preference for natural and clean-label products has driven the demand for safer, more sustainable packaging materials. However, it is important to address concerns about the safety and potential toxicity of the essential oils used, especially in food applications. Consumers may have concerns regarding allergies to specific essential oils or the possibility of chemical residues from packaging components leaching into the food. Therefore, clear communication about the safety and benefits of these materials is necessary to foster consumer trust (Krishna, 2012).

Safety considerations regarding the use of essential oils in food packaging are also governed by regulatory agencies, such as the FDA (Food and Drug Administration) in the United States and the EFSA (European Food Safety Authority) in the European Union. These agencies establish strict guidelines and regulations to ensure the safety of food contact materials. For instance, the FDA regulates substances that come into contact with food under the Food, Drug, and Cosmetic Act, which requires that any food-contact material be proven safe for its intended use. Essential oils used in food packaging must meet specific safety standards, including toxicological assessments to determine acceptable exposure levels. Furthermore, any essential oils or other ingredients used in edible films must be approved for use in food packaging through the FDA's Food Contact Notification (FCN) process or by being listed as Generally Recognized as Safe (GRAS). In the EU, EFSA evaluates the safety of food contact materials through risk assessments, ensuring that substances do not migrate into food at levels that could pose a risk to human health. Both the FDA and EFSA require comprehensive data on the migration behavior of essential oils from the packaging into the food product, ensuring that the concentrations remain within safe limits. Additionally, any claims made about the antimicrobial or antioxidant properties of essential oil-loaded films must be substantiated with scientific evidence to comply with food labeling regulations (Muncke *et al*., 2017). The regulatory approval process for essential oil-loaded Pickering emulsions in food packaging involves rigorous testing to confirm that the films are non-toxic, effective, and meet all safety standards. By meeting these regulatory requirements, manufacturers can ensure that essential oil-based edible films are not only safe for consumers but also meet the quality standards expected by the food industry.

Conclusion and Future Perspectives

Summary of Key Findings

The incorporation of essential oil-loaded Pickering emulsions into edible films has emerged as a promising strategy for enhancing food packaging. The fundamental principles of Pickering emulsions, where solid particles replace traditional surfactants to stabilize oil droplets, have enabled the creation of highly stable, functionalized films with significant antioxidant and antimicrobial properties. These films offer multiple benefits, including extending shelf life, improving food safety, and reducing the need for synthetic preservatives. The ability of essential oils, such as thyme, oregano, and rosemary, to act as natural preservatives due to their potent bioactive components—carvacrol, thymol, and eugenol—has been demonstrated to effectively inhibit the growth of common foodborne pathogens like *E. coli*, *Salmonella* spp., and *Listeria monocytogenes*. Additionally, their antioxidant capabilities prevent the oxidation of fats and oils, preserving the quality and nutritional integrity of food products. The encapsulation of essential oils in Pickering emulsions offers a controlled and sustained release mechanism, enhancing the stability of these volatile compounds, which is critical for their efficacy in food packaging. The use of biopolymers such as chitosan, cellulose, and starch, as well as inorganic nanoparticles like silica, titanium dioxide, and calcium carbonate, as stabilizers for these emulsions, has further improved their applicability in food systems. These biopolymers provide not only structural support but also contribute to the overall biocompatibility and sustainability of the films, making them more suitable for food contact applications. Overall, the integration of essential oil-loaded Pickering emulsions into edible films presents an innovative and environmentally friendly approach to developing active food packaging materials. These films have the potential to reduce food waste, enhance food safety, and meet the growing consumer demand for clean-label products, ultimately advancing the field of food packaging technology.

Challenges and Limitations

While the use of essential oil-loaded Pickering emulsions in edible films shows great promise, several challenges and limitations remain. One of the primary concerns is the sensory impact of essential oils, particularly their strong aroma and flavor, which may not be suitable for all food products. The strong scents of certain essential oils can alter the taste and aroma profile of the food, which may not be desirable for certain types of food products, such as fruits, dairy, or mild-flavored meats. Therefore, selecting appropriate essential oils and optimizing their concentration in the film formulations is crucial to minimize any negative sensory effects. Another challenge is the stability of the emulsions over time, especially during storage and distribution. Although Pickering emulsions are generally more stable than traditional emulsions, issues such as phase separation and changes in droplet size can still occur over time. The stability of these emulsions can be influenced by factors such as temperature, humidity, and the specific nature of the stabilizing agents used. Ensuring the long-term stability of essential oil-loaded emulsions is essential for their effectiveness in real-world applications. The scalability and cost-effectiveness of producing these films on an industrial scale remains another limitation. While laboratory-scale studies have demonstrated the potential of these films, large-scale production requires efficient and cost-effective manufacturing processes. The production of Pickering emulsions involving natural biopolymers and inorganic nanoparticles may incur higher costs compared to traditional plastic-based packaging materials. As a result, further research is needed to optimize production techniques and reduce the cost of raw materials to make these films more economically viable for widespread commercial use. Moreover, regulatory approval for the use of essential oil-loaded films in food packaging remains a critical hurdle. Regulatory agencies such as the FDA and EFSA have strict guidelines regarding the safety and migration of substances from food packaging materials into the food itself. Comprehensive toxicological studies are required to ensure that essential oils and their encapsulating materials do not pose any health risks to consumers. In addition, standardized testing protocols for the performance of active packaging films in different food systems need to be developed to facilitate the approval process.

Future Research Directions

Future research in the field of essential oil-loaded Pickering emulsions for food packaging should focus on several key areas to overcome the existing challenges and expand their applications. One promising avenue for future research is the development of nanoemulsion approaches. Nanoemulsions are similar to Pickering emulsions but involve the use of smaller droplet sizes, typically in the nanometer range, which could offer enhanced stability, increased surface area, and more efficient release of active ingredients. This could improve the performance of essential oil-loaded films in terms of antimicrobial efficacy and antioxidant activity. The use of advanced nanotechnology to tailor the size, distribution, and surface properties of oil droplets could lead to the creation of even more effective and versatile packaging materials. Additionally, smart nanoemulsions, where the release of active compounds is triggered by external stimuli such as temperature, pH, or humidity, could offer enhanced functionality for food preservation. Another exciting direction is the development of smart packaging systems integrated with real-time monitoring sensors. These sensors could provide consumers and food producers with real-time information about the quality and safety of the packaged food. For instance, sensors that detect changes in the pH, gas composition, or temperature within the packaging could signal when the food is nearing the end of its shelf life or when contamination occurs. These sensors could be coupled with the essential oil-loaded Pickering emulsions to create an integrated, multifunctional packaging system that not only preserves the food but also actively monitors and communicates its status. The integration of such sensors into edible films would provide a more proactive approach to food safety, helping to reduce food waste and ensuring that products are consumed at their optimal quality. Furthermore, research should also explore the optimization of essential oil selection and dosage. While essential oils provide significant antimicrobial and antioxidant benefits, their effectiveness can vary depending on the type of food and the particular pathogen or spoilage organism involved. Investigating the synergistic effects of different essential oils and their combinations could lead to more effective formulations for specific food products. Additionally, research in the area of interactions between essential oils and food components such as proteins, lipids, and carbohydrates will be critical for ensuring that the films do not interfere with the sensory qualities or nutritional value of the food.

Author Contributions

H. Mirzaee Moghaddam: Conceptualization, Project administration, Data curation, Visualization, Writing–review & editing. **A. Nahalkar:** Methodology, Data Curation, Writing–Original Draft. **A. Rajaei:** Conceptualization, Supervision, Data curation, Visualization, Writing–review & editing.

Founding Source

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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مقاله مروری
جلد ۲۱، شماره ۳، مرداد- شهریور ۱۴۰۴، ص. ۳۵۷-۳۳۷

فیلم‌های خوراکی زیست‌تخریب‌پذیر حاوی امولسیون‌های پیکرینگ دارای اسانس: مروری بر خواص آنتی‌اکسیدانی و ضدمیکروبی

حسین میرزایی مقدم[1*]- آرین نهالکار[1]- احمد رجایی[1*]

تاریخ دریافت: ۱۴۰۳/۱۲/۲۵
تاریخ پذیرش: ۱۴۰۴/۰۲/۳۱

چکیده

این مقاله به بررسی خواص آنتی‌اکسیدانی و ضدمیکروبی فیلم‌های خوراکی زیست‌تخریب‌پذیر دارای امولسیون‌های پیکرینگ حاوی اسانس می‌پردازد. امروزه، فیلم‌های خوراکی زیست‌تخریب‌پذیر که امولسیون‌های پیکرینگ حاوی اسانس را در خود جای داده‌اند، به‌طور فزاینده‌ای به‌عنوان گزینه‌ای امیدبخش برای بسته‌بندی پایدار مواد غذایی شناخته می‌شوند. افزودن اسانس به ماتریس امولسیون، عملکرد آنتی‌اکسیدانی و ضدمیکروبی این فیلم‌ها را به‌طور قابل‌توجهی افزایش می‌دهد. بر این اساس، ویژگی‌های کلیدی مورد بحث در این مرور شامل فعالیت آنتی‌اکسیدانی، اثربخشی ضدمیکروبی و نقش این فیلم‌ها در افزایش ماندگاری محصولات غذایی است. نتایج نشان داده‌اند که افزودن امولسیون‌های پیکرینگ حاوی اسانس، ظرفیت آنتی‌اکسیدانی فیلم‌ها را به‌طور قابل توجه‌ای افزایش داده و موجب کاهش محسوس در تخریب اکسیداتیو مواد غذایی شده است. علاوه بر این، این فیلم‌ها فعالیت ضدمیکروبی مؤثری در برابر عوامل بیماری‌زای مختلف غذایی مانند اشریشیا کلی و استافیلوکوکوس اورئوس از خود نشان داده‌اند که این عملکرد به خواص زیست‌فعال اسانس‌های افزوده‌شده نسبت داده می‌شود. این فیلم‌ها با مهار مؤثر رشد میکروبی، به بهبود ایمنی مواد غذایی کمک مستقیم می‌کنند. این یافته‌ها، پتانسیل بالای فیلم‌های زیست‌تخریب‌پذیر حاوی امولسیون‌های پیکرینگ را به‌عنوان راهکاری پایدار برای بسته‌بندی مواد غذایی با خواص آنتی‌اکسیدانی و ضدمیکروبی تأکید می‌کنند که به افزایش ماندگاری و ایمنی بیشتر محصولات غذایی بسته‌بندی‌شده منجر می‌شود.

واژه‌های کلیدی: اسانس‌ها، فیلم‌های خوراکی، امولسیون‌های پیکرینگ، فعالیت آنتی‌اکسیدانی، ضدمیکروبی

۱- دانشکده کشاورزی، دانشگاه صنعتی شاهرود، شاهرود، ایران
(*- نویسندگان مسئول: Emails: H_Mirzaee@sharoodut.ac.ir; ahmadrajaee@shahroodut.ac.ir)

https://doi.org/10.22067/ifstrj.2025.92672.1416

بسم الله الرحمن الرحیم

مندرجات

مقالات پژوهشی

مقاله مروری

نشریه پژوهشهای علوم و صنایع غذایی ایران

با شماره پروانه ۱۲۴/۸۴۷ و درجه علمی–پژوهشی شماره ۳/۱۱/۸۱۰ / ۸۸/۵/۱۰ از وزارت علوم، تحقیقات و فناوری

"براساس مصوبه وزارت عتف از سال ۱۳۹۸، کلیه نشریات دارای درجه "علمی–پژوهشی" به نشریه "علمی" تغییر نام یافتند. "

جلد ۲۱ **شماره ۳** **مرداد- شهریور ۱۴۰۴**

ناشر: دانشگاه فردوسی مشهد

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پست الکترونیکی: **ifstrj@um.ac.ir**

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این نشریه به تعداد ۶ شماره در سال و به صورت آنلاین منتشر می‌شود.

نشریه علمی
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