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Incandescent Lamp-assisted Convective Drying of Potato Slices: Optimization Using RSM

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Abstract

This research focuses on the application of response surface methodology (RSM) in the optimization and mathematical modeling of potato slice drying in a laboratory-scale convective dryer assisted by incandescent lamps. The relationships between the independent variables in terms of temperature (°C), incandescent lamp power (W), and slice thickness (mm) were studied in relation to the responses of interest or dependent variables, consisting of drying time (min), overall product acceptance, and effective water diffusivity (m^2s^{-1}). A high value of overall product acceptance is considered to be the optimizing parameter for drying potato sheets. The response surface methodology was applied using a rotational central composite design (RCCD) to optimize the dependent variable. Second-order polynomial regression equations were obtained for each response variable. The optimal drying conditions were established for the maximum value of overall acceptance and were: 69.33 °C, 328.80 W, and 4.40 mm, for temperature, power, and thickness, respectively, with the optimized drying time for the product being approximately 130 min. Drying was carried out during the decreasing drying rate period, and the results show that the addition of energy from incandescent lamps reduces the drying time by 30%. Using the Quasi-Newton Simplex method, the constants of the mathematical models were determined to simulate the drying curve, and the conjugate model of two terms and five constants presented the best fit. Using Fick's law equation, the effective diffusivity of water ranged from 4.48×10^{-10} to $3.38 \times 10^{-9} \text{ m}^2\text{s}^{-1}$, and under optimal drying conditions, it was $2.46 \times 10^{-9} \text{ m}^2\text{s}^{-1}$. The information obtained contributes fundamentally to the development of dryers and the control of drying processes on a commercial and industrial scale.

Keywords: Kinetics, Mathematical modeling, Potato drying, RSM

Introduction

The potato is a tuber that is grown all over the world, the world production of potatoes is approximately 341 million tons and statistically China is the main producer whose production reaches between 66 and 71 million tons, whereas Peru became the first potato producer in Latin America, with a production of 5,7 million tons registered in 2021. The Yungay potato (*Solanum tuberosum* L.) contains interesting nutritional characteristics that make it, according to nutrition specialists, a food of

high nutritional value and is already an alternative for developing countries with food shortages (Midagri, 2023; Flores & León, 2019; FAO, 2023).

High moisture content in foods is a major factor affecting quality, physicochemical properties and nutrients during the postharvest stage and one way to stabilize food and extend its shelf life is to partially remove its water content, thereby reducing the product's water activity (a_w) to a level that prevents the growth



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of microorganisms such as fungi and inactivates enzymes. This is essentially what is achieved in the unit operation of drying used in the food industry (Tahmasebi *et al.*, 2025; Konar, Durmaz, Genc Polat, & Mert, 2022; Kushwah, Kumar, & Gaur, 2023; Madukasi, Onyenanu, Oghenekaro, Nzenwa, & Madu, 2025).

Hot air drying is a widely used method in agroindustry, where it is possible to control drying variables and obtain good quality dry products. This has the additional benefits such as weight and volume reduction that result in a decrease in food transportation and storage costs (García, Muniz, Hernandez, Gonzales, & Fernandez, 2013; Gongora, 2012; Gudiño & Calderon, 2014).

The drying of agro-industrial products requires high energy consumption due to the initial moisture content of the product, so it is necessary to accelerate the drying process due to the significant advantages for both the consumer and the producer. One alternative is to use technological assistance such as microwaves and/or infrared to reduce drying times and improve product quality (Huang, Yang, Tang, Luo, & Sunden, 2021; Bouhile *et al.*, 2025; Ouyang *et al.*, 2025). However, almost no research has been conducted using incandescent lamps.

The product leaving the dryer must be analyzed sensorily to establish its quality as a final product or intermediate manufacturing product. Sensory analysis is an important tool for quality control in food manufacturing processes. One of the tests frequently used to measure the degree of product acceptability is the hedonic scale. The consumer panel (known as consumer analysis) analyzes the respective hedonic attributes of the product: smell, taste, aroma, texture, and overall impression. This test is used to measure the degree of liking or disliking of the product and is acceptable and valid even when untrained tasters and volunteers are used to analyze and define the overall acceptability of the product. (Espinaco, Niizawa, Cuffia, Zorrilla, & Sihufe, 2025; Höhl

& Dreyer, 2025; Tangkham, Vuong, Bui, Noorithi, & Shields, 2025).

In industrial processes, the application of statistical tools for process optimization is common practice. Mathematical modeling is a very important tool that aids in the design, development, optimization, and control of food drying systems, enabling the creation of more sustainable food production systems. Experimental data from agricultural products at various drying temperatures using several well-known mathematical models yield very high R^2 and the lower values of the relative error (Simal, Femenia, Garau, & Rosello, 2005; Midilli, Kucuk, & Yapar, 2002; Vega, Lemus, Tello, Miranda, & Yagnam, 2009; Ravi, Prashant, Nishant, & Om Prakash, 2025; Van der Werf, Cavallera, Delpech, Chapuis, & Courtois, 2023; Chen & Pan, 2023).

Knowledge of optimized drying parameters of biological materials is critical in the design and control of industrial processes. Countless studies have been conducted to investigate their production and utilization of potatoes in general, however, to date, no study has been found and reported on the application of the response surface methodology in hot air drying assisted with incandescent lamps on Yungay potato slices. Therefore, the present study was conducted with the following objectives: 1) To dry Yungay potato slices using the response surface methodology and to investigate the effect of temperature, lamp power and slice thickness on the response variables: drying time, global product acceptance and effective water diffusivity, 2) To determine the optimum parameters of the drying process and 3) to model the drying and calculate the effective diffusivity under the optimized conditions.

Materials and Methods

Raw Materials and Pretreatment

The samples were taken "in situ" in the market of the district of Bellavista Constitutional Province of Callao-Lima-Peru. The Yungay potato with an initial moisture content of 76.97 $\pm$ 0.517 % on a wet basis, was transported to the university city of the National

University of Callao and then placed in a refrigerator and kept at a cold storage temperature of 5°C, the quantity was sufficient for the experimental runs, considering the dimensions and capacity of the dryer tray at the laboratory scale of the Process Engineering and Unit Operations Laboratory, the loads were approximately 140 g of Yungay potato slices on average. The moisture of the potato was determined via oven at 105°C until constant weight according to the AOAC method. The potato samples were washed with running water at room temperature and the damaged samples were separated. The selected samples, once washed, peeled and cut into slices were previously subjected to an immersion treatment in a citric acid solution (0,5% w/w) for 60 seconds to prevent enzymatic browning and then were drained and the remaining surface moisture was removed with soft paper towels. The sample was then placed in a stainless-steel sample holder, the sample holder was previously conditioned so that the slices with an average diameter of 60 mm and thicknesses of 2, 2.81, 4, 5.19 and 6 mm were vertically (Fig. 1) and conveniently separated to create the conditions for "drying on both sides". The initial information was recorded in a spreadsheet (EXCEL) for data collection of the corresponding experimental run.



Fig. 1. Vertical arrangement of potato slices in the sample holder

Hot-air assisted drying system with incandescent lamps

Fig. 2, shows the laboratory-scale dryer used for the experimental runs, designed and built by the researcher of this study, which is installed in the Laboratory of Process Engineering and Unit Operations of the Faculty of Fisheries and Food Engineering of the National University of Callao.

Determination of Global Acceptance

The Global Acceptance Test was conducted with 30 untrained panelists (15 men and 15 women), including students, professors, and administrative staff from the Professional School of Food Engineering at the National University of Callao. A structured 9-point hedonic scale was used, with 9 corresponding to extreme liking and 1 to extreme dislike for the properties of color, aroma, flavor, texture, and overall impression. The organoleptic property evaluations were always carried out at midday and after the experimental drying runs. Panelists were presented with samples labeled with a three-digit code on plastic plates and given an evaluation form to fill out.

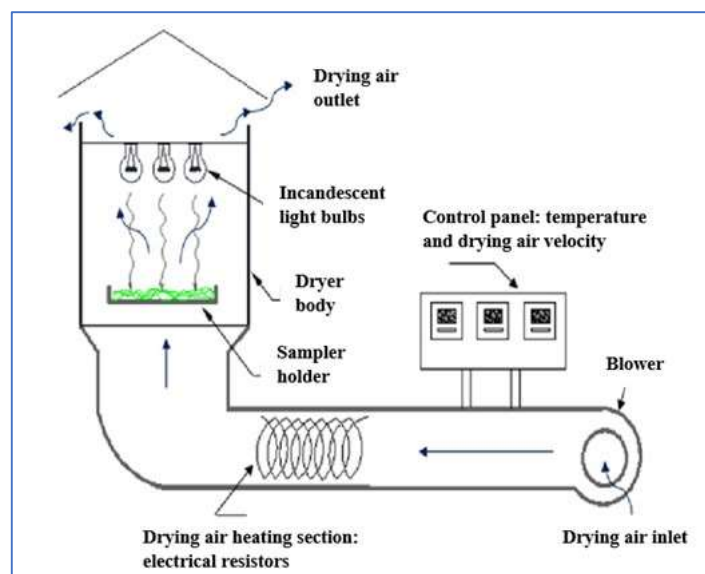


Fig. 2. Schematic of laboratory scale dryer

Response Surface Methodology-Research design

Experimental design (Montgomery, 2004) was used to obtain a quadratic polynomial function mathematical equation, as shown in equation 1, where the response variable of drying time depended on the independent variables and the regression coefficients were calculated using Statistica 7.0 software (Statsoft®) for professionals.

$$y = \beta_0 + \sum_i^k \beta_i x_i + \sum_i^k \beta_{ii} x_i^2 + \sum_{i < j}^k \sum_j \beta_{ij} x_i x_j + \varepsilon \quad (1)$$

Where ε is the error term, β_i are the linear effects, β_{jj} the quadratic effects, and β_{ij} the linear interactions.

In Table 1, 2 and 3, the rotatable central composite design is presented with the coded and actual variables and the values of the response variables of global product acceptance, drying time and effective diffusivity of water, experimental and calculated respectively. The independent variables are: temperature (x_1), power of the

incandescent lamps (x_2) and slice thickness (x_3), which were investigated at five levels each. The complete design consisted of 20 experimental trials, 08 factorial trials (levels -1 and +1), 06 axial (levels -1.68 and +1.68) and 06 center points (level 0), the latter were made for error estimation. They were carried out in duplicate, totaling 40 trials, which were randomized in order to minimize experimental systematic errors.

Effective Diffusivity of Water in Drying under Optimum Conditions

Considering that the solid is a flat plate with both surfaces exposed throughout the drying process, constant diffusivity, negligible volume variation and disregarding the effect of the temperature gradient inside the sample, the following analytical solution is obtained (Crank, 1975; Treybal, 1980):

$$MR = \frac{X - X_e}{X_o - X_e} = \frac{8}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{(2n-1)^2} \exp \left[-(2n-1)^2 \frac{\pi^2 D_{eff} t}{4L^2} \right] \quad (2)$$

Where, is the dimensionless humidity, X , X_o and X_{eq} is the humidity at time t , initial and equilibrium, respectively, D_{eff} is the effective diffusivity, t is the time and L is the sample semi-thickness.

Table 1- Rotatable central composite experimental design with the coded and actual values and the response variable of global product acceptance of dried Yungay potato (*Solanum tuberosum* L.) slices

Test	Coded variables			Real variables			Global acceptance(--)		
	x_1	x_2	x_3	Temperature (°C)	Power (W)	Thickness (mm)	Observed value	Estimated value	Relative deviation (%)
1	-1	-1	-1	48.1	91.7	2.81	6	5.6	6.0
2	1	-1	-1	71.9	91.7	2.81	7	6.3	10.6
3	-1	1	-1	48.1	358.9	2.81	8	7.0	12.3
4	1	1	-1	71.9	358.9	2.81	9	8.6	4.1
5	-1	-1	1	48.1	91.7	5.19	6	6.1	1.4
6	1	-1	1	71.9	91.7	5.19	7	7.7	10.1
7	-1	1	1	48.1	358.9	5.19	6	6.5	7.6
8	1	1	1	71.9	358.9	5.19	9	9.1	0.9
9	-1.68	0	0	40	225	4	5	5.3	6.8
10	+1.68	0	0	80	225	4	8	8.1	0.7
11	0	-1.68	0	60	0	4	6	6.0	0.8
12	0	+1.68	0	60	450	4	8	8.4	4.4
13	0	0	-1.68	60	225	2	6	7.3	22.1
14	0	0	+1.68	60	225	6	9	8.1	10.3
15	0	0	0	60	225	4	9	8.7	3.8
16	0	0	0	60	225	4	8	8.7	8.2
17	0	0	0	60	225	4	9	8.7	3.8
18	0	0	0	60	225	4	9	8.7	3.8
19	0	0	0	60	225	4	8	8.7	8.2
20	0	0	0	60	225	4	9	8.7	3.8

Table 2- Rotatable central composite experimental design with the coded and actual values and the response variable of drying time of Yungay potato (*Solanum tuberosum* L.) slices

Test	Coded variables			Real variables			Drying time (min)		
	x_1	x_2	x_3	Temperature (°C)	Power (W)	Thickness (mm)	Observed value	Estimated value	Relative deviation (%)
1	-1	-1	-1	48.1	91.7	2.81	248	227.7	8.2
2	1	-1	-1	71.9	91.7	2.81	89	92.4	3.8
3	-1	1	-1	48.1	358.9	2.81	245	221.8	9.5
4	1	1	-1	71.9	358.9	2.81	88	70.0	20.4
5	-1	-1	1	48.1	91.7	5.19	350	344.3	1.6
6	1	-1	1	71.9	91.7	5.19	170	169.5	0.3
7	-1	1	1	48.1	358.9	5.19	353	326.0	7.7
8	1	1	1	71.9	358.9	5.19	138	134.6	2.4
9	-1.68	0	0	40	225	4	336	370.0	10.1
10	+1.68	0	0	80	225	4	96	95.6	0.4
11	0	-1.68	0	60	0	4	194	196.4	1.2
12	0	+1.68	0	60	450	4	131	162.2	23.8
13	0	0	-1.68	60	225	2	81	104.2	28.6
14	0	0	+1.68	60	225	6	246	256.4	4.2
15	0	0	0	60	225	4	179	184.0	2.8
16	0	0	0	60	225	4	183	184.0	0.6
17	0	0	0	60	225	4	189	184.0	2.6
18	0	0	0	60	225	4	184	184.0	0.0
19	0	0	0	60	225	4	190	184.0	3.2
20	0	0	0	60	225	4	185	184.0	0.5

Table 3- Rotatable central composite experimental design with the coded and actual values and the response variable of the effective diffusivity of water during drying of Yungay potato (*Solanum tuberosum* L.) slices

Test	Coded variables			Real variables			Effective diffusivity ($10^{10} \text{ m}^2/\text{s}$)		
	x_1	x_2	x_3	Temperature (°C)	Power (W)	Thickness (mm)	Observed value	Estimated value	Relative deviation (%)
1	-1	-1	-1	48.1	91.7	2.81	5.12	5.98	16.8
2	1	-1	-1	71.9	91.7	2.81	13.76	13.68	0.6
3	-1	1	-1	48.1	358.9	2.81	4.48	5.77	28.8
4	1	1	-1	71.9	358.9	2.81	15.68	16.39	4.5
5	-1	-1	1	48.1	91.7	5.19	12.01	11.81	1.6
6	1	-1	1	71.9	91.7	5.19	27.29	26.51	2.8
7	-1	1	1	48.1	358.9	5.19	15.28	15.87	3.9
8	1	1	1	71.9	358.9	5.19	33.84	33.49	1.0
9	-1.68	0	0	40	225	4	7.78	6.51	16.3
10	+1.68	0	0	80	225	4	27.24	27.78	2.0
11	0	-1.68	0	60	0	4	12.32	12.68	2.9
12	0	+1.68	0	60	450	4	19.45	18.36	5.6
13	0	0	-1.68	60	225	2	7.62	6.21	18.5
14	0	0	+1.68	60	225	6	24.80	25.48	2.7
15	0	0	0	60	225	4	14.91	15.88	6.5
16	0	0	0	60	225	4	16.21	15.88	2.1
17	0	0	0	60	225	4	14.27	15.88	11.3
18	0	0	0	60	225	4	16.28	15.88	2.5
19	0	0	0	60	225	4	16.86	15.88	5.8
20	0	0	0	60	225	4	16.60	15.88	4.4

The terms of the infinite series of eq. 2, converge rapidly with increasing drying time and considering only the first term of the series results in small errors even without considering the effect of shrinkage (Celma, Rojas, & Lopez-Rodriguez, 2008), eq. 3, Therefore, linearizing eq. 3, results in eq. 4,

$$MR = \frac{8}{\pi^2} \exp \left[-\frac{\pi^2 \cdot D_{eff} \cdot t}{4 \cdot L^2} \right] \quad (3)$$

$$\ln(MR) = \ln \frac{8}{\pi^2} - \frac{\pi^2 \cdot D_{eff}}{4 \cdot L^2} \cdot t \quad (4)$$

With the experimental data of the drying kinetics at the optimum conditions of the drying process and with the value of the slope of eq. 4, it was possible to estimate the effective diffusivity of water: D_{eff} (m^2s^{-1}).

Mathematical Simulation of the Drying Curve

Ten mathematical models (Table 4) were used to simulate the drying curve of the optimum conditions proposed in the literature sources, such as: Lewis, Diffusional, Henderson-Pabis two-term, Sigmoid model, Page, Page modified I and II, Henderson-Pabis, Midilli *et al.* and Two-term conjugate model

(Uribe *et al.*, 2011; Celma *et al.*, 2008; Erbay & Icier, 2009; Botelho *et al.*, 2011; Sharma, Verma, & Pathare, 2005; Vivanco & Nieto, 2021; Vivanco, 2023).

The adjustment of the constants of the equations used was determined using the SOLVER computer package that estimates the calculation of the nonlinear parameters by the application of the Quasi-Newton Simplex method of the Microsoft Corporation MS-Excel®. The least squares regression technique was applied to study the behavior of the drying kinetics.

Results and Discussion

Experimental Design Result Using the Response Surface Methodology (RSM)

Tables 5, 6, and 7 present the analysis of variance for the sources of variation in the regression equations that generate the response surfaces related to overall acceptance, drying time, and effective diffusivity, respectively. The F values calculated in this relationship are: 4.657, 28.430, and 79.898, respectively. When these values are compared with the F value in Table: F (5%, 9,10) = 3.02; the calculated F

value exceeds the Table F value by ratios of: 1.54, 9.41, and 26.46, respectively. The result indicates that the mathematical regression

models of the response surfaces are statistically valid for predicting the response variables within the ranges established in the research.

Table 4- Mathematical models for the simulation of the drying curve

Model	equation	Bibliographic source
Lewis or Newton model	$MR = e^{(-k \cdot t)}$	Marinos-Kouris & Maurolis (1995)
Diffusional Model	$MR = ae^{(-k_1 \cdot t)} + (1 - a)e^{(-k_2 \cdot t)}$	Verma et al. (1985)
Drying with double kinetics or modified Henderson and Pabis equation with two terms	$MR = ae^{(-k_1 \cdot t)} + be^{(-k_2 \cdot t)}$	Karathanos (1999)
Sigmoid Model	$MR = \frac{a}{1 + e^{\frac{t-b}{c}}}$	Oviedo-Lopera et al (2017)
Page Model	$MR = e^{(-k \cdot t^n)}$	Page (1949)
Modified Page-I	$MR = e^{(-k \cdot t)^n}$	Overhults et al. (1973)
Modified Page-II	$MR = e^{\left[-k \left(\frac{t}{T}\right)^n\right]}$	Diamante & Munro(1993)
Henderson-Pabis model	$MR = a \cdot e^{(-k \cdot t)}$	Henderson-Pabis (1961)
Midilli et al.	$MR = a \cdot e^{(-k \cdot t)} + b \cdot t$	Midilli et al.(2002)
Two-term conjugate model	$MR = a \cdot e^{(-k_1 \cdot t^n)} + b \cdot e^{(-k_2 \cdot t^n)}$	Vivanco (2023)

Table 5- Analysis of variance of global acceptance of dried Yungay potato (*Solanum tuberosum* L.) slices

	Sum of squares	N° degrees of freedom	Quadratic mean	Calculated F-value
Regression	28.10	9	3.122	4.657
Residues	6.70	10	0.670	
Lack of fit	5.371	5	1.074	4.03
Pure erro	1.333	5	0.267	
Total	34.80	19		

Table 6- Analysis of variance of the drying time of dried Yungay potato (*Solanum tuberosum* L.) slices

Source of variance	Sum of squares	N° degrees of freedom	Quadratic mean	Calculated F-value
Regression	125961.00	9	13995.667	28.430
Residues	4922.80	10	492.280	
Lack of fit	4840.80	5	968.160	59.034
Pure erro	82.00	5	16.400	
Total	130883.80	19		

Table 7- Analysis of variance of effective diffusivity of dried Yungay potato (*Solanum tuberosum* L.) slices

Source of variance	Sum of squares	N° degrees of freedom	Quadratic mean	Calculated F-value
Regression	1075.67	9	119.519	79.898
Residues	14.96	10	1.496	
Lack of fit	9.682	5	1.936	1.83
Pure erro	5.277	5	1.055	
Total	1090.63	19		

Tables 1, 2 and 3 show the values of the response variables that were obtained experimentally during the hot air-drying tests assisted with incandescent lamps. Table 8 shows the regression coefficients of the coded models developed in the estimation of the

response variables: global acceptance of the product, drying time and effective diffusivity of water with values of the coefficients of determination of 0,807; 0,962 and 0,986; respectively.

Table 8- Regression coefficients for the response variables of the drying process of Yungay potato (*Solanum tuberosum* L.) slices with hot air assisted by incandescent lamps

Encoded parameter	Regression coefficient		
	Global acceptance (–)	Drying time (min)	Effective diffusivity (m ² /s)
β_0	8,655	184,007	15,877
x_1	0,809	-81,657	6,330
x_2	0,686	-10,175	1,691
x_3	0,223	45,307	5,735
x_1^2	-0,693	17,279	0,450
x_2^2	-0,515	-1,676	-0,126
x_3^2	-0,338	-1,322	-0,011
$x_1 \cdot x_2$	0,250	-4,125	0,730
$x_1 \cdot x_3$	0,250	-9,875	1,750
$x_2 \cdot x_3$	-0,250	-3,125	1,067
R^2	0,8074	0,9624	0,9863
$MQ - \text{pure error}$	0,267	16,400	1,055
$F_{\text{calculated}}/F_{\text{table}}$	1,54	9,41	26,5

Equations 5, 6 and 7 are presented to estimate the values of global product acceptance (AG), drying time (ϑ) and effective water diffusivity (D_{eff}) respectively, are:

AG (5)

$$\begin{aligned}
 &= 8,6548 + 0,8091 \left(\frac{T-60}{11,90} \right) \\
 &- 0,6925 \left(\frac{T-60}{11,90} \right)^2 + 0,6860 \left(\frac{P-225}{133,93} \right) \\
 &- 0,5154 \left(\frac{P-225}{133,93} \right)^2 + 0,2228 \left(\frac{E-4}{1,19} \right) \\
 &- 0,3382 \left(\frac{E-4}{1,19} \right)^2 \\
 &+ 0,2500 \left(\frac{T-60}{11,90} \right) \left(\frac{P-225}{133,93} \right) \\
 &+ 0,2500 \left(\frac{T-60}{11,90} \right) \left(\frac{E-4}{1,19} \right) \\
 &- 0,2500 \left(\frac{P-225}{133,93} \right) \left(\frac{E-4}{1,19} \right) (--)
 \end{aligned}$$

$$\begin{aligned}
 \vartheta &= 184,0069 - 81,6575 \left(\frac{T-60}{11,90} \right) + \quad (6) \\
 &17,2793 \left(\frac{T-60}{11,90} \right)^2 - 10,1753 \left(\frac{P-225}{133,93} \right) - \\
 &1,6762 \left(\frac{P-225}{133,93} \right)^2 + 45,3066 \left(\frac{E-4}{1,19} \right) - \\
 &1,3219 \left(\frac{E-4}{1,19} \right)^2 - 4,1250 \left(\frac{T-60}{11,90} \right) \left(\frac{P-225}{133,93} \right) - \\
 &9,8750 \left(\frac{T-60}{11,90} \right) \left(\frac{E-4}{1,19} \right) - \\
 &3,1250 \left(\frac{P-225}{133,93} \right) \left(\frac{E-4}{1,19} \right) (\text{min})
 \end{aligned}$$

$$\begin{aligned}
 D_{eff} &= \left[15,8766 - 6,330 \left(\frac{T-60}{11,90} \right) \right. \\
 &+ 0,4499 \left(\frac{T-60}{11,90} \right)^2 + 1,6914 \left(\frac{P-225}{133,93} \right) \\
 &- 0,1260 \left(\frac{P-225}{133,93} \right)^2 + 5,7344 \left(\frac{E-4}{1,19} \right) \\
 &- 0,01104 \left(\frac{E-4}{1,19} \right)^2 \\
 &+ 0,730 \left(\frac{T-60}{11,90} \right) \left(\frac{P-225}{133,93} \right) \\
 &+ 1,750 \left(\frac{T-60}{11,90} \right) \left(\frac{E-4}{1,19} \right) \\
 &\left. + 1,0675 \left(\frac{P-225}{133,93} \right) \left(\frac{E-4}{1,19} \right) \right] 10^{-10} (m^2 s^{-1})
 \end{aligned} \quad (7)$$

Results of the Response Variable: Global Product Acceptance

Since the global acceptance of the product is a determining factor in the drying process, the process optimization analysis will be defined with the maximum value reached in the degree of acceptance of the product as the dependent variable in the response surface. It will start by evaluating the effects of the independent

variables: Temperature, Power and Thickness, on the variable: Global Acceptance.

Fig. 3 shows the pareto diagram of the influence of the independent variables on the response variable of global product acceptance. It can be observed that the independent variables of temperature and power of the incandescent lamps in their linear and square form directly influence the global acceptance of the product, but not so much the thickness of the product. Fig. 4 shows a considerable distribution of the experimental values (observed) and the calculated values (estimated) by means of equation 1 of the mathematical model obtained to generate the response surface. An acceptable approximation of the coordinate pair of data to the straight line is visualized, whose coefficient of determination obtained was $R^2 = 0,8074$, being equation (5) that models the behavior of the global acceptance of the potato slices (AG) of the Yungay potato slices of the experimental design achieved.

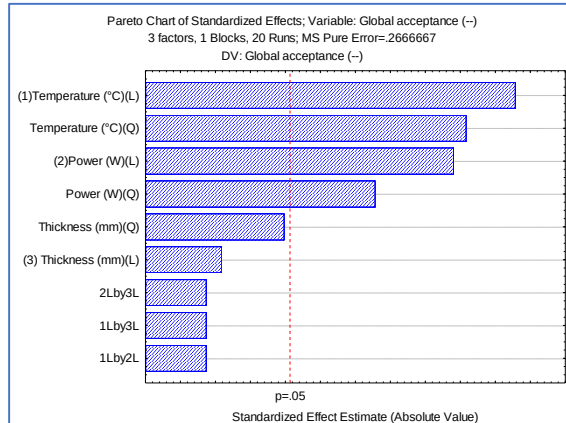


Fig. 3. Pareto diagram of the independent variables on the response variable of global product acceptance of Yungay potato (*Solanum tuberosum* L.) slices

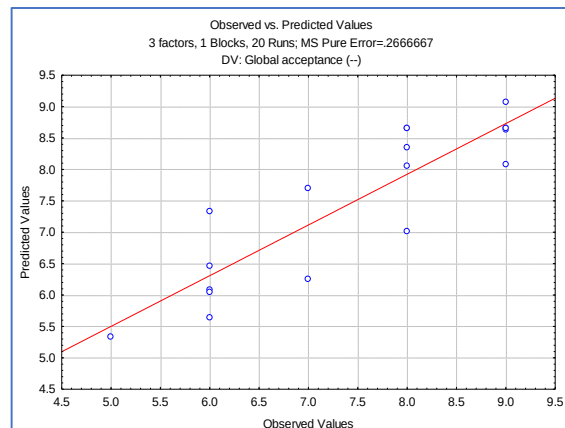


Fig. 4. Observed and calculated values of the global acceptability of Yungay potato (*Solanum tuberosum* L.) slices

In the response and contour surface shown in Fig. 5 (a, b), the maximum critical values correspond to the coded variables of x_1 x_2 x_3 of 0,784; 0,775 and 0,333; which are corresponding to their real variables of:

Temperature= 69,33 °C, Power = 328,8 W and Slice thickness = 4,40 mm respectively. Using the Statistica program, the estimated value of the drying time for these conditions is 130,75min (being the 95% confidence level interval: 125,7-135,8min).

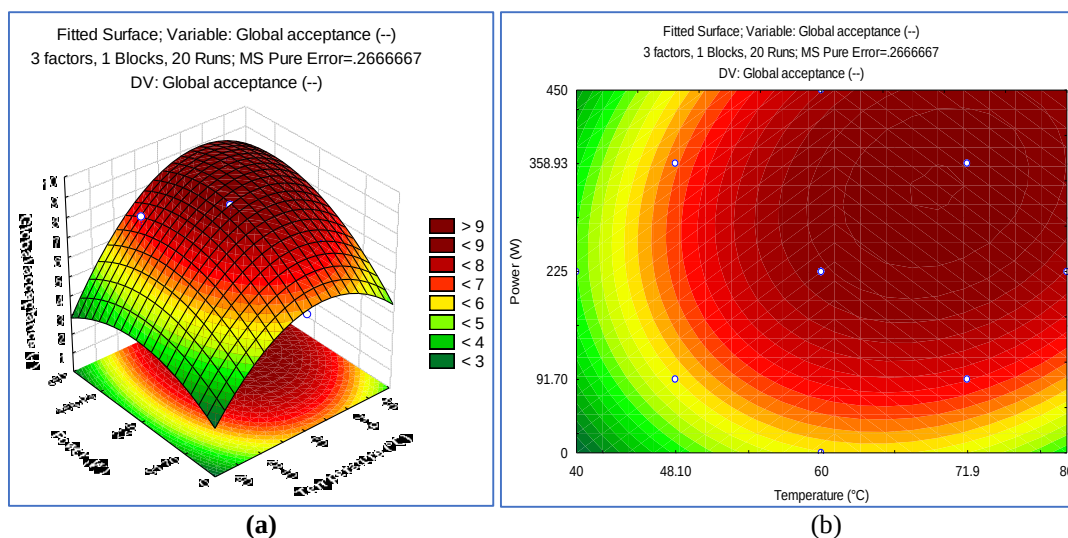


Fig. 5. Response surface (a) and contour surface (b) for the global product acceptance as a function of the power of the incandescent lamps and the air temperature in the drying of Yungay potato slices

Results of the Response Variable: Drying Time

Fig. 6 shows the Pareto plot for the analysis of the effect of the independent variables of the drying air temperature, incandescent lamp power and slice thickness of the Yungay potato on the response variable of drying time (min). It can be observed that the three independent variables have a linear effect on the response variable, as follows: temperature, incandescent lamp power and slice thickness have a significant effect on the drying time of the potato slices.

Likewise, the independent variables in its squared form, temperature, and jointly the

power of the lamps and thickness are in the limit of affectation. Even so, in order that the mathematical regression model does not lose precision, all the variables in linear, conjugate and quadratic form were considered in the equation of the mathematical model. In Fig. 7, the experimental values (observed) and the calculated values (estimated) are distributed in coordinate points very close to the straight line whose coefficient of determination obtained was $R^2 = 0.9624$, being the equation (6) that models the behavior of the drying time (ϑ)(min) of the Yungay potato slices of the experimental plan obtained.

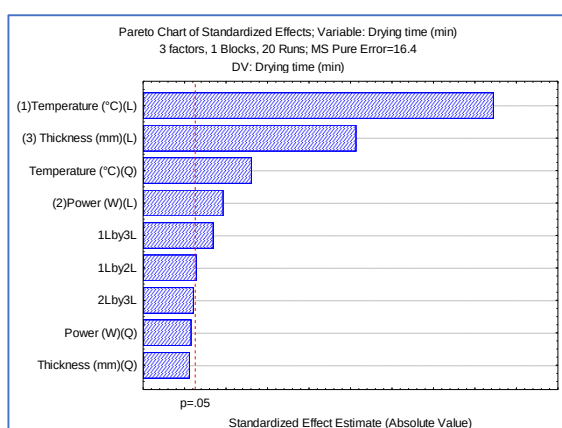


Fig. 6. Pareto diagram of the independent variables on the response variable drying time of Yungay potato (*Solanum tuberosum* L.) slices

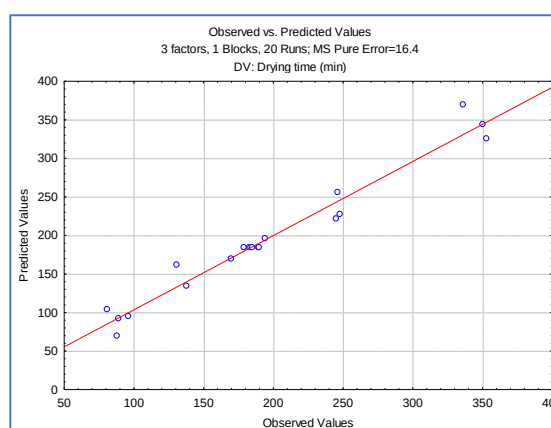


Fig. 7. Observed and calculated values of the drying time of Yungay potato (*Solanum tuberosum* L.) slices

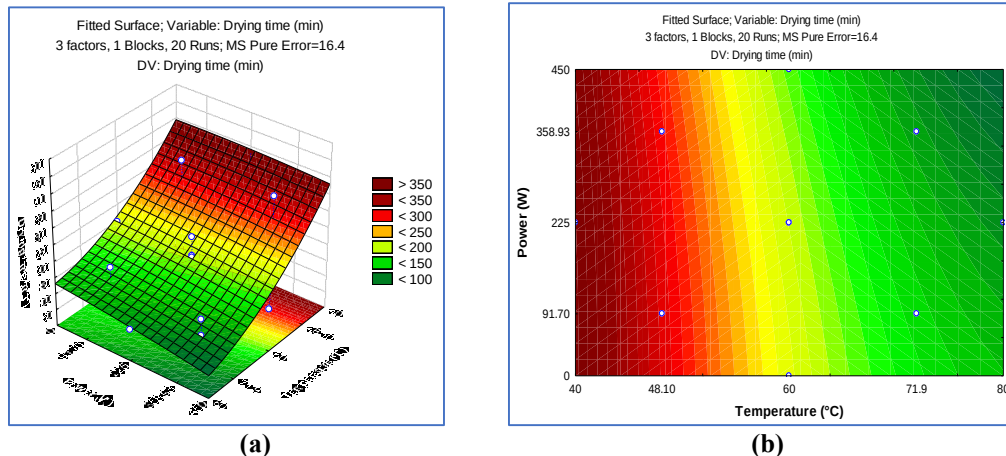


Fig. 8. Response surface (a) and contour (b) of the drying time of Yungay potato (*Solanum tuberosum* L.) slices for the independent variables: drying air temperature and lamp power, maintaining the slice thickness at optimum conditions

Fig. 8 (a and b) shows the response and contour surface of the drying time (min).

For the drying air temperature and power of the lamps for the optimum conditions of 4.40 mm slice thickness (or coded variable of 0,333) to achieve approximately 12% wet basis (13,6% of dry basis), they are reduced to lower values of drying at 150 minutes of drying. The independent variables behave inversely proportional to the drying time, as the values of the independent variables increase the drying times decrease.

Results of the Response Variable: Effective Diffusivity of Water

Fig. 9 shows the Pareto plot for the analysis of the effect of the independent variables of the drying air temperature, power of the incandescent lamps and thickness of the Yungay potato slice on the response variable of the effective diffusivity of water during drying of the Yungay potato slices. It can be observed that the three independent variables linearly affect the response variable, while in the combined-interactive form, temperature with thickness and power with thickness have the greatest effect on the effective diffusivity of water in the drying of Yungay potato slices.

Fig. 10 shows the observed and estimated values of the effective diffusivity of water during the process of drying with hot air assisted by incandescent lamps of Yungay

potato slices according to the experimental design obtained with Equation 7. The pair of values are distributed in coordinate points very close to the straight line whose coefficient of determination obtained was $R^2 = 0,9863$, so a high degree of prediction of this variable has been obtained according to the experimental design.

Fig. 11 (a and b) presents the response and contour surface of the effective diffusivity ($\times 10^{-10} \text{ m}^2 \text{ s}^{-1}$) during the drying process with dry air assisted with incandescent lamps for the proposed experimental design of the drying air temperature and power of the lamps for the optimal conditions of the thickness of the slice cutting of 4,40 mm (or coded variable of 0,333) until the moisture of approximately 12% wet basis is achieved, the values of the effective diffusivity of the drying water show higher values ($> 35 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$) as temperature and power increase, i.e. the relationship is directly proportional.

Drying Curve Result and Effective Diffusivity Value of Water

To characterize the drying curve at optimum conditions ($T = 69,33^\circ \text{C}$, $P = 328,80 \text{ W}$ and $E = 4,40 \text{ mm}$) two experimental runs were carried out with the indicated parameters, whose result or average value represents the experimental drying curve, as shown in Fig. 12 with the mathematical adjustment proposed in

this study, the variation of moisture content on a dry basis as a function of drying time of the Yungay potato slices with hot air assisted by incandescent lamps.

Fig. 13 shows the adjustment of the drying curve obtained with the diffusional equation of

Ficks' second law. From the beginning of drying, it can be observed that the drying rate is decreasing non-linearly, a typical characteristic of this type of agro-industrial product.

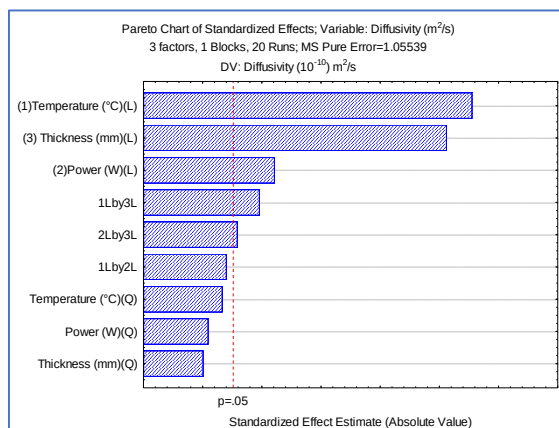


Fig. 9. Pareto diagram of the independent variables on the response variable of the effective diffusivity of water in drying

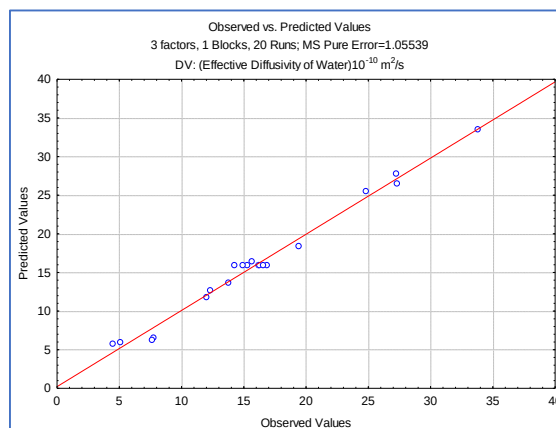
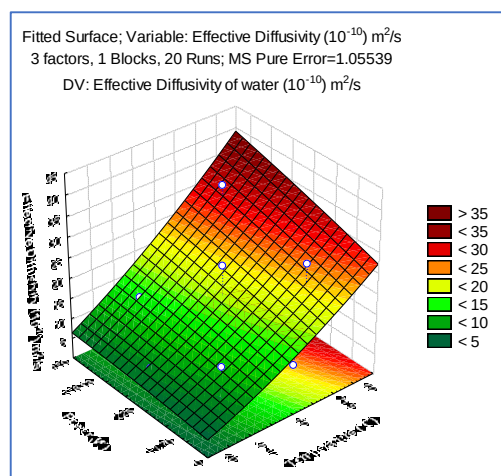
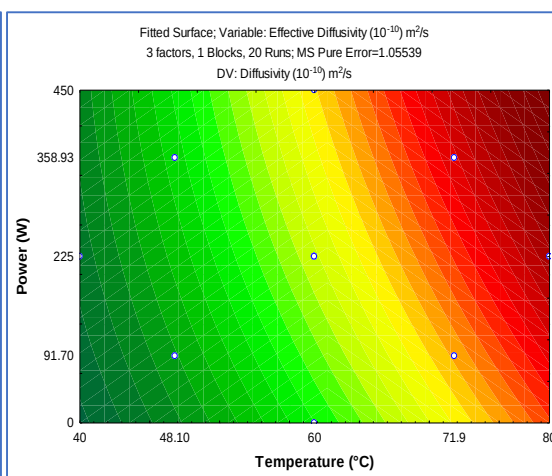


Fig. 10. Observed and calculated values of the effective diffusivity of water in the drying of Yungay potato slices



(a)



(b)

Fig. 11. Response surface (a) and contour (b) of the effective diffusivity of water during the drying process of Yungay potato (*Solanum tuberosum* L.) slices for the independent variables: the drying air temperature and the power of the lamps, maintaining the thickness of the slice in the optimum conditions

From the statistical values obtained (Table 9), it can be affirmed that all the equations are competent in that they present a good fit to the experimental data in the drying of Yungay potato slices, since they are in the order of 0.99.

If we compare the results in the fourth and fifth decimal fraction, we can state that the equation of the Conjugate Model of two terms

and five constants, the sigmoidal model and the modified Page II equation present the best fits due to the high values of the coefficient of determination of 0.99936; 0.99935 and 0.99907 and the lower values of the relative errors calculated as: SSE, RMSE, MPE and χ^2 respectively.

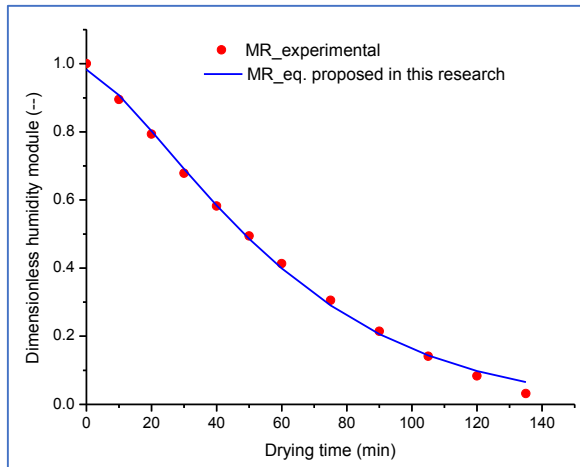


Fig. 12. Drying curve and mathematical model of drying of Yungay potato (*Solanum tuberosum* L.) slices under optimum drying conditions with hot air assisted by incandescent lamps.

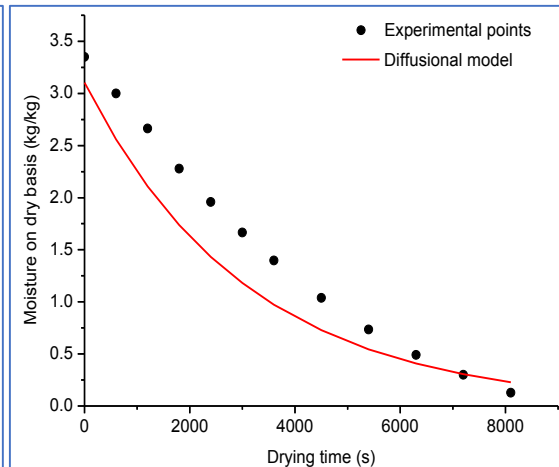


Fig. 13. Drying curve and simulation with the Fick diffusional model for Yungay potato (*Solanum tuberosum* L.) slices under optimum conditions

Using equation 4 and the least squares regression technique, the value of the effective diffusivity of water during the drying of the optimum conditions was determined, as shown in Fig. 12. The coefficient of determination calculated with the diffusional model to the experimental data was $R^2 = 0,9568$. Being the value of the effective diffusivity of $2,46 \cdot 10^{-9} \text{ (m}^2\text{s}^{-1}\text{)}$. The value found in this study is in relation to the values of effective diffusivity reported by different authors for agro-industrial products, as shown in Table 10.

Results of the Additional Heat Load from the Incandescent Lamps during the Drying Period

In Fig. 14, the values and the curve for estimating the drying time of the Yungay potato slices (min) have been simulated. The data were simulated with the regression equation for the values of the optimum conditions: keeping the drying speed of $1,5 \text{ ms}^{-1}$ constant. It is observed that the drying time decreases progressively with the additional radiant energy provided by the incandescent lamps. Fig. 15 shows the moisture distribution on a dry basis of Yungay potato slices in two systems, hot air drying and hot air drying assisted with incandescent lamps,

maintaining a constant drying air temperature of 60°C and a thickness of the slices of 4mm and a drying air speed of $1,50 \text{ ms}^{-1}$, for the conditions, the first without assistance (0W), and the second with assistance (450 W) of power from the incandescent lamps, respectively. It is observed that the drying curve of the Yungay potato slices assisted with incandescent lamps decreases at a faster rate until reaching the final moisture content of 12% wet basis or 0,136 kg/kg in approximately 131 min, while without additional energy assistance the time used was 194 min, that is, using the additional energy assistance in the drying air, for these two cases, the drying times are reduced by approximately 30%.

Bouhile *et al.*, (2025) applied infrared blanching (IRB) on various fruits and vegetables (tomatoes, sweet potatoes, carrots, mangoes, strawberries) found that IRB can reduce drying times by up to 50%, increase drying rates, and reduce energy consumption by approximately 17%, achieving thermal efficiency levels of 80-90%.

Table 9- Results of statistical analysis in modeling the variation of dimensionless moisture modulus and drying time

Mathematical Model	Constantes del modelo	R^2	SSE	$RMSE$	$EMR(\%)$	χ^2
Lewis or Newton's model	k	0,01492	0,99122	0,00259	0,05088	43,20
	a	0,50000				
Diffusional model	k_1	0,01563	0,99365	0,00239	0,04887	37,89
	k_2	0,01563				
Drying with double kinetics or Modified Henderson & Pabis two-term equation	a	0,52280	0,99337	0,00196	0,04428	38,12
	b	0,52280				
	k_1	0,01580				
	k_2	0,01580				
Sigmoidea Model	a	1,46780	0,99935	0,00015	0,01217	10,63
	b	25,8897				
	c	35,1959				
Page's model	k	0,00520	0,99823	0,00033	0,01814	17,32
	n	1,26080				
Page model modified I	k	0,02730	0,99122	0,00259	0,05088	43,20
	n	0,54630				
Page model modified II	k	0,07948	0,99907	0,00025	0,01583	13,02
	n	1,31500				
	l	3,04788				
Henderson-Pabis model	a	1,06052	0,99518	0,00178	0,04218	32,73
	k	0,01671				
Midilli <i>et al.</i> model	a	1,06050	0,99518	0,00178	0,04218	32,73
	b	0,00000				
	k	0,01671				
Conjugated two-term model	a	0,40915	0,99936	0,00022	0,01476	12,01
	b	0,57365				
	k_1	0,00351				
	k_2	0,00350				
	n	1,35549				

Table 10- Some values of the effective diffusivity of water in agro-industrial products

Product	Temperature	Effective Diffusivity (m^2/s)	Reference
Rice husk	30 a 60°C	$8,42 \cdot 10^{-9} - 1,69 \cdot 10^{-8}$	Thakur-Gupta (2006)
Peanut shell powder	50 a 90°C	$9,60 \cdot 10^{-9} - 2,26 \cdot 10^{-8}$	Chen et al. (2012a)
Diced apple 1cm ³	45 a 90°C	$6,7 \cdot 10^{-10} - 2,7 \cdot 10^{-9}$	Lewiski & Korczak (1996)
Blueberries	30 a 70°C	$1,0 \cdot 10^{-10} - 2,0 \cdot 10^{-10}$	Ramaswamy & Nsonzi (1998)
Gracilaria Chilensis Algae	30 a 70°C	$2,76 \cdot 10^{-9} - 22,41 \cdot 10^{-9}$	Vega et al. (2009)
Red algae-Chondracanthus Chamissoi	55°C	$2,03 \cdot 10^{-11}$	Vivanco P. D. (2023)

Results of the Additional Heat Load by Incandescent Lamps on the Value of the Effective Diffusivity of Water

Fig. 16 shows the trend of the values of water diffusivity during the drying of the Yungay potato slices assisted with incandescent lamps. A progressive increase in the values of the effective diffusivity of water during the drying

of the Yungay potato slices is observed as the power values of the incandescent lamps increase. The simulation was made with the regression equation (equation 7) and for values of 0-450W of power, keeping constant the values of the independent variables in the optimum conditions.

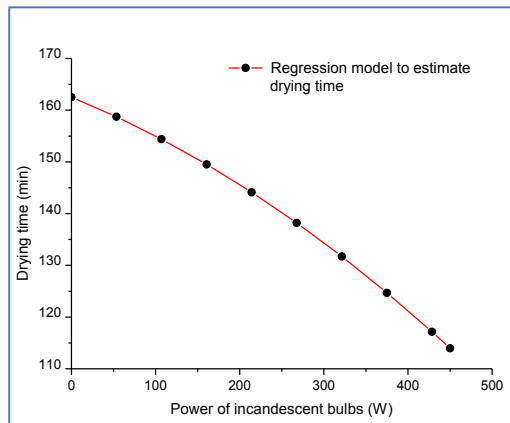


Fig. 14. Graphical simulation of the drying times of the Yungay potato slices for variations in the power of the incandescent lamps

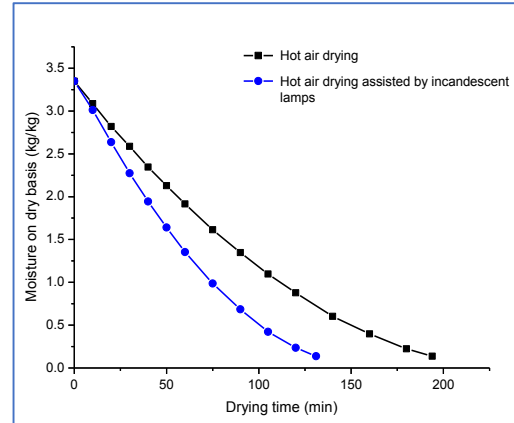


Fig. 15. Drying curves of Yungay potato slices with hot air and assisted with incandescent lamps

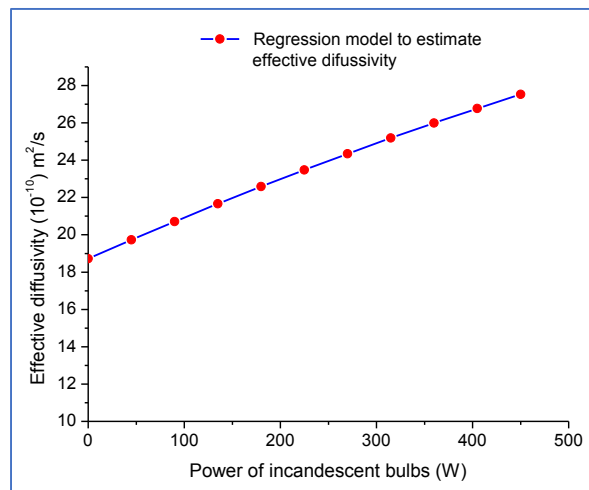


Fig. 16. Graphical simulation of water diffusivity during the drying of Yungay potato slices for variations in the power of the incandescent lamps

For the optimization of the drying process of Yungay potato slices, with hot air assisted by incandescent lamps, using the experimental design of rotatable central composite, a second order polynomial order mathematical equation could be obtained, which allowed estimating the global acceptance of the product, the drying time and the value of water diffusivity. In the optimum drying conditions, the drying times are less than or equal to 135min, the statistical procedures applied indicate that the equations generated by means of the experimental data are reliable due to the high values of the determination coefficients they present, so the regression equations predict the values with

enough precision as observed to obtain the optimized parameters of the drying process.

Conclusion

Through the use of the response surface methodology, in the drying process of Yungay potato slices, the optimized parameters were determined to achieve drying times of less than or approximately 135 minutes, being: Temperature of 64,33 °C, Power of the incandescent lamps of 328,80 W and Thickness of the slices of 4,40 mm, maintaining the drying air speed constant and equal to 1,50 ms^{-1} . The values of the constants of the 10 proposed equations were determined to simulate the drying curve under optimum conditions. It is

confirmed that the mathematical models of Newton, Diffusional, Henderson & Pabis modified, sigmoid model, Page, Page modified I, Page modified II and Henderson-Pabis, Midilli et al., and the equation of conjugate model of two terms and five constants ($R^2 = 0.99936$), have described with quite good quality the behavior of the evolution of the moisture content of the product during the drying time in the optimal conditions and in second place the equation of the sigmoid model ($R^2 = 0.99935$). It was determined that the drying of Yungay potato slices by means of hot air and assisted with incandescent lamps has a period of decreasing rate. The value of the average effective diffusivity of water in the hot air-drying process assisted by incandescent lamps for Yungay potato slices, carried out under optimum conditions, was determined numerically: $2.46 \cdot 10^{-9} \text{ (m}^2\text{s}^{-1}\text{)}$, very similar to those published on the drying of other agro-industrial products. The use of this technological proposal demonstrated that it is possible to obtain dry Yungay potato slices by means of additional energy provided by incandescent lamps, which allows for shorter drying times.

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Author Contributions

- Conceptualization: ideas, formulation of research objectives.
- Data curation: maintenance of data and its interpretation for development and subsequent reuse.
- Formal analysis: application of statistical, mathematical, and computational techniques in the analyses.

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- Funding acquisition: Obtaining financial support from the research fund of the National University of Callao (Resolution No. 469-24R).
- Investigation: Carrying out the research project from which the scientific article is generated.
- Methodology: Development of the methodology and research design.
- Project administration: Responsibility for managing and coordinating the planning and execution of the research activity.
- Resources: Provision of study materials, reagents, equipment and materials, computer resources, and analytical tools.
- Software: Spreadsheet programming for data collection.
- Supervision: Sole responsibility for the supervision, planning, and execution of the research.
- Validation: Verification of the reproducibility of the experimental results.
- Visualization: Preparation of the scientific article for publication.
- Writing – original draft: Drafting of the initial draft of the research work.
- Writing – review and editing: Preparation and presentation of the research work for subsequent publication.

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خشک کردن ورقه‌های سیب‌زمینی با هوای گرم به کمک لامپ رشته‌ای: بهینه‌سازی فرآیند با استفاده از روش

سطح پاسخ

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چکیده

مطالعه حاضر کاربرد روش سطح پاسخ جهت بهینه‌سازی و مدلسازی ریاضی خشک کردن ورقه‌های سیب‌زمینی در یک خشک کن آزمایشگاهی هوای گرم به کمک لامپ‌های رشته‌ای را بیان می‌کند. تأثیر دما، توان لامپ‌های رشته‌ای و ضخامت ورقه به‌عنوان متغیرهای مستقل بر سطح پاسخ متغیرهای وابسته شامل زمان خشک شدن، پذیرش کلی محصول و ضریب نفوذ مؤثر آب مورد بررسی قرار گرفت. ارزش بالای پذیرش کلی محصول به‌عنوان پارامتر بهینه برای خشک کردن ورقه‌های سیب‌زمینی در نظر گرفته شد. روش سطح پاسخ با استفاده از طرح مرکب مرکزی چرخشی برای بهینه‌سازی متغیر وابسته اعمال شد. معادلات رگرسیون چند جمله‌ای درجه دوم برای هر متغیر پاسخ به‌دست آمد. شرایط بهینه خشک کردن جهت دسترسی به بالاترین پذیرش در ارتباط با دما، توان و ضخامت به‌ترتیب برابر ۶۹/۳۳ درجه سانتی‌گراد، ۳۲۸/۸۰ وات و ضخامت ۴/۴۰ میلی‌متر بود. زمان بهینه خشک کردن حدوداً ۱۳۰ دقیقه بود. خشک کردن در مدت زمان کاهش سرعت خشک‌کنندگی صورت گرفت. نتایج نشان داد که افزایش انرژی توسط لامپ‌های رشته‌ای منجر به کاهش ۳۰ درصدی زمان خشک کردن شد. با استفاده از روش شبه نیوتن سیمپلکس، ثابت‌های مدل‌های ریاضی برای شبیه‌سازی منحنی خشک کردن تعیین شدند و مدل مزدوج دو جمله‌ای و پنج ثابت، بهترین برازش را نشان داد. با استفاده از معادله قانون فیک، ضریب نفوذ مؤثر آب از ۱۰-۱۰ تا ۴/۴۸ ۱۰-۹ متر مربع بر ثانیه متغیر بود و در شرایط خشک کردن بهینه ۹-۱۰ ۲/۴۶ متر مربع بر ثانیه بود. اطلاعات به‌دست‌آمده از این پژوهش در توسعه خشک‌کن‌ها و کنترل فرآیندهای خشک کردن در مقیاس تجاری و صنعتی کمک اساسی می‌کند.

واژه‌های کلیدی: خشک کردن سیب‌زمینی، سینتیک، مدل‌سازی ریاضی، RSM

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Research Article

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Quality Evaluation and Sensory Properties of Cookies Prepared from Foxtail Millet (*Setaria italica*) Flour and Orange Peel (*Citrus sinensis*) Powder

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Abstract

The growing demand for functional foods has encouraged with incorporation of natural, nutrient-rich ingredients into traditional products to support health and wellness. Foxtail millet, a gluten-free grain rich in protein, fiber, and micronutrients, and orange peel powder, a by-product abundant in dietary fibre and bioactive compounds, were used to develop cookies with functional properties. This study aimed to develop and evaluate functional cookies prepared from foxtail millet enriched with orange peel powder. Five formulations were produced with 49:1, 48:2, 47:3, 46:4, 45:5 of foxtail millet and orange peel powder for sample 1 to sample 5 while the control sample had 50:0. The samples were analyzed for proximate composition, antioxidant activity, microbial safety, and sensory properties to determine the optimal level of incorporation using standard methods. The results revealed that for proximate composition, protein content ranged from 11.7% to 7.2% for sample 1 to sample 5 while the control sample had 11.9%. Fat content ranged from 20.5% to 19.3% for sample 1 to sample 5 while the control sample had 20.7%. Crude fibre content ranged from 2.8% to 4.8% for sample 1 to sample 5 while the control sample had 2.1%. Ash content ranged from 1.10% to 1.54% for sample 1 to sample 5 while the control sample had 1.04%. Moisture content ranged from 2.61% to 4.25% for sample 1 to sample 5 while the control sample had 2.5%. Nutritional analysis indicated a progressive enhancement in dietary fiber and antioxidant activity, contributing to improved digestive health and free radical scavenging capacity. Sensory evaluation showed the optimal orange peel powder concentration (2%) for sensory overall acceptability. The study demonstrated that incorporating orange peel powder significantly enhances the nutritional and functional attributes of cookies.

Keywords: Antioxidant activity, Foxtail millet, Functional cookies, Orange peel powder



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Introduction

The food industry has introduced a range of new nutritious food products aimed at meeting consumers' needs for both health and convenience. Among these, bakery products especially biscuits have gained notable popularity in the market. Their widespread consumption across various age groups, along with their extended shelf life, makes them a preferred choice for many age-groups (Andaregie *et al.*, 2024; Arepally, Reddy, Coorey, & Goswami, 2023).

In countries like India, policy support and initiatives such as the International Year of Millets 2023 have boosted millet consumption and innovation in processed millet products (Muthamilarasan & Prasad, 2021). Furthermore, Srivastava *et al.* (2020) emphasizing the role of millet-based products in enhancing dietary diversity and addressing micronutrient deficiencies. While challenges remain in terms of consumer awareness and product development, millets show strong potential in reformulating the convenience food sector toward more health-promoting options.

Foxtail millet (*Setaria italica* L.) P. Beauv.) has emerged as a highly significant crop among the various species of millets cultivated across the world. In terms of global production and productivity, it holds the distinction of being the sixth most productive grain crop, making it an important contributor to food security in several regions. This ancient cereal is known for its adaptability and ease of cultivation, thriving even in marginal soils and under low-input agricultural practices. Taxonomically, it is classified within the *Setaria* genus, which falls under the Poaceae family- commonly referred to as the grass family- and is further categorized within the subfamily Panicoideae (Sharma & Niranjana, 2018).

One of the key advantages of foxtail millet is that it is a non-glutinous grain, similar to pseudo cereals like buckwheat and quinoa. Moreover, it does not promote acid formation in the digestive system, making it a gentle and

easily digestible option for individuals with sensitive stomachs or gluten intolerance. From a nutritional standpoint, millets, including foxtail millet, are increasingly recognized as functional food ingredients due to their rich nutrient profile and health-promoting effects. They are especially valued for their relatively high lysine content—a vital amino acid that is often limited in many commonly consumed cereal grains—thus serving as an excellent complementary protein source in cereal-based diets (Singh *et al.*, 2018).

Foxtail millet is known for its rich and diverse composition of health-promoting nutrients, which not only enhance its value as a staple food source but also distinguish it from other cereals due to its well-balanced nutritional profile. This millet contains several essential components that contribute significantly to human health and nutrition. The primary constituents of foxtail millet include complex carbohydrates, particularly starch, as well as a considerable amount of protein, dietary fibre, beneficial fats, a variety of essential vitamins, and important minerals. This unique combination of macro- and micronutrients not only supports its role as a nutritious grain but also contributes to its desirable sensory attributes. The specific nutrient makeup of foxtail millet plays a vital role in defining its taste, aroma, texture, and visual appeal, making it a favourable choice for both traditional and modern dietary applications (Sharma & Niranjana, 2018).

Foxtail millet also includes certain compounds known as anti-nutrients. These anti-nutritional factors can negatively impact the bioavailability and overall absorption of essential nutrients, thereby diminishing the nutritional quality of the grain to some extent. Importantly, the distribution of these anti-nutrients is not uniform throughout the grain, which opens up possibilities for targeted processing interventions. To counteract the adverse effects of these compounds and to enhance the nutritional effectiveness of foxtail millet, a range of traditional and modern food processing techniques can be applied. These

include methods such as milling, cooking, extrusion, germination, and fermentation, all of which have been shown to significantly reduce anti-nutrient levels and improve the availability of micronutrients in the grain (Sharma & Niranjana, 2018).

Orange peel powder, derived from the outer skin of the sweet orange fruit (*Citrus sinensis*), is often considered an underutilized but highly nutritious food component. Rich in dietary fibre, phenolic compounds, flavonoids, β -carotene, vitamins, and antioxidants. Therefore, orange peel powder holds significant potential in functional food applications (Belose, Kotecha, Godase, & Chavan, 2021). Orange peels, which account for nearly 50% of the fruit's mass after juice extraction, are usually discarded as waste in the citrus industry (Zaker, Sawate, Patil, & Sadawarte, 2016). However, research indicates that these peels contain a higher concentration of phenolics and flavonoids than the edible pulp itself (Gorinstein *et al.*, 2001).

Globally, oranges represent a significant horticultural commodity with India contributing about 8608000 tons of the global 122.5 million tons production (Rangnekar, 2024). Despite such high production volumes, orange peels remain significantly underexploited. Sweet orange peels are particularly abundant in bioactive constituents, making them suitable for incorporation into food systems for enhanced nutritional value (Kumar, Tomer, & Kaur, 2018).

In light of the high perishability of orange peels and their rich phytochemical composition, suitable processing techniques are necessary to convert them into stable, functional food ingredients. A pivotal step in orange peel powder preparation involves drying followed by grinding. The drying phase serves to reduce moisture content, thus enhancing shelf life and reducing microbial risks (Sankalpa *et al.*, 2017).

The application of orange peel powder in food products, particularly bakery items like cookies, has received growing attention due to its dual role in enhancing both nutritional and

sensory attributes. Cookies, widely consumed across all age groups, present an ideal medium for nutrient fortification owing to their popularity, convenience, and extended shelf life (Dhankar, 2013).

Studies have demonstrated that incorporating orange peel powder into cookie formulations significantly boosts their dietary fibre, vitamin, and antioxidant levels without adversely affecting taste and texture when used in optimal amounts (Belose *et al.*, 2021). The objective has been to determine the maximum permissible level of inclusion that retains consumer acceptability while offering health benefits. Such enrichment aligns with the rising consumer demand for functional foods that not only satiate but also contribute to disease prevention (Dukwal, 2004).

Furthermore, the fortification of baked products with orange peel powder has broader implications in the Indian food industry, where demand for ready-to-eat snacks is escalating. This development not only satisfies nutritional improvement objectives but also aligns with waste minimization strategies by utilizing agro-industrial by-products (Obafaye & Omoba, 2018). Therefore, this study was carried out to evaluate proximate composition, antioxidant properties, microbial and sensory properties of functional cookies prepared from foxtail millet and orange peel powder.

Materials and Methods

Raw Materials

The major ingredient foxtail millet flour was purchased from Sri Narayanan Herbal shop, Lattice bridge road, Adyar. The Parry's Amrit Brown sugar was purchased from Nilgiris, Kelambakkam. Weikfield Baking powder was procured from Adyar Departmental Stores, Besant Nagar. Amul butter was procured freshly on the day of the product preparation from Pooja's Supermarket, Padur.

Table 1- Formulation of cookie samples

Ingredients	Control (g)	Test Sample 1 (g)	Test Sample 2 (g)	Test Sample 3 (g)	Test Sample 4 (g)	Test Sample 5 (g)
Foxtail millet flour	50	49	48	47	46	45
Orange peel powder	0	1	2	3	4	5
Butter	25	25	25	25	25	25
Sugar	25	25	25	25	25	25
Baking powder	1	1	1	1	1	1

Formulation of cookie samples

Keys:

Sample 1	49:1 Foxtail millet :
Orange peel powder	
Sample 2	48:2 Foxtail millet :
Orange peel powder	
Sample 3	47:3 Foxtail millet :
Orange peel powder	
Sample 4	46:4 Foxtail millet :
Orange peel powder	
Sample 5	45:5 Foxtail millet :
Orange peel powder	
Control Sample	50:0 Foxtail millet :
Orange peel powder	

Preparation of Orange Peel Powder

Oranges were peeled and the peels were washed thoroughly to remove any dirt or debris. The peels were placed in a dehydrator at a low temperature of 57°C for several hours, until they were completely dried. The dried peels were grinded into a fine powder. The powder was sieved through 0.45 mm mesh sieve to ensure very fine powder free of any large pieces. The orange peel powder was stored in an airtight container in a cool, dark place. A dehydrator operating at a low temperature (57°C) was preferred to sun drying because it generally preserves bioactive compounds better than traditional sun drying, which can expose food to high temperatures and UV radiation, potentially degrading these beneficial components. While both methods aim to remove moisture, the controlled and lower temperature of a dehydrator helps retain more of the natural colors, flavors, and

nutritional value, including antioxidants and vitamins.

Preparation of Cookies

The foxtail millet cookies incorporating orange peel powder was prepared in different composition according to the above formulation (Table 1). In a mixing bowl, butter and sugar were weighed and creamed together to a fluffy consistency. Then in a separate bowl, dry ingredients like foxtail millet flour, orange peel powder and baking powder were weighed accurately and mixed together. Now, dry ingredients were transferred to the creamed butter bowl and mixed together to form dough. The dough was then kneaded, rolled and cut with cookie cutter. First, the oven was preheated for 10 minutes at 180 degree Celsius. Then the cookies are baked in the pre-heated oven for 20 to 30 mins at 160° degree Celsius. After baking, the cookies were cooled down at room temperature. Then, they were packaged in Ziploc bags until laboratory analysis (Fig. 1).

Proximate Analysis

Proximate analysis of the cookie samples were determined by the following methods.

Moisture

The moisture content was determined following the procedure outlined in AOAC method, 2005 (Method No. 930.09). Approximately 5 grams of the finely ground sample were accurately weighed into a clean, dry dish. The dish was then subjected for three hours, or until a constant weight was reached,

to a hot air oven set at $105^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Following drying, the sample was weighed after being cooled for 30 minutes in a desiccator. Until the difference between two consecutive weights did not surpass 0.5 mg, indicating consistent weight, the drying, cooling, and weighing procedures were repeated for each sample. The

following formula was used to determine the moisture content, which was then stated as a percentage of the initial sample weight based on the weight loss after drying:

$$\text{Moisture (\%)} = \frac{(\text{Initial weight} - \text{Final weight})}{\text{Initial weight}} \times 100$$

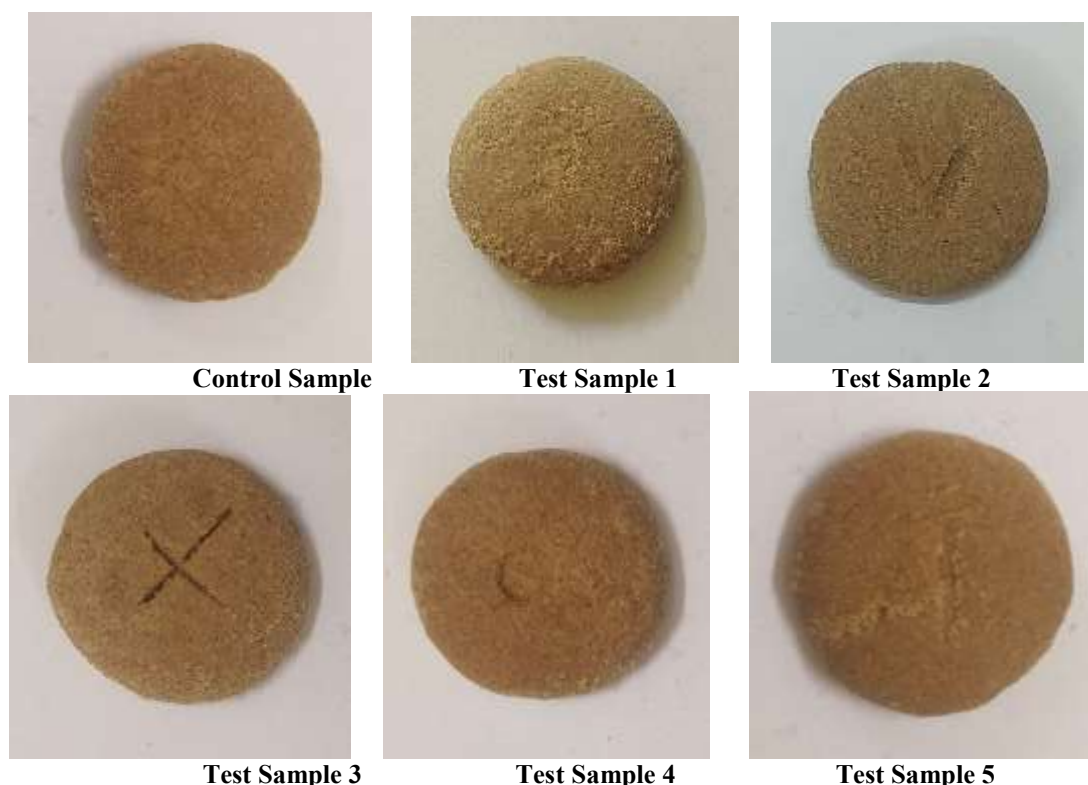


Fig. 1. Foxtail millet cookies incorporated with orange peel powder samples

Protein

The protein content was estimated by the Kjeldahl method as described in [AOAC method, 2005](#) (Method No. 930.09), which determines the total nitrogen content and converts it to protein using a standard conversion factor. Approximately 1 gram of the finely ground sample was weighed accurately and digested with concentrated sulfuric acid in the presence of a catalyst mixture (usually containing potassium sulfate and copper sulfate) to convert organic nitrogen into ammonium sulfate. After complete digestion and the solution becoming clear, it was diluted and made alkaline with sodium hydroxide. The ammonia released was distilled and collected in a known volume of standard

boric acid solution containing a mixed indicator. The trapped ammonia was then titrated with standard hydrochloric acid to determine the amount of nitrogen present. The protein content was calculated by multiplying the nitrogen content by a factor (commonly 6.25 for general food products). The result was expressed as a percentage of the original sample using the following formula:

$$\text{Protein (\%)} = \frac{(\text{Volume of acid} \times \text{Normality} \times 1.4007 \times \text{Conversion factor})}{\text{Weight of sample}}$$

$$\text{Crude Protein (\%)} = \% \text{ Nitrogen} \times 6.25$$

Fat

The total fat content was determined using the Soxhlet extraction method as described in

AOAC method, 2005 (Method No. 930.09). Approximately two grams of the ground and moisture-free sample were weighed accurately and transferred into a thimble. A pre-weighed clean and dry round bottom flask was fixed below the extractor, and the thimble was placed inside the Soxhlet apparatus. Petroleum ether (boiling point 40–60°C) was used as the solvent for fat extraction. The thimble was covered with cotton wool to prevent sample loss. The extraction was carried out continuously for 6–8 hours. After completion, the solvent was evaporated on a water bath, and the flask containing the extracted fat was further dried in a hot air oven at 80°C for 30 minutes to remove any residual solvent. The flask was then cooled in a desiccator and weighed to determine the amount of fat extracted. The total fat content was calculated using the following formula:

$$\text{Fat content (\%)} = \left(\frac{\text{Weight of extracted fat}}{\text{Weight of sample}} \right) \times 100$$

Crude Fiber

The crude fiber content was determined as per AOAC method, 2005 (Method No. 930.09). Two grams of the dried and finely ground sample were weighed and subjected to sequential digestion. The sample was first boiled with 1.25% sulfuric acid for 30 minutes to remove the acid-soluble components. The mixture was then filtered using a muslin cloth, and the residue was washed with hot distilled water until free from acid. This was followed by alkali digestion, where the residue was boiled with 1.25% sodium hydroxide solution for another 30 minutes. After digestion, the residue was filtered again, washed thoroughly with hot water, followed by an alcohol rinse to ensure removal of any remaining alkali. The final residue was then dried in a hot air oven at 105°C to a constant weight. After cooling in a desiccator, the dried residue was transferred to a pre-weighed crucible and ignited in a muffle furnace at 550°C until complete ashing. The crude fiber content was calculated from the loss in weight on ignition and expressed as a

percentage of the original sample weight using the following formula:

$$\text{Crude Fibre (\%)} = \frac{(\text{Weight after drying} - \text{Weight after ashing})}{\text{Weight of sample}} \times 100.$$

Total Ash

The total ash content was determined according to the procedure described in AOAC method, 2005 (Method No. 930.09). About 2 to 5 grams of the dried sample were accurately weighed into a pre-weighed, dry silica crucible. The sample was first charred gently over a low flame until the smoke ceased, preventing any loss of material due to burning. The crucible was then transferred to a muffle furnace and ignited at 550°C ± 25°C for about 4 to 6 hours until a white or light grey ash was obtained, indicating complete oxidation of the organic matter. After ignition, the crucible was carefully removed and cooled in a desiccator to room temperature. It was then weighed to determine the weight of the total ash. The total ash content was calculated using the following formula:

$$\text{Total Ash (\%)} = \left(\frac{\text{Weight of ash}}{\text{Weight of sample}} \right) \times 100$$

Carbohydrate

The carbohydrate content of the sample was determined by the difference method as per AOAC method, 2005 (Method No. 930.09). The moisture, ash, crude fiber, crude protein, and fat contents were first estimated using their respective standard methods. The total carbohydrate percentage was then calculated by subtracting the sum of these components from 100.

Carbohydrate (%) = 100 – (% moisture + % ash + % crude fiber + % crude protein + % fat)

Antioxidant Analysis

DPPH Radical Scavenging Assay The percentage of antioxidant activity (AA %) of each substance was assessed by DPPH free radical scavenging assay (Tailor & Goyal, 2014, Baliyan *et al.*, 2022, Gulcin & Alwasel, 2023). In 1 mL or 3 mL cuvettes, analyses

were carried out using a UV-VIS spectrophotometer. To achieve this, a freshly made stock solution of 10^{-3} M DPPH radicals in ethanol or methanol was made prior to analysis. The DPPH solution was made by diluting 3 mL of the stock solution with 50 mL of methanol in a volumetric flask and covering it with aluminium foil to keep it out of the light. 1.00 ± 0.200 was the specified absorbance value. Next, 3 mL of DPPH working solution was added to the 0.5 mL extract, stirred, and allowed to sit in the dark for half an hour. The presence of an antioxidant agent in the reaction medium causes the purple colour to vanish. A reference sample was made in the same way with 0.5 mL of solvent. At 517 nm, a freshly made DPPH radical solution exhibits maximum absorption. Three repetitions of each analysis were performed, and absorbance at 517 nm was measured. Reaction mixtures without test chemicals are called blanks. Using the following formula, the scavenging activity percentage (AA%) was determined:

% Antioxidant activity = $\{(\text{absorbance at blank}) - (\text{absorbance at test}) / (\text{absorbance at blank})\} \times 100$

Microbial Analysis

Total Plate Count

The total plate count (TPC) was carried out immediately after baking as per [AOAC method, 2005](#) (Method No. 966.23) to determine the number of viable aerobic mesophilic microorganisms in the sample. Ten grams of the sample were aseptically weighed and homogenized in 90 mL of sterile peptone water to prepare a 1:10 dilution. Serial decimal dilutions were then made using sterile diluents. From each selected dilution, 1 mL was transferred aseptically into sterile Petri plates in duplicate, followed by the addition of molten and cooled Plate Count Agar (PCA). The plates were gently swirled to mix and allowed to solidify. The inoculated plates were then incubated at $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 72 ± 3 hours in an upright position. After incubation, colonies were counted manually using a

colony counter, and the results were expressed as colony-forming units per gram (CFU/g) of the sample.

Yeast and Mold Count

The yeast and mold count was carried out as per [AOAC method, 2005](#) (Method No. 966.23) to determine the number of viable fungal organisms in the sample. Ten grams of the sample were aseptically weighed and homogenized in 90 mL of sterile peptone water to make a 1:10 dilution. Serial dilutions were prepared using the same diluent. From the appropriate dilutions, 1 mL was aseptically transferred into sterile Petri plates, followed by pouring of sterile and cooled Potato Dextrose Agar (PDA) acidified with tartaric acid to a pH of around 3.5 to inhibit bacterial growth. The plates were incubated at $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 5 days in an inverted position. After incubation, visible colonies of yeast and mold were counted and recorded separately. Only plates containing 10 to 150 colonies were considered for accuracy, and results were expressed as colony-forming units per gram (CFU/g) of the sample.

Sensory Analysis

The sensory quality of the cookies was conducted with a trained panel of 20 members. The panel members consisted of Faculty Members and Postgraduate Students from the Department of Food Technology from Hindustan Institute of Technology and Science, Padur. Ten grammes of cookie samples were presented to each panellist in a cubicle in randomized order at room temperature. Glass of drinking water was provided as palate cleansers between samples, to ensure unbiased evaluation. Using a 9-point hedonic scale, where 1 stands for "dislike extremely," 2 for "dislike very much," 3 for "dislike moderately," 4 for "dislike slightly," 5 for "neither like nor dislike," 6 for "like slightly," 7 for "like moderately," 8 for "like very much," and 9 for "like extremely," the panellists were asked to rate the cookies'

appearance, color, taste, aroma, mouth feel, texture, and overall acceptability.

Data Analysis

Ten samples were produced from each formulation and analyses were done in triplicates to obtain mean values. One-way analysis of variance was used and Shapiro-Wilk test was used to formally assess the normality of a dataset at $p \geq 0.05$. Data were analysed using SPSS Version 22 for windows (IBM Corporation, New York, USA). Duncan's multiple range tests were used to compare the means at the 5% probability level.

Results

Proximate Analysis of the Cookie Samples

The results of proximate analysis of cookies are presented in [Table 2](#). The moisture content gradually increased from the control (2.5 g/100g) to Sample 5 (4.25 g/100g) as the level of orange peel powder increased. This increase is likely due to the higher water-holding capacity of orange peel powder, which absorbs moisture during the baking process. The moisture in orange peel powder is high due to its high pectin content, which has a strong ability to bind water and form hydrogen bonds. This property allows the powder to absorb and retain significant amounts of water, leading to increased moisture in the surrounding environment or material it's incorporated into. The most notable increase in moisture content occurred between Sample 3 (3.12 g/100g) and Sample 4 (3.74 g/100g), indicating that higher concentrations of orange peel powder further enhance moisture retention. This could affect the texture and shelf-life of the cookies, making them softer and potentially more prone to moisture-related changes during storage ([Gómez et al., 2020](#)). Although, an increase in moisture content may contribute to a softer texture, potentially improving palatability but also increasing susceptibility to microbial spoilage during storage if not properly managed.

The observed decrease in protein content when orange peel powder was added to

the mixture can be attributed to the substitution of protein-rich ingredients with orange peel powder, which has lower protein content ([Natocho, et al., 2024](#), [Abdel Wahab, Abou Elyazeed, & Abdalla, 2018](#)). Specifically, the protein content went from 11.9 g/100g in the control to 7.2 g/100g in Sample 5, coinciding with the increase in orange peel powder. The decrease in protein content is a direct consequence of the dilution effect caused by replacing other ingredients with orange peel powder. Orange peel powder is known to be a good source of fibre and antioxidants, but it is not a primary source of protein. Therefore, as the proportion of orange peel powder increases, the overall protein content of the mixture naturally decreases ([Natocho, Mugabi, & Muyonga, 2024](#), [Abdel Wahab et al., 2018](#)). The most significant drop occurred between Sample 2 (10.2 g/100g) and Sample 3 (8.4 g/100g), indicating that as the inclusion of orange peel powder increased, acidity of the orange peel powder also increased. The addition of orange peel powder to foxtail millet cookies can slightly reduce the protein content due to acidity-induced denaturation. Acidity, introduced by the orange peel powder, can cause proteins to unfold and lose their functional structure. While the protein content may be slightly lower than in cookies without orange peel powder, the cookies still maintain a moderate protein level. This makes them a suitable option for those looking for a protein-rich snack or part of a balanced diet ([Sharma, Mahato, & Lee, 2017](#)).

The fat content showed a slight decrease from the control (20.7 g/100g) to Sample 5 (19.3 g/100g) with increasing levels of orange peel powder. This reduction in fat may be due to the replacement of fat-containing ingredients with orange peel powder, which contains negligible fat. The decrease was gradual, with the smallest drop observed between Sample 1 (20.5 g/100g) and Sample 2 (20.2 g/100g). The reduction in fat is minimal but suggests that incorporating orange peel powder can slightly lower the overall fat

content of the cookies while maintaining a relatively balanced nutritional profile.

The fat content exhibited a mild reduction from 20.7 g/100g in the control to 19.3 g/100g in Sample 5. This is attributable to the partial replacement of higher-fat ingredients with orange peel powder, which contains negligible fat. Such modifications are beneficial for consumers seeking lower-fat options, especially when integrated into functional food development without compromising too much on sensory attributes (Adams & Foster, 2021).

The crude fiber content increased progressively from the control (2.1 g/100g) to Sample 5 (4.89 g/100g) as the percentage of orange peel powder increased. This rise is expected, as orange peel powder is a rich source of dietary fiber, particularly soluble and insoluble fibers. The gradual increase in fiber content reflects the substitution of other ingredients with orange peel powder, which enhanced the fiber content in the cookies. The most significant rise occurred between Sample 4 (4.3 g/100g) and Sample 5 (4.89 g/100g), indicating that higher levels of orange peel powder have a strong impact on the fiber content. This increase in fiber makes the cookies more functional, promoting digestive health and contributing to their nutritional value.

Crude fiber content rose significantly from 2.1 g/100g in the control to 4.89 g/100g in Sample 5, reflecting the high fiber density of orange peel powder. This increment enhances the functional value of the cookies, contributing to improved digestive health and increased satiety. The rich presence of soluble and insoluble fiber in citrus peels supports these outcomes and has been previously linked to improved gastrointestinal function and potential prebiotic effects (Gómez *et al.*, 2020).

The crude ash content showed a gradual increase from the control (1.04 g/100g) to Sample 5 (1.54 g/100g) as the level of orange peel powder increased. Ash content reflects the mineral content in the samples, and the increase is likely due to the higher mineral

content in orange peel powder compared to the other ingredients. As the concentration of orange peel powder rises, more minerals such as calcium, potassium, and magnesium are incorporated into the cookies, leading to a higher ash content. The most significant increase in ash content occurred between Sample 1 (1.1 g/100g) and Sample 2 (1.4 g/100g), suggesting that the early levels of orange peel powder contribute notably to the mineral composition of the product. This indicates that higher levels of orange peel powder can enhance the mineral profile of the cookies.

Ash content, which serves as an indicator of mineral content, also increased with rising levels of orange peel powder, moving from 1.04 g/100g in the control to 1.54 g/100g in Sample 5. This is consistent with existing literature that notes the mineral richness of orange peel, including potassium, calcium, and magnesium, thereby enhancing the micronutrient profile of the cookies (Gorinstein *et al.*, 2021).

The carbohydrate content increased slightly from the control (61.9 g/100g) to Sample 5 (64.0 g/100g) as the level of orange peel powder increased. This gradual rise in carbohydrates is likely due to the additional carbohydrate content provided by the orange peel powder itself, which contains soluble and insoluble fibres that contribute to the total carbohydrate count. Although the orange peel powder is rich in fiber, it still adds some carbohydrates, which may explain the slight increase in the samples with higher concentrations of orange peel powder. The increase in carbohydrates is not significant but suggests that the inclusion of orange peel powder can contribute to the overall carbohydrate content while also enriching the product with beneficial dietary fiber.

Carbohydrate content showed a slight rise from 61.9 g/100g to 64.0 g/100g across the samples, which may be explained by the carbohydrate component of orange peel itself, including free sugars and complex polysaccharides (Kha, Nguyen, & Roach,

2014). Although modest, this increase complements the rising fiber content and contributes to the energy yield of the product.

The energy content decreased gradually from the control (481.9 kcal/100g) to Sample 5 (458.5 kcal/100g) with increasing levels of orange peel powder. This decline is attributed to the substitution of higher-calorie ingredients with orange peel powder, which is naturally lower in energy and rich in dietary fiber. As the fiber content rises, the proportion of digestible nutrients decreases, leading to a reduction in total energy value. A noticeable drop in energy was observed particularly between Sample 3 and Sample 4, indicating a

stronger effect at higher inclusion levels. These findings suggest that orange peel powder can be effectively used to develop lower-calorie, fiber-enriched functional cookies.

Energy values declined from 481.9 kcal/100g in the control to 458.5 kcal/100g in Sample 5, likely due to the lower caloric density of orange peel powder compared to conventional baking ingredients. This reduction positions the cookies as a viable option for calorie-conscious consumers, without significantly compromising other macronutrients (Boukroufa, Boutekedjiret, Petigny, Rakotomanomana, & Chemat, 2015).

Table 2- Proximate analysis of the cookies

Parameter	Control	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Moisture (g/100g)	2.5±0.1 ^a	2.6±0.1 ^a	2.9±0.1 ^a	3.1±0.1 ^a	3.7±0.1 ^a	4.2±0.1 ^a
Protein (g/100g)	11.9±0.2 ^a	11.7±0.2 ^a	10.2±0.2 ^a	8.4±0.2 ^b	8.4±0.2 ^b	7.2±0.2 ^b
Fat (g/100g)	20.7±0.2 ^a	20.5±0.2 ^a	20.2±0.2 ^a	19.7±0.2 ^b	19.4±0.2 ^b	19.3±0.2 ^b
Fibre (g/100g)	2.1±0.1 ^a	2.8±0.1 ^a	3.1±0.1 ^a	3.9±0.1 ^b	4.3±0.1 ^b	4.9±0.1 ^b
Ash (g/100g)	1.0±0.1 ^a	1.1±0.1 ^a	1.4±0.1 ^a	1.5±0.1 ^a	1.5±0.1 ^a	1.5±0.1 ^a
Carbohydrates (g/100g)	61.9±0.2 ^a	62.3±0.2 ^a	62.7±0.2 ^a	63.4±0.2 ^a	63.8±0.2 ^a	64.0±0.2 ^a
Energy (kcal/100g)	481.0±0.5 ^a	480.5±0.5 ^a	478.4±0.5 ^a	476.2±0.5 ^a	463.4±0.5 ^a	458.5±0.5 ^a

In order to determine mean values, ten samples were produced from each formulation, and analyses were conducted in duplicate. Duncan's multiple range tests was used to separate means, and significance was accepted at a 5% level of confidence ($p < 0.05$). * The column's mean $\pm$ standard deviation, which is indicated by the same superscript, does not differ significantly at $p \leq 0.05$.

Keys:

Sample 1	49:1 Foxtail millet:
Orange peel powder	
Sample 2	48:2 Foxtail millet:
Orange peel powder	
Sample 3	47:3 Foxtail millet:
Orange peel powder	

Sample 4	46:4 Foxtail millet:
Orange peel powder	
Sample 5	45:5 Foxtail millet:
Orange peel powder	
Control Sample	50:0 Foxtail millet:
Orange peel powder	

Antioxidant Analysis

The antioxidant activity increased significantly from the control (3% inhibition) to Sample 5 (34.8% inhibition) with rising levels of orange peel powder (Fig. 2). This value directly quantifies the antioxidant activity by measuring the decrease in DPPH absorbance after the addition of the antioxidant. A higher percentage inhibition indicates a stronger antioxidant capacity, as

more DPPH radicals have been neutralized (Zhou *et al.*, 2022, Hofmann *et al.*, 2024). Absorbance values at 517 nm are used to calculate the percentage inhibition. The DPPH assay works by measuring the decrease in absorbance of a DPPH solution (typically purple) when it reacts with an antioxidant. The reaction produces a colourless or yellowish compound (DPPH-H), and the extent of this discoloration (indicated by a decrease in absorbance) is directly related to the antioxidant's activity (Zhou *et al.*, 2022, Hofmann *et al.*, 2024).

While the percentage inhibition is the key metric, absorbance values can provide additional information, especially when considering the reaction mechanism or if there are unusual results (Zhou *et al.*, 2022, Hofmann *et al.*, 2024). For example: If the absorbance does not reach zero even at high concentrations of the antioxidant, it might indicate that not all DPPH is being scavenged or that the reaction products also absorb at 517 nm. Tracking absorbance over time can provide insights into the speed of the reaction. If other compounds in the sample absorb at or near 517 nm, they can interfere with the assay,

and tracking absorbance can help identify and potentially account for this interference (Zhou *et al.*, 2022, Hofmann *et al.*, 2024).

This sharp increase is attributed to the high concentration of bioactive compounds in orange peel powder, such as flavonoids, polyphenols, and vitamin C, all of which exhibit strong antioxidant properties. As more orange peel powder was incorporated, these compounds contributed more effectively to scavenging free radicals. The most notable jump occurred between Sample 3 (19.6%) and Sample 4 (25.2%), indicating a dose-dependent enhancement. These results highlight the functional benefit of using orange peel powder in cookie formulations, making them not just nutritious but also potentially health-protective. This significant rise underscores the functional potential of orange peel powder, attributed to its rich content of phenolics, flavonoids, and vitamin C, which are known for their free radical scavenging properties (Gulcin & Alwasel, 2023, Baliyan *et al.*, 2022, M'hiri, Ioannou, Ghoul, & Boudhrioua, 2017). This antioxidant enrichment supports the development of health-promoting bakery products.

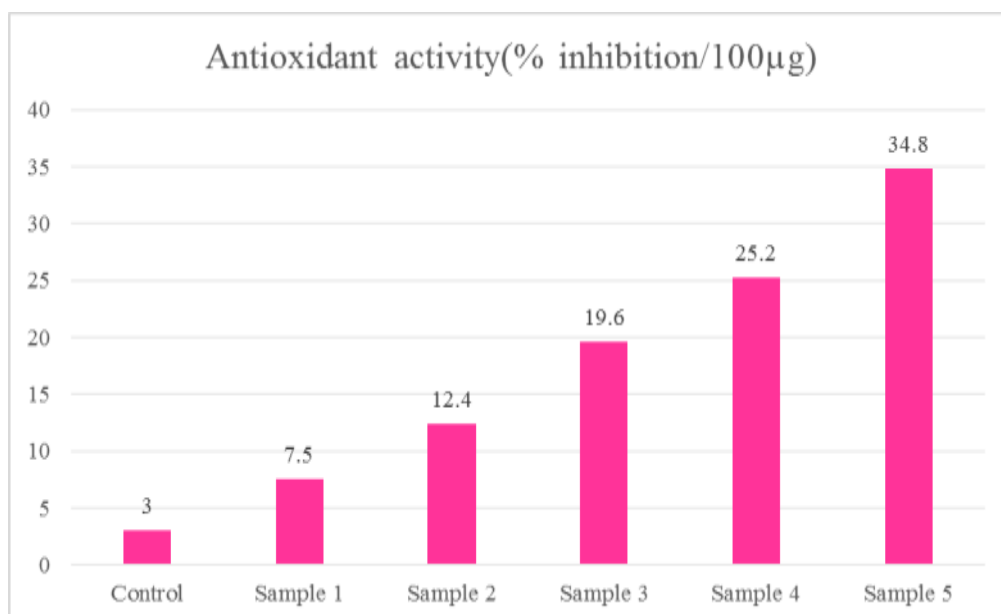


Fig. 2. Antioxidant activity (% inhibition) of cookie samples

Microbial Analysis

The total plate count increased gradually from the control (326 CFU/g) to Sample 5 (458 CFU/g) with the rising levels of orange peel powder (Table 3). This increase could be due to the natural microbial load present in dried orange peel powder, which, despite drying, may still retain some microbial spores. Additionally, the higher moisture content observed in samples with more orange peel powder can create a more favorable environment for microbial growth. However, the microbial counts remained within acceptable safety limits for baked products, suggesting no spoilage risk. Proper drying and hygienic handling of orange peel powder during processing are essential to minimize microbial load in future applications.

The yeast and mold count was consistently low across all samples, including the control. All samples, from the control (CS) to the highest concentration of 5% orange peel powder (S5), recorded a Yeast and Mold count

of less than 10 CFU/g. These results indicate that there was minimal fungal contamination in all the cookies, demonstrating that orange peel powder does not promote yeast and mould growth. The consistently low Yeast and Mold counts suggest the cookies possess good stability and are less prone to fungal contamination, which is critical for ensuring the quality and safety of the product throughout its shelf life.

Microbial analysis revealed a gradual increase in the total plate count from 350 CFU/g in the control to 500 CFU/g in Sample 5. The increase is plausibly due to residual microbial spores in dried orange peel, although the levels remained within safe limits for baked goods (IS (Indian Standard) 5402:2021). Conversely, yeast and mold counts remained below 10 CFU/g across all samples, indicating effective control of fungal contamination and affirming the hygienic preparation conditions and good manufacturing practices (GMP)

Table 3- Microbial profile of cookies

Parameter	Control	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Total Plate Count (CFU/g)	350	400	450	470	490	500
Yeast and Mould (CFU/g)	<10	<10	<10	<10	<10	<10

In order to determine mean values, ten samples were produced from each formulation, and analyses were conducted in duplicate. Duncan's multiple range tests was used to separate means, and significance was accepted at a 5% level of confidence ($p < 0.05$).

Keys:

Sample 1 49:1 Foxtail millet :
Orange peel powder
Sample 2 48:2 Foxtail millet :
Orange peel powder
Sample 3 47:3 Foxtail millet :
Orange peel powder
Sample 4 46:4 Foxtail millet :
Orange peel powder
Sample 5 45:5 Foxtail millet :
Orange peel powder

Control Sample 50:0 Foxtail millet :
Orange peel powder

Sensory Analysis

The average of the hedonic score given to the cookies by the sensory panel members are represented in Table 4 to show comparative results and the mean average values. According to the results of the sensory evaluation, there was significant differences at $p \leq 0.05$ between samples and the sample's flavor, consistency, and general acceptability vary significantly depending on the amount of supplementation.

The cookies made with orange peel powder maintained an attractive look, likely due to the balanced color and consistent surface texture. From Sample 2 score (8.3) to Sample 5 score (7.7), a gradual decline in appearance scores

was noted. This suggests that increasing the OPP concentration subtly altered the external features of the cookies. Orange peel powder, being high in fiber and naturally coarse in texture, may have affected the smoothness and uniformity of the dough surface during baking. Despite these changes, the appearance remained moderately acceptable even at 5% orange peel powder, suggesting that while higher levels of orange peel powder do influence the visual presentation, they do not render the product visually unappealing.

The control and Sample 1 scored 9.0 and 8.9, reflecting good color attributes and high visual appeal. From Sample 2 (8.7) to Sample 5 (8.0), the color scores showed a gradual downward trend, suggesting that higher concentrations of orange peel powder slightly affected the visual appearance of the cookies. This is likely due to the natural pigmentation of orange peel powder, which contains carotenoids and flavonoids that can deepen the color of baked products. At lower levels, these pigments may enhance visual appeal by imparting a warm, golden hue. Despite the decline, the scores remain relatively high, indicating that the visual changes introduced by orange peel powder were not drastically unfavorable but may have slightly influenced consumer perception as the intensity of color increased.

The samples with less concentration of orange peel powder, reflects high taste acceptability among the panellists. This indicates that the base formulation using foxtail millet flour, with no or minimal incorporation of orange peel powder (1%), maintained a pleasing flavor profile. From Sample 2 (8.0) to Sample 5 (6.8), a gradual decline in taste scores was observed. This downward trend suggests that increasing levels of orange peel powder introduced stronger citrus notes and possibly a mild bitterness, characteristic of citrus peel. While orange peel powder contributes beneficial bioactive compounds, at higher concentrations, its phenolic content and essential oils (like limonene) can impart astringency or

overpowering flavors (Adams & Foster, 2021, Zhou *et al.*, 2022, Hofmann *et al.*, 2024). These sensory shifts likely contributed to reduced taste acceptability in higher orange peel powder samples.

The initial samples with less concentration of orange peel powder preserved a pleasant and appealing aroma (Adams & Foster, 2021, Zhou *et al.*, 2022, Hofmann *et al.*, 2024). This suggests that a small quantity of orange peel powder may complement the natural aroma of foxtail millet and other cookie ingredients, potentially enhancing the citrusy fragrance without overpowering it. From Sample 2 (8.1) to Sample 5 (7.0), a noticeable decline in aroma scores was observed. These trend likely results from the increasing intensity of the volatile compounds present in orange peel powder, such as limonene, linalool, and other essential oils (Adams & Foster, 2021, Zhou *et al.*, 2022, Hofmann *et al.*, 2024). While these components are aromatic and beneficial at lower concentrations, their strong citrus scent can become overwhelming or less pleasant as the level increases, especially when combined with the earthy aroma of foxtail millet. Moreover, during baking, the heat may alter the aromatic compounds in orange peel powder, sometimes resulting in slightly bitter or acidic or rancid notes that can affect the overall aroma perception (Adams & Foster, 2021, Zhou *et al.*, 2022, Hofmann *et al.*, 2024).

The initial samples received the highest mouth feel scores, indicating a soft and pleasant texture. Foxtail millet flour likely contributed to the light and crisp bite in these samples. As orange peel powder levels increased, mouth feel scores declined from 7.8 in Sample 2 to 7.1 in Sample 5. This drop may be due to the high fiber content and coarse texture of orange peel powder. At higher levels, orange peel powder can make the cookies feel drier or grainier. The absorption of moisture by fiber may also have led to a firmer, less smooth bite (Adams & Foster, 2021, Zhou *et al.*, 2022, Hofmann *et al.*, 2024).

The control (9.0) and Sample 1 (8.8) had the highest texture scores, indicating a desirable crispness and consistency. The use of foxtail millet flour likely helped achieve a light, crumbly texture. From Sample 2 (8.3) to Sample 5 (7.6), a gradual decline in scores was observed, with a slight recovery in Sample 5. The increased addition of orange peel powder, rich in fiber, may have made the cookies denser or slightly coarse. This could lead to less uniform texture, affecting the bite and chewiness (Adams & Foster, 2021, Zhou *et al.*, 2022, Hofmann *et al.*, 2024). However, the minor rise in Sample 5 suggests the fiber may have contributed some structural benefit at that level.

The initial samples with the minimal concentration of orange peel powder did not affect overall acceptability. From Sample 2 (7.95) to Sample 5 (7.0), a steady decline in acceptability was observed. This reflects the combined effect of changes in taste, aroma,

texture, and mouth feel with increasing orange peel powder. Higher levels of orange peel powder likely introduced bitterness, coarseness, and stronger aroma, which reduced panel preference. Lower levels of orange peel powder (1–2%) appeared optimal for maintaining product quality and consumer appeal.

Sensory evaluation revealed a general decline in scores for parameters such as taste, aroma, texture, and overall acceptability as the level of orange peel powder increased. While initial levels of inclusion maintained favorable sensory properties, higher concentrations introduced bitterness, coarseness, and intensified citrus notes that were less preferred by the panelists. Despite this, even the highest inclusion level maintained moderate acceptability, indicating that formulation optimization could balance functional benefits with sensory quality (Adams & Foster, 2021, Zhou *et al.*, 2022, Hofmann *et al.*, 2024).

Table 4- Sensory analysis of cookie samples

Parameters	Control	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Appearance	9.0±0.2 ^a	9.0±0.2 ^a	8.3±0.2 ^b	8.2±0.2 ^b	8.2±0.2 ^b	7.7±0.2 ^c
Colour	9.0±0.2 ^a	8.9±0.2 ^a	8.7±0.2 ^a	8.3±0.2 ^b	8.3±0.2 ^b	7.5±0.2 ^c
Taste	9.0±0.2 ^a	8.7±0.2 ^a	8.0±0.2 ^b	7.6±0.2 ^c	7.6±0.2 ^c	6.8±0.2 ^d
Aroma	9.0±0.2 ^a	9.0±0.2 ^a	8.1±0.2 ^b	7.7±0.2 ^c	7.7±0.2 ^c	7.0±0.2 ^c
Mouth feel	9.0±0.2 ^a	8.7±0.2 ^a	7.8±0.2 ^b	7.5±0.2 ^c	7.4±0.2 ^c	7.1±0.2 ^c
Texture	9.0±0.2 ^a	8.8±0.2 ^a	8.3±0.2 ^b	8.2±0.2 ^b	7.4±0.2 ^c	7.6±0.2 ^c
Overall acceptability	9.0±0.2 ^a	8.7±0.2 ^a	8.0±0.2 ^b	7.9±0.2 ^b	7.8±0.2 ^b	7.0±0.2 ^c

In order to determine mean values, ten samples were produced from each formulation, and analyses were conducted in duplicate. Duncan's multiple range tests was used to separate means, and significance was accepted at a 5% level of confidence ($p < 0.05$). * The column's mean $\pm$ standard deviation, which is indicated by the same superscript, does not differ significantly at $p \leq 0.05$.

Keys:

Sample 1 49:1 Foxtail millet :
Orange peel powder
Sample 2 48:2 Foxtail millet :
Orange peel powder

Sample 3 47:3 Foxtail millet :
Orange peel powder
Sample 4 46:4 Foxtail millet :
Orange peel powder
Sample 5 45:5 Foxtail millet :
Orange peel powder
Control Sample 50:0 Foxtail millet :
Orange peel powder

Conclusion

Functional foods are increasingly recognized for their potential to improve overall health by delivering nutritional and bioactive components from natural and nutrient-dense ingredients. This study

successfully formulated cookies by incorporating orange peel powder at concentrations ranging from 1% to 5% into a foxtail millet base. The addition of orange peel powder significantly improved the fiber content and antioxidant activity of the cookies, contributing to potential health benefits such as enhanced digestion and oxidative stress reduction. Among the tested formulations the optimal orange peel powder concentration of 2% (Sample 2) for overall acceptability and best sensory scores, offering a desirable balance of texture, taste, appearance, aroma, and mouth feel. Though higher orange peel powder levels further increased functional properties, they adversely affected sensory qualities, particularly in terms of bitterness and mouth feel. Microbial analysis confirmed that all samples met safety standards, supporting the feasibility of the product. The successful integration of foxtail millet, a nutrient-rich, underutilized grain and orange peel powder, a valuable agro-industrial by-product demonstrates a sustainable approach to developing, functional bakery products. These cookies made of foxtail millet and orange peel powder that is completely free of gluten, with enhanced nutritional attributes serves as a good snack option for population with celiac

disease, as it contributes well to their diet. These align with modern dietary trends focused on wellness, plant-based nutrition, and waste valorization, making them a viable option for health-conscious consumers and food innovators.

Author Contribution

Yogalakshmi J.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing— original draft.
Adeyeye, S.A.O.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Resources, Software, Validation, Visualization, Writing— original draft, Writing – review and editing

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Conflict of Interest

The authors certify that they have no conflict of interest.

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مقاله پژوهشی

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ارزیابی کیفی و ویژگی‌های حسی کوکی‌های تهیه‌شده از آرد ارزن دم‌روباهی و پودر پوست پرتقال

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چکیده

تقاضای روزافزون برای غذاهای فراسودمند، باعث شده است که مواد طبیعی و غنی از مواد مغذی در محصولات سنتی به‌منظور حمایت از سلامت و تندرستی گنجانده شوند. ارزن دم‌روباهی، یک غله بدون گلوتن غنی از پروتئین، فیبر و ریزمغذی‌ها، و پودر پوست پرتقال، یک محصول جانبی سرشار از فیبر غذایی و ترکیبات زیست‌فعال، برای تولید کوکی‌هایی با خواص فراسودمند استفاده شدند. این پژوهش با هدف تولید و ارزیابی کوکی‌های سلامتی بخش تهیه‌شده از ارزن دم‌روباهی غنی‌شده با پودر پوست پرتقال انجام گردید. پنج فرمولاسیون با نسبت‌های ۴۹:۱، ۴۸:۲، ۴۷:۳، ۴۶:۴، ۴۵:۵ از ارزن دم‌روباهی و پودر پوست پرتقال برای نمونه ۱ تا نمونه ۵ تولید شد، در حالی که نمونه کنترل نسبت ۵۰:۰ داشت. نمونه‌ها از نظر آنالیز شیمیایی، فعالیت آنتی‌اکسیدانی، ایمنی میکروبی و خواص حسی مورد تجزیه و تحلیل قرار گرفتند تا سطح بهینه ترکیب با استفاده از روش‌های استاندارد تعیین شود. نتایج آنالیز شیمیایی نشان داد که محتوای پروتئین از ۱۱/۷٪ تا ۷/۲٪ برای نمونه ۱ تا نمونه ۵ متغیر بود، در حالی که نمونه کنترل ۱۱/۹٪ پروتئین داشت. میزان چربی برای نمونه ۱ تا ۵ از ۲۰/۵٪ تا ۱۹/۳٪ متغیر بود، در حالی که در نمونه کنترل ۲۰/۷٪ بود. میزان فیبر خام برای نمونه ۱ تا ۵ از ۲/۸٪ تا ۴/۸٪ متغیر بود، در حالی که برای نمونه کنترل ۲/۱٪ بود. میزان خاکستر برای نمونه ۱ تا ۵ از ۱/۱۰٪ تا ۱/۵۴٪ متغیر بود، در حالی که در نمونه کنترل ۱/۰۴٪ بود. میزان رطوبت برای نمونه ۱ تا ۵ از ۲/۶۱٪ تا ۴/۲۵٪ متغیر بود، در حالی که در نمونه کنترل ۲/۵٪ بود. از نقطه نظر تغذیه‌ای، افزایش تدریجی فیبر غذایی و فعالیت آنتی‌اکسیدانی در نمونه‌ها را شاهد بودیم که به بهبود سلامت گوارش و ظرفیت مهار رادیکال‌های آزاد کمک می‌کند. ارزیابی حسی میزان بهینه افزودن پودر پوست پرتقال (۲٪) مناسب برای پذیرش کلی حسی نشان داد. نتایج این پژوهش نشان داد که افزودن پودر پوست پرتقال به‌طور قابل توجهی ویژگی‌های تغذیه‌ای و سلامتی بخشی کوکی‌ها را افزایش می‌دهد.

واژه‌های کلیدی: ارزن دم‌روباهی، بیسکویت عملکردی، پودر پوست پرتقال، فعالیت آنتی‌اکسیدانی

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Research Article

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Improving the Shelf Life of Red Grapes Using Polyethylene Nanocomposite Packaging and Modified Atmosphere Techniques

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Abstract

Red ruby grapes are known for their rich anthocyanin content and nutritional and medicinal properties, but their high perishability limits postharvest shelf life. Current research highlights the potential of advanced packaging techniques, such as modified atmosphere packaging (MAP), and nanotechnology to enhance the quality and shelf life of fresh products. This study aims to investigate the combined use of these technologies to maintain the quality and increase the shelf life of red ruby grapes. This study evaluated the effectiveness of polyethylene packaging films containing silver nanoparticles and titanium dioxide with modified atmosphere packaging (50% CO₂ + 5% O₂ + 45% N₂). The experiments were conducted under three temperature conditions (25°C, 15°C, and 4°C) and over a 28-day storage period. Samples were assessed at five intervals (0, 7, 14, 21, and 28 days) for quality attributes and microbial loads. This approach was chosen to address the challenges of microbial growth and quality deterioration in grapes stored at different temperatures. The findings showed a continuous decrease in anthocyanin content and color intensity during the storage period, alongside an increase in soluble solids. Microbial analysis showed higher mold and yeast counts in grapes stored at 25°C and 15°C compared to those stored at 4°C. Packaging with MAP and nanocomposite films containing silver nanoparticles and titanium dioxide effectively preserved the quality of grapes, particularly at 4°C, where superior results were observed over the 28 days. This study demonstrates the integration of MAP and films containing nanoparticles to address the limitations of conventional grape storage methods. This approach offers practical solutions for the horticultural industry and contributes to the advancement of storage and preservation technologies.

Keywords: Modified atmosphere packaging, Nanosilver, Nanotitanium dioxide, Polyethylene, Ruby grape

Introduction

Grapes are among the most essential horticultural products, valued for their taste, nutritional properties, and economic importance. However, their high moisture content and susceptibility to fungal infections make them highly perishable and difficult to preserve after harvest. This is primarily due to respiration, transpiration, and other biochemical activities (Ngcobo *et al.*, 2012).

Additionally, fungal pathogens such as *Botrytis cinerea* (gray mold) and *Rhizopus stolonifer* are significantly contribute to grape decay, causing severe losses in both pre-and postharvest conditions (Teles, Benedetti, Gubler, & Crisosto, 2014). Preserving grape quality during storage is critical to minimizing waste and maintaining market value (Shin, Hai, Nock, Holliday, & Watkins, 2007). Grapes hold a



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unique position among horticultural products due to their widespread consumption, economic value, and sensitivity to post-harvest spoilage. Compared to other fruits, grapes are particularly challenging to preserve because of their high moisture content and susceptibility to fungal infections, which significantly impact both quality and marketability. Addressing these challenges is critical, not only to reduce economic losses but also to ensure the availability of high-quality produce for consumers.

Historically, sulfur dioxide (SO₂) was widely used to preserve grapes (Ahmed *et al.*, 2018; Franck, Latorre, Torres, & Zoffoli, 2005; Sortino *et al.*, 2017). However, its corrosive effects on metals, as well as adverse impacts on grape flavor and toxicity concerns, have limited its acceptability. Regulatory bodies, including the U.S. Food and Drug Administration, have restricted its use, prompting the development of safer, more sustainable alternatives. In recent years, innovative packaging solutions such as MAP and nanoparticle-based films have gained attention for their potential to extend the shelf life of perishable commodities like grapes (Artés-Hernández, Aguayo, & Artés, 2004; Candir *et al.*, 2012; Deng *et al.*, 2019; Kumar, Boro, Ray, Mukherjee, & Dutta, 2019).

Antimicrobial packaging is a type of active packaging designed to inhibit microbial growth by incorporating antimicrobial agents into packaging films and utilizing active polymers. These systems effectively minimize or eliminate microbial activity in food by creating an environment hostile to microorganisms. When the packaging system is impregnated with antimicrobial properties, it effectively retards microbial growth by lengthening the lag phase and reducing the growth rate or lifespan of microorganisms (Alizadeh-Sani, Rhim, Azizi-Lalabadi, Hemmati-Dinarvand, & Ehsani, 2020; Kumar *et al.*, 2019).

One promising approach to preventing microbial growth in food packaging is the incorporation of nanotechnology into polymer films. Silver nanoparticles (SNPs) are particularly effective due to their antibacterial

and antifungal properties. When incorporated into packaging films, SNPs have been shown to reduce bacterial growth by up to 98% within the first 24 hours compared to conventional packaging films (Joshaghanizade, Shahriari, Hoseini Ghiasvand, & Salehifar, 2024). The antimicrobial activity of SNPs stems from the release of positively charged silver ions upon decomposition, with nanoparticles typically ranging in size from 10 to 20 nm (Darab, Shahriari, & Mozafari, 2021). Titanium dioxide (TiO₂) is one of the most commonly used photocatalysts, exhibiting photocatalytic properties when activated by ultraviolet radiation. These properties make TiO₂ valuable in environmental applications, such as removing pollutants from air and water (Gelover, Gómez, Reyes, & Leal, 2006). TiO₂ is effective against both Gram-positive and Gram-negative bacteria, as well as viruses. Its antimicrobial efficiency depends on factors like particle size, light intensity, and wavelength, and typically requires concentrations ranging from 100 to 1000 ppm (Hajkova, Spatenka, Horsky, Horska, & Kolouch, 2007; Villatte *et al.*, 2015). Silver nanoparticles have been shown to enhance the photocatalytic activity of TiO₂ under visible light. For instance, incorporating 1% silver by weight into TiO₂ can significantly reduce the time needed to eliminate *Escherichia coli* (10⁷ CFU/ml) from 65 minutes to 16 minutes under ultraviolet light exposure (Sarkar, Jana, Samanta, & Mostafa, 2007). The combination of silver and TiO₂ demonstrates exceptional photocatalytic performance, attributed to their synergistic optical activity and visible light reactivity. TiO₂ nanoparticles are widely used in large-scale applications, particularly for packaging films. This is due to their light color and resistance to ultraviolet radiation penetration. Additionally, nano-sized compounds like SiO₂ and TiO₂ are extensively applied in food coatings, offering innovative solutions for improving food preservation (Thiyagu, Gokilakrishnan, Uvaraja, Maridurai, & Prakash, 2022).

In food packaging, other methods beyond films incorporating silver nanoparticles (SNPs)

and titanium dioxide (TiO_2) are also utilized. One widely recognized approach is modified atmosphere packaging (MAP), which has been employed for many years to preserve perishable food products and fruits by extending shelf life and maintaining quality (Ehsani, Shahriari, & Famil, 2018). The core principle of MAP involves reducing the oxygen content within the packaging environment. By decreasing oxygen levels from approximately 20% to near zero, MAP helps to stabilize the packaging atmosphere, thereby slowing down the growth of aerobic microorganisms and mitigating oxidation reactions (Pasha *et al.*, 2023). This process significantly extends the shelf life of food products. Typically, the removed oxygen is replaced with inert nitrogen gas, sometimes supplemented with carbon dioxide to achieve the desired atmosphere (Sobhani, Zamindar, & Aarabi Najvani, 2022).

The present study addresses grape spoilage by integrating the advantages of nanotechnology-based packaging and MAP. This strategy aims to extend the shelf life of grapes through the application of nanocomposite polyethylene (PE) films infused with silver nanoparticles (SNPs) and titanium dioxide (TiO_2), while simultaneously maintaining controlled atmospheric conditions using MAP. The antimicrobial properties of SNPs, coupled with the photocatalytic activity of TiO_2 , work synergistically to inhibit microbial growth and oxidative reactions, which are the primary causes of spoilage. The main objective of this study is to evaluate the efficacy of nanocomposite PE films containing SNPs and TiO_2 when used under MAP conditions, examining the impact of varying nanoparticle concentrations and storage at three distinct temperatures (25°C, 15°C, and 4°C) over five storage periods (0, 7, 14, 21, and 28 days).

Materials and Methods

Materials

Ruby grapes were sourced from the gardens of Qazvin City, Iran. The grapes were screened for color and ripeness, ensuring that any unripe

or damaged specimens were excluded. For the evaluation, 100 grams of intact grapes, free from blemishes or physical defects, were carefully weighed using an electronic balance (FX-300i, Japan). The grape packaging was designed to evaluate the effect of two different types of nanocomposite packaging (silver and titanium dioxide) in comparison to conventional grape packaging, as detailed below.

Polyethylene Film Containing SNP/ TiO_2

Low-density polyethylene (LDPE) granules (LH0075) were sourced from Fakour Nano Bespar Company, located in Shiraz, Iran. These granules possess a melt flow index of 2 g/10 min and a density of 0.921 g/cm³. Titanium dioxide powder, boasting a purity of 99.5%, was acquired from Merck in Germany. According to the manufacturer (Merck), the titanium dioxide nanoparticles had a particle size of <100 nm as measured by BET and <50 nm as measured by XRD. Additionally, silver nanoparticles (SNPs) with purity of 99% and an average diameter of 35 nm were procured from Neutrino Company, China. The nanocomposite film was prepared by blending SNPs and titanium dioxide powder in ratios of 65:35 and 75:25, respectively. Before commencing the mixing process, all materials underwent 24 hours of humidification under vacuum conditions at 60°C. Subsequently, the three constituent materials were combined using the direct mixing method, with the final percentage of SNPs in 1 kg of nanocomposite determined within the mixer. Mixing was conducted at a rotor speed of 60 rpm and 170°C for 15 minutes. During this phase, the nanocomposite underwent mixing utilizing a twin-screw extruder (Cincinnati Milacron, Batavia, OH, USA), featuring a 55 mm extruder diameter and a screw length-to-diameter ratio of 30. The extruder operated at a spiral rotation speed of 100 rpm, with a thermal program incorporating four temperature zones set at 155°C, 160°C, 165°C, and 170°C, respectively. Nanocomposite films with a total nanoparticle loading of 2 wt% and thickness of 50 µm were

prepared, with SNP and TiO₂ distributed as 1.30 wt% SNP + 0.70 wt% TiO₂ for the 65:35 formulation, and 1.50 wt% SNP + 0.50 wt% TiO₂ for the 75:25 formulation. The nanocomposite film was made according to the method reported by (Kubacka *et al.*, 2009; Zhang & Chen, 2009).

Scanning Electron Microscope (SEM) & Transmission Electron Microscope (TEM)

To investigate the effect of SNP on polyethylene films, surface analysis was performed using a Zeiss SEM (scanning electron microscope), model VP1430, Germany. The film samples, measuring 6x6 cm, were securely attached to the pins of the device using specialized techniques. A reflective layer consisting of gold was carefully deposited on the prepared samples using a Bal-Tec layering machine. In addition, to gain deeper insight into other relevant aspects, TEM (Transmission Electron Microscope) with an HMG400 model from Eindhoven, Netherlands was used. For TEM investigation, 6x6 cm film samples are coated with an ultra-thin carbon shield with a thickness between 20 and 40 nm. This shield is thin and electron transparent, ensuring accurate and insightful observations. The method employed is in line with previous reports as outlined in the cited articles (Youssef & Abdel-Aziz, 2013).

Fourier Transform Infrared Spectroscopy Analysis

Chemical structure recognition of the compounds and functional groups was carried out through Fourier Transform Infrared Spectroscopy (FTIR spectroscopy) under ambient conditions (Metak, Nabhani, & Connolly, 2015; Zielińska, Skwarek, Zaleska, Gazda, & Hupka, 2009). FT-IR spectra of the

film were recorded within the wavelength range of 400-4500 cm⁻¹. The FTIR infrared spectroscopic analysis was conducted using an Avatar 320 device manufactured by Nicolet Company, USA.

Grape Packaging

Packaging films containing SNP and titanium dioxide with dimensions of 20x15 cm were used for grape packaging. The thickness of the film was 0.07 mm and each package contained 100±10 grams of grapes. A 2x3 cm sachet containing 20 grams of potassium permanganate as an ethylene absorbent and 20 grams of silica gel as moisture absorbent placed inside each package. The packages were thermally sealed using a MAP machine (A-200 PLC, Henkelman, Holland) with a gas pressure of 5.5 bar, and a gas mixture consisting of 50% carbon dioxide + 5% oxygen + 45% nitrogen at a speed of 28 L.min⁻¹. The control samples were placed in common polyethylene bags under normal atmospheric conditions, without vacuum or the addition of ethylene (potassium permanganate) or moisture (silica gel) absorbents. The packed grapes were stored for 28 days at three distinct temperatures: 4, 15, and 25 °C. A series of physical, chemical, and microbiological tests were performed on the grapes at regular intervals of 7 days, to assess their quality. Table shows the coded samples that were analyzed in this research.

Headspace Gas Analysis

The O₂ and CO₂ percentages in the headspace were determined utilizing the Oxybaby gas analyzer model, manufactured by Wittgas in Germany. Approximately 8 mL of headspace gas was taken for analysis from the package (Antmann, Ares, Lema, & Lareo, 2008; Kargwal, Garg, Singh, Garg, & Kumar, 2020).

Table 1- Specification of coded samples ratios of SNP to TiO₂

Sample code	Temperature (°C)	(SNP + nanotitanium dioxide)
Conventional packaging or Control Samples (CO)		
COT4	4	-
COT15	15	-
COT25	25	-
Conventional Packaging with Silver Nanoparticles (SNP)		
C65T4	4	65:35
C65T15	15	65:35
C65T25	25	65:35
C75T4	4	75:25
C75T15	15	75:25
C75T25	25	75:25
Modified atmosphere packaging with SNP		
M65T4	4	65:35
M65T15	15	65:35
M65T25	25	65:35
M75T4	4	75:25
M75T15	15	75:25
M75T25	25	75:25

Microbial Analyses

Microbiological analyses, including standard plate counts and assessment of yeast and molds, were conducted following established protocols outlined in the American Public Health Association (APHA) guidelines of 1992 (Akhondzadeh Basti, 2006; Saxena, Bawa, & Srinivas Raju, 2008). Plate count agar (PCA) medium was employed to determine standard plate counts, while acidified potato dextrose agar (PDA) was utilized for yeast and mold counts. Colonies were then counted and expressed as log CFU/g of the sample. The inoculated PDA plates were incubated for 3 to 5 days at a 25°C, and the colony counts subsequently reported as Log CFU/g of the sample.

Color Measurement of Grapes

Grape color analysis was conducted using a Minolta Chromameter CR-400 (Minolta Co., Ltd., Osaka, Japan). The Hunter color scale was employed, and parameters including lightness (L) and chromaticity values for a (red-green) and b (yellow-blue) were recorded. The experiments were performed by placing the grape samples on a standard white plate to ensure consistent lighting. The samples were homogenized in a mixer grinder before color measurement (Candir *et al.*, 2012).

Total Soluble Solids

Five grapes were handpicked from each cluster, pooled together, and finely ground to obtain a uniform sample. The concentration of total soluble solids (TSS) was assessed using a refractometer (REF108, China) at a consistent temperature of 27°C, with an accuracy of $\pm 0.2\%$. The results were expressed as % Brix (Ayhan, Eştürk, & Taş, 2008).

Measurement of Anthocyanin Concentration

The quantification of anthocyanin concentration was conducted utilizing the differential pH method (Teng, Jiang, He, & Bai, 2020). In this method, initial grape samples were subjected to dewatering. Subsequently, 2 ml of grape juice was diluted to a final volume of 25 ml using a pH=1 buffer solution consisting of a 0.2 M potassium chloride and 0.2 M hydrochloric acid mixture. Simultaneously, another 2 ml of grape juice was similarly diluted to 25 ml but with a pH=4.5 buffer solution, composed of a mixture of 1 M sodium acetate and 1 M hydrochloric acid. The absorbance of the resultant solutions was measured at 510 nm employing a spectrophotometer (Model: (AA) PGLtd, Manufacturer: PG-Instruments, USA). The concentration of anthocyanin was subsequently determined using the following equation:

$$C = \frac{A \times MW \times DF \times 10^3}{\epsilon \times L} \quad (1)$$

Where:

C = Anthocyanin concentration in mg/L ;

A = ($Abs_{pH=1} - Abs_{pH=4.5}$)

MW (molecular weight) = 484.82 g/mol for cyanidin-3-glucoside (CYD-3-Glu)

DF =1 (dilution factor); L = path length in cm; ϵ = 24825 molar extinction coefficient

Statistical Analyses

Data of physical and chemical parameters underwent an analysis of variance (ANOVA). To assess the significance of differences between treatments and storage time, a post-hoc analysis was conducted utilizing Duncan's test, and significance was determined at a threshold of $P < 0.05$. All statistical analyses were performed using the SPSS software package, version 20, for Windows.

Results and Discussion

SEM & TEM

In Fig. 1, SEM images show two distinct nanocomposite samples obtained from

polyethylene injected with SNP and TiO₂ in different ratios: 65:35 (left image) and 75:25 (right image). The films produced with a ratio of 75:25 of SNP to TiO₂ show homogeneous dispersion of SNP in the matrix.

Fig. 2 shows TEM images of polyethylene films containing different ratios of SNP to TiO₂ (65:35, left image; 75:25 right image). While differences are subtle, the film with a ratio of 75: 25 of SNP to TiO₂ has more uniform structure. Structures with a convex asymmetric state are observed, which is probably related to the time of polyethylene film formation.

FTIR

Fig. 3 displays the FTIR spectra of polyethylene films with different SNP to TiO₂ ratios, specifically 65:35 and 75:25. In these spectra, distinctive absorption bands originating from the polyethylene film are evident, predominantly arising from the vibrations of carbon-carbon (C-C) and carbon-hydrogen (C-H) bonds.

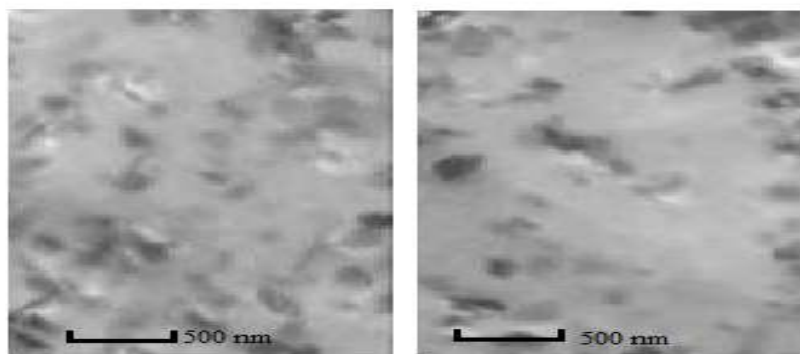


Fig. 1. SEM images of polyethylene films with different ratios of SNP to TiO₂, with the left image showing a 65:35 ratio and the right image showing a 75:25 ratio

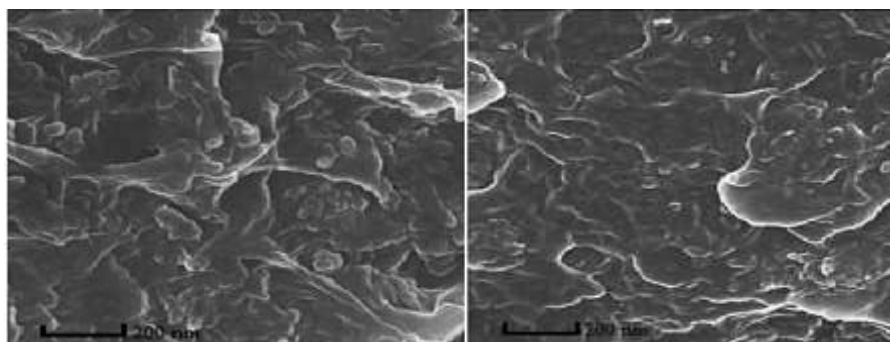


Fig. 2. TEM images of polyethylene films with different ratios of SNP to TiO_2 , the left image showing a 65:35 ratio and the right image showing a 75:25 ratio

This spectral region, commonly termed the "CH stretching region," encompasses the vibrational modes of both C-C and C-H bonds. It is essential to underscore that the precise positions and intensities of the C-C and C-H bands can exhibit variations contingent on the unique properties and composition of the analyzed sample. Additionally, the presence of long-chain hydrocarbons within the prepared films is discernible in the spectrum. This observation is supported by the identification of peaks at frequencies of 1264, 1418, 1517, 1856, 2069, and 3373 cm^{-1} , primarily attributed to methylene deformation, carbon-hydrogen aromatic tension, carbon-carbon, and methylene displacement.

This observation suggests that there is no evidence of chemical bonding or substantial

interaction between polyethylene and silver nanoparticles within the resulting structures. Instead, the silver nanoparticles are dispersed within the polyethylene matrix and on its surfaces, contributing to the antimicrobial properties of the polyethylene nanocomposite containing silver and titanium dioxide. Notably, rutile bands characteristic of titanium dioxide were not detected in the polyethylene film. This absence could be attributed to the high concentration of polyethylene, which may have masked the presence of other components at lower concentrations (León *et al.*, 2017). This phenomenon has been observed in other studies where the dominant polymer matrix conceals the characteristic peaks of embedded nanoparticles (Sarkar *et al.*, 2017).

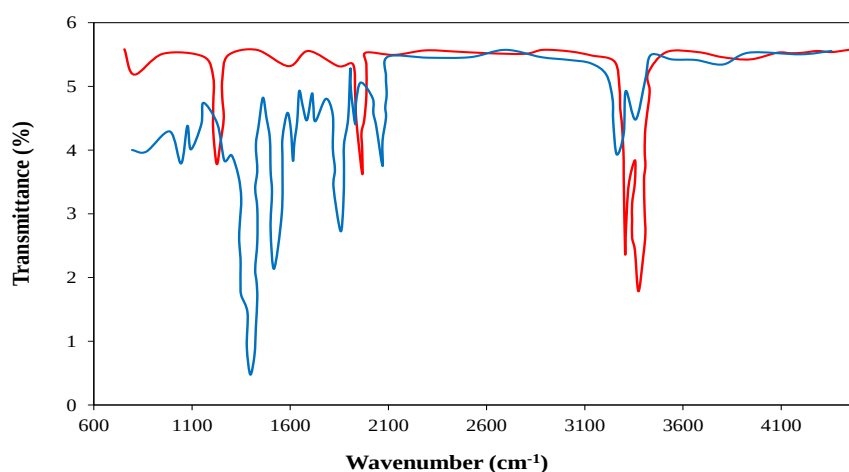


Fig. 3. FTIR spectra polyethylene films with different ratios of SNP to TiO_2 ; the red spectrum shows a 65:35 ratio and the blue spectrum shows a 75:25 ratio

Mold and Yeast Analysis

It should be noted that total mold and yeast counts provide a general indication of fungal activity and spoilage. Although grape spoilage is mainly caused by *Botrytis cinerea* and *Rhizopus stolonifer*, in this study only the overall mold and yeast counts were measured. The results of mold and yeast counts in various packaging samples can be found in Table 1. According to the findings, softening and moldy spoilage were observed in the control samples at 25°C after 7 days, and at 4°C and 15°C after 14 days. Consequently, mold and yeast counts were exclusively measured in the control sample at 25°C on first day, and at 4°C and 15°C only up to the seventh day of storage. As the storage period extended, mold and yeast count in samples stored at 15°C and 25°C significantly increased ($P < 0.05$). Consequently, mold and yeast counts were at their lowest on first day and reached their highest levels in all samples on the 28th day of storage ($P < 0.05$). In contrast, at 4°C, mold and yeast counts decreased with prolonged storage, and on the seventh day, there was no significant difference compared to first day ($P > 0.05$). At 4°C, the highest mold and yeast counts were observed on both first day and the seventh day, while the lowest counts were recorded in all samples on the 28th day of storage ($P < 0.05$). Statistical analysis revealed no significant differences between samples in various packaging on first day ($P > 0.05$). Mold and yeast counts were notably higher at 25°C compared to other samples during most storage periods, while at 4°C, they were significantly lower. An increase in the presence of SNP in packaging films corresponded to lower mold and yeast counts during most storage periods. The utilization of a modified atmosphere in packaging also led to decreased mold and yeast counts. By the end of the storage period, the C65T25 sample exhibited the highest mold and yeast counts (3.78 log CFU/g), while the M75T4 sample had the lowest counts (2.32 log CFU/g) ($P < 0.05$). These findings are in agreement with those reported by Sobhani et al., where the

application of nano-based packaging and modified atmosphere similarly resulted in lower microbial counts during storage (Sobhani et al., 2022).

The findings suggest that integrating silver nanoparticles (SNPs) alongside titanium dioxide into packaging films yields positive outcomes in prolonging the storage time of grape samples. Proposed mechanisms associated with the antimicrobial properties of SNP and titanium dioxide in this context are primarily related to the release of silver ions at the film surface, which can interact with microbial cells and inhibit their growth (Efati, Ahari, Shahbazzadeh, Nowruzi, & Yousefi, 2021). This effect is enhanced when combined with MAP, which reduces microbial proliferation through altering gas composition. Consistent with the findings of the present study, Danglu et al. (2020) demonstrated that polyethylene films incorporated with silver nanoparticles (SNPs) effectively reduced microbial growth and extended the shelf life of mushrooms during storage. Similarly, Mihaly Cozmuta et al. (2015) reported that coating wheat bread with SNPs significantly inhibited the growth of mold and yeast. A similar pattern of microbial behavior, akin to that observed for total mesophilic bacteria, was also noted in the case of molds and yeasts (Lok et al., 2006).

On the other hand, the use of MAP also reduced the count of mold and yeast. The gases used in the modified atmosphere, such as carbon dioxide, have antimicrobial properties, and the mechanism is that they dissolve in the water in the food tissue and produce carbonic acid, which enters the cell membrane of the microorganism and enters the cell. It is then ionized and causes the death of microorganisms by disturbing the electrical balance inside the cell. Another reason is that samples packaged under a modified atmosphere show a lower level of mold and yeast compared to those in conventional packaging, mainly due to the antibacterial and antifungal effects of carbon dioxide. (Beigmohammadi et al., 2016; He, Li, Fei, & Peng, 2021).

While the number of yeast and mold (log CFU/g) in different packaged samples such as C65T4 and M65T4, C65T15 and M65T15 at different storage time showed some variability, the combined use of nanomaterial-modified PE and MAP did exhibit an overall trend towards reduced microbial growth. Specifically, samples stored with MAP and higher SNP

concentrations consistently showed lower counts of yeast and mold, indicating the efficacy of these packaging strategies in maintaining grape quality over time. These results underline the potential of SNP/TiO₂-modified films in enhancing food safety and extending shelf life.

Table 1- Changes in the number of yeast and mold (log CFU/g) in the different packaged samples at different storage times

Sample code	Storage day				
	0	7	14	21	28
COT4	3.12±0.04 ^{Aa}	3.10±0.02 ^{BCa}	-	-	-
COT15	3.11±0.02 ^{Ab}	3.67±0.02 ^{Aa}	-	-	-
COT25	3.15±0.02 ^{Aa}	-	-	-	-
C65T25	3.15±0.04 ^{Ad}	3.25±0.02 ^{BCd}	3.34±0.04 ^{Ac}	3.47±0.07 ^{Ab}	3.78±0.07 ^{Aa}
C75T25	3.12±0.04 ^{Ac}	3.21±0.01 ^{ABc}	3.27±0.14 ^{ABc}	3.46±0.02 ^{Ab}	3.63±0.04 ^{ABa}
M65T25	3.16±0.04 ^{Ac}	3.21±0.01 ^{ABbc}	3.19±0.06 ^{ABCb}	3.29±0.05 ^{Bb}	3.54±0.05 ^{BCa}
M75T25	3.13±0.02 ^{Ac}	3.2±0.01 ^{ABb}	3.17±0.02 ^{BCb}	3.19±0.08 ^{Bb}	3.45±0.03 ^{BCa}
C65T15	3.13±0.02 ^{Ac}	3.18±0.03 ^{BCb}	3.21±0.04 ^{ABCb}	3.25±0.04 ^{Bb}	3.42±0.08 ^{CDa}
C75T15	3.12±0.02 ^{Ac}	3.18±0.03 ^{BCb}	3.19±0.04 ^{ABCb}	3.17±0.01 ^{Bb}	3.24±0.03 ^{DEa}
M65T15	3.11±0.03 ^{Ac}	3.17±0.04 ^{BCb}	3.18±0.03 ^{ABCb}	3.2±0.03 ^{Bb}	3.26±0.03 ^{Ea}
M75T15	3.11±0.02 ^{Ac}	3.17±0.03 ^{BCb}	3.18±0.03 ^{ABCb}	3.18±0.06 ^{Bb}	3.21±0.03 ^{Ea}
C65T4	3.15±0.03 ^{Aa}	3.17±0.03 ^{BCa}	3.11±0.07 ^{BCb}	3±0.13 ^{Cb}	2.73±0.12 ^{Fc}
C75T4	3.15±0.04 ^{Aa}	3.15±0.04 ^{BCa}	2.94±0.07 ^{DEbc}	2.86±0.12 ^{Dc}	2.72±0.18 ^{Fc}
M65T4	3.15±0.04 ^{Aa}	3.14±0.03 ^{BCa}	3.05±0.1 ^{CDb}	2.68±0.03 ^{Ec}	2.58±0.02 ^{Gc}
M75T4	3.15±0.04 ^{Aa}	3.12±0.03 ^{BCa}	2.88±0.09 ^{Eb}	2.51±0.02 ^{Fc}	2.32±0.07 ^{Hc}

Different uppercase letters in the same row and lowercase letters in the same column indicate significant differences ($p < 0.05$).

Anthocyanins Content

The red color of Ruby grapes is due to the presence of anthocyanins, a prominent group of pigments found in these delicious fruits. Among the multitude of phenolic compounds found in grapes, anthocyanins predominate, and cyanidin-3-O-glucoside appears as the most common among them. This particular compound plays an essential role in giving grapes strong antioxidant capabilities. Notably, anthocyanin pigments are very sensitive and prone to degradation, especially when exposed to storage conditions (Enaru, Drețcanu, Pop, Stănilă, & Diaconeasa, 2021). The results of anthocyanin levels under different temperature conditions in all packaging samples are presented in Fig. 4.

It is worth mentioning that the initial anthocyanin content in the red grapes investigated in this research was 210.07 mg of

cyanidin 3-glucoside/kg of grape. However, it's crucial to recognize that the quantity of anthocyanin in grapes is influenced by a multitude of factors, including grape variety, soil composition, climate, geographical location, agricultural practices, latitude, growing season, storage conditions, and the use of growth regulators (Hernández-Jiménez, Gómez-Plaza, Martínez-Cutillas, & Kennedy, 2009; Lambri *et al.*, 2015; Theodorou *et al.*, 2019). Consequently, it can be asserted that the content of anthocyanin in red grapes is predominantly determined by the diversity in cultivars and species. In all samples, an evident decrease in anthocyanin content was observed as storage duration increased, regardless of the temperature namely, 4°C, 15°C, and 25°C. It should be noted that in the control sample stored at 25°C after 7 days and at 4 and 15°C after 14 days of storage, signs of softening and

moldy spoilage were observed. Consequently, anthocyanin measurements were only conducted on the control sample at 25°C on the first day, and for control samples stored at 4°C and 15°C, assessments were limited to the first 7 days. As storage days progressed, a significant decline in anthocyanin levels was consistently observed across all packaging samples ($P < 0.05$). The highest amount of anthocyanin was recorded on the first day, while a significant decline was observed throughout the storage period. The lowest amount of anthocyanin was detected in the control sample stored at 25°C on the 28th day of storage. Statistical analysis showed that the anthocyanin values on the first day did not exhibit significant differences ($P > 0.05$).

The observation of anthocyanin content at various temperatures during storage reveals intriguing patterns. Notably, at 25°C, anthocyanin content consistently remained lower than in the other packaging samples throughout most of the storage period. Conversely, at 4°C, anthocyanin content exhibited a noticeable increase. This trend aligns with published research, which has illuminated temperature as a pivotal influencer on anthocyanin accumulation in fruits, both pre-and post-harvest. Elevating the storage temperature is found to hinder the build-up of anthocyanin pigments in fruits. In simpler terms, temperature increments during the growth phase result in heightened respiration and carbohydrate consumption, subsequently leading to a decrease in the available sugar required for anthocyanin formation (Marszałek, Woźniak, Kruszewski, & Skąpska, 2017; Muche, Speers, & Rupasinghe, 2018).

In investigating the effect of packaging type on the preservation of anthocyanin content of grapes, it was observed that packaging films with higher SNP led to a relatively slower decrease in anthocyanin content during the storage period. The use of MAP also showed the ability to maintain the anthocyanin content in the samples. Ultimately, upon the conclusion of the storage period, the sample labeled as

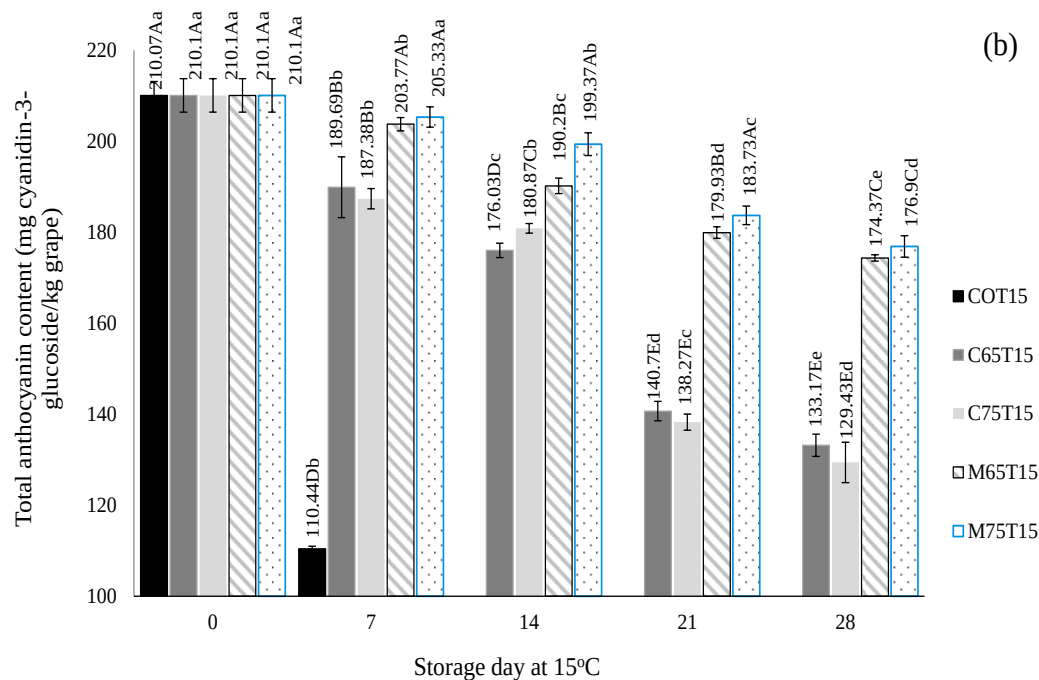
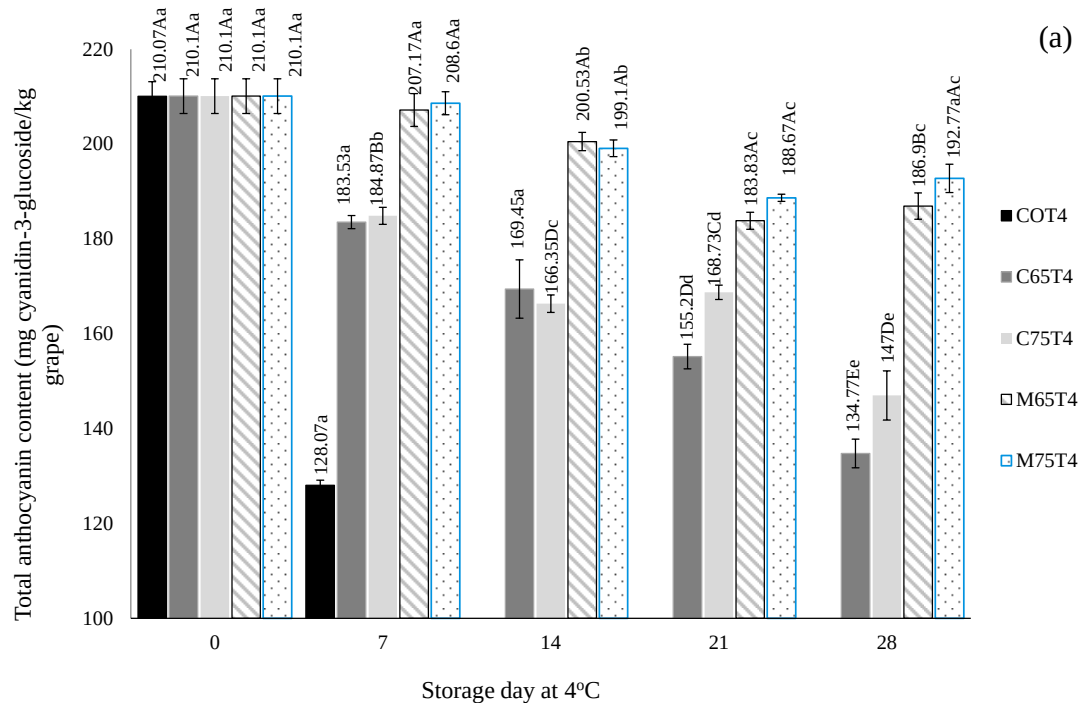
C65T25 exhibited the lowest anthocyanin content (111.14 mg of cyanidin 3-glucoside/ kg of grape) at 25°C, while the sample labeled as M75T4 displayed the highest anthocyanin content (192.77 mg of cyanidin 3-glucoside/kg of grape) at 4°C. It's worth mentioning that the initial anthocyanin content in the red grapes investigated in this research was 210.07 mg of cyanidin 3-glucoside/ kg of grape. This value exhibited an 8.2% reduction after 28 days of storage in the M75T4 packaging. Therefore, the results indicated that the use of 75% SNPs in the packaging film along with the use of MAP has a good ability to maintain the content of anthocyanin after 28 days at 4°C.

The use of SNPs in packaging film and the use of MAP have advantages over conventional packaging in maintaining grape anthocyanin content during storage for several reasons: a) SNPs integrated into the packaging film create a more effective barrier against the penetration of oxygen and moisture. This limits the exposure of the grapes to external factors that can lead to the degradation of anthocyanins. In conventional packaging, which may not have the same inhibitory properties, grapes are more vulnerable to environmental elements that can accelerate anthocyanin degradation (Rajakannu, Shankar, Perumal, Subramanian, & Dhakshinamoorthy, 2015). MAP allows for the regulation of internal gas composition, often by reducing oxygen levels. This controlled atmosphere can slow down the rate of grape respiration. Slower respiration means that fewer metabolic processes, including anthocyanin degradation, occur during storage. Standard packaging lacks this level of control, leading to higher respiration rates and faster anthocyanin loss (Enaru *et al.*, 2021). MAP, along with SNPs, can inhibit microorganism growth and delay enzymatic spoilage. Microorganisms and enzymes can help degrade anthocyanins. These packaging methods help preserve anthocyanins for longer by reducing their activity.

In summary, the use of SNPs in packaging films along with MAP provides a more controlled and protective environment for

grapes during storage. This results in reduced respiration, reduced exposure to oxygen and moisture, and inhibition of degradation factors, all of which collectively help preserve the

grape's anthocyanin content for longer than conventional packaging.



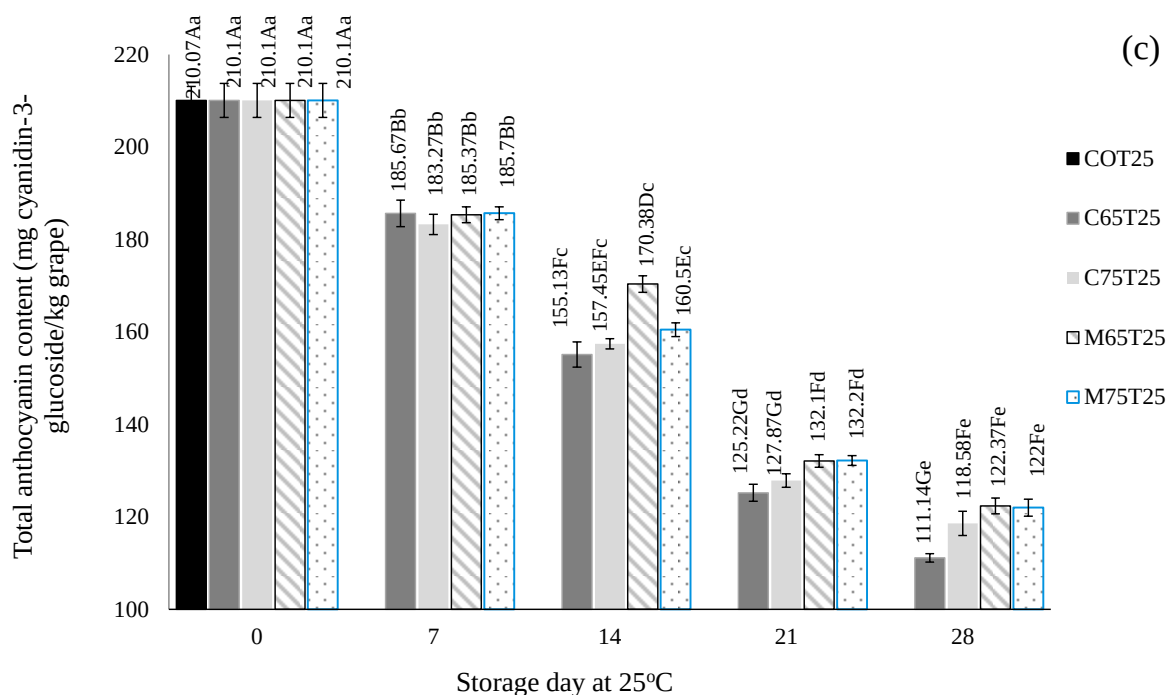


Fig. 4. The results of anthocyanin levels in different temperature conditions and different types of packaging (a) 4°C; (b) 15°C; (c) 25°C. Error bars represent the standard deviation

Total Soluble Solids

The results concerning the total soluble solids (TSS) content of grapes in various packaging types at three different temperatures (4°C, 15°C, and 25°C) are illustrated in Fig. 5. It's worth noting that in the control sample stored at 25°C for 7 days and at 4°C and 15°C for 14 days, evident signs of softening and moldy spoilage were observed, rendering the measurement of TSS content in these samples unfeasible. According to the experimental data in Fig. 5, with the increase in storage day, a significant increase in the content of TSS was observed in all samples in different packaging methods ($P < 0.05$). The lowest soluble solids content was recorded on first day, while the highest content was observed on the 28th day of storage. Notably, statistical analysis revealed no significant differences among the samples on first day ($P > 0.05$). The increasing trend of TSS in grapes during storage in this study was in good agreement with the report by Sobhani et al. during storage of cherry tomatoes (Sobhani et al., 2022).

Due to the increase in soluble solid content in grape samples with increasing days of

storage, the reason can be explained as follows. As grapes continue to ripen even after harvest, the sugar content in grapes can increase. This sugar accumulation is a natural part of fruit development and is driven by enzymatic processes. The longer the grapes are stored, the more time there is for these processes to continue, resulting in higher soluble solids content. During storage, some of the water in the grapes can evaporate or transpire, especially if the storage conditions are dry or the storage temperature is increased. This loss of water without a corresponding loss of solutes (sugars, acids, and other compounds) causes the remaining liquid to thicken and increase the soluble solids content (Hudina, Stampar, Orazem, Petkovsek, & Veberic, 2012; Khorram, Ramezani, & Hosseini, 2017).

In this current research, the percentage of SNP during the days of storage did not show a significant effect on the content of soluble solids. However, the use of MAP effectively reduced the rate of change in soluble solids content. As a result, on the last day of storage, the lowest amount of soluble solids was recorded in samples M65T4 (18.37%) and

M75T4 (18.36%), and no significant difference was observed between them ($P>0.05$).

Finally, MAP was shown to be effective in stabilizing the soluble solids content during grape storage because it allows control of the storage environment, especially the gas composition surrounding the grapes. This respiration control helps maintain quality and prevent loss of soluble solids content. Here, we have interpreted how MAP achieves this as follows: One of our primary goals in choosing MAP was to reduce the oxygen content in the packaging. Therefore, by reducing the oxygen level and maintaining the reduced oxygen atmosphere, the rate of grape respiration can be reduced. On the other hand, by increasing the concentration of CO_2 and decreasing the concentration of oxygen, the respiration of grapes can be inhibited in packaging and the enzymatic processes that break down carbohydrates can be slowed down. This helps maintain the soluble solids content and increases the shelf life of the grapes. MAP can help maintain optimal humidity levels for grape storage. Moisture control is important because excessive moisture loss can lead to dehydration of grapes and increased soluble solids contents, while excessive moisture can promote mold growth and spoilage. MAP helps maintain the ideal balance and preserve the quality of the grapes (Pasha *et al.*, 2023).

Overall, as previous research has shown, MAP is an effective way to create an optimal storage environment for fruits, preventing loss of soluble solids by controlling gas composition, humidity, and exposure to factors that can destroy the fruit. This results in longer shelf life, better fruit quality, and reduced risk of economic loss for producers and distributors (Sabır, Selçuk, & Unal, 2020).

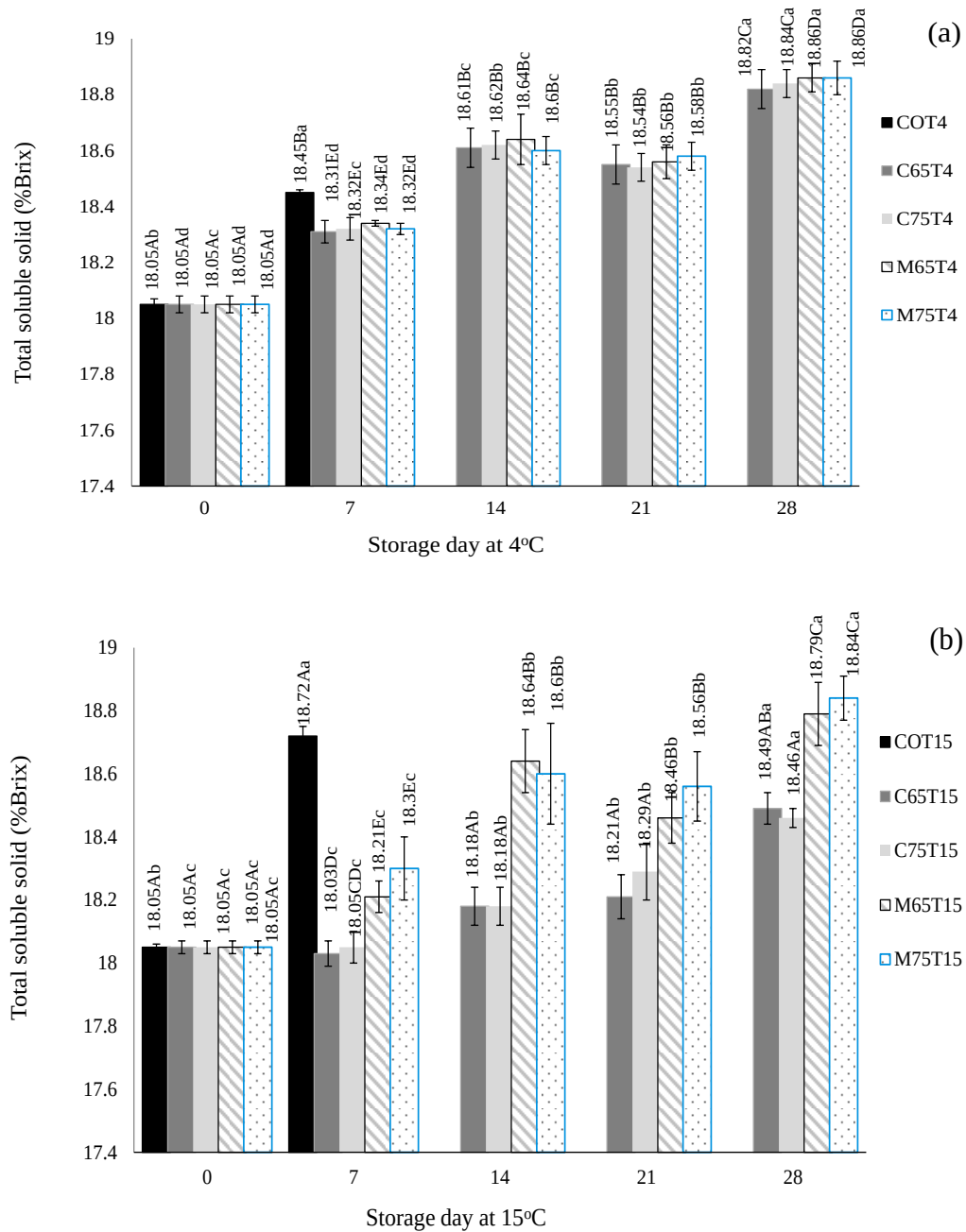
The Color Index of Grapes

The research findings, as outlined in Tables 3, 4, and 5, highlight a consistent decreasing trend in the L, a, and b indices over the storage

period. In the control sample at 25°C, softening and moldy spoilage occurred after 7 days, while at 4°C and 15°C, these issues emerged after 14 days. Consequently, the color indices in the control sample at 25°C were only observed on the first day, whereas at 4°C and 15°C were observed on the seventh day. Notably, at 25°C, the L, a, and b indices consistently exhibited lower values than those of other samples throughout most storage period. Conversely, at 4°C, these indices were significantly higher than those at 15 and 25°C.

Examining the L index specifically (Table 2), a decline was observed during the storage period, attributed to the grape's chlorophyll content. Notably, neither the SNP concentration nor the type of packaging demonstrated a statistically significant effect on the L color index throughout the storage days ($p>0.05$). However, at 4°C, MAP exhibited a positive impact on the L color index. Coded samples M65T4 and M75T4 showed the highest L color index values at the end of the storage period, recording 37.81 and 37.84, respectively.

The results regarding the color index b, presented in Table 3, revealed a significant decrease in all treatments with an increase in storage days ($P<0.05$). The highest values belonged to the first day, while the lowest values were noted across all samples on the 28th day. At 25°C, the values of color index b were significantly lower than other samples during most storage times, whereas at 4°C, they were notably higher. In samples at 25°C, SNP concentration and packaging type had no significant effect on the color index b ($P>0.05$), but at 4 and 15°C, due to increasing SNP concentration and using MAP, leads to decreasing trend of index b. The M75T4 sample exhibited the highest values of color index b (1.92) at the end of the storage period ($P < 0.05$).



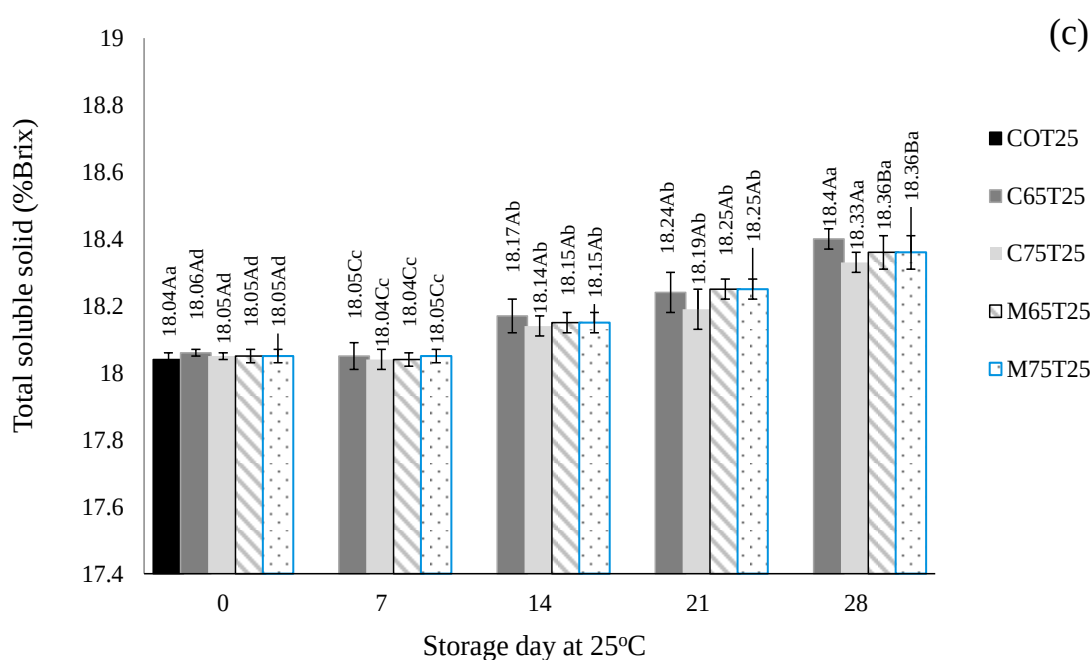


Fig. 5. The results of soluble solids content in different temperature conditions and different types of packaging (a) 4°C; (b) 15°C; (c) 25°C. Error bars represent the standard deviation

Similarly, results of the color index *a* in different samples (Table 4) showed a significant decrease with increasing storage days ($P < 0.05$). The highest values were observed on the first day, while the lowest index occurred on the 28th day across all samples. No significant differences were found between samples on the first day ($P > 0.05$). As the SNP concentration increased at different storage temperatures and times, the decrease in color index “a” became less pronounced. The use of MAP also reduced the variation of color index *a*. It is worth mentioning that the lowest value of color index was observed in the C65T25 sample (7.01) and the highest value was observed in the M75T4 sample (7.74) at the end of the storage time ($P < 0.05$).

In the concluding remarks of this section, it became evident that a significant correlation exists between the color index values and the onset of grape browning. The accumulation of pigmented compounds, such as melanin, contributing to the browning of tissues, results

in a diminished brightness of grape color. Anthocyanin, the principal pigment in grapes, appears to undergo degradative change during the storage period at various temperatures, showcasing a connection with alterations in color indicators (Muche *et al.*, 2018).

The quantity of anthocyanin compounds in grapes is intricately linked to its antioxidant activity. Notably, by the end of the storage period, there is a noticeable decrease in the concentration of anthocyanin compounds. This reduction is attributed to the oxidation reactions that transpire within the pigments during the storage period. The influence of enzymatic browning reactions on the color of grapes is noteworthy, contributing to a reduction in the transparency of grape samples (Pantelidis, Vasilakakis, Manganaris, & Diamantidis, 2007). Additionally, it is crucial to acknowledge the works referenced, which likely provide further insights into the broader context of the discussed phenomena (Moon, Kwon, Lee, & Kim, 2020).

Table 2- Rate of color index (L) in different packages of grapes

Sample code	Storage day				
	0	7	14	21	28
COT4	45.12±0.04 ^{Aa}	34.91±0.81 ^{Db}	-	-	-
COT15	45.14±0.05 ^{Aa}	36.66±0.86 ^{Eb}	-	-	-
COT25	45.13±0.06 ^{Aa}	-	-	-	-
C65T25	45.12±0.07 ^{Aa}	40.01±0.11 ^{BCb}	37.60±0.14 ^{Cc}	34.96±0.01 ^{Ed}	33.21±0.13 ^{De}
C75T25	45.11±0.02 ^{Aa}	40.05±0.09 ^{Cb}	37.58±0.18 ^{Cc}	34.94±0.08 ^{Ed}	33.27±0.17 ^{De}
M65T25	45.11±0.04 ^{Aa}	40.05±0.09 ^{Cb}	37.63±0.14 ^{Cc}	34.95±0.09 ^{Ed}	33.25±0.18 ^{De}
M75T25	45.12±0.07 ^{Aa}	40.03±0.12 ^{Cb}	37.55±0.16 ^{Cc}	34.98±0.11 ^{Ed}	33.21±0.13 ^{De}
C65T15	45.12±0.07 ^{Aa}	42.18±0.18 ^{Bb}	39.56±0.26 ^{Bc}	36.79±0.17 ^{Cd}	35.32±0.17 ^{Cc}
C75T15	45.11±0.04 ^{Aa}	42.22±0.16 ^{Bb}	39.60±0.33 ^{Bc}	35.81±0.22 ^{Dd}	35.29±0.14 ^{Cc}
M65T15	45.12±0.03 ^{Aa}	42.19±0.15 ^{Bb}	39.53±0.53 ^{Bc}	36.74±0.34 ^{Cd}	35.25±0.14 ^{Cc}
M75T15	45.11±0.02 ^{Aa}	42.20±0.14 ^{Bb}	39.56±0.56 ^{Bc}	36.78±0.24 ^{Cd}	35.22±0.12 ^{Cc}
C65T4	45.11±0.05 ^{Aa}	44.84±0.29 ^{Aa}	42.14±0.14 ^{Bb}	39.17±0.19 ^{Bc}	36.79±0.26 ^{Bd}
C75T4	45.12±0.03 ^{Aa}	44.86±0.32 ^{Aa}	42.16±0.16 ^{Bb}	39.20±0.15 ^{Bc}	36.85±0.27 ^{Bd}
M65T4	45.12±0.03 ^{Aa}	44.90±0.33 ^{Aa}	43.18±0.18 ^{Ab}	40.20±0.15 ^{Ac}	37.81±0.23 ^{Ad}
M75T4	45.11±0.02 ^{Aa}	44.88±0.32 ^{Aa}	43.17±0.17 ^{Ab}	40.25±0.16 ^{Ac}	37.84±0.31 ^{Ad}

Different uppercase letters in the same row and lowercase letters in the same column indicate significant differences ($p < 0.05$).

Table 3- Rate of color index (b) in different packages of grapes

Sample code	Storage day				
	0	7	14	21	28
COT4	2.33±0.08 ^{Aa}	1.67±0.08 ^{Bb}	-	-	-
COT15	2.35±0.06 ^{Aa}	1.39±0.05 ^{Cb}	-	-	-
COT25	2.38±0.06 ^{Aa}	-	-	-	-
C65T25	2.37±0.02 ^{Aa}	2.18±0.08 ^{Ab}	1.83±0.10 ^{Bc}	1.63±0.15 ^{Bcd}	1.33±0.09 ^{Ee}
C75T25	2.37±0.02 ^{Aa}	2.20±0.06 ^{Aa}	1.85±0.25 ^{Bb}	1.66±0.17 ^{BCc}	1.34±0.11 ^{Ed}
M65T25	2.37±0.02 ^{Aa}	2.13±0.12 ^{Ab}	1.75±0.14 ^{Cc}	1.57±0.09 ^{BCd}	1.34±0.07 ^{Ee}
M75T25	2.37±0.02 ^{Aa}	2.11±0.08 ^{Ab}	1.72±0.16	1.58±0.15 ^{BCcd}	1.38±0.11 ^{ECcd}
C65T15	2.37±0.02 ^{Aa}	2.22±0.07 ^{Aa}	1.87±0.07 ^{Bb}	1.60±0.05 ^{BCc}	1.38±0.11 ^{Ed}
C75T15	2.37±0.02 ^{Aa}	2.11±0.08 ^{Ab}	1.86±0.06 ^{Bc}	1.54±0.05 ^{Cd}	1.43±0.07 ^{De}
M65T15	2.37±0.02 ^{Aa}	2.09±0.08 ^{Ab}	1.66±0.25 ^{Dc}	1.51±0.07 ^{Cc}	1.52±0.08 ^{CDd}
M75T15	2.37±0.02 ^{Aa}	2.25±0.08 ^{Ab}	2.18±0.13 ^{Abc}	1.99±0.12 ^{Ac}	1.81±0.05 ^{Bd}
C65T4	2.37±0.02 ^{Aa}	2.19±0.06 ^{Ab}	2.12±0.05 ^{Ab}	1.94±0.15 ^{Ac}	1.77±0.05 ^{Cd}
C75T4	2.37±0.02 ^{Aa}	2.16±0.10 ^{Aab}	2.09±0.20 ^{Ab}	1.75±0.08 ^{Bc}	1.86±0.05 ^{Bd}
M65T4	2.37±0.02 ^{Aa}	2.15±0.07 ^{Ab}	2.12±0.10 ^{Abc}	1.96±0.07 ^{Ac}	1.86±0.07 ^{Bd}
M75T4	2.37±0.02 ^{Aa}	2.11±0.08 ^{Ab}	2.02±0.07 ^{Ac}	1.95±0.07 ^{Ac}	1.92±0.07 ^{Ad}

Different uppercase letters in the same row and lowercase letters in the same column indicate significant differences ($p < 0.05$).

Table 4- Rate of the color index (a) in different packages of grapes

Sample code	Storage day				
	0	7	14	21	28
COT4	8.26±0.08 ^{Aa}	7.11±0.1 ^{Bb}	-	-	-
COT15	8.32±0.07 ^{Aa}	6.8±0.04 ^{Bb}	-	-	-
COT25	8.28±0.09 ^{Aa}	-	-	-	-
C65T25	8.31±0.09 ^{Aa}	8.19±0.12 ^{Aa}	7.64±0.07 ^{Db}	7.31±0.07 ^{Fc}	7.01±0.08 ^{Fd}
C75T25	8.31±0.09 ^{Aa}	8.17±0.17 ^{Aa}	7.66±0.09 ^{Db}	7.31±0.06 ^{Fc}	7.10±0.05 ^{Ed}
M65T25	8.31±0.09 ^{Aa}	8.08±0.05 ^{Ab}	7.80±0.05 ^{CDc}	7.49±0.04 ^{DEd}	7.13±0.02 ^{Ee}
M75T25	8.31±0.09 ^{Aa}	8.05±0.22 ^{Aa}	7.68±0.13 ^{Db}	7.39±0.06 ^{DEFc}	7.21±0.07 ^{Dc}
C65T15	8.31±0.09 ^{Aa}	8.21±0.10 ^{Aa}	7.76±0.06 ^{CDb}	7.46±0.07 ^{DEc}	7.28±0.09 ^{Dc}
C75T15	8.31±0.09 ^{Aa}	8.14±0.16 ^{Aa}	7.77±0.15 ^{CDb}	7.33±0.07 ^{EFc}	7.20±0.06 ^{Dc}
M65T15	8.31±0.09 ^{Aa}	8.18±0.06 ^{Aa}	7.91±0.10 ^{BCb}	7.60±0.05 ^{CDc}	7.47±0.06 ^{Cc}
M75T15	8.31±0.09 ^{Aa}	8.05±0.06 ^{Aa}	7.95±0.08 ^{Bb}	7.67±0.04 ^{BCc}	7.54±0.04 ^{Cc}
C65T4	8.31±0.09 ^{Aa}	8.06±0.25 ^{Aa}	7.65±0.09 ^{Cb}	7.40±0.07 ^{DEFc}	7.10±0.06 ^{Ed}
C75T4	8.31±0.09 ^{Aa}	8.02±0.14 ^{Ab}	7.67±0.06 ^{Cc}	7.30±0.09 ^{Fd}	7.16±0.05 ^{DEe}
M65T4	8.31±0.09 ^{Aa}	8.16±0.16 ^{Aa}	7.93±0.10 ^{Bb}	7.77±0.04 ^{ABc}	7.67±0.03 ^{Bd}
M75T4	8.31±0.09 ^{Aa}	8.20±0.06 ^{Aa}	8.01±0.06 ^{Ab}	7.88±0.03 ^{Ac}	7.74±0.05 ^{Ad}

Different uppercase letters in the same row and lowercase letters in the same column indicate significant differences ($p < 0.05$).

Conclusion

In this study, we aimed to evaluate the effect of MAP and silver/titanium dioxide nanocomposite films (with ratios of 35:65 or 25:75) under different storage conditions (25°C, 15°C, and 4°C) and durations (0, 7, 14, 21, and 28 days) regarding the chemical and microbial characteristics of grapes. Our findings show that the use of nanocomposite films containing silver/titanium dioxide particles and MAP techniques during the storage of red ruby grapes led to significant improvements compared to the control samples. A key result of this research emphasizes the effectiveness of using a MAP along with silver/titanium dioxide nanocomposite films (75:25 ratio) and keeping grapes at 4°C. This combination showed reduced mold formation and improved overall color retention. Overall, our research emphasizes the potential of modified atmosphere packaging and low-density polyethylene films with silver/titanium dioxide particles as effective antifungal measures for grape storage, thereby increasing food safety standards. In addition, their implementation promises significant benefits in terms of long-term durability and cost-effectiveness. This study was limited to a single grape variety and specific storage conditions, which may affect the generalizability of the results. Future research could explore the effects of these packaging strategies on different grape cultivars and other perishable fruits. Additionally, investigating the long-term environmental impact, potential migration of

nanoparticles into fruits, and consumer safety aspects would provide valuable insights for practical applications. Further studies could also optimize nanocomposite formulations and MAP gas compositions to enhance storage efficiency and shelf life.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest for the research, authorship, and/or publication of this article.

Author Contributions

Anahita Norouzi Tafreshi: conducted the experiments, collected and processed the data, and prepared a draft of the manuscript. **Shahla Shahriari:** supervised and designed the experiments, interpreted the data, contributed to the writing of the original draft, and revised the manuscript. **Toktam Mostaghim:** co-supervisor on this project; provided general guidance throughout the development of the work.

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بهبود ماندگاری انگور قرمز با استفاده از بسته‌بندی نانوکامپوزیتی پلی‌اتیلن و فناوری اتمسفر اصلاح‌شده

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چکیده

انگورهای یاقوتی قرمز به دلیل محتوای غنی آنتوسیانین و خواص تغذیه‌ای و دارویی شناخته شده‌اند، اما فسادپذیری بسیار بالا آنها ماندگاری‌شان را محدود می‌کند. تحقیقات نشان می‌دهد تکنیک‌های پیشرفته بسته‌بندی، مانند بسته‌بندی با اتمسفر اصلاح‌شده (MAP) و فناوری نانو می‌تواند کیفیت و ماندگاری این محصولات تازه را افزایش دهند. هدف این مطالعه بررسی استفاده ترکیبی از این فناوری‌ها برای حفظ کیفیت و افزایش ماندگاری انگورهای یاقوتی است. این مطالعه اثربخشی فیلم‌های بسته‌بندی پلی‌اتیلن حاوی نانوذرات نقره و دی‌اکسید تیتانیوم را در ترکیب با بسته‌بندی با اتمسفر اصلاح‌شده ($50\% \text{CO}_2 + 5\% \text{O}_2 + 45\% \text{N}_2$) ارزیابی کرد. آزمایش‌ها تحت سه شرایط دمایی (۲۵ درجه سلسیوس، ۱۵ درجه سلسیوس و ۴ درجه سلسیوس) در یک دوره نگهداری ۲۸ روزه انجام شد. نمونه‌ها در پنج بازه زمانی (۰، ۷، ۱۴، ۲۱ و ۲۸ روز) از نظر ویژگی‌های کیفی و بار میکروبی ارزیابی شدند. این رویکرد به هدف بررسی رشد میکروبی و کاهش کیفیت در انگورهای نگهداری شده در دماهای مختلف انتخاب شد. یافته‌ها نشان‌دهنده کاهش مداوم محتوای آنتوسیانین و شدت رنگ در طول دوره نگهداری، در کنار افزایش مواد جامد محلول بود. بررسی رشد میکروبی، تعداد کپک و مخمر بیشتری را در انگورهای نگهداری شده در دمای ۲۵ و ۱۵ درجه سلسیوس در مقایسه با انگورهای نگهداری شده در دمای ۴ درجه سلسیوس نشان داد. بسته‌بندی با اتمسفر اصلاح‌شده و فیلم‌های نانوکامپوزیتی حاوی نانوذرات نقره و دی‌اکسید تیتانیوم به‌طور مؤثری کیفیت انگور را به‌ویژه در دمای ۴ درجه سلسیوس حفظ کرد، که در طول ۲۸ روز نتایج بهتری مشاهده شد. این مطالعه، پتانسیل استفاده هم‌زمان بسته‌بندی با اتمسفر اصلاح‌شده و فیلم‌های با نانوذرات را برای رفع محدودیت‌های روش‌های سنتی نگهداری انگور نشان می‌دهد. این دستاورد جدید، راه‌حل‌های عملی برای صنعت باغبانی ارائه می‌دهد و راه را برای بهبود فناوری‌های ذخیره‌سازی و نگهداری هموار می‌کند.

واژه‌های کلیدی: انگور یاقوتی، بسته‌بندی با اتمسفر اصلاح‌شده، پلی‌اتیلن، نانوذرات نقره، نانودی‌اکسید تیتانیوم

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Research Article

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Heavy Metals in Some Vegetables, Soil and Water in Industrial and Non-Industrial Zones of Central Region of Iran

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Abstract

The presence of heavy metals in water, soil, and agricultural products is considered a significant threat to human health. This study aimed to evaluate different heavy metals such as Mercury (Hg), Lead (Pb), Arsenic (As), Cadmium (Cd), Zinc (Zn) and Cobalt (Co), in some vegetable varieties like cucumber, tomato and eggplant and also in the soil and water resources, in greenhouses around the mega industrial zones vicinity (1-10 Km) and comparing it with a greenhouse located in suburb of Isfahan, Iran. Measurement of heavy metals was carried out using ICP-OES in replicate. Concentrations of heavy metals in soil varied by region and generally following the descending pattern: Co > Pb > As > Hg > Zn > Cd. Heavy metals transfer factor were in the order of Cd > Hg > Zn > Pb > Co > As. According to the results, the mega industrial zones of Isfahan province showed a hazardous situation, and notably, heavy metal levels in the suburban region of Isfahan exceeded the health risk index (HRI). Based on the findings, it is recommended that producers adopt regular monitoring of irrigation and soil quality, apply phytoremediation and organic soil amendments to reduce heavy metal uptake, and select low-accumulating crop varieties in order to minimize health risks and ensure sustainable greenhouse production near industrial zones.

Keywords: Heavy metals pollution, ICP-OES, Health risk assessment, Industrial zones, Vegetable

Introduction

Heavy metals (HMs) contamination in soil and consequently in vegetable is considered a major health hazard. Several factors influence heavy metal uptake in plants, such as climatic conditions, environmental pollution, soil characteristics, and plant maturity at harvest (Voutsas, Grimanis, & Samara, 1996). Vegetables are an essential component of a healthy diet and provide vital nutrients such as potassium, fiber, folate, and vitamins A and E. In Iran, vegetable consumption is relatively high, with an average daily intake of 278 g,

which exceeding many other countries (Gharib, Fatoorechian, & Ahmadianiar, 2003). Therefore, assessing the accumulation of heavy metals (HMs) in food products is crucial for evaluating related health risks. Heavy metals, defined as elements with a specific density greater than 5 g/cm³, are harmful to the environment and living organisms (Järup, 2003). They are persistent in nature due to their long biological half-lives and persistence to degradation, resulting in their accumulation within human tissues over time (Singh, Sharma, Agrawal, & Marshall, 2010; Nabulo, Black, & Young,



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2011; Briffa, Sinagra, & Blundell, 2020; Radkhah, Eagderi, & Sadeghinejad Masouleh, 2022; Radkhah, Eagderi, & Atash Afrazah, 2025). Studies have confirmed that soil acidification can enhance Cd accumulation in vegetable tissues (Yang *et al.*, 2014). Greenhouse production, which is expanding globally, has also been associated with increased level of metals such as Cd and Zn in soil, particularly due to manure application in organic systems (Yang *et al.*, 2014; Cai *et al.*, 2015). HMs can adversely affect multiple organs, including the nervous, skeletal, circulatory, enzymatic, endocrine, and immune systems, while some are carcinogenic and impair nutrient absorption, leading to disorders and organ damage (Khan *et al.*, 2013). Sources of heavy metals in agricultural systems include industrial wastewater discharge, pesticide application, and other anthropogenic activities (Al-Hossainy, Mohamed, Hassan, & Abd Allah, 2017).

In Iran, several studies have investigated heavy metal accumulation in plants, water and environmental samples in order to determine significant levels of HMs in vegetables from contaminated farmlands, highlighting the potential risk to human health (Rezaei Malidareh, Shokrzadeh, Khasi, Rouhi, & Zaboli, 2016; Sadegh, Rezaei, & Sayadi Anari, 2019; Ranjbar, Najafpoor, & Dehghan, 2020; Kakouei Dinaki, Cheraghi, Lorestani, Sobhan Ardakani, & Chamani, 2021; Sabourmoghaddam, 2024). Similar concerns

have been raised in other countries: for instance, Rehman *et al.* (2018) measured Zn, Cr, Hg, Cu, and Ni in soil of Pakistan and found levels below critical limits, while Bi, Zhou, Chen, Jia, & Bao, (2018) reported differential transfer factors for Cd, Zn, Cu, As, Hg, and Pb in vegetables grown in China.

Isfahan, the third populated city in Iran, faces increasing industrial activity, which may contribute to environmental heavy metal pollution. Therefore, the present study aimed to determine the concentrations of heavy metals such as arsenic (As), cadmium (Cd), lead (Pb), zinc (Zn), cobalt (Co), and mercury (Hg) in cucumber, tomato, and eggplant, as well as in soil and water resources, from greenhouses located near mega-industrial zones of Isfahan province and a suburban reference site.

Materials and Methods

Study area

According to Fig. 1, the study area encompasses 4 greenhouses, with tomato (*Solanum lycopersicum*), cucumber (*Cucumis sativus*), and eggplant (*Solanum melongena*) cultivation. The first one was located at a distance of 3 km from the western gate of Mobarakeh Steel Company, the second one 8 km from Isfahan Steel company, the third one 1 km from Isfahan Cement Factory, the forth one 1 km from Sepahan Cement Factory and the fifth one 15 km from Isfahan in Dorcheh zone (control).



Fig. 1. Location of obtained samples near Mega-Factories and Dorcheh City

Sample Collection

Soil sampling: Soil samples (~500 g) composed of 10–15 sub-samples (each ~30–50 g), excluding the top 2–3 cm, were collected using an X-pattern sampling strategy and stored in clean, sealed plastic bags (Ferri *et al.*, 2015).

Water sampling: Samples from well water used for irrigation practices were collected in pre-cleaned 500 ml high-density polyethylene bottles with two replications from each site (Gebrekidan, Weldegebriel, Hadera, & Van der Bruggen, 2013).

Plant sampling: Four samples per vegetable variety (tomato, cucumber, and eggplant) were collected from each greenhouse, washed with distilled water, then dried, and stored in sealed polyethylene bags at (-20)°C for future analysis.

Pretreatment and Digestion Procedures

Soil Pretreatment and Digestion Procedures

All composite soil samples were thoroughly homogenized, oven-dried at 65°C for one day, and then sieved to obtain a particle size of 150µm. A 0.25g soil sample was weighed and 6 ml of concentrated nitric acid and 3 ml of concentrated hydrochloric acid were then added. The mixture was heated up to 210°C on a hot-plate until near drying. After cooling off, the sample was diluted to a specific volume (100 mL). Then 6 ml concentrated hydrochloric

acid added and the mixture was placed on a hot-plate until it became honey colored indicating dissolution of the elements. Finally, the resulting solution was diluted to the final volume of 250 mL (Ferri *et al.*, 2015; Beccaloni, Vanni, Beccaloni, & Carere, 2013).

Water Digestion Method for Injection

Water was filtered through syringe filters (pore size = 0.45µm) prior to analysis. This filtration step effectively removes particulate matter, ensuring that the water samples are suitable for ICP-OES analysis without additional digestion, pH adjustment was performed (by addition of 5 ml concentrated nitric acid or 2 ml of concentrated hydrochloric acid) before injection to ICP-OES instrument (Noli & Tsamos, 2016).

Plant Pretreatment and Digestion Procedures

After removal of inedible parts (except the skin), vegetables were rinsed with distilled water, excessive water was then removed and the vegetables were cut into smaller pieces and homogenized. For digestion, 2 g (wet basis) of each sample was placed in a polytetrafluoroethylene vessel with 5 mL of concentrated nitric acid in addition to 2 mL of hydrogen peroxide (Khillare, Jyethi, & Sarkar, 2012). This solution was then diluted to a final volume of 50 mL.

Transfer Factor

Transfer factor represents how readily a metal is absorbed by a specific plant from the surrounding soil environment. The transfer factor (TF) values from soil to plants were calculated using the following equation:

$$\text{Eq (1): } MTF = C_{\text{plant}} / C_{\text{soil}}$$

C_{plant} and C_{soil} indicate the levels of a given element in plant tissues and corresponding soil samples, respectively (Khan *et al.*, 2013).

Sample Analysis

Inductively coupled plasma optical emission spectrometry (ICP-OES) was employed to determine heavy metal concentrations in all collected samples (vista pro, Varian, Australia). The instrument was calibrated with a standard solution containing individual heavy metals.

Statistical Analysis

All statistical evaluations were initially tested for normal distribution and variance homogeneity using the Kolmogorov–Smirnov test. Where applicable, data were subjected to one-way analysis of variance (ANOVA). Further analysis was conducted using a General Linear Model (GLM) under a nested design framework, implemented via SAS software (version 9, Windows platform). Mean comparisons were performed using the Least Significant Difference (LSD) test at a significance level of $p < 0.05$. Additionally, Pearson correlation coefficients were computed to assess relationships between metal concentrations in soil, water, and plant samples.

Results and Discussion

Heavy metal concentrations varied significantly in soil samples and were determined in the following order: $\text{Co} > \text{Pb} > \text{As} > \text{Hg} > \text{Zn} > \text{Cd}$. Similar trend was found in water and plant as follow: $\text{Co} > \text{Pb} > \text{Hg} > \text{As} > \text{Zn} > \text{Cd}$. In water, Co and Cd

showed the highest and the lowest range, respectively. Our findings indicated that metal concentrations in plants are more influenced by levels in irrigation water compared to those in soil; possibly due to higher solubility and bioavailability of metals in water which was in agreement with other reports (Khan, Cao, Zheng, Huang, & Zhu, 2008).

Table 1 provides a summary of heavy metal concentrations measured in soil samples taken from the five greenhouse sites under investigation. The results show that the mean concentration of arsenic in soil samples collected from the five locations of the studied areas varied from 59.585 mg/kg to 82.372 mg/kg; the lowest belonging to the soil of Dorcheh, and the highest to Sepahan Cement Company. Sepahan Cement Factory and Dorcheh had the lowest mercury (26.96 and 33.499 mg/kg) and Isfahan Steel Company and Mobarakeh Steel company showed the highest (50.363 and 44.107 mg/kg) mercury concentration, respectively. Hg concentrations were found to be highest in the soils of Isfahan Steel and Mobarakeh Steel companies ($p < 0.05$). The Zn concentrations also varied in different sampling locations. Isfahan Cement Company had the lowest zinc concentration (4.114 mg/kg). The mean concentration of Co varied from 669.823 mg/kg to 674.405 mg/kg. The lowest as shown in Table 1 was from the soil of Isfahan Steel Company and the highest was from Dorcheh area but the differences were not significant ($p > 0.05$). Similarly, the highest amount of Cd was from Isfahan Cement Factory (1.065 mg/kg) (Table 1).

Data presented in Table 1 clearly showed that concentrations of heavy metals in the soil samples collected from the five different locations were not all the same. Heavy metal build-up in soil is caused by applications of agrochemicals, treated wastewater, and agricultural waste.

Table 1- Heavy metal concentrations (mg/kg) in the soil sampled from the study area

Study area	Pb	As	Hg	Zn	Co	Cd
ISC*	107.163±2.39 ^a	74.625±12.182 ^b	50.363±11.43 ^a	5.117±0.446 ^{ab}	669.823±3.542 ^b	0.279±0.215 ^c
MSC	108.235±0.758 ^a	71.831±7.374 ^b	44.107±20.806 ^{ab}	5.7±0.288 ^a	671.628±3.525 ^{ab}	0.394±0.234 ^{bc}
SCF	107.722±2.295 ^a	69.02±9.31 ^b	26.96±14.938 ^c	5.657±0.496 ^a	673.125±1.783 ^{ab}	0.819±0.618 ^{ab}
ICF	108.497±1.724 ^a	82.372±8.027 ^a	42.31±34.289 ^b	4.114±1.442 ^b	673.146±4.739 ^{ab}	1.065±0.568 ^a
D	108.656±2.471 ^a	59.585±4.125 ^c	33.499±15.156 ^c	4.739±0.203 ^{ab}	674.405±3.265 ^a	0.28±0.258 ^c

*ISC: Isfahan Steel Company ; MSC: Mobarakeh Steel company ;SCF: Sepahan Cement Factory; ICF: Isfahan Cement Factory ; D: Dorcheh

Results are mean ± SD. In each column means followed by the same letter are not significantly different at P<0.05 using LSD test.

Analysis of the variances (ANOVA) showed significantly different amounts of As, Hg and Zn in water from different areas. Concentration of the heavy metals found in the water used for irrigation in the studied areas are presented in Table 2. Analysis of Dorcheh water showed the presence of a higher amount of arsenic than other areas (0.076 mg/l). Isfahan Steel and Mobarakeh Steel companies had the lowest

amount of As (0.054 mg/l and 0.057 mg/l, respectively). Water used in the greenhouse in the Isfahan Cement Factory zone carried the highest amount of Hg (0.095 mg/l), and the water used in the greenhouse near Mobarakeh Steel Company had the highest amount of Zn (0.066 mg/l). There were no significant variations for Zn in other areas.

Table 2- Heavy metal concentration (mg/L) in water for irrigation sampled from the study area

Study area	Pb	As	Hg	Zn	Co	Cd
WISC*	0.11±0.003 ^a	0.054±0.007 ^c	0.072±0.003 ^c	0.008±0.001 ^b	0.675±0.006 ^{ab}	0.002±0.001 ^a
WMSC	0.112±0.002 ^a	0.057±0.01 ^{bc}	0.074±0.009 ^{bc}	0.066±0.007 ^a	0.68±0.006 ^{ab}	0.003±0 ^a
WSCF	0.111±0.004 ^a	0.06±0.011 ^b	0.078±0.005 ^b	0.029±0.003 ^b	0.679±0.005 ^{ab}	0.002±0.001 ^a
WICF	0.11±0.003 ^a	0.06±0.008 ^b	0.095±0.075 ^a	0.029±0.003 ^b	0.682±0.008 ^a	0.001±0.001 ^a
WD	0.11±0.004 ^a	0.076±0.021 ^a	0.075±0.006 ^{bc}	0.028±0.003 ^b	0.672±0.006 ^b	0.003±0.002 ^a

*WISC: Water used for irrigation in Isfahan Steel Company; WMSC: water used for irrigation in Mobarakeh Steel Company;

WSCF: water used for irrigation in Sepahan cement Factory; WICF: water used for irrigation in Isfahan cement Factory; WD: water used for irrigation in Dorcheh

Results are mean ± SD. In each column means followed by the same letter are not significantly different at p<0.05 using LSD test.

The level of the heavy metals in tomato, cucumber and eggplant collected from the study areas were analyzed. The results as shown in Table 3 indicate that the highest concentration of Pb was observed in eggplant (8.101 mg/kg) and the lowest in cucumber (1.922 mg/kg). The highest Arsenic was measured in eggplant (1.440 mg/kg). Cucumber and tomato carried comparatively lower levels

of As. Hg and Co in cucumber had the lowest amount (1.891 mg/kg) and (17.585 mg/kg), respectively. There was no significant difference in Hg and Co levels of tomato and eggplant. The mean concentration of Zn was the highest in tomato (0.544 mg/kg). The mean concentration of Cd was the highest in tomato (0.157 mg/kg) and the lowest in cucumber (0.044 mg/kg).

Table 3- Heavy metal concentration (mg/kg) in plant sampled from the study area

Vegetables	Pb	As	Hg	Zn	Co	Cd
Tomato	3.95±0.312 ^b	1.048±0.316 ^b	1.891±0.113 ^a	0.544±0.042 ^a	17.585±0.464 ^a	0.157±0.071 ^a
Cucumber	1.922±0.824 ^c	0.882±0.352 ^b	1.116±0.453 ^b	0.089±0.038 ^b	11.139±4.381 ^b	0.044±0.031 ^c
Eggplant	8.101±4.162 ^a	1.44±0.343 ^a	2.165±0.139 ^a	0.102±0.037 ^b	17.373±0.225 ^a	0.105±0.028 ^b

Results are mean ± SD. In each column means followed by the same letter are not significantly different at p<0.05 using LSD test.

The transfer factors from soil to plants for the examined vegetables were calculated; the results as presented in Table 4 show different

transfer factors for different vegetables and locations. Cd had the higher transfer factor in all vegetable varieties and ranged from 0.683 in

tomato cultivated near the Isfahan Steel Company to 0.005 in cucumber, which was cultivated near the Sepahan Cement Factory. Pb had the highest transfer factor in eggplants cultivated near Isfahan Cement Factory (0.122).

Tomato had the higher Zn transfer factor than eggplant and cucumber. Tomato cultivated in a green- house near Isfahan Steel Company had the highest Zn transfer factors (0.366).

Table 4- Transfer factors from soil to vegetables in study area

Study area	Plant	Pb	As	Hg	Zn	Co	Cd
ISC	Tomato	0.04±0.001 ^d	0.019±0.002 ^b	0.035±0.007 ^d	0.015±0.006 ^c	0.026±0 ^a	0.683±0.297 ^a
ISC	Cucumber	0.012±0 ^h	0.012± 0.002 ^{cde}	0.02±0.003 ^d	0.012±0 ^c	0.013±0.001 ^b	0.402± 0.078 ^{abc}
ISC	Eggplant	0.055±0 ^c	0.019±0.004 ^b	0.04±0.11 ^d	0.02±0.003 ^c	0.026±0 ^a	0.333±0.109 ^{abc}
MSC	Tomato	0.035±0 ^{de}	0.011± 0.001 ^{cde}	0.066± 0.011 ^{cd}	0.017±0.004 ^c	0.026±0.001 ^a	0.448± 0.026 ^{abc}
MSC	Cucumber	0.012±0 ^h	0.011± 0.001 ^{cde}	0.015±0.004 ^d	0.012±0.002 ^c	0.012±0 ^b	0.263±0.095 ^{abc}
SCF	Tomato	0.036±0 ^{de}	0.016± 0.001 ^{bcd}	0.042± 0.003 ^{cd}	0.366± 0.034 ^a	0.027± 0.0001 ^a	0.614±0.363 ^{ab}
SCF	Cucumber	0.019± 0.009 ^{gh}	0.016± 0.008 ^{bcd}	0.065± 0.034 ^{cd}	0.021±0.013 ^c	0.019±0.009 ^{ab}	0.005±0.002 ^c
SCF	Eggplant	0.097±0.002 ^b	0.017±0.002 ^{bc}	0.162±0.08 ^{ab}	0.028±0.006 ^c	0.026±0 ^b	0.28±0.232 ^{abc}
ICF	Tomato	0.033± 0.002 ^{def}	0.008±0 ^e	0.195±0.008 ^a	0.028±0.027 ^c	0.027±0.001 ^a	0.116± 0.065 ^{abc}
ICF	Cucumber	0.025±0.11 ^{fg}	0.009±0.00 ^{de3}	0.046±0.017 ^d	0.021±0.005 ^c	0.02±0.001 ^a	0.098±0.06 ^{bc}
ICF	Eggplant	0.122±0.001 ^a	0.022±0.001 ^{ab}	0.026±0.003 ^d	0.02±0.003 ^c	0.02±0.003 ^c	0.202±0.08 ^{abc}
D	Tomato	0.04±0.002 ^d	0.015±0 ^{bcd--}	0.129± 0.013 ^{bc}	0.079±0 ^b	0.026±0 ^{ab}	0.341± 0.106 ^{abc}
D	Cucumber	0.019± 0.009 ^{gh}	0.019±0.009 ^b	0.028±0.013 ^d	0.023±0.009 ^c	0.018±0.009 ^a	0.4±0.168 ^{abc}
D	Eggplant	0.028± 0.002 ^{efg}	0.028±0.003 ^{deg}	0.048±0.002 ^d	0.012±0.004 ^c	0.026±0 ^a	0.238± 0.124 ^{abc}

ISC: Isfahan Steel Company; MSC: Mobarakeh Steel Company; SCF: Sepahan Cement Factory; ICF: Isfahan Cement Factory; D: Dorcheh

Results are mean ± SD. In each column means followed by the same letter are not significantly different at p<0.05 using LSD test

In a study carried out by [Khan et al. \(2013\)](#), the concentration of different heavy metals namely Cd, Ni, Zn, Mn and Cr were measured in agricultural soil and crops in Pakistan ([Khan et al., 2013](#)). Contradictory to our results none of measured HMs were higher than Codex and Iran standard except for Cd (0.05 mg/kg). Maximum limit for Pb and Cd is 0.05 mg/kg of food samples like the plants of this study ([Codex No. 193](#); [National Standard No. 12968](#); [EC No. 1881](#)). Since Arsenic is used in pesticides formula and Pb is the major pollutant of steel companies, hence there levels are remarkably higher in Isfahan Province in comparison with Swat province of Pakistan (with no steel factory up to 100Km). Also, in comparison with the current study, [Rehman et al. \(2018\)](#) measured Zn, Cu, Ni, Mn and Cr concentrations in agricultural soils of Pakistan.

Although climate characteristics of Isfahan and Khyber province are approximately similar, results were totally different. All measured HMs by [Rehman et al. \(2018\)](#) were lower than the legal limit, however the current study proved that As, Hg and Cd were higher than legal limits in most of the zones. [Table 5](#) summarizes the amount of different heavy metals in vegetables cultivated in studied zones. Tomatoes cultivated in greenhouse near Sepahan Cement Factory had the highest value for Zn and Co. Eggplants picked up from the green house near Isfahan Cement Factory contained very high value of Pb. According to the results, food-crop cultivated on contaminated agricultural soils can easily absorb high amounts of heavy metals, which will cause entrance of HMs to human food chain.

Since different regions showed relatively different amounts of HMs, therefore, as a result

absorbed amount of HMs were different for each site.

Table 5- Evaluation of the interaction effects of Region and plant on heavy metals (mg/kg)

Region	Plant	Cd	Co	Zn	Hg	As	Pb
ISC	Tomato	0.13±0.04 ^{bcd}	17.33±0.02 ^a	0.07±0.02 ^c	1.9±0.04 ^{ab}	1.54±0.11 ^{ab}	4.18±0.07 ^{de}
ISC	Cucumber	0.06±0.01 ^{gh}	8.71±0.51 ^b	0.06±0 ^c	0.77±0.12 ^d	0.68±0.03 ^d	1.37±0.04 ^h
ISC	Eggplant	0.07±0.01 ^{gh}	17.29±0.08 ^a	0.1±0 ^c	2.15±0.13 ^a	1.53±0.27 ^{ab}	5.79±0.02 ^c
MSC	Tomato	0.26±0 ^a	17.26±0.23 ^a	0.1±0.03 ^c	1.81±0.05 ^{abc}	0.78±0.02 ^{cd}	3.82±0.03 ^{def}
MSC	Cucumber	0.04±0.01 ^{gh}	8.36±0.01 ^b	0.07±0.01 ^c	0.86±0.08 ^d	0.74±0.02 ^{cd}	1.34±0.02 ^h
SCF	Tomato	0.14±0.06 ^{bc}	18.05±0.66 ^a	2.12±0.18 ^a	1.94±0.14 ^{ab}	1.2±0.09 ^{bc}	3.78±0.13 ^{def}
SCF	Cucumber	0.01±0.003 ^h	12.53±3.89 ^{ab}	0.12±0.07 ^c	1.32±0.69 ^{bcd}	1.16±0.56 ^{bcd}	2.08±0.97 ^{gh}
SCF	Eggplant	0.11±0.002 ^{cde}	17.4±0.14 ^a	0.15±0.03 ^c	2.19±0.33 ^a	0.95±0.07 ^{cd}	10.53±0.02 ^b
ICF	Tomato	0.17±0.04 ^b	17.75±0.75 ^a	0.05±0.01 ^c	1.99±0.08 ^{ab}	0.74±0.02 ^{cd}	3.6±0.16 ^{def}
ICF	Cucumber	0.08±0.02 ^{defg}	13.63±4.5 ^{ab}	0.09±0.03 ^c	1.49±0.57 ^{abcd}	0.71±0.26 ^d	2.74±1.23 ^{fg}
ICF	Eggplant	0.14±0.04 ^{bc}	17.52±0.49 ^a	0.1±0.02 ^c	2.16±0.04 ^a	1.72±0.1 ^a	13.01±0.05 ^a
D	Tomato	0.07±0.01 ^{efg}	17.54±0.24 ^a	0.37±0.08 ^b	1.81±0.19 ^{abc}	0.97±0.08 ^{cd}	4.37±0.17 ^d
D	Cucumber	0.04±0.02 ^{gh}	12.47±3.87 ^{ab}	0.11±0.04 ^c	1.15±0.54 ^{cd}	1.12±0.54 ^{bcd}	2.08±0.97 ^{gh}
D	Eggplant	0.1±0.02 ^{cdef}	17.28±0.08 ^a	0.06±0.01 ^c	2.16±0.08 ^a	1.55±0.25 ^{ab}	3.08±0.07 ^{efg}

ISC: Isfahan Steel Company; MSC: Mobarakeh Steel Company; SCF: Sepahan Cement Factory; ICF: Isfahan Cement Factory; D: Dorcheh

Results are mean ± SD. In each column means followed by the same letter are not significantly different at p<0.05 using LSD test

Data presented in Table 6 show the Pearson correlation coefficients of heavy metals in soil and the plants studied in this research. Soil mercury and plant arsenic showed a positive and significant correlation of 0.521. Lead content in the plant had a positive and significant correlation with arsenic (0.466), mercury (0.639), and cobalt (0.520) in the plant.

Plant arsenic also had a positive and significant correlation with mercury (0.521) in soil and cobalt (0.464) in plant. The highest correlation between the elements measured in the plant was observed between mercury and cobalt (0.937). Mercury also showed a positive and significant correlation with cadmium (0.506).

Table 6- Pearson correlation coefficients of elements measured in soil and plants (tomato, eggplant and cucumber)

	Pb (soil)	As (soil)	Hg (soil)	Zn (soil)	Co (soil)	Cd (soil)	Pb (plants)	As (plants)	Hg (plants)	Zn (plants)	Co (plants)	Cd (plants)
Pb (soil)	1											
As (soil)	-0/310	1										
Hg (soil)	-0/036*	0/156	1									
Zn (soil)	0/010	-0/246	0/161	1								
Co (soil)	0/284	-0/407*	0/074	0/089	1							
Cd (soil)	0/109	0/421*	-0/389*	-0/112	-0/218	1						
Pb (plants)	-0/216	0/106	0/273	0/044	0/186	0/072	1					
As (plants)	-0/536**	0/113	0/521**	0/105	-0/158	-0/050	0/464*	1				
Hg (plants)	-0/335*	0/280	0/001	-0/114	-0/300	0/181	0/639**	0/618**	1			
Zn (plants)	-0/292	0/103	0/034	0/237	-0/061	-0/176	-0/026	0/078	0/165	1		
Co (plants)	-0/297	0/312	-0/084	-0/067	-0/263	0/171	0/520**	0/563**	0/937**	0/246	1	
Cd (plants)	-0/167	0/384*	-0/048	-0/088	-0/297	0/124	0/306	0/017	0/506**	0/147	0/569	1

* and ** are significant at 5 percent and 1 percent probability levels, respectively

Conclusion

In this study, different heavy metals namely Pb, As, Cd, Co, and Hg in cucumber (*Cucumis sativus*), eggplant (*Solanum melongena*), tomato (*Solanum lycopersicum*), water, and soil of greenhouses near (1–10 km) mega-industrial zones were determined and compared with a

greenhouse located in the suburb of Isfahan. The Pb values of the three vegetables examined in this experiment were above the standard limits. Eggplant showed the highest Pb content, which was 10 times higher than the standard level. Pb and Hg transfer factors in eggplant were higher compared to the other both studied

vegetables. Eggplant and tomato absorbed the highest amounts of Pb and Co, indicating higher transfer factors in these plants. Tomato absorbed the highest levels of Zn and Cd, indicating higher Zn and Cd transfer factors. Among the three studied vegetables, cucumber was less susceptible to heavy metal absorption. Based on these findings, it is recommended to monitor soil and irrigation water quality regularly, apply soil amendments and phytoremediation techniques to reduce heavy metal uptake, and select plant varieties with lower metal accumulation to minimize health risks. For future research, studies should focus on long-term monitoring of heavy metals in different crop varieties, air, water and soil, assessment of seasonal variations, and evaluation of mitigation strategies to improve food safety and environmental sustainability. These efforts will contribute to more informed

management practices in greenhouse production near industrial zones.

Author Contributions

Ashkan Nasresfahani: Study conception and design, data collection, draft manuscript preparation. **Nafiseh Zamindar:** Study conception and design, data collection, analysis and interpretation of results, Investigation, Validation, Supervision, draft manuscript preparation, Project administration, Conceptualization. **Saeed Paidari:** Writing – review & editing, Data curation. **Haniyeh Rezaee Barzani:** Interpretation of results, draft manuscript preparation, review & editing.

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فلزات سنگین در برخی سبزیجات، خاک و آب در مناطق صنعتی و غیرصنعتی منطقه مرکزی ایران

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چکیده

وجود فلزات سنگین در آب، خاک و محصولات کشاورزی تهدید قابل توجهی برای سلامت انسان به شمار می‌آید. هدف از این مطالعه، ارزیابی فلزات سنگینی همچون جیوه (Hg)، سرب (Pb)، آرسنیک (As)، کادمیم (Cd)، روی (Zn) و کبالت (Co) در برخی سبزیجات مانند خیار، گوجه‌فرنگی و بادمجان و همچنین در منابع خاک و آب گلخانه‌ها واقع در فاصله نزدیک (۱-۱۰ کیلومتر) از مناطق صنعتی بزرگ و مقایسه آن با یک گلخانه واقع در حومه استان اصفهان بود. اندازه‌گیری فلزات سنگین با استفاده از روش ICP-OES و با تکرار انجام شد. غلظت فلزات سنگین در خاک بر اساس منطقه متفاوت بود و به‌طور کلی الگوی نزولی $Cd > Hg > Zn > Pb > As > Co$ را نشان داد. فاکتور انتقال فلزات سنگین نیز به‌ترتیب $Cd > Hg > Zn > Pb > As > Co$ بود. نتایج نشان داد که مناطق صنعتی بزرگ استان اصفهان وضعیت مخاطره‌آمیزی دارند و قابل توجه است که سطح فلزات سنگین در منطقه حومه اصفهان نیز از شاخص ریسک سلامت (HRI) فراتر رفته است. بر اساس یافته‌ها، پیشنهاد می‌شود که تولیدکنندگان نظارت منظم بر کیفیت آب آبیاری و خاک را انجام دهند، از روش‌های گیاه‌پالایی و بهبود دهنده‌های آلی خاک برای کاهش جذب فلزات سنگین استفاده کنند و گونه‌های گیاهی کم‌جذب را انتخاب نمایند تا خطرات سلامت کاهش یابد و تولید پایدار در گلخانه‌های نزدیک مناطق صنعتی تضمین شود.

واژه‌های کلیدی: آلودگی فلزات سنگین، سبزیجات، شاخص ریسک سلامت، مناطق صنعتی، ICP-OES

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Research Article

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Effects of Cold Plasma and Nano-zinc Oxide on Modification of Corn Starch as a Food Packaging Material

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Abstract

This study explores the effects of cold plasma (CP) treatment on eco-friendly starch/ZnO (SZ) bio-nanocomposite films, incorporating 3 wt% nano-ZnO (ZnO NPs) and varying CP exposure times (0, 30, 60, 90 s). The results indicate that prolonged CP treatment increases film thickness, viscosity, water solubility, moisture absorption, and surface roughness, while reducing contact angle and moisture content. No significant changes were observed in water vapor permeability, density, or UV-Vis properties. CP treatment enhanced tensile strength, elongation at break, and tensile energy to break, while decreasing lightness and whiteness indices without altering color difference. The study highlights CP as a rapid, eco-friendly method for modifying films, with greater efficacy when applied to aqueous starch solutions, offering potential for industrial-scale applications in packaging materials.

Keywords: Cold plasma, Dual-modification, Green packaging, Nanocomposite, Starch, ZnO

Introduction

Packaging serves a vital function in preserving the integrity of food products, prolonging their shelf life, conveying essential information to consumers, and ensuring efficient distribution and transportation. These roles underscore its significance in maintaining food quality, safety, and accessibility throughout the supply chain (Venkateshaiah *et al.*, 2021). However, the widespread reliance on petroleum-derived plastics in the packaging sector has resulted in persistent environmental contamination, as these materials are neither biodegradable nor compostable. This has significantly contributed to the accumulation of

non-degradable waste, posing serious ecological challenges (Cheng *et al.*, 2021). To address this issue, researchers have been focusing on developing biodegradable alternatives, with starch being considered one of the most promising biopolymers due to its abundance, availability, cost-effectiveness, and film-forming properties (Shahabi-Ghahfarrokhi, Almasi, & Babaei-Ghazvini, 2020).

Despite its advantages, starch-based packaging materials have limitations such as high sensitivity to moisture, excessive water vapor permeability (WVP), and poor mechanical properties (Babaei-Ghazvini,



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Shahabi-Ghahfarrokhi, & Goudarzi, 2018; Goudarzi, Shahabi-Ghahfarrokhi, & Babaei-Ghazvini, 2017). To overcome these drawbacks, various approaches have been explored, including incorporation with other biopolymers (Goudarzi & Shahabi-Ghahfarrokhi, 2018a), chemical modification (Wihodo & Moraru, 2013), the use of different plasticizers (Almasi, Ghanbarzadeh, & Entezami, 2010), nanocomposite formation (Goudarzi & Shahabi-Ghahfarrokhi, 2018a; Goudarzi *et al.*, 2017; Shahabi-Ghahfarrokhi & Babaei-Ghazvini, 2019), UV irradiation (Goudarzi & Shahabi-Ghahfarrokhi, 2018b; Shahabi-Ghahfarrokhi, Goudarzi, & Babaei-Ghazvini, 2019), and γ -irradiation (Ali & Ghaffar, 2017). Among these strategies, the integration of nanoparticles into the biopolymer matrix has demonstrated significant potential in enhancing the functional properties of starch-based nanocomposites. Achieving optimal performance relies heavily on the uniform dispersion of nanoparticles within the matrix, as the aggregation of nanoparticles can compromise the overall efficiency and effectiveness of the nanocomposites (Babaei-Ghazvini *et al.*, 2018; Goudarzi & Shahabi-Ghahfarrokhi, 2018a, 2018b; Salarbashi *et al.*, 2016; Shahabi-Ghahfarrokhi & Babaei-Ghazvini, 2019; Shahabi-Ghahfarrokhi, Khodaiyan, Mousavi, & Yousefi, 2015b). Moreover, nanocomposites with antimicrobial properties (Salarbashi *et al.*, 2016) and UV-barrier properties (Babaei-Ghazvini *et al.*, 2018; Goudarzi *et al.*, 2017; Shahabi-Ghahfarrokhi *et al.*, 2015b) have emerged as active packaging technology.

ZnO NPs offers several desirable properties, including photoactivity, antimicrobial activity, cost-effectiveness, non-toxicity, UV protection, and biocompatibility, making it suitable for active food packaging applications (Babaei-Ghazvini *et al.*, 2018; El Fawal *et al.*, 2020; Liu *et al.*, 2021; Salarbashi *et al.*, 2016; Shahabi-Ghahfarrokhi & Babaei-Ghazvini, 2019; Shahabi-Ghahfarrokhi *et al.*, 2015b). The nutritional quality, flavor, and safety of food can be compromised by UV radiation through

the process of lipid oxidation, which results in the development of toxic compounds. By using UV-protective food packaging materials, the need for synthetic antioxidants can be reduced (Shahabi-Ghahfarrokhi *et al.*, 2015b).

However, the migration of nanoparticles into food and the potential ingestion of these nano compounds raise concerns. To minimize the risk, researchers have proposed enhancing the compatibility between the nanoparticles and the polymer matrix (Souza & Fernando, 2016). A previous study used UV rays to improve the compatibility of ZnO NPs in a bio nanocomposite (Shahabi-Ghahfarrokhi & Babaei-Ghazvini, 2019); however, this process is time-consuming and unsuitable for industrial-scale production.

Plasma, characterized as a quasi-neutral ionized gas and recognized as the fourth state of matter, has gained prominence as an environmentally sustainable technology with diverse applications. These include food sanitization, biomedical advancements, semiconductor manufacturing, and innovative packaging solutions (Dimitrakellis & Gogolides, 2018). Plasma exists as hot and CP. Thermal plasma is a state of plasma in which all constituent species—electrons, ions, and neutrals—exist in a condition of local thermodynamic equilibrium, resulting in a single, uniform temperature that can exceed 10,000 K. In contrast, non-thermal plasma, often referred to as CP, is characterized by a significant thermodynamic non-equilibrium state. In this state, electrons possess a very high temperature (energy), while heavy particles, such as ions and neutral species, remain close to ambient temperature (Sharma, 2020). CP is a particularly promising technique due to its cost-effectiveness, non-thermal nature, ease of access, and environmental friendliness. CP can be used to modify biopolymers and improve their properties, such as adhesion and printability (Heidemann, Dotto, Laurindo, Carciofi, & Costa, 2019). Key factors that influence the CP process include treatment duration, feed gas, electric field strength, and the applied electrical power (Maniglia,

Castanha, Rojas, & Augusto, 2021). Although CP has been utilized in prior study to modify starch-based films, the process has been reported to be time-intensive (Goiana *et al.*, 2021), often requiring treatment times ranging from 10 to 20 min. Therefore, for industrial applications, a rapid and efficient CP procedure is necessary.

This study employed CP as a rapid (<90 s) and environmentally benign treatment to modify starch-based films, thereby improving the compatibility of ZnO NPs with the biopolymer matrix. The resulting modified SZ bionanocomposite was investigated as a food packaging material. This approach provides a promising pathway for the design and production of eco-friendly packaging materials with enhanced performance characteristics.

Materials and Methods

Materials

Corn starch, was sourced from Zarrin Corn Company (Shahrud, Iran). ZnO NPs with average size of 30–40 nm was acquired from Tecnan (Spain). High-purity analytical grade reagents were procured from commercial suppliers as follows: magnesium nitrite and glycerol were obtained from Merck KGaA (Darmstadt, Germany), while sodium chloride and calcium chloride were sourced from Dr. Mojalali Co. (Tehran, Iran).

Film Preparation

A 5% (wt%) starch solution was prepared in distilled water (DW) and heated to 80°C while stirring continuously for 30 min using a heater magnetic stirrer. Glycerol, added at 45% of the dry weight, was incorporated as a plasticizer and stirred for an additional 10 min to improve the flexibility of the films. A suspension of ZnO NPs was prepared in DW at a final concentration of 3% (w/w, dry basis). The suspension was stirred for 10 min and subsequently sonicated for 30 min in an ultrasonic bath (vCLEAN1 – L2, Viera Tejarat Backer Co, Iran). This ZnO NP suspension was then introduced gradually into the film-forming solution under continuous stirring for 10 min. The resulting mixture was placed 2.5 cm from the nozzle of a jet CP system (Plasmatech-18A, Super arc plasma, Iran). Using air as the feed gas at a power output of 2100 W, the solution was subjected to plasma irradiation (Fig. 1) while being agitated continuously with a magnetic stirrer to ensure homogeneity. Following exposure to CP for 30, 60, and 90 s, the film-forming solutions were cast onto level, non-sticking polystyrene plates (15 cm diameter) and dried for 48 h at ambient temperature. The resulting films were subsequently peeled from the casting surface. Prior to analysis, all samples were conditioned for 48 h at 50–55% relative humidity (RH).



Fig. 1. The experimental setup for the cold plasma irradiation of starch/ZnO NPs film-forming solutions using a jet cold plasma system

Physical Characteristics Microstructure

The surface and cross-sectional morphology of the film specimens, along with the dispersion of ZnO NPs, were characterized using a field emission scanning electron microscope (FE-SEM; TESCAN MIRA3, Czech Republic). To prepare cross-sectional views, the films were cryogenically fractured in liquid nitrogen. All samples were mounted on aluminum stubs with double-sided carbon tape and sputter-coated with a thin layer of gold to ensure electrical conductivity prior to imaging.

Intrinsic Viscosity

The intrinsic viscosity, $[\eta]$, of the samples was determined by extrapolating the reduced and inherent viscosity to infinite dilution using the Huggins (Eq. 1) and Kraemer (Eq. 2) equations (Ghasemlou, Khodaiyan, Jahanbin, Gharibzahedi, & Taheri, 2012; Goudarzi &

Shahabi-Ghahfarrokhi, 2018b). Intrinsic viscosity was measured in triplicate.

$$\frac{\eta_{sp}}{C} = [\eta] + k_H [\eta]^2 C \quad (1)$$

$$\frac{\ln(\eta_{rel})}{C} = [\eta] + k_K [\eta]^2 C \quad (2)$$

The intrinsic viscosity ($[\eta]$), specific viscosity (η_{sp}), and relative viscosity (η_{rel}) are fundamental parameters in this analysis. The values of η_{sp} and η_{rel} were calculated using Eq. (3) and (4), respectively. The Huggins (K_H) and Kraemer (K_K) coefficients, alongside the polymer concentration (C), are key constants in the respective models. Measurements of intrinsic viscosity were conducted at a constant temperature of 24 °C using a Cannon-Fenske routine capillary viscometer.

$$\eta_{rel} = \frac{t_{slu}}{t_{slv}} \quad (3)$$

$$\eta_{sp} = \eta_{rel} - 1 \quad (4)$$

Where t_{slv} and t_{slu} are average efflux time of water and aqueous solution of polymer, respectively.

Density

The density of the film samples was determined in triplicate using a pycnometric method with n-hexane as the displacement solvent. The procedure was conducted at a constant ambient temperature of 20 °C. A clean, dry pycnometer was first weighed to obtain its tare mass (W_p). Several pieces of the film were then introduced into the pycnometer, and the combined mass was recorded (W_{ps}). The pycnometer containing the film was subsequently filled with n-hexane, ensuring complete immersion of the samples, and weighed again (W_{psh}). Following this, the clean, dry pycnometer was filled solely with n-hexane and weighed (W_{ph}). Finally, the pycnometer was filled with DW and weighed (W_{pw}) to allow for calibration, given the known density of DW (0.9982 g/cm³ at 20 °C). The densities of n-hexane (D_h) and the film samples (D_s) were subsequently calculated using Eqs. (5) through (7).

$$D_h = \frac{(w_{ph} - w_p)}{(w_{pw} - w_p)/D_w} \quad (5)$$

$$V_s = \frac{(w_{ph} - w_p) - (w_{psh} - w_{ps})}{D_h} \quad (6)$$

$$D_s = \frac{(w_{ps} - w_p)}{V_s} \quad (7)$$

Thickness

The thickness of the films was measured by a routine micrometer with an accuracy of 0.01 mm at 10 random points (Salarbashi et al., 2017).

Moisture Content

To determine the moisture content (MC), film samples were dried in a laboratory oven (Pars Azma Co., Iran) at a constant temperature of 105 ± 1 °C. The mass loss was measured, and the moisture content was subsequently calculated in triplicate using Eq. (8).

$$MC (\%) = \frac{(m_1 - m_2)}{m_1} \times 100 \quad (8)$$

The initial weight of the specimen was recorded as m_1 , while the weight of the specimen following oven drying was recorded as m_2 .

Film Solubility in Water

Water solubility (SW) was measured in triplicate and defined as the percentage of water-soluble dry matter lost from the initial dry weight of the film. Briefly, 2 cm × 2 cm sections of each film were prepared and dried at 105°C until a constant mass (m_3) was achieved. Subsequently, the dried samples were immersed in 50 mL of DW and agitated gently for 6 h at a controlled temperature of 25°C (Hassannia-Kolaei, Khodaiyan, Pourahmad, & Shahabi-Ghahfarrokhi, 2016). Following immersion, the films were dried again at 105 ± 1°C until a constant weight (m_4) was achieved. The SW of each specimen was determined in triplicate using Eq (9).

$$SW(\%) = \frac{(m_3 - m_4)}{m_4} \times 100 \quad (9)$$

Moisture Absorption

The moisture absorption (MA) properties of the films were determined gravimetrically according to a standard protocol (Goudarzi et al., 2017). Film samples (2 cm × 2 cm) were first desiccated over calcium chloride (0% RH) until a constant initial mass (m_5) was achieved. Subsequently, the samples were equilibrated in a desiccator containing a saturated magnesium nitrate solution, maintaining a RH of 50–55%, until a constant final mass (m_6) was attained. The experiment was conducted in triplicate. MA was then calculated using Eq. (10), based on the mass difference before and after conditioning (Goudarzi et al., 2017).

$$MA (\%) = \frac{(m_6 - m_5)}{m_5} \times 100 \quad (10)$$

Contact Angle

Surface wettability is characterized by static water contact angle measurements, determined by the sessile drop method. In this procedure, a 5 µl droplet of distilled water was dispensed onto the sample surface. A profile image of the droplet was then acquired with a digital

microscope (AM2111, Dino-Lite, Taiwan) employing backlight illumination to ensure high contrast. The contact angle was then calculated using the CA plugin in ImageJ software (version Java 1.8.0_172) (Goudarzi & Shahabi-Ghahfarokhi, 2018a). This was performed in triplicate.

Water vapor permeability

The water vapor permeability (WVP) of the films was evaluated in triplicate using the container weight change method, following the ASTM E96-95 standard (Standard, 1989). Glass vials, fabricated to precise specifications (13 mm internal diameter × 45 mm height), were prepared as permeation cells. Each vial contained a charge of calcium chloride desiccant to maintain a 0% RH environment within the internal headspace. Film specimens were mounted hermetically over the mouth of each vial using a double-sided adhesive tape. The prepared assemblies were then equilibrated in a controlled-humidity desiccator, which contained a saturated sodium chloride solution to maintain a constant 75% RH atmosphere. The mass of each vial assembly was monitored gravimetrically to track water vapor permeation. Measurements were recorded periodically using an analytical balance with a precision of ±0.0001 g. Data acquisition was performed at hourly intervals for the initial 8-hour period and continued thereafter until a steady-state mass gain was observed, indicating the attainment of diffusion equilibrium. The steady-state water vapor flux was determined from the linear portion of the mass gain versus time plot, where the slope (S) was calculated by linear regression analysis. The water vapor transmission rate (WVTR) and the WVP were then derived from this slope using Equations (11) and (12), respectively.

$$WVTR = \frac{S}{A} \quad (11)$$

$$WVP = \frac{WVTR \times X}{\Delta P} \quad (12)$$

where A denotes the effective film area (m²), X represents the mean film thickness (m), and ΔP is the vapor pressure differential (1753.55 Pa).

Mechanical Properties

The mechanical properties of the films—specifically tensile strength (TS), elongation at break (EB), and tensile energy to break (TEB)—were evaluated in accordance with ASTM standard method D882-02 (Standard, 2004). Measurements were performed in triplicate using a universal testing machine (STM-5, Santam Co., Iran). Prior to testing, rectangular film specimens (100 mm × 10 mm) were conditioned for at least 48 hours at 50–55% RH within a desiccator charged with a saturated magnesium nitrate solution. For analysis, specimens were mounted in the grips with an initial gauge length of 70 mm and elongated at a constant crosshead speed of 50 mm/min. The resultant data were used to calculate the mechanical properties based on Equations (13) through (15).

$$TS = \frac{F_{Max}}{A_{Min}} \quad (13)$$

$$EB = \frac{L_{Max}}{L_0} \times 100 \quad (14)$$

$$TEB = A_{Stress-Strain} \quad (15)$$

Where F_{max} is the maximum load (N), A_{min} is the minimum original cross-sectional area (mm²), L_{max} is the elongation at fracture (mm), L_0 is the initial gauge length (mm), and $A_{Stress-Strain}$ is the area beneath the engineering stress-strain curve (MPa), representing the material's toughness.

Color

The color properties of the film samples were analyzed using a portable colorimeter (TES 135A, Electronic CORP, Taiwan) in triplicate. The film specimens were placed on a white standard plate, which provided the calibration background ($L^* = 96.93$, $a^* = -0.95$, $b^* = 1.86$) (Babaei-Ghazvini *et al.*, 2018). From these measurements, the whiteness index (WI) and the total color difference (ΔE) were computed in accordance with Eq. (17) and (16), respectively.

$$WI = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (16)$$

$$\Delta E = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2} \quad (17)$$

UV-Vis Spectroscopy

The ultraviolet-visible (UV-Vis) absorption profiles of the nanocomposite films were conducted using a SPECORD 250 UV/VIS spectrophotometer (Analytik Jena, Germany). Spectra were recorded across a wavelength range of 190–800 nm. For analysis, the films were sectioned into 1×2 cm² segments and mounted in the sample holder against an empty quartz cuvette, which was used to establish the baseline reference. All measurements were performed in triplicate.

Statistical Analysis

The experimental data, arranged in a completely randomized design, were subjected to a one-way analysis of variance (ANOVA) using SPSS software (v. 23, SPSS Inc., USA). To compare mean values across treatment groups, Duncan's multiple range test was applied, with statistical significance established at the $p < 0.05$ level.

Results and Discussion

Microstructure

The microstructure of the film specimens was investigated using FE-SEM. Fig. 2 and Fig. 3 show microstructure of the surface and the cross-section of SZ and cold plasma-irradiated starch/ZnO (CPSZs) at different exposure times of 30 s, 60 s, and 90 s. The surface of the SZ films is almost uniform with homogenous distribution of ZnO NPs on it, while the ZnO NPs spots were not clear in the cross-section of the SZ films. On the other hand, the etched surfaces by CP have made the rougher surface on the CPSZs compared to the SZ film and created some cavities and fine particles on them. The etching effect on the starch solution was caused by the bombardment of energetic CP species. These species include electrons, ions, radicals, and UV radiation. The interaction of these energetic CP components with the starch solution led to an etching or

eroding effect on the starch (Chen *et al.*, 2020; S.K. Pankaj *et al.*, 2015). The roughness of the CPSZs films is drastically higher than the virgin SZ film which agrees with previous studies (Bastos, Santos, da Silva, & Simão, 2009; Heidemann *et al.*, 2019). Although the formation of etching on biopolymeric films in the solid state has been reported (Chen *et al.*, 2020; S.K. Pankaj *et al.*, 2015), the formation of etching in the liquid state is confusing. We also observed this phenomenon in our previous report (Bayanloo, Shahabi-Ghahfarrokhi, & Hagh Nazari, 2024). It seems that CP created a new structure in the matrix of the bionanocomposite. The new structure is a long chain (Table 1-Intrinsic viscosity) with low density (Table 1-Density) and appropriate affinity with the matrix of the bionanocomposite (Fig. 2 and Fig. 3). On the other hand, the new SZ structure was broken down to a finer structure with increasing plasma processing times, which is clearly visible in Fig. 3.

Intrinsic Viscosity

The intrinsic viscosity of a polymer is influenced by factors such as its average molecular weight, molecular rigidity (determined by bond types and quantity), and molecular configuration (e.g., random coil or rod-like structures) (Momeni *et al.*, 2018). These changes offer valuable information regarding the molecular size and structural characteristics of the polymer.

As shown in Table 1, the intrinsic viscosity of the film-forming solutions increased significantly with longer plasma treatment times. This suggests that reactive plasma species facilitated the formation of a higher molecular weight polymer network in the CPSZs solutions compared to the untreated SZ control.

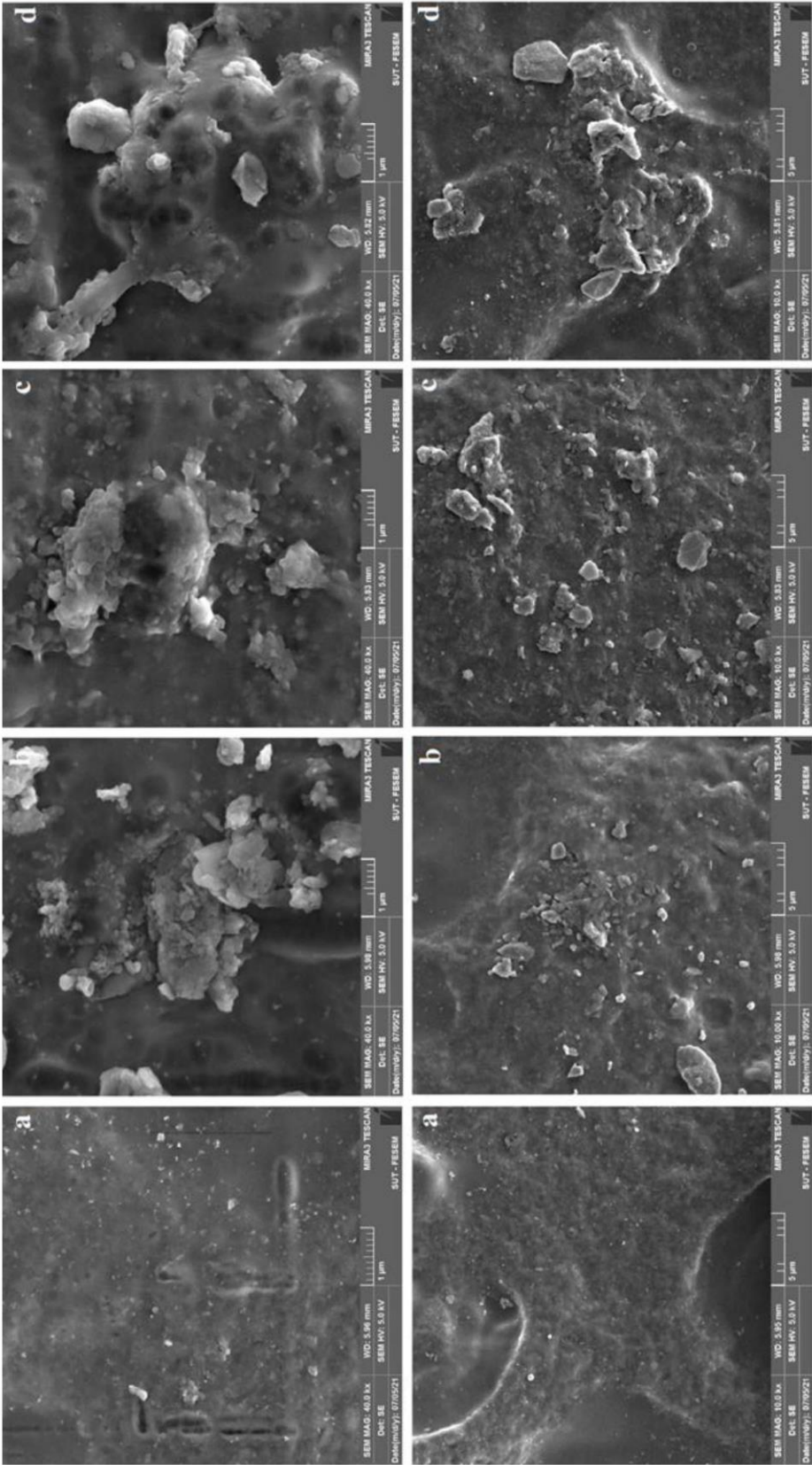


Fig. 2. The surface microstructure of starch/ZnO NPs (SZ) (a) and modified SZ treated with cold plasma at varying exposure times of 30 s (b), 60 s (c), and 90 s (d), shown at different magnifications

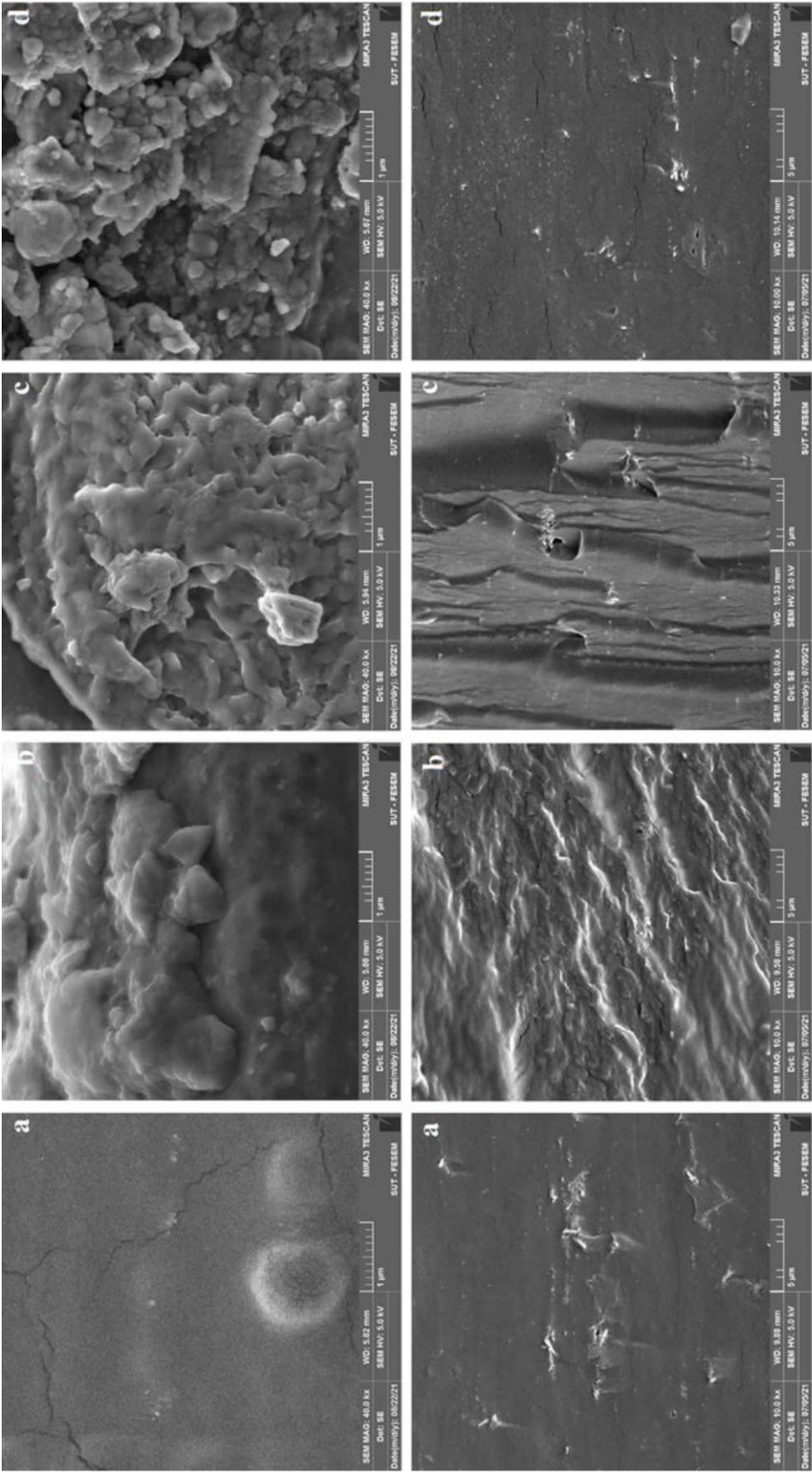


Fig. 3. The cross-sectional microstructure of starch/ZnO NPs (SZ) (a) and modified SZ subjected to CP at exposure times of 30 s (b), 60 s (c), and 90 s (d), depicted at different magnifications.

These results align with previous studies demonstrating that the rheological modifications imparted by CP on biopolymers are contingent upon factors including the biopolymer's origin, the plasma generator design, and the working gas composition (Momeni *et al.*, 2018; Shahabi-Ghahfarrokhi, Khodaiyan, Mousavi, & Yousefi, 2015a; Sheikhi *et al.*, 2021). However, they contradict our previous studies, which employed UV (Goudarzi & Shahabi-Ghahfarrokhi, 2018a, 2018b; Shahabi-Ghahfarrokhi & Babaei-Ghazvini, 2019; Shahabi-Ghahfarrokhi *et al.*, 2019) and gamma ray (Shahabi-Ghahfarrokhi *et al.*, 2015a) methods for biopolymer modification. Unlike the UV and gamma ray approaches, which reduced the molecular weight of the biopolymers, the current study using CP resulted in an increase in molecular weight.

Density

Polymer density is a function of multiple critical parameters. These include the molecular weight of the constituent chains, the material's crystallinity, the packing efficiency of the polymer segments, and the properties of incorporated particles, such as their size, distribution, and morphological shape (Bulbul, Bhushette, Zambare, Deshmukh, & Annapure, 2019). A reduction in the density of biopolymers is often associated with an increase in free volume or voids within their structural framework (Mohammadi-Alamuti, Shahabi-Ghahfarrokhi, & Shaterian, 2023). The densities of SZ and CPSZs are presented in Table 1., no significant difference was observed between samples. Previous studies have shown that the density of CP-modified starch and xanthan-based films decreased with longer processing times, up to 20 min. This effect was observed across treatments applied at two different power levels (50 W and 60 W) (Bulbul *et al.*, 2019; Honarvar *et al.*, 2017). It appears that the high density of ZnO NPs counteracted the density reduction observed in modified starch through CP treatment.

Contact Angle

The CA between a water droplet and a film surface serves as an indicator of surface wettability (Honarvar *et al.*, 2017). The Wenzel model is commonly applied to describe CA behavior on rough surfaces with chemical homogeneity (Wenzel, 1936). According to this model, surface roughness intensifies the intrinsic hydrophilic or hydrophobic characteristics of the surface. Specifically, roughness can enhance either wetting or non-wetting behavior, depending on the surface's chemical properties (Cassie & Baxter, 1944; Seo & Kim, 2015).

The CA values for water droplets on the film surfaces are presented in Table 1. A significant reduction in CA was observed for SZ films as the plasma processing times increased. In line with the Wenzel model, the development of a rough surface (Fig. 2) and the modification of the surface chemical structure of SZ through CP treatment contributed to this phenomenon. CP treatment typically results in a notable increase in surface free energy, primarily due to the introduction of polar functional groups on the polymer surface (Pankaj & Thomas, 2016). Additionally, CP generated in an air atmosphere is particularly effective in reducing CA, as the radicals formed on the polymer surface react rapidly with oxygen-containing species in the air (De Geyter, Morent, Leys, Gengembre, & Payen, 2007). These observations align with prior studies investigating the impact of CP treatment on polymer surface wettability (Bayanloo *et al.*, 2024; Ulbin-Figlewicz, Zimoch-Korzycka, & Jarmoluk, 2014; Wiącek, Jurak, Gozdecka, & Worzakowska, 2017).

Thickness

Film thickness is an essential parameter when calculating the mechanical properties and WVP of films. The thickness of a film is dependent on the preparation methods used to make the film, the drying conditions applied during the film formation process, (Hosseini,

Rezaei, Zandi, & Farahmandghavi, 2015) and biopolymers type. Table 1 shows the thickness of the SZ nanocomposite before and after CP irradiation. The thickness of the films was significantly increased by plasma processing times. As shown in Fig. 3, a porous structure was formed by CP in SZ nanocomposites, lead to extension of the thickness of the CPSZs. This change was also observed in previous studies (Li et al., 2011; Wu et al., 2020).

Moisture Content

Table 1 presents the MC values for SZ and CPSZs. The MC of the films decreased after undergoing CP treatment. This reduction in MC was found to be independent of plasma processing times. MC is a parameter that indicates the total void volume occupied by water molecules in the microstructure of biopolymers (Li et al., 2011). However, it appears that the porous structure formed in CPSZs facilitates the escape of moisture during the drying process of the films. This observation aligns with the formation of a porous microstructure (Fig. 3) and the decrease in CA as indicated in Table 1, which was also observed in previous studies (Moosavi et al., 2020; Moradi et al., 2020).

Solubility in Water

The SW is a critical factor influencing the suitability of films for packaging applications, as these films often serve as protective layers for food products (Moosavi et al., 2020). Table 1 presents the SW values for SZ and CPSZs films. The SW of CPSZs films exhibited an increase compared to SZ films, a trend that was independent of plasma processing times. This change can be attributed to the formation of particles through CP etching and the development of a porous microstructure (Fig. 2 and Fig. 3), which enhanced the affinity between water and the biopolymer. This observation is consistent with the changes in CA (Table 1) observed with increasing plasma processing times. The formation of hydrophilic groups on the film surface likely contributed to the increased SW of the biopolymers. Factors

such as nanoparticle content, nanoparticle type, biopolymer type, and the gas used for CP generation appear to influence the water sensitivity of bionanocomposites. Furthermore, the ratio of crystalline to amorphous structures disrupted by CP treatment plays a significant role in modifying the moisture sensitivity of biopolymers, as supported by previous studies (Sheikhi et al., 2021).

Moisture Absorption

The water resistance properties of packaging materials are critical in influencing the shelf life of food products (Dong et al., 2018). Consequently, numerous studies have investigated the MA of biopolymers, with a primary focus on improving water resistance (Goudarzi & Shahabi-Ghahfarrokhi, 2018a; Goudarzi et al., 2017; Hassannia-Kolae, Khodaiyan, Pourahmad, et al., 2016; Hassannia-Kolae, Khodaiyan, & Shahabi-Ghahfarrokhi, 2016; Shahabi-Ghahfarrokhi et al., 2015a). Table 1 presents the MA values for SZ and CPSZs films. The MA of the films increased following CP treatment, though no significant differences were observed across varying plasma processing times. The hydrophilicity of the films is largely determined by surface morphology, surface chemical composition (including the presence of polar groups), and surface free energy (S. Pankaj et al., 2015). Hence, these polar groups make the surfaces of plasma-treated polymer more hydrophilic compared to the virgin polymer surface (Yang, Chen, Guo, & Zhang, 2009). It appears that the formation of polar groups through CP and the porous microstructure of CPSZs (Fig. 2 and Fig. 3) contribute to this change. This interpretation is supported by the corresponding CA results, which is in agreement with previous studies (Bayanloo et al., 2024).

Water Vapor Permeability

Starch-based films are hydrophilic, leading to high WVP due to water clustering and diffusion through micro-cavities. This high WVP, driven by vapor pressure differences and

the film's inherent structure, is a major limitation for commercial applications like food packaging. Effective packaging requires films that act as barriers to humidity exchange, a property measured directly by WVP testing (Bayanloo *et al.*, 2024; S.K. Pankaj *et al.*, 2015; Romani *et al.*, 2019).

Table 1 shows WVP of the SZ and CPSZs. There was no significant difference between the WVP of SZ and CPSZ. It was anticipated that the porosity, roughness of the surface and cross-section (as depicted in Fig. 2 and Fig. 3), and the formation of polar groups on the biopolymer surface through CP treatment (Table 1-CA) would lead to an increase in WVP. The use of ZnO NPs seems to fill voids and pores within the biopolymer matrix, and create an indirect path for water molecules to pass through. Consequently, the effects of porosity and roughness are nullified. Similar trends have been reported in prior studies (S.K. Pankaj *et al.*, 2015).

Mechanical Properties

The mechanical properties of biopolymers, specifically strength and flexibility, are critical for preventing structural failure and maintaining product integrity (Ghanbarzadeh & Oromiehi, 2009). These properties were evaluated by measuring TS (the maximum stress before fracture), EB (a measure of flexibility), and TEB (toughness or the total energy absorbed per unit volume before rupture, representing overall durability) (Mohammadi-Alamuti *et al.*, 2023).

The mechanical properties of SZ and CPSZs films are detailed in Table 2. The observed enhancement in these properties can be linked to alterations in molecular weight, rigidity, and/or molecular configuration induced by CP modification, as evidenced by intrinsic viscosity measurements provided in Table 1. It

is plausible that the generation of free radicals during CP treatment promotes the formation of cross-links within the film structure. Consequently, the mechanical properties of the film are significantly affected by the quantity and nature of these cross-links, as well as the presence of mono- and disaccharides produced during irradiation, which further modulate these properties. Prior research has also confirmed the efficacy of CP in altering the mechanical characteristics of films (Bayanloo *et al.*, 2024; Moosavi *et al.*, 2020).

Color

As shown in Fig. 4, the SZ films demonstrated a transparent appearance. The addition of CP to form CPSZs films did not compromise this visual transparency. Quantitative support for these visual characteristics is provided by the colorimetric data (L^* , a^* , b^* , ΔE , and WI) detailed in Table 3 for both film types. The results indicate that CP significantly influences certain visual properties of CPSZs films, specifically L^* , ΔE , and WI, whereas the parameters a and b remain unaffected. Notably, increasing the plasma processing times led to a reduction in L and WI values, while ΔE values showed a corresponding increase. These findings highlight the impact of CP irradiation on the optical properties of the films.

The development of color in the food packaging materials was likely due to the Maillard reaction occurring between the available carbohydrates and the residual protein (present in the starch). Previous studies have also observed similar color changes in their findings (Bayanloo *et al.*, 2024; Romani *et al.*, 2019).

Table 1- Physical characteristics of starch/ZnO NPs (SZ) and cold plasma-treated SZ films at varying plasma processing durations (30, 60, and 90 s)^{†,*}

Time (s)	Thickness (mm)	Moisture Content (%)	Moisture absorption (%)	Solubility in water (%)	WVP ($\times 10^{-10} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$)	Density (g/cm^3)	Contact angle (degree)	Intrinsic viscosity (dl/g)
0	0.14±0.01d	20.83±0.19a	7.30±0.41b	16.01±0.16b	3.24±0.01a	1.42±0.16a	90.39±0.50a	0.12±0.01c
30	0.15±0.02c	20.51±0.11ab	8.43±0.07a	16.36±0.29ab	3.27±0.02a	1.24±0.03a	87.59±0.75b	0.13±0.01bc
60	0.16±0.01b	20.47±0.15b	8.63±0.13a	16.46±0.18a	3.35±0.01a	1.23±0.23a	85.22±0.57c	0.14±0.01ab
90	0.17±0.01a	20.33±0.22b	8.86±0.16a	16.70±0.26a	3.43±0.03a	1.21±0.13a	83.24±0.90d	0.15±0.01a

[†] Values in the same column sharing identical letters indicate no significant difference (P<0.05)

* Data are presented as means ± SD

Table 2- Mechanical characteristics of starch/ZnO NPs (SZ) films and cold plasma-treated SZ films at varying plasma exposure durations (30, 60, and 90 s)

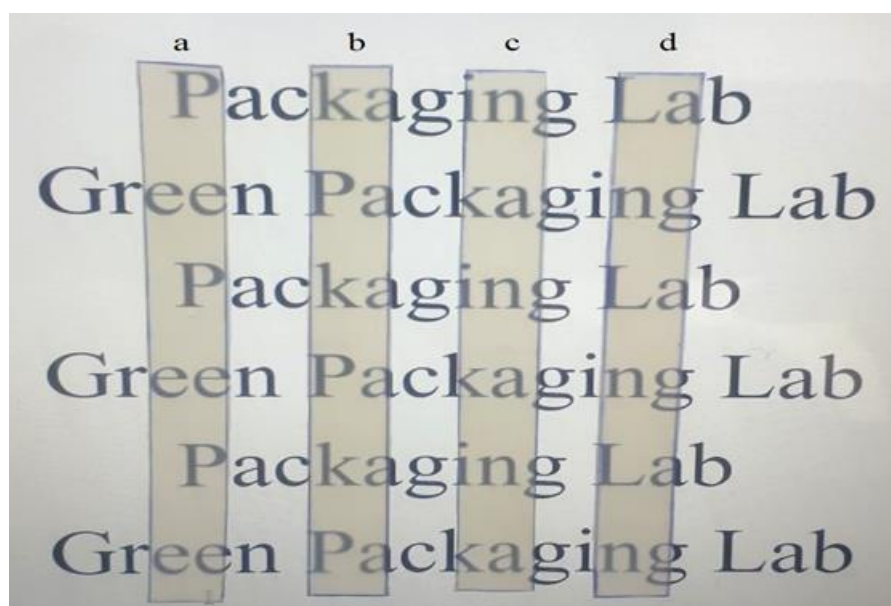
Time (s)	Tensile strength (MPa)	Elongation at break (%)	Tensile energy to break (MJ/m ³)
0	4.54 ± 0.30 c	41.09 ± 5.63 c	1.54 ± 0.29 c
30	4.92 ± 0.20 bc	47.95 ± 7.19 bc	1.97 ± 0.25 bc
60	5.41 ± 0.30 ab	55.89 ± 0.52 ab	2.33 ± 0.31 ab
90	5.85 ± 0.28 a	59.75 ± 5.10 a	2.72 ± 0.35 a

‡ Values in the same column sharing identical letters indicate no significant difference (P<0.05)
 * Data are presented as means ± SD

Table 3- Color parameters (L^* , a^* , b^*), color difference (ΔE), and whiteness index (WI) of starch/ZnO NPs (SZ) and cold plasma-irradiated SZ films, measured at varying plasma processing times of 30, 60, and 90 s

Time (s)	L^*	a^*	b^*	ΔE	WI
0	92.69 ± 0.30a	-0.67 ± 0.18a	-1.83 ± 0.19a	5.63 ± 0.28c	92.42 ± 0.29a
30	92.33 ± 0.29b	-0.86 ± 0.19a	-1.79 ± 0.01a	5.87 ± 0.22bc	92.08 ± 0.30b
60	91.99 ± 0.43c	-0.90 ± 0.35a	-1.73 ± 0.36a	6.13 ± 0.26ab	91.74 ± 0.40c
90	91.60 ± 0.41d	-0.91 ± 0.18a	-1.67 ± 0.17a	6.39 ± 0.39a	91.38 ± 0.41d

‡ Values in the same column sharing identical letters indicate no significant difference (P<0.05)
 * Data are presented as means ± SD

**Fig.4. The visual properties of starch/ZnO NPs (SZ) (a) and cold plasma-irradiated SZ films treated at different plasma processing times of 30 s (b), 60 s (c), and 90 s (d).**

UV-Vis Spectroscopy

The transmission of UV and visible light through the packaging materials is an important characteristic that can influence the aesthetic appeal of the package and protect the packaged

contents from photochemical degradation. This factor can sometimes be more critical than the oxygen permeability of the packaging (Mohammadi-Alamuti *et al.*, 2023). Exposure to UV rays speeds up food spoilage by causing

the formation of free radicals, destroying antioxidants, oxidizing lipids, degrading nutrients, and leading to discoloration and off-flavors (Mohammadi-Alamuti *et al.*, 2023). The opacity of the film influences how much light can pass through it, which can be essential for regulating light exposure in certain products (Romani *et al.*, 2020). The efficacy of ZnO as a UV-blocking agent is attributed to its strong absorption of ultraviolet radiation (Babaei-Ghazvini *et al.*, 2018; Shahabi-Ghahfarrokhi & Babaei-Ghazvini, 2019). The incorporation of ZnO into biopolymer packaging compositions can provide protection against photo-oxidation. The UV-Vis spectra of SZ and CPSZs are illustrated in Fig. 5. The CPSZs-90s sample

exhibited the highest absorbance among the tested spectra, albeit without statistical significance. This spectral behavior aligns with the visible characteristics of the SZ and CPSZs films presented in Fig 4. The observed effect is consistent with previous research demonstrating that UV irradiation is an effective modification technique for altering the UV-Vis spectral properties of starch-based films (Babaei-Ghazvini *et al.*, 2018; Shahabi-Ghahfarrokhi & Babaei-Ghazvini, 2019; Shahabi-Ghahfarrokhi *et al.*, 2019). It appears that the wavelength, intensity, and exposure time of the applied UV rays significantly affect the transparency of the modified SZ films.

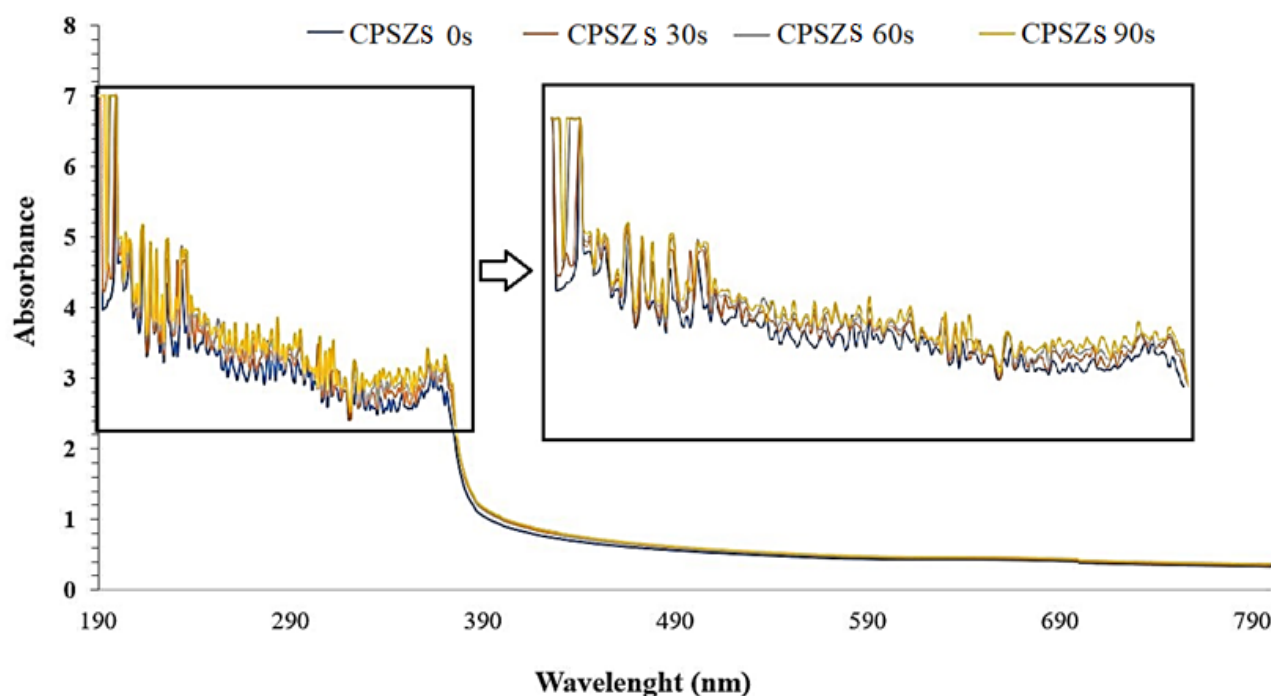


Fig. 5. The UV-Vis absorption spectra of starch/ZnO NPs (SZ) and cold plasma-irradiated SZ films processed at different exposure times of 30 s (CPSZs 30s), 60 s (CPSZs 60s), and 90 s (CPSZs 90s).

Conclusion

Despite the impressive properties of petroleum-based plastics in food packaging, there is growing concern over their environmental impact. As a result, starch-based packaging materials have gained attention as potential substitutes for plastics. In the current

study, two modification methods (i.e., nano reinforcement and CP) were employed to address the limitations of starch-based packaging materials.

Previous studies have utilized CP to modify biopolymers, but it has been a time-consuming process, making it impractical for the

packaging industry. However, in this study, simultaneous CP in an aqueous solution with the addition of ZnO was found to effectively modify the packaging properties of starch in a shorter time (e.g., 30 to 90 seconds). The high moisture content of the film-forming solution and the presence of photo-active nanoparticles (i.e., ZnO) were found to intensify the formation of reactive plasma species (e.g., free radicals, ions, electrons). As a result, the modification of starch-based nanocomposites was accelerated in the current study compared to previous studies.

The application of CP was observed to improve the mechanical properties (e.g., TS, EB, TEB) of SZ films without negatively affecting their visual characteristics. However, no significant alterations were noted in the UV-blocking capabilities of the SZ films. While CP did not substantially influence the water sensitivity of SZ films, it increased the hydrophilicity of the film surface. This effect can be linked to the formation of polar groups and the development of a rougher surface texture due to etching in CPSZs films. The uniform dispersion of ZnO achieved through CP was identified as a critical factor contributing to the enhanced packaging properties of CPSZs films.

Based on the bibliography and the results of this study, it is evident that the simultaneous application of CP and SZ is beneficial for coating and surface pasteurization of food products such as nuts, meats, and fruits.

Subsequent investigations should examine the application of CP with alternative gases for starch modification. Concurrently, research must quantify the active species produced during plasma treatment and assess the impact of any residual compounds on the sensory and physicochemical properties of the treated food products.

Author Contributions

Amir-Hossein Bayanloo: methodology, validation, formal analysis, investigation, writing—original draft preparation, and visualization. **Iman Shahabi-Ghahfarrokhi:** conceptualization, methodology, validation, formal analysis, writing—review and editing, supervision, funding acquisition, and project administration. **Simin Hagh-Nazari:** methodology, validation, formal analysis, and supervision.

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اثر پلاسما سرد و نانواکسید روی بر اصلاح نشاسته ذرت به عنوان ماده بسته بندی غذایی

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چکیده

این پژوهش به بررسی اثر پلاسما سرد بر فیلم های بیونانو کامپوزیت دوستدار محیط زیست نشاسته / اکسید روی می پردازد که شامل ۳ درصد وزنی نانوذره اکسید روی و زمان های مختلف اعمال پلاسما سرد (۰، ۳۰، ۶۰ و ۹۰ ثانیه) هستند. نتایج نشان می دهد که افزایش مدت زمان تیمار پلاسما سرد موجب افزایش ضخامت فیلم، ویسکوزیته، حلالیت در آب، جذب رطوبت و زبری سطح می شود؛ در حالی که زاویه تماس و میزان رطوبت کاهش می یابد. ولی تغییر قابل توجهی در نفوذپذیری بخار آب، چگالی و یا ارزیابی تراوایی به طیف های UV-Vis مشاهده نشد. تیمار پلاسما سرد باعث بهبود استحکام کششی، درصد افزایش طول در نقطه شکست و انرژی کششی تا نقطه شکست در نمونه ها شد، در حالی که شاخص های روشنایی و سفیدی کاهش یافتند بدون آنکه تفاوت رنگی ایجاد شود. این مطالعه پلاسما سرد را به عنوان روشی سریع و سازگار با محیط زیست برای اصلاح فیلم ها معرفی می کند که کارایی بیشتری هنگام اعمال بر محلول های نشاسته ای دارد و پتانسیل بالایی برای کاربردهای صنعتی در مواد بسته بندی فراهم می آورد.

واژه های کلیدی: اصلاح دوگانه، اکسید روی، بسته بندی سبز، پلاسما سرد، نانوکامپوزیت، نشاسته

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Research Article

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Influence of Cooking and Fermentation on Nutrient and Anti-nutrient Profiles of Millet and Rice

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Abstract

This study investigates how the traditional processing methods, such as cooking and fermentation, affect the nutritional, anti-nutritional, and mineral composition of the six edible grains, including the three rice varieties (*Oryza sativa*: Matta, Boiled and Brown rice) and the three millets (foxtail, jowar and pearl millet). The grains were analyzed in their raw, cooked, and fermented forms. The carbohydrates and the protein content were determined along with the anti-nutritional compounds such as the flavonoids, oxalates, phenolics, phytates and the tannins. The mineral concentrations of calcium, potassium, iron, magnesium, manganese, and zinc were determined using Atomic Absorption Spectroscopy. The results showed that cooking significantly reduced carbohydrate content by 85-90% across all grains, while fermentation caused an even greater reduction of up to 95%. Protein levels were grain-specific, and fermentation generally enhanced the protein concentration by 20-50%. Flavonoid content was reduced by 70-90% while phytates, and oxalates were reduced substantially by 60-90% through both treatments due to leaching and thermal degradation, while the phenolic content increased by 25-40%, particularly in the foxtail millet. The tannin levels decreased with cooking by 40-60%, but they increased after the fermentation, likely due to the enzymatic release of the bound compounds. Mineral concentrations were consistently declined in the cooking and fermented forms, yet fermentation improved bioavailability by reducing the anti-nutrients. Overall, the cooking was more effective in lowering the anti-nutritional factors, whereas the fermentation enhanced the protein and improved the accessibility of the essential minerals such as iron and zinc. These findings emphasize the importance of the traditional household processing methods in enhancing the nutritional quality of rice and millet-based diets, particularly in the regions dependent on cereal staples.

Keywords: Bioavailability, Fermentation, Millet, Mineral content, Rice varieties



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Introduction

Whole grains are widely known as an essential part of a healthy diet. Despite this understanding, many people consistently fail to meet the recommended intake of at least three servings per day (Slavin, Jacobs, Marquart, & Wiemer, 2001). Dietary choices are strongly influenced by regional biodiversity and local food availability, which significantly impact the mineral content of the consumed diets (Penafiel, Lachat, Espinel, Van Damme, & Kolsteren, 2011). Humans require at least 49 nutrients to maintain their optimal physiological functions, including 23 mineral elements that participate in various metabolic and biochemical processes (Fraga, 2005; Welch & Graham, 2004). Although needed in smaller quantities, these minerals must be consumed regularly (Welch & Graham, 2004).

Macrominerals such as sodium (Na), potassium (K), and chloride (Cl) are principally important as they maintain and regulate intracellular compounds. The electrochemical gradient is maintained by these elements across the cell membrane, which is crucial for signal transmission, metabolic transport and cell extractability (Quintaes & Diez-Garcia, 2015). It is also said that depending on the kind of food matrix composition, the bio-accessibility of minerals such as iron (Fe), zinc (Zn), calcium (Ca), and magnesium (Mg) varies significantly (Suliburska & Krejpcio, 2014).

Millets are rich source of micro and macro nutrients, and they also contain a higher concentration of essential amino acids and minerals compared to commonly consumed cereals such as wheat and rice (Nakandalage *et al.*, 2016). Health benefits attributed to millets include antioxidant, hypoglycaemic, anti-inflammatory, anti-ulcer, and cholesterol-lowering effects.

For over half of the global population, rice (*Oryza sativa*) is the common principal staple food, which offers and provides energy from carbohydrates and also provides essential

macro and micronutrients for human health (Nakandalage *et al.*, 2016). Rice provides a primary source of energy through complex carbohydrates that meet metabolic energy needs (Shrestha, Baruah, Subhash, Ananthan, & Longvah, 2020). Furthermore, it also provides moderate amounts of protein, which are primarily rich in amino acids such as lysine and methionine (Nakandalage *et al.*, 2016). Rice is also rich in critical micronutrients such as Fe, Zn, Mg, Mn and B-complex vitamins, which play an important role in redox balance and enzymatic functions (Perera, Seneweera, & Hirotsu, 2018; Wairich, Ricachenevsky, & Lee, 2022). However, the bioavailability of these micronutrients is often affected and compromised by the anti-nutritional factors for instance the phytates and the oxalates (Fukushima *et al.*, 2020) and therefore understanding its nutritional composition and methods to retain these micronutrients through cooking and fermentation is important to enhance the dietary quality of rice-based foods, especially in the regions where micronutrient deficiency are prevalent and where rice is also a primary staple food (Kumari & Roy, 2023; Wairich *et al.*, 2022).

The traditional method of processing food, such as cooking and fermentation, plays an important role in enhancing the nutritional quality of staple foods like rice, especially for economically vulnerable people. These methods happen to be accessible and inexpensive, making them highly suitable for the low-resource setting. Cooking is said to increase the digestibility of the macronutrients, soften the fibrous structure, and reduce microbial contamination. It also causes the thermal degradation of anti-nutritional compounds such as phytates and tannins, causing an increase in the bioavailability of minerals (Kumari & Roy, 2023; Savard *et al.*, 2019). Moreover, the fermentation can enrich foods with probiotics and the B-vitamins, making it a low-cost nutritional strategy for people who have limited access to diversified diets (Domán *et al.*, 2023; Neocleous,

Shammas, Phedonos, Phylactou, & Skordis, 2014).

This comparative analysis of the grains in their raw, cooked, and fermented forms is essential to understand how the traditional processing of the grains influences their nutritional and anti-nutritional profiles. As cooking is a universal household practice, it induces the thermal and structural changes that affect the nutrient content by leaching and degradation (Savard *et al.*, 2019). Fermentation is a time-tested preservation and enhancement technique that reduces the anti-nutritional factors and improves the bioavailability of the grains, which is important in cereal-based diets (Siqueira *et al.*, 2020). Fermentation is regarded as a transformative method that increases the nutritional quality of food. It facilitates the combination of different plant-based proteins and supports the development of more sustainable dietary options (Wairich *et al.*, 2022). Thus, this study aimed to analyze and compare the level of carbohydrates and protein content, anti-nutritional factors like flavonoids, oxalates, tannins, phenolics and phytates along with the mineral profile of six edible grains, matta rice, boiled rice, brown rice, foxtail millet (*Setaria italica*), jowar millet (*Sorghum bicolor*), and pearl millet (*Pennisetum glaucum*) in their raw, cooked and fermented form.

Materials and Methods

Sample Collection and Identification

Three commonly consumed rice (*Oryza sativa*) varieties, such as matta rice (OsMR), boiled rice (OsBoR), and brown rice (OsBR), and three millets varieties such as foxtail millet (FM) (*Setaria italica*), jowar millet (JW) (*Sorghum bicolor*), and pearl millet (PM) (*Pennisetum glaucum*) were procured from a local market in Bengaluru, Karnataka, India and were subjected to the estimation of nutritional and anti-nutritional and mineral content at three different form like raw, cooked and fermentation. The raw form refers to cleaned and dried uncooked grains and is

directly used for the analysis. For the cooked form, about 40 g of each grain was boiled in 400 mL of water at 100 °C for a specific duration, depending on the grain type. The rice samples were generally cooked within 20-25 minutes, while the millets required about 30-40 minutes for complete softening. Cooking was performed in a semi-closed vessel, simulating the typical household cooking practices. After cooking, starch-rich water (leachate) was decanted, and the softened grains were used for further analysis. For the fermented state, the above cooked grains were subjected to a fermentation process naturally. Briefly, the cooked and decanted grains were transferred to sterile containers, separately, and loosely covered with a lid to allow air exchange and incubated at ambient room temperature (27–30 °C) for 12 hours. No microbial starter culture was added, allowing spontaneous fermentation. The process relied solely on the native microbiota present on the grains and in the surrounding environment. This method aimed to facilitate natural fermentation, representing traditional household practices, rather than any controlled ageing or preservation. Following fermentation, the grains were subjected to nutritional, phytochemical, and anti-nutritional analyses and compared with corresponding raw and cooked forms to evaluate the impact of processing on their compositional characters.

Nutrient and Antinutrient Analysis

Nutrient content, such as carbohydrate (Nielsen, 2010) and protein (Lowry, Rosebrough, Farr, & Randall, 1951) contents, was estimated in all six grains of their three different forms of processing. Similarly, anti-nutritional factors such as total flavonoid content, oxalate (Domán *et al.*, 2023), total phenolic content (Siqueira *et al.*, 2020), phytate (Siqueira *et al.*, 2020) and total tannin content were estimated using standard protocols, in all three forms.

The grain samples of 0.2g each were soaked in 5 ml of methanol and covered with aluminium foil for 48 hours for the complete exudation of the phytochemicals. This solution was then filtered using the Whatman filter paper, and 1ml of filtrate was mixed with 10% aluminium chloride and 1M sodium acetate. After 45 minutes of incubation in the dark at room temperature, the absorbance was measured at 415nm. Quercetin (0.1 mg/ml) was used as the standard (Bag, Grihanjali Devi, & Bhaigyabati, 2015).

Oxalate estimation

Oxalate estimation was performed by following the method of Abaza (1967). The sample was digested in 6N HCl in a boiling water bath for 1 hour. After filtration and evaporation, concentrated ammonia was added until the solution turned faint yellow. The mixture was reheated at 90 °C, and 5% CaCl₂ was added. After overnight settling, the precipitate was dissolved while boiling. The solution was then left overnight and filtered, and the precipitate was dissolved in 10ml of 20% H₂SO₄, heated to 70°C for 10min and titrated against N/20 of KMnO₄ to near endpoint (colorless to pink).

$$\text{Oxalate (\%)} = \frac{\text{ml of KMnO}_4 \times \text{Molecular weight of KMnO}_4 \times \text{Equivalent weight of oxalate}}{\text{Weight of the sample}} \times 100$$

Total Phenolics estimation

Approximately 1g of each grain was extracted with 10 mL of ethanol (80%) for 1 hour and centrifuged at 10,000 rpm for 20 minutes. To the supernatant, Folin-Ciocalteu reagent and distilled water were added. After 6 min, sodium carbonate (7%) was added, and the volume was then adjusted to 6ml with distilled water. The mixture was incubated at room temperature for 90 minutes in the dark. Absorbance was measured at 760nm using gallic acid (0.2 mg/ml) as the standard (Dewanto, Xianzhong, Adom, & Liu, 2002).

Phytate Estimation

For phytate estimation, 2g of each grain was treated with 2% hydrochloric acid and

incubated for 3 hours. After filtration, the extract was treated with ferric chloride (0.00195g/ml) after adding 0.3% ammonium thiocyanate (0.3%) until a brownish-yellow color persisted for ~5 min (Harris & Mosher, 1934).

$$\text{Total phytic acid} = \frac{\text{Titre value} \times 0.00195 \times 1.9 \times 100}{2}$$

Total Tannins estimation

About 500mg of grain was shaken with 50ml of distilled water for 1 hour. A 1ml aliquot of the filtrate was mixed with 0.05 M ferric chloride in 0.1 N hydrochloric acid and 0.008 M potassium ferrocyanide. Absorbance was read at 760nm within 10 minutes. A standard was prepared using tannic acid to get 1mg/L and measured (Okwu & Orji, 2007; Van Buren & Robinson, 1969).

Mineral Content Analysis

The mineral content in the selected grain samples, such as zinc (Zn), iron (Fe), copper (Cu), calcium (Ca), magnesium (Mg), manganese (Mn), and potassium (K), was estimated using Atomic Absorption Spectroscopy in all three forms (PerkinElmer Analyst 400, USA). For digestion, 10 ml of concentrated nitric acid was added to each form and allowed to pre-digest for 30 minutes at room temperature. Samples were then heated until initial digestion was complete, followed by the addition of 2-3 mL perchloric acid. Heating continued until the solution became clear (PerkinElmer Analyst 400, USA)

Statistical Analysis

All experiments were performed in triplicate, and the obtained data were then expressed as mean ± standard deviation (SD). The data obtained were then subjected to statistical analysis using SPSS software (version 24) to identify significant differences among the treatments. The values were considered statistically significant at p-value < 0.05.

Results and Discussion

Nutrient Analysis

Carbohydrate and protein contents were assessed across all six selected grains in their

raw, cooked, and fermented forms. The results revealed significant changes in the carbohydrate level which was highest in the raw state across all grains. Among the rice varieties, OsMR showed the highest carbohydrate content ($10.71 \pm 0.12\%$), followed by OsBoR ($10.44 \pm 0.05\%$) and OsBR ($10.01 \pm 0.12\%$). The millet varieties JW, PM, and FM followed in descending order, with FM registering the lowest ($6.58 \pm 0.13\%$) (Table 1). However, in the cooked and fermented state, a marked reduction in carbohydrate content was observed. The loss is due to the leaching of soluble starch during cooking and decanting (Agrawal, Hidame, & Gurla, 2011). The loss during fermentation is likely due to the utilization of simple sugars by lactic acid bacteria, which consume carbohydrates during their metabolic activity (Nielsen, 2010). Similarly, protein content varied significantly with the state of processing. In the raw state, millet varieties registered higher protein levels than rice varieties. PM registered the highest protein content ($35.71 \pm 0.13\%$), followed by FM > JW (Table 1). Among the rice varieties, OsBoR had the highest protein content ($32.39 \pm 0.17\%$), while OsMR showed relatively lower values.

Cooking affected the protein content variably, possibly due to the reduction in moisture content, leading to a higher concentration of protein (Bastías, Balladares, Acuña, Quevedo, & Muñoz, 2017; Liu, Zheng, & Chen, 2019). OsBoR registered the highest post-cooking protein content, followed by OsMR and OsBR. However, JW and FM showed substantial reductions in protein

content post-cooking, indicating potential protein denaturation or leaching.

Fermentation generally led to an increase in protein content across most of the selected grains in the current study. The highest protein concentration was found in OsBR, followed by OsBoR > JW. The increase in the protein content is likely attributed to the reduction of carbohydrates and fats during the fermentation process, resulting in a proportional enrichment of protein in the dry matter (Nkhata, Ayua, Kamau, & Shingiro, 2018). However, the relatively high protein values obtained in the present study may also reflect methodological limitations (Lowry's method) (Everette *et al.*, 2010). This method is known to overestimate protein concentrations due to interference from phenolic and other reducing compounds present in grain extract, which can yield inflated protein (Rodríguez, Bianchi, Simonato, Rizzi, & Tironi, 2025; Everette *et al.*, 2010; Marks, Buchsbaum, & Swain, 1985; Smith, 1982). Comparative evaluations of protein estimation techniques such as Dumas, Kjeldahl and Lowry methods have reported that indirect and colorimetric assays may overstate true protein values by a substantial margin. Therefore, the higher protein content observed in the present study may partly result from matrix interference rather than actual biological protein levels (~1-2%) (Mæhre, Dalheim, Edvinsen, Elvevoll, & Jensen, 2018; Hayes, 2020; Redmile-Gordon, Brookes, Goulding, & Hirsch, 2013). Thus, these findings indicate that both cooking and fermentation processes, with the extent of change being grain-specific and process-dependent.

Table 1- Nutritional values in three different states of the selected grain

Grains	Processing stage of grains					
	Carbohydrate (%)			Protein (%)		
	Raw	Cooked	Fermented	Raw	Cooked	Fermented
OsMR	10.71±0.12 ^a	1.16±0.06 ^b	0.91±0.11 ^a	29.43±0.09 ^a	17.82±0.19 ^c	25.31±0.90 ^b
OsBR	10.01±0.03 ^c	1.14±0.06 ^b	0.47±0.04 ^c	29.24±0.10 ^b	16.61±0.65 ^c	49.14±0.10 ^a
OsBoR	10.44±0.05 ^b	0.69±0.10 ^c	0.36±0.01 ^d	32.39±0.17 ^b	28.50±0.40 ^c	38.53±0.30 ^a
JW	9.97±0.04 ^c	1.51±0.09 ^a	0.36±0.01 ^d	31.99±0.11 ^a	7.60±0.11 ^c	31.15±0.31 ^b
PM	9.22±0.04 ^d	1.24±0.09 ^b	0.89±0.05 ^a	35.71±0.13 ^a	9.48±0.15 ^c	28.27±0.17 ^b
FM	6.58±0.13 ^e	0.71±0.16 ^c	0.61±0.07 ^b	33.39±0.07 ^a	5.66±0.22 ^c	29.10±0.58 ^b

Different superscripts in each column indicate a significant difference ($p < 0.05$)

Anti-nutrient Analysis

Cooking and fermentation significantly influenced the levels of flavonoids, oxalates, phenolics, phytates and tannins across the six selected grains. Cooking led to a consistent reduction in flavonoid content in all grain varieties (Fig. 1), primarily due to the leaching of the water-soluble compounds into the cooking medium and thermal degradation of the flavonoid compounds (Jiménez-Monreal, García-Diz, Martínez-Tomé, Mariscal, & Murcia, 2009; Turkmen, Sari, & Velioglu, 2005). PM retained the highest amount of flavonoids post-cooking (0.70 ± 0.03), followed by $\text{OSMR} = \text{OsBR} = \text{JW} > \text{FM} > \text{OsBoR}$. A significant difference was among the cooked grains of PM and FM, showing distinctive profiles. In millet varieties, fermentation further decreased the flavonoid content, with a significant difference ($P < 0.05$). While in rice varieties OsBoR and OSMR exhibited the highest flavonoid content, with a significant difference after the fermentation (1.17 ± 0.11 and 1.11 ± 0.04), followed by $\text{OsBR} > \text{PM} = \text{FM} > \text{JW}$, a decrease that can be directly correlated with the enzymatic degradation and microbial metabolism (Adebo *et al.*, 2021)

Similarly, cooking resulted in a considerable decrease in the oxalate levels of particularly PM (Fig. 2). This decrease can be attributed to the rupture of the grain (plant cell) during the process of boiling, allowing the oxalates to leach into the water, which is normally discarded. High heat is also said to break down the cell walls and release the oxalates (Maqbool *et al.*, 2021). In general, cooking is said to reduce the oxalate content by 60-90% (Shi, Arntfield, & Nickerson, 2018). After cooking, the JW retained the highest level of oxalate, followed by $\text{OSMR} > \text{OsBoR} > \text{FM} > \text{PM} = \text{OsBR}$ (Fig. 2). In contrast, the fermentation had a minimum impact on the oxalate levels. OSMR, FM, PM and JW continued to show the highest oxalate content, followed by $\text{OsBR} = \text{OsBoR}$ (Fig. 2). The limited fermentation of the oxalate is

likely due to the short fermentation time (< 24 hours), which might not have led to microbial degradation of oxalates. The reason is that the oxalate degradation is time and pH-sensitive (Adebo *et al.*, 2021). The presence of tannins and oxalate content in the rice samples of the present study aligns with previous reports, suggesting that these compounds are naturally concentrated in the bran and husk layers of rice grains and are retained to varying degrees in brown and minimally polished varieties (Alotaibi, Babiker, Alshammari, & Yahya, 2024; Irakli, Lazaridou, & Biliaderis, 2020). The comparatively higher levels in the OSMR, OsBR, and OsBoR can be attributed to their limited polishing and their partial retention of the bran and the aleurone layers (Bhat *et al.*, 2020). These outer layers are rich in phenolic compounds, tannins and oxalates that remain bound to the cell wall components such as cellulose, hemicellulose and lignin (Bhat *et al.*, 2020; Wisetkomolmat *et al.*, 2022). In contrast to the flavonoids and the oxalates, the grains are said to have a notable increase in the phenolic content upon cooking ($P < 0.05$) (Fig. 3). This is more likely due to the disruption of the cell walls and cleavage of the ester and the ester bonds during thermal treatment which results in the release of the phenolics that are more extractable. Cooking is also said to inactivate the polyphenol oxidase enzymes, which are generally said to degrade the phenols (Limberger-Bayer, De Francisco, Chan, Oro, Ogliari, & Barreto, 2014; Turkmen *et al.*, 2005). FM has recorded the highest phenolic content in the cooked state, followed by $\text{OsBR} > \text{JW} = \text{OSMR} > \text{OsBoR} = \text{PM}$ (Fig. 3). Among all varieties, FM was significantly different from the other grains, while OSMR and OsBoR formed the lowest group. Nevertheless, fermentation led to a slight reduction in the phenolic content due to the microbial utilization of phenolics as the carbon source and their conversion into the volatile forms that are not captured by the Folin-Ciocalteu assay (Arguedas-Villa, Kovacevic, Allen, Stephan, & Tasara, 2014). In the

fermented state, PM recorded the highest phenolic content, followed by OsBoR>JW=FM=OsMR=OsBR, showing the lowest values (Fig. 3). Cooking also led to a reduction in the phytate levels in all the grains, although the difference was not statistically significant among the cooked samples ($P>0.05$). This reduction is again due to the leaching of the water-soluble phytates into the cooking medium and partial breakdown of the phytic acids at the elevated temperature (Lang, Whitney, & Steinberg, 1982; Li, Beta, Sun, & Corke, 2006). In the cooked state, all the grains showed a decrease in the phytate levels (Fig. 4). Among the raw grains, JW exhibited the highest phytate content, followed by the OsBR=OsMR=OsBoR=FM=PM (Fig. 4). After the fermentation, there is only a marginal reduction in the phytate content, particularly in PM, JW. FM maintained the highest level, followed by the OsBR>OsMR>OsBoR=FM=PM. Although there is a difference among the grains, this is not statistically significant ($P>0.05$). This effect is due to the insufficient endogenous phytate activity under the suboptimal fermentation conditions (Sheethal *et al.*, 2022).

In the case of tannins, the cooking caused a significant decrease in all the grains. The highest post-cooking tannin content was observed in OsMR, followed by PM>FM>OsBoR>JW (Fig. 5). This decrease is also attributed to the leaching of the water-soluble tannins into cooking water and thermal polymerisation of tannins, which makes them less reactive in spectrophotometric assays. Remarkably, there was a sharp increase in the tannin content by fermentation, with OsBR and FM exhibiting the highest value, followed by OsBoR=JW>OsMR>PM (Fig. 5). This increase in the tannin content is due to the microbial enzyme action, such as the cellulases and the proteases, which liberate the bonded tannins, making them more extractable. Moreover, fermentation can also generate phenolic compounds that bond with protein and carbohydrates to form mimic tannin-like structures that are more quantifiable in spectrophotometric assays (Chung, Wong, Wei, Huang, & Lin, 1998; Olives Barba, Cámara Hurtado, Sánchez Mata, Fernández Ruiz, & López Sáenz De Tejada, 2006).

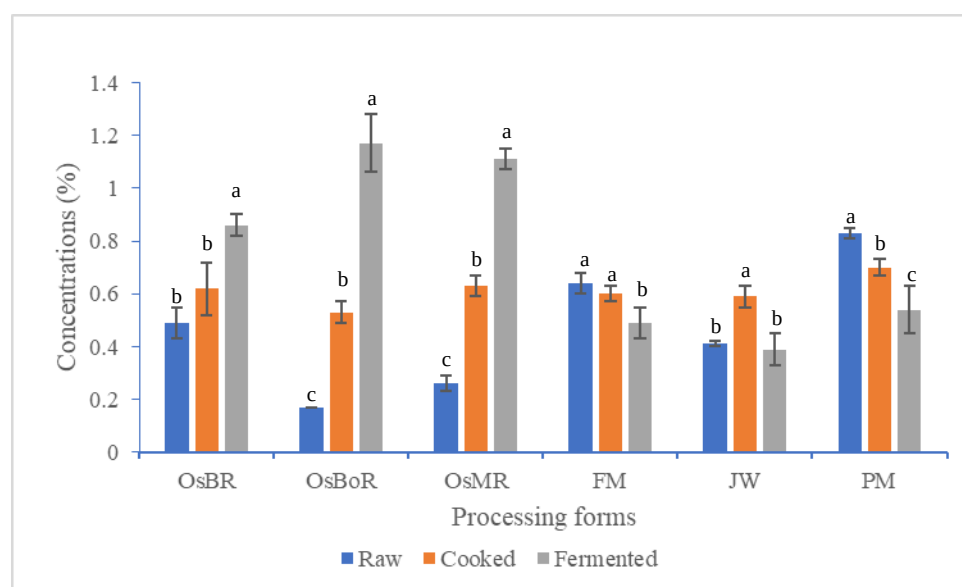


Fig. 1. Concentration of flavonoids present in different states of cooking
Different alphabets within each group indicate a statistically significant difference ($p < 0.05$)

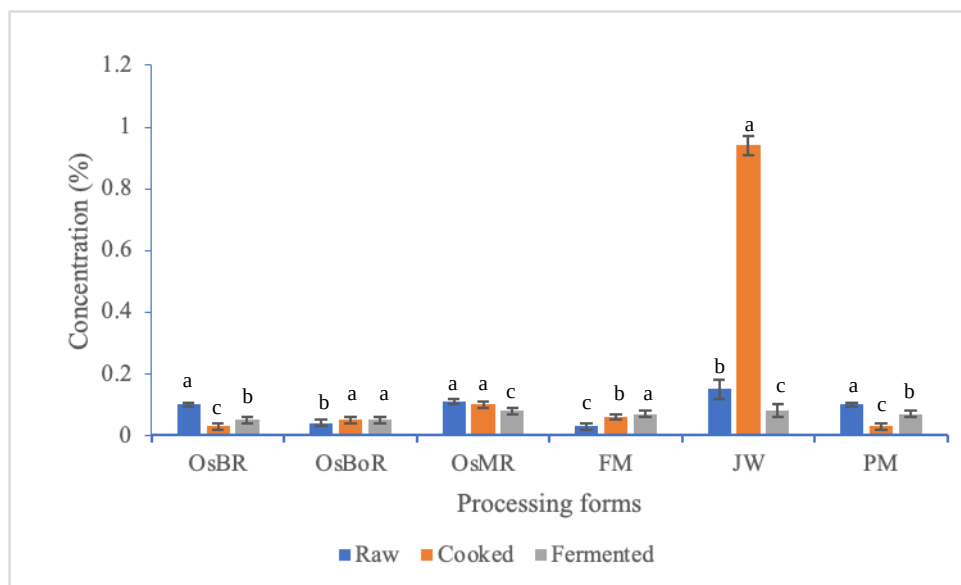


Fig. 2. Concentration of Oxalates in raw, cooked, and fermented forms of selected grains
Different alphabets within each group indicate a statistically significant difference ($p < 0.05$)

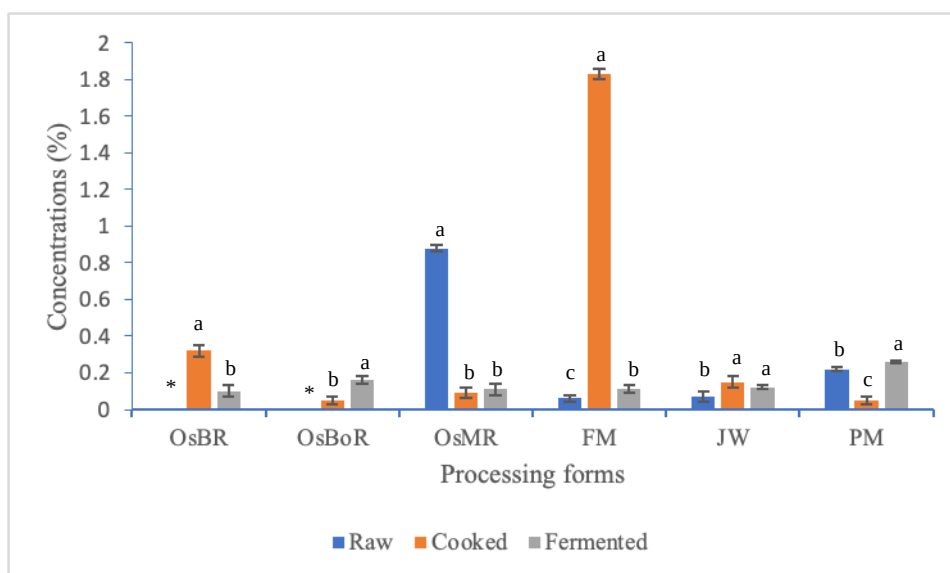


Fig. 3. Concentration of Phenolic in raw, cooked, and fermented forms of selected grain
Different alphabets within each group indicate a statistically significant difference ($p < 0.05$). *Indicate below detectable values.

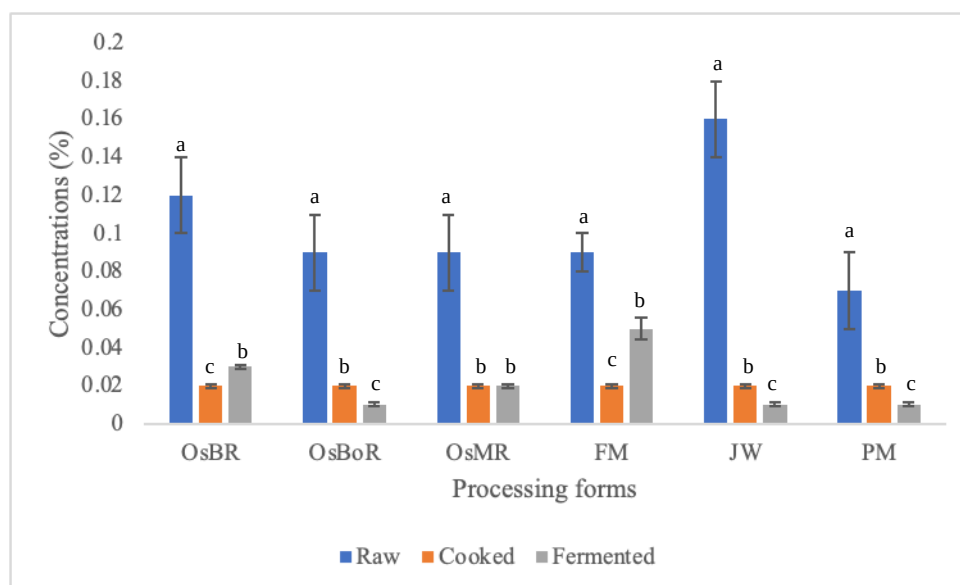


Fig. 4. Concentration of Phytates in raw, cooked, and fermented forms of selected grains
Different alphabets within each group indicate a statistically significant difference ($p < 0.05$)

This decrease is also attributed to the leaching of the water-soluble tannins into cooking water and thermal polymerisation of tannins, which makes them less reactive in spectrophotometric assays. Remarkably, there was a sharp increase in the tannin content by fermentation, with OsBR and FM exhibiting the highest value, followed by OsBoR=JW>OsMR>PM (Fig. 5). This increase in the tannin content is due to the microbial enzyme action, such as the

cellulases and the proteases, which liberate the bonded tannins, making them more extractable. Moreover, fermentation can also generate phenolic compounds that bond with protein and carbohydrates to form mimic tannin-like structures that are more quantifiable in spectrophotometric assays (Chung *et al.*, 1998; Olives Barba *et al.*, 2006).

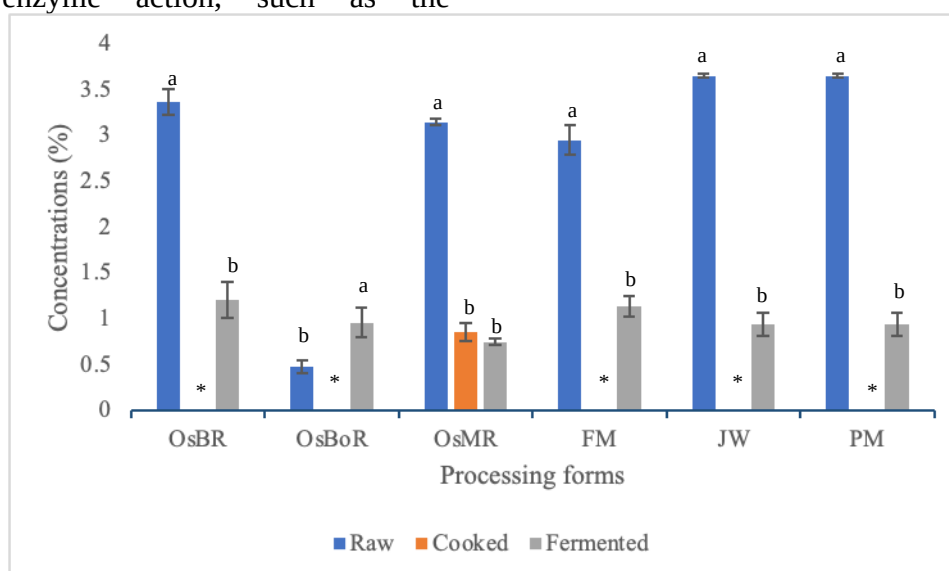


Fig. 5. Concentration of Tannins in raw, cooked, and fermented forms of selected grains
Different alphabets within each group indicate a statistically significant difference ($p < 0.05$); * indicate below detectable values.

Mineral Content Analysis

We have also analyzed the elemental content of Calcium, Iron, Potassium, Magnesium, Manganese, and Zinc across all six selected grains in their raw, cooked, and fermented states. Across the six elements, the raw grain consistently exhibited the highest concentrations, followed by cooked and fermented, with a statistically significant reduction seen among the grains ($P < 0.05$). In the unprocessed grains, the minerals are found in their native forms and are bound to phytic acid, fiber or the protein complexes, although their bioavailability is less, their total availability remains the highest. In the raw state of the grains, FM had recorded the highest content of Ca (0.06264 ± 0.00046), followed by $PM > JW > OsBR > OsBoR > OsMR$ (Fig. 6), while FM had the highest Fe content (0.00723 ± 0.02), followed by $JW > OsBR > OsBoR > PM > OsMR$ (Fig. 7). K was found to be at a higher level in PM and JW compared to other grains with significant difference ($P < 0.05$), followed by $FM > OsBR > OsBoR > OsMR$ (Fig. 10). Mg followed a similar pattern: $FM > OsBR > JW > PM > OsBoR > OsMR$ (Fig. 8). Mn was highest in OsBR and FM followed by $JW > PM > OsMR > OsBoR$ (Fig. 9). Zn content followed the trend $FM > PM > JW > OsBR = OsMR > OsBoR$ (Fig. 11). Copper was

below detectable limits (BDL) in most samples. Upon cooking, there was a significant reduction in the elemental content, which showed a significant statistical difference ($P < 0.05$), except for magnesium in its raw state. Here, the boiling or steaming can cause the minerals to leach out into the cooking water, which explains the drop in the mineral content (Meiners *et al.*, 1976; Moyo, 2024). OsMR was found to have retained the highest calcium content after post-cooking, while the OsBR and OsMR had relatively high Fe and K content. Fermentation further reduced the elemental levels, where OsMR showed high retention of Zn and Fe, while JW retained calcium. This reduction is due to the solubilization and microbial uptake. Fermentation is also reported to break down the antinutritional factors, increasing the bioavailability of the minerals even if their total content is low (Gibson, 2007; Hemalatha, Platel, & Srinivasan, 2005). When compared to all the other treatments, elemental degradation was found to follow the trend $Raw > cooked > fermented$, which shows a notable statistical difference among the grains and the treatment, suggesting that cooking and fermentation influence the bioavailability of the elements in a grain-specific manner.

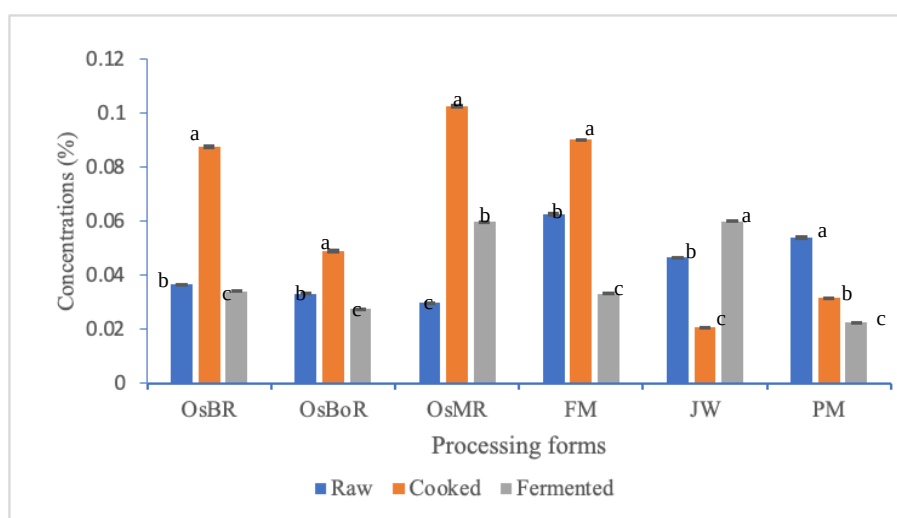


Fig. 6. Concentration of Calcium in raw, cooked, and fermented forms of selected grains
Different alphabets within each group indicate a statistically significant difference ($p < 0.05$)

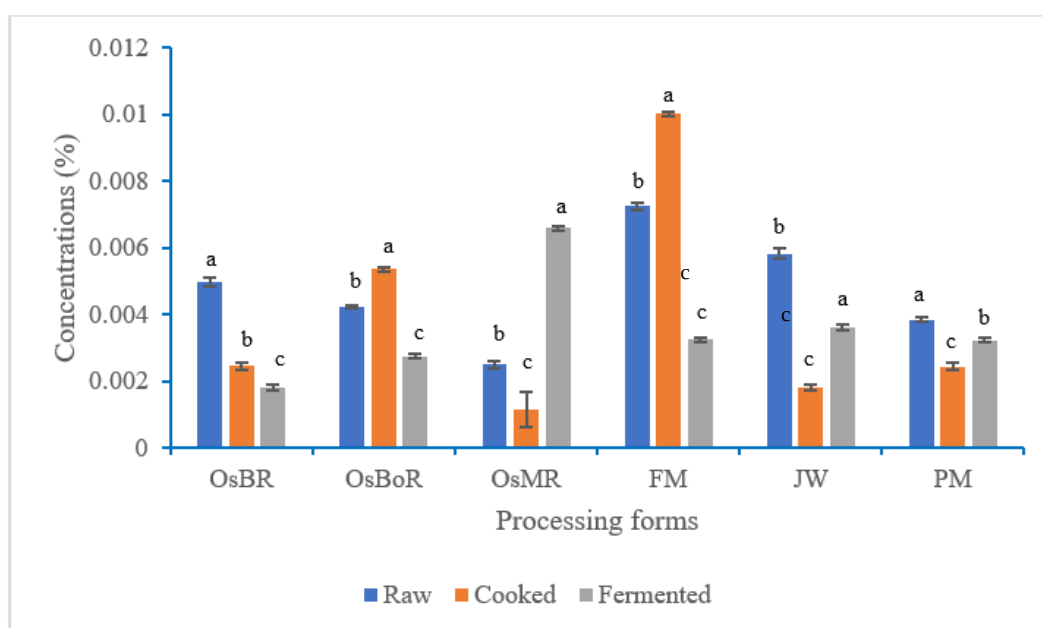


Fig. 7. Concentration of iron in raw, cooked, and fermented forms of selected grains
Different alphabets within each group indicates a statistically significant difference ($p < 0.05$)

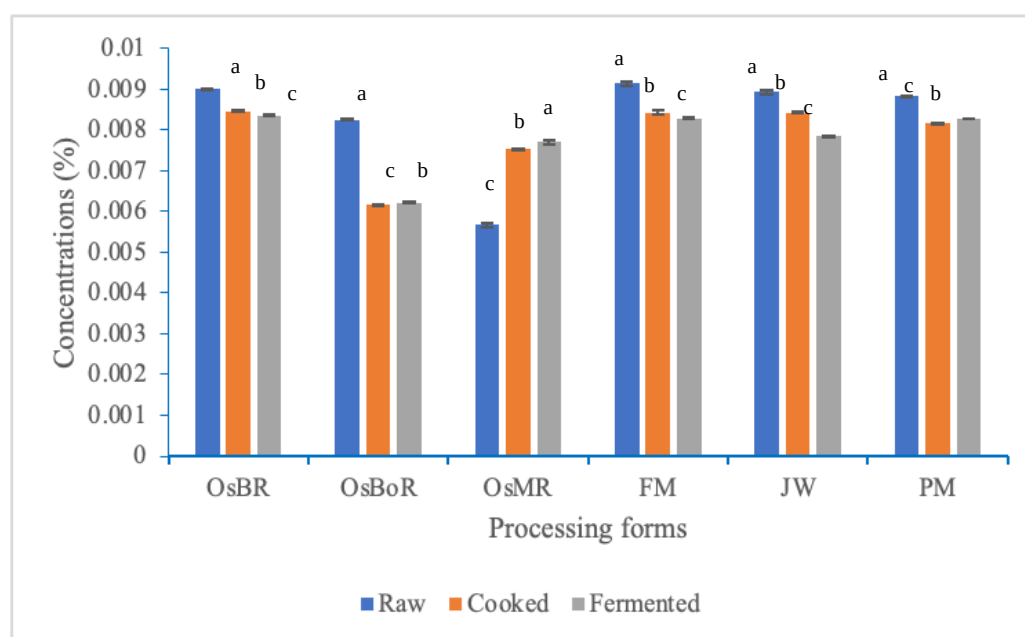


Fig. 8. Concentration of magnesium in raw, cooked, and fermented forms of selected grains
Different alphabets within each group indicate a statistically significant difference ($p < 0.05$)

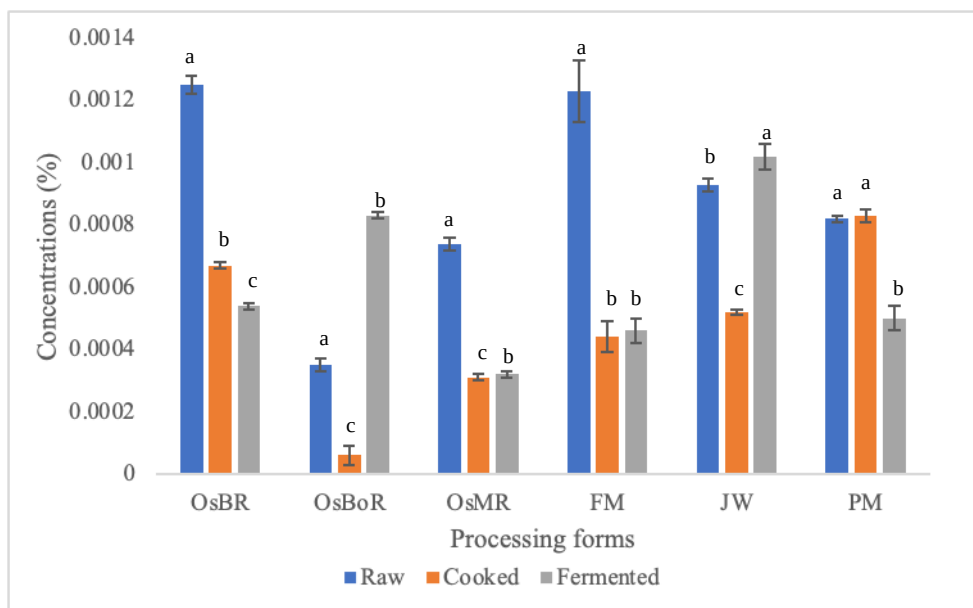


Fig. 9. Concentration of Manganese in raw, cooked, and fermented forms of selected grain
Different alphabets within each group indicate a statistically significant difference ($p < 0.05$).

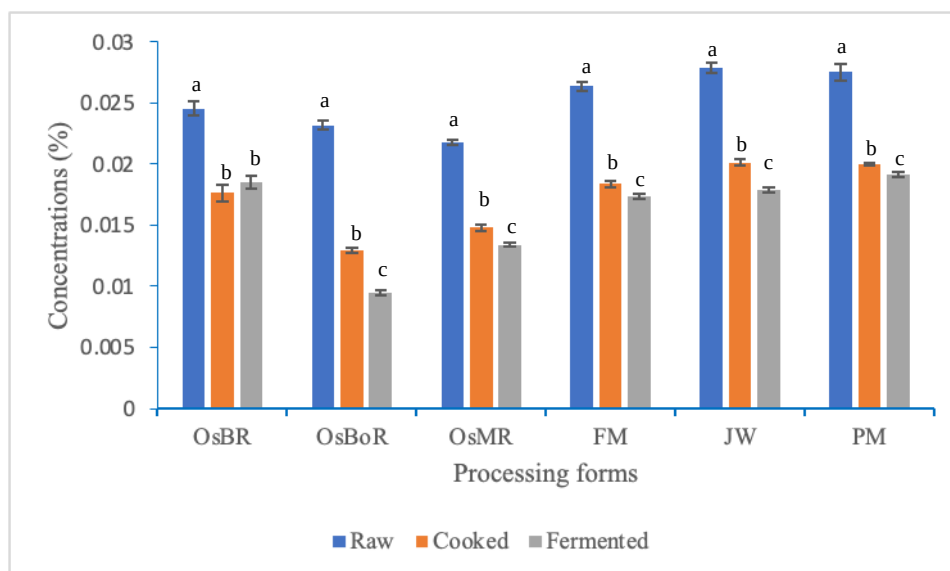


Fig. 10. Concentration of Potassium in raw, cooked, and fermented forms of selected grains
Different alphabets within each group indicate a statistically significant difference ($p < 0.05$).

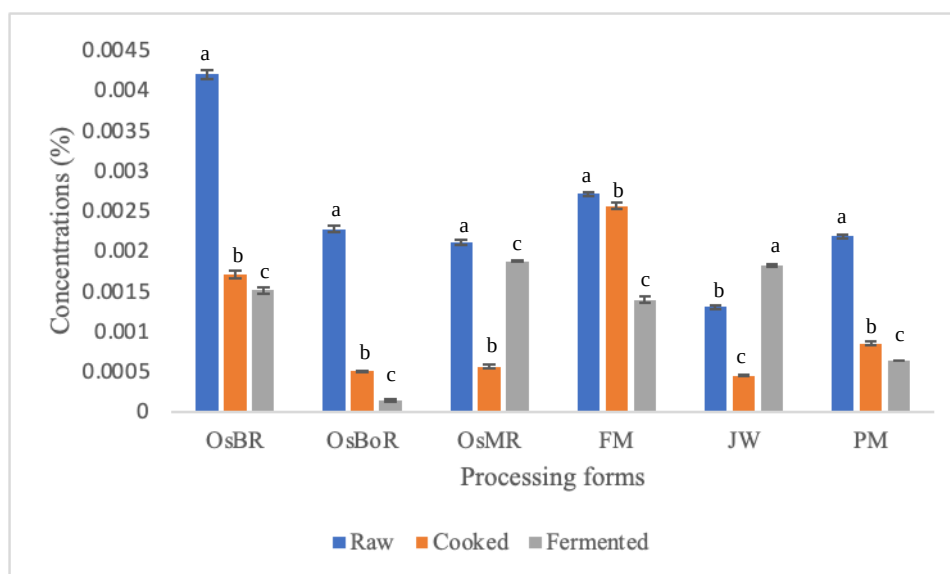


Fig. 11. Concentration of Zinc in raw, cooked, and fermented forms of selected grains
Different alphabets within each group indicate a statistically significant difference ($p < 0.05$)

Conclusion

The current study demonstrated that both cooking and fermentation notably influenced the nutritional and anti-nutritional compositions of the selected grains. Cooking primarily resulted in a marked reduction of carbohydrate, oxalates, phytates and tannins, mainly due to the leaching and thermal degradation. Fermentation, on the other hand, caused an improvement in the protein level and also caused a reduction in several anti-nutrients; a decline in the mineral content was also observed. In spite of the apparent decline in the mineral content, the breakdown of the anti-nutrients caused an increase in the bioavailability of the minerals. Importantly, the RDA (recommended dietary allowance) have recommended that the essential nutrients, such as protein intake, have to be at least 46g/day for women and 56g/day for men, while Fe intake is of 18mg/day for women of age 19-50 and 8mg/day for men and Zn levels are 8-11 mg/day, i.e 8mg/day for women and 11mg/day for men (DeLoughery, 2017). In this study, fermentation has been shown to be the most effective method in enhancing the bioavailability of protein and minerals such as Fe and Zn. Cooking was found to be more effective in reducing the anti-nutritional factors which tend to hinder the nutritional

uptake (Evans & Lawrenson, 2017; Quintaes & Diez-Garcia, 2015). Although a decline in the mineral content was observed, the bioavailability was increased due to the breakdown of the anti-nutritional factors. These findings highlight the importance of traditional fermentation technique as a promising approach for the nutrient uptake from the grains and support better dietary outcomes (Hur, Lee, Kim, Choi, & Kim, 2014). To build on the findings of this study, one can explore the bioavailability studies, microbial profiling, extended fermentation durations, comparison of natural fermentation with that of starter cultures, sensory and shelf-life evaluation and application in functional foods.

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Authors Contribution

Merin Rose Jeeson: Data curation, Formal analysis, Investigation, Methodology, Visualization, writing – original draft.
Krishnakumar Velayudhannair: Conceptualization, Formal analysis, Project

administration, Supervision, Validation,
Writing – review and editing.

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تأثیر پخت و تخمیر بر پروفایل‌های مواد مغذی و ضد‌مغذی در ارزن‌ها و برنج

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چکیده

این مطالعه به بررسی تأثیر روش‌های سنتی فرآوری، شامل پخت و تخمیر، بر ترکیب مواد مغذی، ضد‌مغذی و مواد معدنی شش غله خوراکی می‌پردازد؛ سه رقم برنج (*Oryza sativa*، مآتا، برنج پخته و برنج قهوه‌ای) و سه نوع ارزن (ارزن دمروباهی، جوار و ارزن مرواریدی). غلات در سه حالت خام، پخته و تخمیرشده مورد تجزیه و تحلیل قرار گرفتند. میزان کربوهیدرات و پروتئین همراه با ترکیبات ضد‌مغذی شامل فلاونوئیدها، اگزالات‌ها، فنولیک‌ها، فیتات‌ها و تانن‌ها اندازه‌گیری شد. غلظت مواد معدنی شامل کلسیم، پتاسیم، آهن، منیزیم، منگنز و زینک با استفاده از دستگاه جذب اتمی تعیین گردید. نتایج نشان داد پخت، میزان کربوهیدرات را در تمامی غلات به‌طور معنی‌داری بین ۸۵ تا ۹۰ درصد کاهش می‌دهد، در حالی‌که تخمیر کاهش بیشتری تا حدود ۹۵ درصد ایجاد می‌کند. مقدار پروتئین وابسته به نوع غله بود و تخمیر به‌طور کلی موجب افزایش ۲۰ تا ۵۰ درصدی پروتئین می‌شد. میزان فلاونوئیدها بین ۷۰ تا ۹۰ درصد کاهش یافت و فیتات‌ها و اگزالات‌ها نیز در اثر هر دو تیمار، به‌دلیل نفوذپذیری و تخریب حرارتی، به‌طور قابل‌توجهی بین ۶۰ تا ۹۰ درصد کاهش پیدا کردند؛ در مقابل، محتوای ترکیبات فنولیک به‌ویژه در ارزن دمروباهی، ۲۵ تا ۴۰ درصد افزایش یافت. سطح تانن‌ها در اثر پخت بین ۴۰ تا ۶۰ درصد کاهش یافت، اما پس از تخمیر افزایش نشان داد که احتمالاً ناشی از آزادسازی آنزیمی ترکیبات متصل است. غلظت مواد معدنی در هر دو حالت پخته و تخمیرشده کاهش یافت؛ با این حال، تخمیر با کاهش ضد‌مغذی‌ها، قابلیت زیست‌فراهمی مواد معدنی را بهبود بخشید. در مجموع، پخت در کاهش عوامل ضد‌مغذی مؤثرتر بود، در حالی‌که تخمیر موجب افزایش پروتئین و بهبود دسترسی‌پذیری مواد معدنی ضروری مانند آهن و روی شد. این یافته‌ها اهمیت روش‌های سنتی خانگی در بهبود کیفیت تغذیه‌ای رژیم‌های غذایی مبتنی بر برنج و ارزن را به‌ویژه در مناطقی که وابسته به غلات هستند، نشان می‌دهد.

واژه‌های کلیدی: ارزن، ارقام برنج، تخمیر، زیست‌فراهمی، محتوای معدنی

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Review Article

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Ascorbic Acid: Rationale and Applications in Inhibiting Enzymatic Browning of Fruits and Vegetables– A Comprehensive ReviewO. Al-abbasy^{1*}, Z. Alsarraf², N. Mahdi³, A. Al-barwari¹

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Available Online: 02.12.2025**How to cite this article:**Al-abbasy, O., Alsarraf, Z., Mahdi, N., & Al-barwari, A. (2025). Ascorbic acid: Rationale and applications in inhibiting enzymatic browning of fruits and vegetables– A comprehensive review. *Iranian Food Science and Technology Research Journal*, 21(6), 697-715. <https://doi.org/10.22067/ifstrj.2025.91193.1391>**Abstract**

Enzymatic browning mostly happens in fresh fruits and vegetables and is critical in determining the product's shelf life. A class of enzymes known as polyphenol oxidases are responsible for this color alteration. Polyphenol oxidase is the main enzyme that catalyzes the oxidation of phenolic compounds in the presence of oxygen, forming brown pigment. Therefore, several methods are required to prevent these reactions. Natural ascorbic acid is considered among the highly effective chemicals in stopping this reaction and preventing browning. It is a non-toxic and effective alternative to synthetic chemicals. It is also characterized by having powerful antioxidant and free radical scavenging properties. This review offers a focused and novel contribution to the scientific literature by exclusively investigating AA as an anti-browning agent (ABA), enabling a detailed understanding of its specific mechanisms, efficacy, and practical applications. Unlike broader reviews that cover many inhibitors, this work provides a comparative analysis of the performance of AA in various foods, highlighting its strengths and limitations in different contexts. By integrating research from past years, we highlight different approaches, such as the combination with synergists and integration with edible coatings and packaging. Importantly, we not only describe its optimal conditions and benefits, but also assess its limitations, such as its instability and susceptibility. Finally, the evidence and future directions are organized in a way that helps food technologists identify promising protocols, design preservation strategies, and avoid previously documented limitations of future research.

Keywords: Ascorbic acid, Browning control, Enzymatic browning, Fruits, Vegetables**Introduction**

Browning in fruits and vegetables (FV) is a major challenge to the food manufacturing industry due to the influence on appearance, nutritional value, and safety of the food product (Tinello & Lante, 2018). Consumers most often rely on visual cues to assess food quality and make informed decisions about freshness. The discoloration of the product contributes to decreased market viability and greater consumer rejection (Dias *et al.*, 2020). In addition to the visual impact of discoloration,

browning also negatively impacts the utilization of produce and losses of production resources across the entire agricultural supply chain, from producers to retailers (Moon, Kwon, Lee, & Kim, 2020). Two major types of biochemical reactions cause this undesirable discoloration: non-enzymatic browning and enzymatic browning. Non-enzymatic browning which is similar to the Maillard reaction, is more common in heat processing and less relevant for fresh produce. On the other hand, enzymatic browning (EB) dominates in fresh

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and minimally processed fruits and vegetables, where it causes tissue damage and rapid exposure to oxygen (Alrushdi, Mohammed *et al.*, 2025; Paravisini & Peterson, 2019). The effects of EB on quality are particularly significant during postharvest handling, storage, and minimal processing. These operations result in significant quality loss and economic cost (Moon *et al.*, 2020). EB is associated with three enzymes: phenylalanine ammonia-lyase (PAL), peroxidase (POD) and polyphenol oxidase (PPO) (Arnold & Gramza-Michałowska, 2022). PPO (EC 1.10.3.1) catalyzes the EB reaction in plants, fungi, and bacteria. It is a copper-dependent cofactor protein, which can be activated as tyrosinase (monophenolase) or catechol-oxidase (diphenolase) (Sommano, Chanasut, & Kumpoun, 2020).

This enzyme converts phenols to quinones, which combine with other substances to produce melanoid, a brown pigment (Lim & Wong, 2018; Singh *et al.*, 2018). EB affects color retention and leads to nutritional and sensory losses, weakening marketability and reducing consumer acceptance. Therefore, it may be responsible for food waste (Shrestha *et al.*, 2020). To mitigate the effects of EB, the fresh produce industry has traditionally relied on chemical inhibitors, but the rising concerns regarding the safety and regulatory status of synthetic additives, due to their possible health risks (e. g. allergic reactions), have increased focus on natural, food-safe alternatives (Bobo-García, Arroqui, Merino, & Vírveda, 2020; Favre, Dos Santos, López-Fernández, Mazzobre, & del Pilar Buera, 2018). Considering this, exploring novel natural additives for food without negative side effects that have anti-browning abilities is critically desirable for food-processors (Al-Abbasy, Ali, Rashan, & Al-Bajari, 2021). Ascorbic acid (AA), is a crucial natural substance found in abundance in plant sources. It can replace artificial additives to preserve FV, avoid costly losses, and extend shelf life. The use of AA in this field is due to its non-toxicity and lack of

reported adverse effects in typical food applications (Dias *et al.*, 2020; Paudel *et al.*, 2020). AA has been selectively applied to various fresh foods (e.g, apples, potatoes, mushrooms, etc) and its potential use as a preservative. This review provides a comprehensive summary by collecting and analyzing data exclusively on AA applications as an anti-browning agent in fresh products. Thus, it offers a scientific basis for food technologists to develop safe and effective anti-browning strategies.

Search Criteria

Approximately 220 articles have been surveyed for this review. These articles were selected by a search process based on keywords such as ascorbic acid, browning, enzymatic browning, polyphenol oxidase, peroxidase, and browning control on electronic database systems such as PubMed, John Thompson, Scopus, Science Direct, and Google Scholar. After analyzing all the articles, many were excluded, and the research papers that were useful for this study were identified.

Browning

The Browning phenomenon is widely considered a major problem facing the global economy. It is a biological process that often occurs in food and under different circumstances, which can have beneficial and harmful effects. For fruits, vegetables, and nuts, browning is considered undesirable. These foods lose their nutritional value and produce undesirable color, flavor, and taste. However, this process can be advantageous in processing other products such as chocolate, coffee, black tea (Debelo, Li, & Ferruzzi, 2020), bread, and soy sauce (Nooshkam, Varidi, & Bashash, 2019). It is necessary to create a distinctive color and flavor in these foods. The EB reaction (Fig. 1) is mainly caused by PPO (Arnold & Gramza-Michałowska, 2022). PPO oxidizes diphenol to quinone, a highly sensitive active molecule subsequently polymerize into brown pigments by reacting with other phenolics, sugars, amino acids, or soluble proteins.

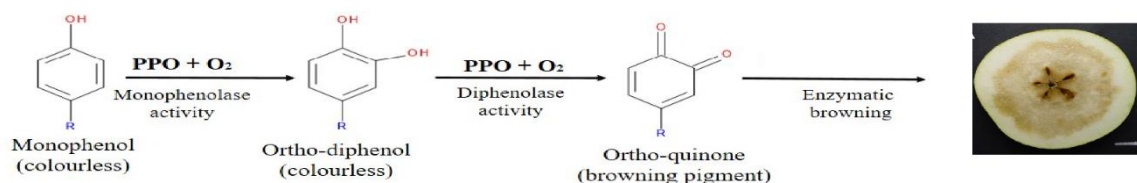


Fig. 1. The schematic of the enzymatic browning process (Moon *et al.*, 2020) (modified)

It mostly happens in products during harvesting, storage, and processing (Queiroz, Mendes Lopes, Fialho, & Valente-Mesquita, 2008). The presence of EB degrades color, flavor, and nutritional content, resulting in reduced shelf life (Rashan & Al-abbasy, 2021). The rate of EB depends on the phenolic substrate contents, pH, oxygen accessibility, temperature, concentration, and source of an enzyme (Lante, Tinello, & Nicoletto, 2016).

Ascorbic Acid

AA, commonly known as vitamin C, is a vital nutrient that can be acquired from various sources like fruits, vegetables, and multivitamin supplements (Ali *et al.*, 2024; Murererehe, Uwitonze, Nikuze, Patel, & Razzaque, 2022). It plays a crucial role in numerous physiological functions within human health. AA is a six-carbon lactone naturally produced from glucose and other sugars (Kükürt & Gelen, 2024; Mittu *et al.*, 2022). Chemically, AA is a weak organic acid with an enediol structure conjugated with the carbonyl group in the lactone ring (Al-burgus, Ali, & Al-abbasy, 2024; Atta, Mohamed, & Abdelgawad, 2017). The

compound's acidity and antioxidant properties are attributed to the two enolic hydrogen atoms supplying the electrons. Dehydroascorbic acid, which has the same vitamin activity as AA, is created when oxygen is present (Fig. 2) (Li, Guo, & Wang, 2008; Rico, Martin-Diana, Barat, & Barry-Ryan, 2007).

It is a natural component and broadly spread in FV (Kabasakalis, Siopidou, & Moshatou, 2000; Pernice *et al.*, 2009), such as citrus fruits, strawberries, potatoes, tomato, broccoli, gooseberry, cabbage, turnip, red peppers, green peppers, and many green veggies. So, plants are becoming important sources of vitamin C (Malo & Wilson, 2000). It plays a role in numerous mono and dioxygenases to conserve metals in a reduced state. For example, mono and dioxygenases commonly comprise copper or iron as redox cofactors. The food sector uses AA extensively because of its conservatory benefits, namely as the most potent and non-toxic antioxidant and a free-radical scavenger (Santos *et al.*, 2022; Tu, Njus, & Schlegel, 2017).

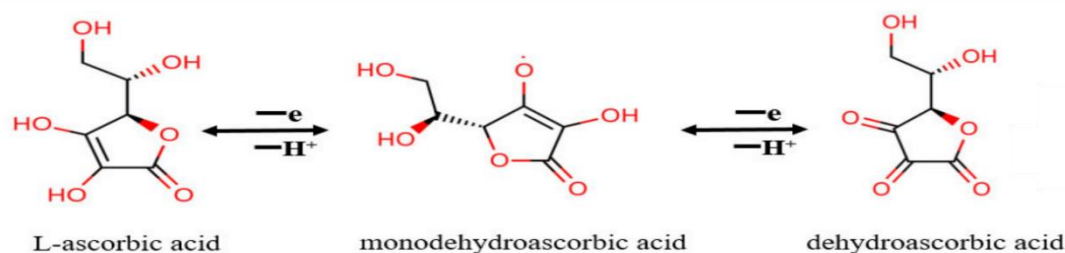


Fig. 2. Degradation of L-ascorbic acid to dehydroascorbic acid (Yin *et al.*, 2022)

Since humans can not synthesize AA, it must be obtained through diet; it is considered an important nutrient. However, during food storage, AA is quickly oxidized, which causes considerable losses (Hassan, Haddad, & Sultan,

2020). It is also acknowledged as the most important hydrophilic antioxidant for different types of enzymatic reactions (Bratovic, 2020; Telang, 2013). This atypical antioxidant produces monodehydro ascorbate, a radical that

combines preferentially with radicals rather than non-radical substances, and it gives a single reducing equivalent. This occurs because monodehydro ascorbate would form an energetically undesirable tricarbonyl structure if an electron were removed. AA oxidizes exclusively to monodehydroascorbate rather than producing this structure, and when monodehydroascorbate combines with other radicals, it oxidizes through processes that might prevent the creation of this undesirable structure (Du, Cullen, & Buettner, 2012). Reactive nitrogen and oxygen species, including hydroperoxyl radicals and superoxide, aqueous peroxy radicals, and singlet oxygen, are easily scavenged by AA. (Yates, Schlicker, & Suitor, 1998).

Browning Control

Browning control has always been a challenge for the food industry. Since phenols, oxygen, and enzymes are the three primary components contributing to browning processes, the primary goal of anti-browning agent treatments is to eliminate one of these variables and thus control browning (Younus, Mahdi, Al-Abbasy, & Sheej Ahmad, 2025). This control will extend the shelf life, improve customer acceptance of the food, and reduce economic losses (Bobo-García *et al.*, 2020). To attenuate this undesirable process, industries employ chemical, physical, or biological techniques and treatments (Feng, Liu, Liu, Shi, & Wang, 2020; Zhang, He, & Simpson, 2018). These techniques have drawbacks, including changes in the sensory and nutritional qualities of the final products and potential health risks (Levaj *et al.*, 2023). An efficient and widely used method for managing the EB of various fresh-cut FV is chemical treatment with anti-browning agents. These agents can be classified based on their mechanisms of action into several categories as chelating agents, reducing agents, acidulants, complexing agents, and enzyme inhibitors (Altunkaya & Gökmen, 2008, 2009). Successful browning inhibition by reducing agents, such as AA, erythorbic acid, ascorbyl-2-phosphate, ascorbyl-triphosphate,

and cystein was observed (Farouk *et al.*, 2020; Sikora & Świeca, 2018).

Anti-browning Mechanism of AA

Color is the key factor in appraising the consumer's quality of FV. Many natural and synthetic chemical compounds are commonly used to obstruct the browning rate of fresh FV through various mechanisms (Rodrigues, Asquiere, & Orsi, 2014). This treatment thus provides temporary protection to preserve these products from damage (Eleni & Theodoros, 2011). One of these important and extensively used compounds for this purpose is AA. Due to its safety and nutritional value, it is frequently employed as an anti-browning agent in dipping solutions (Pizzocaro, Torreggiani, & Gilardi, 1993; Veltman, Kho, Van Schaik, Sanders, & Oosterhaven, 2000). It has been investigated both alone and in conjunction with other antioxidants or preservatives, such as some phenols that function as competitive inhibitors like resorcinols (Yoruk & Marshall, 2003). This status is generally recognized as safe (GRAS). The Thailand Food and Drug Administration claims that there hasn't been any restriction for the upper limit of AA consumption (Suttirak & Manurakchinakorn, 2010). Understanding the kinetic and mechanistic pathways through which AA inhibits enzymatic browning is crucial for optimizing its application in food preservation. Kinetics studies on the rate of PPO inhibition by AA have shown that AA interacts with the enzyme's active site, preventing the oxidation of phenolic compounds (Ndakidemi, Mneney, & Ndakidemi, 2014; Yoruk & Marshall, 2003). The inhibitory action is concentration-dependent, with higher AA concentrations leading to more significant inhibition of PPO activity. Several studies have focused on elucidating these mechanisms:

1. Reduction of o-quinones back to phenolic substrates: As a strong reducing agent, AA converts the reactive o-quinones, the intermediate compounds produced during the PPO, back into their original phenolic forms before they can polymerize. This

reaction prevents the accumulation of pigmented compounds and effectively halts browning at an early stage (Lee & Whitaker, 1995; Tang, Guo, & Zhu, 2023).

2. Reduction of Cu^{2+} to mononuclear copper Cu^+ at the PPO active site (Suttirak & Manurakchinakorn, 2010).
3. Consumption of oxygen: AA can also react non-enzymatically with O_2 , reducing the oxygen availability required for PPO activity. This secondary mechanism further slows down quinone production and subsequently browning progression (Sapers & Miller, 1998).
4. AA acts as a competitive inhibitor of PPO by binding to the active center of the enzyme and competing with its natural substrates (Gao, Li, & Li, 2013).

On the other hand, AA may decrease the pH of crushed food products and thus inhibits the activities of POD and PPO or activates phenylpropanoid metabolism to raise total phenolic content (Landi, Degl'Innocenti, Guglielminetti, & Guidi, 2013; Li, Ding, Li, & Yan, 2023; Zhou *et al.*, 2021). The efficiency of AA derivatives, containing dehydroascorbic acid, ascorbic acid-2-phosphate, isoascorbic acid, and ascorbic acid-2-sulfate, was associated with that of AA (Hsu, Shieh, Bills, & White, 1988). Based on kinetic studies, AA and isoascorbic acid are the utmost effective inhibitors of mushroom PPO, followed by dehydroascorbic acid. According to (Özoğlu & Bayındırlı, 2002), AA is more effective than its isomer isoascorbic acid.

Essential Motives for Using AA as an Anti-browning Agent

Brown pigment on food products decreases consumer tolerability and affects their taste and nutritional status. Healthcare awareness has led to an increased focus on finding solutions to food browning (Singh *et al.*, 2018). Consumers evaluate food products based on color, appearance, taste, and nutritional value (Peanparkdee & Iwamoto, 2019). Chemical-based techniques were employed to prevent FV from browning. However, the application of

this technique may pose health risks because some chemicals may include hazardous residues (Moon *et al.*, 2020). Therefore, researchers resorted to using AA as an effective inhibitor of various PPOs (Arslan, Erzengin, Sinan, & Ozensoy, 2004; Chazarra, García-Carmona, & Cabanes, 2001). AA is a natural, abundantly available, and non-toxic material. Because of its great browning-inhibiting efficiency and the lack of deleterious effects in the foods to which it is introduced, AA is used as a reference or comparison to natural and synthetic chemicals, as well as natural extracts.

Effect of AA on Individual Commodities

AA has been widely studied for its antioxidant effect against enzymatic browning in fruits and vegetables. This process is important for the maintenance of quality, color, and nutrient value of the commodity. AA efficacy on different commodities varies significantly with commodity type, processing type, and concentration of AA used. Literature has proven the effectiveness of AA in affecting the quality of apples and preventing fresh apples from turning brown. Granny Smith was less susceptible to browning upon immersion in (3% w/v) compared to other types (Yi *et al.*, 2017). Similarly, another study on fresh apples (*Malus pumila*) concluded that PPO was inhibited by a large amount of AA (Wen *et al.*, 2021). The impact of added AA stored at 4°C was investigated on POD and PPO reaction in light pretreated watermelon (*Cucumis melo L. reticulatus* Naud), and POD activity declined with time (And & Watson, 2001). The inhibitory efficacy of potatoes' PPO by chili pepper and lemon extracts was 70% and 54%, respectively. Anti-browning activity could be attributed to its high content of AA (Mercimek *et al.*, 2015). In addition, the AA application to freshly harvested potatoes can postpone browning by inhibiting PAL (Zhou *et al.*, 2021). AA is a potent competitive inhibitor of PPO's active site with catechol as a substrate to reduce the intensity of browning. This has been demonstrated in certain plants such as lettuce (*Lactuca sativa*) (Altunkaya &

Gökmen, 2008), Peppermint (*Mentha piperita*) (Kavrayan & Aydemir, 2001), and tea leaves (*Camellia sinensis*) (Öztürk, Aksoy, & Küfrevioğlu, 2020). Table 1 summarizes the inhibition of PPO activity in different vegetables and fruits using AA.

Synergistic Treatments

There have been various reports on the AA combination with other factors having a synergistic effect in inhibiting the action of the PPO and thus enhancing the control of the browning of fresh foods. These synergists may be plant extracts, chemical compounds, or physical treatments. For instance, a synergistic influence of AA (0.05% w/v) and cinnamon essential oil nanoemulsion (0.1–0.4% w/v) was found to completely inactivate PPO at 4 °C after two days of storage (Xu *et al.*, 2020). AA with sodium dihydrogen phosphate controls the browning of yellow noodles by slightly reducing the PPO activity but significantly decreasing the free quinone content (Tang *et al.*, 2023). The mango fruits were immersed in calcium nanoparticles with different

concentrations of AA and stored for 35 days at 6 °C. The browning in kiwifruit segments can be effectively prevented by adding 0.3 g/L polyvinylpyrrolidone to the medium, which is followed by 0.2 mg/L AA. A mixture of 1.2% yerba mate leaf extract and 1% AA was proven to optimally suppress EB in fresh-cut Granny Smith apples (Rodríguez-Arzuaga & Piagentini, 2018). Another study indicated that *Rosa roxburghii* extract, rich in AA and coumaric acid, inhibits the activity of PPO in Red Fuji cloudy juice (Yu *et al.*, 2021). According to (Abdelwahd, Hakam, Labhilili, & Udupa, 2008; Babaei, Abdullah, Saleh, & Abdullah, 2013), the most effective method for managing EB was to pre-treat the tip of Faba bean seeds and *Curculigo latifolia* using polyvinylpyrrolidone and AA for nine hours. The PPO and POD activities responsible for browning of sugarcane juice were entirely inhibited when AA was combined with bentonite (Hithamani, Medappa, Chakkaravarthi, Ramalakshmi, & Raghavarao, 2018).

Table 1- Inhibitory effect of AA treatments on PPO activity in fruits and vegetables
(* (w/v) and **mmol/L)

Fruit/Vegetable	AA Concentration	% PPO inhibition	References
Abiu	0.176% *	80%	(Susanto, Arif, Widayanti, & Matra, 2023)
Apple (Red Delicious)	0.4 *	45%	(Arnold & Gramza-Michałowska, 2022)
Apple puree	0.35% *	100	(Vercammen <i>et al.</i> , 2012)
Apple slices (Golden Delicious)	1% *	~25%	(Watada, Izumi, Luo, & Rodov, 2005)
Apple slices (Newton Pippin)	1% *	~59%	(Watada <i>et al.</i> , 2005)
Apricot	2 **	100%	(Derardja, Pretzler, Kampatsikas, Barkat, & Rompel, 2019)
Apricot	10 **	99%	(Morsy & Rayan, 2019)
Banana	1% *	~85%	(Danyen, Boodia, & Ruggoo, 2009)
Banana	0.5% *	~60%	(Moline, Buta, & Newman, 1999)
Blackberry	0.1 **	100%	(Schwartz, Olgin, & Klinman, 2001)
Blueberry	Varied	99.7%	(Siddiq & Dolan, 2017)
Eggplant	0.5–1% *	15–25%	(Barbagallo, Chisarib, & Patanè, 2012)
Guava puree	1% *	~72%	(Khedr & Ali, 2017)
Lettuce	5 **	~90%	(Landi <i>et al.</i> , 2013)
Lotus Rhizome	0.08**	86.21%	(Li, Li, Yan, & Wang, 2022)
Mango	0.1% *	60%	(Arafat, 2009)
Mango	1%*	75%	(Prasad, Sharma, & Srivastav, 2016)
Mango	2% *	85-90%	(Marín <i>et al.</i> , 2021)

Mango Puree	Varied	91.1% - 95.5%	(Guerrero-Beltrán, Swanson, & Barbosa-Cánovas, 2005)
Mung Bean	2–20**	51–60%	(Sikora & Świeca, 2018)
Mushroom	0.04 **	50%	(Hsu <i>et al.</i> , 1988)
Peach	1**	95.4%	(Jia, Jiang, Chen, Wei, & Shao, 2022)
Pear slices	1%*	75%	(Kader, Zagory, Kerbel, & Wang, 1989)
Pineapple	0.05**	70%	(Auon-Reyna <i>et al.</i> , 2019)
Pineapple slices	0.8% *	~50%	(Pérez-Gago, González-Aguilar, & Olivas, 2010)
Pomegranate	150 **	100%	(Patil, Dhande, Thigale, & Rajput, 2011)
Potato	1%*	65%	(Li <i>et al.</i> , 2023)
Potato slices	0.5%*	~65%	(AL-abbasy <i>et al.</i> , 2025)
Strawberry	2% *	~88%	(Nazoori, Poraziz, Mirdehghan, Esmailizadeh, & Zamani Bahramabadi, 2020)

AA and its isomer, erythorbic acid, were found to be equally effective in preventing browning in pineapple slices. The EB inhibition in apricots was investigated by inactivating their PPO using plant proteases and AA. Apricot PPO activity significantly declined to 66 and 55 % when treated with (0.1 and 0.2 mM mmol/L) of AA, respectively, compared to the untreated samples (Derardja *et al.*, 2019). A combination of AA and citric acid has been shown to significantly reduce the browning of fresh fruits such as Chinese Yam (*Dioscorea polystachya* Turczaninow). Citric acid can lower the pH of food products, hence increasing the efficacy of AA (Yang, Song, Wang, Wang, & Zhao, 2024). Alternatively, the combination of AA with chitosan (a natural polymer derived from chitin) has increased the ability to inhibit browning of plums and fresh-cut apples. Chitosan acts as a protective environmental barrier, allowing AA to work more effectively, protecting the food from external air (Liu, Yuan, Chen, Li, & Liu, 2014; Özdemir & Gökmen, 2019). The ultrasound

and AA showed a synergistic inhibitory effect on fresh-cut apple PPO activity during storage (Jang & Moon, 2011). The combination of AA (2g/L) with modified atmosphere packing inhibited the PPO activity and browning intensity of fresh-cut eggplant treated for four days (Sarengaowa *et al.*, 2022). (Almeida & Nogueira, 1995) reported that PPO activity in banana, pear, and peach fruits was controlled using AA and heat treatment at 70°C for 2 minutes. The researchers highlighted the applicability of this method to substitute for the use of sulfur dioxide to control PPO activity.

Application in Juices

The juice industry faces significant challenges with EB during the storage of fresh fruit juices. AA is one of the most extensively used natural preservatives to combat this issue. It has been shown through many studies that AA is effective in preventing this phenomenon that occurs in fruit juices. These effects are summarized in Table 2.

Table 2- Inhibitory effect of AA on different fruit juices

Fruit juice	AA concentration or combination	References
Banana	(1000 – 1200 ppm)	(Tapre & Jain, 2016)
Banana	(470 mg/L)	(Koffi, Sims, & Bates, 1991)
Apple	(1.8 mmol/L)	(Hope, 1961)
Apple	ascorbic and cyclodextrins	(Hicks, Sapers, & Seib, 1990)
Apple	ascorbic acid and acidic polyphosphate	(Sapers <i>et al.</i> , 1989)
Apple	0.2% w/v	(Komthong, Igura, & Shimoda, 2007)
Apple	-	(Ribárszki <i>et al.</i> , 2022)
Cloudy apple	-	(Özoğlu & Bayındırlı, 2002)
Pear	-	(Montgomery & Petropakis, 1980)
Pear	0.24% (w/v)	(Jiang, Kim, Nam, Yim, & Eun, 2016)

Common Trends, Optimal Conditions, and Knowledge Gaps

In compiling recent applications and developments of AA preservation, certain trends are common. First among these is the strong focus on multifunctional natural preservation techniques that accommodate consumer demand for sustainable and clean solutions. Synergistic mixtures and their use in edible or active packaging systems reflect a broader trend towards comprehensive antioxidant protection, combining physical barriers and biochemical activity to maintain freshness and prevent spoilage (Chua, Chan, Tay, & Soo, 2023; Oliveira *et al.*, 2025). Optimum conditions for the efficacy of AA vary across commodities, but certain factors are universal in their implication. The concentration of AA tends to be in the range of 0.1-1%, being more potent for the browning controlling (Rasane *et al.*, 2024). The pH of the processed product is a crucial factor, with the optimum value being slightly acidic (4-6), which is the most suitable environment for AA action (Nicolas, Richard-Forget, Goupy, Amiot, & Aubert, 1994). Moreover, the exposure to the light and temperature may affect AA stability, with studies showing that it is most effective when used at low storage temperature (0-10°C), thereby inhibiting rapid degradation and maintaining its anti-browning activity (Oms-Oliu *et al.*, 2010; Paull, 1999). However, gaps in knowledge must be filled by conducting further research on long-term stability under conditions of fluctuating storage, compatibility with other natural preservatives, and scalability of smart packaging and packaging technologies. Although genetic approaches to increasing endogenous AA levels in crops hold much promise. However, regulatory concerns and

public acceptability remain significant barriers (Oms-Oliu *et al.*, 2010; Paull, 1999).

A Comparative Study

AA is widely recognized as one of the most effective natural anti-browning agents due to its strong reducing ability and non-toxicity. However, its effectiveness varies when compared to other chemical and natural inhibitors (Table 3), particularly in terms of mechanism of action, stability, cost, and sensory impact (Kim *et al.*, 2019). For example, citric acid acts as a metal chelator in preventing EB, through binding to the copper in PPO or pH-lowering, as mentioned by (Altunkaya & Gökmen, 2008). This applies to most carboxylic acids. However, the amino acid L-cysteine can inhibit the browning process principally by reducing POD and PPO activities via forming a stable complex with copper (Li *et al.*, 2023; Sadoon & Ahmad, 2020).

Toxicologically, certain compounds, such as sulfites, especially in some sensitive persons, like asthmatics, are recognized to cause adverse health effects. Among this extremely sensitive group, many deaths have occurred as a result of consuming sulfur-containing foods. On the other hand, sulfates can also release SO₂ gas in some foods, which harms the flavor of the product being treated (Taylor *et al.*, 1988). These effects were not observed when using AA. Natural extracts are highly concentration-dependent in inhibiting enzymatic browning, which limits their use due to their strong taste or color (Gómez-López, 2002). In contrast, ascorbic acid is used at lower concentrations, is colorless and tasteless, and is consistently effective in a wide range of products (Rico *et al.*, 2007).

Table 3- Comparison of anti-browning agents with ascorbic acid

ABA	Mechanism of action	Effectiveness	Side effects/ Limitations
Ascorbic acid	Reduces quinones back to phenols, acts as an antioxidant, and an oxygen scavenger	High short-term effectiveness, often combined with other agents for stability	Unstable, rapidly oxidizes, leading to browning rebound (re-browning)
Citric acid	Lowers pH, reducing PPO activity, and chelates copper at the active site of PPO	Moderate, best when combined with other agents	Alters flavor due to acidity, limited by pH tolerance of the product
Sodium metabisulfite	Reduces quinones, a strong PPO inhibitor, and reacts with o-quinones to form colorless products	Very high	Can cause allergic reactions (sulfite sensitivity), banned or limited in some countries
Calcium ascorbate	Combining ascorbic acid's reduction with calcium's firming effect	Better than pure ascorbic acid in maintaining texture and color	Costly, limited by calcium-induced hardness in some products
EDTA	Chelates copper ions from PPO, inhibiting enzyme activity	Moderate	Not approved for all food uses; may require labeling, synthetic additives
Hexylresorcinol	Directly inhibits PPO; prevents formation of melanins	Very high	More expensive, limited regulatory approval in some regions
Honey	Natural antioxidant, mild antimicrobial, and PPO inhibition	Low to moderate	May impart unwanted flavor/sweetness, variable composition
L- Cysteine	Reacts with o-quinones to form colorless products; an antioxidant	High	May cause off-odors (sulfurous), allergen concerns
Acetylated monoglycerides	Form coatings that limit oxygen contact, a physical barrier	Moderate (indirect)	Less effective alone, usually used with other agents

Future Trends in the Use of Ascorbic Acid as an Anti-Browning Agent

Changes in technology have expanded the potential uses of AA in food preservation, particularly when combined with other antioxidants or antimicrobials to produce blends with enhanced efficacy (Baghi, Gharsallaoui, Dumas, & Ghnimi, 2022). To increase its stability and extend its functional life, packaging systems such as nanoemulsions, biopolymer coatings, edible films, and biodegradable coatings are being developed. This feature has oxygen-binding capabilities and provides continuous antioxidant protection, enhancing food quality and sustainability (Aziz, Ali, Haddad, & Abdullah, 2024; Kuai *et al.*, 2021). On the processing front, treatments such as ultrasound-assisted dipping, vacuum impregnation, and spraying are being optimized to improve AA delivery and minimize waste (Díaz-Montes & Castro-Muñoz, 2021). In the long term, plant breeding and genetic engineering methods seek to improve the

natural growth or retention of AA in crops, reducing the need for external additives (Zhu, Wu, & Sun, 2019). Finally, with growing consumer demand for organic products with clean labels, AA's natural and safe properties are gaining popularity, and future applications are expected to focus on regulatory compliance and clear labeling.

Limitation

AA is also frequently used in food applications but faces limitations due to its instability and high susceptibility to oxidation, especially when exposed to air, light, and temperature (Comunian, Abbaspourrad, Favaro-Trindade, & Weitz, 2014; Wagner & Buettner, 2023). It may reduce its effectiveness over time and cause temporary effect. It can affect flavor and pH to become sour or tart, which is undesirable in fruits with delicate or low-acid flavors. This change in sensory properties requires maximum dosage adjustment to be made carefully, especially in

sensitive juice products where flavor balance is of the highest priority. These limitations underscore the importance of considering AA's chemical stability and sensory effect in well-designed systems (Taqi, AL-shahery, & Al-Abbasy, 2024; Yin *et al.*, 2022).

Conclusion

The global food consumption landscape is rapidly transforming. Consumer preferences are driving a swift evolution in food choices worldwide. Color is one of the most important factors influencing consumer purchasing decisions. However, repeat purchases depend on the consumer's satisfaction with the product's texture, flavor, and nutritional value. EB is one of the serious problems to the food industry and represents the second most vital reason for the negative impact on color, which leads to deterioration. EB's progress has been related to PPO activity. Therefore, inhibiting this enzyme will certainly limit the development of this phenomenon. Applying anti-browning agents is considered the best effective way to control the EB reaction in fresh FV. In this regard, it is preferable to have natural agents such as AA. The widespread use of AA is due to its generally abundant, non-toxic, safe, and healthy properties compared to other chemicals. The capability of AA to prevent EB is represented by: reducing o-quinones to diphenols or Cu^{2+} to Cu^+ at the PPO active site, while also decreasing crushed food pH. Based on the research considered, we conclude that AA was a good inhibitor for PPO and could prevent EB of FV. The anti-browning

efficiency of AA is related to two main factors: the types and cultivars of produce, and its concentration when it is alone or the concentrations of its synergetic factors. But often elevated concentrations had a greater effect than low ones. Also, the low pH at which AA works has a better effect in preventing the formation of brown pigmentation. After conducting this review and evaluating the general nutritional situation, we recommend using AA to prevent this phenomenon from forming on its own without combining it with other substances. This is to avoid the side effects of the combined substances on human health after consuming food products.

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Authors Contribution

O.Y.A: Conceptualization, Writing– review and editing, Supervision. **Z.H.A:** Data curation, Writing– original draft, Investigation. **N.M.M:** Formal analysis, Writing– original draft. **A.S.A:** Validation, Data analysis, Software.

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Abbreviation

Anti-browning agent (ABA); Ascorbic acid (AA); Enzymatic browning (EB); fruits and vegetables (FV); Peroxidase (POD); Phenylalanine ammonia-lyase (PAL), Polyphenol oxidase (PPO).

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مقاله مروری

جلد ۲۱، شماره ۶، بهمن - اسفند ۱۴۰۴، ص. ۷۱۵-۶۹۷

اسید اسکوربیک: ضرورت‌ها و کاربردها در مهار قهوه‌ای شدن آنزیمی میوه‌ها و سبزیجات - یک بررسی جامع

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چکیده

قهوه‌ای شدن آنزیمی بیشتر در میوه‌ها و سبزیجات تازه اتفاق می‌افتد و در تعیین ماندگاری محصول بسیار مهم است. دسته‌ای از آنزیم‌ها که به‌عنوان پلی‌فنول اکسیداز شناخته می‌شوند، مسئول این تغییر رنگ هستند. پلی‌فنول اکسیداز آنزیم اصلی است که اکسیداسیون ترکیبات فنلی را در حضور اکسیژن کاتالیز می‌کند و رنگدانه قهوه‌ای تشکیل می‌دهد. بنابراین، روش‌های مختلفی برای جلوگیری از این واکنش‌ها مورد نیاز است. اسید اسکوربیک طبیعی از جمله مهمترین مواد شیمیایی در متوقف کردن این واکنش و جلوگیری از قهوه‌ای شدن است. این ماده یک جایگزین غیرسمی و مؤثر برای مواد شیمیایی مصنوعی است. همچنین با داشتن خواص آنتی‌اکسیدانی قوی و مهار رادیکال‌های آزاد مشخص می‌شود. این مقاله مروری، مطالعات انجام شده در مورد استفاده از اسید اسکوربیک به‌عنوان یک عامل ضد قهوه‌ای شدن در منابع غذایی مختلف را خلاصه می‌کند. این مقاله ممکن است بینشی در مورد پتانسیل توسعه استفاده از اسید اسکوربیک در عملیات قهوه‌ای شدن مواد غذایی و کاهش ضررهای اقتصادی در بخش مواد غذایی ارائه دهد.

واژه‌های کلیدی: اسید اسکوربیک، سبزیجات، کنترل قهوه‌ای شدن، قهوه‌ای شدن آنزیمی، میوه‌ها

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مندرجات

مقالات پژوهشی

- ۶۰۴ خشک کردن ورقه‌های سیب‌زمینی با هوای گرم به کمک لامپ رشته‌ای: بهینه‌سازی فرآیند با استفاده از روش سطح پاسخ
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مقالات مروری

- ۷۱۵ اسید اسکوربیک: ضرورت‌ها و کاربردها در مهار قهوه‌ای شدن آنزیمی میوه‌ها و سبزیجات- یک بررسی جامع
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بهمن - اسفند ۱۴۰۴

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