Ferdowsi University of Mashhad

Iranian Food Science and Technology Research Journal

Vol.20 No.3

2024

ISSN:1735-4161

Contents

Iranian Food Science and Technology Research Journal

Vol. 20 No. 3 2024

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

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

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

Editorial Board:

Mortazavi, Seyed A.	Professor, Department of Food Science and Technology, Ferdowsi University of Mashhad, Iran
Shahidi, F.	Professor. Department of Food Science and Technology, Ferdowsi University of Mashhad, Mashhad, Iran
Habibi najafi, M.	Professor, Department of Food Science and Technology, Ferdowsi University of Mashhad, Mashhad, Iran
Razavi, Seyed M. A.	Professor, Department of Food Science and Technology, Ferdowsi University of Mashhad, Mashhad, Iran
Kashaninejad, M.	Professor, Department of Food Science and Technology, Agricultural Sciences & Natural Resources University of Gorgan, Gorgan, Iran
Khomeiri, M.	Professor, Department of Food Science and Technology, Agricultural Sciences & Natural Resources University of Gorgan, Gorgan, Iran
Farhoosh, R.	Professor, Department of Food Science and Technology, Ferdowsi University of Mashhad, Mashhad, Iran
Fazli Bazzaz, S.	Professor, Department of Pharmaceutical Chemistry, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
Koocheki, A.	Professor, Department of Food Science and Technology, Ferdowsi University of Mashhad, Mashhad, Iran
Mohebbi, M.	Professor, Department of Food Science and Technology, Ferdowsi University of Mashhad, Mashhad, Iran
Ghanbarzadeh, B.	Professor, Department of Food Science and Technology, Faculty of Agriculture, Tabriz University, Tabriz, Iran
Alemzadeh, I.	Professor, Department of Food Chemical Engineering, Faculty of Chemical and Petroleum Engineering, Sharif University of Technology, Tehran, Iran
Rajabzadeh, GH.	Associate Professor, Department of Food Nanotechnology, Research Institute of Food Science and Technology, Mashhad, Iran
Heydarpour, M.	Associate Professor, Brigham and Women's Hospital, Boston, Massachusetts. United States America
Ghoddusi, H. B.	Associate Professor, School of Human Sciences, London Metropolitan University, England
Khosravidarani, K.	Professor, Department of Food Industry, School of Nutrition Sciences & Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Abbaszadegan, M.	Professor, Director Water & Environmental Technology Center, Arizona State University, United States of America
Mohammadifar, M. A.	Associate Professor, Research Group for Food Production Engineering, Technical University of Denmark, Denmark
Vosoughi, M.	Professor, Department of Food Chemical Engineering, Faculty of Chemical and Petroleum Engineering, Sharif University of Technology, Tehran, Iran
Almasi, H.	Associate Professor, Department of Food Science and Technology, Urmia University, Urmia , Iran
Fathi, M.	Associate Professor, Department of Food Science and Technology, Isfahan University of Technology Isfahan, Iran
Abbasi, S.	Professor, Department of Food Science and Technology, Tarbiat Modares University, Tehran, Iran
Borges, N.	Professor, Faculty of Nutrition and Food Sciences, University of Porto; Portugal
Moazzami, Ali A.	Doctor of Philosophy, Department of Molecular Sciences, Swedish University, Sweden
Dr. Nkemnaso Obi C.	Department of Microbiology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria
Dr, Olalekan Adeyeye S.A.	Department of Food Technology, Hindustan Institute of Technology and Science, Chennai, Tamil Nadu, India

Publisher Ferdowsi University of Mashhad

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

P.O.BOX: 91775- 1163

Fax: (98)051-38787430

E-Mail: ifstrj@um.ac.ir

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

Contents

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

Research Article
Vol. 20, No. 3, Aug.-Sep. 2024, p. 1-15

The Effect of Xanthan-based Edible Coatings Enriched with Oleic Acid on the Storage Quality and Antioxidant Properties of Sapodilla (*Manilkara zapota*) Fruit

D. Rezakhani Nejad[1], A. Mirzaalian Dastjerdi[2], S. Rastegar[2]*

1 and 2- Ph.D. Student and Associate Professor, Department of Horticultural Sciences, Faculty of Agriculture and Natural Resources, University of Hormozgan, Bandar Abbas, Iran, respectively.
(*- Corresponding Author Email: rastegarhort@gmail.com)

Received: 01.01.2024
Revised: 15.02.2024
Accepted: 28.02.2024
Available Online: 01.06.2024

How to cite this article:
Rezakhani Nejad, D., Mirzaalian Dastjerdi, A., & Rastegar, S. (2024). The effect of xanthan-based edible coatings enriched with oleic acid on the storage quality and antioxidant properties of sapodilla (*Manilkara zapota*) fruit. *Iranian Food Science and Technology Research Journal*, *20*(3), 1-15. https://doi.org/10.22067/ifstrj.2024.86132.1307

Abstract

The sapodilla fruit has a limited shelf life due to its perishability and rapid moisture loss. The application of edible coatings has attracted much interest because they are effective in prolonging the shelf life of fruits. This study aims to evaluate the effectiveness of an edible coating made from xanthan gum (XG) (0.1% and 0.2%) combined with oleic acid (Ol) (1%) in prolonging the shelf life of sapodilla fruit at 8 ± 1 °c and a relative humidity (RH) of 85-90%. Weight loss was significantly reduced in the treated fruits, with the minimum weight loss observed in the Xan 0.2% + Ol treatment. Except for the Ol treatment, the other treatments showed a higher level of firmness compared to the control. At the end of the experiment, the treatments significantly reduced fruit respiration. The treated fruits also showed significantly increased antioxidant capacity and higher levels of ascorbic acid compared to the control. The lowest TSS (22.8%) level was noted in the Xan 0.2 + Ol treatment. Moreover, the results showed that fruit treated with Xan 0.1% + Ol coating exhibited higher activity in the superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) enzymes compared to the fruit treated with Xan 0.2 + Ol coating and the control samples. In general, fruits treated with Xan 0.2 + Ol and Xan 0.1% + Ol demonstrated the highest overall quality compared to the control and other treatments. Therefore, the application of these treatments is recommended for maintaining the quality of sapodilla fruit.

Keywords: Coatings, Oleic acid, Sapodilla, Storage, Xanthan

Introduction

Sapodilla, *Manilkara zapota*, is the most well-known and the most widely used fruit of the Sapotaceae family. Sapodilla is a rich source of nutrients (carbohydrates, organic acids, proteins, amino acids), minerals (potassium, calcium, iron), and includes numerous bioactive compounds primarily composed of allergitannins, galotannins, phenolic acids, and flavonoids. Due to its rich phytochemical profile in both edible and non-edible parts, Sapodilla has various medicinal potential through different biological activities. Due to its perishability and quick moisture loss, the fruit of Sapodilla has a short shelf life, but post-harvest technologies can enhance fruit storage to some extent. There are several methods for preserving and improving the post-harvest life of fruits and vegetables, among which the use of biodegradable films and coatings is very promising (Khalil *et al*., 2020). Since the edible coating is made from natural

©2024 The author(s). This is an open access article distributed under Creative Commons Attribution 4.0 International License (CC BY 4.0).
https://doi.org/10.22067/ifstrj.2024.86132.1307

materials, it is safe and suitable for human consumption (Paidari *et al*., 2021). Furthermore, edible coatings have many advantages, including the ability to form a semi-permeable barrier against gases and water vapor, help maintain firmness, add gloss to coated fruits while improving market acceptability, enhancing mechanical properties, and preventing the loss of volatile compounds (Galus & Kadzi´nska, 2015). Xanthan gum is an extracellular high molecular weight polysaccharide produced by *Xanthomonas campestris* bacteria and is one of the most important commercial microbial hydrocolloids used in the food industry as a thickening and stabilizing agent (Zheng *et al*., 2019). Xanthan gum-based edible coating is a long-chain polymeric substance containing polymeric functional groups that exhibit unique properties under specific conditions, which can improve the mechanical properties of biodegradable materials. Xanthan can improve the tensile behavior of starch layers without reducing their water absorption capacity and water vapor permeability (Sapper *et al*., 2019). Gelatin-carboxymethyl cellulose (CMC) films with xanthan gum showed improved physical and mechanical properties (Nur Hazirah *et al*., 2016). A composite film of nanocapsules/xanthan gum can prolong the storage of freshly cut apples by reducing the initial respiration rate (Galindo-P´erez *et al*., 2015). Lipid-based coatings (oleic acid) hinder moisture transfer due to their relatively low polarity. Therefore, a new approach is proposed to increase the shelf life of perishable fruits with minimal processing (Md. Sharif *et al*., 2017; Mladenoska *et al*., 2012; Karunanayake *et al*., 2020; Mitelut *et al*., 2021). The content of oleic acid has been demonstrated to serve as an emulsifying agent and a base for preserving fruits (Butar-Butar *et al*., 2021). In a study by Setianingsih *et al*. (2023), a combination of palmitic, stearic, and oleic acids was employed in an emulsion, leading to enhanced appearance and prolonged shelf life of orange fruits.

Numerous studies have investigated the effectiveness of coatings in preserving the quality of various fruits during storage. These studies have consistently shown positive results. For instance, in a study conducted by Wani *et al*. (2021), it was found that the use of Arabic gum, carrageenan, and xanthan gum combined with lemon grass essential oil proved to be effective in maintaining the quality parameters of strawberries during storage. This coating treatment demonstrated superior results compared to the control, indicating its potential for extending the shelf life and preserving the quality of strawberries. In another study, guava fruits were coated with a mixture of Arabic gum (10%), oleic acid (1%), and cinnamon oil (1%). This coating significantly delayed browning development in guava compared to other treatments, while preserving fruit firmness and reducing weight loss. Additionally, it prevented lipid peroxidation and electrolyte leakage at the end of the storage period, indicating its effectiveness in maintaining fruit quality (Vargas *et al*., 2006). Previous studies have extensively explored the use of fruit coatings; however, this research introduces an innovative approach by utilizing a combination of xanthan gum and oleic acid to preserve the quality of sapodilla fruits. To the best of our knowledge, there is no previous report on the application of this compound for maintaining the quality of sapodilla fruit. Therefore, this research represents the first investigation to assess the effects of xanthan gum and its combination with oleic acid on the shelf life of sapodilla.

Materials and Methods

Fruit Treatment and Edible Coating Preparation

For this study, mature stage fruits of the Alano variety of Sapodilla (Chico) were harvested from the Minab Agricultural Research Station. Immediately after harvest, the fruits were transported to the physiology laboratory. Healthy and uniform fruits were carefully selected for the experiment. The selected fruits were subjected to various experimental treatments, which included the application of edible coatings comprising xanthan gum at concentrations of 0.1% and

0.2%, along with oleic acid at a concentration of 1.0%. Following the application of the coatings, the treated fruits were stored at 8±1 °c and a relative humidity of 90-85% for 10, 30, 20, and 40 days.

Weight Loss

The weight loss of the fruits was measured using a digital scale by weighing each individual fruit on the first day and at regular intervals (every 10 days). The percentage of weight loss was calculated using the following formula (Juhaimi *et al*., 2012).

Weight loss (%) = [(Fruit initial weight-fruit weight at each sampling time)/Fruit initial weight] ×100

Firmness

The firmness of the fruits was measured at two points in the middle section (without the peel). Two points on the surface of each fruit were selected and the firmness was reported in N (Juhaimi *et al*., 2012).

Respiration

A specific weight of fruit was placed in a plastic container. The initial CO_2 level (D1) was measured during the first instance, and again after 20 minutes (D2), using a respirometer device called the STEP Respiratory Sensor. The respiration rate was expressed in mL/kg.h according to the following formula (Xing *et al*., 2008).

$RCO_2 = (D2- D1) \times 10^6 \times$ Volume of container$/$(Time × Fruit weight)

Total Antioxidant Activity

The antioxidant activity was calculated based on the method described by Brand-Williams *et al*. (1995), and the absorbance of the samples was measured using a microplate reader at 517 nanometers. The antioxidant activity was then calculated using the following formula.

Antioxidant activity (%)= [1 - (Abs sample/ (Abs control)] × 100

Soluble Solids Content

The soluble solids content of the fruit juice was measured in terms of the Brix^o using a digital refractometer (DBR95, Taiwan).

Ascorbic Acid Content

The ascorbic acid content was measured using a spectrophotometer (O'Grady *et al*., 2014). The absorbance of the samples was read at 510 nanometers using a microplate reader instrument (Epoch, Bio Tek® Instruments, VT, USA).

Catalase

The measurement of catalase activity in the samples was performed using the method described by Chance & Maehly (1955). The absorbance at 240 nanometers was read using a UV-Visible spectrophotometer model UNICO 2150 for a duration of one minute.

Ascorbate Peroxidase (APX)

The enzyme activity was measured using the method described by Nakano & Asada (1981). The activity of this enzyme was measured at 290 nanometers for a duration of two minutes using a spectrophotometer. The enzyme activity was then calculated in terms of units per gram of fresh weight (U/g FW min) of the flesh or peel.

Superoxide Dismutase (SOD)

The SOD activity was measured using the method described by Giannopolitis & Ries (1977). The absorbance of the samples was read at 560 nanometers using a spectrophotometer. The activity of this enzyme was expressed as units per gram of fresh weight (U/g FW).

Results and Discussion

Weight Loss

The fruits covered with edible coatings exhibited lower weight loss compared to the control throughout the storage. At the end of the storage period, the Xan 0.2 % + Ol treatment had a significant reduction in fruit weight loss by 28.51% compared to the control (Fig. 1). Weight loss is an important indicator of fruit

quality during the post-harvest stage and is influenced by transpiration due to differences in vapor pressure between the fruit and the environment (Yaman & Bayonidirli, 2002). It is also influenced by respiration and various physiological mechanisms (Juhaimi *et al*., 2012). The application of an edible coating composed of chitosan and essential oils helps minimize water loss in fruits by reducing the rate of water vapor transmission. This coating acts as a barrier, effectively blocking the escape of water vapor and inhibiting excessive transpiration. By regulating the loss of moisture, the coating helps maintain the fruit's water content and prevents dehydration. The reduction in water vapor transmission rate achieved through the coating contributes to the preservation of fruit quality and freshness (Widyastuti *et al*., 2023). Similar results were obtained in the study conducted by Kumar *et al*. (2021). They found that a bilayer edible coating consisting of xanthan gum and beeswax on tomato resulted in increased shelf life and improved resistance to water vapor transmission.

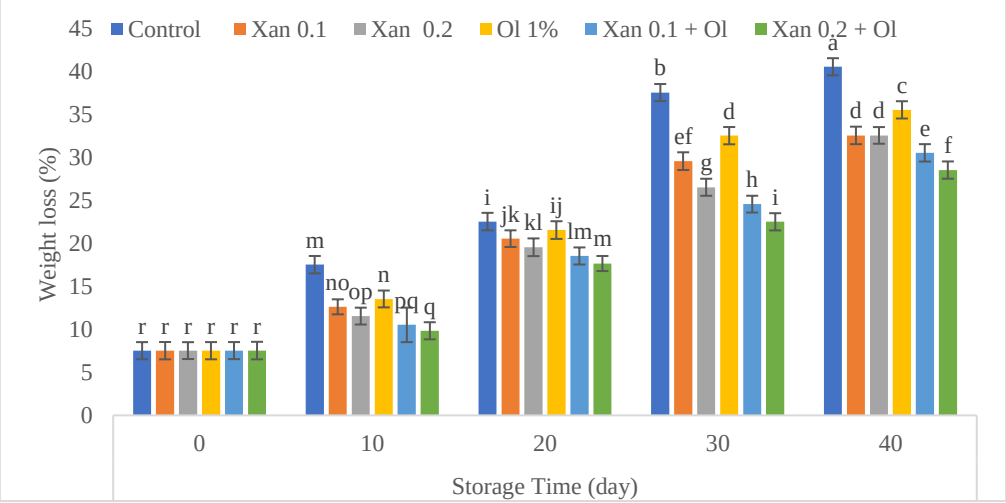


Fig. 1. The interaction effect between treatments (Control, Xanthan 0.1 %, Xanthan 0.2 %, Oleic acid 1%, Xanthan 0.1 % + Oleic acid 1 %, and Xanthan 0.2 % + Oleic acid 1 %) and storage periods on the weight loss of sapodilla fruit during four periods (10 days) of storage at 8 ± 1℃ and 85% relative humidity (RH)
The values represent mean ± SD from three replicates (n=3). Statistical analysis was performed using LSD test.

Firmness

Fruit firmness gradually decreased during storage (Fig. 2-A). The highest level of firmness was observed in the Xan 0.2 % + Ol and Xan 0.1 % + Ol treatments, respectively (Fig. 2-B). Fruit firmness is an important parameter in fresh horticultural products, and it decreases as the storage time increases. The loss of fruit firmness is concurrent with changes that occur in the cell wall structure. Pectin substances are responsible for the integrity of fruits. They are the main components of the middle lamella and predominantly form the initial cell wall structure. The effects of coatings on fruits and their storage conditions vary significantly, as evidenced by the considerable impact on fruit firmness. In the case of sapota fruits, the reduction in firmness can be attributed to several factors, including a decrease in cellular turgor pressure, the release of extracellular and vascular air, and the degradation and breakdown of cell walls (Shah *et al*., 2016). The addition of lipids to the polymer composition can increase the water repellency behavior of the coating and consequently reduce water permeability. Typically, the permeability values increase linearly with a decrease in the concentration of the essence (Sánchez-González *et al*.,

2010). In this regard, Vargas *et al.* (2006) reported that the combination of oleic acid with chitosan coating resulted in decreased permeability and respiration rate due to surface solid density.

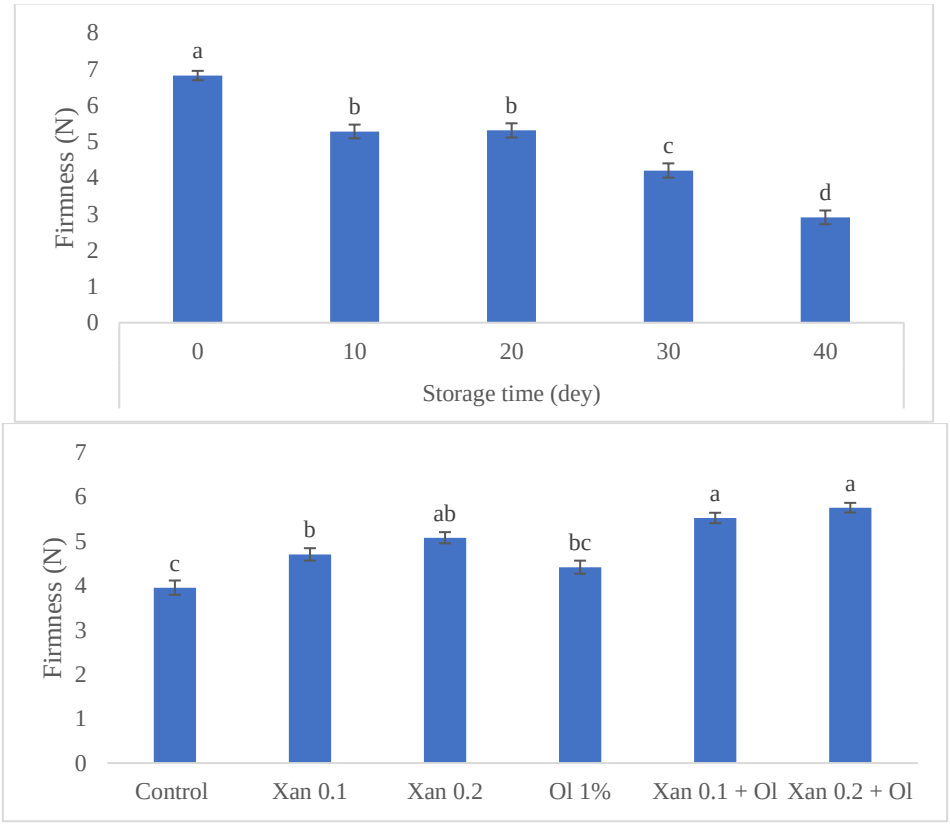


Fig. 2. The effect of storage periods and different coatings (Control, Xanthan 0.1%, Xanthan 0.2 %, Oleic acid 1%, Xanthan 0.1 % + Oleic acid, and Xanthan 0.2 % + Oleic acid) on the firmness of sapodilla fruit during four periods (10 days) of storage at 8 ± 1℃ and 85% relative humidity (RH)

The values represent mean ± SD from three replicates (n=3). Statistical analysis was performed using LSD test.

Respiration

As shown in Fig. 3, the fruits treated with Xan 0.2 % + Ol and Xan 0.1 % + Ol showed the lowest respiration rate compared to other samples. Edible coatings could modify gas transfer (carbon dioxide, oxygen, and ethylene) and consequently delay respiration rate and physiological processes, thus extending the shelf life of fruits and vegetables. Furthermore, previous studies have shown that the delay in respiration rate in fruits can be attributed to the inhibition of ethylene production (Hassan *et al.*, 2018). The results of this study were consistent with the findings reported by Naveed *et al.* (2024), who documented a significant reduction in the respiration rate of jujube fruits covered with xanthan gum coating.

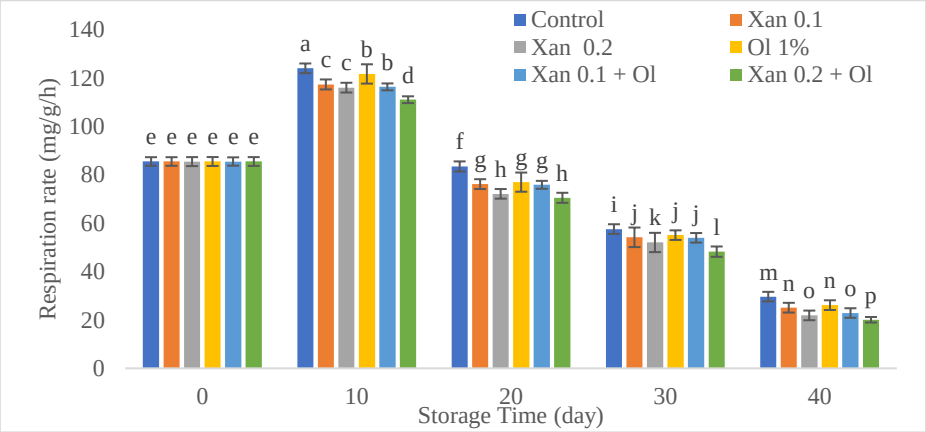


Fig. 3. The interaction effect between treatments (Control, Xanthan 0.1 %, Xanthan 0.2 %, Oleic acid 1%, Xanthan 0.1 % + Oleic acid, and Xanthan 0.2 % + Oleic acid) and storage periods on the respiration rate of sapodilla fruit during four periods (10 days) of storage at 8 ± 1℃ and 85% relative humidity (RH)

The values represent mean ± SD from three replicates (n=3). Statistical analysis was performed using LSD test.

Total Antioxidant Activity

The fruits treated with Xan 0.1% + Ol exhibited the highest antioxidant activity, which was 5.23 times greater than the activity observed in the control (Fig. 4). Usually, the production of reactive oxygen species (ROS) increases during fruit ripening and storage, leading to oxidative stress and fruit decay. Reports have shown that an increase in total phenolic content correlates with an increase in antioxidant capacity (Etemadipoor *et al*., 2020). In this study, since the combined xanthan coating with oleic acid disrupts the ripening process, it results in higher antioxidant activity in the fruit. Additionally, several changes in vitamin content throughout the ripening process can influence antioxidant activity.

Ascorbic Acid Content

The highest content of ascorbic acid was observed in the fruit treated with Xan 0.1 % + Ol (137.86 mg/100 g FW) compared to the control (100.41 mg/100 g FW) (Fig. 5). Ascorbic acid acts as an antioxidant in fruits and reduces fruit damage. This action is achieved through the elimination of free radicals produced during the ripening and oxidation process. The presence of oxygen can have a negative impact on the ascorbic acid content in fruits (Ayranci & Tunc, 2004). The presence of an edible coating on the fruit reduces the detrimental effects of oxygen, and this is accomplished through the performance of antioxidant compounds present in the coating and their role as a barrier against oxygen transfer (Oliveira *et al*., 2017).

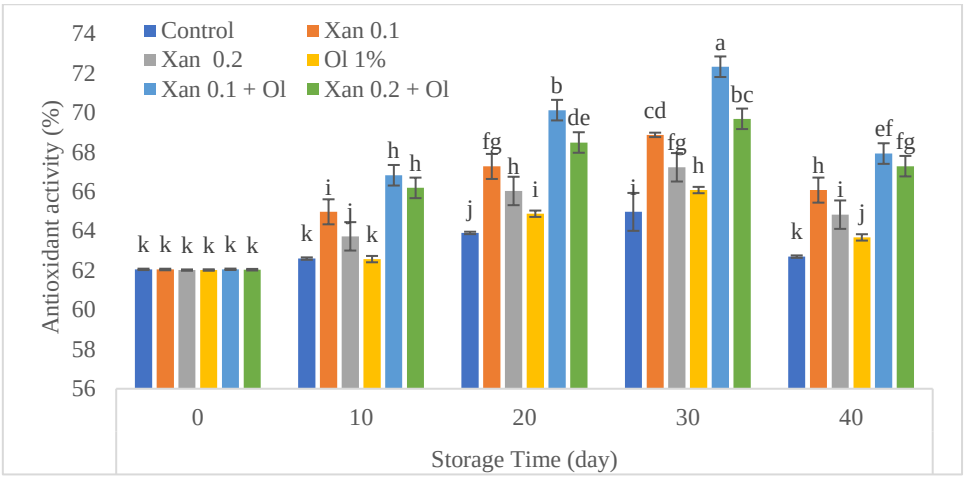


Fig. 4. The interaction effect between treatments (Control, Xanthan 0.1 %, Xanthan 0.2 %, Oleic acid 1%, Xanthan 0.1 % + Oleic acid, and Xanthan 0.2 % + Oleic acid) and storage periods on the total antioxidant activity of sapodilla fruit during four periods (10 days) of storage at 8 ± 1℃ and 85% relative humidity (RH)
The values represent mean ± SD from three replicates (n=3). Statistical analysis was performed using LSD test.

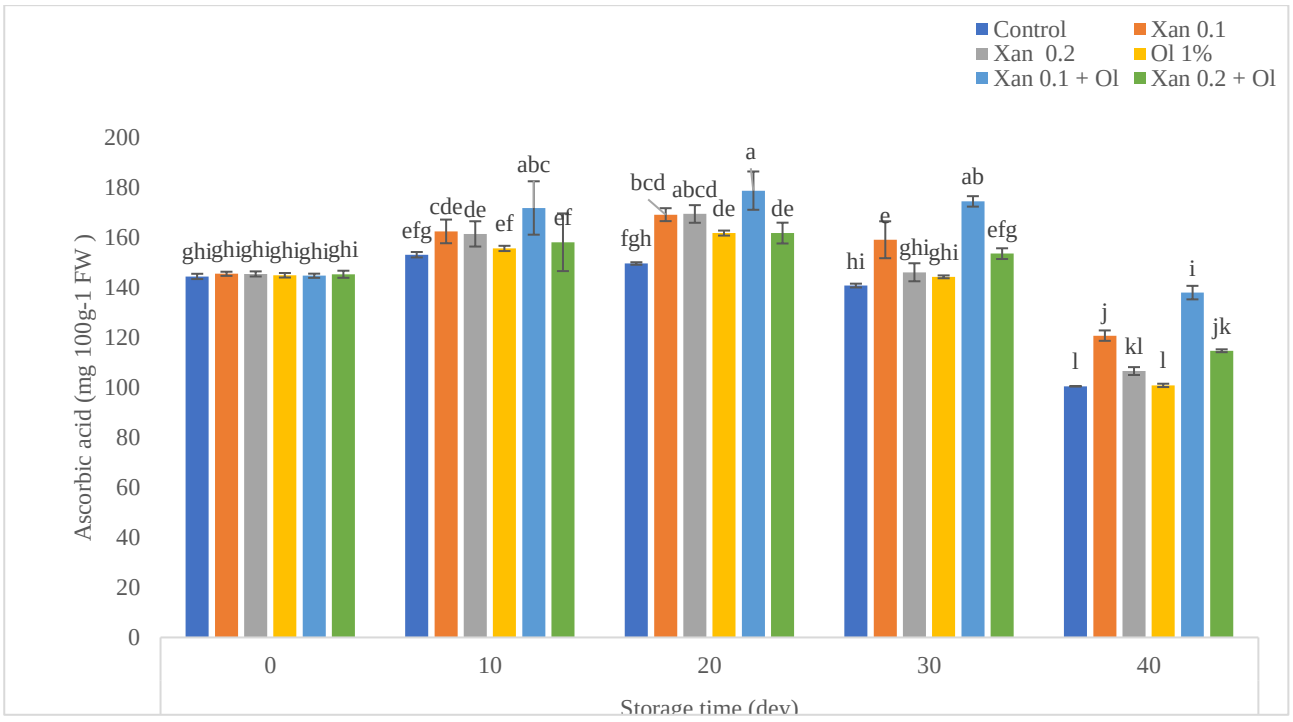
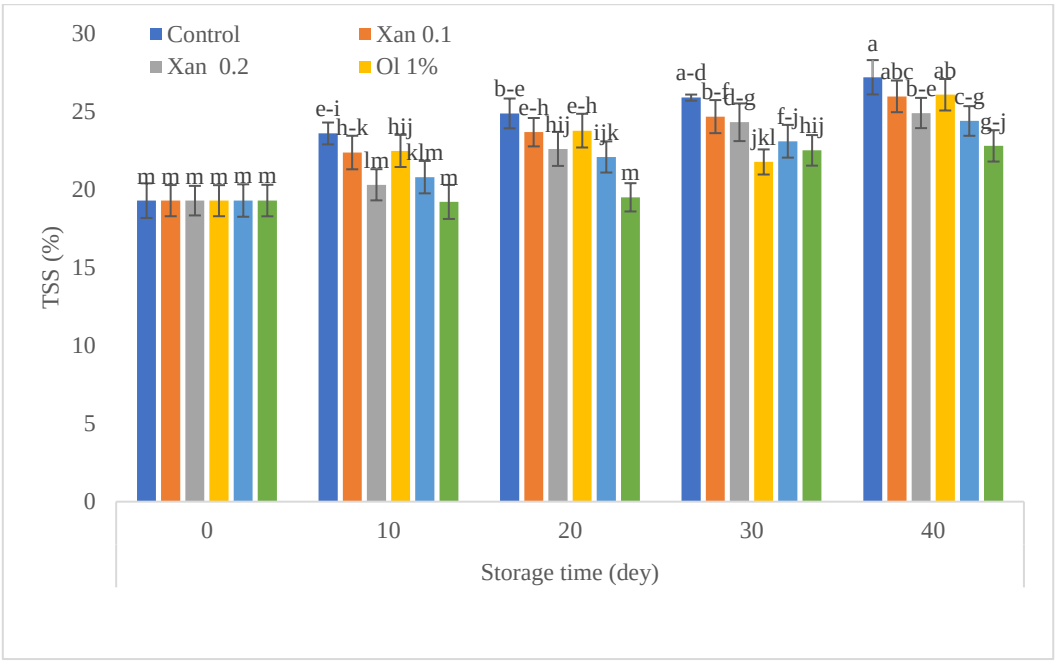
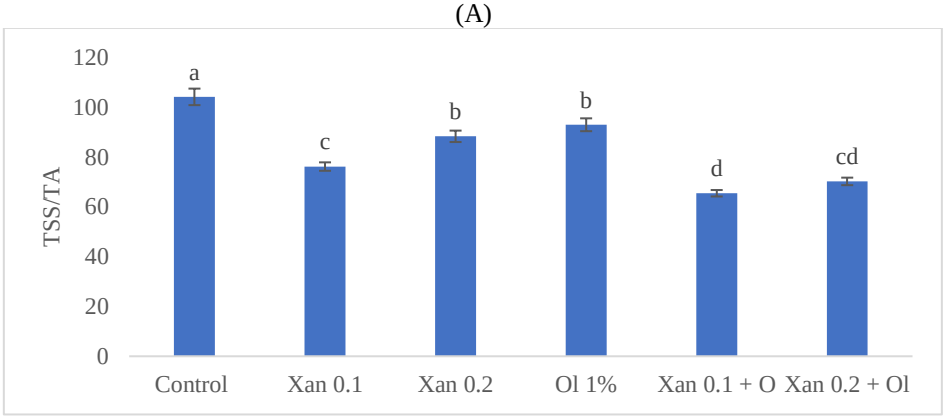


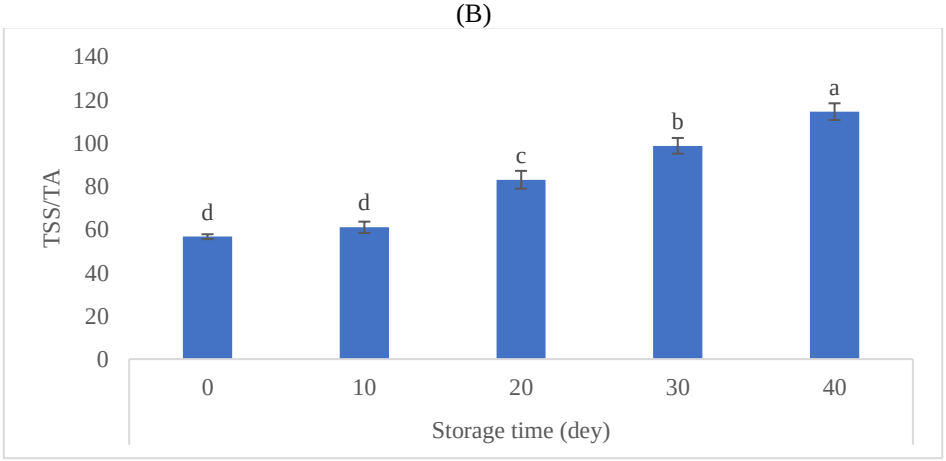
Fig. 5. The interaction effect between treatments (Control, Xanthan 0.1 %, Xanthan 0.2 %, Oleic acid 1%, Xanthan 0.1 % + Oleic acid, and Xanthan 0.2 % + Oleic acid) and storage periods on the ascorbic acid content of sapodilla fruit during four periods (10 days) of storage at 8 ± 1℃ and 85% relative humidity (RH)
The values represent mean ± SD from three replicates (n=3). Statistical analysis was performed using LSD test.



(A)



(B)



(C)

Fig. 6. The interaction effect between treatments (Control, Xanthan 0.1 %, Xanthan 0.2 %, Oleic acid 1%, Xanthan 0.1 % + Oleic acid, and Xanthan 0.2 % + Oleic acid) and storage periods on the total soluble solids (TSS) (A), and TSS/TA ratio (B-C) of sapodilla fruit during four periods (10 days) of storage at 8 ± 1℃ and 85% relative humidity (RH)

The values represent mean ± SD from three replicates (n=3). Statistical analysis was performed using LSD test.

Therefore, the combined xanthan coating with oleic acid enabled the fruits to retain higher levels of ascorbic acid compared to other treatments. This was accompanied by reduced oxygen permeability and limited moisture transfer from the fruit surface. In a similar report, Kumar *et al*. (2023) reported that an edible coating based on xanthan gum and pomegranate peel extract on mango fruit enhanced the physical and antioxidant properties of the fruit due to providing increased flexibility.

Total Soluble Solids and TSS/TA Ratio

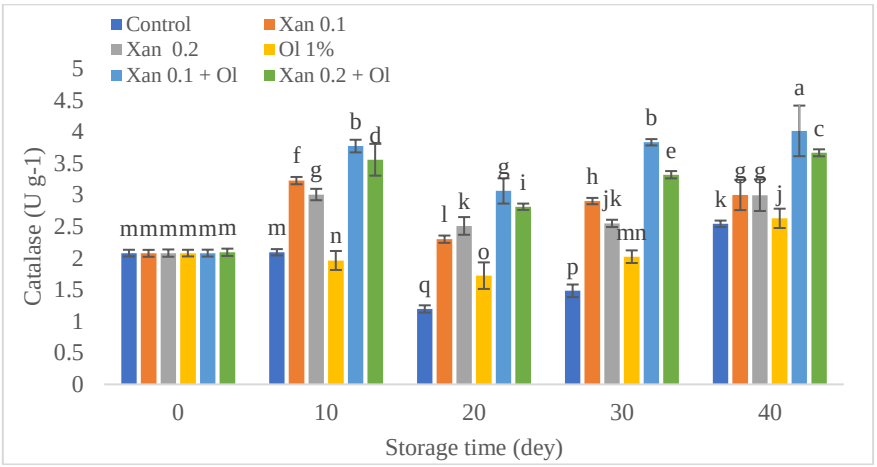
The highest increase in TSS (27.2 %) was observed in the control fruit at the end of storage, while the lowest increase (22.80 %) was observed in fruits coated with Xan 0.2 % + Ol (Fig. 6-A). Generally, the TSS of fruits gradually accumulates during ripening. This phenomenon may be due to the hydrolysis reaction and conversion of starch into simple sugars. At the end of storage, the control fruit showed an increase in the TSS/TA ratio by 104.099 compared to its initial state, while the Xan 0.1 % + Ol coating resulted in a decrease in the TSS/TA ratio (Fig. 6-B). The TSS/TA ratio gradually increased during storage (Fig. 6-C). Etemadipoor *et al*. (2020) reported similar results on coated guava fruits with a combination of Arabic gum, oleic acid, and cinnamon essential oil.

Enzymatic Activity

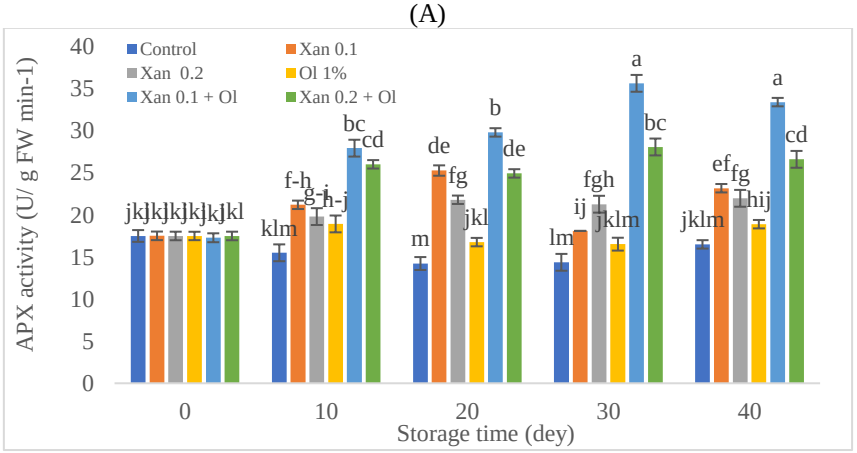
The activity of CAT enzyme showed a significant increase within the first 20 days of storage. The highest enzyme activity was observed in fruits treated with a combination of 0.1% Xan + Ol during the second storage period (Fig. 7-A). Similar findings were reported for other fruits such as chickoo (Camargo *et al*., 2016) and lychee (Zhang *et al*., 2018). The peak increase in APX activity was observed on the tenth day in Xan 0.1 % + Ol treatment, followed by a decrease (Fig. 7-B). Our findings are consistent with a previous study conducted by Ali *et al.* (2021), who found that using a CMC coating prior to storage preserved higher enzymatic activities and deactivated free radicals, reducing senescence in 'Kinnow' mandarin fruit under low-temperature conditions. The maximum activity of SOD enzyme in the coated fruits was observed 40 days after storage, and the peak in Xan 0.1 % + Ol-coated fruits was significantly higher than the control ($P < 0.05$) (Fig. 7-C). These results are similar to study conducted by Yuan *et al*. (2023), which examined the effect of a combined treatment of 1-methylcyclopropene and melatonin on the quality characteristics and active oxygen metabolism of stored mango fruit. An oxidative stress occurred throughout the entire storage period, characterized by an increase in active oxygen species such as superoxide, hydrogen peroxide, and hydroxyl radicals. The antioxidant system, which includes enzymes such as POD, SOD, CAT, and APX, plays an important role in preventing or reducing damage caused by ROS (Wang & Gao, 2013).

Conclusion

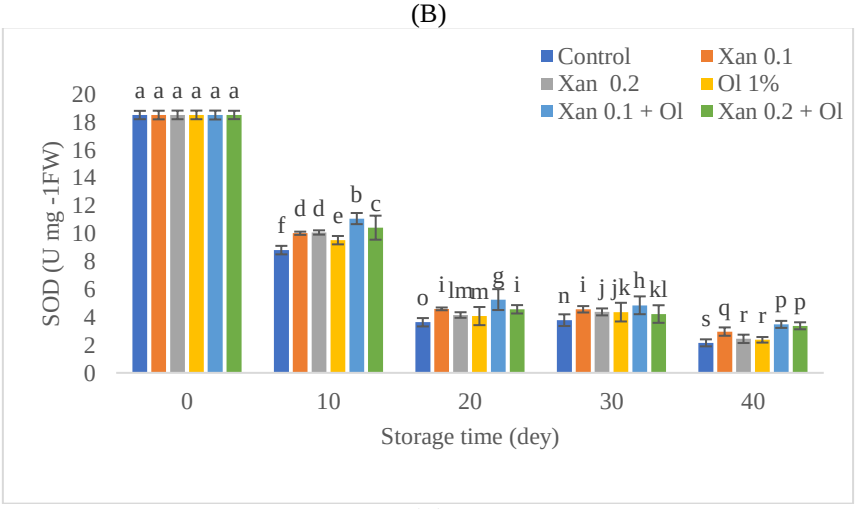
In conclusion, this study demonstrated that the application of edible coatings made from xanthan gum (XG) at concentrations of 0.1% and 0.2%, combined with oleic acid (Ol) at a concentration of 1%, effectively prolonged the shelf life of sapodilla fruit. The treated fruits exhibited reduced weight loss, with Xan 0.2% + Ol treatment showing the minimum weight loss. Additionally, the treated fruits maintained higher firmness levels compared to the control, except for the Ol treatment. The coatings also significantly reduced fruit respiration and enhanced antioxidant capacity, as well as increased levels of ascorbic acid. Xan 0.2% + Ol treatment resulted in the lowest total soluble solids (TSS) level. Furthermore, Xan 0.1% + Ol coating demonstrated higher activity levels in the superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) enzymes compared to Xan 0.2% + Ol coating and the control.



(A)



(B)



(C)

Fig. 7. The interaction effect between treatments (Control, Xanthan 0.1 %, Xanthan 0.2 %, Oleic acid 1%, Xanthan 0.1 % + Oleic acid, and Xanthan 0.2 % + Oleic acid) and storage periods on catalase (A), ascorbate peroxidase (B), and superoxide dismutase (C) activities of sapodilla fruit during four periods (10 days) of storage at 8 ± 1℃ and 85% relative humidity (RH)

The values represent mean ± SD from three replicates (n=3). Statistical analysis was performed using LSD test.

As a conclusion, Xan 0.2% + Ol and Xan 0.1% + Ol treatments exhibited the highest overall quality among the examined treatments. Given the challenges associated with postharvest preservation of this fruit, the application of these coatings presents a suitable method for extending the storage period and maintaining the quality.

Author Contributions

D. Rezakhani Nejad: Data curation, investigation, methodology, software, writing–original draft. **A. Mirzaalian Dastjerdi, S. Rastegar:** Conceptualization, data curation, project administration, supervision, writing–review and editing.

Funding Sources

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

References

1. Ali, S., Anjum, M.A., Ejaz, S., Hussain, S., Ercisli, S., Saleem, M.S., & Sardar, H. (2021). Carboxymethyl cellulose coating delays chilling injury development and maintains eating quality of 'Kinnow'mandarin fruits during low temperature storage. *International Journal of Biological Macromolecules, 168*, 77-85. https://doi.org/10.1016/j.ijbiomac.2020.12.028
2. Ayranci, E., & Tunc, S. (2004). The effect of edible coatings on water and vitamin C loss of apricots (*Armeniaca vulgaris* Lam.) and green peppers (*Capsicum annuum* L.). *Food Chemistry, 87*(3), 339-342. https://doi.org/10.1016/j.foodchem.2003.12.003
3. Brand-Williams, W., Cuvelier, M.E., & Berset, C.L.W.T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology, 28*(1), 25-30. https://doi.org/10.1016/S0023-6438(95)80008-5
4. Butar-Butar, M.E.T., Sukawaty, Y., & Sa'adah, H. (2021). Formulation and evaluation lotion of Tengkawang seed fat (*Shorea mecistopteryx* Ridley) and cocoa fat (*Theobroma cacao* L.) as a base. *Jurnal Farmasi Etam (JFE), 1*(1), 1-9. https://doi.org/10.52841/jfe.v1i1.188
5. Camargo, J. M., Dunoyer, A. T., & García-Zapateiro, L. A. (2016). The effect of storage temperature and time on total phenolics and enzymatic activity of sapodilla (*Achras sapota* L.). Revista Facultad Nacional de Agronomía Medellín, 69(2), 7955-7963. https://doi.org/10.15446/rfna.v69n2.59140
6. Chance, B., & Maehly, A.C. (1955). [136] Assay of catalases and peroxidases. https://doi.org/10.1016/S0076-6879(55)02300-8
7. Etemadipoor, R., Dastjerdi, A.M., Ramezanian, A., & Ehteshami, S. (2020). Ameliorative effect of gum arabic, oleic acid and/or cinnamon essential oil on chilling injury and quality loss of guava fruit. *Scientia Horticulturae, 266*, 109255. https://doi.org/10.1016/j.scienta.2020.109255
8. Galindo-Pérez, M.J., Quintanar-Guerrero, D., Mercado-Silva, E., Real-Sandoval, S.A., & Zambrano-Zaragoza, M.L. (2015). The effects of tocopherol nanocapsules/xanthan gum coatings on the preservation of fresh-cut apples: Evaluation of phenol metabolism. *Food and Bioprocess Technology, 8*, 1791-1799. https://doi.org/10.1007/s11947-015-1523-y
9. Galus, S., & Kadzińska, J. (2015). Food applications of emulsion-based edible films and coatings. *Trends in Food Science & Technology, 45*(2), 273-283. https://doi.org/10.1016/j.tifs.2015.07.011
10. Giannopolitis, C.N., & Ries, S.K. (1977). Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiology, 59*(2), 309-314. https://doi.org/10.1104/pp.59.2.309

11. Hassan, B., Chatha, S.A.S., Hussain, A.I., Zia, K.M., & Akhtar, N. (2018). Recent advances on polysaccharides, lipids and protein based edible films and coatings: A review. *International Journal of Biological Macromolecules, 109,* 1095-1107. https://doi.org/10.1016/j.ijbiomac.2017.11.097
12. Hazirah, M.N., Isa, M.I.N., & Sarbon, N.M. (2016). Effect of xanthan gum on the physical and mechanical properties of gelatin-carboxymethyl cellulose film blends. *Food Packaging and Shelf Life, 9,* 55-63. https://doi.org/10.1016/j.fpsl.2016.05.008
13. Juhaimi, F.A., Ghafoor, K., & Özcan, M.M. (2012). Physical and chemical properties, antioxidant activity, total phenol and mineral profile of seeds of seven different date fruit (*Phoenix dactylifera* L.) varieties. *International Journal of Food Sciences and Nutrition, 63*(1), 84-89. https://doi.org/10.3109/09637486.2011.598851
14. Karunanayake, K.O.L.C., Liyanage, K.C.M., Jayakody, L.K.R.R., & Somaratne, S. (2020). Basil oil incorporated beeswax coating to increase shelf life and reduce anthracnose development in mango cv. Willard. *Ceylon Journal of Science, 49*(5), 355-361. https://doi.org/10.4038/cjs.v49i5.7802
15. Khajeh-Ali, S.H., Shahidi, F., Megahed, & Sedaghat, N. (2022). Evaluation of the effect of carboxy methyl cellulose edible coating containing Astragalus honey (*Astragalus gossypinus*) on the shelf-life of pistachio kernel. *Food Control, 139,* 109094. https://doi.org/10.1016/j.foodcont.2022.109094
16. Khalil, O.A., Mounir, A.M., & Hassanien, R.A. (2020). Effect of gamma irradiated *Lactobacillus* bacteria as an edible coating on enhancing the storage of tomato under cold storage conditions. *Journal of Radiation Research and Applied Sciences, 13*(1), 318-330. https://doi.org/10.1080/16878507.2020.1723886
17. Kumar, A., & Saini, C.S. (2021). Edible composite bi-layer coating based on whey protein isolate, xanthan gum and clove oil for prolonging shelf life of tomatoes. *Measurement Food, 2,* 100005. https://doi.org/10.1016/j.meafoo.2021.100005
18. Kumar, N., Pratibha, Upadhyay, A., Trajkovska Petkoska, A., Gniewosz, M., & Kieliszek, M. (2023). Extending the shelf life of mango (*Mangifera indica* L.) fruits by using edible coating based on xanthan gum and pomegranate peel extract. *Journal of Food Measurement and Characterization, 17*(2), 1300-1308. https://doi.org/10.1007/s11694-022-01706-6
19. Miteluț, A.C., Popa, E.E., Drăghici, M.C., Popescu, P.A., Popa, V.I., Bujor, O.C., & Popa, M.E. (2021). Latest developments in edible coatings on minimally processed fruits and vegetables: A review. *Foods, 10*(11), 2821. https://doi.org/10.3390/foods10112821
20. Mladenoska, I. (2012). The potential application of novel beeswax edible coatings containing coconut oil in the minimal processing of fruits. *Advanced Technologies, 1*(2), 26-34.
21. Nakano, Y., & Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and Cell Physiology, 22*(5), 867-880. https://doi.org/10.1093/oxfordjournals.pcp.a076232
22. Naveed, F., Nawaz, A., Ali, S., & Ejaz, S. (2024). Xanthan gum coating delays ripening and softening of jujube fruit by reducing oxidative stress and suppressing cell wall polysaccharides disassembly. *Postharvest Biology and Technology, 209,* 112689. https://doi.org/10.1016/j.postharvbio.2023.112689
23. O'grady, L., Sigge, G., Caleb, O.J., & Opara, U.L. (2014). Effects of storage temperature and duration on chemical properties, proximate composition and selected bioactive components of pomegranate (*Punica granatum* L.) arils. *LWT-Food Science and Technology, 57*(2), 508-515. https://doi.org/10.1016/j.lwt.2014.02.030
24. Oliveira, L.S., Rodrigues, D.C., Lopes, M.M.A., Moura, C.F.H., Oliveira, A.B., & Miranda, M.R.A. (2017). Changes in postharvest quality and antioxidant metabolism during development and

ripening of sapodilla (*Manilkara zapota* L.). *International Food Research Journal*, *24*(6), 2427-2434.

25. Paidari, S., Zamindar, N., Tahergorabi, R., Kargar, M., Ezzati, S., Shirani, N., & Musavi, S.H. (2021). Edible coating and films as promising packaging: a mini review. *Journal of Food Measurement and Characterization*, *15*(5), 4205-4214. https://doi.org/10.1007/s11694-021-00979-7
26. Sánchez-González, L., González-Martínez, C., Chiralt, A., & Cháfer, M. (2010). Physical and antimicrobial properties of chitosan–tea tree essential oil composite films. *Journal of Food Engineering*, *98*(4), 443-452. https://doi.org/10.1016/j.jfoodeng.2010.01.026
27. Sapper, M., Talens, P., & Chiralt, A. (2019). Improving functional properties of cassava starch-based films by incorporating xanthan, gellan, or pullulan gums. *International Journal of Polymer Science*. https://doi.org/10.1155/2019/5367164
28. Setianingsih, N.L., Suaniti, N.M., Wirawan, I.G. (2023) Increased attractiveness and shelf life of siamese tangerines (*Citrus nobilis* L.) using various palmitic, stearic, and oleic acid emulsions and mixtures. *AJARCDE (Asian Journal of Applied Research for Community Development and Empowerment)*, *13,7*(2), 87-96. https://doi.org/10.29165/ajarcde.v7i2.204
29. Shah, N.N., Vishwasrao, C., Singhal, R.S., & Ananthanarayan, L. (2016). n-Octenyl succinylation of pullulan: Effect on its physico-mechanical and thermal properties and application as an edible coating on fruits. *Food Hydrocolloids*, *55*, 179-188. https://doi.org/10.1016/j.foodhyd.2015.11.026
30. Sharif, Z.I.M., Mustapha, F.A., Jai, J., & Zaki, N.A.M. (2017). Review on methods for preservation and natural preservatives for extending the food longevity. *Chemical Engineering Research Bulletin, 19*. https://doi.org/10.3329/cerb.v19i0.33809
31. Vargas, M., Albors, A., Chiralt, A., & González-Martínez, C. (2006). Quality of cold-stored strawberries as affected by chitosan–oleic acid edible coatings. *Postharvest Biology and Technology*, *41*(2), 164-171. https://doi.org/10.1016/j.postharvbio.2006.03.016
32. Wang, S.Y., & Gao, H. (2013). Effect of chitosan-based edible coating on antioxidants, antioxidant enzyme system, and postharvest fruit quality of strawberries (*Fragaria x aranassa* Duch.). *LWT-Food Science and Technology*, *52*(2), 71-79. https://doi.org/10.1016/j.lwt.2012.05.003
33. Wani, S.M., Gull, A., Ahad, T., Malik, A.R., Ganaie, T.A., Masoodi, F.A., & Gani, A. (2021). Effect of gum Arabic, xanthan and carrageenan coatings containing antimicrobial agent on postharvest quality of strawberry: Assessing the physicochemical, enzyme activity and bioactive properties. *International Journal of Biological Macromolecules*. https://doi.org/10.1016/j.ijbiomac.2021.06.008
34. Widyastuti, T., Dewi, S.S., & Mudawy, A.A. (2023). Use of chitosan and essential oils as edible coating for Sapodilla fruit (*Manilkara zapota*). In IOP Conference Series: Earth and Environmental Science (Vol. 1172, No. 1, p. 012057). IOP Publishing. https://doi.org/10.1088/1755-1315/1172/1/012057
35. Xing, Z., Wang, Y., Feng, Z., & Tan, Q. (2008). Effect of different packaging films on postharvest quality and selected enzyme activities of *Hypsizygus marmoreus* mushrooms. *Journal of Agricultural and Food Chemistry*, *56*(24), 11838-11844. https://doi.org/10.1021/jf8024387
36. Yaman, Ö., & Bayoındırlı, L. (2002). Effects of an edible coating and cold storage on shelf-life and quality of cherries. *LWT-Food Science and Technology*, *35*(2), 146-150. https://doi.org/10.1006/fstl.2001.0827
37. Yuan, F., Wang, C., Yi, P., Li, L., Wu, G., Huang, F., & Gan, T. (2023). The effects of combined 1-methylcyclopropene and melatonin treatment on the quality characteristics and active oxygen

metabolism of mango fruit during storage. *Foods*, *12*(10), 1979. https://doi.org/10.3390/foods12101979

38. Zhang, Y., Huber, D.J., Hu, M., Jiang, G., Gao, Z., Xu, X., & Zhang, Z. (2018). Delay of postharvest browning in litchi fruit by melatonin via the enhancing of antioxidative processes and oxidation repair. *Journal of Agricultural and Food Chemistry*, *66*(28), 7475-7484. https://doi.org/10.1021/acs.jafc.8b01922
39. Zheng, M., Lian, F., Zhu, Y., Zhang, Y., Liu, B., Zhang, L., & Zheng, B. (2019). pH-responsive poly (xanthan gum-g-acrylamide-g-acrylic acid) hydrogel: Preparation, characterization, and application. *Carbohydrate Polymers*, *210*, 38-46. https://doi.org/10.1016/j.carbpol.2019.01.052

نشریه پژوهشهای علوم و صنایع غذایی ایران
https://ifstrj.um.ac.ir

مقاله پژوهشی

جلد ۲۰، شماره ۳، مرداد-شهریور ۱۴۰۳، ص. ۱۵-۱

تأثیر پوشش خوراکی بر پایه صمغ زانتان غنی‌شده با اسید اولئیک بر کیفیت انبارمانی و خواص آنتی‌اکسیدانی میوه چیکو (*Manilkara zapota*)

دارا رضاخانی نژاد[۱]- عبدالمجید میرزاعلیان دستجردی[۲]- سمیه رستگار[۲*]

تاریخ دریافت: ۱۴۰۲/۱۰/۱۱
تاریخ پذیرش: ۱۴۰۲/۱۲/۰۹

چکیده

میوه‌ی چیکو یا (sapodilla) به‌دلیل فسادپذیری بالا و از دست دادن سریع رطوبت، عمر مفید محدودی دارد. استفاده از پوشش‌های خوراکی به‌دلیل تأثیرگذاری در افزایش طول عمر مفید میوه‌ها، مورد توجه زیادی قرار گرفته است. هدف از این مطالعه ارزیابی اثربخشی پوشش خوراکی ساخته شده از صمغ زانتان (۰/۱ و ۰/۲ درصد) همراه با اسید اولئیک (۱ درصد) در افزایش طول عمر مفید میوه‌ی چیکو در دمای ۱ ± ۸ درجه سانتی‌گراد و رطوبت نسبی ۸۵-۹۰ درصد است. کاهش وزن در میوه‌های تحت تیمار به‌طور قابل توجهی کمتر بود، به‌طوری‌که کمترین کاهش وزن در تیمار (صمغ زانتان ۰/۲ درصد + اسید اولئیک) مشاهده شد. بجز تیمار اسید اولئیک، سایر تیمارها در مقایسه با شاهد، سفتی بالاتری را نشان دادند. در پایان آزمایش، تیمارها به‌طور قابل توجهی تنفس میوه را کاهش دادند. در میوه‌های تحت تیمار همچنین در مقایسه با شاهد، ظرفیت آنتی‌اکسیدانی به‌طور قابل توجهی افزایش یافته و سطوح بالاتری از اسید آسکوربیک را نشان دادند. پایین‌ترین سطح TSS (۲۲/۸ ٪) در تیمار (صمغ زانتان ۰/۲ درصد+ اسید اولئیک) مشاهده شد. علاوه بر این، نتایج نشان داد که میوه‌های تحت پوشش (صمغ زانتان ۰/۱ درصد + اسید اولئیک) فعالیت بالاتری را در آنزیم‌های سوپراکسید دسموتاز (SOD)، کاتالاز (CAT) و آسکوربات پراکسیداز (APX) نسبت به میوه‌های تیمار شده با (صمغ زانتان ۰/۲ درصد + اسید اولئیک) و نمونه‌های شاهد نشان دادند. به طور کلی، میوه‌های تحت تیمار با (صمغ زانتان ۰/۲ درصد + اسید اولئیک) و (صمغ زانتان ۰/۱ درصد + اسید اولئیک) در مقایسه با شاهد و سایر تیمارها، بالاترین کیفیت کلی را نشان دادند. بنابراین، استفاده از این تیمارها برای حفظ کیفیت میوه‌ی چیکو توصیه می‌شود.

واژه‌های کلیدی: انبار، اولئیک اسید، پوشش خوراکی، زانتان، ساپودیلا (چیکو)

۱ و ۲- به‌ترتیب دانشجوی دکتری و دانشیار گروه علوم باغبانی، دانشکده کشاورزی و منابع طبیعی دانشگاه هرمزگان، هرمزگان، ایران
(*- نویسنده مسئول: Email: rastegarhort@gmail.com)

https://doi.org/10.22067/ifstrj.2024.86132.1307

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

Research Article
Vol. 20, No. 3, Aug.-Sep. 2024, p. 17-31

Application of FT-IR Spectroscopy with Various Classification and Regression Models for Detection and Quantification of Sodium Hydrosulfite in Iranian Wheat Flour

A. Kazemi[1*], A. Mahmoudi[2], M. Khojastehnazhand[3], S.H. Fattahi[4]

1 and 2- Ph.D. Student and Professor, Department of Biosystems Engineering, University of Tabriz, Tabriz, Iran
(*- Corresponding Author Email: A.Kazemi@tabrizu.ac.ir)
3- Assistant Professor, Department of Mechanical Engineering, University of Bonab, Bonab, Iran
4- Assistant Professor, Department of Biosystems Engineering, University of Maragheh, Maragheh, Iran

Received: 30.01.2024
Revised: 06.04.2024
Accepted: 09.04.2024
Available Online: 01.06.2024

How to cite this article:
Kazemi, A., Mahmoudi, A., Khojastehnazhand, M., & Fattahi, S.H. (2024). Application of FT-IR spectroscopy with various classification and regression models for detection and quantification of sodium hydrosulfite in Iranian wheat flour. *Iranian Food Science and Technology Research Journal*, *20*(3), 17-31. https://doi.org/10.22067/ifstrj.2024.86585.1312

Abstract

Wheat flour is one of the most important and strategic food resources especially in developing countries. The addition of Sodium hydrosulfite to flour for improving some appearance features can have dangerous impacts on the consumer health. Therefore, detection of this harmful substance is great practical significance. In the present study, the potential of Fourier transform-mid infrared (FT-MIR) spectroscopy in 400-4000 cm^{-1} for the fast detection of Sodium hydrosulfite powder in wheat flour was investigated. After getting the spectral data from samples, firstly some preprocessing methods were used to correct harmful and unwanted effects on spectral data, and then Principal Component Analysis (PCA) as unsupervised and Support Vector Machine (SVM) and Artificial Neural Network (ANN) models as supervised classification models and Partial Least Square Regression (PLSR) as regression model were applied to detect and quantify the adulteration in pure flour samples. The best outcomes were the accuracy of 86.66 and 86.70 for SVM and ANN models with S-G + D2 + SNV preprocessing, respectively and R^2_p = 0.99 For PLSR model.

Keywords: Adulteration, Chemometrics, Sodium hydrosulfite, Spectroscopy, Wheat flour

Introduction

Bread as one of the most significant sources of daily requirement components for body (such as proteins, minerals and vitamins) is one of the staple foods for many countries, particularly in Iran (Ahamadabadi *et al.*, 2016; GhR, Yunesian, Vaezi, Nabizadeh, & GhA, 2006; Sabeghi, 2004). The consumption of bread in Iran is five times more than Europe (Malakootian & Dowlatshahi, 2005; Sabeghi, 2004). Among the main ingredients of bread, wheat flour has a special place and has direct relation to the quality of bread and also to the health of consumers. Therefore, it should get the certificate of Iranian national standard. Wheat contains 78.10% carbohydrate, 14.70% protein, 2.10% fat, 2.10% minerals and noticeable proportion of vitamins (Adams, Lombi, Zhao, & McGrath, 2002; Shewry, 2009; Shewry *et al.*, 2006; Topping, 2007). According to the statistics of world Health Organization and Food and Agriculture

©2024 The author(s). This is an open access article distributed under Creative Commons Attribution 4.0 International License (CC BY 4.0), which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author (s) and the source.
https://doi.org/10.22067/ifstrj.2024.86585.1312

Organization of United Nations, 25 types of food additives are used in each country according to the food safety policy (Martins, Sentanin, & De Souza, 2019). The maximum acceptable amount of them and also the Assurance of avoidance of any unauthorized additives should be considered. Sodium hydrosulfite also known as Blankit is a white crystalline powder containing inorganic sulfur compounds (Reza *et al*., 2014). In food industry, this material is applied for nuts, sugar, etc. to avoid browning and bleaching and regeneration of cellulose fibers (de Carvalho & Schwedt, 2005). Sodium hydrosulfite has been utilized in Iranian bread industry to hide visible defects of bread by affecting the velocity of production process and compensating some visible results of lack of natural fermentation and poor flour quality (Asgari, SeidMohammadi, Faradmal, Moradi, & Yari, 2018). This material has so dangerous effects on human health. Adverse effects of Blankit include the elimination and damage of villi in the stomach and intestines in the long term, therefore, it can cause the development of gastrointestinal cancer. It is also known to be an effective factor in developing diabetes (Karami, Alikord, Mokhtari, Sadighara, & Jahed-Khaniki, 2021). Therefore, detection of this harmful material in the human's diet is essential. In general, different approaches have been applied to quantify sulfur factors in food, such as titration (Monnier & Wiliams, 1972), liquid and gas chromatography (Rethmeier, Rabenstein, Langer, & Fischer, 1997), high performance ion chromatography (Lavigne-Delcroix, Tusseau, & Proix, 1996), electroanalysis methods include the study of the electrical activity of sulfites, voltammetry (Govaert, Temmerman, & Kiekens, 1999), and amperometry, potentiometric and the method of general evaluation of sulfites in the automated system (Pisoschi et al., 2020). The mentioned techniques encompass some drawbacks such as being high-cost, laborious, and destructive. Therefore, some other nondestructive, inexpensive and fast methods are required. Fourier Transform infrared (FT-IR) spectroscopy is one of the fingerprint techniques which is widely used to identify components of food and determine possible impurities. FT-IR spectroscopy can be adjusted in the middle range (450-4000 cm^{-1}, FT-MIR) or near range (4000-10000 cm^{-1}, FT-NIR) (Pallone, dos Santos Caramês, & Alamar, 2018). FT-MIR comes up with more structural and chemical information than Fourier Transform-Near Infrared (FT-NIR) by the ability of displaying vibrational and rotary stretching process of covalent bonds (Lohumi, Lee, Lee, & Cho, 2015). Some researchers have explored the applicability of spectroscopic techniques to investigate chemical information of materials. Mohamed *et al*. explored classification of five food powder types (wheat flour, organic wheat flour, rice flour, corn starch, and tapioca starch) and reported that Support Vector Machine (SVM) model had acceptable outcomes for classification of mentioned powders (Mohamed, Solihin, Astuti, Ang, & Zailah, 2019). In another study, Girolma *et al.* applied FT-IR techniques in different ranges (FT-MIR and FT-NIR) to detect the adulteration of durum wheat pasta with common wheat. Linear Discriminant Analysis (LDA) and Partial Least Square–Discriminant Analysis (PLS-DA) had the results of 80 and 95% for three class dataset and 91 and 97% for two class datasets (De Girolamo *et al*., 2020). However as far as our knowledge, the applicability of FT-MIR spectroscopy method with combination of ANN for classification and PLSR model for quantification of adulteration of this harmful material in Iranian wheat flour has not been investigated. In the present study, the applicability of FT-MIR spectroscopy combined with chemometric methods and various preprocessing algorithms for detection and quantification of sodium hydrosulfite in wheat flour in Iran was studied.

Materials and Methods

In the present research, after preparing samples, spectral data were acquired and preprocessed. The both supervised and

unsupervised models were applied. Afterward, the results were analyzed for detection and quantification of pure and adulterated samples. Fig. 1. represents the flowchart of flour adulteration detection procedure by FT-MIR spectroscopy.

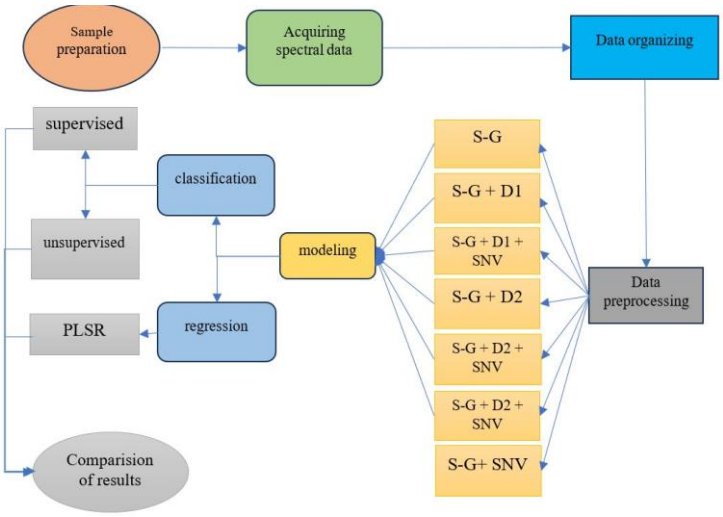


Fig. 1. The schematic flowchart of the steps of present study

Sample Preparation

Sardari wheat seeds (harvested in 2021) were purchased from a seed modifying center in Bonab, East Azerbaijan, Iran. The seeds were then harvested in four distinct places in Iran, taking in consideration the geographical variation of samples. Sardari wheat was selected because it is the highest under-harvest wheat variety in Iran. Sodium hydrosulfite (with the purity of 90%) was acquired from a supermarket in Bonab, Iran. First, wheat seeds were milled by a laboratory benchtop mill to get the wheat flours. Then the flour was passed through a sieve (mesh 420µm) to get homogenous flour sample. The considered adulterant concentrations (w/w) were 10, 15, 20, and 25%. Totally, 150 samples were prepared (25 for pure flour, 25 for sodium hydrosulfite, and 25 samples for 4 adulterant groups). After mixing the adulterant to pure flour with the mentioned levels, they were blended intensely to get the homogenous samples as much as possible. Finally, the prepared samples were transferred to microtubes to transfer to the laboratory.

Spectra Acquisition

Spectral data were acquired at the central laboratory of Tabriz University with FT-MIR spectrometer (TENSOR 27, Bruker, Germany) in transmittance mode and with resolution of 1 cm^{-1}. The scanning speed was 20 kHz and with 64 scans. Each powdered sample was placed on the ATR (single bounce) crystal and pressed until the desired signal density acquired. The crystal was washed with 100% ethanol after testing each sample. For each individual sample, 3 transmittance spectra were acquired and mean spectrum of three replicates was used for further analysis. Finally, the mean spectra were transferred to Excel 2019 version to be prepared for statistical analysis. Multivariate statistical analysis was conducted with Unscrambler v 10.4 (Camo software As, Oslo, Norway, 2011) for PCA and SVM and classification Toolbox in Matlab (Mathworks, Inc., Natick, Massachusetts, USA) for Artificial Neural Networks (ANN).

Preprocessing

Before classification or regression modeling, pretreatment of spectral data is an essential step to remove the unwanted and uninformative data. This can be due to large amount of water in samples, different

conditions of samples, and noise in spectra that comes from electronic components in the system (Boysworth & Booksh, 2008; Christy & Kvalheim, 2007; Varmuza & Filzmoser, 2016). The most common applied preprocessing techniques in spectroscopy is divided in to two categories: spectral normalization and spectral derivatives (Rinnan *et al*., 2009). Spectral normalization techniques which contain Standard Normal Variate (SNV), Multiplicative Scatter Correction (MSC), and de-trending (DT) can be used for correction of scattering effects. While spectral derivatives including (first and second derivatives and smoothing techniques) are applied for correction of peak overlap and baseline drifts (López-Maestresalas *et al.*, 2019). Both SNV and MSC are the most commonly used algorithms to correct the scatter effects. The difference between SNV and MSC methods is based on the fact that the scatter correction in SNV method is based on the average value of every individual spectra, but in MSC technique a reference spectra (average spectra) is required to contrast the whole spectra with that (Dhanoa, Lister, Sanderson, & Barnes, 1994; Zeaiter, Roger, & Bellon-Maurel, 2005). Among the spectral derivative methods, Savitzky-Golay (S-G) is the most common algorithm for derivation (Savitzky & Golay, 1964). By this method, the data with a window size chosen are fitted by a polynomial for which the degree must also be chosen (Barak, 1995). In present study, S-G (with the window size of ten), SNV, MSV, first and second derivatives and combination of them were applied.

Classification

Spectra contain high volumes of information, which are very difficult to interpret by visual inspection only. Chemometrics is a tool for extracting this information from the multivariate chemical data, using mathematics. Chemometrics is generally applied to explore patterns of association in data; track properties of materials on a continuous basis or to prepare and use multivariate classification models. By utilizing diverse preprocessing techniques, the generation of principal models is triggered and subsequently produces output data. Both unsupervised and supervised techniques for classification were utilized in this study.

Unsupervised Classification

In the first step of data exploration, Principal Component Analysis (PCA) is usually applied to recognize any possible separated groups. The main objective of PCA model as an unsupervised modelling method is decreasing the dimensionality of data and preservation of the present variation (Jolliffe, 1986). The reduction of dimensionality is done by defining new variables, principal Components (PCs) that consists linear combinations of the original data (Kamruzzaman, Barbin, ElMasry, Sun, & Allen, 2012). First PC represents the most variance of dataset and the next PCs which are orthogonal to the preceding ones contain the most of the remaining variance (Fodor, 2002). Application of data matrix for PCA model in this study consists of 1886 columns (corresponding to the recorded wavenumbers) and 150 rows (corresponding to the number of samples).

Supervised Classification

SVM Model

The Support Vector Machine classification (SVMC) is a supervised classification technique that utilizes kernel functions to represents the original space in the format of feature space. It determines the best separation between classes by applying a unique hyperplane to the dataset (Ballabio & Todeschini, 2009; Fletcher, 2009; Vapnik, 1999). The final classification outcomes of SVM are determined by a small number of Support Vectors that are the samples lying on the margins of the model. For building classification model and evaluation of their performance, calibration and test datasets were used, respectively. 70% of data was appointed as training and 30% of data was considered as test dataset. SVs lie to the closest boundaries between classes. In SVM model various kernel

functions encompassing linear, Radial Basis Function (RBF), Sigmoid, and polynomial could be employed (Chandrasekaran, Panigrahi, Ravikanth, & Singh, 2019). It is necessary for the correct selection of functions since the type of kernel function directly impacts the model's performance and outcomes (Kazemi, Mahmoudi, & Khojastehnazhand, 2023).

ANN Model

Recently, Artificial Neural Network (ANN) elucidated from human brain function has been one of the most commonly used modeling technique for classification. The functioning of Neural Networks relies on input, hidden, and output layers, each containing varying numbers of neurons. Neurons have a weight assigned to them based on the model's training and serve as storage for the model's inputs and calculation layers. Randomly assigning weights to neurons sets the foundation for training an ANN model. The present study employed a feed-forward network, a type of neural network methodology, where 70% of data was initially used for training purposes and the remaining 30% for testing purposes.

Regression Modeling

After classification of samples, the prediction of adulterated level was done by using partial least squares regression (PLSR). The utilization of PLSR helps to enhance the interconnection between spectral data and the features that need to be quantified. By distinguishing between X and Y variables, PLSR defines a set of new features named latent variables, which are characterized as orthogonal and linear combinations of X variables (Peng, Cheng, Wang, & Zhu, 2020). In present study, the PLSR model was applied to the FT-MIR spectra to investigate the possibility to predict the percentage of sodium hydrosulfite adulteration in wheat flour. The reliability of the acquired predicted model is explored by using external validation data. 70% of the dataset were used to build calibration model and 30% of the dataset was used for testing the created model.

The assessment of acquired models are done by sensitivity and specificity according to equations 1 and 2 (Kazemi, Mahmoudi, Veladi, Javanmard, & Khojastehnazhand, 2022):

$$\text{Sensitivity (\%)} = \frac{TP}{TP+FN} * 100 \quad (1)$$

$$\text{Specificity (\%)} = \frac{TN}{TN+FP} * 100 \quad (2)$$

Where TP (True Positive) is the number of samples belonging to either pure flour correctly classified as pure samples; FP (False Positive) is the number of mixed samples wrongly classified as pure samples; TN (True Negative) is the number of mixed samples correctly classified as mixed; FN (False Negative) is those pure samples classified as mixed. These two statistical parameters take values between 0 and 1. The higher their values, the better the classification performance of models.in the regression modeling, Root Mean Square Error (RMSE) of calibration (RMSEC), prediction (RMSEP), and coefficient of determination (R^2) values are important parameters which evaluate the predictive power of a PLS calibration model. Higher predictive power is represented with higher R^2 and lower RMSE (Pebriana, Rohman, Lukitaningsih, & Sudjadi, 2017; Rohman & Salamah, 2018). For PLS calibration models developed to predict the amount of adulteration in adulterated flour, RMSEC and RMSEP can be calculated using equations 3 and 4, where Y_i and $\hat{Y}_i$ are the actual and predicted values of an adulterated samples, respectively. M and N are the number of data in calibration and prediction set, respectively (Sikorska, Khmelinskii, & Sikorski, 2014).

$$\text{RMSEC} = \sqrt{\frac{\sum(\hat{Y}_i - Y_i)^2}{M-1}} \quad (3)$$

$$\text{RMSEP} = \sqrt{\frac{\sum(\hat{Y}_i - Y_i)^2}{N}} \quad (4)$$

$$\text{CCR} = \frac{TP+TN}{TP+FN+TN+FN} \quad (5)$$

Results and Discussion

Spectra Interpretation

FT-MIR spectra of pure wheat flour, sodium hydrosulfite, and adulterated samples with different adulteration levels is displayed in Fig. 2. Due to some peaks overlaps, chemometric tools is necessary to extract information. In most of peaks almost all of the classes showed similar peaks except Blankit. We had major peaks at 1050 cm^{-1}, 1730 cm^{-1}, 2950 cm^{-1}, and 3400 cm^{-1}. But the wavelength of pure Blankit was different and except some peaks like 1050 cm^{-1}, in the majority of peaks of other adulterated classes, it did not have peaks and also in some cases like 1950 cm^{-1} and 2050 cm^{-1} it showed peaks but other classes did not have. The basic bands at 2800-3040 cm^{-1} are related to C-H and C-H_2 symmetric and asymmetric stretching and mainly attributed to band vibrations of the lipids in the flours (Roa, Santagapita, Buera, & Tolaba, 2014). The bands with the maximum at 1640 cm^{-1} are associated to protein band vibrations (Guzmán-Ortiz *et al.*, 2015). Furthermore, spectra show a strong absorption band, from 900-1200 cm^{-1} and C-H bending (1000 cm^{-1}), mainly related to carbohydrates (Rodríguez, Rolandelli, & Buera, 2019).

PCA Model

PCA model as unsupervised modeling was applied to dataset to decrease the dimensions of data as preserving the original variables. The acquired FT-MIR data was processed by PCA model to explore the probable similarities and differences among pure and adulterated flour samples. With the comparison of different applied preprocessing techniques, the result of PCA model with (S-G + D1 + SNV) was the best.

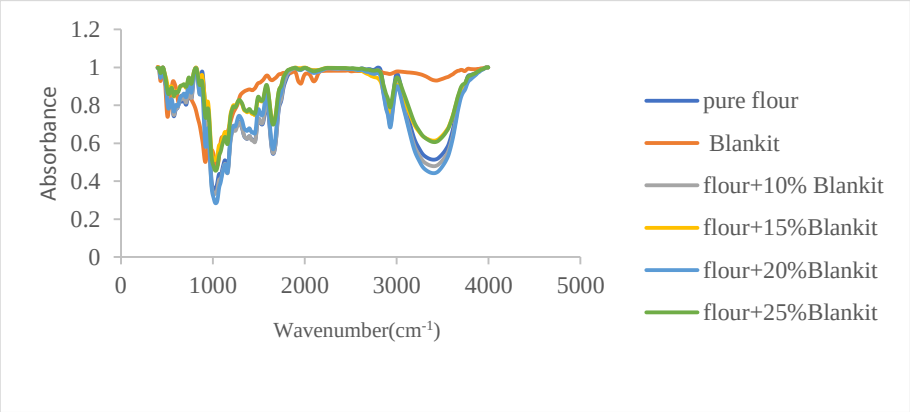


Fig. 2. The acquired FT-MIR spectra for flour samples

The obtained score plot of first two PCs (PC1=88% and PC2 = 4%) is shown in fig.3. this figure displays that, all the pure samples were projected on PC1 negative values. Thus, PC1 provided a fairly discrimination between pure and adulterated samples. As it is observable from Fig. 3, the pure flour samples were gathered and separated well from the adulterated samples. Due to high chemical composition difference of pure Sodium hydrosulfite, the hydrosulfite samples were well-separated and were located on the other side of PC1. Because of similarities of the chemical composition of adulterated samples, there were some misclassifications between different adulterated level groups. Similarities of compositional structure of samples with different adulteration levels can be a reason for misclassification of adulterated samples. Mishra *et al.* applied PCA model combined with hyperspectral imaging method to detect peanut traces in wheat flour with the presentation of 99.43% of variance, pure and adulterated samples were well-distinguished along PC1 similar to the present study (Mishra *et al.*, 2015). In addition, the results of PCA

model in the present study was in agreement with the result of PCA model for discrimination of wheat flour with other cereal flours (barley, rye, and triticale flour). The score plot represented good discrimination of barley flour samples from wheat flour. However, one type of wheat flour was located very close to other flours (Nur Arslan, 2020).

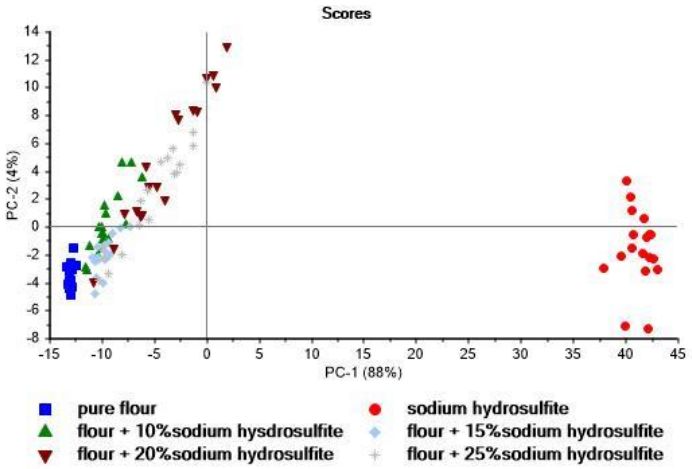


Fig. 3. The score plot of PCA model

SVM and ANN Models

Table 1 represents the accuracy of SVM and ANN models as supervised classification methods after applying various kinds of preprocessing methods and combination of them for training datasets. The SVM model was implemented in four different kernel functions (linear, polynomial, Radial Basis Function (RBF), and Sigmoids). In both models, 70% of data was randomly assigned to model training and other 30% were used for model testing. In addition, 5% of 70% neural network model training data was used to validate them. As depicted in Table 1, the accuracy of SVM model with linear kernel function with S-G + D2 + SNV preprocessing was 86.66% and also 86.70% for ANN models. Based on the applied preprocessing methods, the ANN model also yielded acceptable results based on the optimal neural network structure shown in Fig. 4.

Fig. 5 represents SVM graphical model after employing S-G + D2 + SNV preprocessing.

As shown in Table 1, linear kernel had better results for all preprocessing techniques.

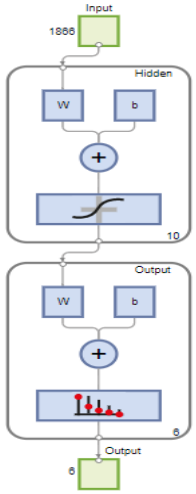


Fig. 4. The structure of the optimal artificial neural network

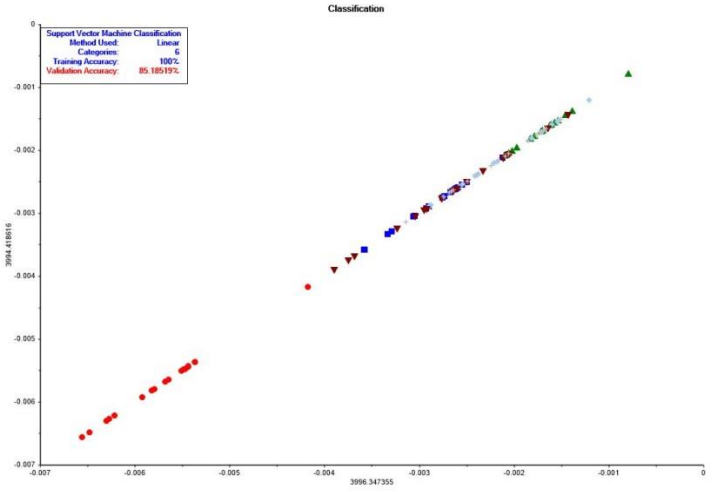


Fig. 5. SVM graphical model for classification of samples

Table 1- The accuracy of SVM and ANN models

Model	SVM												ANN		
Kernel function	Linear			Polynomial			RBF			Sigmoid			-		
Preprocessing	Train	Val	Test	Train	Val	Test	Train	Val	Test	Train	Val	Test	Train	Val	Test
S-G	78.75	68.75	75.55	12.50	11.25	6.66	10	13.75	6.66	17.5	18.75	20	88.9	80	80
S-G +D1	20	17.5	20	20	20	20	20	20	20	20	20	20	84.4	80	75.6
S-G + D1 + SNV	100	81.25	84.44	67.5	50	71.11	52.5	43.75	51.11	1.25	10	2.22	98.9	100	80
S-G + D2	20	20	20	20	20	20	20	20	20	20	20	20	50	60	46.7
S-G + D2 + SNV	100	81.25	86.66	68.75	53.75	68.88	61.25	45	55.55	1.25	23.75	4.44	100	100	86.7
S-G + D2 + MSC	20	20	20	20	20	20	20	20	20	20	20	20	98.9	100	80
S-G + SNV	93.75	70	80	30	30	30	30	28.75	26.66	10	12.5	8.88	85.6	86.7	73.3

Linear kernels work well when the underlying relationship between the input features and the target variable is approximately linear. The better performance of linear kernel maybe due to the nature of the dataset, which is separated or modeled effectively by linear boundaries. Furthermore, in high-dimensional spaces, like spectroscopic data, linear kernels can perform better than more complex kernels. This is because complex kernels can exacerbate the curse of dimensionality making it harder to find a suitable decision boundary. In addition, according to the better outcomes of polynomial kernel in comparison with other kernels, it can be concluded that the structure and nature of dataset tends to simple and linear form. In another study, Yuan et al, employed NIR spectroscopy to detect Sodium hydroxymethanesulfonate in wheat flour. Three algorithms including PLS-DA, advanced K-means dynamic clustering, and LS-SVM were used to establish the calibration models. The outcomes of LS-SVM outperformed other two methods, with the classification accuracy of 94.70% for the prediction (Yuan, Xiang, Yu, & Xu, 2011). However, the outcomes of SVM model in the mentioned research was better than our present study but this point should be

mentioned that, the applied SVM model in that study was for classification of two classes but 86.66% result of present study was for classification of five classes. In spite of the fact that FTIR spectroscopy combined with chemometric methods confirmed its application to detect the adulteration of Sodium hydrosulfite in wheat flour, but there were some limitations in the present study which we hope to be solved in the future studies. The environmental effects like moisture are different from the bakeries or industrial places. Although, due to the fact that the moisture was similar in all samples, this issue was solved. But for application of this study method in other situations, definitely the conditions of system should be calibrated again. Besides, the applied technique in the present study can be studied for detection of other adulterants in wheat flour simultaneously. In order to assess the classification ability of each class in SVM model, the confusion matrix was investigated for test dataset (Table 2). The results were assessed by calculation of sensitivity, specificity, and accuracy statistical parameters. As it was expected, the highest classification accuracy was for class 1 (pure wheat flour). Also, the accuracy of class C (adulterated with 15% level) was 100%. The weakest classification result was for class D (20% adulterated). In class E (25% adulterated) 6 samples were classified correctly, and 3 samples were classified for class D. The difference of these classes is 5% adulteration level. Then, the classification result of this class was acceptable too.

Table 2- The confusion matrix of SVM model for test dataset

	A	**B**	**C**	**D**	**E**
A	9	0	0	0	0
B	0	9	0	0	0
C	0	1	8	0	0
D	0	1	0	7	1
E	0	0	0	3	6
Sensitivity	1	1	0.88	0.77	0.66
Specificity	1	0.94	1	0.91	0.97
CCR (%)	100	81	100	70	85

PLSR Model

In order to quantify the adulterant in wheat flour, FT-MIR spectroscopy-based regression model (PLSR) was built. This regression model is based on developing algebraic correlation between the quantity of adulteration in wheat flour samples and absorption of sample along different wavelengths. The ideal calibration model was determined based on lowest RMSEC, RMSECV, and highest R_c^2 and R_{cv}^2. The value of mentioned statistical parameters as well as the number of "Latent Variables" (LVs) are presented in Table 3.

Table 3- The results of PLSR model in predicting the adulteration level using different preprocessing methods

Preprocessing	**LV**	**Calibration**		**Test**	
		R^2	**RMSE**	**R^2**	**RMSE**
SG	7	0.995	0.118	0.994	0.123
S-G + D1	7	1.00	2.46	1.00	2.39
S-G + D1 + SNV	7	0.987	0.36	0.979	0.82
S-G + D2	7	1.00	3.94	1.00	2.87
SG + D2 + SNV	7	0.975	0.265	0.967	0.312
SG + D2 + MSC	7	1.00	2.87	1.00	2.50
S-G+ SNV	7	0.992	0.15	0.989	0.18

As shown in Table 3, the best PLS model was obtained with the preprocessing method of S-G using seven LVs, which showed the prediction performance (R_{cv}^2 = 0.994 and RMSECV = 0.123). The similarity of train and test results represent of model's good ability for prediction of precise levels of adulteration. The performance of PLSR was also externally validated by using the test set of samples, as shown in Fig. 6. The PLS prediction plot illustrates that PLSR model displayed a very good prediction ability (R_p^2 = 0.992). Fig. 6 shows the relationship between reference data and predicted values obtained in the laboratory.

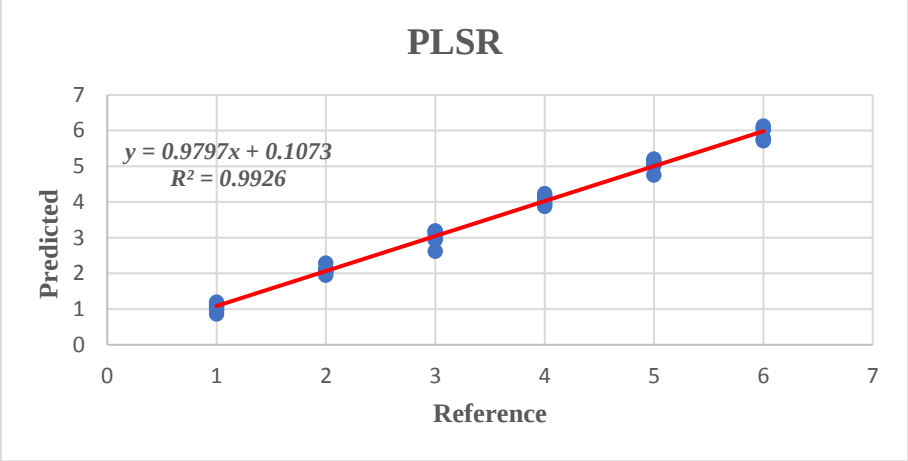


Fig. 6. The performance of PLSR model for prediction of adulteration levels

Recently, Martins et al, predicted the presence of whey protein in wheat flour by FT-IR spectroscopy and multivariate analysis. The PLSR model was applied to the acquired spectra and the best model of obtained spectra had R_{cal}^2 = 0.99, R_{pre}^2 = 0.98, RMSEC = 3.5, and RMSEP = 3.00 (Martins *et al*., 2022). However, the R^2 results were in agreement with this research, but RMSE results were weaker. In other research, Nur Arslan, applied PLSR model to explore the amount of barely flour in wheat flour. The statistical parameters of this study were close to the results of present study (R^2 values were at least 0.994 and RMSECV result was in the range 0.36-1.50%) (Arslan *et al*., 2020). In another study, the prediction of Azodicarbonamide in wheat flour by visible/near-infrared spectroscopy was investigated by Che *et al*. By comparing 3 applied models in this research (PLSR, Back Propagation Neural Network, and Radial Basis Function), Radial Basis Function model had the best prediction results with Correlation Coefficient ®, RMSEP 0.99 and 0.54, respectively (Che *et al*., 2017), and were in agreement with the outcomes of present study.

Conclusion

The presence of Sodium hydrosulfite (Blankit) in wheat flour was investigated by FT-MIR spectroscopy. PCA as unsupervised and SVM and ANN as supervised models were applied to detect the adulteration and PLSR model as regression model was applied to quantify the amount of adulteration. The mentioned chemometric models were built after some preprocessing techniques. The acquired results for detection and quantification of Sodium Hydrosulfite proved that FT-IR spectroscopy can be a reliable method to detect and quantify Sodium hydrosulfite in wheat flour.

Declarations

Conflict of Interest

There is no potential conflict of interest between the authors.

Author Contribution

A. Kazemi: Conceptualization, data curation, formal analysis, investigation, methodology, project administration, software, supervision, validation, writing-original drift. **A. Mahmoudi**: Formal analysis, methodology, supervision, visualization. **M. Khojastehnazhand**: Software, supervision, writing-review and editing, formal analysis. **S.H. Fattahi**: Visualization, validation, writing-review and editing, supervision.

Funding Resources

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

References

1. Adams, M.L., Lombi, E., Zhao, F.J., & McGrath, S.P. (2002). Evidence of low selenium concentrations in UK bread-making wheat grain. *Journal of the Science of Food and Agriculture, 82*(10), 1160-1165. https://doi.org/10.1002/jsfa.1167
2. Ahamadabadi, M., Saeidi, M., Rahdar, S., Narooie, M.R., Salimi, A., Alipour, V., Biglari, H. (2016). Amount of baking soda and salt in the bread baked in city of zabol. *Iioab Journal, 7*, 518-522.
3. Arslan, F. N., Akin, G., Karuk Elmas, Ş. N., Üner, B., Yilmaz, I., Janssen, H.-G., & Kenar, A. (2020). FT-IR spectroscopy with chemometrics for rapid detection of wheat flour adulteration with barley flour. *Journal of Consumer Protection and Food Safety, 15*, 245-261. https://doi.org/10.1007/s00003-019-01267-9
4. Asgari, G., SeidMohammadi, A., Faradmal, J., Moradi, M.J., & Yari, K. (2018). The study of Blankit concentration as an unauthorized additive in hamadan bakeries and risk assessment of food consumption. *Pajouhan Scientific Journal*, *16*(4), 21-27.
5. Ballabio, D., & Todeschini, R. (2009). Multivariate classification for qualitative analysis *Infrared spectroscopy for food quality analysis and control* (pp. 83-104): Elsevier. https://doi.org/10.1016/B978-0-12-374136-3.00004-3
6. Barak, P. (1995). Smoothing and differentiation by an adaptive-degree polynomial filter. *Analytical Chemistry, 67*(17), 2758-2762. https://doi.org/10.1021/ac00113a006
7. Boysworth, M., & Booksh, K. (2008). Aspects of multivariate calibration applied to near-infrared spectroscopy. *PRACTICAL SPECTROSCOPY SERIES, 35*, 207.
8. Chandrasekaran, I., Panigrahi, S. S., Ravikanth, L., & Singh, C. B. (2019). Potential of near-infrared (NIR) spectroscopy and hyperspectral imaging for quality and safety assessment of fruits: An overview. *Food Analytical Methods, 12*, 2438-2458. https://doi.org/10.1007/s12161-019-01609-1
9. Che, W., Sun, L., Zhang, Q., Zhang, D., Ye, D., Tan, W., Dai, C. (2017). Application of visible/near-infrared spectroscopy in the prediction of azodicarbonamide in wheat flour. *Journal of Food Science, 82*(10), 2516-2525. https://doi.org/10.1111/1750-3841.13859
10. Christy, A.A., & Kvalheim, O.M. (2007). Latent-variable analysis of multivariate data in infrared spectrometry. *Near-infrared Spectroscopy in Food Science and Technology*, 145-162. https://doi.org/10.1002/0470047704
11. de Carvalho, L.M., & Schwedt, G. (2005). Sulfur speciation by capillary zone electrophoresis: Determination of dithionite and its decomposition products sulfite, sulfate and thiosulfate in commercial bleaching agents. *Journal of Chromatography A, 1099*(1-2), 185-190. https://doi.org/10.1016/j.chroma.2005.08.084
12. De Girolamo, A., Cervellieri, S., Mancini, E., Pascale, M., Logrieco, A. F., & Lippolis, V. (2020). Rapid authentication of 100% italian durum wheat pasta by FT-NIR spectroscopy combined with chemometric tools. *Foods, 9*(11), 1551. https://doi.org/10.3390/foods9111551

13. Dhanoa, M., Lister, S., Sanderson, R., & Barnes, R. (1994). The link between multiplicative scatter correction (MSC) and standard normal variate (SNV) transformations of NIR spectra. *Journal of Near Infrared Spectroscopy, 2*(1), 43-47. https://doi.org/10.1255/jnirs.30
14. Fletcher, T. (2009). Support vector machines explained. *Tutorial paper*, 1-19.
15. Fodor, I.K. (2002). *A survey of dimension reduction techniques*. Retrieved from.
16. GhR, J. K., Yunesian, M., Vaezi, F., Nabizadeh, R., & GhA, P. (2006). A survey on baking Soda elimination from Iranian flat breads in bakeries of Islamshahr city in 2005. *Research Journal School Health, Yazd, 1*(2), 21-31.
17. Govaert, F., Temmerman, E., & Kiekens, P. (1999). Development of voltammetric sensors for the determination of sodium dithionite and indanthrene/indigo dyes in alkaline solutions. *Analytica chimica acta, 385*(1-3), 307-314. https://doi.org/10.1016/S0003-2670(98)00591-1
18. Guzmán-Ortiz, F. A., Hernández-Sánchez, H., Yee-Madeira, H., San Martín-Martínez, E., Robles-Ramírez, M.D.C., Rojas-López, M., Mora-Escobedo, R. (2015). Physico-chemical, nutritional and infrared spectroscopy evaluation of an optimized soybean/corn flour extrudate. *Journal of food science and technology, 52*, 4066-4077. https://doi.org/10.1007/s13197-014-1485-5
19. Jolliffe, I. T. (1986). Principal Component Analysis and Factor Analysis. In I. T. Jolliffe (Ed.), *Principal Component Analysis* (pp. 115-128). New York, NY: Springer New York.
20. Kamruzzaman, M., Barbin, D., ElMasry, G., Sun, D.-W., & Allen, P. (2012). Potential of hyperspectral imaging and pattern recognition for categorization and authentication of red meat. *Innovative Food Science & Emerging Technologies, 16*, 316-325. https://doi.org/10.1016/j.ifset.2012.07.007
21. Karami, M., Alikord, M., Mokhtari, Z., Sadighara, P., & Jahed-Khaniki, G. (2021). Sodium Hydrosulfite (Blankit) in Iranian food as a threat to human health: a review. *Journal of Food Safety and Hygiene*. https://doi.org/10.18502/jfsh.v7i2.8398
22. Kazemi, A., Mahmoudi, A., & Khojastehnazhand, M. (2023). Detection of sodium hydrosulfite adulteration in wheat flour by FT-MIR spectroscopy. *Journal of Food Measurement and Characterization, 17*(2), 1932-1939. https://doi.org/10.1007/s11694-022-01763-x
23. Kazemi, A., Mahmoudi, A., Veladi, H., Javanmard, A., & Khojastehnazhand, M. (2022). Rapid identification and quantification of intramuscular fat adulteration in lamb meat with VIS–NIR spectroscopy and chemometrics methods. *Journal of Food Measurement and Characterization, 16*(3), 2400-2410. https://doi.org/10.1007/s11694-022-01352-y
24. Lavigne-Delcroix, A., Tusseau, D., & Proix, M. (1996). Validation of a chromatographic chemiluminescence detector. *Sciences des Aliments, 16*, 267-280.
25. Lohumi, S., Lee, S., Lee, H., & Cho, B.-K. (2015). A review of vibrational spectroscopic techniques for the detection of food authenticity and adulteration. *Trends in Food Science & Technology, 46*(1), 85-98. https://doi.org/10.1016/j.tifs.2015.08.003
26. López-Maestresalas, A., Insausti, K., Jarén, C., Pérez-Roncal, C., Urrutia, O., Beriain, M.J., & Arazuri, S. (2019). Detection of minced lamb and beef fraud using NIR spectroscopy. *Food Control, 98*, 465-473. https://doi.org/10.1016/j.foodcont.2018.12.003
27. Malakootian, M., & Dowlatshahi, S. (2005). The quality of the manufactured bread and hygienic conditions of bakeries. *Journal of Environmental Health Science & Engineering, 2*(2), 72-78.
28. Martins, F.C., Sentanin, M.A., & De Souza, D. (2019). Analytical methods in food additives determination: Compounds with functional applications. *Food Chemistry, 272*, 732-750. https://doi.org/10.1016/j.foodchem.2018.08.060
29. Martins, M.S., Nascimento, M.H., Barbosa, L.L., Campos, L.C., Singh, M.N., Martin, F.L., Barauna, V. G. (2022). Detection and quantification using ATR-FTIR spectroscopy of whey

protein concentrate adulteration with wheat flour. *LWT, 172*, 114161. https://doi.org/10.1016/j.lwt.2022.114161

30. Mishra, P., Herrero-Langreo, A., Barreiro, P., Roger, J.M., Diezma, B., Gorretta, N., & Lleó, L. (2015). Detection and quantification of peanut traces in wheat flour by near infrared hyperspectral imaging spectroscopy using principal-component analysis. *Journal of Near Infrared Spectroscopy, 23*(1), 15-22.
31. Mohamed, M.Y., Solihin, M.I., Astuti, W., Ang, C.K., & Zailah, W. (2019). *Food powders classification using handheld near-infrared spectroscopy and support vector machine.* Paper presented at the Journal of Physics: Conference Series. https://doi.org/10.1088/1742-6596/1367/1/012029
32. Monnier, G., & Wiliams, S. (1972). Determination of sulfur dioxide. (AOAC Official Method 990.28). *Analyst, 95*, 119.
33. Pallone, J.A.L., dos Santos Caramês, E.T., & Alamar, P.D. (2018). Green analytical chemistry applied in food analysis: alternative techniques. *Current Opinion in Food Science, 22*, 115-121. https://doi.org/10.1016/j.cofs.2018.01.009
34. Pebriana, R.B., Rohman, A., Lukitaningsih, E., & Sudjadi. (2017). Development of FTIR spectroscopy in combination with chemometrics for analysis of rat meat in beef sausage employing three lipid extraction systems. *International Journal of Food Properties, 20*(sup2), 1995-2005.
35. Peng, L., Cheng, H., Wang, L.-J., & Zhu, D. (2020). Comparisons of the prediction results of soil properties based on fuzzy c-means clustering and expert knowledge from laboratory Visible–Near-Infrared reflectance spectroscopy data. *Canadian Journal of Soil Science, 101*(1), 33-44. https://doi.org/10.1139/cjss-2020-0025
36. Pisoschi, A.M., Pop, A., Gajaila, I., Iordache, F., Dobre, R., Cazimir, I., & Serban, A.I. (2020). Analytical methods applied to the assay of sulfur-containing preserving agents. *Microchemical Journal, 155*, 104681. https://doi.org/10.1016/j.microc.2020.104681
37. Rethmeier, J., Rabenstein, A., Langer, M., & Fischer, U. (1997). Detection of traces of oxidized and reduced sulfur compounds in small samples by combination of different high-performance liquid chromatography methods. *Journal of Chromatography A, 760*(2), 295-302. https://doi.org/10.1016/S0021-9673(96)00809-6
38. Reza, T., Aktar, S., Al Amin, H., Rahman, M., Arefin, A., Mohanto, N.C., Nikkon, F. (2014). In vivo analysis of toxic effect of hydrose used in food preparations in Bangladesh. *Asian Pacific Journal of Tropical Biomedicine, 4*(11), 884-889. https://doi.org/10.12980/APJTB.4.201414B366
39. Rinnan, Å., Nørgaard, L., van den Berg, F., Thygesen, J., Bro, R., & Engelsen, S.B. (2009). Data pre-processing. *Infrared spectroscopy for food quality analysis and control*, 29-50.
40. Roa, D.F., Santagapita, P.R., Buera, M.P., & Tolaba, M.P. (2014). Ball milling of Amaranth starch-enriched fraction. Changes on particle size, starch crystallinity, and functionality as a function of milling energy. *Food and Bioprocess Technology, 7*, 2723-2731. https://doi.org/10.1007/s11947-014-1283-0
41. Rodríguez, S.D., Rolandelli, G., & Buera, M.P. (2019). Detection of quinoa flour adulteration by means of FT-MIR spectroscopy combined with chemometric methods. *Food Chemistry, 274*, 392-401. https://doi.org/10.1016/j.foodchem.2018.08.140
42. Rohman, A., & Salamah, N. (2018). The employment of spectroscopic techniques coupled with chemometrics for authentication analysis of halal pharmaceuticals. *Journal of Applied Pharmaceutical Science, 8*(10), 063-068. https://doi.org/10.7324/JAPS.2018.81009
43. Sabeghi, M. (2004). Interview with Dean of Faculity of flour and Bread. *Journal of Iran dough-baked, 3*, 5-6.

44. Savitzky, A., & Golay, M.J. (1964). Smoothing and differentiation of data by simplified least squares procedures. *Analytical Chemistry*, *36*(8), 1627-1639. https://doi.org/10.1021/ac60214a047
45. Shewry, P. (2009). The HEALTHGRAIN programme opens new opportunities for improving wheat for nutrition and health: Wiley Online Library.
46. Shewry, P.R., Powers, S., Field, J.M., Fido, R.J., Jones, H.D., Arnold, G.M., Barro, F. (2006). Comparative field performance over 3 years and two sites of transgenic wheat lines expressing HMW subunit transgenes. *Theoretical and Applied Genetics*, *113*, 128-136. https://doi.org/10.1007/s00122-006-0279-1
47. Sikorska, E., Khmelinskii, I., & Sikorski, M. (2014). Vibrational and electronic spectroscopy and chemometrics in analysis of edible oils. *Methods in Food Analysis; Cruz, RMS, Khmelinskii, I., Vieira, M., Eds*, 201-234.
48. Topping, D. (2007). Cereal complex carbohydrates and their contribution to human health. *Journal of Cereal Science*, *46*(3), 220-229. https://doi.org/10.1016/j.jcs.2007.06.004
49. Vapnik, V. (1999). *The nature of statistical learning theory*: Springer science & business media. https://doi.org/10.1109/72.788640
50. Varmuza, K., & Filzmoser, P. (2016). *Introduction to multivariate statistical analysis in chemometrics*: CRC press. https://doi.org/10.1201/9781420059496
51. Yuan, W., Xiang, B., Yu, L., & Xu, J. (2011). A non-invasive method for screening sodium hydroxymethanesulfonate in wheat flour by near-infrared spectroscopy. *Food Analytical Methods*, *4*, 550-558. https://doi.org/10.1007/s12161-011-9198-0
52. Zeaiter, M., Roger, J.-M., & Bellon-Maurel, V. (2005). Robustness of models developed by multivariate calibration. Part II: The influence of pre-processing methods. *TrAC Trends in Analytical Chemistry*, *24*(5), 437-445. https://doi.org/10.1016/j.trac.2004.11.023

نشریه پژوهشهای علوم و صنایع غذایی ایران
https://ifstrj.um.ac.ir

مقاله پژوهشی

جلد ۲۰، شماره ۳، مرداد-شهریور ۱۴۰۳، ص. ۳۱-۱۷

کاربرد طیف‌سنجی FT-IR با مدل‌های طبقه‌بندی و رگرسیون مختلف برای تشخیص و کمی‌سازی هیدروسولفیت سدیم در آرد گندم ایران

امیر کاظمی[۱*]- اصغر محمودی[۲]- مصطفی خجسته‌نژند[۳]- سید حسین فتاحی[۴]

تاریخ دریافت: ۱۴۰۲/۱۱/۱۰

تاریخ پذیرش: ۱۴۰۳/۰۱/۲۱

چکیده

آرد گندم یکی از مهم‌ترین و استراتژیک‌ترین منابع غذایی به‌ویژه در کشورهای در حال توسعه است. افزودن هیدروسولفیت سدیم به آرد برای بهبود برخی ویژگی‌های ظاهری می‌تواند اثرات خطرناکی بر سلامت مصرف‌کننده داشته باشد. بنابراین تشخیص این ماده مضر از اهمیت عملی بالایی برخوردار است. در مطالعه حاضر، پتانسیل طیف سنجی مادون قرمز تبدیل فوریه (FT-MIR) در ۴۰۰۰-۴۰۰ cm^{-1} برای تشخیص سریع پودر هیدرو سولفیت سدیم در آرد گندم مورد بررسی قرار گرفت. پس از گرفتن داده‌های طیفی از نمونه‌ها، ابتدا از برخی روش‌های پیش‌پردازش برای تصحیح اثرات مضر و ناخواسته بر داده‌های طیفی استفاده شد و سپس از آنالیز مؤلفه‌های اصلی (PCA) به‌عنوان مدل بدون نظارت و از مدل‌های ماشین‌بردار بدون نظارت و پشتیبانی (SVM) و شبکه عصبی مصنوعی (ANN) به‌عنوان مدل‌های بانظارت استفاده شد. همچنین از مدل رگرسیون حداقل مربعات جزئی (PLSR) به‌عنوان مدل رگرسیونی برای تشخیص و تعیین کمیت تقلب در نمونه‌های آرد خالص استفاده شد. بهترین نتایج به‌ترتیب بادقت ۸۶٫۶۶ و ۸۶٫۷۰ برای مدل‌های SVM و ANN با پیش‌پردازش S-G + D2 + SNV و $R^2_p = 0.99$ برای مدل PLSR بود.

واژه‌های کلیدی: آرد گندم، تقلب، سدیم هیدروسولفیت، طیف‌سنجی، کمومتریکس (شیمی آماری)

۱ و ۲- به‌ترتیب دانشجوی دکتری و استاد، گروه مهندسی بیوسیستم، دانشکده کشاورزی، دانشگاه تبریز، تبریز، ایران
(*- نویسنده مسئول: Email: A.Kazemi@tabrizu.ac.ir)
۳- استادیار، گروه مهندسی مکانیک، دانشکده فنی و مهندسی، دانشگاه بناب، بناب، ایران
۴- استادیار، گروه مهندسی بیوسیستم، دانشکده کشاورزی، دانشگاه مراغه، مراغه، ایران

https://doi.org/10.22067/ifstrj.2024.86585.1312

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

Research Article
Vol. 20, No. 3, Aug.-Sep. 2024, p. 33-47

Investigating the Effect of Protease Enzyme Type and Hydrolysis Time on the Antioxidant Properties of Flaxseed Meal (*Linum usitatissimum*) Protein Hydrolysates

M. Hashemi[1], S.H. Hosseini Ghaboos[2]*, A. Seraj[3]

1- M.Sc. Student, Department of Food Science and Engineering, Azadshahr Branch, Islamic Azad University, Azadshahr, Iran
2- Assistant Professor, Food Science and Technology Research Center of East Golestan, Azadshahr Branch, Islamic Azad University, Azadshahr, Iran
(*- Corresponding Author Email: SH.Hosseinighaboos@iau.ac.ir)
3- Assistant Professor, Department of Agriculture and Animal Science, Azadshahr Branch, Islamic Azad University, Azadshahr, Iran

Received: 06.02.2024
Revised: 30.04.2024
Accepted: 18.05.2024
Available Online: 01.06.2024

How to cite this article:
Hashemi, M., Hosseini Qhaboos, S.H., & Seraj, A. (2024). Investigating the effect of protease enzyme type and hydrolysis time on the antioxidant properties of flaxseed meal (*Linum usitatissimum*) protein hydrolysates. *Iranian Food Science and Technology Research Journal*, *20*(3), 33-47. https://doi.org/10.22067/ifstrj.2024.86726.1314

Abstract

In this research, the effect of protease enzyme type (pepsin and pancreatin) and hydrolysis time (40-200 minutes) on the degree of hydrolysis and antioxidant properties (DPPH radical scavenging activity, Fe chelating activity, Fe reducing power and total antioxidant capacity) of flaxseed meal protein hydrolysates was investigated. The results showed that increasing the hydrolysis time increased the degree of hydrolysis, and the samples obtained from pancreatin had a higher degree of hydrolysis than pepsin. The highest activity of Fe^{2+} chelating (53.71 ± 0.45%) and Fe^{3+} reduction (1.32 ± 0.02, absorbance at 700 nm) was achieved by pancreatin after 200 minutes of hydrolysis. Pancreatin samples were more capable of inhibiting DPPH free radicals than pepsin, and their activity increased with increasing time up to 160 minutes. The highest total antioxidant capacity (1.36 ± 0.08 absorbance at 695 nm) among the samples was obtained after 160 minutes of hydrolysis with pancreatin. The antioxidant capacity of flax seed protein hydrolysates in inhibiting DPPH radical, Fe chelating activity, and total antioxidant capacity was lower than the antioxidant capacity of vitamin C at a concentration of 50 (mg/ml), but it had more Fe reducing power than vitamin C. Therefore, it can be concluded that compared to pepsin, pancreatin had a greater ability to produce flaxseed protein hydrolysates with significant antioxidant properties. According to the results, flaxseed protein hydrolysates from pancreatin enzyme and a hydrolysis time of 160 minutes have the ability to be used in food formulations to produce functional products.

Keywords: Antioxidant, Enzymatic hydrolysi, Flax seed, Pancreatin, Pepsin

Introduction

Oxidation of lipids and the production of free radicals play an undeniable role in dangerous diseases such as atherosclerosis, cancer, and cardiovascular diseases. Free radicals are produced in aerobic organs during respiration. They are extremely unstable, quickly react with biological molecules, and lead to irreparable damage to cells (Fiaschi & Chiarugi, 2012). Also, this phenomenon is one of the most important problems of the food industry, because, in addition to the adverse and irreparable effects on the health of consumers, it causes the production of dangerous

©2024 The author(s). This is an open access article distributed under Creative Commons Attribution 4.0 International License (CC BY 4.0).
https://doi.org/10.22067/ifstrj.2024.86726.1314

compounds and unpleasant odors and tastes, which causes heavy and irreparable economic losses to the manufacturer (Kaveh *et al*., 2019b). In the food industry, synthetic antioxidants such as BHA[1], BHT[2], PG[3] and TBHQ[4] are used, which have favorable antioxidant potential and reasonable price, but in recent decades, their adverse effects on human health have caused concern among scientists. As a result, in some countries, their application in food formulations has been limited or banned (Sadeghi Mahoonak & Kaveh, 2022).

On the other side, in recent years, public awareness regarding the direct effect of diet on human health has increased, which has increased the demand for food products without synthetic preservatives (Rezazadeh-bari *et al*., 2019). Therefore, identifying natural compounds with antioxidant potential has become a research priority for researchers. Among the natural antioxidants, bioactive peptides are suitable options, which usually have 2-20 amino acid residues and a molecular weight of less than 6000 da. These peptides can be produced by three methods: microbial fermentation, enzymatic hydrolysis, and chemical synthesis (Ulug *et al*., 2021). Recently, protein hydrolysates have been produced using various plant sources such as wheat germ (Karami *et al*., 2019) ،orange seed (Mazloomi *et al*., 2020), soybean (Islam *et al*., 2022), grape seed (Ding *et al*., 2018) and fenugreek (Kaveh *et al*., 2022). One of the suitable vegetable options for production protein hydrolysates is flaxseed, which is a rich source of protein that contains 35-45% oil. Flaxseed contains about 20% of unsaturated fatty acids such as oleic acid and more than 70% of alpha-linoleic acid (omega-3) fatty acids (Martinchik *et al*., 2012).

Among the suitable methods for production protein hydrolysates, enzymatic hydrolysis with protease enzymes is a suitable method that has been proven in various studies such as Farzanfar *et al*., (Farzanfar *et al*., 2024), Alvand *et al*., (Alvand *et al*., 2022), Xia *et al*., (Xia *et al*., 2023) and Islam et al., (Islam *et al*., 2023). Enzymatic hydrolysis is a process usually carried out under controlled conditions (pH, temperature, time, and enzyme concentration) and is the most common method for the production of bioactive peptides (Mora & Toldrá, 2023). In this regard, it has been reported that enzymatic hydrolysis is one of the most effective methods of producing bioactive peptides and protein hydrolysates because it has many advantages, such as high yield, no adverse effect on the nutritional value of proteins, and low side reactions (Habinshuti *et al*., 2023). Therefore, the aim of this research was to investigate the effect of protease enzyme type (pancreatin and pepsin) and hydrolysis time (40-210 minutes) on antioxidant properties (DPPH radical scavenging activity, Fe reducing power, Fe chelating activity, and total antioxidant capacity) and the degree of hydrolysis of hydrolyzed flaxseed protein and comparing it with the antioxidant activity of vitamin C as a synthetic antioxidant and unhydrolyzed flaxseed protein.

Materials and Methods

Pancreatin, pepsin, ammonium molybdate, iron dichloride, ferric chloride, trichloroacetic acid, ferrozine, ascorbic acid, and DPPH from Sigma, ethanol, sodium triphosphate, sulfuric acid, soda, hydrochloric acid, potassium dihydrogen phosphate, and dipotassium hydrogen phosphate were purchased from Merck, and flax seeds were purchased from a local store in the center of Tehran. The devices used in this research are listed in Table 1.

1- butylated hydroxyanisole
2- butylatedhydroxytoluene
3- propyl gallate
4- Tertiary butylhydroquinone

Table 1- The used devices

Device	Producer Company	Country
Water bath	Memmert, WNE45	Germany
Centrifuge	HERMLE, 36 HK	Germany
Shaker incubator	Wiggens, WS-600R	South Korea
Hot plate	VELP	Italy
Spectrophotometer	LABNICS, NUVS100	England
pH meter	inoLab, 7110	Germany
Scale	Sartorius	Germany
Freeze dryer	Chrtist	Germany

Fat Removal

To extract protein, flaxseed was first milled with an electric miller. The resulting powder was mixed with hexane at a ratio of 1:4 (w/v) and continuously stirred for 3 hours at room temperature. In the next step, hexane was separated using a Buchner funnel. The defatted powder was dried at 35°C and finally passed through a 40 mesh sieve (Kaveh *et al*., 2022).

Protein Extraction

In order to extract protein from the defatted powder, the resulting powder was mixed with distilled water at a ratio of 1:10 and the pH was adjusted to pH=10 using 1 N NaOH and stirred continuously for 2 hours at room temperature. The resulting solution was then centrifuged at 5000×g for 30 minutes. Then, the pH of the supernatant was adjusted to pH= 4 (the isoelectric pH of flaxseed protein) using 1 N HCl. In the next step, to separate the proteins, the resulting solution was centrifuged at 5000 ×g for 20 minutes, and the pellet was washed twice with distilled water and centrifuged again at 5000 ×g for 5 minutes. Then, the resulting protein isolate was dried with a freeze dryer and kept away from light at 4°C until further analysis (Kaveh *et al*., 2023).

Production of Flaxseed Protein Hydrolysate

Flax seed protein isolate obtained from the previous step was mixed at 5% concentration with 0.2 M phosphate buffer solution pH=7.4 for pancreatin activity, and for pepsin activity, it was mixed with distilled water, and the pH was adjusted to pH=2 using 1 N HCl. The resulting suspensions were continuously stirred for 30 minutes at room temperature in order to ensure complete hydration. Then, the samples were placed in a shaker incubator (40°C for pancreatin and 37°C for pepsin). The amount of enzyme was added at an enzyme-to-protein ratio of 1% (w/w). The reaction time was 40-200 minutes. After each time interval, the samples were transferred into a water bath of 90°C to inactivate the protease enzymes, and after 10 minutes, placed in an ice container to reach the ambient temperature. Then the samples were centrifuged for 20 minutes at 8000 × g, and the resulting supernatant was dried using a freeze dryer and kept in dark containers away from light at -20°C (Fadimu *et al*., 2021).

Degree of Hydrolysis

To evaluate the degree of hydrolysis of the resulting protein hydrolysates, the suspension of hydrolyzed protein and trichloroacetic acid (0.44 M) were mixed in a volume ratio of 1:1 and incubated for 15 minutes at 4°C. Then, the resulting mixture was centrifuged at 10000 rpm for 10 minutes. The amount of protein in the supernatant containing trichloroacetic acid 0.22 M was determined by the Bradford method. Bovine Serum albumin (BSA) was used as a standard (Sarabandi *et al*., 2019). The degree of hydrolysis was determined using Equation 1:

$$DH\ (\%) = \frac{\text{Protein (TCA+Supernatant)}}{\text{Protein (Flax seed hydrolysate suspension)}} \times 100 \quad (1)$$

Evaluation of Antioxidant Properties of Hydrolyzed Protein

DPPH Radical Scavenging Activity

To evaluate the DPPH radical scavenging activity of hydrolyzed flaxseed protein, the samples were dissolved in distilled water at a concentration of 40 mg/ml and vitamin C at a concentration of 50 mg/ml. Then, they were mixed with DPPH ethanol solution at a concentration of 0.15 mM in a ratio of 1:1 and vortexed for 20 seconds for complete mixing. In the next step, the resulting mixture was kept in the dark for 30 minutes and finally centrifuged at 4000 rpm for 15 minutes. The absorbance of the produced supernatant was read at 517 nm. DPPH radical scavenging activity of the samples was calculated using equation 2. A blank is the absorption of the control (DPPH ethanolic solution without hydrolyzed protein), and A sample is the absorption of the sample (Zhang *et al.*, 2015) .

$$I\ (\%) = [\frac{Ablank - Asample}{Ablank}] \times 100 \qquad (2)$$

Total Antioxidant Capacity

To evaluate the antioxidant capacity of protein hydrolysates, 0.1 ml of each hydrolyzed protein was dissolved in distilled water at a concentration of 40 mg/ml or vitamin C at a concentration of 50 mg/ml and mixed with 1 ml of the reagent (sulfuric acid 0.6 M, sodium phosphate 28 mM and ammonium molybdate 4 mM,100 ml of the reagent contained 3.25 ml of sulfuric acid, 0.49 g of ammonium molybdate and 1.064 g of sodium phosphate), then the resulted mixture was incubated in a water bath at 90°C. Finally, after cooling the samples and reaching the ambient temperature, the absorbance of the samples was read at 695 nm. Higher absorbance indicates stronger total antioxidant capacity (Kaveh *et al.*, 2022).

Fe chelating Activity

To evaluate the Fe chelating activity of hydrolyzed protein samples, 1 ml of hydrolyzed protein dissolved in distilled water at a concentration of (40 mg/ml) or vitamin C at a concentration of 50 mg/ml, then 0.05 ml of $FeCl_2$ (2 mM) and 1.85 ml of double distilled water were added. In the next step, 0.1 ml of ferrozine solution (5 mM) was added and the mixture was vigorously vortexed. Finally, it was stored for 10 minutes at ambient temperature and its absorbance was read at 562 nm. The chelating activity of the samples was calculated using equation (3). A blank (the absorbance of the control sample without antioxidant compound and containing distilled water) and A sample (the absorbance of the hydrolyzed sample) (Jamdar *et al.*, 2010).

$$\text{Chelating effect}\ (\%) = [(A_{blank} - A_{sample}/A_{blank})] \times 100 \qquad (3)$$

The Fe Reducing Power

In order to evaluate the Fe reducing power of hydrolyzed proteins, 0.5 ml of hydrolyzed protein solution in distilled water at a concentration of 40 mg/ml or vitamin C at a concentration of 50 mg/ml was mixed with 0.5 ml of 0.2 M phosphate buffer (pH=6.6) and 0.5 ml potassium ferricyanide (1% w/v) and kept in water bath at 50 °C for 20 minutes. Then, 0.5 ml of 10% trichloroacetic acid solution was added to the mixture and centrifuged at 2500 rpm for 10 minutes. Finally, 1 ml of the resulting supernatant was mixed with 1 ml of distilled water and 0.2 ml of ferric chloride (0.1% w/v) and kept at room temperature for 10 minutes. The absorbance of the samples was read at 700 nm. An increase in the absorbance of the mixture indicates an increase in the reducing power (Kaveh *et al.*, 2023).

Statistical Analysis

The statistical analysis of data was carried out using the SPSS 16.0 software (SPSS Inc., Chicago, IL). A means comparison was ascertained by Duncan's test at the 5% significance level using analysis of variance (ANOVA). All experiments were performed in triplicate, and the results were reported as means ± standard deviation.

Results and Discussion

Degree of Hydrolysis

Degree of hydrolysis is a measure of protein hydrolysis by protease enzyme. Fig. 1 shows the effect of hydrolysis time and the type of protease enzyme on the degree of hydrolysis of flaxseed protein. The results of statistical

analysis showed that both factors of hydrolysis time and type of enzyme had a significant effect on the degree of hydrolysis ($p<0.05$), so that after 200 minutes of the hydrolysis process with pancreatin, the degree of hydrolysis was 25.41 ± 0.2% and with pepsin enzyme, it was 24.15 ± 0.18%. The degree of hydrolysis increased significantly with the activity of pepsin and pancreatin enzymes in the first 160 minutes. However, increasing the time of hydrolysis afterward did not have a significant effect on the degree of hydrolysis and the rate of degree of hydrolysis decreased. This trend comes from excessive decomposition of the substrate and the inhibitory effect of the final product, which negatively affects the protease enzyme activity. On the other hand, the decrease in the rate of enzymatic hydrolysis might be as a result of the decrease in the number of peptide bonds available for hydrolysis, as well as the deactivation of the protease enzyme (Kaveh *et al.*, 2024). Our findings are similar to reports of Sherafat *et al.* (Sherafat *et al.*, 2018) and Yasmi *et al.* (Yasemi *et al.*, 2013) in the hydrolysis of after-cooking waste of skipjack fish and carp fish, respectively.

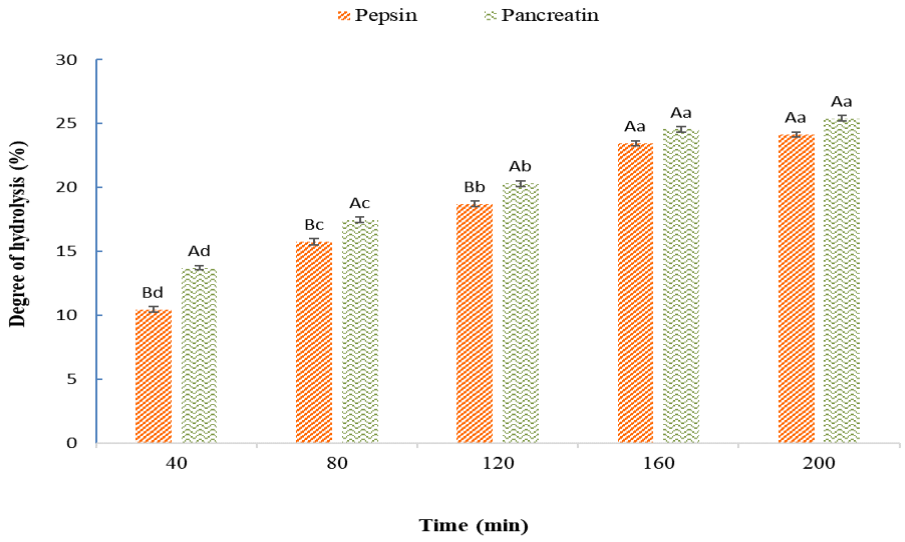


Fig. 1. The effect of hydrolysis time and protease type on the degree of hydrolysis of flax seed protein hydrolysate
(Lowercase letters indicate a significant difference between hydrolyzed samples with the same enzyme at different hydrolysis times, and uppercase letters indicate a significant difference between hydrolyzed samples with different enzymes at the same hydrolysis time, $p < 0.05$)

DPPH Radical Scavenging Activity

DPPH is a fat-soluble free radical that has the highest absorbance at 517 nm, and by receiving hydrogen from compounds with antioxidant activity, it becomes a stable compound, and its absorbance rate decreases (Hashemi *et al.*, 2022). The results of the hydrolysis time investigation showed (Fig. 2) that the lowest level of DPPH radical scavenging activity was related to non-hydrolyzed protein, and increasing the hydrolysis time caused a significant increase in the DPPH radical scavenging activity of hydrolyzed proteins ($p<0.05$). The DPPH radical scavenging activity of protein hydrolysates from pancreatin and pepsin were in the range of 37.54±0.46-51.24±0.37% and 33.12±0.33-45.30±0.26, respectively. DPPH radical scavenging activity for vitamin C at the concentration of 50 (mg/ml) was 70.35 ± 0.94%. Therefore, it can be concluded that increasing the time and degree of hydrolysis causes the release of proton-donating peptides that are capable of reacting with the DPPH free

radical and transforming it into stable compounds, and finally, the radical chain reactions are terminated. On the other hand, in the samples obtained from pancreatin activity, increasing the hydrolysis time more than 160 minutes did not make a significant difference in the inhibitory activity of the resulting hydrolyzed protein, and by pepsin enzyme activity, increasing the hydrolysis time more than 120 minutes caused a decrease in the ability of produced protein hydrolysate in DPPH radical scavenging activity. On the other hand, the negative effect of the excessive increase in enzymatic hydrolysis time can cause the greater effect of the protease enzyme, which causes the breaking and decomposition of a number of antioxidant peptides produced in the initial stages of hydrolysis, as a result of this process, the ability of protein hydrolysates in inhibition of DPPH free radical is reduced (Mazloomi-Kiyapey *et al*., 2019). On the other side, there was a significant difference between the DPPH radical scavenging activity of hydrolyzed proteins obtained from pancreatin and pepsin enzymes. Samples obtained from pancreatin activity had more DPPH radical scavenging activity, so after 200 minutes of hydrolysis by pancreatin enzyme, the DPPH radical scavenging activity was 51.24 ± 0.37%, while the DPPH radical scavenging activity of the hydrolyzed protein resulting from pepsin activity was 42.71 ± 0.42%, this result can be due to the difference in the composition, the amino acid sequence and molecular weight and as a result, the difference in the degree of hydrolysis of the peptides resulting from the activity of these two enzymes (Chalamaiah *et al*., 2015). In this regard, Batista *et al*. (Batista *et al*., 2010) reported on hydrolyzing black crab fish[1] waste protein, stated that DPPH radical scavenging activity increases with increasing degree of hydrolysis. They attributed this finding to the increase of hydrogen-donating peptides with an increase in the degree of hydrolysis, which has a great ability to react with free radicals. Also, Kaveh *et al*. (Kaveh *et al*., 2019a) reported on hydrolysis of fenugreek seed protein, that increasing the enzyme hydrolysis time up to 160 minutes increased the activity of the hydrolyzed protein in inhibiting DPPH free radical by 48%, but increasing the hydrolysis time did not have a significant effect on the inhibitory ability of the resulting hydrolyzed proteins. In accordance with these findings, Mazloomi *et al*. (Mazloomi-Kiyapey *et al*., 2019) and Kaveh *et al*. (Kaveh *et al*., 2022) reported that there is a direct relationship between the DPPH radical scavenging activity and the degree of hydrolysis of both orange seed and fenugreek seed proteins.

Total Antioxidant Capacity

The total antioxidant capacity evaluation test (phosphomolybdenum evaluation) is a quantitative method to evaluate water-soluble and fat-soluble antioxidant capacity; in fact, the total antioxidant capacity of a compound with antioxidant potential. This method based on the reduction of Mo^{6+} to Mo^{5+}, and as a result of this reaction, a green phosphomolybdenum complex is formed in an acidic environment.

1- black scabbardfish

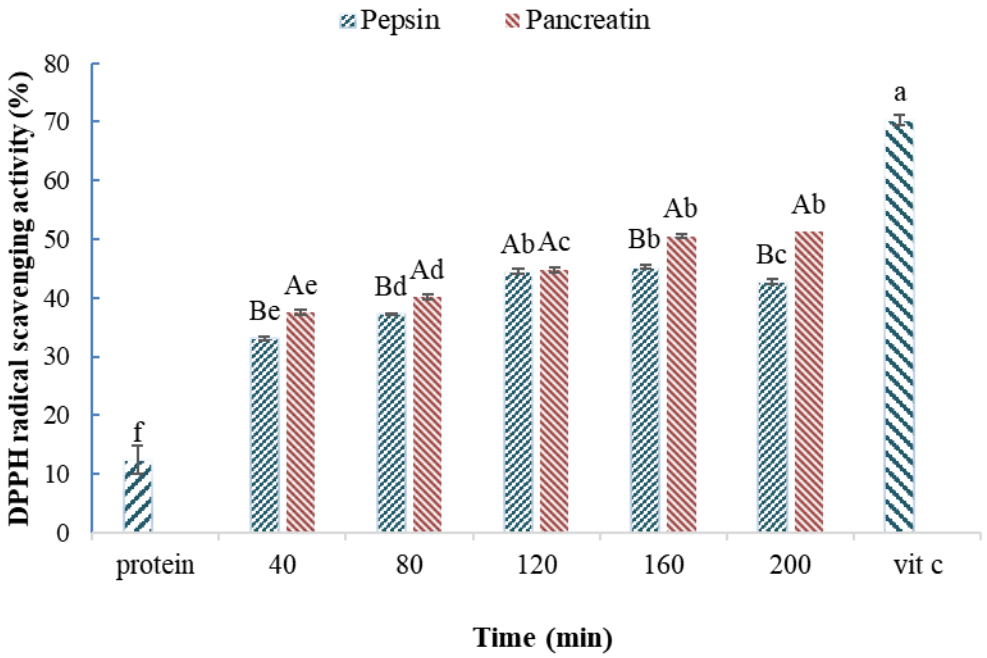


Fig. 2. The effect of hydrolysis time and protease type on DPPH radical scavenging activity of flax seed protein hydrolysate compared to unhydrolyzed protein (40 mg/ml) and Vitamin C (50 mg/ml)
(Lowercase letters indicate a significant difference between hydrolyzed samples with the same enzyme at different hydrolysis times, and uppercase letters indicate a significant difference between hydrolyzed samples with different enzymes at the same hydrolysis time, $p < 0.05$)

As can be seen in Fig. 3, in this research, enzymatic hydrolysis significantly increased the antioxidant capacity of the flaxseed protein, and increasing the hydrolysis time with pepsin and pancreatin enzymes significantly increased the antioxidant activity of samples. For example, after 40 minutes of hydrolysis, the antioxidant capacity of samples obtained from pepsin and pancreatin was 0.710 ± 0.06 and 0.860 ± 0.05 respectively (absorbance at 695 nm), which by progressing the hydrolysis process up to 200 minutes for pepsin enzyme and 160 minutes for pancreatin enzyme, the antioxidant capacity increased significantly and reached to 1.1 ± 0.08 and 1.36 ± 0.08 (absorbance at 695 nm), respectively ($p<0.05$). The total antioxidant activity of all hydrolysates was lower than the total antioxidant capacity of vitamin C (1.65 ± 0.06, absorbance at 695 nm). In this regard, it can be stated that with the increase in the hydrolysis time of the pepsin and pancreatin, the release of peptides with electron-donating properties has increased. These peptides have been able to transform free radicals into stable compounds with less reactivity, which ultimately increased the antioxidant activity of all samples with increasing time (Arabshahi-Delouee & Urooj, 2007). In accordance to these results, Umayaparvathi *et al.* (Umayaparvathi *et al.*, 2014) revealed that the antioxidant capacity of oyster protein hydrolysates was concentration dependent and the highest total antioxidant activity of the hydrolysates was obtained at a concentration of 1 mg/ml, but it was significantly lower than the control sample (vitamin C). Also, Bougatef *et al.* (Bougatef *et al.*, 2009) reported that increasing in the concentration of fish protein hydrolysate had a positive effect on the increasing the total antioxidant capacity of the samples and the hydrolysates obtained from trypsin had higher total antioxidant capacity than samples obtained from pepsin.

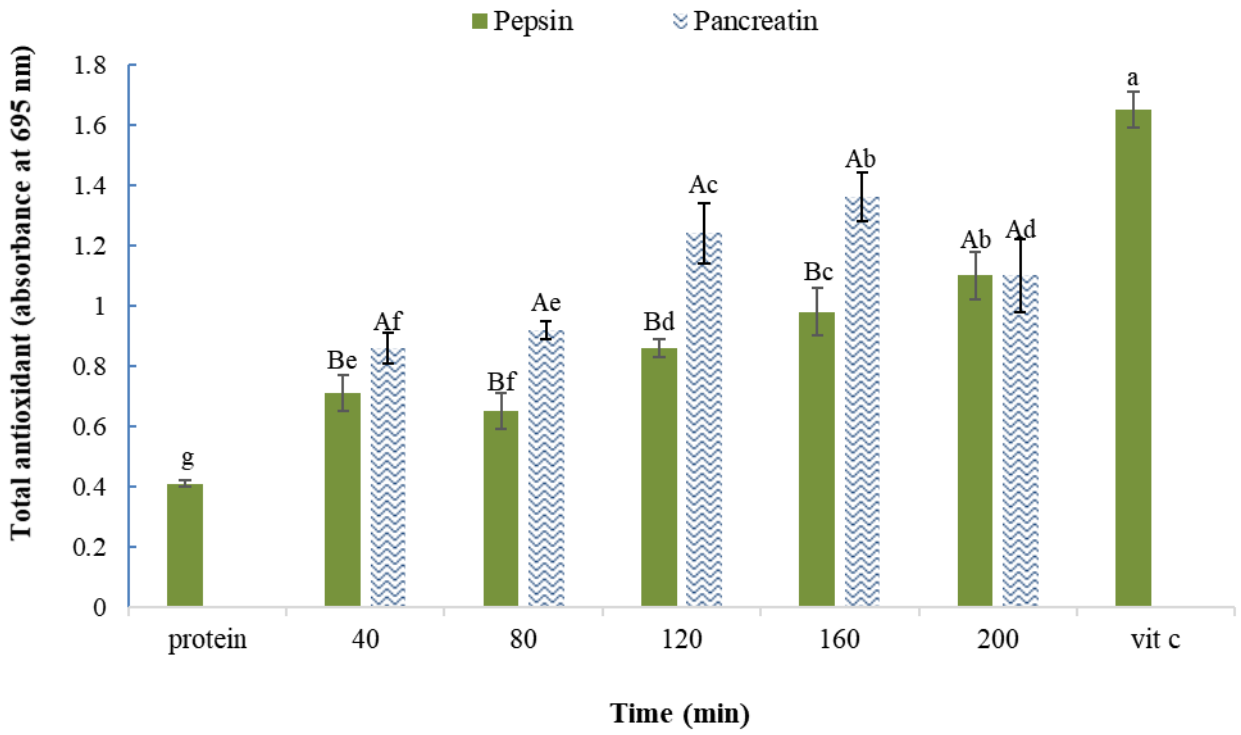


Fig. 3. The effect of hydrolysis time and protease type on total antioxidant capacity of flax seed protein hydrolysate compared to unhydrolyzed protein (40 mg/ml) and Vitamin C (50 mg/ml)
(Lower case letters indicate a significant difference between hydrolyzed samples with the same enzyme at different hydrolysis times and uppercase letters indicate a significant difference between hydrolyzed samples with different enzymes at the same hydrolysis time, $p < 0.05$)

Fe Chelating Activity

In the occurrence of lipid oxidation, metal ions such as Fe^{2+} play an essential role as a catalyst that causes the production and release of dangerous hydroxyl radicals from hydrogen superoxide. These free radical compounds react with the nearby biomolecules at a high speed and lead to the damage of body cells and tissues. Therefore, inhibiting and chelating metal ions plays an essential role in preventing oxidation (Olennikov *et al*., 2014). According to Fig. 4, the chelating activity of hydrolyzed proteins resulting from the activity of pepsin and pancreatin enzymes was significantly higher than the original (non-hydrolyzed) flax seed protein ($p<0.05$); This result indicates the appropriate performance of the enzymes used in this research in the production of peptides with the ability to chelate Fe^{2+} ions. The amount of chelation in hydrolyzed proteins resulting from the activity of pepsin and pancreatin was between 32.21 ± 0.28 ± 46.51 ± 0.28 and 30.76 ± 0.44 - 53.71 ± 0.45, respectively. It should be noted that the level of Fe^{2+} chelating activity of the produced hydrolysates was lower than vitamin C chelating activity at all hydrolysis times. In general, compared to pepsin, the pancreatin enzyme led to the production of a hydrolyzed protein with more Fe^{2+} chelating ability in all hydrolysis times except 40 and 120 minutes. Various studies have shown that the chelating ability of hydrolyzed proteins depends on the type of enzyme, the time and degree of hydrolysis, and the amino acid sequences of the original protein (Zarei *et al*., 2016). Similar to these results, also Sarabandi *et al*. (Sarabandi *et al*., 2018) and Jamdar *et al.* (Jamdar *et al*., 2010) reported an increase in Fe chelating activity of casein and peanut hydrolyzed proteins with increasing time and degree of hydrolysis.

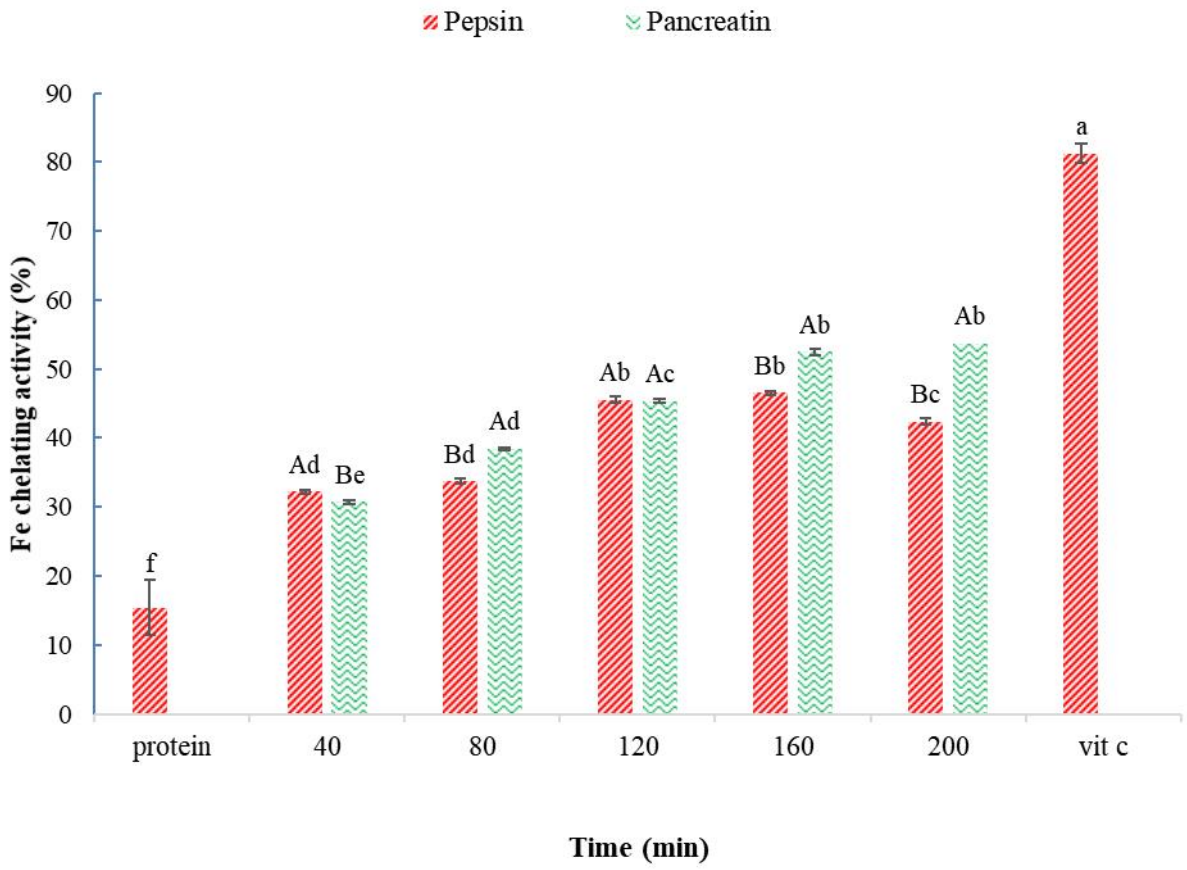


Fig. 4. The effect of hydrolysis time and protease type on Fe chelating activity of flax seed protein hydrolysate compared to unhydrolyzed protein (40 mg/ml) and Vitamin C (50 mg/ml)
(Lower case letters indicate a significant difference between hydrolyzed samples with the same enzyme at different hydrolysis times and uppercase letters indicate a significant difference between hydrolyzed samples with different enzymes at the same hydrolysis time, $p < 0.05$)

Fe Reducing Power

The reducing power test evaluates the electron-donating ability of antioxidant compounds. In other words, this test evaluates a compound's antioxidant ability to donate electrons and convert Fe^{3+} ions to Fe^{2+}. Various studies have shown that there is a direct relationship between the reducing power of bioactive compounds and their antioxidant activity (Vavrusova *et al.*, 2015). Fig. 5 shows the reducing power of hydrolyzed protein resulting from the activity of pepsin and pancreatin compared to unhydrolyzed flaxseed protein and vitamin C. The non-hydrolyzed protein had the lowest reducing power of 0.318 ± 0.02 (absorbance at 700 nm), and all the samples obtained from the activity of pancreatin and pepsin at all hydrolysis times had the reducing power. They had more activity than vitamin C as a positive control sample ($p<0.05$). These results show the positive effect of hydrolysis with pepsin and pancreatin enzymes on increasing the Fe reducing power of unhydrolyzed flax seed protein. In general, it can be stated that increasing the time and degree of hydrolysis caused a significant increase in the reducing power, this finding can have various reasons, including, increasing the amount of hydrolysis with protease enzyme can lead to the release of free amino acids, which

play their role as an additional source of electrons and protons (Zhu *et al*., 2008). On the other side, increasing the degree of hydrolysis increases the availability of electron-donating amino acids such as lysine, histidine, methionine, and tryptophan. As a result, the Fe-reducing power of hydrolyzed protein increases significantly (Jamdar *et al*., 2010). On the other side, the results showed that the Fe reducing power of the hydrolyzed proteins obtained from pancreatin was higher than that of pepsin, which indicates the better performance of pancreatin compared to pepsin in increasing the Fe reducing power. In this regard, Ambigaipalan *et al*. (Ambigaipalan *et al*., 2015) reported that hydrolyzing date kernel protein with alcalase, flavorzyme and thermolysin protease enzymes, and the peptides obtained from alcalase enzyme had lower Fe reducing power. You *et al*. (You *et al*., 2009) and Mazloomi *et al*. (Mazloomi-Kiyapey *et al*., 2019) reported an increase in the reducing power of the hydrolyzed proteins obtained from tian fish and orange seed after the enzymatic hydrolysis process.

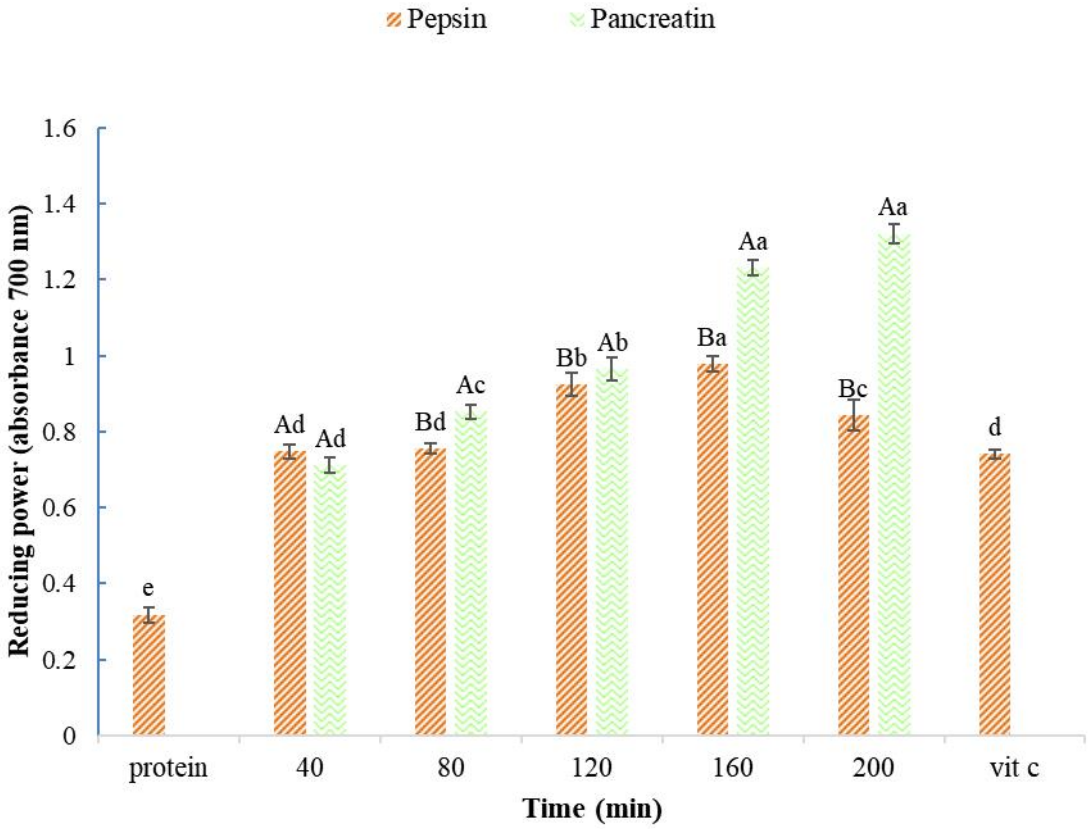


Fig. 5. The effect of hydrolysis time and protease type on Fe reducing power of flax seed protein hydrolysate compared to unhydrolyzed protein (40 mg/ml) and Vitamin C (50 mg/ml)
(Lower case letters indicate a significant difference between hydrolyzed samples with the same enzyme at different hydrolysis times and uppercase letters indicate a significant difference between hydrolyzed samples with different enzymes at the same hydrolysis time, $p < 0.05$)

Conclusion

The application of compounds with antioxidant properties to prevent oxidation is common in the food industry, but the concerns about the adverse effects of synthetic antioxidants have increased the attention of researchers to identify and extract natural compounds with antioxidant properties.

Hydrolyzed proteins are among the natural compounds that have significant antioxidant properties. In this research, the effect of hydrolysis time and type of protease enzyme on antioxidant activity and degree of hydrolysis of flaxseed protein was investigated. The results showed that the antioxidant properties of hydrolyzed flaxseed proteins with pepsin and pancreatin enzymes depend on the degree of hydrolysis of the samples, and with the increase of the degree of hydrolysis, the antioxidant activity of the samples (DPPH radical scavenging activity, Fe chelating activity, Fe reducing power and total antioxidant capacity) increased significantly. After hydrolysis for 200 minutes, the highest DPPH radical scavenging activity (51.24 ± 0.37%), Fe reducing power (1.32 ± 0.02, absorbance at 700 nm) and Fe chelating activity (53.71 ± 0.45%) was related to the samples obtained from the activity of pancreatin enzyme and had no significant difference with the antioxidant activity of the hydrolyzed protein obtained from pancreatin after 160 minutes of hydrolysis; Also, the highest amount of total antioxidant capacity (1.36 ± 0.08, absorbance at 695 nm) was related to the hydrolysates obtained from pancreatin up to 160 minutes of hydrolysis. Therefore, it can be concluded that enzymatic hydrolysis of flaxseed meal protein with pancreatin enzyme after 160 minutes of hydrolysis is a suitable solution for producing peptides with desirable antioxidant properties. Due to the appropriate antioxidant capacity, the resulting hydrolyzed proteins have the potential to be used in food formulations to produce functional products and can compete with synthetic antioxidants, so by conducting in vivo tests, the produced flax seed hydrolyzed protein can be used in the food industry as a substitute for synthetic antioxidants, effectively.

Author Contributions

S.H. Hosseini Ghaboos: Conceptualization, Supervision, Validation, Writing–review and editing; **M. Hashemi:** Formal analysis, Writing–original draft, Methodology, Software; **A. Seraj:** Validation, Visualization, Investigation.

Funding Sources

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

References

1. Alvand, M., Sadeghi Mahoonak, A., Ghorbani, M., Shahiri Tabarestani, H., & Kaveh, S. (2022). Comparison of the antioxidant properties of hydrolyzed Turkmen melon seed protein by pancreatin and alcalase. *Food Engineering Research*, *21*(2), 75–90. https://doi.org/10.22092/fooder.2023.354314.1303
2. Ambigaipalan, P., Al-Khalifa, A.S., & Shahidi, F. (2015). Antioxidant and angiotensin I converting enzyme (ACE) inhibitory activities of date seed protein hydrolysates prepared using alcalase, flavourzyme and thermolysin. *Journal of Functional Foods*, *18*, 1125–1137. https://doi.org/10.1016/j.jff.2015.01.021
3. Arabshahi-Delouee, S., & Urooj, A. (2007). Antioxidant properties of various solvent extracts of mulberry (*Morus indica* L.) leaves. *Food Chemistry*, *102*(4), 1233–1240. https://doi.org/10.1016/j.foodchem.2006.07.013
4. Batista, I., Ramos, C., Coutinho, J., Bandarra, N.M., & Nunes, M.L. (2010). Characterization of protein hydrolysates and lipids obtained from black scabbardfish (*Aphanopus carbo*) by-products and antioxidative activity of the hydrolysates produced. *Process Biochemistry*, *45*(1), 18–24. https://doi.org/10.1016/j.procbio.2009.07.019
5. Bougatef, A., Hajji, M., Balti, R., Lassoued, I., Triki-Ellouz, Y., & Nasri, M. (2009). Antioxidant and free radical-scavenging activities of smooth hound (*Mustelus mustelus*) muscle protein

hydrolysates obtained by gastrointestinal proteases. *Food Chemistry*, *114*(4), 1198–1205. https://doi.org/10.1016/j.foodchem.2008.10.075
6. Chalamaiah, M., Hemalatha, R., Jyothirmayi, T., Diwan, P.V, Bhaskarachary, K., Vajreswari, A., Kumar, R. R., & Kumar, B.D. (2015). Chemical composition and immunomodulatory effects of enzymatic protein hydrolysates from common carp (*Cyprinus carpio*) egg. *Nutrition*, *31*(2), 388–398. https://doi.org/https://doi.org/10.1016/j.nut.2014.08.006
7. Ding, Q., Zhang, T., Niu, S., Cao, F., Wu-Chen, R.A., Luo, L., & Ma, H. (2018). Impact of ultrasound pretreatment on hydrolysate and digestion products of grape seed protein. *Ultrasonics Sonochemistry*, *42*, 704–713. https://doi.org/10.1016/j.ultsonch.2017.11.027
8. Fadimu, G.J., Gill, H., Farahnaky, A., & Truong, T. (2021). Investigating the impact of ultrasound pretreatment on the physicochemical, structural, and antioxidant properties of lupin protein hydrolysates. *Food and Bioprocess Technology*, *14*, 2004–2019. https://doi.org/10.1007/s11947-021-02700-4
9. Farzanfar, F., Sadeghi Mahoonak, A., Ghorbani, M., Hosseini Qaboos, S.H., & Kaveh, S. (2024). The effect of ultrasound pretreatment on the antioxidant properties of hydrolyzed protein from flaxseed meal using alcalase and pancreatin enzymes by response surface methodology. *Journal of Food Science and Technology (Iran)*, *21*(147), 187–205.
10. Fiaschi, T., & Chiarugi, P. (2012). Oxidative stress, tumor microenvironment, and metabolic reprogramming: a diabolic liaison. *International Journal of Cell Biology*, *2012*. https://doi.org/10.1155/2012/762825
11. Habinshuti, I., Nsengumuremyi, D., Muhoza, B., Ebenezer, F., Aregbe, A.Y., & Ndisanze, M.A. (2023). Recent and novel processing technologies coupled with enzymatic hydrolysis to enhance the production of antioxidant peptides from food proteins: A review. *Food Chemistry*, 136313. https://doi.org/10.1016/j.foodchem.2023.136313
12. Hashemi, S.M.B., Abedi, E., Kaveh, S., & Mousavifard, M. (2022). Hypocholesterolemic, antidiabetic and bioactive properties of ultrasound-stimulated exopolysaccharide produced by *Lactiplantibacillus plantarum* strains. *Bioactive Carbohydrates and Dietary Fibre*, *28*, 100334. https://doi.org/10.1016/j.bcdf.2022.100334
13. Islam, M., Huang, Y., Islam, S., Fan, B., Tong, L., & Wang, F. (2022). Influence of the degree of hydrolysis on functional properties and antioxidant activity of enzymatic soybean protein hydrolysates. *Molecules*, *27*(18), 6110. https://doi.org/10.3390/molecules27186110
14. Islam, M., Huang, Y., Jain, P., Fan, B., Tong, L., & Wang, F. (2023). Enzymatic hydrolysis of soy protein to high moisture textured meat analogue with emphasis on antioxidant effects: As a tool to improve techno-functional property. *Biocatalysis and Agricultural Biotechnology*, 102700. https://doi.org/10.1016/j.bcab.2023.102700
15. Jamdar, S.N., Rajalakshmi, V., Pednekar, M.D., Juan, F., Yardi, V., & Sharma, A. (2010). Influence of degree of hydrolysis on functional properties, antioxidant activity and ACE inhibitory activity of peanut protein hydrolysate. *Food Chemistry*, *121*(1), 178–184. https://doi.org/10.1016/j.foodchem.2009.12.027
16. Karami, Z., Peighambardoust, S.H., Hesari, J., Akbari-Adergani, B., & Andreu, D. (2019). Antioxidant, anticancer and ACE-inhibitory activities of bioactive peptides from wheat germ protein hydrolysates. *Food Bioscience*, *32*, 100450. https://doi.org/10.1016/j.fbio.2019.100450
17. Kaveh, S., Mahoonak, A.S., Ghorbani, M., & Jafari, S.M. (2022). Fenugreek seed (*Trigonella foenum graecum*) protein hydrolysate loaded in nanosized liposomes: Characteristic, storage stability, controlled release and retention of antioxidant activity. *Industrial Crops and Products*, *182*, 114908. https://doi.org/10.1016/j.indcrop.2022.114908
18. Kaveh, S., Sadeghi Mahoonak, A., Erfani Moghadam, V., Ghorbani, M., Gholamhosseinpour, A., & Raeisi, M. (2023). Evaluation the antioxidant properties of purified bioactive peptides from the

wastes of skipjack fish (*Katsuwonus pelamis*) processing, by pepsin and trypsin digestive enzymes. *Journal of Food Science and Technology (Iran)*, *20*(141), 200–222. https://doi.org/10.22034/FSCT.20.141.200

19. Kaveh, S., Sadeghi Mahoonak, A., Erfanimoghaddam, V., Ghorbani, M., Gholamhossein pour, A.A., & Raeisi, M. (2024). Optimization of the effect of hydrolysis conditions and type of protease on the degree of hydrolysis and antioxidant properties of the protein hydrolysate from the skipjack fish (*Katsuwonus pelamis*) viscera by the response surface methodology. *Journal of Food Science and Technology (Iran)*, *20*(144), 131–152. https://doi.org/10.22034/FSCT.20.144.131
20. Kaveh, S., Sadeghi Mahoonak, A., Ghorbani, M., Jafari, S.M., & Sarabandi, K. (2019a). Antioxidant properties of fenugreek bioactive peptides prepared with pancreatin enzyme. *Food Engineering Research*, *18*(67), 103–122. https://doi.org/10.22092/fooder.2020.124616.1190
21. Kaveh, S., Sadeghi Mahoonak, A., Ghorbani, M., Jafari, S.M., & Sarabandi, K. (2019b). Optimization of factors affecting the antioxidant activity of fenugreek seed's protein hydrolysate by response surface methodology. *Iranian Journal of Nutrition Sciences & Food Technology*, *14*(1).
22. Martinchik, A.N., Baturin, A.K., Zubtsov, V.V, & VIu, M. (2012). Nutritional value and functional properties of flaxseed. *Voprosy Pitaniia*, *81*(3), 4–10.
23. Mazloomi-Kiyapey, S.N., Sadeghi-Mahoonak, A., Ranjbar-Nedamani, E., & Nourmohammadi, E. (2019). Production of antioxidant peptides through hydrolysis of medicinal pumpkin seed protein using pepsin enzyme and the evaluation of their functional and nutritional properties. *Arya Atherosclerosis*, *15*(5), 218.
24. Mazloomi, S.N., Sadeghi Mahoonak, A., Ghorbani, M., & Houshmand, G. (2020). Physicochemical properties of chitosan-coated nanoliposome loaded with orange seed protein hydrolysate. *Journal of Food Engineering*, *280*, 109976. https://doi.org/10.1016/j.jfoodeng.2020.109976
25. Mora, L., & Toldrá, F. (2023). Advanced enzymatic hydrolysis of food proteins for the production of bioactive peptides. *Current Opinion in Food Science*, *49*, 100973. https://doi.org/10.1016/j.cofs.2022.100973
26. Olennikov, D.N., Kashchenko, N.I., & Chirikova, N.K. (2014). A novel HPLC-assisted method for investigation of the Fe^{2+}-chelating activity of flavonoids and plant extracts. *Molecules*, *19*(11), 18296–18316. https://doi.org/10.3390/molecules191118296
27. Rezazadeh-bari, M., Najafi-Darmian, Y., Alizadeh, M., & Amiri, S. (2019). Numerical optimization of probiotic Ayran production based on whey containing transglutaminase and Aloe vera gel. *Journal of Food Science and Technology*, *56*(7), 3502–3512. https://doi.org/10.1007/s13197-019-03841-3
28. Sadeghi Mahoonak, A.R., & Kaveh, S. (2022). Assessment of ACE-inhibitory and antioxidant activities of the peptide fragments from pumpkin seeds. *Iranian-Journal Nutrition Science Food Technology*, *17*(3), 45–56.
29. Sarabandi, K., Mahoonak, A.S., Hamishehkar, H., Ghorbani, M., & Jafari, S.M. (2019). Protection of casein hydrolysates within nanoliposomes: Antioxidant and stability characterization. *Journal of Food Engineering*, *251*, 19–28. https://doi.org/10.1016/j.jfoodeng.2019.02.004
30. Sarabandi, K., Mahoonak, A. S., Hamishekar, H., Ghorbani, M., & Jafari, S. M. (2018). Microencapsulation of casein hydrolysates: Physicochemical, antioxidant and microstructure properties. *Journal of Food Engineering*, *237*, 86–95. https://doi.org/10.1016/j.jfoodeng.2018.05.036

31. Sherafat, N., Motamedzadegan, A., & Safari, R. (2013). The effect of hydrolyzing time on cooked tuna fish (*Skipjack tuna*) wastes by alcalase on protein recovery and the hydrolysate molecular weight. *Journal of Innovation in Food Science and Technology*, *5*(3).
32. Ulug, S.K., Jahandideh, F., & Wu, J. (2021). Novel technologies for the production of bioactive peptides. *Trends in Food Science & Technology*, *108*, 27–39. https://doi.org/10.1016/j.tifs.2020.12.002
33. Umayaparvathi, S., Meenakshi, S., Vimalraj, V., Arumugam, M., Sivagami, G., & Balasubramanian, T. (2014). Antioxidant activity and anticancer effect of bioactive peptide from enzymatic hydrolysate of oyster (*Saccostrea cucullata*). *Biomedicine & Preventive Nutrition*, *4*(3), 343–353. https://doi.org/10.1016/j.bionut.2014.04.006
34. Vavrusova, M., Pindstrup, H., Johansen, L.B., Andersen, M.L., Andersen, H.J., & Skibsted, L.H. (2015). Characterisation of a whey protein hydrolysate as antioxidant. *International Dairy Journal*, *47*, 86–93. https://doi.org/10.1016/j.idairyj.2015.02.012
35. Xia, X., Song, S., Zhou, T., Zhang, H., Cui, H., Zhang, F., Hayat, K., Zhang, X., & Ho, C.-T. (2023). Preparation of saltiness-enhancing enzymatic hydrolyzed pea protein and identification of the functional small peptides of salt reduction. *Journal of Agricultural and Food Chemistry*, *71*(21), 8140–8149. https://doi.org/10.1021/acs.jafc.3c02046
36. Yasemi, M., Ghomi Marzdashti, M.R., Darnahal, T., Mohammadzadeh, B., & Amini, H. (2013). Yield of protein recovery and degree of hydrolysis associated protein hydrolysates from Bighead Carp (*Aristichthys nobilis*) by using enzymes. *Iranian Scientific Fisheries Journal*.
37. You, L., Zhao, M., Cui, C., Zhao, H., & Yang, B. (2009). Effect of degree of hydrolysis on the antioxidant activity of loach (*Misgurnus anguillicaudatus*) protein hydrolysates. *Innovative Food Science & Emerging Technologies*, *10*(2), 235–240. https://doi.org/10.1016/j.ifset.2008.08.007
38. Zarei, M., Ghanbari, R., Tajabadi, N., Abdul-Hamid, A., Bakar, F.A., & Saari, N. (2016). Generation, fractionation, and characterization of iron-chelating protein hydrolysate from palm kernel cake proteins. *Journal of Food Science*, *81*(2), C341–C347. https://doi.org/10.1111/1750-3841.13200
39. Zhang, Y., Sun, W., Zhao, M., Su, G., Ning, Z., & Sun-Waterhouse, D. (2015). Improvement of the ACE-inhibitory and DPPH radical scavenging activities of soya protein hydrolysates through pepsin pretreatment. *International Journal of Food Science & Technology*, *50*(10), 2175–2182. https://doi.org/10.1111/ijfs.12856
40. Zhu, L., Chen, J., Tang, X., & Xiong, Y.L. (2008). Reducing, radical scavenging, and chelation properties of in vitro digests of alcalase-treated zein hydrolysate. *Journal of Agricultural and Food Chemistry*, *56*(8), 2714–2721. https://doi.org/10.1021/jf703697e

نشریه پژوهشهای علوم و صنایع غذایی ایران
https://ifstrj.um.ac.ir

مقاله پژوهشی

جلد ۲۰، شماره ۳، مرداد-شهریور ۱۴۰۳، ص. ۴۷-۳۳

بررسی تأثیر نوع آنزیم پروتئازی و زمان هیدرولیز بر ویژگی‌های آنتی‌اکسیدانی پروتئین هیدرولیز شده کنجاله بذر کتان (*Linum usitatissimum*)

مریم هاشمی[۱]- سید حسین حسینی قابوس[۲*]- ابولقاسم سراج[۳]

تاریخ دریافت: ۱۴۰۲/۱۱/۱۷
تاریخ پذیرش: ۱۴۰۳/۰۲/۲۹

چکیده

در این پژوهش بررسی تأثیر نوع آنزیم پروتئازی (پپسین و پانکراتین) و زمان هیدرولیز (۲۰۰-۴۰ دقیقه) بر درجه هیدرولیز و ویژگی‌های آنتی‌اکسیدانی (فعالیت مهار رادیکال DPPH، قدرت شلاته‌کنندگی آهن، قدرت احیاءکنندگی یون آهن و فعالیت آنتی‌اکسیدانی کل) پروتئین هیدرولیز شده کنجاله بذر کتان انجام شد. نتایج نشان داد که افزایش زمان هیدرولیز باعث افزایش درجه‌ی هیدرولیز شد و نمونه‌های حاصل از پانکراتین نسبت به پپسین دارای درجه‌ی هیدرولیز بالاتری بودند. بیشترین میزان فعالیت شلاته‌کنندگی Fe^{2+} (۰/۴۵ ± ۵۳/۷۱ درصد) و احیاءکنندگی Fe^{3+} (۰/۰۲ ± ۱/۳۲، جذب در ۷۰۰ نانومتر) توسط پانکراتین و پس از ۲۰۰ دقیقه هیدرولیز حاصل شد. نمونه‌های حاصل از پانکراتین نسبت به پپسین توانایی بیشتری در مهار رادیکال آزاد DPPH داشتند و فعالیت آن‌ها با افزایش زمان تا ۱۶۰ دقیقه افزایش یافت. بیشترین ظرفیت آنتی‌اکسیدانی کل (۰/۰۸ ± ۱/۳۶ جذب در ۶۹۵ نانومتر) در بین نمونه‌ها پس از ۱۶۰ دقیقه هیدرولیز با پانکراتین به‌دست آمد. قابلیت آنتی‌اکسیدانی پروتئین هیدرولیز شده بذر کتان در مهار رادیکال DPPH، شلاته کنندگی یون آهن و آنتی‌اکسیدانی کل از قابلیت آنتی‌اکسیدانی ویتامین ث در غلظت (mg/ml) ۵۰، کمتر بود اما از خاصیت احیاءکنندگی یون آهن بیشتری نسبت به ویتامین ث برخوردار بود. بنابراین می‌توان نتیجه گرفت که پانکراتین در مقایسه با پپسین توانایی بیشتری در تولید پروتئین هیدرولیز شده بذر کتان با خاصیت آنتی‌اکسیدانی قابل توجه داشت. با توجه به نتایج پروتئین‌های هیدرولیز شده بذر کتان با استفاده از آنزیم پانکراتین و زمان هیدرولیز ۱۶۰ دقیقه، قابلیت استفاده در فرمولاسیون مواد غذایی برای تولید محصولات فراسودمند را دارند.

واژه‌های کلیدی: آنتی‌اکسیدان، بذر کتان، پپسین، پانکراتین، هیدرولیز آنزیمی

۱- دانشجوی کارشناسی ارشد گروه علوم و مهندسی صنایع غذایی، واحد آزادشهر، دانشگاه آزاد اسلامی، آزادشهر، ایران
۲- استادیار مرکز تحقیقات صنایع غذایی شرق گلستان، واحد آزاد شهر، دانشگاه آزاد اسلامی، آزادشهر، ایران
(*- نویسنده مسئول: Email: SH.Hosseinighaboos@iau.ac.ir)
۳- استادیار گروه کشاورزی و دامپروری، واحد آزاد شهر، دانشگاه آزاد اسلامی، آزادشهر، ایران

https://doi.org/10.22067/ifstrj.2024.86726.1314

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

Research Article
Vol. 20, No. 3, Aug.-Sep. 2024, p. 49-64

Preharvest Melatonin and Postharvest Xanthan Gum Coating Maintain the Quality of Orlando Tangelos during Storage

S. Mollaei Mohammad Abadi[1], S. Rastegar[2*]

1 and 2- Master's Student and Associate Professor, Department of Horticultural Sciences, Faculty of Agriculture and Natural Resources, University of Hormozgan, Bandar Abbas, Iran, respectively.
(*- Corresponding Author Email: s.rastegar@hormozgan.ac.ir)

Received: 09.04.2024	**How to cite this article:**
Revised: 02.06.2024	Mollaei Mohammad Abadi, S., & Rastegar, S. (2024). Preharvest melatonin and postharvest xanthan gum coating maintain the quality of Orlando tangelos during storage. *Iranian Food Science and Technology Research Journal*, *20*(4), 49-64. https://doi.org/10.22067/ifstrj.2024.87553.1324
Accepted: 02.06.2024	
Available Online: 02.06.2024	

Abstract

This study aimed to determine the effects of preharvest spraying of melatonin and postharvest immersion in xanthan gum on the quality and postharvest performance of Orlando tangelo mandarin fruits. After selecting suitable and uniform trees, melatonin foliar spraying was performed at three different concentrations: 0,100 µM, and 200 µM. Foliar spraying was performed one month before harvest and was repeated three times at weekly intervals. Furthermore, the fruits were immersed in two different concentrations of xanthan gum (0.1% and 0.2%) postharvest, these fruits were stored in a cold room at 5 ± 1 °C. Evaluation of fruit characteristics was carried out at the time of harvest and after 45 and 90 days of cold storage. The results showed that foliar spraying of melatonin at a concentration of 100 µM showed the highest weight and pulp of the fruit. Furthermore, melatonin treatment resulted in higher levels of ascorbic acid and increased fruit acidity compared to the control. During storage, fruits treated with melatonin and xanthan coatings showed better quality than those of the control. At the end of the experiment, the lowest weight loss was observed in fruits treated with 200 µM melatonin + 0.1% xanthan. The highest ascorbic acid content was observed in the 100 µM melatonin +0.1% xanthan. The maximum antioxidant activity was observed in 100 µM and 200 µM +0.1% xanthan and also 100 µM melatonin alone. In general, the findings suggest that preharvest foliar spraying and the postharvest application of xanthan coatings can be effective strategies for maintaining Orlando tangelo quality during cold storage.

Keywords: Citrus, Melatonin, Spraying, Storage, Xanthan gum

Introduction

Citrus is one of the most important fruit trees in several countries, including Iran. Citrus fruits contain abundant beneficial phytochemicals, including vitamins A, C, and E, mineral elements, flavonoids, coumarins, limonoids, carotenoids, pectins, and other compounds. These compounds contribute to the high nutritional value in humans (Saini *et al*. 2022). While there are various tangerine species, tangelo mandarin (*Citrus*×*tangelo*) has become a significant player in the citrus industry (Traore *et al*., 2023). Orlando tangerine is a hybrid of the Duncan grapefruit and Dancy tangerines. It is characterized by large, round, medium-ripe fruits that are juicy and fleshy, and have a relatively high seed count. In addition, they exhibited good heat resistance. It is estimated that 20-30% of fresh fruits are lost after harvest. This is mainly because of its perishable nature. To mitigate this, cold storage is used to delay ripening changes including ethylene formation, softening, pigment changes, respiration rate, acidity changes, and

©2024 The author(s). This is an open access article distributed under Creative Commons Attribution 4.0 International License (CC BY 4.0).
https://doi.org/10.22067/ifstrj.2024.87553.1324

weight loss. However, because citrus fruits have a subtropical nature, they are susceptible to chilling when stored at low temperatures. Consequently, appropriate postharvest treatments in conjunction with cold storage are necessary to ensure the preservation of fruits at the desired levels of quality.

Melatonin, a natural endogenous plant hormone found in various crops, has been increasingly recognized for its positive effects on postharvest fruit preservation. Studies have shown that melatonin treatment can improve the content of bioactive compounds and the antioxidant activity in different fruits. Moreover, the environmentally friendly use of natural substances such as melatonin has emerged as an important approach to modulate the biosynthetic pathways that influence fruit quality during the ripening and postharvest stages (Arabia *et al.*, 2022). In pomegranate trees of the "Mollar de Elche" cultivar, melatonin treatment has shown promising results in increasing fruit quality traits, such as anthocyanin and phenolic content, as well as overall fruit quality during storage. Treatment with preharvest melatonin at concentrations of 0.1 or 1 mM has demonstrated a significant impact on yield and quality improvement in pomegranate fruits during both harvest and postharvest storage (Medina-Santamarin *et al.*, 2021). These findings highlight the potential of melatonin as a beneficial treatment for improving fruit quality and preservation, providing opportunities to optimize postharvest strategies and maintain fruit freshness and nutritional value. (Arabia *et al.*, 2022; Lorente-Mento 2021).

Xanthan gum (XG), produced by *Xanthomonas campestris*, is generally recognized as safe (GRAS) by the Food and Drug Administration (FAO, 2020). Natural hydrocolloid-based edible coatings and films offer additional protection to fresh or blanched fruits and vegetables. Edible coatings made from natural gums show promise for improving the quality and extending the shelf life of fruits and vegetables (Salehi, 2020). Edible coatings, particularly those enhanced with xanthan gum, play a crucial role in preventing quality deterioration by selectively regulating gas exchange between food and its external environment. This application not only extends the shelf life but also preserves the overall appearance and quality of fruits during storage (Tripathi *et al.*, 2021). In a study on guava fruit, a mixture of 1% xanthan gum and 0.2% chitosan nanoparticles, used as a coating, was reported to improve the overall quality of guava fruits during long-term cold storage and extend their shelf life (Gad & Zagzog, 2017). Recently, Rastegar *et al*. (2024) reported that the postharvest application of melatonin and melatonin combined with γ-aminobutyric acid (GABA) plays an effective role in mitigating chilling damage in Orlando mandarin fruits stored at 3 ± 0.5 °C for 90 days. However, the literature lacks reports on the effects of preharvest application of melatonin and postharvest immersion in xanthan gum on the quantitative and qualitative characteristics of Orlando mandarin fruit. In light of the importance of preserving citrus fruits and the potential benefits of melatonin and xanthan gum-based edible coatings, this study aims to investigate the combined effect of preharvest melatonin treatment and postharvest xanthan gum-based edible coatings on the postharvest quality and preservation of Orlando tangerine fruits during cold storage. By examining the physicochemical and biochemical attributes of the treated fruits, we aim to provide valuable insights into the novel strategies for enhancing the postharvest quality and extending the shelf life of Orlando tangerine fruits, thus contributing to the citrus industry's efforts to minimize postharvest losses and meet consumer demands for high-quality fruits.

Materials and Methods

Fruit Spray and Harvest

In a citrus orchard located in Rudan city, an experiment involving foliar spraying of melatonin was conducted on 9-year-old Orlando tangerine trees (*Citrus paradisi* × *C. reticulata*). The experiment consisted of three melatonin concentrations: 0 (distilled water as

a control treatment), 100 µM, and 200 µM. The spray was applied one month before harvest and repeated three times with a one-week interval between each application. Each treatment included 3 replicates and each replicate included one tree. Upon reaching commercial maturity in December, the fruits were harvested and transported to the laboratory for evaluation of their quantitative and qualitative characteristics. The collected fruits were divided into two distinct groups for the purposes of analysis. The initial group was subjected to an assessment in order to examine the impact of pre-harvest melatonin application on the quality attributes of the fruit. The subsequent group was utilized to investigate the post-harvest treatment involving xanthan, as well as the subsequent storage conditions on the fruits.

Preparation of Xanthan Gum Solution

To prepare xanthan gum solution, the gum was gradually dissolved in water and stirred for 30 min at room temperature. The resulting solution was then refrigerated for 24 h. Two concentrations of xanthan gum were prepared: 0.1% and 0.2%.

Coating Fruit and Storage

The selected fruits were subjected to disinfection with 0.05% sodium hypochlorite for one min, followed by washing with distilled water. The fruits were coated with xanthan gum solution using the immersion method. The fruits were immersed in the solution at room temperature for 5 min. The treatments and their abbreviations used in the experiment are shown in (Table 1). After the surface coating was completely dried, the coated fruits were transferred to a fruit basket and stored for 90 days at 5 ± 1 °C. The main factors and characteristics were evaluated after 45 and 90 days of storage to assess any changes or effects resulting from the treatments (Fig. 1).

Table 1- Treatments and their abbreviations used in the experiment

Treatments	Abbreviation
Distilled water	Control
Melatonin 100 µM	M 100 µM
Melatonin 200 µM	M 200 µM
Xanthan gum 0.1%	XG 0.1%
Xanthan gum 0.2%	XG 0.2%
Melatonin 100 Mm+ Xanthan gum 0.1%	M 100 Mm+ XG 0.1%
Melatonin 200 µM + Xanthan gum 0.1%	M 200 µM + XG 0.1%
Melatonin 100 µM + Xanthan gum 0.2%	M 100 µM + XG 0.2%
Melatonin 200 µM + Xanthan gum 0.2%	M 200 µM + XG 0.2%

Fig. 1. Different stages of experiment and measurement of fruit traits

Length and Diameter of the Fruit

In order to quantify the dimensions of the fruit, a sample of 10 fruits was randomly selected from each treatment. The length and diameter of the selected fruits were then measured using a metal ruler, and the recorded measurements were expressed in centimeters.

Weight of the Fruits

The weight of the fruit was assessed using a digital scale (SHS, Japan) with a precision of 0.1 gr. At the time of harvest (0 days), the weight of the fruit was assessed using a digital scale with a precision of 0.1 gr.

Fruit Volume

A graduated cylinder was used to measure fruit volume. A certain portion of the graduated cylinder was filled with water and the fruit was placed in it. The change in the water level indicated the volume of the fruit (Omid *et al*., 2010).

Weight Loss

Weight loss was determined by comparing the weight of the fruit at the time of harvest (0 days) with the weight measured on sampling days 45 and 90 during the storage period. The weight of the fruits was recorded using a digital scale with an accuracy of 0.1 gr. The percentage weight loss was calculated using the following formula:

Percentage of weight loss = [(Initial weight - Final weight) / Initial weight] x 100

The measurements and calculations were conducted according to the methodology described by (Dong & Wang, 2018).

Total Soluble Solids (TSS)

To measure the total soluble solids (TSS) in fruit juice, a few drops of tangerine juice were placed on the screen of a digital refractometer (DBR95, Taiwan). The refractometer measured the TSS in Brix degrees.

Titratable Acidity (TA)

For TA quantitation, 5 mL of fruit juice was mixed with 20 mL of distilled water and titrated with 0.1 N NaOH to pH 8.2. The result was expressed as a percentage of citric acid.

Ascorbic Acid Content

A titration method was employed to measure ascorbic acid content. An aliqout (0.05 ml) of fruit juice was mixed with 5 ml of 1% cold metaphosphoric acid. Subsequently, a solution containing indophenol (sodium-2,6-dichlorophenol-indophenol) was titrated until a purple color appeared. The amount of titrant used was indicative of ascorbic acid content, which was expressed as milligrams per 100 ml of juice (Bor *et al*., 2006).

Total Phenol

The total phenol content of the fruit juice was measured using the Folin-Ciocalteu reagent. A methanolic extract was prepared by mixing tangerine juice (0.5 ml of tangerine juice) with 3 ml of 85% methanol and refrigerating for 24 h. Next, 60 µL of the methanolic extract was mixed with 300 µL of Folin's reagent (1:10), followed by the addition of 240 µL of 7% sodium carbonate. After leaving the solution for two-hour in darkness, the absorbance of the samples was measured using a microplate reader (Biotek model EPOCH2) at a specific wavelength of 760 nm (Ordonez *et al*., 2006).

Total Flavonoid

Total flavonoid content was determined by adding 180 µL 85% methanol, 12 µL 10% aluminum chloride, 12 µL potassium acetate, and 336 µL distilled water to 60 µL methanolic extract. The mixture was left at room temperature for half an hour and the absorbance was measured with microplate reader at 415 nm.

Determination of the Antioxidant Activity

The scavenging activity of the DPPH (2, 2-Diphenyl-1picrylidrazil) radical was assessed using the method outlined by Brand-Williams *et al*. (1995) with some modification. To carry

out the test, 30 µL of the methanolic extract was mixed with 150 µL DPPH solution, which contained 0.025 g DPPH in 100 mL of 85% methanol. The mixture was then left in the dark at room temperature for 40 min, and a microplate reader was used to measure the absorbance at 517 nm.

Statistical Analysis

The experiment was conducted as a factorial design in a completely randomized block design. Statistical analyses were performed using the SAS version 9.4. Average data were compared using the LSD test at a significance level of 5%. Graphs were plotted using Excel 2019 software.

Results and Discussion

Effect of melatonin pre-harvest spray on fruit characteristics at harvest time

The application of melatonin at a concentration of 100 µM resulted in a significant increase in the weight and pulp of the fruit compared to the control group. No significant effects were observed on other physical characteristics of the fruit. Ascorbic acid at 200 µM and titratable acid at both concentrations showed a significant increase compared with the control, but other traits were not affected by melatonin and had no any significant effects on fruit size and volume. This implies that melatonin application did not have a noticeable impact on the overall size and volume of Orlando tangelo (Table 2). However, it should be noted that the effect of melatonin on fruit characteristics can vary depending on the fruit variety and growing conditions. Melatonin plays a role in various physiological processes related to fruit enlargement and crop yield. Liu *et al*. (2019) reported that melatonin contributes to the enlargement of pear fruits. Similarly, in pomegranate trees treated with 0.1 mM melatonin, Medina-Santamarin *et al.* (2021) observed an increase in crop yield, including the number of fruits per tree and overall weight per tree. This effect was attributed to the improved development of the aril part of the fruit. The effects of melatonin on apple growth and ripening were investigated by Verde *et al*. (2022). This study demonstrated that melatonin treatment results in an increase in apple fruit size and weight. Additionally, in a separate study involving blackberries, the external application of melatonin increased the number, size, weight, and color of fruits (Verde *et al*., 2022). The observed effects of melatonin on fruit size and weight can be attributed to its ability to promote cell growth and enlargement, as reported by Zhao *et al*. (2023). Collectively, these studies suggest that melatonin has the potential to positively influence fruit characteristics, such as size, weight, and yield of various fruits. However, it is important to note that the specific effects of melatonin can vary depending on fruit species, cultivar, and experimental conditions.

Our findings revealed that the application of a high concentration of melatonin led to a significant increase in the ascorbic acid content of the fruit compared with both the control and a lower concentration of melatonin. This indicates that higher concentrations of melatonin, when applied by foliar spraying, have a pronounced effect on increasing the ascorbic acid levels in Orlando Tangelo mandarin fruits. Ascorbic acid, also known as vitamin C, is a vital antioxidant that plays a crucial role in various physiological processes and contributes to the nutritional quality of fruit. In addition to the increased ascorbic acid content, the treated fruits also exhibited a higher titratable acidity than the control. This suggests that melatonin application, particularly at higher concentrations, influenced the acidity of the fruit. The higher titratable acidity observed in melatonin- treated fruits indicates the possible impact of melatonin on the acid balance of the fruit. Fruits treated with 200 µM melatonin showed higher TSS than control. Similarly, it has been shown that in a study focusing on pear fruit, the foliar application of melatonin at a concentration of 100 µM resulted in an increase in the total soluble solids (TSS) of the fruit (Table 3).

Table 2- The effect of melatonin foliar spraying on the physical characteristics of Orlando tangelo fruit

Treatments	Fruit weight (g)	Peel weight (g)	Pulp weight (g)	Water volume (ml)	Fruit length (cm)	Fruit diameter (cm)	Fruit volume
Control	168 ^{b}	35.5 ^{a}	130 ^{b}	83.3 ^{a}	6.3 ^{a}	6.76 ^{a}	147 ^{a}
Melatonin 100 µM	218 ^{a}	48 ^{a}	166 ^{a}	90 ^{a}	6.4 ^{a}	6.7 ^{a}	182 ^{a}
Melatonin 200 µM	163 ^{b}	37.5 ^{a}	126 ^{b}	86 ^{a}	6.1 ^{a}	6.6 ^{a}	179 ^{a}

In each column, the numbers with the same letters are statistically not significantly different from each other at the 5% probability level.

Table 3- The effect of melatonin foliar spraying on the biochemicals characteristics of Orlando tangelo fruit

Treatment	Antioxidants (%)	Flavonoid (mg/gFW)	Phenol (mg/gFW)	Ascorbic acid (mg/100 ml)	pH	TA (%)	TSS (%)
Control	79^{bc}	3.88^{a}	9^{ab}	56de	3.32$^{c–d}$	0.39^{c}	9.5^{c}
Melatonin 100 µM	82.1^{ab}	3.56^{a}	9.12^{a}	62^{d}	3.43$^{a–c}$	0.52^{a}	9.7^{c}
Melatonin 200 µM	76^{c}	3.69^{a}	7.63^{b}	89^{a}	3.18^{f}	0.51^{a}	10.4^{bc}

In each column, the numbers with the same letters are statistically not significantly different from each other at the 5% probability level.

Postharvest Study

Weight Loss

Minimal weight loss was observed in sample treated with 200 µM melatonin + 0.1% xanthan, as well as the sample treated with 0.2% xanthan alone after 90 days of storage,. The decrease in weight loss was found to be statistically significant when compared to the control group (Fig. 2).

Weight loss in fruits can occur because of water loss caused by changes in surface transfer resistance against water vapor, transpiration, and the rate of fruit respiration, as described by Shahid and Abbasi, (2011). To prevent weight loss, edible coatings are found to be effective in minimizing transpiration. Edible coatings create a barrier on the surface of the fruit, partially or completely covering the openings and small pores, thereby reducing the gas exchange and transpiration (Vignesh & Nair, 2019). The ability of some coatings to limit water loss and maintain fruit weight during storage has been well documented (Kittur *et al*., 2001). The use of guar gum coating in tomatoes controlled the fruit weight loss, which is similar to the results obtained in our study (Ruelas-Chacon *et al*., 2017). Therefore, the lower weight loss observed in fruits treated with melatonin than in the control group can be attributed to the effect of melatonin in increasing the thickness of the cuticle (Bal, 2019). Pre-harvest melatonin treatment during cherry fruit growth in trees has also been shown to reduce fruit weight loss during cold storage (Carrión-Antol, 2022). In another study, postharvest application of melatonin delayed weight loss and fruit rot in blueberries (Shang *et al*., 2021). Additionally, treating pear slices with xanthan gum and calcium ascorbate was found to reduce fruit weight loss compared to the control group, a finding that aligns with our results (Guccione *et al*., 2023).

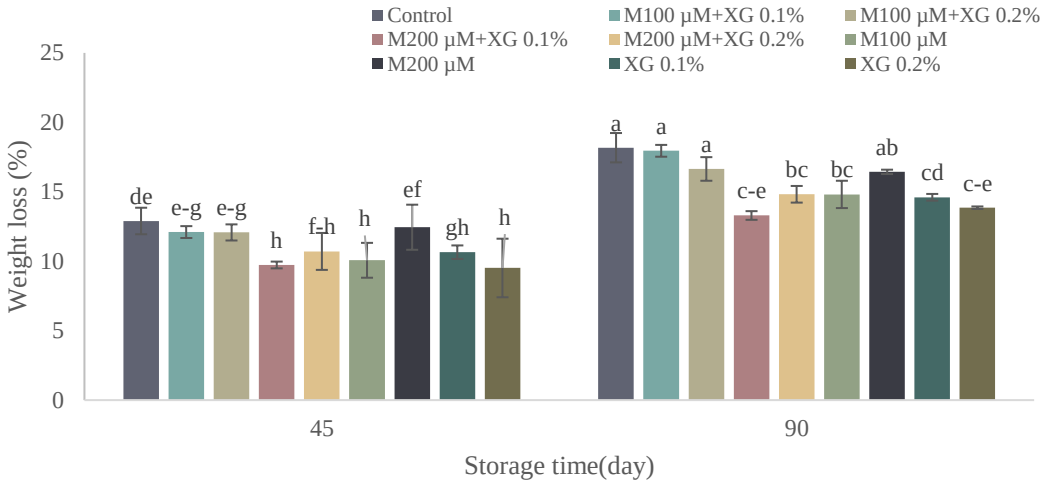


Fig. 2. The effect of different levels of melatonin spray and xanthan gum immersion on the weight loss percentage of Orlando tangelo

Total Soluble Solids (TSS) and Titratable Acidity (TA)

During fruit storage, the lowest TSS was observed in melatonin 100 µM+ xanthan 0.2% and melatonin 200 µM+ xanthan 0.1% treatments, which was significantly lower than that in the control and other treatments (Fig. 3 a). Furthermore, composite coatings prepared from xanthan gum and lemongrass essential oil were found to be effective in maintaining the soluble solid content (SSC) and reducing oxidative stress in Kinnow mandarin fruits during long-term storage, as reported by Bajaj *et al.* (2024). This indicates that xanthan gum and lemongrass essential oil coatings can preserve the nutritional quality of fruit, which includes TSS parameters. In grapes, melatonin treatment increased the content of soluble solids, as demonstrated by Xull *et al.* (2017). In contrast, the combination of xanthan gum and olive oil in grapefruits reduced the accumulation of total soluble solids and total sugars, as reported by Baraiya *et al.* (2016). These findings highlight the potential role of different treatments, including melatonin and coatings, in modulating sugar content and TSS parameters of fruits.

The results showed that melatonin 100 µM+ xanthan 0.2%, melatonin 200 µM+ xanthan 0.2% and xanthan 0.1% treatments exhibited a significantly higher titratable acid (TA) content (Fig. 3b). In tomato fruits, the combined effect of coatings based on whey protein isolate, xanthan gum, and clove oil resulted in less degradation of titratable acidity compared to uncoated samples, as reported by Kumar and Saini, (2021). This indicates that the coatings helped maintain the titratable acidity of tomatoes. Furthermore, in a study involving preharvest treatments in strawberries, including melatonin application, Xia *et al.* (2020) found that treatments applied one week before harvest resulted in fruit with higher total soluble solids (TSS) and lower titratable acidity (TA). This suggests that the timing of treatment can influence the acid content of the fruits. It is important to consider the specific fruit species, treatment conditions, and experimental context when interpreting the effects of melatonin and other treatments on titratable acidity. More research is needed to fully understand the mechanisms underlying these effects and optimize treatment strategies for different fruit crops.

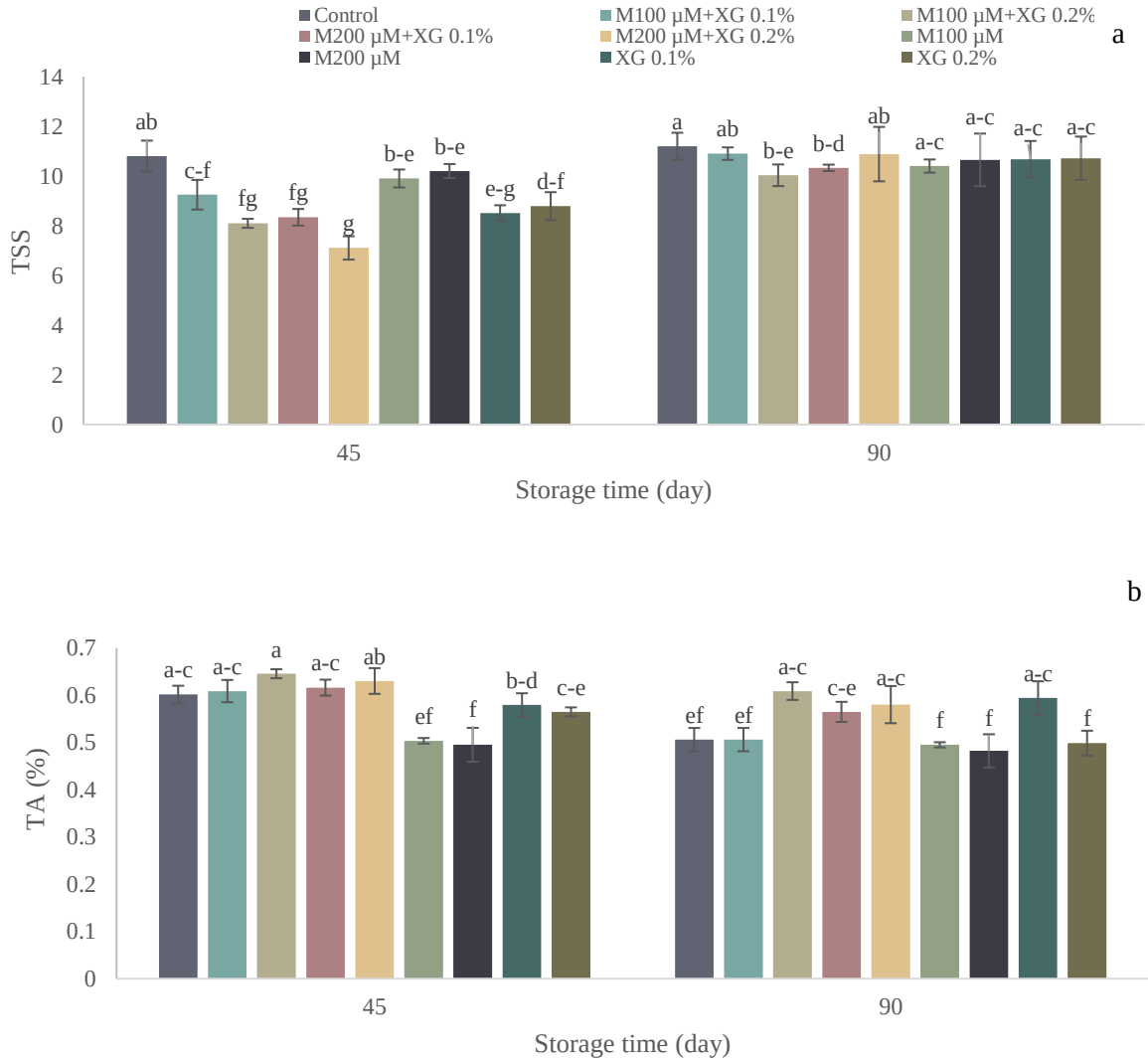


Fig. 3. The effect of different levels of melatonin spray and xanthan gum immersion on the content of total soluble solids (TSS) and TA of Orlando tangelo fruit

Ascorbic Acid

After 45 days of storage, melatonin 200 µM+ xanthan 0.1% and xanthan 0.1% showed the highest ascorbic acid content, which was significantly different from the control. At the end of the experiment, melatonin 100 µM+ xanthan 0.1% treatment showed a significantly higher level of ascorbic acid than the control, while the other treatments did not show a significant difference with the control (Fig. 4). Ascorbic acid is a potent antioxidant that protects fruits from the harmful effects of reactive oxygen species, as mentioned by Blokhina *et al*. (2003). Ascorbic acid acts as an antioxidant and undergoes decomposition upon reacting with oxygen. Consequently, the application of edible coatings that minimize oxygen contact has been found to effectively delay the oxidative degradation of ascorbic acid in fruits (Ayranci & Tunc 2004). Edible coatings are effective in delaying ascorbic acid degradation during storage. This is attributed to the formation of a protective layer that reduces the exposure of fruit skin to oxygen *(Tigist et al*., 2013). Studies have shown that pomegranate fruits from trees treated with melatonin contain more ascorbic acid compared to those from control trees. Furthermore, the coated samples exhibited less degradation of

ascorbic acid than the uncoated samples, as reported by Kumar and Saini (2021). Furthermore, according to Xia *et al*., (2020) the application of 0.05 or 0.1 mM melatonin improved the concentration of ascorbic acid in cherry fruits. The study conducted by *Shang et al.* (2021) supports the positive effect of melatonin on ascorbic acid, although specific details have not been provided. These findings highlight the potential of melatonin treatment and edible coatings to preserve the ascorbic acid content in fruits by reducing oxidative degradation.

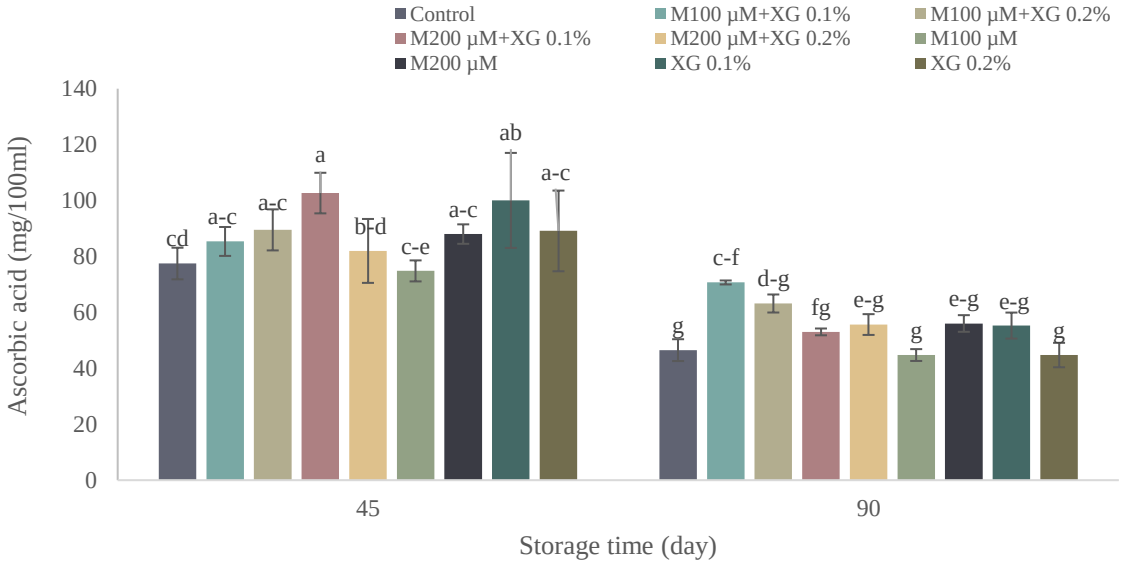


Fig. 4. The effect of different levels of melatonin spray and xanthan gum immersion on the ascorbic acid of Orlando tangelo

Total Phenol, Flavonoid and Antioxidant Capacity

After 45 days of storage, the phenolic content of the treated fruits was found to be higher than that of the control in most of the treatments. However, during the subsequent 90 days of storage, there was a significant decline in the phenolic content among all treatments, and no notable difference was observed between the control and the treated groups (Fig. 5a). Phenolic compounds are synthesized at a faster rate during the ripening process of fruits but tend to decrease as the fruit approaches senescence. These compounds play crucial roles in determining fruit quality and phytochemical levels (Shamloo *et al.*, 2015). Phenolic compounds are known for their antioxidant properties and contribute to the sensory attributes, nutritional value, and potential health benefits of fruit. Phenolic compounds play a crucial role in maintaining the nutritional quality of fruits and vegetables by influencing their color, firmness, taste, and bitterness. These compounds represent a diverse group of secondary metabolites, with documented beneficial effects on human health. Phenolic compounds act as antioxidants and have various biological activities. The role of melatonin in modulating the total phenolic content of citrus fruits has been studied, and it has been found that melatonin treatment in oranges leads to maintain the total phenols during storage (Ma *et al.*, 2021). For grapes, xanthan gum has been shown to preserve phenolic compounds by creating a protective coating around the grapes, which restricts oxygen supply and helps maintain phenolic content (Quoc *et al.*, 2014). Furthermore, research has shown that melatonin treatment in pomegranate trees helps to maintain higher

levels of phenolic compounds in fruits than in the control group throughout the storage period. Similarly, the application of melatonin has been reported to increase the concentration of phenolic compounds in grapes (Wang *et al*., 2020).

Based on the data presented in (Fig. 5b) , the flavonoid content during the storage period was consistently higher in the treated groups compared to the control. In particular, after 90 days of storage, the xanthan 0.2% treatment exhibited the highest flavonoid content among all the treatments. Flavonoids are a group of polyphenolic compounds that are released as part of the defense mechanisms of plants (Uckoo *et al*., 2015). These secondary metabolites enhance the antioxidant capabilities of fruits by eliminating free radicals. Additionally, a study conducted on Kinnow tangerines examined the impact of coatings on fruit quality. It was found that, , the total flavonoid content and juice content were better preserved than the control, indicating that the coatings helped maintain the flavonoid content of tangerines (Bajaj *et al*., 2024). These findings highlight the potential of melatonin and xanthan gum treatments to promote flavonoid accumulation in fruits.

Based on the data presented in (Fig. 5c), fruit treated with melatonin 200 µM and 100 µM showed the highest antioxidant activity than other treatments in 45th day of storage. However, at the end of storage, melatonin 100 µM+ xanthan 0.1%, melatonin 200 µM+ xanthan 0.1% and + melatonin 100 µM showed higher antioxidant activity than the control. Melatonin improves the content of bioactive compounds and antioxidant activity in various fruit products (Wu *et al*., 2021). In grapes, coatings enriched with xanthan gum and acid phytochemicals were found to preserve the antioxidant and tissue properties of grapes during cold storage (Golly *et al*., 2019). Furthermore, studies have also shown that melatonin enhances the antioxidant activities of sweet cherry (Xia *et al*., 2020).

Conclusion

This work demonstrated that spray of melatonin at a concentration of 100 µM had significant effects on the characteristics of Orlando tangelo mandarin fruits. These effects included an increase in fruit weight and fruit pulp weight, as well as higher levels of ascorbic acid and increased fruit acidity, compared to the control. Throughout the experiment, the fruits treated with a combination of 200 µM melatonin and 0.1% xanthan gum exhibited the lowest weight loss. Furthermore, treatment with 100 µM melatonin and 0.1% xanthan gum showed the highest ascorbic acid content, while the treatments with 100 µM and 200 µM melatonin, both with 0.1% xanthan gum, exhibited the highest antioxidant activity. Overall, our findings suggest that preharvest spray of melatonin and the application of xanthan gum coating may be an effective strategy to maintain the quality of Orlando tangelo mandarin fruits during storage. These techniques have the potential to improve various characteristics such as weight, ascorbic acid content, acidity, and antioxidant activity.

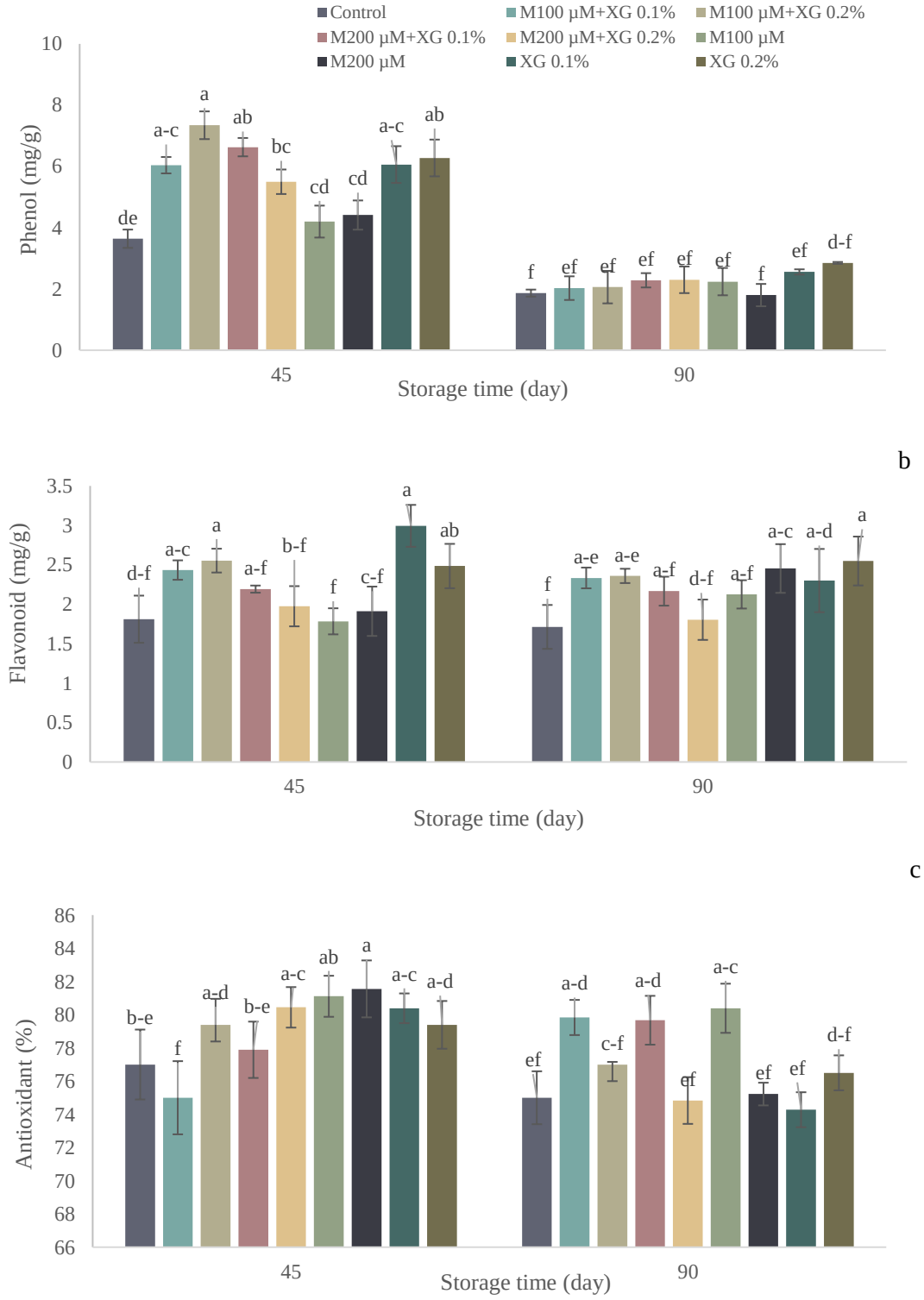


Fig. 5. The effect of different levels of melatonin spray and xanthan gum immersion on the total phenol (a), flavonoid (b) and (c) Orlando Tangelo

Author Contributions

S. Mollaei Mohammad Abadi: Data curation, investigation, methodology, software, writing–original draft. **S. Rastegar:** Conceptualization, data curation, project

administration, supervision, writing–review and editing.

Funding Sources

Part of the funding for this research was provided by Hormozgan Province Science & Technology Park.

Acknowledgments

This study as a research project was financially supported by the University of Hormozgan and Hormozgan Science and Technology Park, Iran.

References

1. Arabia, A., Munne-Bosch, S., & Muñoz, P. (2022). Melatonin triggers tissue-specific changes in anthocyanin and hormonal contents during postharvest decay of Angeleno plums. *Plant Science*, *320*, 111287. https://doi.org/10.1016/j.plantsci.2022.111287
2. Ayranci, E., & Tunc, S. (2004). The effect of edible coatings on water and vitamin C loss of apricots (*Armeniaca vulgaris* Lam.) and green peppers (*Capsicum annuum* L.). *Food Chemistry*, *87*(3), 339-342. https://doi.org/10.1016/j.foodchem.2003.12.003
3. Bajaj, K., Kumar, A., Gill, P.P.S., Jawandha, S.K., & Kaur, N. (2024). Xanthan gum coatings augmented with lemongrass oil preserve postharvest quality and antioxidant defense system of Kinnow fruit under low-temperature storage. *International Journal of Biological Macromolecules*, 129776. https://doi.org/10.1016/j.ijbiomac.2024.129776
4. Bal, E. (2019). Physicochemical changes in 'Santa Rosa' plum fruit treated with melatonin during cold storage. *Journal of Food Measurement and Characterization*, *13*, 1713-1720. https://doi.org/10.1007/s11694-019-00088-6
5. Baraiya, N.S., Ramana Rao, T.V., & Thakkar, V.R. (2016). Composite coating as a carrier of antioxidants improves the postharvest shelf life and quality of table grapes (*Vitis vinifera* L. var. Thompson Seedless). *Journal of Agricultural Science and Technology*, *18*(1), 93-107.
6. Blokhina, O., Virolainen, E., & Fagerstedt, K.V. (2003). Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Annals of Botany*, *91*(2), 179-194. https://doi.org/10.1093/aob/mcf118
7. Bor, J.Y., Chen, H.Y., & Yen, G.C. (2006). Evaluation of antioxidant activity and inhibitory effect on nitric oxide production of some common vegetables. *Journal of Agricultural and Food Chemistry*, *54*(5), 1680-1686. https://doi.org/10.1021/jf0527448
8. Brand-Williams, W., Cuvelier, M., & Berset, C. (1995). Antioxidative activity of phenolic composition of commercial extracts of sage and rosemary. *Lwt*, *28*, 25–30.
9. Carrión-Antolí, A., Martínez-Romero, D., Guillén, F., Zapata, P.J., Serrano, M., & Valero, D. (2022). Melatonin pre-harvest treatments leads to maintenance of sweet cherry quality during storage by increasing antioxidant systems. *Frontiers in Plant Science*, *13*, 863467. https://doi.org/10.3389/fpls.2022.863467
10. Dong, F., & Wang, X. (2018). Guar gum and ginseng extract coatings maintain the quality of sweet cherry. *Lwt*, *89*, 117-122. https://doi.org/10.1016/j.lwt.2017.10.035
11. Food and Agriculture Organization – FAO. (2020). International year of fruits and vegetables Retrieved from http://www.fao.org/documents/card/en/c/cb2395en/. Food and Drug Approvals-FD. 2020. https://www.fda.gov
12. Gad, M.M., & Zagzog, O.A. (2017). Mixing xanthan gum and chitosan nano particles to form new coating for maintaining storage life and quality of elmamoura guava fruits. *International Journal of Current Microbiology and Applied Sciences*, *6*(11), 1582-1591. https://doi.org/10.20546/ijcmas.2017.611.190

13. Golly, M.K., Ma, H., Sarpong, F., Dotse, B.P., Oteng-Darko, P., & Dong, Y. (2019). Shelf-life extension of grape (*Pinot noir*) by xanthan gum enriched with ascorbic and citric acid during cold temperature storage. *Journal of Food Science and Technology*, *56*, 4867-4878.
14. Guccione, E., Allegra, A., Farina, V., Inglese, P., & Sortino, G. (2023). Use of xanthan gum and calcium ascorbate to prolong cv. Butirra pear slices shelf life during storage. *Advances in Horticultural Science*, *37*(1), 59-66. https://doi.org/10.36253/ahsc-13872
15. Khatri, D., Panigrahi, J., Prajapati, A., & Bariya, H. (2020). Attributes of *Aloe vera* gel and chitosan treatments on the quality and biochemical traits of post-harvest tomatoes. *Scientia Horticulturae*, *259*, 108837. https://doi.org/10.1016/j.scienta.2019.108837
16. Kittur, F.S., Saroja, N., Habibunnisa, & Tharanathan, R. (2001). Polysaccharide-based composite coating formulations for shelf-life extension of fresh banana and mango. *European Food Research and Technology*, *213*, 306-311. https://doi.org/10.1007/s002170100363
17. Kumar, A., & Saini, C.S. (2021). Edible composite bi-layer coating based on whey protein isolate, xanthan gum and clove oil for prolonging shelf life of tomatoes. *Measurement: Food*, *2*, 100005. https://doi.org/10.1016/j.meafoo.2021.100005
18. Liu, J., Yue, R., Si, M., Wu, M., Cong, L., Zhai, R., Yang, C., Wang, Z., Ma, F., & Xu, L. (2019). Effects of exogenous application of melatonin on quality and sugar metabolism in 'Zaosu' pear fruit. *Journal of Plant Growth Regulation*, *38*, 1161-1169. https://doi.org/10.1007/s00344-019-09921-0
19. Lorente-Mento, J.M., Guillén, F., Castillo, S., Martínez-Romero, D., Valverde, J.M., Valero, D., & Serrano, M. (2021). Melatonin treatment to pomegranate trees enhances fruit bioactive compounds and quality traits at harvest and during postharvest storage. *Antioxidants*, *10*(6), 820. https://doi.org/10.3390/antiox10060820
20. Ma, Q., Lin, X., Wei, Q., Yang, X., Zhang, Y.N., & Chen, J. (2021). Melatonin treatment delays postharvest senescence and maintains the organoleptic quality of 'Newhall' navel orange (*Citrus sinensis* (L.) Osbeck) by inhibiting respiration and enhancing antioxidant capacity. *Scientia Horticulturae*, *286*, 110236. https://doi.org/10.1016/j.scienta.2021.110236
21. Medina-Santamarina, J., Zapata, P.J., Valverde, J.M., Valero, D., Serrano, M., & Guillén, F. (2021). Melatonin treatment of apricot trees leads to maintenance of fruit quality attributes during storage at chilling and non-chilling temperatures. *Agronomy*, *11*(5), 917. https://doi.org/10.3390/agronomy11050917
22. Omid, M., Khojastehnazhand, M., & Tabatabaeefar, A. (2010). Estimating volume and mass of citrus fruits by image processing technique. *Journal of Food Engineering*, *100*(2), 315-321. https://doi.org/10.1016/j.jfoodeng.2010.04.015
23. Ordonez, A.A.L., Gomez, J.D., & Vattuone, M.A. (2006). Antioxidant activities of Sechium edule (Jacq.) Swartz extracts. *Food Chemistry*, *97*(3), 452-458. https://doi.org/10.1016/j.foodchem.2005.05.024
24. Quoc, L., Hoa, D., Ngoc, H., & Phi, T. (2015). Effect of xanthan gum solution on the preservation of acerola (*Malpighia glabra* L.). *Cercetari Agronomice in Moldova*, *48*, 89–97. https://doi.org/10.1515/cerce-2015-0045
25. Rastegar, S., Aghaei Dargiri, S., & Mohammadi, M. (2024). Mitigating postharvest chilling injury in Orlando tangelo fruit: potential of melatonin and GABA in enhancing the antioxidant system. *Acta Physiologiae Plantarum*, *46*(3), 30. https://doi.org/10.1007/s11738-024-03653-9
26. Ruelas-Chacon, X., Contreras-Esquivel, J.C., Montañez, J., Aguilera-Carbo, A.F., Reyes-Vega, M.L., Peralta-Rodriguez, R.D., & Sanchéz-Brambila, G. (2017). Guar gum as an edible coating for enhancing shelf-life and improving postharvest quality of roma tomato (*Solanum lycopersicum* L.). *Journal of Food Quality*, *2017*. https://doi.org/10.1155/2017/8608304

27. Saini, R.K., Ranjit, A., Sharma, K., Prasad, P., Shang, X., Gowda, K.G.M., & Keum, Y.S. (2022). Bioactive compounds of citrus fruits: A review of composition and health benefits of carotenoids, flavonoids, limonoids, and terpenes. *Antioxidants*, *11*(2), 239. https://doi.org/10.3390/antiox11020239
28. Salehi, F. (2020). Edible coating of fruits and vegetables using natural gums: A review. *International Journal of Fruit Science*, *20*(sup2), S570-S589. https://doi.org/10.1080/15538362.2020.1746730
29. Shahid, M.N., & Abbasi, N.A. (2011). Effect of bee wax coatings on physiological changes in fruits of sweet orange CV. "blood red". *Sarhad Journal of Agriculture*, *27*(3), 385-394.
30. Shamloo, M.M., Sharifani, M., Daraei Garmakhany, A., & Seifi, E. (2015). Alternation of secondary metabolites and quality attributes in Valencia Orange fruit (*Citrus sinensis*) as influenced by storage period and edible covers. *Journal of Food Science and Technology*, *52*(4), 1936-1947. https://doi.org/10.1007%2Fs13197-013-1207-4
31. Shang, F., Liu, R., Wu, W., Han, Y., Fang, X., Chen, H., & Gao, H. (2021). Effects of melatonin on the components, quality and antioxidant activities of blueberry fruits. *Lwt*, *147*, 111582. https://doi.org/10.1016/j.lwt.2021.111582
32. Traore, M., Diarra, S., Sissoko, S., Diawara, M. O., Traore, M., Traore, B.M., Samake, M., & Sidibe, A. (2023). Determinism of some phytohormones and potassium nitrate on the vegetative growth of Tangelo (*Citrus x tangelo*) in the Sudano-Sahelian area of Mali. *GSC Biological and Pharmaceutical Sciences*, *23*(1), 160-167. https://doi.org/10.30574/gscbps.2023.23.1.0147
33. Tigist, M., Workneh, T.S., & Woldetsadik, K. (2013). Effects of variety on the quality of tomato stored under ambient conditions. *Journal of Food Science and Technology*, *50*, 477-486. https://doi.org/10.1007/s13197-011-0378-0
34. Tripathi, A.D., Sharma, R., Agarwal, A., & Haleem, D.R. (2021). Nanoemulsions based edible coatings with potential food applications. *International Journal of Biobased Plastics*, *3*(1), 112-125. https://doi.org/10.1080/24759651.2021.1875615
35. Uckoo, R.M., Jayaprakasha, G.K., & Patil, B.S. (2015). Phytochemical analysis of organic and conventionally cultivated Meyer limes (*Citrus meyeri* Tan.) during refrigerated storage. *Journal of Food Composition and Analysis*, *42*, 63-70. https://doi.org/10.1016/j.jfca.2015.01.009
36. Verde, A., Míguez, J. M., & Gallardo, M. (2022). Role of melatonin in apple fruit during growth and ripening: possible interaction with ethylene. *Plants*, *11*(5), 688. https://doi.org/10.3390/plants11050688
37. Vignesh, R.M., & Nair, B.R. (2019). Improvement of shelf life quality of tomatoes using a novel edible coating formulation. *Plant Science Today*, *6*(2), 84-90. https://doi.org/10.14719/pst.2019.6.2.443
38. Wang, L., Luo, Z., Yang, M., Li, D., Qi, M., Xu, Y., & Li, L.I. (2020). Role of exogenous melatonin in table grapes: First evidence on contribution to the phenolics-oriented response. *Food Chemistry*, *329*, 127155. https://doi.org/10.1016/j.foodchem.2020.127155
39. Wu, X., Ren, J., Huang, X., Zheng, X., Tian, Y., Shi, L. Dong, P., & Li, Z. (2021). Melatonin: Biosynthesis, content, and function in horticultural plants and potential application. *Scientia Horticulturae*, *288*, 110392. https://doi.org/10.1016/j.scienta.2021.110392
40. Xia, H., Shen, Y., Shen, T., Wang, X., Zhang, X., Hu, P., Liang, D., Lin, L., Deng, H., Wang, J., Deng, Q., & Lv, X. (2020). Melatonin accumulation in sweet cherry and its influence on fruit quality and antioxidant properties. *Molecules*, *25*(3), 753. https://doi.org/10.3390/molecules25030753
41. Xu, L.L., Yue, Q.Y., Bian, F.E., Zhai, H., & Yao, Y.X. (2017). Effects of melatonin treatment on grape berry ripening and contents of ethylene and ABA. *Acta Phytophysiology Sinica*, *53*, 2181-2188. https://doi.org/10.3389/fpls.2017.01426

42. Zhao, L., Yan, S., Wang, Y., Xu, G., & Zhao, D. (2023). Evaluation of the effect of preharvest melatonin spraying on fruit quality of 'Yuluxiang' pear based on principal component analysis. *Foods*, *12*(18), 3507. https://doi.org/10.3390/foods12183507

نشریه پژوهشهای علوم و صنایع غذایی ایران
https://ifstrj.um.ac.ir

مقاله پژوهشی
جلد ۲۰، شماره ۳، مرداد-شهریور ۱۴۰۳، ص. ۶۴-۴۹

حفظ کیفیت اورلاندو تانجلو طی انبار با کاربرد قبل از برداشت ملاتونین و پس از برداشت پوشش زانتان

سکینه ملائی محمدآبادی[۱]- سمیه رستگار[۲*]

تاریخ دریافت: ۱۴۰۳/۰۱/۲۱
تاریخ پذیرش: ۱۴۰۳/۰۳/۱۳

چکیده

این مطالعه با هدف تعیین تأثیر محلول‌پاشی قبل از برداشت ملاتونین و غوطه‌وری پس از برداشت صمغ زانتان بر کیفیت پس از برداشت میوه نارنگی Orlando tangelo انجام شد. پس از انتخاب درختان مناسب و یکنواخت، محلول‌پاشی ملاتونین در سه غلظت۰ میکرومولار ۱۰۰ میکرومولار و ۲۰۰ میکرومولار انجام شد. محلول‌پاشی یک ماه قبل از برداشت انجام شد و سه بار در فواصل یک هفته تکرار شد. علاوه بر این، میوه‌ها پس از برداشت در دو غلظت مختلف صمغ زانتان (۰/۱ و ۰/۲ درصد) غوطه‌ور شدند، میوه‌ها در انبار سرد در دمای ۱ ± ۵ درجه سانتی‌گراد نگهداری شدند. ارزیابی خصوصیات میوه در زمان برداشت و پس از ۴۵ و ۹۰ روز نگهداری در سردخانه انجام شد. نتایج نشان داد که محلول‌پاشی ملاتونین با غلظت ۱۰۰ میکرومولار بیشترین وزن میوه و گوشت را نشان داد. علاوه بر این، تیمار ملاتونین منجر به سطوح بالاتر اسید اسکوربیک و افزایش اسیدیته میوه نسبت به شاهد شد. در طول نگهداری، میوه‌های تیمار شده با پوشش ملاتونین و زانتان کیفیت بهتری نسبت به شاهد نشان دادند. در پایان آزمایش، کمترین کاهش وزن در میوه‌های تیمار شده با ۲۰۰ میکرومولار ملاتونین + ۰/۱ درصد زانتان مشاهده شد. بیشترین مقدار اسید اسکوربیک در ۱۰۰ میکرومولار ملاتونین + ۰/۱ درصد زانتان مشاهده شد. حداکثر فعالیت آنتی‌اکسیدانی در ۱۰۰ میکرومولار و ۲۰۰ میکرومولار + ۰/۱ درصد زانتان و همچنین ۱۰۰ میکرومولار ملاتونین به تنهایی مشاهده شد. به‌طور کلی، یافته‌ها نشان می‌دهند که محلول‌پاشی قبل از برداشت و استفاده از پوشش زانتان می‌تواند استراتژی‌های مؤثری برای حفظ کیفیت Orlando tangelo در طول انبارمانی سرد باشد.

واژه‌های کلیدی: اسپری، انبارمانی، صمغ زانتان، مرکبات، ملاتونین

۱ و ۲- به‌ترتیب دانشجوی کارشناسی ارشد و دانشیار، گروه علوم باغبانی، دانشکده کشاورزی و منابع طبیعی، دانشگاه هرمزگان، بندرعباس، ایران
(*- نویسنده مسئول: Email: rastegarhort@gmail.com)

https://doi.org/10.22067/ifstrj.2024.87553.1324

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

Research Article
Vol. 20, No. 3, Aug.-Sep. 2024, p. 65-79

Enhancement of Antioxidant Activity and Bioactive Compounds in Soy Whey Fermented with *Lactiplantibacillus plantarum* and *Weissella confusa*

Sh. Atashgahi[1], A. Moayedi[2*], A. Sadeghi Mahoonak[3], H. Shahiri Tabarestani[4], A.R. Sadeghi[2]

1, 2, 3 and 4- M.Sc. Graduate, Associate Professor, Professor and Assistant Professor, Department of Food Science and Technology, Faculty of Food Science, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran, respectively.
(*- Corresponding Author Email: amoayedi@gau.ac.ir)

Received:	13.04.2024	**How to cite this article:**
Revised:	22.06.2024	Atashgahi, Sh., Moayedi, A., Sadeghi Mahoonak, A., Shahiri Tabarestani, H., & Sadeghi, A.R. (2024). Enhancement of antioxidant activity and bioactive compounds in soy whey fermented with *Lactiplantibacillus plantarum* and *Weissella confusa*. *Iranian Food Science and Technology Research Journal*, *20*(4), 65-79. https://doi.org/10.22067/ifstrj.2024.87477.1325
Accepted:	22.06.2024	
Available Online:	22.06.2024	

Abstract

Soy whey (SW) is a byproduct from tofu and soy protein isolate (SPI) production that contains various nutrients such as protein, amino acids, minerals, carbohydrates, isoflavones. In this study, SW was fermented with lactic acid bacteria (LAB) with the aim to enhance total phenolic contents (TPC), Gamma amino butyric acid (GABA) and antioxidant activity. Eight different LAB strains were selected and the activity and cell counts of the most potent strains were investigated during fermentation. The results showed that all the isolates were able to grow in SW and the increase in incubation time led to significantly ($p<0.05$) decrease the pH of all samples from 5.75 to 4.5. Among eight LAB isolates, *Lactiplantibacillus plantarum* MCM4 and *Weissella confusa* MDM8 showed higher activity in terms of acid production, increase in TPC content and proteolytic activity. The sample fermented by *L. plantarum* MCM4 had the highest content of free amino acids (1.73 mg/ml) and the unfermented sample with 0.9 mg/ml had the lowest content. GABA concentration varied from 6.15 mg/mL (unfermented) to 24.175 mg/100 mL (SW fermented with *L. plantarum* MCM4). In this research, it was found that fermentation increased the antioxidant capacity of SW in such a way that the highest amount was observed in sample fermented with *Lactiplantibacillus plantarum* MCM4. A positive correlation (R^2= +0.72) was found between viable cell counts and proteolysis. It can be concluded that, fermentation with *L. plantarum* MCM4 and *W. confusa* MDM8 can be applied as an approach to valorize SW.

Keywords: Biorefinery, Fermentation, Gamma aminobutyric acid, Lactic acid bacteria

Introduction

Soy whey (SW) is a by-product from Tufo cheese and soy protein isolate (SPI) production that contains various nutrients such as proteins, amino acids, carbohydrates, isoflavones and Gamma-aminobutyric acid (GABA) (Belén *et al*., 2013). Nine kilograms of SW is produced from 1 kg soy used for Tufo production, and 20 tonnes of SW is produced per 1 ton of SPI. SW as a rich nutritional components can be used for the production of functional beverages, bioactive peptides, enzymes and biogas. Because of its high BOD and COD, SW disposal as a waste cause environmental problems (Wang & Ying, 2007). Therefore, it needs further treatments before disposal which

©2024 The author(s). This is an open access article distributed under Creative Commons Attribution 4.0 International License (CC BY 4.0).
https://doi.org/10.22067/ifstrj.2024.87477.1325

is an expensive operation. Because of the lack of appropriate technology and enough economic motivation for SW recycling, most of produced SW is disposed as the waste water that cause environmental challenges and water contamination (Candow, Burke, Smith-Palmer, & Burke, 2006). Regarding to the production of high amounts of SW from Tufo and SPI processing, it is vital to find a solution for its valorization and efficient management (Chua & Liu, 2019).

The use of agricultural waste and recycling the industrial by-products have gained much attention during the recent decades. In this regard, bio-refinery is referred to the bioconversion of agricultural and industrial wastes to the value-added products by using biological elements (Kumar *et al.*, 2022). Among the methods applied for the waste valorization, microbial fermentation has a unique place as it may result in the formation of health-promoting compounds. Microbial fermentation has been widely used for the valorization of cheese whey into fermented beverages. In the bio-refinery projects, lactic acid bacteria (LAB) are of great importance mainly because of their safety and adaptation to various ecosystems.

When grown in a nutrient media, (LAB) synthesize low-molecular weight compounds that contribute to the improvement of aroma and sensorial properties of the final product (König, Unden, & Fröhlich, 2009). Lactic fermentation can be applied for the valorization of SW to high value-added products, or recycling its nutrients. Recently, alcoholic beverages have been produced from SW using *saccharomyces* and non-*saccharomyces* yeasts (Chua, Lu, & Liu, 2017, 2018). In addition, a SW-based beverage has been developed using *Lactobacillus plantarum* B1-6 (Xiao *et al.*, 2015) and *Lactobacillus amylolyticus* L-6 (Fei *et al.*, 2017). The recent study by Tu *et al.* (2019) has shown that SW can be fermented to a functional Kombucha (Tu, Tang, Azi, Hu, & Dong, 2019). However, compared to the cheese whey, few studies have been done on SW fermentation. In the current study, the effects of proteolytic LAB fermentation on the antioxidant activity and bioactive compounds of SW have been investigated.

Materials and Methods

Materials and Microbial Cultures

SW used in this study was obtained from Donya factory (Golestan province, Iran). The proteolytic LAB used in this study (MDM8, MDM21, MCM4, BRM3, SRM2, ORT2, ORM4, ORM3) were previously isolated from pickled cabbage, sour dough and raw milk (Table 1) (Karimian, Moayedi, Khomeiri, Aalami, & Mahoonak, 2020; Khanlari, Moayedi, Ebrahimi, Khomeiri, & Sadeghi, 2021; Moayedi, Mahmoudi, Khomeiri, & Loghman, 2019). All the proteolytic LAB used in this study were kept as frozen cultures in the microbial bank.

Screening of Bacterial Isolates in Terms of Acidifying Activity in SW

For the determination of acidifying capacity of bacterial cultures, SW was sterilized at 108 °C for 15 min in an autoclave. Then it was inoculated (2%, v/v) with each bacterial culture (turbidity around 0.25 at 600 nm) and incubated at 37 °C. The pH value of incubated samples were measured at time intervals 0, 12, 24 and 48 h.

Viable Cell Counts during Fermentation

Viable cell counts of bacterial isolates added to the media was determined using pour plate method at 0, 12, 24 and 36 h of incubation as described by (Gül, Özçelik, Sağdıç, & Certel, 2005). Two serial dilution was used and the cell counts was determined according to the following formula (Moslemi, Moayedi, Khomeiri, & Maghsoudlou, 2023):

Table 1- LAB strains used in this study

Code	Similarity (%)	Name (NCBI)	Source	Reference
MCM4	98.4	*Lactiplantibacillus plantarum*	Pickled cabbage	Karimian *et al*. (2020)
MDM8	98.8	*Weissella confusa*	Sourdough	Khanlari *et al*. (2021)
MDM21	99.2	*Entrococcus faecium*	Sourdough	Khanlari *et al*. (2021)
BRM3	99	*Lactococcus lactis* subsp. *lactis*	Raw milk	Moayedi *et al*. (2019)
SRM2	99	*Lactobacillus reuteri*	Raw milk	Moayedi *et al*. (2019)
ORT2	98	*Lactobacillus delbrueckii*	Raw milk	Moayedi *et al*. (2019)
ORM3	97	*L. delbrueckii* subsp. *bulgaricus*	Raw milk	Moayedi *et al*. (2019)
ORM4	99	*Lactobacillus curvatus*	Raw milk	Moayedi *et al*. (2019)

$$N = \frac{\sum Ci}{V(n1 + 0.1n2)d}$$

Where $\sum Ci$ is total colony counted, V volume, n1 the cell counts from the first dilution, n2 the cell counts in the second dilution, and d the least dilution used for cell counts determination.

Determination of Free Amino Acid Content (Proteolytic Activity)

The method described by Church *et al.* (1983) was used for the determination of proteolytic activity. In this method, amino groups reacted with OPA that results in increase in absorbance at 340 nm. A standard curve was prepared using L-Serine as the standard amino acid (Church, Swaisgood, Porter, & Catignani, 1983).

Determination of Total Phenolic Compounds (TPC)

The TPC was determined by using Foline Ciacalteau method (Xiao *et al*., 2015). The results were expressed as mg of Gallic acid equivalents per ml (mg GAE/ml).

Selection of the Potent Isolates to Achieve the Maximum Bioactivity

After the screening, LAB with the highest capacity to increase TPC and proteolytic activity were selected to achieve the highest bioactivity of fermented SW. The type of bacterial culture and fermentation time were considered as the variables, while DPPH scavenging activity, TPC, GABA content, total antioxidant capacity and ferric reducing antioxidant potential (FRAP) were the responses.

Determination of GABA Concentration

GABA concentration was determined using high performance liquid chromatography equipped with UV detector according to the method of Karimian *et al*. (2020). The filtrate containing GABA was derivatized with phenylisothiocyanate (PITC) followed by detection at 254 nm (Karimian *et al*., 2020).

Determination of DPPH Scavenging Activity, FRAP and TAC

For the determination of DPPH scavenging activity, 650 µm of fermented sample was added to 1000 µm deionized water and 1000 µm DPPH solution (0.15 mM) and kept at a dark place (room temperature) for 20 min. Then the absorbance was read at 517 nm using UV-Visible spectrophotometer. Deionized water was used as the blank and DPPH scavenging activity was calculated according to following equation:

$$DPPH\ scavenging\ activity\ (\%) = (\mathrm{Ac} - \frac{\mathrm{As}}{\mathrm{Ac}}) \times 100$$

Where Ac and As were the absorbance for the sample and control (DPPH solution), respectively.

FRAP was evaluated similar to method described by (Yıldırım, Uğur, & Kutlu, 2017). TAC was determined according to the method of Meshginfar *et al*. 2018 with slight modification. For the preparation of TAC solution, 3.25 ml H_2SO_4 (0.6 M), 1.064 g Na_2SO_4 and 0.49 g ammonium molibdate were mixed and made up the volume to 125 ml. Aliqoute of 15 µl of each sample was added to 1 mL of TAC solution and incubated at 90 °C for 60 min. After cooling, the absorbance was read at 695 nm. Deionized water was used as the blank and ascorbic acid as the standard

(Meshginfar, Sadeghi Mahoonak, Hosseinian, Ghorbani, & Tsopmo, 2018).

Statistical Analysis

Statistical analysis was performed with factorial experiments (completely randomized design) using SAS software. The mean values were compared to each other using Duncan's multiple range test (95 % confidence interval).

Results and Discussion

Bacterial Culture Screening

The acidifying capacity of eight different LAB isolates (BRM3, MDM21, MCM4, SRM2, MDM8, ORM3, ORM4 and ORT2) in SW was investigated at different time intervals (0, 4, 12 and 24 h) of incubation (Fig. 1). All the examined isolates had the ability to grow in SW that resulted in reduction in pH from 5.75 to 4.5. The effects of incubation time and isolates were significant on pH changes ($P \leq 0.05$). Similar results have been reported on the growth ability of LAB strains in soy milk (Xu *et al*., 2019) and the media containing soy protein isolate (Yang, Ke, & Li, 2021) followed by pH reduction.

Total Phenolic Compounds (TPC)

The results of TPC change in SW after 24 h of fermentation are shown in Fig. 2. Fermentation caused significant changes in TPC of all fermented samples, and there was significant difference among various LAB tested.

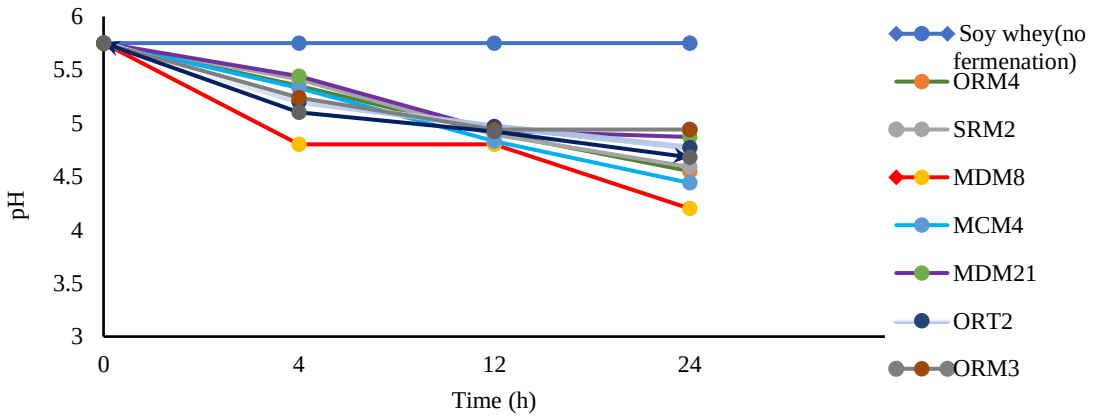


Fig. 1. pH levels in soy whey fermented by different LAB isolates

The lowest TPC content was found in unfermented SW (0.49 mg GAE/ml) and the highest TPC in the sample fermented with *Weissella confusa* MDM8 (1.27 mg GAE/ml). It has been reported that beta-glucosidase produced by LAB during fermentation is responsible for increase in TPC (Lee, Hung, & Chou, 2008). Moreover, some phenolic compounds in insoluble fibers may be released as affected by fermentation (Chandrasekara & Shahidi, 2012). Phenolic compounds have different biological activities such as antioxidant, anticancer, antibacterial, anti-atherosclerosis, and anti-carcinogenic effects (Chung, Seo, Ahn, & Kim, 2011).

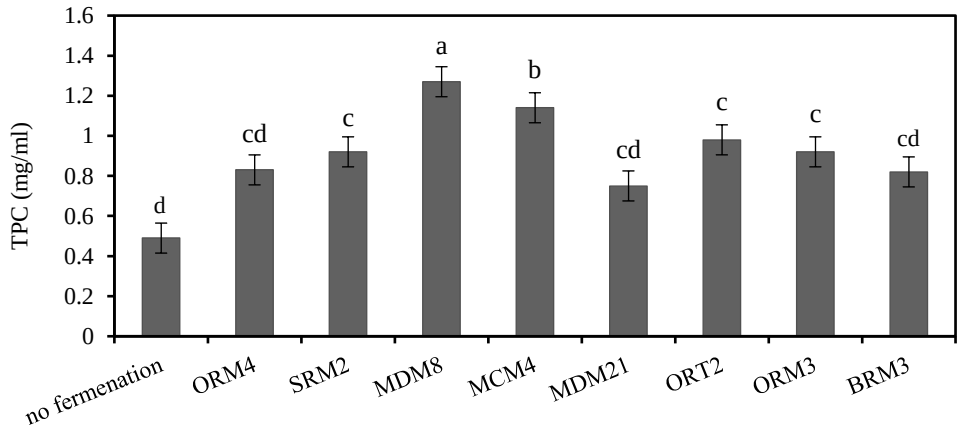


Fig. 2. Variations in the total phenolic content (TPC) of fermented soy whey (after 24 hours) by eight lactic acid bacteria isolates

Different letters on each column indicate significant differences between the samples ($p < 0.05$).

Free Amino Acid (Proteolysis)

As shown in Fig. 3, the type of inoculated LAB had significant effects on free amino acid content in fermented SW. SW fermented by *Lactiplantibacillus plantarum* MCM4 contained the highest FAA content (1.73 mg/ml), while unfermented SW contained the lowest content (0.9 mg/ml). It is clear that LAB tested in this study had the affinity to soy proteins which resulted in protein degradation into small peptides and free amino acids (Sharma, Garg, Kumar, Bhatia, & Kulshrestha, 2020). FAA content has been shown to be increased in parallel with increase in fermentation time (Baumann & Bisping, 1995; Bekiroglu *et al*., 2023). In addition, it has been reported that in tempe fermentation, bacteria with high proteolytic activity release amino acids five times higher than others, and such activity is affected by relative humidity and fermentation temperature (Baumann & Bisping, 1995). Peptides released during fermentation may have various functional activity such as antioxidant, antihypertensive, antibacterial, anticancer, anti-diabetic activities (Li & Wang, 2021).

According to the results obtained from screening the tested LAB in SW in terms of acidifying activity (Fig. 1), effects on TPC, and proteolysis, *L. plantarum* MCM4 and *W. confusa* MDM8 showed better activity and were selected for further investigations.

Effects of fermentation with selected strains on bioactivity and bioactive compounds of SW

Cell Counts

Changes in viable cell counts of *L. plantarum* MCM4 and *W. confusa* MDM8 during SW fermentation are shown in Fig. 4. Both tested LAB strains grew well in SW, and cell counts increased as fermentation time increased up to 24 h (Fig. 4-a), however it remained constant after 24 h. During soy fermentation, cell counts of LAB increased significantly ($p<0.05$) when fermentation time increased from 24 h to 36 h, and then it became constant (Zhang *et al*., 2014). In another study, all tested LAB isolates entered stationary phase after 12 to 18 h of soy milk fermentation (Undhad Trupti, Das, Solanki, Kinariwala, & Hati, 2021). In addition, Gan *et al*. (2017) stated that cell counts of *L. plantarum* increased markedly during 9 h of fermentation in soy milk (Gan, Shah, Wang, Lui, & Corke, 2017). In the initial stages of fermentation, an increase in bacterial cell counts is observed due to the presence of fermentable raw materials and desirable conditions. When fermentation time is extended, viable cell counts will decrease because of undesirable conditions such as oxygen reduction and enhanced acidity (Liu *et al*., 2021).

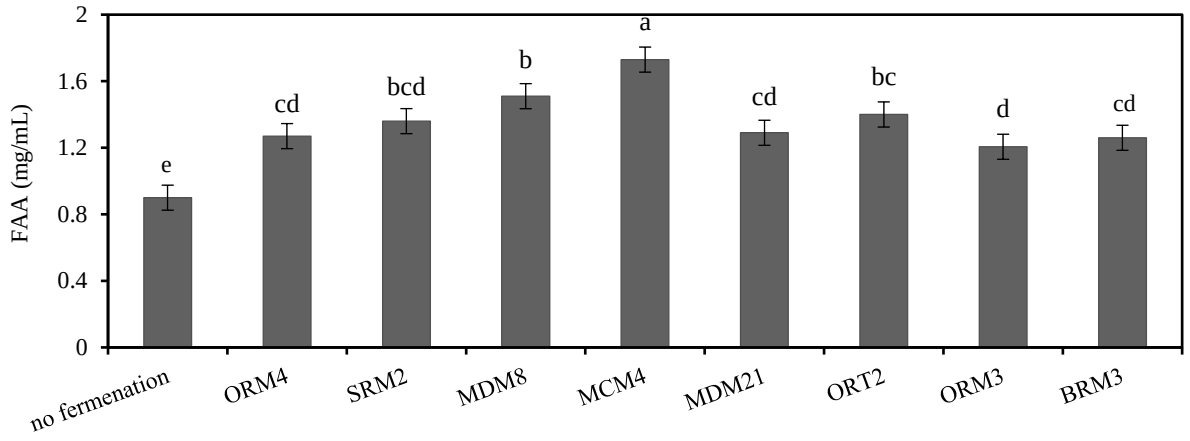


Fig. 3. The content of free amino group in soy whey fermented by 8 isolates of lactic acid bacteria during 24 hours of fermentation

Different letters on each column indicate significant differences between samples ($p<0.05$).

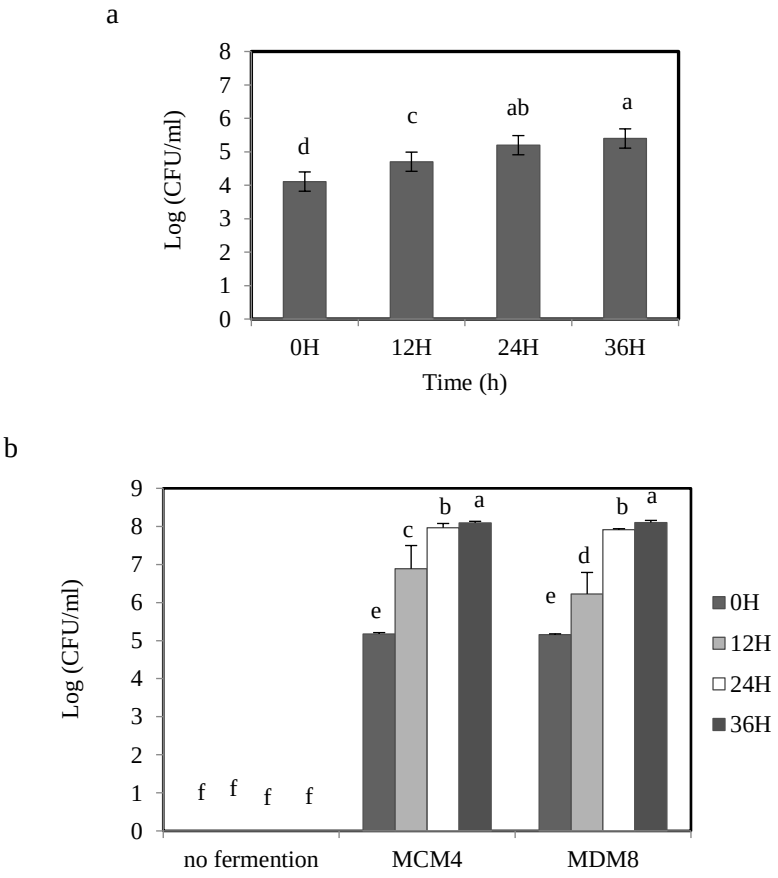


Fig. 4. Cell count in soy whey fermented by 2 isolates of *Lactiplantibacillus plantarum* MCM4 and *Weissella confusa* MDM8 (a) main effect of time and (b) interaction effect of isolate type and time on cell counts

Different letters on each column indicate significant differences between samples ($p<0.05$).

Effect of Fermentation on Free Amino Acid Content (Proteolysis)

Proteolytic activity of LAB has been widely studied because of its industrial importance and

essential role in bacteria (Lim, Foo, Loh, Mohamad, & Abdullah, 2019). In the current study, proteolytic activity of two LAB strains was investigated in SW during 36 h of incubation at 37 °C (Fig. 5). It was found that FAA content is affected by LAB strains, as the highest proteolysis was observed in the sample fermented by *W. confusa* MDM8 (0.9 mg/ml) and the lowest amount in unfermented sample (0.7 mg/ml). As can be seen in Fig. 5-a, fermentation longer than 24 h, did not increase proteolysis in the sample fermented with *L. plantarum* MCM4, while there was no significant difference between the samples fermented with *W. confusa* MDM8 after 12, 24 and 36 h of incubation. In the previous studies it has been reported that there was a positive correlation between fermentation time and FAA content (Baumann & Bisping, 1995; Bekiroglu *et al.*, 2023).

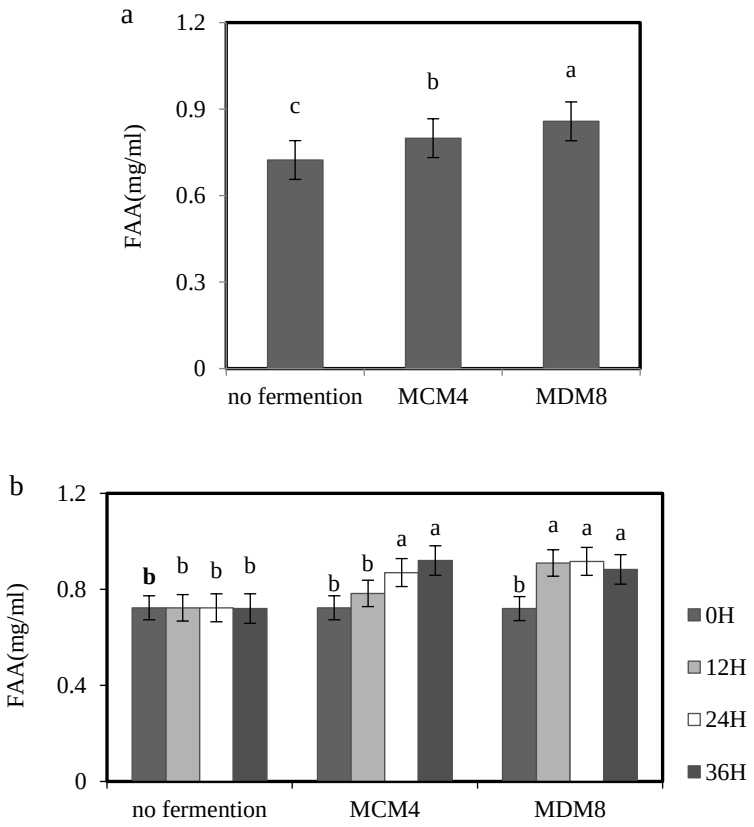


Fig. 5. Free amino acid (FAA) content in soy whey fermented with *L. plantarum* MCM4 and *W. confusa* MDM8. (a) The main effect of bacteria and (b) the interaction effect of bacteria and time on free amino group content
Different letters on each column indicate significant differences among the samples ($p<0.05$).

Effect of Fermentation on Total Phenolic Compounds (TPC)

The TPC of the samples is shown in Fig. 6. It is observed that the phenolic compound content significantly increases with the fermentation time ($p < 0.05$), as the highest TPC was observed in the sample fermented by *L. plantarum* MCM4 (1 mg GAE/ ml) and the lowest amount in unfermented sample (0.7 mg GAE/ ml). It has been reported that fermenting soy with various microorganisms, including *Lactiplanti-bacillus plantarum*, leads to an increase in phenolic compound content (Fernandez-Orozco *et al*., 2007). Additionally, an increase in phenolic and flavonoid content in soy flour fermented with *Lactobacillus casei* has been reported (Li *et al*., 2020).

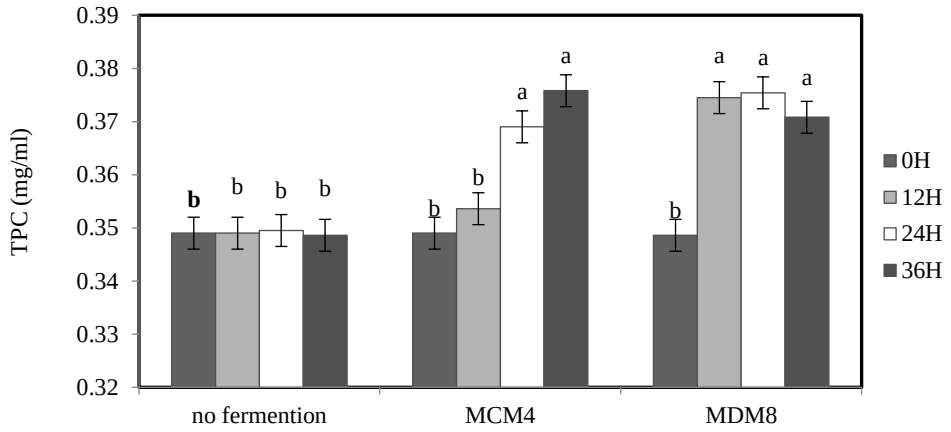


Fig. 6. The phenolic compound content in soy whey fermented with *L. plantarum* MCM4 and *W. confusa* MDM8 during fermentation

Different letters on each column indicate significant differences between the samples ($p < 0.05$).

Effect of Fermentation on GABA Content

GABA is a non-protein amino acid with a wide distribution in the nature that has been comprehensively studied because of its various physico-chemical functions and its positive effects on metabolic disorders (Pannerchelvan, Rios-Solis, *et al*., 2023). Among the organisms, LAB are one the most important GABA producers, and therefore the processes in which LAB strains are applied are highly considered (Pannerchelvan, Muhamad, *et al*., 2023). In this study, all samples contained GABA, and its concentration varied from 6.5 mg/ml (unfermented SW) to 24.18 mg/ml (SW fermented with *L. plantarum* MCM4) (Fig. 7). As shown in Fig. 7-a, there was significant differences between *L. plantarum* MCM4 and *W. confuse* MDM8 in terms of their ability to produce GABA. Moreover, it was found that fermentation time had significant effect on GABA content, as it was increased when fermentation time increased (Fig. 7-b). This can be attributed to increase in viable cell counts, and subsequently increase in bioconversion of glutamic acid to GABA (Moayedi, Zareie, Yaghoubi, & Khomeiri, 2022). GABA concentration in the fermented foods may be influenced by different factors such as pH, temperature, media composition (for example GABA precursors) and inoculation volume (Khanlari *et al.*, 2021). Aoki et al. (2013) reported that GABA content in fermented soy increased with increase in fermentation time (Aoki *et al*., 2003). Also, Han *et al*. (2020), showed that addition of 4% soy protein isolate to soy milk and then fermentation with *Streptococcus thermophillus* caused an increase in GABA content by 1.5 fold higher than the sample without SPI addition (Han, Liao, Wu, Gong, & Bai, 2020). Karimian *et al*. (2020) reported that inoculation of proteolytic LAB and addition of SPI to the cheese whey resulted in increase in GABA content in the fermented whey. Proteolytic activity of starter cultures not only increased the release of GABA precursors, but also reduce the fermentation time to reach to a desired pH (Karimian *et al*., 2020).

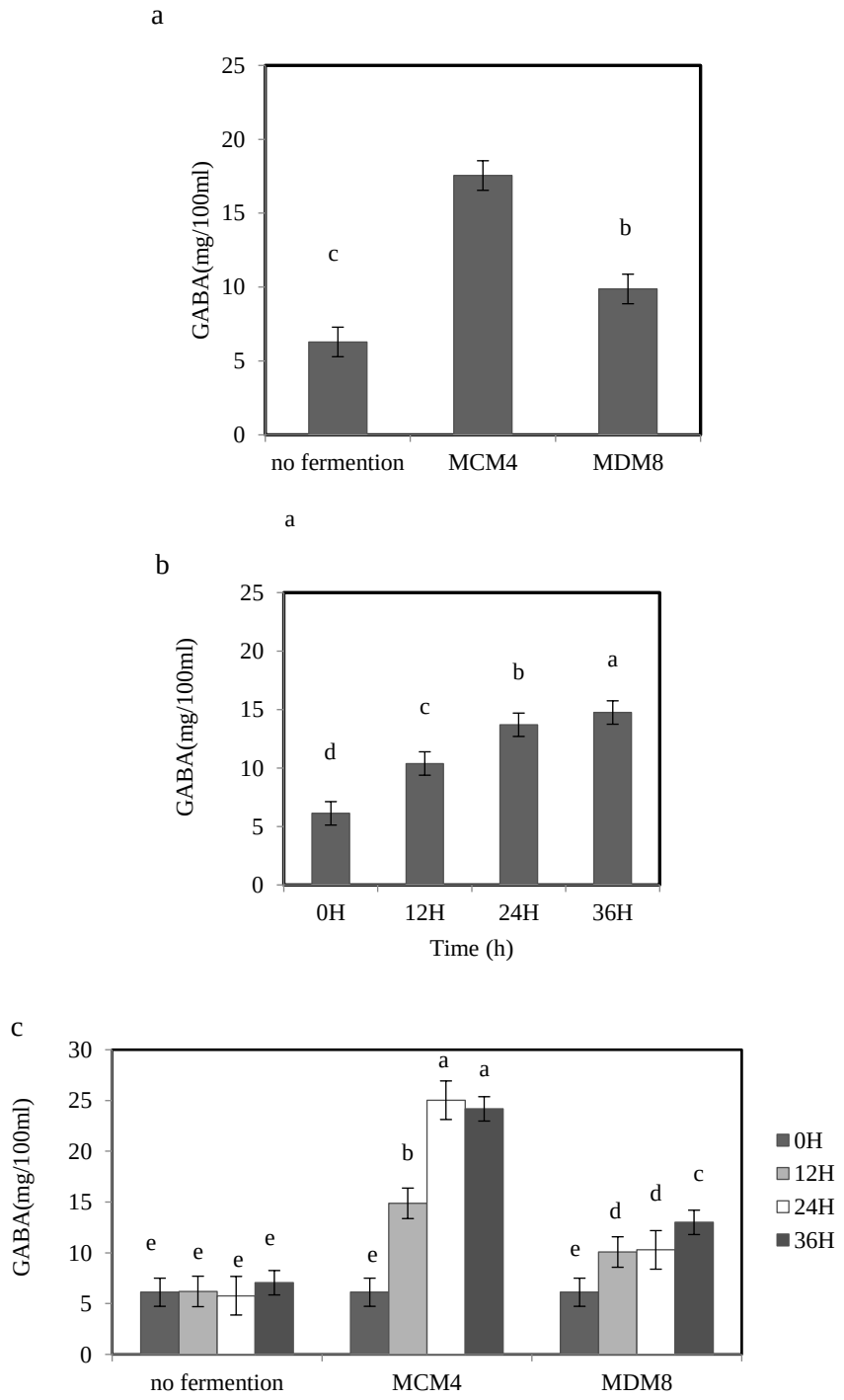


Fig. 7. The content of GABA in soy whey fermented with *L. plantarum* MCM4 and *W. confusa* MDM8 (a) main effect of bacteria, (b) main effect of time and (c) interaction effect of bacteria and time on GABA content
Different letters on each column indicate significant differences between samples ($p<0.05$).

Effect of Fermentation on Antioxidant Activity

Different antioxidant compounds may act against oxidizing agents through distinct mechanisms. Consequently, a single method cannot comprehensively evaluate the antioxidant capacity of complex matrices (Xiao *et al.*, 2015). Therefore, three antioxidant capacity assays with various approaches and mechanisms were employed to assess the antioxidant capacities of fermented soy whey,

and the results are presented in Fig. 8. Overall, significant differences were observed between the two bacterial species examined, and the ferric reducing power of the samples increased with fermentation time (Fig. 8-a). According to the results shown in this figure, the highest ferric reducing power at 36 hours was observed in *L. plantarum* MCM4 sample, while the lowest ferric reducing power was noted in the non-fermented sample.

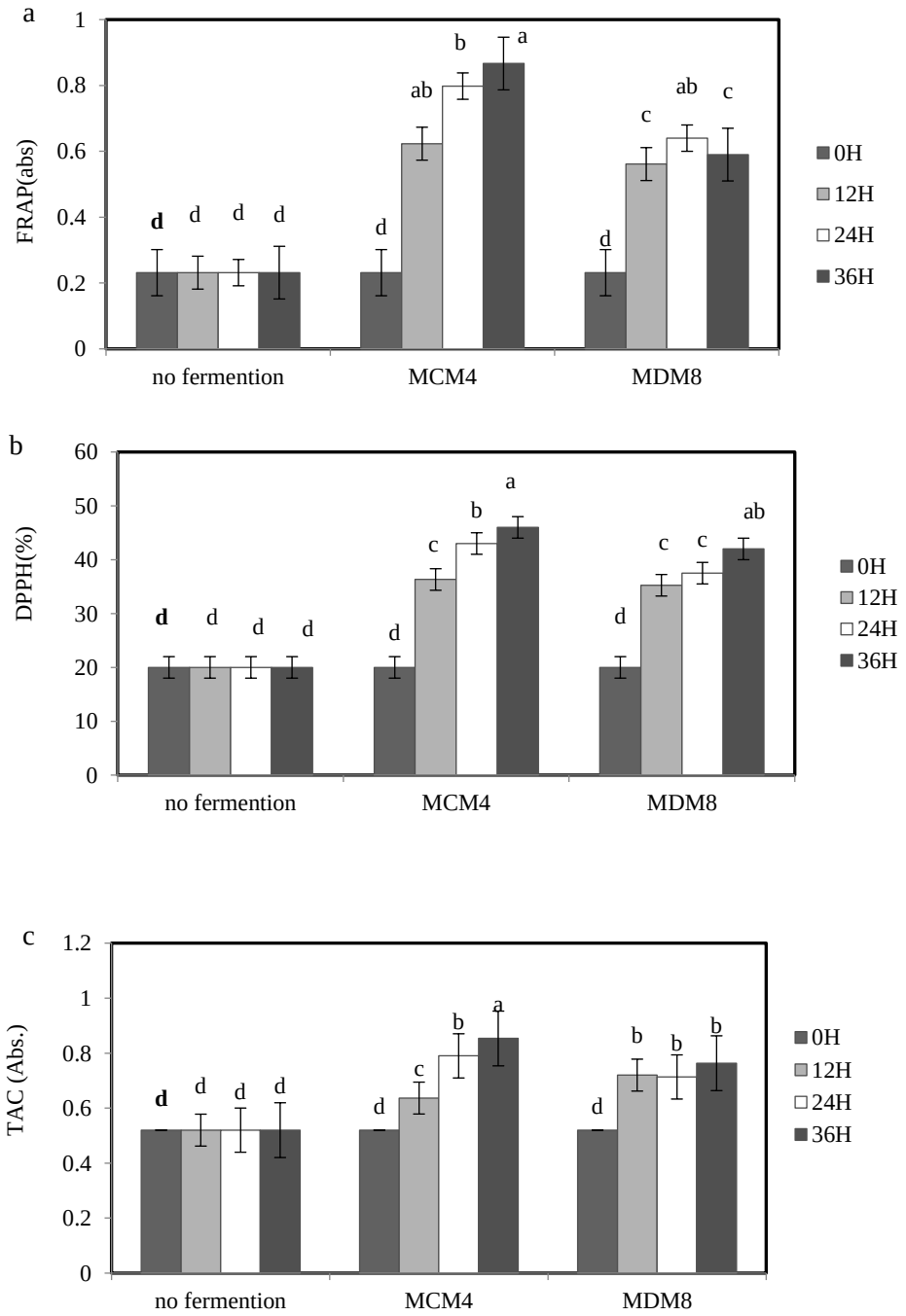


Fig. 8. Effect of fermentation on antioxidant activity in fermented soy whey: ferric reducing antioxidant power (FRAP) (A), DPPH radical scavenging activity (B), and total antioxidant activity (C)

Different letters on each column indicate significant differences between the samples ($p < 0.05$).

The results of the DPPH free radical scavenging assay are shown in Fig. 8-b. As viewed, the fermentation process significantly increased DPPH radical scavenging activity, influenced by both the bacterial species and the fermentation time. Extending the fermentation time up to 24 hours led to an increase in DPPH radical scavenging, while no significant change in scavenging activity was observed beyond 24 hours. Additionally, *L. plantarum* MCM4 demonstrated better performance compared to *W. confusa*.

Regarding the total antioxidant capacity, both the bacterial species and the fermentation time had a significant effect on the dependent variable ($p < 0.05$). Additionally, *L. plantarum* MCM4 exhibited better performance than *W. confusa* after 36 hours of fermentation (Fig. 8-c).

Overall, it was observed that fermentation positively affects total phenolic content and antioxidant activity. However, the degree of impact depends on the species of microorganisms used. It has been shown that lactic acid bacteria increased the aglycone isoflavone content in fermented soy whey due to high beta-glucosidase activity, and the released aglycone form can act as an antioxidant (Hur, Lee, Kim, Choi, & Kim, 2014). In the fermentation of soy whey by various lactic acid bacteria species, including *L. plantarum* and *S. thermophilus*, it has been reported that extending the fermentation time from 24 hours to 48 hours leads to increased reducing power and DPPH free radical scavenging activity in the fermented sample (Monajjemi, Aminin, Ilkhani, & Mollaamin, 2012). Xiao *et al*. (2015) reported that fermenting soy whey with *L. plantarum* results in an increased ferric reducing power. These researchers attributed the increased reducing power in the fermented sample compared to the control to the release of iron-chelating compounds and the production of phenolic compounds during fermentation (Xiao *et al*., 2015). Additionally, in another study, soy samples fermented using two different proteolytic *Bacillus subtilis* isolates, *B. subtilis* MTCC5480 and *B. subtilis* MTCC1747, showed increased DPPH radical scavenging activity and reducing power compared to non-fermented soy. This was attributed to the high level of protein hydrolysis, increased TPC, and free amino acid content during fermentation (Sanjukta, Rai, Muhammed, Jeyaram, & Talukdar, 2015).

Conclusion

This study aimed to enhance the amounts of bioactive compounds in SW using LAB. At first, the growth ability of eight LAB isolated from sourdough, raw milk, cabbage pickle and fermented olive in SW was investigated. From all examined isolates, *L. plantarum* MCM4 and *W. confuse* MDM8 displayed better performance in terms of acidifying capacity, and enhancing TPC and FAA content. The mentioned strains grew well in SW and when inoculated to SW caused an increase in FAA content, TPC, GABA content and antioxidant activity. Regarding to the potential of the mentioned LAB strains, and their growth ability in SW, they can be used for the development of soy-based fermented products. For the better understanding of the mechanism behind bioactivity of SW, and optimization of fermentation conditions, it would be useful to identify phenolic compounds and isoflavons released during fermentation.

Author Contributions

S. Atashgahi: Funding acquisition, investigation, writing-original draft; **A. Moayedi:** Project administration, supervision, conceptualization, writing–review and editing; **A. Sadeghi Mahoonak:** Data curation, methodology; **H. Shahiri Tabarestani:** Formal analysis, software, writing–review and editing; **A.R. Sadeghi:** Data curation, validation.

Funding Sources

This research was funded by Gorgan University of Agricultural Sciences and Natural Resources (Gorgan, Iran).

References

1. Aoki, H., Uda, I., Tagami, K., Furuya, Y., Endo, Y., & Fujimoto, K. (2003). The production of a new Tempeh-like fermented soybean containing a high level of & gamma;-aminobutyric acid by anaerobic incubation with *Rhizopus*. *Bioscience, Biotechnology, and Biochemistry, 67*(5), 1018-1023. https://doi.org/10.1271/bbb.67.1018
2. Baumann, U., & Bisping, B. (1995). Proteolysis during tempe fermentation. *Food Microbiology, 12*, 39-47. https://doi.org/10.1016/S0740-0020(95)80077-8
3. Bekiroglu, H., Karimidastjerd, A., Ozmen, D., Toker, O.S., Inan, M., Sagdic, O., & Dertli, E. (2023). Improvement of some techno-functional properties of aquafaba by pre-fermentation with *Lactobacillus plantarum* MA2. *Food Bioscience, 54*, 102807. https://doi.org/10.1016/j.fbio.2023.١٠٢٨٠٧
4. Belén, F., Benedetti, S., Sánchez, J., Hernández, E., Auleda, J.M., Prudêncio, E.S., & Raventós, M. (2013). Behavior of functional compounds during freeze concentration of tofu whey. *Journal of Food Engineering, 116*(3), 681-688. https://doi.org/10.1016/j.jfoodeng.2013.01.019
5. Candow, D.G., Burke, N.C., Smith-Palmer, T., & Burke, D.G. (2006). Effect of whey and soy protein supplementation combined with resistance training in young adults. *International Journal of Sport Nutrition and Exercise Metabolism, 16*(3), 233-244. https://doi.org/10.1123/ijsnem.16.3.233
6. Chandrasekara, A., & Shahidi, F. (2012). Bioaccessibility and antioxidant potential of millet grain phenolics as affected by simulated in vitro digestion and microbial fermentation. *Journal of Functional Foods, 4*(1), 226-237. https://doi.org/10.1016/j.jff.2011.11.001
7. Chua, J.-Y., & Liu, S.-Q. (2019). Soy whey: More than just wastewater from tofu and soy protein isolate industry. *Trends in Food Science & Technology, 91*, 24-32. https://doi.org/10.1016/j.tifs.2019.06.016
8. Chua, J.-Y., Lu, Y., & Liu, S.-Q. (2017). Biotransformation of soy whey into soy alcoholic beverage by four commercial strains of *Saccharomyces cerevisiae*. *International Journal of Food Microbiology, 262*, 14-22. https//:doi.org/10.1016/j.ijfoodmicro.2017.09.007
9. Chua, J.-Y., Lu, Y., & Liu, S.-Q. (2018). Evaluation of five commercial non-*Saccharomyces* yeasts in fermentation of soy (tofu) whey into an alcoholic beverage. *Food Microbiology, 76*, 533-542. https://doi.org/1/٠,١٠١٦j.fm.2018.07.016
10. Chung, I.-M., Seo, S.-H., Ahn, J.-K., & Kim, S.-H. (2011). Effect of processing, fermentation, and aging treatment to content and profile of phenolic compounds in soybean seed, soy curd and soy paste. *Food Chemistry, 127*(3), 960-96 .٧. https://doi.org/10.1016/j.foodchem.2011.01.065
11. Church, F.C., Swaisgood, H.E., Porter, D.H., & Catignani, G.L. (1983). Spectrophotometric assay using o-phthaldialdehyde for determination of proteolysis in milk and isolated milk proteins1. *Journal of Dairy Science, 66*(6), 1219-1227. https://doi.org/10.3168/jds.S0022-0302(83)81926-2
12. Fei, Y., Liu, L., Liu, D., Chen, L., Tan, B., Fu, L., & Li, L. (2017). Investigation on the safety of *Lactobacillus amylolyticus* L6 and its fermentation properties of tofu whey. *LWT, 84*, 314-322. https://doi.org/10.1016/j.lwt.2017.05.072
13. Fernandez-Orozco, R., Frias, J., Muñoz, R., Zielinski, H., Piskula, M.K., Kozlowska, H., & Vidal-Valverde, C. (2007). Fermentation as a bio-process to obtain functional soybean flours. *Journal of Agricultural and Food Chemistry, 55*(22), 8972-8979. https://doi.org/10.1021/jf071823b
14. Gan, R.Y., Shah, N.P., Wang, M.F., Lui, W.Y., & Corke, H. (2017). *Lactobacillus plantarum* WCFS1 fermentation differentially affects antioxidant capacity and polyphenol content in mung bean (*Vigna radiata*) and soya bean (*Glycine max*) milks. *Journal of Food processing and Preservation, 41*(1), e12944. https://doi.org/10.1111/jfpp.12944
15. Gül, H., Özçelik, S., Sağdıç, O., & Certel, M. (2005). Sourdough bread production with *Lactobacilli* and *S. cerevisiae* isolated from sourdoughs. *Process Biochemistry, 40*(2), 691-697. https://doi.org/10.1016/j.procbio.2004.01.044
16. Han, M., Liao, W.-y., Wu, S.-m., Gong, X., & Bai, C. (2020). Use of *Streptococcus thermophilus* for the in situ production of γ-aminobutyric acid-enriched fermented milk. *Journal of Dairy Science, 103*(1), 98-105. https://doi.org/10.3168/jds.2019-16856
17. Hur, S.J., Lee, S.Y., Kim, Y.-C., Choi, I., & Kim, G.-B. (2014). Effect of fermentation on the antioxidant activity in plant-based foods. *Food Chemistry, 160*, 346-356. https://doi.org/10.1016/j.foodchem.2014.03.112

18. Karimian, E., Moayedi, A., Khomeiri, M., Aalami, M., & Mahoonak, A.S. (2020). Application of high-GABA producing *Lactobacillus plantarum* isolated from traditional cabbage pickle in the production of functional fermented whey-based formulate. *Journal of Food Measurement and Characterization, 14*, 3408-3416. https://doi.org/10.1007/s11694-020-00587-x
19. Khanlari, Z., Moayedi, A., Ebrahimi, P., Khomeiri, M., & Sadeghi, A. (2021). Enhancement of γ-aminobutyric acid (GABA) content in fermented milk by using *Enterococcus faecium* and *Weissella confusa* isolated from sourdough. *Journal of Food Processing and Preservation, 45*(10), e15869. https://doi.org/10.1111/jfpp١٥٨٦٩
20. König, H., Unden, G., & Fröhlich, J. (2009). *Lactic acid bacteria: biology of microorganisms on grapes, in must and in wine*: Springer-Verlag Berlin Heidelberg.
21. Kumar, V., Sharma, N., Umesh, M., Selvaraj, M., Al-Shehri, B.M., Chakraborty, P., & Maitra, S.S. (2022). Emerging challenges for the agro-industrial food waste utilization: A review on food waste biorefinery. *Bioresource Technology, 362*, 127790. https://doi.org/10.1016/j.biortech.2022.127790
22. Lee, I.H., Hung, Y.-H., & Chou, C.-C. (2008). Solid-state fermentation with fungi to enhance the antioxidative activity, total phenolic and anthocyanin contents of black bean. *International Journal of Food Microbiology, 121*(2), 150-156. https://doi.org/10.1016/j.ijfoodmicro.2007.09.008
23. Li, S., Jin, Z., Hu, D., Yang, W., Yan, Y., Nie, X., Chen, X. (2020). Effect of solid-state fermentation with *Lactobacillus casei* on the nutritional value, isoflavones, phenolic acids and antioxidant activity of whole soybean flour. *LWT, 125*, 109264. https://doi.org/10.1016/j.lwt.2020.109264
24. Li, W., & Wang, T. (2021). Effect of solid-state fermentation with *Bacillus subtilis* lwo on the proteolysis and the antioxidative properties of chickpeas. *International Journal of Food Microbiology, 338*, 108988. https://doi.org/10.1016/j.ijfoodmicro.2020.108988
25. Lim, Y.H., Foo, H.L., Loh, T.C., Mohamad, R., & Abdullah, N. (2019). Comparative studies of versatile extracellular proteolytic activities of lactic acid bacteria and their potential for extracellular amino acid productions as feed supplements. *Journal of Animal Science and Biotechnology, 10*(1), 15. https://doi.org/10.1186/s40104-019-0323-z
26. Liu, X., Qian, M., Shen, Y., Qin, X., Huang, H., Yang, H., Bai, W. (2021). An high-throughput sequencing approach to the preliminary analysis of bacterial communities associated with changes in amino acid nitrogen, organic acid and reducing sugar contents during soy sauce fermentation. *Food Chemistry, 349*, 129131. https://doi.org/10.1016/j.foodchem.2021.129131
27. Meshginfar, N., Sadeghi Mahoonak, A., Hosseinian, F., Ghorbani, M., & Tsopmo, A. (2018). Production of antioxidant peptide fractions from a by-product of tomato processing: mass spectrometry identification of peptides and stability to gastrointestinal digestion. *Journal of Food Science and Technology, 55*(9), 3498-3507. https://doi.org/10.1007/s13197-018-3274-z
28. Moayedi, A., Mahmoudi, M., Khomeiri, M., & Loghman, S. (2019). Isolation, molecular identification and safety assessment of proteolytic lactic acid bacteria obtained from different raw milks. *Journal of Food Science and Technology (Iran), 16*(89), 59-69.
29. Moayedi, A., Zareie, Z., Yaghoubi, F., & Khomeiri, M. (2022). Optimization of gamma aminobutyric acid production from various protein hydrolysates by *Lactiplantibacillus plantarum* MCM4. *Applied Food Biotechnology, 9*(4), 287-296. https://doi.org/10.22037/afb.v9i4.38553
30. Monajjemi, M., Aminin, A., Ilkhani, A., & Mollaamin, F. (2012). Nano study of antioxidant activities of fermented soy whey prepared with lactic acid bacteria and kefir. *African Journal of Microbiology Research, 6*(2), 426-430. https://doi.org/10.5897/AJMR11.1249
31. Moslemi, M., Moayedi, A., Khomeiri, M., & Maghsoudlou, Y. (2023). Development of a whey-based beverage with enhanced levels of conjugated linoleic acid (CLA) as facilitated by endogenous walnut lipase. *Food Chemistry: X, 17*, 100547. https://doi.org/10.1016/j.fochx.2022.100547
32. Pannerchelvan, S., Muhamad, F. N., Wasoh, H., Mohamed, M.S., Wong, F.W.F., Mohamad, R., & Halim, M. (2023). Improvement of ɣ-aminobutyric acid production and cell viability of *Lactiplantibacillus plantarum* B7 via whole-cell immobilisation in repeated batch fermentation system. *Probiotics and Antimicrobial Proteins*. https://doi.org/10.1007/s12602-023-10200-4
33. Pannerchelvan, S., Rios-Solis, L., Faizal Wong, F.W., Zaidan, U.H., Wasoh, H., Mohamed, M.S., & Halim, M. (2023). Strategies for improvement of gamma-aminobutyric acid (GABA) biosynthesis via

lactic acid bacteria (LAB) fermentation. *Food & Function, 14*(9), 3929-3948. https://doi.org/10.1039/D2FO03936B
34. Sanjukta, S., Rai, A.K., Muhammed, A., Jeyaram, K., & Talukdar, N.C. (2015). Enhancement of antioxidant properties of two soybean varieties of Sikkim Himalayan region by proteolytic *Bacillus subtilis* fermentation. *Journal of Functional Foods, 14*, 650-6658. https://doi.org/10.1016/j.jff.2015.02.033
35. Sharma, R., Garg, P., Kumar, P., Bhatia, S.K., & Kulshrestha, S. (2020). Microbial fermentation and its role in quality improvement of fermented foods. *Fermentation, 6*(4). https://doi.org/10.3390/fermentation6040106
36. Tu, C., Tang, S., Azi, F., Hu, W., & Dong, M. (2019). Use of kombucha consortium to transform soy whey into a novel functional beverage. *Journal of Functional Foods, 52*, 81-89. https://doi.org/10.1016/j.jff.2018.10.024
37. Undhad Trupti, J., Das, S., Solanki, D., Kinariwala, D., & Hati, S. (2021). Bioactivities and ACE-inhibitory peptides releasing potential of lactic acid bacteria in fermented soy milk. *Food Production, Processing and Nutrition, 3*, 1-14. https://doi.org/10.1186/s43014-021-00056-y
38. Wang, Q., & Ying, T. (2007). Research progress on waste water utilization in soybean processing. *Food Science, 28*(9), 594-599.
39. Xiao, Y., Wang, L., Rui, X., Li, W., Chen, X., Jiang, M., & Dong, M. (2015). Enhancement of the antioxidant capacity of soy whey by fermentation with *Lactobacillus plantarum* B1–6. *Journal of Functional Foods, 12*, 33-44. https://doi.org/10.1016/j.jff.2014.10.033
40. Xu, Y., Ye, Q., Zhang, H., Yu, Y., Li, X., Zhang, Z., & Zhang, L. (2019). Naturally fermented acid slurry of soy whey: high-throughput sequencing-based characterization of microbial flora and mechanism of tofu coagulation. *Frontiers in Microbiology, 10*, 1088.
41. Yang, X., Ke, C., & Li, L. (2021). Physicochemical, rheological and digestive characteristics of soy protein isolate gel induced by lactic acid bacteria. *Journal of Food Engineering, 292*, 110243. https://doi.org/10.1016/j.jfoodeng.2020.110243
42. Yıldırım, I., Uğur, Y., & Kutlu, T. (2017). Investigation of antioxidant activity and phytochemical compositions of *Celtis tournefortii*. *Free Radicals and Antioxidants, 7*(2), 160-165.
43. Zhang, S., Shi, Y., Zhang, S., Shang, W., Gao, X., & Wang, H. (2014). Whole soybean as probiotic lactic acid bacteria carrier food in solid-state fermentation. *Food Control, 41*, 1-6. https://doi.org/10.10/١٦j.foodcont.2013.12.026

نشریه پژوهشهای علوم و صنایع غذایی ایران
https://ifstrj.um.ac.ir

مقاله پژوهشی

جلد ۲۰، شماره ۳، مرداد-شهریور ۱۴۰۳، ص. ۷۹-۶۵

افزایش فعالیت آنتی‌اکسیدانی و محتوای ترکیبات زیست‌فعال در آب‌پنیر سویا تخمیر شده با *Lactiplantibacillus plantarum* و *Weissella confusa*

شادی آتشگاهی[۱]- علی مؤیدی[۲*]- علیرضا صادقی ماهونک[۳]- هدی شهیری طبرستانی[۴]- علیرضا صادقی[۲]

تاریخ دریافت: ۱۴۰۳/۰۱/۲۵

تاریخ پذیرش: ۱۴۰۳/۰۴/۰۲

چکیده

آب‌پنیر سویا محصول جانبی فرآیند تولید پنیر توفو و ایزوله پروتئین سویا است که دارای مقدار قابل توجهی ترکیبات مغذی مانند پروتئین، آمینواسید، اولیگوساکارید و ایزوفلاون می‌باشد. در این پژوهش، تخمیر آب‌پنیر سویا با استفاده از باکتری‌های اسید لاکتیک با هدف افزایش محتوای ترکیبات فنلی و گاما-آمینو بوتیریک اسید و فعالیت آنتی‌اکسیدانی محصول تخمیر شده صورت گرفت. برای این منظور، ابتدا ۸ سویه لاکتیکی مختلف غربال‌گری شدند و در مرحله بعد فعالیت مؤثرترین سویه‌ها و شمارش سلولی آن‌ها در طول تخمیر بررسی شد. نتایج نشان داد تمامی جدایه‌ها قادر به رشد در آب‌پنیر سویا بودند و افزایش زمان گرمخانه‌گذاری باعث کاهش معنی‌دار pH تمام نمونه‌ها از ۵/۷۵ به ۴/۵ شد. از بین ۸ جدایه LAB، *Lactiplantibacillus plantarum* MCM4 و *Weissella confusa* MDM8 فعالیت بالاتری از نظر تولید اسید، افزایش محتوای TPC و فعالیت پروتئولیتیک نشان دادند. نمونه تخمیر شده توسط *L. plantarum* MCM4 بیشترین محتوای آمینو اسیدهای آزاد (۱/۷۳ میلی‌گرم در میلی‌لیتر) و نمونه تخمیر نشده با ۰/۹ میلی‌گرم در میلی‌لیتر کمترین مقدار را داشت. علاوه بر این، بیشترین میزان آمینو اسید آزاد پس از تخمیر ۳۶ و ۲۴ ساعت در نمونه تخمیر شده با *L. plantarum* MCM4 مشاهده شد. غلظت گابا از ۶/۱۵ میلی-گرم در ۱۰۰ میلی‌لیتر (تخمیر نشده) تا ۲۴/۱۷۵ میلی‌گرم در ۱۰۰ میلی-لیتر (SW تخمیر شده با *L. plantarum* MCM4) متغیر بود. همچنین همبستگی مثبتی بین شمارش سلولی و شدت پروتئولیز مشاهده شد. به‌طور کلی *L. plantarum* و *W. confusa* به خوبی در آب‌پنیر سویا رشد کردند و منجر به افزایش ترکیبات بالقوه زیست‌فعال در محصول نهایی شدند. بنابراین، تخمیر با *L. plantarum* MCM4 و *W. confusa* MDM8 می‌تواند به‌عنوان روشی برای ایجاد ارزش افزوده در آب‌پنیر سویا در نظر گرفته شود.

واژه‌های کلیدی: باکتری‌های لاکتیک اسید، تخمیر، زیست پالایش، گاما-آمینوبوتیریک اسید

۱، ۲، ۳ و ۴- به‌ترتیب دانش‌آموخته کارشناسی ارشد، دانشیار، استاد و استادیار گروه علوم و صنایع غذایی، دانشکده صنایع غذایی، دانشگاه علوم کشاورزی و منابع طبیعی گرگان، گرگان، ایران

(*- نویسنده مسئول: Email: amoayedi@gau.ac.ir)

https://doi.org/10.22067/ifstrj.2024.87477.1325

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

Review Article
Vol. 20, No. 3, Aug.-Sep. 2024, p. 81-103

Exploring the Potential of Cultured Meat: Technological Advancements, Sustainability Prospects, and Challenges

P. Ramezani[1]*, A. Motamedzadegan[2]*

1 and 2- M.Sc. Student and Full Professor of Department of Food Science and Technology, Sari Agricultural Sciences and Natural Resources University, Sari, Iran, respectively.
(*- Corresponding Author Email: p.ramezani@sanru.ac.ir, amotgan@yahoo.com)

Received:	28.04.2024	**How to cite this article:**
Revised:	16.06.2024	Ramezani, P., & Motamedzadegan, A. (2024). Exploring the potential of cultured meat: Technological advancements, sustainability prospects, and challenges. *Iranian Food Science and Technology Research Journal*, *20*(4), 81-103. https://doi.org/10.22067/ifstrj.2024.87796.1329
Accepted:	29.06.2024	
Available Online:	29.06.2024	

Abstract

The effects of traditional livestock farming on the environment and its limited scalability contribute to the persistent worldwide dilemma of food insecurity. Growing animal cells under regulated conditions has given rise to cultured meat, which might be a more ethical and ecological option. The potential of cultured meat to solve issues with food security is critically examined in this review article, which does so by thoroughly analyzing its effects on global food systems, sustainability prospects, technical breakthroughs, and related obstacles. Life cycle analyses show that the environmental impact of producing cultured meat is much lower than that of producing traditional meat. Significant scientific advancements have moved the production of cultured meat closer to commercial viability, including scaffold advances, tissue engineering, bioreactor design, and cell line optimization. There are still a number of formidable obstacles to overcome, including establishing large-scale manufacturing at a reasonable cost, negotiating intricate regulatory environments, guaranteeing product safety, and cultivating customer acceptability. To overcome these challenges and realize the promise of cultured meat to improve food and nutrition security while promoting environmental sustainability and animal welfare, an interdisciplinary strategy incorporating scientific, technical, regulatory, and social views is essential.

Keywords: Bioreactor design, Cultured meat, Food security, Environmental sustainability, Scaffolding

Introduction

A significant problem facing the world today is food insecurity since millions of people lack access to enough food that is safe and nourished. The conventional livestock production industry, which plays a vital role in the world's food systems, is confronted with many issues such as resource depletion, environmental degradation, and ethical concerns over animal care. Cultured meat has gained a lot of interest as a possible more ethical and sustainable meat substitute for conventional meat production. Cultured meat is produced by cultivating animal cells in carefully regulated lab settings to create products that resemble meat.

In 2013, the first cultured meat burger patty was developed, leading to the establishment of many firms dedicated to marketing cultured meat products. These enterprises are geographically dispersed and specialize in distinct meat products (Choudhury *et al*., 2020). Memphis Meats, now known as Upside Foods, is a pioneering firm that successfully created the world's first cultured meatball and chicken strip. Eat Just Company introduced the first

©2024 The author(s). This is an open access article distributed under Creative Commons Attribution 4.0 International License (CC BY 4.0).

https://doi.org/10.22067/ifstrj.2024.87796.1329

cultured chicken nuggets and obtained authorization to market cultured chicken meat in Singapore. In 2013, Mosa Meat, a company that emerged from research conducted at Maastricht University, successfully created the first-ever cultured beef burger. This groundbreaking achievement came at a significant expense of $330,000 (Stephens *et al*., 2018).

We critically evaluate cultured meat's ability to solve issues with food security in this review. We examine the implications of producing cultured meat for the world's food systems, sustainability, and related possibilities and problems. The means of an extensive assessment of the literature.

The World's Food Systems and Sustainability

A viable substitute for conventional cattle farming, cultured meat, also referred to as lab-grown or in vitro meat, has the ability to address a number of the environmental problems related to conventional meat production. Growing meat from animal cells in a controlled environment is the process of producing cultured meat, which has the potential to significantly lower greenhouse gas emissions as well as land and water usage.

Compared to traditional animal farming, cultured meat production drastically reduces water and land use by as much as 90% and 99%, respectively (Penn, 2018). According to Munteanu *et al*. (2021) cultured meat also can lessen greenhouse gas emissions, which are a significant problem since cattle production is primarily to blame. Cultured meat production, however, may use more energy than usual since technological processes are supplanting biological ones. Cultured meat can potentially reduce soil erosion and water pollution, two of the main environmental problems caused by cattle farming. Another advantage of cultured meat is that it may be produced in places where conventional cattle would not thrive.

According to a life cycle assessment (LCA) research, compared to traditional European meat production, producing 1000 kg of cultured meat uses a lot less land and water and produces a lot less greenhouse gas emissions. According to Tuomisto and Teixeira de Mattos (2011), cultured meat may specifically lead to 78–96% reductions in greenhouse gas emissions, 99% reductions in land usage, and 82–96% reductions in water use. Energy consumption, on the other hand, maybe comparable to or slightly lower, except chicken which has a lower energy use.

A multidisciplinary assessment of the research on cultured meat reveals that it has the potential to reduce pollution and the amount of agricultural area used for farming, both of which might be beneficial to human health. The assessment also notes that when certain biological activities are replaced by artificial processes, there is a possibility that the energy required for the creation of cultured meat might be more significant. To completely comprehend the sustainability and effectiveness of cultured meat production, further experimental research is needed (Munteanu *et al*., 2021).

Spirulina, a type of microalgae, is renowned for its substantial protein content, ranging from 46% to 63% of its dry weight. This protein concentration is comparable to meat and soybeans (Lupatini *et al*., 2017). Additionally, it has indispensable amino acids, rendering it a protein source with a high biological value. Spirulina is regarded as a sustainable protein source since it grows rapidly and utilizes resources efficiently. Compared to conventional protein sources, it necessitates a smaller amount of land and water (Manzocchi *et al*., 2020). Additionally, its cultivation can help reduce nitrogen waste, making it environmentally friendly (Mullenix *et al*., 2021).

Proteins obtained from yeasts and other microorganisms, known as single-cell proteins, are also rich in protein content. Yeast-based SCPs can yield a significant quantity of protein, however the exact proportions may differ depending on the specific microorganism employed. SCPs are produced using fermentation techniques that can effectively utilize agricultural and industrial by-products,

making them a viable and environmentally friendly choice. Vertical farming necessitates smaller amounts of land and water in contrast to conventional agriculture and can be cultivated in controlled surroundings, hence minimizing the influence on natural ecosystems. (Aragão *et al*., 2022).

Proteins derived from legumes, grains, and seeds, which are acquired from plants, exhibit varying protein content. Soybeans are a well-known source of plant-based protein, known for their high protein concentration and sometimes used as a benchmark for comparing other plant proteins. Plant-based proteins often have a lower environmental footprint compared to animal-based proteins. They possess a reduced need for water, land, energy and produce a smaller amount of greenhouse emissions. The uptake of plant-based proteins is driven by the imperative to develop more sustainable food systems (López-Martínez *et al*., 2022).

Mealworms (*Tenebrio molitor*) and lesser mealworms (*Alphitobius diaperinus*) have high protein content and a favorable amino acid profile, making them suitable for human and animal consumption (Kröncke & Benning, 2023; Roncolini *et al.*, 2020). Earthworms (*Eisenia fetida*) also offer high protein levels and a proper amino acid profile. Using worms as protein sources can reduce the environmental impact associated with traditional livestock feed, contributing to more sustainable production processes (Musyoka *et al*., 2019). Despite challenges related to biosafety, consumer acceptance, and market price, there is promising potential for large-scale manufacturing of this type of products. Snacks can be enhanced with lesser mealworm powder to significantly boost their protein and mineral content, while maintaining the enjoyable sensory characteristics of the snacks (Roncolini *et al*., 2020). Moreover, the hydrolysates derived from these worms can be employed as a growth factor in the production of cultured meat.

Comparative investigation has shown that Spirulina and cultured beef have the highest land use efficiency per unit of protein and calories, outperforming other protein sources. Cultured meat exhibits comparable energy consumption levels to conventional animal products, while showcasing lower greenhouse gas emissions. In contrast, crops demonstrate optimal energy utilization and minimal greenhouse gas emissions per unit of energy and protein. They can serve as feedstock for cultured meat production or as ingredients for plant-based meat. Additionally, crops supply essential nutrients and proteins for cellular growth and development (Newton & Blaustein-Rejto, 2021). Substituting animal products with cultured meat can improve food security and yield positive environmental results (Chriki & Hocquette, 2020; Tzachor *et al*., 2022).

Last but not least, the production of cattle contributes significantly to the utilization of land, water, and greenhouse gas emissions. As an alternative, cultured beef has the potential to mitigate several environmental impacts associated with animal production. It uses 99% less land, 90% less water, and 45% less energy (Penn, 2018).

Due to its novelty, obtaining regulatory permission for the production and sale of cultured meat is a crucial hurdle that must be tackled. The European Union has incorporated cultured beef into its Novel Foods Regulation, establishing a lawful framework for its future development and commercialization. The manufacturing of cultured meat in the United States is being regulated by both the FDA and USDA in collaboration. In 2020, Singapore achieved the distinction of becoming the first jurisdiction to provide regulatory approval for a cultured beef product. Nevertheless, there is currently a global absence of a comprehensive regulatory framework that encompasses all aspects, including safety review of media components, scaffolds, prospective use of gene editing techniques, as well as guidelines for assessing food safety concerns, toxicity, and correct labeling (Guan *et al*., 2021).

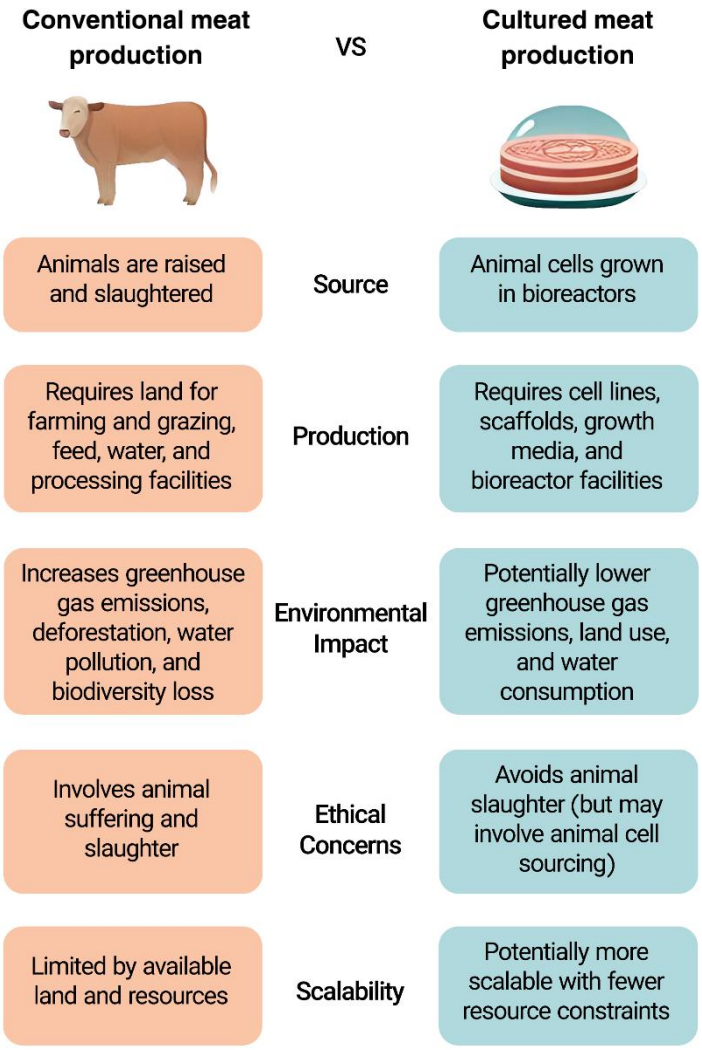


Fig. 1. Comparison between conventional and cultured meat production

In conclusion, producing meat using cultured means offers a practical way to lessen the environmental effect of meat consumption. It provides a significant reduction in land, water, and greenhouse gas use, all of which are essential for the sustainability of food production systems. However, in order to fully reap the advantages of cultured meat, further investigation is required, along with changes to regulations.

A comparison of the resource efficiency of traditional livestock production to cultured meat may be made by looking at metrics like feed conversion ratios and water footprint. The water footprint (WF) of animal products is a crucial resource usage indicator, with meat having a greater WF than milk or eggs, according to the literature. In particular, compared to other animals like sheep, goats, pigs, and chickens, beef has a far higher WF. The leading cause of this variance is the different feed conversion ratios between monogastric species like poultry and swine and ruminants like cattle, sheep, and goats, which have lower feed conversion ratios (Ibidhi & Ben Salem, 2020). Additionally, since more water is needed for feed and animal upkeep, the water footprint of livestock products is often more significant than that of plant-based diets (Mekonnen & Hoekstra, 2012).

The emergence of cultured meat signals a profound change in the way humans may grow and prepare meat in the future. This invention may help to resolve a number of moral issues related to conventional cattle production methods.

The way animals are treated in traditional livestock production is one of the main issues with animal welfare. Animals raised in traditional agricultural ways may be subjected to cruel handling techniques, cramped quarters, and painful methods of killing. These problems may be resolved by using cultured meat, which does away with the need of raising and killing animals for nourishment. Because cultured meat is made from animal cells in a lab, it has the potential to significantly minimize the animal suffering involved in the meat industry (Penn, 2018).

Furthermore, animal dignity is a factor in the ethical discussion surrounding cultured meat. Similar to vegetarianism, some claim that cultured meat might cause farm animals to become extinct, which could be seen as an insult to their dignity. The argument that created meat does not inherently diminish animal dignity any more than existing techniques does, however, cast doubt on this viewpoint. Alternatively, Chauvet (2018) suggests that it may be seen as a means of averting the agony and sacrificing of nonhuman creatures.

Culture, religion, health, and epidemiology concerns may all play a role in determining whether or not cultured meat is seen as an acceptable alternative to regular beef. As an example, Muslims hold the ceremonial slaughter of animals in high regard. Due to the steadfast nature of specific religious directives, the commercialization of cultured meat could not entirely eradicate current practices. A possible marketable alternative might be cultured beef that abides by Shariah rules (Hamdan *et al.*, 2021). Another example is that cultured meat, as opposed to traditional meat from killed animals, may lower the risk of transmissible spongiform encephalopathies (TSEs), such as mad cow disease (bovine spongiform encephalopathy, BSE). Cultured meat is made by cultivating cells of animals in a controlled lab setting, without using any parts of the animal's neurological system. The brain and other organs of afflicted animals' neurological systems are the primary sites of improperly folded prion proteins, which cause TSEs such as mad cow disease. Additionally, contamination from other sources is minimized in the controlled laboratory setting where cultured beef is produced (Schaefer & Savulescu, 2014).

Furthermore, since cultured meat production is not constrained by land availability or the biological limitations of animal reproduction, it can be scaled up more effectively than conventional livestock farming. This scaling potential may make it possible to more sustainably supply the rising demand for beef products worldwide. However, according to Stephens *et al.* (2018), the development of cultured meat permits the possibility of modifying the nutritional makeup, texture, and taste of meat products.

Although the manufacturing of cultured meat exhibits promises for environmental sustainability, several obstacles need to be overcome to fulfill its potential fully: (1) The energy source used has a significant influence on the environmental effects of producing cultured meat. When compared to fossil fuel-based energy sources, renewable energy sources like solar, wind, or hydroelectric electricity would dramatically lower the carbon footprint (Smetana *et al.*, 2015), (2) Reducing environmental effects and reaching sustainability objectives depend on obtaining economies of scale and increasing the effectiveness of cultured meat production procedures (Mattick *et al.*, 2015), (3) For cultured meat products to be widely adopted and sustainably produced, suitable regulatory frameworks must be developed and public concerns about their acceptability and safety must be addressed (Bryant & Barnett, 2018; Stephens *et al.*, 2018). (4) To reduce environmental effects and increase sustainability, ongoing research and

optimization of the whole life cycle of cultured beef production, from cell line generation to bioreactor design and waste management, are required (Mattick *et al*., 2015).

Cultured meat production has the potential to greatly aid in the attainment of sustainability objectives and the reduction of the environmental effects linked to traditional livestock production systems by effectively tackling the obstacles and capitalizing on the available prospects.

Technological Advancements in the Production of Cultured Meat

Cell Line Development

An essential part of producing cultured meat is the creation and refinement of cell lines. For this reason, stem cells from a variety of origins have been investigated:

Animal-derived stem cells, such as muscle satellite cells or embryonic stem cells from cattle, have been used in early studies on the generation of cultured meat (Post, 2012). These cells may divide and specialize into numerous types of muscle fibers. The use of iPSCs, somatic cells that have been reprogrammed to a pluripotent state, as a result of advancements in stem cell technology also presents a viable and moral alternative for the scalable and ethical creation of cultured meat, as opposed to using stem cells sourced from animals (Lee *et al*., 2023). The use of immortalized cell lines, which can proliferate continuously and be kept in culture for long periods, has also been investigated by other researchers (Wang *et al*., 2024). These cell lines may provide a reliable and scalable source for the creation of cultured meat.

The utilization of pluripotent cells in cultured meat entails the conversion of these cells into distinct muscle and adipose cells that are necessary for meat generation. This procedure is essential for the development of sustainable and practical techniques to manufacture cultured meat, which has the potential to overcome the limits of conventional meat production.

Porcine induced pluripotent stem cells (piPSCs) can be effectively transformed into skeletal muscle cells by employing a combination of a GSK3B inhibitor (glycogen synthase kinase-3β) and a DNA methylation inhibitor (5-aza-cytidine), followed by the activation of MYOD1. Within a span of 11 days, this technique leads to the development of myotubes that possess the functional attributes of muscle cells (Genovese *et al*., 2017).

Stem cells, such as progenitor stem cells derived from muscle tissues, mesenchymal stem cells, and induced pluripotent stem cells (iPSCs), are very suitable for producing muscle cells for cultured meat. These cells possess the ability to renew themselves and differentiate into numerous cell types, making them well-suited for extensive growth in a laboratory setting while keeping their stem cell characteristics. Stem cells show great potential for the production of lab-grown meat, but they encounter difficulties associated with the cultivation process, including the need to retain a large number of cells while ensuring their excellent quality. Methods to address these constraints involve improving the environment in which the culture takes place and utilizing specialized inhibitors and activators to direct the process of differentiation (Ozhava *et al*., 2022).

Pluripotent stem cells, specifically piPSCs, can be efficiently transformed into muscle cells by the use of specific inhibitors and activators. These cells, in addition to other types of stem cells, possess substantial potential for the generation of grown meat. Nevertheless, the obstacles in preserving the quality and quantity of cells during in vitro culturing must be resolved by the use of optimum procedures.

For dependable and effective cultured meat production, cell lines must be stable and behave consistently. To evaluate the stability and usefulness of cell lines, characterization approaches such as metabolic profiling, karyotyping, and gene expression analysis are used (Lee *et al*., 2023)

One of the main obstacles in the development of cultured meat is optimizing the

proliferation and differentiation of stem cells into muscle fibers. Numerous methods and approaches have been investigated. In order to facilitate efficient cell proliferation and differentiation, researchers have concentrated on creating specialized culture media formulations. In addition, sophisticated bioreactor systems have been developed to offer controlled environments for cell growth, nutrient delivery, and waste removal, allowing for scalable production (Edelman *et al*., 2005). To imitate the texture and organoleptic qualities of conventional meat, researchers have looked into using three-dimensional scaffolds and tissue engineering techniques to direct the organization and structure of cultured muscle fibers (Ben-Arye & Levenberg, 2019; Stephens *et al*., 2018).

It is essential to understand that cultured meat currently has distinct organoleptic features compared to regular meat. Due to its lack of postmortem changes, it has different sensory and nutritional qualities than regular meat. The texture of uncooked cultured meat can be challenging to achieve and may need co-cultivation of various cell types and electrical or mechanical stimulation. However, processed meat products may need ingredients as additives to improve texture. Without myoglobin, chemical colorants may be required to produce the right red. Postmortem metabolism lacks crucial flavor precursors, hence artificial flavors like plant-based meat replacements are used. Without appropriate supplementation, meat may lack vitamins, minerals, fatty acids, and bioactive compounds, lowering consumer satisfaction. Without adding exogenous chemicals, cultured meat cannot match the sensory experience and nutritional content of regular meat (Fraeye *et al*., 2020).

Ong *et al*. (2023) have identified genetic drift in the cell lines used for cultured meat production as a potential food safety hazard. During prolonged culture periods, cells have the potential to a mass genetic mutations and experience phenotypic alterations as a result of several stimuli such as physical pressures, biochemical exposures, excessive cell division, or contamination events like mycoplasma infection. It is crucial to monitor the stability of cell lines and analyze any changes in gene expression or metabolite profiles to guarantee safety and maintain product consistency. In addition, regulators have expressed concerns about the potential risk of tumor formation from consuming immortalized or continuously reproducing cell lines. Expert panels have determined that the likelihood of immortalized cells surviving digestion and developing tumors is exceedingly low according to existing scientific knowledge. However, the authors suggest further studies to confirm these assumptions experimentally. It may be necessary to communicate the risks carefully in order to address any remaining consumer beliefs that connect uncontrolled cell growth to concerns about cancer. Identifying primary research goals includes creating reliable techniques to identify and manage genetic drift, as well as defining safe thresholds for the maximum number of times, cells can be passed.

The discovery, characterization, and optimization of cell lines via ongoing research and technical breakthroughs are essential for enhancing the commercial viability, scalability, and efficiency of cultured meat production.

Bioreactor Design

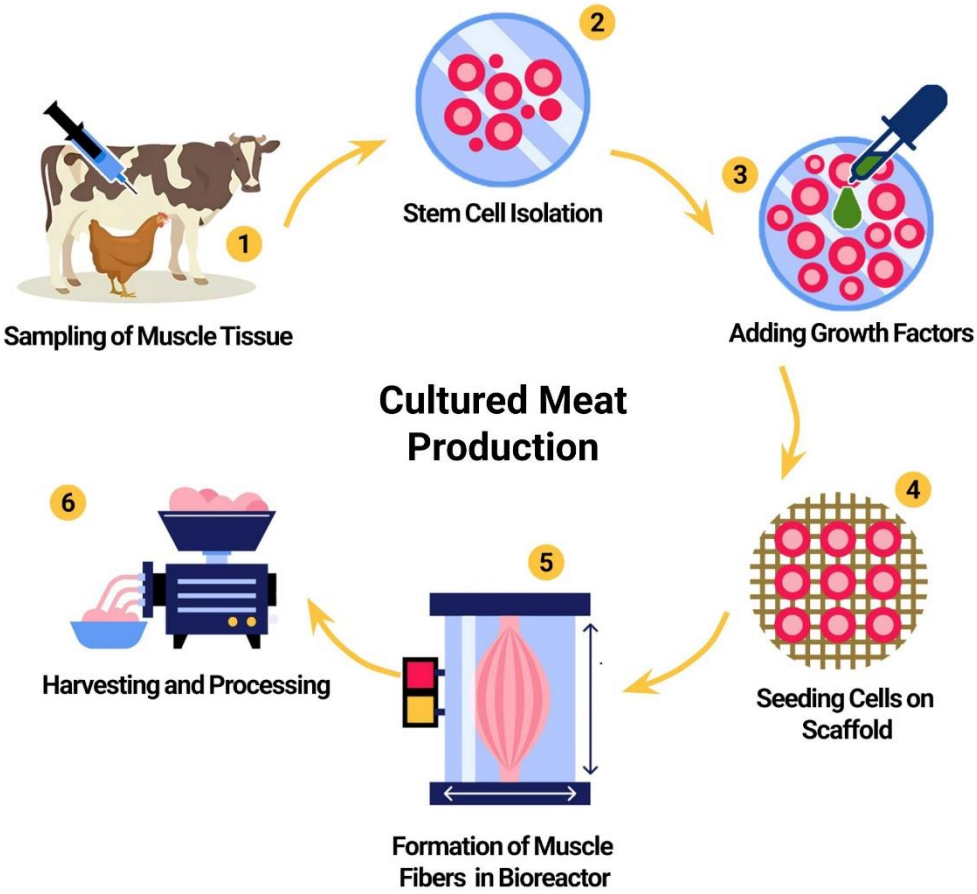


Fig. 2. A schematic showing the steps used to make cultured meat

The progress of cultured meat production heavily depends on developing scalable bioreactor systems. Animal stem cells are multiplied and differentiated in bioreactors to make cultured meat, which offers a sustainable substitute for conventional livestock production.

Bioreactors provide a regulated and adequate substitute for animal husbandry by improving efficiency and scalability in cell treatment and the production of cultured meat (Ge *et al*., 2023).

The potential of stirred tank bioreactors (STRs) for large-scale cultured meat production has been assessed. Larger reactors may lower the cost of goods sold (COGS), according to research that analyzed facilities with various STR sizes. A ~211,000 L STR, for example, might reduce the COGS to $25/kg, but a ~42,000 L STR had a base case COGS of $35/kg. Moreover, a ~262,000 L airlift reactor (ALR) would lower the COGS to $17/kg, suggesting that more expansive and unconventional bioreactor designs might be more economical (Negulescu *et al*., 2023)

In the context of cell expansion, whereby bovine adipose-derived stem cells (bASCs) were grown on microcarriers in spinner flasks, the scalability of bioreactors is also investigated. The results of the research showed that an 80% medium exchange in conjunction with decreased cell seeding densities led to a 28-fold growth without affecting the cells' capacity to differentiate into distinct lineages (Hanga *et al*., 2020). This shows that using microcarrier-based methods to scale up cell culture to produce cultured meat may be a feasible alternative.

Microcarriers are matrices that provide support for adherent cells in bioreactor systems. They have a high surface area-to-volume ratio, which allows for efficient cell growth and expansion. This feature also makes cell manufacturing more cost-effective (Chen *et al*., 2020). Within the realm of cultured meat production, they have a vital function in expanding muscle cell culture, perhaps acting as a temporary surface for cell growth and as a consumable material integrated into the end result (Bodiou *et al*., 2020).

In contrast to conventional fermentation procedures, the design of expansion bioreactors for producing cultured meat presents particular difficulties. A review highlights how crucial it is to take into account essential elements and basic cell biology characteristics when creating a procedure that is both economically competitive and practical. It emphasizes how vital details that are essential to the process' success are often overlooked in the design of cultured meat bioreactors (Allan *et al*., 2019).

The possibility for effective large-scale production of cultured beef using alternatives to typical bioreactor technologies, such as microcarrier cultures in suspension or packed bed bioreactors, is highlighted. It is expected that these systems' optimization would result in resource- and money-efficient manufacturing techniques (Moritz *et al*., 2015).

Perfusion bioreactors, like hollow fiber bioreactors (HFBs), are designed to create cultured meat with perfectly aligned, densely packed muscle fibers at the centimeter scale. The HFB method makes use of semipermeable hollow fibers to evenly distribute nutrients and oxygen, two essential elements for tissue growth and development. The texture and taste of conventional beef may be almost perfectly replicated with this technique (Nie *et al*., 2023).

Even with these developments, there are still issues and constraints to be resolved. Significant barriers include the high cost of cell culture medium, the difficulty of scaling up bioreactors, and the need to preserve cell quality throughout expansion (Allan *et al*., 2019; Hanga *et al*., 2020; Negulescu *et al*., 2023). In addition, the adaption of bioreactor technologies for industrial-sized cell culture is driven by the requirement for automation, strict management of production conditions, and increased productivity potential.

There are still obstacles to be solved even if scalable bioreactor technologies like STRs, microcarrier-based systems, and HFBs can support large-scale cell and tissue growth for the production of cultured meat. These include media costs, scaling up technological obstacles, and making sure the bioprocess design is both economical and efficient. In order to optimize these systems for the commercial-scale production of cultured meat, further investigation and development are required.

For the purpose of producing cultured meat, it is crucial to optimize the growth conditions in bioreactors to maximize cell proliferation, differentiation, and tissue creation.

The design of the bioreactor and the way mass transfer and fluid flow interact are crucial for establishing a consistent environment for tissue development. Specific geometries, like the radial-flow-type bioreactor, provide a more consistent environment for parenchymal cells to develop and differentiate ex vivo, according to the research performed on several bioreactor designs. This is because areas with slow-flowing conditions that are unfavorable to uniform cell proliferation may result from the lack of barriers parallel to the flow routes (Peng & Palsson, 2000).

The practical and scalable production of cultured meat is another use for bioreactors. They provide the oxygen and nutrients and the regulated environments required for cell division, maturation, and proliferation. The assessment of bioreactor technologies in cell treatment and the production of cultured meat emphasizes the significance of bioreactor types and their uses, highlighting the need for further study to go beyond present constraints and difficulties (Ge *et al*., 2023).

The last topic discussed is the growth of embryonic stem cells (ESCs) in bioreactors, emphasizing the effects of metabolic stress brought on by ineffective feeding. Perfusion

cultures were shown to be able to sustain metabolite concentrations below hazardous limits, leading to an intense proliferation of high-quality 'naive' ESCs in research that used a perfusion bioreactor and a mathematical model. According to Yeo *et al*. (2013), this work emphasizes how crucial it is to regulate cellular metabolism in order to preserve pluripotency and enhance ESC bioprocesses.

An encouraging substitute for conventional cattle farming is the production of cultured meat in bioreactors, which has the potential to both lessen environmental effects and meet the world's expanding food need. However, the capacity to sustain ideal growth circumstances is what will determine this technology's success, and that means putting in place efficient monitoring and control mechanisms.

Controlled process conditions may considerably limit variability in product output and quality, which is especially important for the production of cultured meat, as shown by bioreactors developed for plant cell and tissue cultures (Eibl & Eibl, 2008). Similar to this, micro-bioreactors equipped with microfluidic devices and integrated online monitoring have shown the capacity to monitor biomass and regulate pH, improving the results of fermentation (Buchenauer *et al*., 2009). The application of these ideas to cultured meat bioreactors may guarantee a consistent and repeatable production process.

Sensor monitoring is crucial for preserving the most crucial parameters in the context of cultured meat production (Djisalov *et al*., 2021). To cultivate adherent cells in closed bioreactors, innovative process control systems that integrate monitoring and control technologies for ideal environmental conditions have been created (Das *et al*., 2014). This method may be modified to improve quality and repeatability in cultured meat bioreactors.

Last but not least, the use of a single-use pneumatic bioreactor system for mammalian cells highlights the significance of reducing the creation of nutritional gradients and hydrodynamic shear, while allowing real-time monitoring and modification of culture conditions (Obom *et al*., 2014). The uniform proliferation of meat cells might be ensured by using this technique in the manufacturing of cultured meat.

To sum up, the precise control of growth conditions, which is necessary to ensure the quality, safety, and scalability of cultured meat products, is made possible by the integration of advanced sensors, monitoring devices, and automated control systems, all of which are essential for the efficient operation of bioreactors in the production of cultured meat.

Growth Factors

The utilization of growth factors (GFs), which are necessary for cell proliferation and differentiation in culture conditions, is a key part of cultured meat production. The unique composition of fetal bovine serum (FBS) and the difficulty in replicating its effects with serum-free media has made its replacement in cultured meat production challenging. FBS contains a complex mixture of proteins, growth factors, and other nutrients essential for cell growth (Lee *et al*., 2022). It is crucial to optimize the content of FBS or an alternative in the media for cultured meat production, since it directly affects cell development. Higher concentrations of FBS promote increased cell proliferation (Ikasari *et al*., 2022).

The utilization of fetal bovine serum (FBS) throughout the manufacturing procedure gives rise to ethical apprehensions and possible health hazards, specifically the transfer of zoonotic diseases. Fetal bovine serum (FBS) is obtained by performing a cardiac puncture on bovine fetuses, without the use of anesthetic. This procedure has the potential to cause agony and anguish to the fetuses, rendering the technique cruel (Jochems *et al*., 2002).

Bovine viral diarrhea virus (BVDV) is a prominent pathogen in the cattle industry, recognized for its ability to induce many reproductive and developmental complications in afflicted animals. Recent research has brought attention to the possibility of fetal bovine serum (FBS) being contaminated with BVDV, which has raised concerns regarding its

impact on the health of both animals and humans. A recent investigation examined commercially accessible FBS samples gathered from 2017 to 2021 to assess the presence of BVDV contamination. The results were concerning, since 82.9% of the samples tested positive for pestivirus-specific RT-PCR, and a considerable number exhibited seropositivity for BVDV1 and BVDV2 (Nakamura *et al*., 2022). The significant level of contamination emphasizes the necessity for rigorous quality control protocols in the manufacturing of FBS to minimize the hazards linked to BVDV.

Although BVDV mainly affects cattle, its existence in FBS utilized in many biotechnological and pharmacological applications, including its role as a growth factor in cultured meat production, poses significant zoonotic risks. Theoretically, if FBS is contaminated, it could transfer BVDV into cell cultures and biological products, which could potentially endanger human health.

Protein hydrolysates as a cost-efficient substitute for fetal bovine serum in cultured meat media shows great potential. Taheri *et al*. (2011) demonstrated that protein hydrolysates obtained from fish waste have an appropriate amino acid composition. These hydrolysates can be used as a nitrogen source in fish diets and also as functional additives in the food industry. Thus, protein hydrolysates could serve as a cost-effective alternative to replace fetal bovine serum in the manufacturing of cultured meat.

The research conducted by Hamzeh *et al*. (2018) investigated the bioactive properties of protein hydrolysates derived from the mantle of cuttlefish (*Sepia pharaonis*), with a specific emphasis on their antioxidant and antiproliferative activities. They found that cuttlefish protein hydrolysates with degrees of hydrolysis (DH) of 20%, 30%, and 40% exhibited the highest levels of DPPH radical scavenging activity, reducing power, and overall antioxidant capacity. The observed values in the cuttlefish mantle protein isolate were markedly inferior compared to these values. Moreover, the protein hydrolysate with a degree of hydrolysis (DH) of 20% had the most pronounced inhibitory impact on the proliferation of MDA-231 and T47D cancer cell lines. The predominant amino acids in the cuttlefish protein hydrolysates were glutamine, constituting 15.7% of the total, and asparagine, comprising 10.9%. The findings suggest that protein hydrolysates derived from marine sources, particularly cuttlefish can serve as functional constituents in the production medium of cultured meat. This utilization would enhance the stability of antioxidants and promote cellular proliferation, thereby diminishing the requirement for mammalian serum or growth factors. Mirzakhani *et al*. (2018) investigated the apparent protein digestibility (APD) and degree of protein hydrolysis (DPH) of several feed ingredients for Siberian sturgeon (*Acipenser baeri*) in both a live animal context and a laboratory setting, respectively. A strong correlation was found between the length of action potential in living organisms and the use of enzyme extracts taken from the digestive system of the fish to study diphenyl hydrazine in a laboratory setting. The study demonstrates that the *in vitro* DPH method, which employs species-specific enzymes, can be a valuable tool for assessing protein digestibility in feed materials. This method can determine the suitability of various protein hydrolysates and growth factors derived from different sources for incorporation into the production media of cultured meat. It takes into account the particular requirements of the cell lines being cultivated.

According to Ahmad *et al*. (2023), growth factors, including FGF-2, IGF-1, PDGF, and TGF-β1, as well as hormones like insulin and testosterone, are vital for the proliferation and differentiation of MSCs, which are essential for the generation of cultured meat. Research conducted by Yu *et al*. (2023) found that muscle satellite cell proliferation was enhanced in commercial serum-free medium containing high concentrations of FGF2. This finding highlights the significance of FGF2 and its receptor FGFR1 in advancing effective cell-cultivated meat production. Stout *et al*. (2024) found that engineering muscle satellite cells to

make their own FGF2 via autocrine signaling is a feasible technique to reduce the cost of cultured meat production by eliminating the requirement for this expensive growth factor in the medium. Epidermal growth factor (EGF) may be helpful to cultured meat production medium because it improves the cleavage and development rates of cow embryos *in vitro* when added to media (Prasad *et al*., 2018).

Lugworms, which are frequently encountered in marine habitats, offer a unique and encouraging reservoir of protein hydrolysates that can be utilized to facilitate the production of cultured meat. In a recent study conducted by Batish *et al*. (2022), the researchers investigated the ability of lugworm protein hydrolysates to decrease or substitute fetal bovine serum (FBS) in cell culture conditions used for fish cell lines. Surprisingly, lugworm hydrolysates at low concentrations of 0.001-0.1 mg/mL achieved a significant 90% decrease in FBS levels. This reduction was achieved without compromising the proliferation, survival, and morphology of zebrafish embryonic stem cells, which remained comparable to those under conventional conditions with 10% serum. The hydrolysates derived from lugworms demonstrated significant yields (30.05%) and productivity (100.16 mg/mL), indicating their feasibility for large-scale production. Furthermore, lactate dehydrogenase experiments verified that these hydrolysates did not damage the integrity of the cell membrane. Lugworm protein hydrolysates are a viable option for creating cost-effective and sustainable media formulations for cultured aquatic meat products. They have the ability to support serum-free or low-serum cell culture conditions, making them an attractive contender for this purpose.

Ashizawa *et al*. (2022) proposed a method to reduce the costs of cultured meat production by using insect cell lines. This requires including growth factors obtained from insects in the culture media. The cost of the culture media significantly rises when standard mammalian cell culture employs expensive recombinant growth agents like FGF-2 and TGF-β. To assess the potential cost reduction of generating meat from insect cells, the scientists conducted a simulation using IDGF-2, a growth factor present in *Drosophila* species that promotes the development of imaginal discs. Although the exact cost is still uncertain, the simulation indicates that including IDGF-2 into the mixture might potentially reduce the cost to $7.78 per kilogram. This highlights the possibility of using insect-derived macromolecules as more affordable alternatives to expensive mammalian growth factors in cultured meat production systems.

In addition to this discovery, research conducted by Kim *et al*. (2023) examined the possibility of using edible hydrolysates obtained from fermented soybean meals and edible insects (mealworm and cricket) as substitutes for fetal bovine serum (FBS) in the growth of pig muscle stem cells. The hydrolysates exhibited antioxidant activity and created an appropriate cell culture environment, maintaining the medium pH within an acceptable range. Cell proliferation was enhanced by supplementing the medium containing 10% FBS with hydrolysates (0.01-5% FAB-H (Fermented soybean meal with *Aspergillus oryzae* and *Bacillus subtilis* hydrolysate) and FB-H (Fermented soybean meal with *Bacillus licheniformis* hydrolysate), 0.01-1% TM-H (*Tenebrio molitor* larvae hydrolysate), or 0.01-0.1% GB-H (*Gryllus bimaculatus* imago hydrolysate)). Significantly, concentrations of 0.01% and 0.1% of FAB-H, FB-H, and TM-H demonstrated the ability to substitute for up to 50% of FBS while preserving the ability to proliferate and differentiate. Occasionally, the presence of 0.1% FB-H and TM-H in 50% FBS-reduced medium resulted in even greater differentiation than 10% FBS media. Although more research is required to understand the long-term effects fully, this study indicates that substituting a portion of fetal bovine serum (FBS) with three edible and affordable natural substances (FAB-H, FB-H, and TM-H) might

substantially decrease the expenses associated with producing cultured meat.

Scaffolding Technology

In order to produce cultured meat that tastes, feels, and is nutritionally similar to regular meat, biomaterials and scaffold design play a critical part in the process. To create cultured meat, cells must grow, proliferate, and differentiate into muscle tissue. Scaffolds provide these processes the support they need.

Collagen and gelatin, mainly derived from animals, are the predominant components used in scaffolds for cultured meat research. Gelatin is a biopolymer protein that can form a gel and is used for its functional properties. Tabarestani *et al*. (2010) conducted a study that demonstrated the efficient extraction of gelatin from rainbow trout skin and confirmed its desirable physico-chemical properties. The extracted gelatin had a favorable molecular weight distribution, characterized by a high ratio of α1/α2 chains and a significant number of β chains. Additionally, it displayed exceptional gel strength, viscosity, and melting point. In the context of cultured meat production, fish-derived gelatin, namely from rainbow trout skin, is an ideal biomaterial. This is because it has the capacity to form a gel and has molecular properties that make it suitable for providing structural support for muscle cell adhesion, proliferation, and differentiation.

Moreover, the utilization of scaffold biomaterials derived from fish waste has potential in aligning cultured meat production with the objectives of animal welfare and sustainability. In their study, Shaviklo *et al*., (2016) investigated the use of protein derived from tuna red flesh as a substitute raw material in the production of silver carp fish burgers. The researchers discovered that adding 20% tuna protein isolate to minced silver carp enhanced the sensory characteristics and overall approval of the product. The results indicated that proteins derived from discarded fish parts have the potential to be used as alternative ingredients for building the structure of cultured fish meat. This could lead to enhanced sustainability and quality of the end product.

Furthermore, the characteristics of biomaterials may be enhanced for the manufacturing of cultured meat by the process of crosslinking. Crosslinking methods play a crucial role in the creation of scaffolds for cultured meat and tissue engineering. These techniques are essential for providing the required support for cell survival, proliferation, and differentiation. The mechanical characteristics of alginate hydrogels can be improved and muscle cell development can be supported by dual-crosslinking employing visible light and covalent bonding. This suggests that these hydrogels have the potential to be used as scaffolds for cultured meat (Tahir & Floreani, 2022). The combination of physical gelation and chemical crosslinking in gelatin methacryloyl (GelMA) hydrogels leads to a variety of mechanical characteristics, which have an impact on cellular behavior and enable accurate photopatterning of structures containing cells (Young *et al*., 2020). The process of radiation crosslinking gelatin scaffolds provides excellent transparency and effective crosslinking, which helps maintain cell adhesion motifs and amino acid content. This is advantageous for tissue engineering (Kimura *et al*., 2021).

However, there is rising interest in plant-derived biomaterials for scaffolding to better fit with the objectives of animal welfare and environmental conservation. Better tissue formation, differentiation, and cell proliferation are possible with these materials (Seah *et al*., 2022).

The difficulties in designing scaffolds for generating cultured meat are distinct from those encountered in biomedical tissue engineering. Critical factors include the size and expense of the manufacturing process as well as the characteristics of the finished product, such as food safety and texture. For a cultured meat product to be successful, the scaffold has to mimic the characteristics of vertebrate skeletal muscle. Future research is focused on scaffolds that enable high-quality meat development

while reducing production costs. The farmed meat business is seeing promising advances in scaffolding technology at this time (Bomkamp *et al*., 2022).

For the development of cultured meat, combining biomaterials with food biopolymers is another strategy under consideration. The goal of this integration is to solve limitations related to scalability, sustainability, and edibility. Ng and Kurisawa (2020) conducted a study of existing biomaterial methodologies for the engineering of muscle and adipose tissue, highlighting the need for solutions that address these new limitations.

Biomaterials for decellularized scaffolds are a highly biocompatible and biodegradable substitute for synthetic scaffolds. Research on the use of decellularized scaffolds produced from plants and animals in the production of cultured meat is ongoing, and it has the potential to have a major impact on cellular agriculture and future food applications (Lu *et al*., 2022).

The development of biomaterials and technologies that facilitate the organization and culture of muscle stem cells in a way that emulates the normal tissue structure of animals has dominated recent advancements in the engineering of three-dimensional scaffolds. This is essential to produce cultured meat that tastes and feels like real animal flesh (Wang *et al*., 2023).

It has been shown that textured soy protein works well as a scaffold to create three-dimensional skeletal muscle tissue in cows. This biomaterial is edible and rich in nutrients, which promotes cell adhesion and proliferation to produce a meat-like product with desirable sensory qualities (Ben-Arye *et al*., 2020).

To sum up, the effective development of cultured meat depends on the design and material composition of the scaffolds. With a significant emphasis on sustainability, scalability, and the capacity to mimic the taste and nutritional attributes of traditional meat, innovations in this sector are developing quickly.

The production of cultured meat with the texture and organoleptic qualities of real meat is a difficult task that calls for a variety of methods and strategies. In order to achieve the necessary texture features and sensory properties that meet customer expectations, scaffold design plays a critical role.

Technological advancements in scaffolding are necessary to overcome the particular challenges associated with producing grown meat, including scale, affordability, and quality aspects, including texture and food safety (Bomkamp *et al*., 2022). Promising scaffold materials and methods that may be used for cultivated meat development are revealed by a study of recent advancements in scaffolding within the cultivated meat sector. These include a range of tissue engineering techniques, including cell sheet engineering, molding, bioprinting, textured scaffolds, and 3D bioprinting (Wang *et al*., 2022). The fact that the materials used in these tactics must be appropriate for food production and consumption makes them another vital factor to consider.

Tissue engineering methods, which were first performed for biomedical applications, provide new ways to modify the characteristics of meat when it comes to cultured meat production. The architecture of the scaffold, for example, may be precisely controlled by 3D bioprinting and can be tailored to resemble the fibrous structure of muscle tissue, which will affect the final product's texture (Wang *et al*., 2022). Textured scaffolds may be designed to mimic the mouthfeel and chewiness of regular meat while still providing the required mechanical support.

To sum up, in order to replicate the texture and organoleptic qualities of traditional meat, scaffold design plays a crucial role in the manufacturing of cultured meat. Achieving the desired textural and sensory attributes may be facilitated by using suitable materials and sophisticated tissue engineering techniques. It is advised that further study be done in this area to develop scaffolds that can assist the

development of premium meat while lowering manufacturing costs.

Challenges and Prospects for Future Development

There are many issues surrounding the commercialization of cultured meat in science, law, and society. Achieving large-scale manufacturing at a reasonable cost, negotiating intricate regulatory environments, guaranteeing safety, and promoting customer acceptability are the main obstacles.

Large-scale manufacturing, significant progress in cell culture techniques, biomanufacturing techniques, and culture medium optimization are needed to produce economically cultured meat at a commercially feasible scale (Post *et al*., 2020). The efficiency and robustness of current technologies are insufficient to rival traditional meat production. To increase output while cutting expenses, advancements in tissue and bioreactor engineering are essential (Zhang *et al*., 2020). To make cultured meat a viable alternative, it is also necessary to produce affordable culture medium and bioreactor designs (Lee *et al*., 2023). Moreover, to address these issues, it is suggested that interdisciplinary research be integrated, including sophisticated bioreactor engineering and synthetic biology (Zhang *et al*., 2020).

There are a lot of regulatory obstacles to overcome, including uncertainty over how cultured meat will be regulated under current laws. The implementation of a well-defined regulatory framework is vital to guarantee both consumer trust and safety. Furthermore, for regulatory compliance and customer acceptability, developing sensitive and specialized analytical instruments is essential, such as sensors for food safety monitoring (Djisalov *et al*., 2021). The social and political environment must also be navigated by technology, considering issues like ethics, media coverage, religious beliefs, and possible economic effects (Bryant, 2020).

The flavor and sensory assessment of cultured meat, as well as education and addressing ethical and environmental issues, are all critical factors in the complicated problem of consumer acceptability (Hong *et al*., 2021). Neophobia, technophobia, and the idea that cultured meat is healthier all have an impact on public acceptability (Gaydhane *et al*., 2018). Transparent information and instruction on the advantages of cultured meat, such as its ability to prevent illness, preserve the environment, and improve animal welfare, are to allay these worries (Hong *et al*., 2021). Consumer acceptability also depends on developing scaffolding materials and 3D printing techniques that can create muscle cells with a texture and flavor more like to that of traditional meat (Lee *et al*., 2023).

The multidisciplinary character of these problems emphasizes how different stakeholders, such as scientists, engineers, legislators, and social scientists, must work together. For cultured meat production to be viable and widely accepted, a comprehensive strategy that takes into account the technological, socio-political, and economic components of the process is necessary (Jairath *et al*., 2021). The intricate problems of producing cultured meat need the fusion of many scientific fields, including tissue engineering, food science, material science, and sensor technology.

The commercialization of cultured meat is a lofty objective that calls for coordinated efforts from many academic fields. The successful integration of cultured meat into the food system will depend heavily on addressing the issues of cost-effective manufacturing, regulatory compliance, safety, and customer acceptability. Research and development in cultured meat is promising because of its potential advantages for environmental sustainability, animal welfare, and food and nutrition security.

Conclusion

Cultured meat has great promise for addressing issues related to global food security and environmental sustainability. Cultured meat provides a solution to satisfy the

increasing need for protein while reducing the harmful effects of traditional animal agriculture by separating the production of meat from regular livestock husbandry.

Achieving this promise will require overcoming several scientific, technical, social, and regulatory obstacles. Developments in scaffold engineering, bioreactor design, cell line creation, and culture medium optimization are necessary to achieve large-scale manufacturing at a reasonable cost. Gaining the confidence of consumers and facilitating the commercialization of cultured meat products requires navigating complicated regulatory environments and putting in place robust safety procedures.

Promoting customer acceptability is perhaps the biggest obstacle. Concerns about cultured meat's perceived naturalness, safety, and sensory appeal will need to be addressed by open communication, education, and ongoing product development. To address these complex issues holistically, interdisciplinary cooperation between scientists, engineers, politicians, and social scientists is crucial.

Prioritizing sustainability, scalability, and the capacity to mimic the sensory and nutritional attributes of traditional meat is essential as research in this area advances. Tissue engineering, biomaterials, and bioreactor technological innovations are critical to producing cultured meat products that satisfy consumers and have the least negative environmental effects.

In conclusion, the development of cultured meat offers a viable solution to the problems of environmental sustainability and global food security. Even if there are still many obstacles to overcome, the potential advantages of this cutting-edge technology make it worthwhile to carry out further study, make investments, and work together to realize its full potential.

Author contributions

P. Ramezani: Writing–original draft, writing–editing, visualization. **A. Motamedzadegan:** Supervision, validation, writing–review and editing.

Funding Sources

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

References

1. Ahmad, S.S., Hee Jin, Ch., Khurshid, A., Sibhghatulla, Sh.,, Jeong Ho, L., Shahid, A., & Sung Soo, H., (2023). The roles of growth factors and hormones in the regulation of muscle satellite cells for cultured meat production. *Journal of Animal Science and Technology, 65*(1), 16–31. https://doi.org/10.5187/jast.2022.e114
2. Ashizawa, R., Natalie, R., Sophia, L., Avery, P., Victoria, D., & Kaplan, D.L. (2022). Entomoculture: A preliminary techno-economic assessment. *Foods, 11*(19), 3037. https://doi.org/10.3390/foods11193037
3. Allan, S.J., De Bank, P.A., & Ellis, M.J. (2019). Bioprocess design considerations for cultured meat production with a focus on the expansion bioreactor. *Frontiers in Sustainable Food Systems, 3*. https://doi.org/10.3389/fsufs.2019.00044
4. Aragão, C., Gonçalves, A.T., Costas, B., Azeredo, R., Xavier, M.J., & Engrola, S. (2022). Alternative proteins for fish diets: Implications beyond growth. *Animals, 12*(9), 1211. https://doi.org/10.3390/ani12091211
5. Batish, I., Zarei, M., Nitin, N., & Ovissipour, R. (2022). Evaluating the potential of marine invertebrate and insect protein hydrolysates to reduce fetal bovine serum in cell culture media for cultivated fish production. *Biomolecules, 12*(11), 1697. https://doi.org/10.3390/biom12111697

6. Ben-Arye, T., & Levenberg, S. (2019). Tissue engineering for clean meat production. *Frontiers in Sustainable Food Systems*, *3*. https://doi.org/10.3389/fsufs.2019.00046
7. Ben-Arye, T., Shandalov, Y., Ben-Shaul, S., Landau, S., Zagury, Y., Ianovici, I., Lavon, N., & Levenberg, S. (2020). Textured soy protein scaffolds enable the generation of three-dimensional bovine skeletal muscle tissue for cell-based meat. *Nature Food*, *1*(4), 210–220. https://doi.org/10.1038/s43016-020-0046-5
8. Benjaminson, M.A., Gilchriest, J.A., & Lorenz, M. (2002). In vitro edible muscle protein production system (MPPS): Stage 1, fish. *Acta Astronautica*, *51*(12). https://doi.org/10.1016/S0094-5765(02)00033-4
9. Bodiou, V., Moutsatsou, P., & Post, M.J. (2020). Microcarriers for upscaling cultured meat production. *Frontiers in Nutrition*, *7*. https://doi.org/10.3389/fnut.2020.00010
10. Bomkamp, C., Skaalure, S.C., Fernando, G.F., Ben-Arye, T., Swartz, E.W., & Specht, E.A. (2022a). Scaffolding biomaterials for 3D cultivated meat: Prospects and challenges. *Advanced Science*, *9*(3). https://doi.org/10.1002/advs.202102908
11. Bryant, C., & Barnett, J. (2018). Consumer acceptance of cultured meat: A systematic review. In *Meat Science* (Vol. 143). https://doi.org/10.1016/j.meatsci.2018.04.008
12. Bryant, C.J. (2020). Culture, meat, and cultured meat. *Journal of Animal Science*, *98*(8). https://doi.org/10.1093/jas/skaa172
13. Buchenauer, A., Hofmann, M.C., Funke, M., Büchs, J., Mokwa, W., & Schnakenberg, U. (2009). Micro-bioreactors for fed-batch fermentations with integrated online monitoring and microfluidic devices. *Biosensors and Bioelectronics*, *24*(5), 1411–1416. https://doi.org/10.1016/j.bios.2008.08.043
14. Chauvet, D.J. (2018). Should culture meat be refused in the name of animal dignity? *Ethical Theory and Moral Practice*, *21*(2), 387–411. https://doi.org/10.1007/s10677-018-9888-4
15. Chen, X.-Y., Chen, J.-Y., Tong, X.-M., Mei, J.-G., Chen, Y.-F., & Mou, X.-Z. (2020). Recent advances in the use of microcarriers for cell cultures and their ex vivo and in vivo applications. *Biotechnology Letters*, *42*(1), 1–10. https://doi.org/10.1007/s10529-019-02738-7
16. Choudhury, D., Tseng, T.W., & Swartz, E. (2020). The business of cultured meat. *In Trends in Biotechnology*, *38*(6), 573–577. https://doi.org/10.1016/j.tibtech.2020.02.012
17. Chriki, S., & Jean-François, H. (2020). The myth of cultured meat: A review. *Frontiers in Nutrition*, *7*. https://doi.org/10.3389/fnut.2020.00007
18. Das, R., Roosloot, R., van Santen, P., & de Bruijn, J. (2014). Novel process control in a closed system bioreactor for culture of adherent cells. *Cytotherapy*, *16*(4), S106–S107. https://doi.org/10.1016/j.jcyt.2014.01.394
19. Djisalov, M., Knežić, T., Podunavac, I., Živojević, K., Radonic, V., Knežević, N.Ž., Bobrinetskiy, I., & Gadjanski, I. (2021). Cultivating multidisciplinarity: Manufacturing and sensing challenges in cultured meat production. *Biology*, *10*(3), 204. https://doi.org/10.3390/biology10030204
20. Edelman, P.D., McFarland, D.C., Mironov, V.A., & Matheny, J.G. (2005). Commentary: *In Vitro* -cultured meat production. *Tissue Engineering*, *11*(5–6), 659–662. https://doi.org/10.1089/ten.2005.11.659
21. Eibl, R., & Eibl, D. (2008). Design of bioreactors suitable for plant cell and tissue cultures. *Phytochemistry Reviews*, *7*(3), 593–598. https://doi.org/10.1007/s11101-007-9083-z
22. Fraeye, I., Kratka, M., Vandenburgh, H., & Thorrez, L. (2020). Sensorial and nutritional aspects of cultured meat in comparison to traditional meat: Much to be inferred. *Frontiers in Nutrition*, *7*. https://doi.org/10.3389/fnut.2020.00035
23. Gaydhane, M.K., Mahanta, U., Sharma, C.S., Khandelwal, M., & Ramakrishna, S. (2018). Cultured meat: state of the art and future. *Biomanufacturing Reviews*, *3*(1), 1. https://doi.org/10.1007/s40898-018-0005-1

24. Ge, C., Selvaganapathy, P.R., & Geng, F. (2023). Advancing our understanding of bioreactors for industrial-sized cell culture: health care and cellular agriculture implications. *American Journal of Physiology-Cell Physiology*, *325*(3), C580–C591. https://doi.org/10.1152/ajpcell.00408.2022
25. Genovese, N.J., Domeier, T.L., Telugu, B.P.V.L., & Roberts, R.M. (2017). Enhanced development of skeletal myotubes from porcine induced pluripotent stem cells. *Scientific Reports, 7*. https://doi.org/10.1038/srep41833
26. Guan, X., Lei, Q., Yan, Q., Li, X., Zhou, J., Du, G., & Chen, J. (2021). Trends and ideas in technology, regulation and public acceptance of cultured meat. *Future Foods, 3*, 100032. https://doi.org/10.1016/j.fufo.2021.100032
27. Hamdan, M.N., Ramli, M.A., Zaman Huri, N.M.F., Abd Rahman, N.N.H., & Abdullah, A. (2021). Will Muslim consumers replace livestock slaughter with cultured meat in the market? In *Trends in Food Science and Technology*, *109*. https://doi.org/10.1016/j.tifs.2021.01.034
28. Hamzeh, A., Rezaei, M., Khodabandeh, S., Motamedzadegan, A., & Noruzinia, M. (2018). Antiproliferative and antioxidative activities of cuttlefish (*Sepia pharaonis*) protein hydrolysates as affected by degree of hydrolysis. *Journal of Food Measurement and Characterization, 12*(2), 721–727. https://doi.org/10.1007/s11694-017-9685-0
29. Hanga, M.P., Ali, J., Moutsatsou, P., de la Raga, F.A., Hewitt, C.J., Nienow, A., & Wall, I. (2020). Bioprocess development for scalable production of cultivated meat. *Biotechnology and Bioengineering*, *117*(10), 3029–3039. https://doi.org/10.1002/bit.27469
30. Hong, T.K., Shin, D.-M., Choi, J., Do, J.T., & Han, S.G. (2021). Current issues and technical advances in cultured meat production: A review. *Food Science of Animal Resources*, *41*(3), 355–372. https://doi.org/10.5851/kosfa.2021.e14
31. Ibidhi, R., & Ben Salem, H. (2020). Water footprint of livestock products and production systems: a review. *Animal Production Science*, *60*(11), 1369. https://doi.org/10.1071/AN17705
32. Ikasari, B.N., Alfarizi, S., Fauziyah, Sh., Wardhani, P., Soegeng Soegijanto, A., & Sucipto, T. (2022). Effect of fetal bovine serum concentration towards vero cells growth on culture in DMEM medium. *Jurnal Teknologi Laboratorium, 11*(2), 73–77. https://doi.org/10.29238/teknolabjournal.v11i2.313
33. Jairath, G., Mal, G., Gopinath, D., & Singh, B. (2021). A holistic approach to access the viability of cultured meat: A review. *Trends in Food Science & Technology*, *110*, 700–710. https://doi.org/10.1016/j.tifs.2021.02.024
34. Jochems, C.E.A., van der Valk, J.B.F., Stafleu, F.R., & Baumans, V. (2002). The use of fetal bovine serum: Ethical or scientific problem? *Alternatives to Laboratory Animals*, *30*(2), 219–227. https://doi.org/10.1177/026119290203000208
35. Kim, Cho H., Lee, H.J., Jung, D.Y., Kim, M., Jung, H.Y., Hong, H., Choi, Y.S., In Yong, H., & Jo, Ch. (2023). Evaluation of fermented soybean meal and edible insect hydrolysates as potential serum replacement in pig muscle stem cell culture. *Food Bioscience*, *54*, 102923. https://doi.org/10.1016/j.fbio.2023.102923
36. Kimura, A., Yoshida, F., Ueno, M., & Taguchi, M. (2021). Application of radiation crosslinking technique to development of gelatin scaffold for tissue engineering. *Radiation Physics and Chemistry*, *180*, 109287. https://doi.org/10.1016/j.radphyschem.2020.109287
37. Kröncke, N., & Benning, R. (2023). Influence of dietary protein content on the nutritional composition of mealworm larvae (*Tenebrio molitor* L.). *Insects*, *14*(3), 261. https://doi.org/10.3390/insects14030261
38. Lee, D.Y., Lee, S.Y., Yun, S.H., Jeong, J.W., Kim, J.H., Kim, H.W., & Choi, J.S. (2022). Review of the current research on fetal bovine serum and the development of cultured meat. *Food Science of Animal Resources*, *42*(5), 775–99. https://doi.org/10.5851/kosfa.2022.e46

39. Lee, D.K., Kim, M., Jeong, J., Lee, Y.S., Yoon, J.W., An, M.J., Jung, H.Y., Kim, C.H., Ahn, Y., Choi, K.H., Jo, C., & Lee, C.K. (2023). Unlocking the potential of stem cells: Their crucial role in the production of cultivated meat. In *Current Research in Food Science, 7*. https://doi.org/10.1016/j.crfs.2023.100551
40. Lee, D.Y., Lee, S.Y., Jung, J.W., Kim, J.H., Oh, D.H., Kim, H.W., Kang, J.H., Choi, J.S., Kim, G.-D., Joo, S.-T., & Hur, S.J. (2023). Review of technology and materials for the development of cultured meat. *Critical Reviews in Food Science and Nutrition*, *63*(27), 8591–8615. https://doi.org/10.1080/10408398.2022.2063249
41. López-Martínez, M.I., Miguel, M., & Garcés-Rimón, M. (2022). Protein and sport: Alternative sources and strategies for bioactive and sustainable sports nutrition. *Frontiers in Nutrition, 9*. https://doi.org/10.3389/fnut.2022.926043
42. Lu, H., Ying, K., Shi, Y., Liu, D., & Chen, Q. (2022). Bioprocessing by decellularized scaffold biomaterials in cultured meat: A review. *Bioengineering*, *9*(12), 787. https://doi.org/10.3390/bioengineering9120787
43. Lupatini, A.L., Colla, L.M., Canan, C., & Colla, E. (2017). Potential application of microalga *Spirulina platensis* as a protein source. *Journal of the Science of Food and Agriculture*, *97*(3), 724–732. https://doi.org/10.1002/jsfa.7987
44. Manzocchi, E., Guggenbühl, B., Kreuzer, M., & Giller, K. (2020). Effects of the substitution of soybean meal by spirulina in a hay-based diet for dairy cows on milk composition and sensory perception. *Journal of Dairy Science*, *103*(12), 11349–11362. https://doi.org/10.3168/jds.2020-18602
45. Mattick, C.S., Landis, A.E., Allenby, B.R., & Genovese, N.J. (2015). Anticipatory life cycle analysis of in vitro biomass cultivation for cultured meat production in the United States. *Environmental Science and Technology*, *49*(19), 11941–11949. https://doi.org/10.1021/ACS.EST.5B01614
46. Mekonnen, M.M., & Hoekstra, A.Y. (2012). A global assessment of the water footprint of farm animal products. *Ecosystems*, *15*(3). https://doi.org/10.1007/s10021-011-9517-8
47. Minghao, N., Shima, A., & Takeuchi, Sh. (2023). Centimeter-scale perfusable cultured meat with densely packed, highly aligned muscle fibers via hollow fiber bioreactor. *Biorxiv (Preprint)*.
48. Mirzakhani, M.K., Abedian Kenari, A., & Motamedzadegan, A. (2018). Prediction of apparent protein digestibility by in vitro pH-stat degree of protein hydrolysis with species-specific enzymes for Siberian sturgeon (*Acipenser baeri*, Brandt 1869). *Aquaculture, 496*, 73–78. https://doi.org/10.1016/j.aquaculture.2018.07.014
49. Moritz, M.S.M., Verbruggen, S.E.L., & Post, M.J. (2015). Alternatives for large-scale production of cultured beef: A review. *Journal of Integrative Agriculture, 14*(2), 208–216. https://doi.org/10.1016/S2095-3119(14)60889-3
50. Mullenix, G.J., Greene, E.S., Emami, N.K., Tellez-Isaias, G., Bottje, W.G., Erf, G.F., Kidd, M.T., & Dridi, S. (2021). *Spirulina platensis* inclusion reverses circulating pro-inflammatory (Chemo) cytokine profiles in broilers fed low-protein diets. *Frontiers in Veterinary Science, 8*. https://doi.org/10.3389/fvets.2021.640968
51. Munteanu, C., Mireşan, V., Răducu, C., Ihuţ, A., Uiuiu, P., Pop, D., Neacşu, A., Cenariu, M., & Groza, I. (2021). Can cultured meat be an alternative to farm animal production for a sustainable and healthier lifestyle? *Frontiers in Nutrition, 8*. https://doi.org/10.3389/fnut.2021.749298
52. Musyoka, S.N., Liti, D.M., Ogello, E., & Waidbacher, H. (2019). Utilization of the earthworm, *Eisenia fetida* (Savigny, 1826) as an alternative protein source in fish feeds processing: A review. *Aquaculture Research, 50*(9), 2301–2315. https://doi.org/10.1111/are.14091
53. Nakamura, M., Tomochi, H., Andoh, K., Nishimori, A., Suda, Y., Matsuura, Y., & Iwamaru, Y. (2022). Inspection of commercially available fetal bovine Sera collected between 2017 and 2021

for the contamination of bovine viral diarrhea virus. *Journal of the Japan Veterinary Medical Association*, *75*(7), e139–e144. https://doi.org/10.12935/jvma.75.e139
54. Negulescu, P.G., Risner, D., Spang, E.S., Sumner, D., Block, D., Nandi, S., & McDonald, K.A. (2023). Techno-economic modeling and assessment of cultivated meat: Impact of production bioreactor scale. *Biotechnology and Bioengineering*, *120*(4), 1055–1067. https://doi.org/10.1002/bit.28324
55. Newton, P., & Blaustein-Rejto, D. (2021). Social and economic opportunities and challenges of plant-based and cultured meat for rural producers in the US. *Frontiers in Sustainable Food Systems*, *5*. https://doi.org/10.3389/fsufs.2021.624270
56. Ng, S., & Kurisawa, M. (2020). Integrating biomaterials and food biopolymers for cultured meat production. *SSRN Electronic Journal*. https://doi.org/10.2139/ssrn.3692010
57. Obom, K.M., Cummings, P.J., Ciafardoni, J.A., Hashimura, Y., & Giroux, D. (2014). Cultivation of mammalian cells using a single-use pneumatic bioreactor system. *Journal of Visualized Experiments*, *92*. https://doi.org/10.3791/52008
58. Ong, K.J., Tejeda-Saldana, Y., Duffy, B., Holmes, D., Kukk, K., & Shatkin, J.A. (2023). Cultured meat safety research priorities: Regulatory and governmental perspectives. *Foods*, *12*(14). https://doi.org/10.3390/foods12142645
59. Ozhava, D., Bhatia, M., Freman, J., & Mao, Y. (2022). Sustainable cell sources for cultivated meat. *Journal of Biomedical Research & Environmental Sciences*, *3*(12). https://doi.org/10.37871/jbres1607
60. Peng, C.-A., & Palsson, B.Ø. (2000). Cell growth and differentiation on feeder layers is predicted to be influenced by bioreactor geometry. *Biotechnology and Bioengineering*, *50*(5), 479–492. https://doi.org/10.1002/(SICI)1097-0290(19960605)50
61. Penn, J. (2018). Cultured meat: Lab-grown beef and regulating the future meat market. *UCLA Journal of Environmental Law and Policy*, *36*(1). https://doi.org/10.5070/l5361039902
62. Post, M.J. (2012). Cultured meat from stem cells: Challenges and prospects. In *Meat Science*, *92*(3). https://doi.org/10.1016/j.meatsci.2012.04.008
63. Post, M.J., Levenberg, S., Kaplan, D.L., Genovese, N., Fu, J., Bryant, C.J., Negowetti, N., Verzijden, K., & Moutsatsou, P. (2020). Scientific, sustainability and regulatory challenges of cultured meat. *Nature Food*, *1*(7), 403–415. https://doi.org/10.1038/s43016-020-0112-z
64. Prasad, Sh., Prakash, C., Rohit, K., Karunakaran, M., Santra, A., & Subrata, K.D. (2018). Development of cattle embryo through in vitro technique using epidermal growth factor as a media supplement. *International Journal of Bio-resource and Stress Management*, *9*(6), 691–94. https://doi.org/10.23910/IJBSM/2018.9.6.1923
65. Roncolini, A., Milanović, V., Aquilanti, L., Cardinali, F., Garofalo, C., Sabbatini, R., Clementi, F., Belleggia, L., Pasquini, M., Mozzon, M., Foligni, R., Federica Trombetta, M., Haouet, M.N., Serena Altissimi, M., Di Bella, S., Piersanti, A., Griffoni, F., Reale, A., Niro, S., & Osimani, A. (2020). Lesser mealworm (*Alphitobius diaperinus*) powder as a novel baking ingredient for manufacturing high-protein, mineral-dense snacks. *Food Research International*, *131*, 109031. https://doi.org/10.1016/j.foodres.2020.109031
66. Schaefer Owen, G., & Savulescu, J. (2014). The ethics of producing in vitro meat. *Journal of Applied Philosophy*, *31*(2), 188–202. https://doi.org/10.1111/japp.12056
67. Seah, J.S.H., Singh, S., Tan, L.P., & Choudhury, D. (2022). Scaffolds for the manufacture of cultured meat. *Critical Reviews in Biotechnology*, *42*(2), 311–323. https://doi.org/10.1080/07388551.2021.1931803
68. Shaviklo, A.R., Moradinezhad, N., Abolghasemi, S.J., Motamedzadegan, A., Kamali-Damavandi, N., & Rafipour, F. (2016). Product optimization of fish burger containing tuna

protein isolates for better sensory quality and frozen storage stability. *Turkish Journal of Fisheries and Aquatic Sciences*, *16*(4). https://doi.org/10.4194/1303-2712-v16_4_20

69. Smetana, S., Mathys, A., Knoch, A., & Heinz, V. (2015). Meat alternatives: life cycle assessment of most known meat substitutes. *International Journal of Life Cycle Assessment*, *20*(9). https://doi.org/10.1007/s11367-015-0931-6
70. Stephens, N., Di Silvio, L., Dunsford, I., Ellis, M., Glencross, A., & Sexton, A. (2018). Bringing cultured meat to market: Technical, socio-political, and regulatory challenges in cellular agriculture. In *Trends in Food Science and Technology*, 78. https://doi.org/10.1016/j.tifs.2018.04.010
71. Stout, A.J., Zhang, X., Letcher, S.M., Rittenberg, M.L., Shub, M., Chai, K.M., Kaul, M., & Kaplan, D.L. (2024). Engineered autocrine signaling eliminates muscle cell FGF2 requirements for cultured meat production. *Cell Reports Sustainability*, *1*(1), 100009. https://doi.org/10.1016/j.crsus.2023.100009
72. Tabarestani, Shahiri, H., Maghsoudlou, Y., Motamedzadegan, A., & Sadeghi Mahoonak, A.R. (2010). Optimization of physico-chemical properties of gelatin extracted from fish skin of rainbow trout (*Onchorhynchus mykiss*). *Bioresource Technology*, *101*(15), 6207–14. https://doi.org/10.1016/j.biortech.2010.02.071
73. Taheri, A., Abedian Kenari, A., Motamedzadegan, A., & Habibi Rezaie, M. (2011). Optimization of goldstripe sardine (*Sardinella gibbosa*) protein hydrolysate using Alcalase® 2.4L by response surface methodology Optimización de hidrolisato de proteína de Sardinela dorada (*Sardinella gibbosa*) usando Alcalase® 2.4L a través de RSM. CyTA. *Journal of Food*, *9*(2), 114–120. https://doi.org/10.1080/19476337.2010.484551
74. Tahir, I., & Floreani, R. (2022). Dual-crosslinked alginate-based hydrogels with tunable mechanical properties for cultured meat. *Foods*, *11*(18), 2829. https://doi.org/10.3390/foods11182829
75. Tuomisto, H.L., & Teixeira de Mattos, M.J. (2011). Environmental impacts of cultured meat production. *Environmental Science & Technology*, *45*(14), 6117–6123. https://doi.org/10.1021/es200130u
76. Tzachor, A., Smidt-Jensen, A., Ramel, A., & Geirsdóttir, M. (2022). Environmental impacts of large-scale spirulina (*Arthrospira platensis*) production in Hellisheidi Geothermal Park Iceland: Life cycle assessment. *Marine Biotechnology*, *24*(5), 991–1001. https://doi.org/10.1007/s10126-022-10162-8
77. Wang, J., Ding, X., & Zhou, G. (2022). Cutting-edge tissue engineering strategies for cultured meat. *Food Materials Research*, *2*(1), 1–5. https://doi.org/10.48130/FMR-2022-0020
78. Wang, Y., Ji, H., He, L., Niu, Y., Zhang, Y., Liu, Y., Tian, Y., Liu, X., Li, H., Kang, X., Gao, Y., & Li, Z. (2024). Establishment and analysis of immortalized chicken skeletal muscle satellite cell lines1. *Journal of Integrative Agriculture*. https://doi.org/10.1016/j.jia.2024.01.034
79. Wang, Y., Zou, L., Liu, W., & Chen, X. (2023). An overview of recent progress in engineering three-dimensional scaffolds for cultured meat production. *Foods*, *12*(13), 2614. https://doi.org/10.3390/foods12132614
80. Yeo, D., Kiparissides, A., Cha, J.M., Aguilar-Gallardo, C., Polak, J.M., Tsiridis, E., Pistikopoulos, E. N., & Mantalaris, A. (2013). Improving embryonic stem cell expansion through the combination of perfusion and bioprocess model design. *PLoS ONE*, *8*(12), e81728. https://doi.org/10.1371/journal.pone.0081728
81. Young, Ashlyn T., White, O.C., & Daniele, M.A. (2020). Rheological properties of coordinated physical gelation and chemical crosslinking in gelatin methacryloyl (GelMA) hydrogels. *Macromolecular Bioscience*, *20*(12). https://doi.org/10.1002/mabi.202000183

82. Yu, I., Choi, J., Kim, M.K., & Kim, M.J. (2023). The comparison of commercial serum-free media for Hanwoo satellite cell proliferation and the role of fibroblast growth factor 2. *Food Science of Animal Resources*, *43*(6), 1017–30. https://doi.org/10.5851/kosfa.2023.e68
83. Zhang, G., Zhao, X., Li, X., Du, G., Zhou, J., & Chen, J. (2020). Challenges and possibilities for bio-manufacturing cultured meat. *Trends in Food Science & Technology*, *97*, 443–450. https://doi.org/10.1016/j.tifs.2020.01.026

نشریه پژوهشهای علوم و صنایع غذایی ایران
https://ifstrj.um.ac.ir

مقاله مروری

جلد ۲۰، شماره ۳، مرداد-شهریور ۱۴۰۳، ص. ۱۰۳-۸۱

بررسی پتانسیل گوشت کشت شده: پیشرفت‌های فناورانه، چشم‌انداز پایداری و چالش‌ها

محمدپویا رمضانی[۱*] - علی معتمدزادگان[۲*]

تاریخ دریافت: ۱۴۰۳/۰۲/۰۹
تاریخ پذیرش: ۱۴۰۳/۰۴/۰۹

چکیده

دامپروری سنتی به‌دلیل اثرات زیانبار بر محیط‌زیست و محدودیت در امکان توسعه مقیاس تولید، یکی از عوامل اصلی چالش جهانی عدم امنیت غذایی محسوب می‌شود. کشت سلول‌های جانوری در شرایط کنترل شده، منجر به تولید گوشت کشت داده شده یا گوشت مصنوعی گردیده که می‌تواند گزینه‌ای اخلاقی‌تر و سازگارتر با محیط‌زیست برای تأمین امنیت غذایی باشد. این مقاله مروری، پتانسیل گوشت مصنوعی را با تحلیل دقیق تأثیرات آن بر سیستم‌های جهانی تولید و توزیع مواد غذایی، چشم‌انداز پایداری آن، پیشرفت‌های تکنولوژیکی حاصل شده و موانع پیش رو، برای رفع معضل امنیت غذایی مورد ارزیابی قرار می‌دهد. تحلیل چرخه حیات نشان می‌دهد تولید گوشت مصنوعی، اثرات زیست‌محیطی به مراتب کمتری نسبت به گوشت سنتی دارد. پیشرفت‌های علمی مهم در زمینه‌های فناوری داربست‌ها، مهندسی بافت و طراحی بیوراکتورها، تولید گوشت مصنوعی را به تجاری‌سازی نزدیک‌تر کرده است. با این حال، موانع قابل توجهی نیز وجود دارد که باید مرتفع شوند؛ از جمله امکان تولید انبوه در مقیاس بزرگ با هزینه مقرون به صرفه، تطبیق با چارچوب‌های پیچیده قانون‌گذاری و نظارتی، تضمین ایمنی و سلامت محصول و افزایش پذیرش آن از سوی مصرف‌کنندگان. برای غلبه بر این چالش‌ها و تحقق وعده گوشت مصنوعی در راستای بهبود امنیت غذایی و تغذیه، حفظ پایداری محیط‌زیست و رعایت رفاه حیوانات، اتخاذ رویکردی میان‌رشته‌ای که ابعاد علمی، فنی، قانونی و اجتماعی را در نظر می‌گیرد، امری ضروری است.

واژه‌های کلیدی: امنیت غذایی، پایداری، داربست‌سازی، طراحی بیوراکتور، گوشت کشت‌شده

۱ و ۲- به‌ترتیب دانشجوی کارشناسی ارشد و استاد تمام گروه علوم و صنایع غذایی، دانشگاه علوم کشاورزی و منابع طبیعی ساری، ساری، ایران
(*- نویسنده مسئول: Email: p.ramezani@sanru.ac.ir, amotgan@yahoo.com)

https://doi.org/10.22067/ifstrj.2024.87796.1329

مندرجات

نشریه پژوهشهای علوم و صنایع غذایی ایران

با شماره پروانه ۱۲۴/۸۴۷ و درجه علمی–پژوهشی شماره ۳/۱۱/۸۱۰ از وزارت علوم، تحقیقات و فناوری ۸۸/۵/۱۰
"براساس مصوبه وزارت عتف از سال ۱۳۹۸، کلیه نشریات دارای درجه "علمی–پژوهشی" به نشریه "علمی" تغییر نام یافتند. "

جلد ۲۰ **شماره ۳** **مرداد- شهریور ۱۴۰۳**

صاحب امتیاز: دانشگاه فردوسی مشهد

مدیر مسئول: دکتر ناصر شاهنوشی — استاد، اقتصاد کشاورزی، دانشگاه فردوسی مشهد

سردبیر: دکتر مسعود یاورمنش — دانشیار، میکروبیولوژی و بیوتکنولوژی، دانشگاه فردوسی مشهد

اعضای هیئت تحریریه:

دکتر سید علی مرتضوی	استاد، میکروبیولوژی و بیوتکنولوژی، دانشگاه فردوسی مشهد
دکتر فخری شهیدی	استاد، میکروبیولوژی مواد غذایی، دانشگاه فردوسی مشهد
دکتر محمدباقر حبیبی نجفی	استاد، میکروبیولوژی، دانشگاه فردوسی مشهد
دکتر مرتضی خمیری	دانشیار، میکروبیولوژی، دانشگاه علوم کشاورزی و منابع طبیعی گرگان
دکتر سید محمد علی رضوی	استاد، مهندسی و خواص بیوفیزیک مواد غذایی، دانشگاه فردوسی مشهد
دکتر رضا فرهوش	استاد، شیمی مواد غذایی، دانشگاه فردوسی مشهد
دکتر بی بی صدیقه فضلی بزاز	استاد، میکروبیولوژی، دانشکده داروسازی، دانشگاه علوم پزشکی مشهد
دکتر مهدی کاشانی نژاد	استاد، مهندسی مواد غذایی، دانشگاه علوم کشاورزی و منابع طبیعی گرگان
دکتر آرش کوچکی	استاد، تکنولوژی مواد غذایی، دانشگاه فردوسی مشهد
دکتر محبت محبی	استاد، مهندسی مواد غذایی، دانشگاه فردوسی مشهد
دکتر بابک قنبرزاده	استاد، مهندسی مواد غذایی، دانشگاه تبریز
دکتر ایران عالمزاده	استاد، بیوتکنولوژی مواد غذایی، دانشگاه صنعتی شریف
دکتر قدیر رجب‌زاده اوغاز	دانشیار، نانو فناوری مواد غذایی، مؤسسه پژوهشی علوم و صنایع غذایی، مشهد
دکتر مهیار حیدرپور	دانشیار، زیست مولکولی، بیمارستان زنان و بریگام، ایالت متحده آمریکا
دکتر حمید بهادر قدوسی	دانشیار، میکروبیولوژی غذایی، دانشگاه متروپولیتن لندن
دکتر کیانوش خسروی	استاد، بیوتکنولوژی مواد غذایی، دانشگاه علوم پزشکی شهید بهشتی
دکتر مرتضی عباس‌زادگان	استاد، مهندسی عمران و محیط زیست، دانشگاه آریزونا
دکتر محمدامین محمدی‌فر	استاد، مهندسی تولید مواد غذایی، دانشگاه فنی دانمارک
دکتر منوچهر وثوقی	استاد، بیوتکنولوژی مواد غذایی، دانشگاه صنعتی شریف
دکتر هادی الماسی	دانشیار، گروه علوم و صنایع غذایی، دانشکده کشاورزی، دانشگاه ارومیه
دکتر میلاد فتحی	دانشیار، گروه علوم و صنایع غذایی، دانشگاه صنعتی اصفهان
دکتر سلیمان عباسی	استاد، گروه علوم و صنایع غذایی، دانشگاه تربیت مدرس.
دکتر نونو بورخس	استاد، گروه علوم تغذیه و مواد غذایی، دانشگاه پورتو، پرتغال
دکتر علی عطا معظمی	دانشیار، گروه علوم مولکولی، دانشکده منابع طبیعی و علوم کشاورزی، دانشگاه سوئد
دکتر کلیفورد نکمناسو اوبی	دانشیار گروه میکروبیولوژی، دانشگاه کشاورزی مایکل اوکپارا، ایالت ابیا، نیجریه
دکتر ساموئل ایوفمی اولالکان آدییه	دانشیار گروه فناوری غذایی، موسسه تکنولوژی و علم هندوستان، چنای، تامیل نادو، هند

ناشر: دانشگاه فردوسی مشهد

این نشریه درپایگاه‌های زیر نمایه شده است:

AGRIS، Scopus، CABI، DOAJ، EBSCO، Google scholar، Internet Archive، پایگاه استنادی جهان اسلام (ISC)، سامانه نشریات علمی ایران، پایگاه اطلاعات علمی جهاد دانشگاهی (SID)، بانک اطلاعات نشریات کشور (MAGIRAN)، مرجع دانش CIVILICA

پست الکترونیکی: **ifstrj@um.ac.ir**

مقالات این شماره در سایت https://ifstrj.um.ac.ir به صورت مقاله کامل نمایه شده است.

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

نشریه علمی

پژوهشهای علوم و صنایع غذایی ایران

جلد ۲۰ شماره ۳

سال ۱۴۰۳

شماره پیاپی ۸۷

شاپا: ۴۱۶۱-۱۷۳۵

عنوان مقالات

نشریه علمی پژوهشهای علوم و صنایع غذایی ایران جلد ۲۰ شماره ۳ سال ۱۴۰۳ 2024 Vol.20 No.3 Iranian Food Science and Technology Research Journal