Ferdowsi University of Mashhad

Iranian Food Science and Technology Research Journal

Vol.19 No.3

2023

ISSN:1735-4161

Contents

Iranian Food Science and Technology Research Journal

Vol. 19 No. 3 2023

Published by: Ferdowsi University of Mashhad

Executive Manager: Shahnoushi, N.

Editor-in-Chief: Tabatabaei yazdi, F.

Printed by: Ferdowsi University of Mashhad Press, Iran.

Address: The Iranian Food Science & Technology Research Journal, Scientific Publication Office, Food Science and Technology Department, Agriculture Faculty, Ferdowsi University of Mashhad, Iran.

P.O.BOX: 91775- 1163

Phone: (98)511-8795618-20(321)

Fax: (98)511-8787430

E-Mail: ifstrj@um.ac.ir

Web Site: http://jm.um.ac.ir/index.php/food_tech/index

This journal is indexed in ISC, SID, and MAGIRAN.

Contents

Iranian Food Science and Technology Research Journal
Vol. 19, No. 3, Aug.-Sep. 2023, p. 1-14

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد ۱۹، شماره ۳، مرداد- شهریور ۱۴۰۲ ص. ۱۴-۱

Research Article
Homepage: http://ifstrj.um.ac.ir

The Effect of Different Types of Coating and Packaging on the Physical Properties of Persimmon Fruit under Load

S. Jafarzadeh[1], M. Azadbakht[2]*, F. Varasteh[3], M. Vahedi Torshizi[4]

Received: 2023.03.17
Revised: 2023.05.26
Accepted: 2023.05.27
Available Online: 2023.05.27

How to cite this article:
Jafarzadeh, S., Azadbakht, M., Varasteh, F., & Vahedi Torshizi, M. (2023). The effect of different types of coating and packaging on the physical properties of persimmon fruit under load. *Iranian Food Science and Technology Research Journal*, *19*(3), 1-14. https://doi.org/10.22067/ifstrj.2023.81571.1242

Abstract

Since persimmon is a pressure-sensitive fruit and it is difficult to store this fruit in warehouses, in this research, an attempt has been made to examine the parameters affecting the reduction of changes in its physical properties. The samples were loaded at 150 and 250 N, three types of foam container packaging with polyolefin film, polyethylene-terephthalate, and ordinary box, and four types of polyamine putrescine coating with concentrations of 1 and 2 mM, distilled water and uncoated. Properties such as Physiological Weight Loss, volume, and the density of persimmon fruit, as well as the firmness of this fruit in the prepost-storage stage were examined. The results showed, the highest firmness was obtained in the treatment of putrescine at a concentration of 1 mM and a foam container with polyethylene film with a value of 6.5 N, which was almost three times the firmness of uncoated fruits. The lowest Physiological Weight Loss, volume, and density were obtained in the same type of coating and packaging. The values of these parameters were 2.458%, 1.82, and 0.833%, respectively, compared to the first day of storage. Overall, the use of polyamine treatment showed a significant effect on changes in the physical properties of persimmon fruit, and foam containers with polyolefin film emerged as the optimal packaging option, resulting in the least amount of change among the different types of packaging used.

Keywords: Loading force, Packaging, Persimmon, Physical properties, Polyamine, Storage

Introduction

Persimmon is a good source of secondary metabolites including antioxidants, carotenoids, and polyphenols, and is a popular fruit in temperate and tropical regions (Veberic *et al*., 2010). Given the popularity of this fruit, it is important to pay special attention to its appearance, as agricultural products are typically subjected to various factors and processes from harvest to consumption. These processes can be simple processes such as cleaning, sorting, washing, moving, and weighing, or they can be complementary or conversion processes that somehow affect the characteristics of the product and sometimes damage the fruit (Azadbakht *et al*., 2019a). If the damage to agricultural products on farms and orchards is added to it, it will cause a large amount of waste in agricultural products. Mechanical damage to crops between harvest

1, 2 and 3- MSc Graduated, Associate Professor and Assistant Professor of Department of Bio-System Mechanical Engineering, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran, respectively.

(*- Corresponding Author Email: azadbakht@gau.ac.ir)
4- Ph. D. Candidate of Department of Bio-System Engineering, Tarbiat Modares University, Tehran, Iran
DOI: 10.22067/ifstrj.2023.81571.1242

and consumption is a major factor in declining quality and marketability (Azadbakht *et al*., 2016; Yurtlu *et al*., 2005). Therefore, recognizing the different physical, mechanical, chemical, and biological properties and how to maintain or change them in order to achieve the desired goals of the process can have a great impact on maintaining the quantity and quality of the product. Every year, a significant quantity of agricultural and horticultural products goes to waste at various stages, especially after harvest. So that the amount of this waste in third-world countries due to the lack of attention to the principles of storage of agricultural products and lack of development and the evolution of scientific methods of warehousing and damage caused by warehousing pests is more than industrialized countries (Azadbakht *et al*., 2019b; Azadbakht *et al*., 2019c). To reduce this wastes, packaging and post-harvest processes can be used. For packaging, it is necessary to know some physical characteristics of fruits. The volume and mass of the fruit are the most significant and easily identifiable physical properties. These factors serve as a basis for various considerations and decisions related to the fruit. Fruits may require containers specially designed for them The volume and surface area of the fruit are useful in predicting the desired amount of drying and therefore in reducing the drying time in the dryer (Azadbakht *et al*., 2016; Azabakht *et al*., 2019d). Also, recognizing the characteristics of different types of fruits under different conditions allows us to modify and design machines and industrial processes with new qualitative characteristics, as a result, their losses can be minimized, and operational efficiency can be enhanced. The first step in meeting the quality standards of fruits and improving the different processing lines of these products is to know the different properties of these products and the types of their changes due to various factors (Masoudi *et al*., 2006). However, the use of coatings to increase the productivity of agricultural products is crucial. These coatings are used to control the rate of product respiration, control physiological diseases and reduce the growth of microorganisms, and most importantly, reduce crop water loss. These coatings are non-toxic substances and a type of wax that is safe for the environment and human health, with no negative effects, which prevents the growth of fungi and maintains the appearance of the fruit for a longer period of time, while they are easy to use (Ardakani *et al*., 2010). On the other hand, fruit coating in the warehouse may affect the antioxidant capacity and nutritional value of the fruit. In this context, it has been reported that a slight increase in the amount of internal carbon dioxide in the fruit due to the use of coatings increases the synthesis of antioxidant compounds (Jeong et al., 2004). Various studies have been conducted on the effect of coating and packaging factors in different warehouses.

Azadbakht *et al*. (2019e) conducted a study on the effect of loading and storage of pear fruit, which concluded that loading had a significant effect on deteriorating the physical attributes of pear fruit. Mirdehghan *et al*. (2007) conducted a study on the effect of using polyamine coating treatment on pomegranate during storage. The results of this study showed that using this coating in warehousing prevents the reduction of firmness and respiration of pomegranate fruit. Additionally, it has been observed to have an impact on the chemical properties of the fruit. Mirdehghan *et al*. (2016) conducted a study using a coating on grapes, which showed that the application of polyamine treatment during warehousing resulted in lower weight loss compared to control samples and other coatings. Zahedi *et al*. (2018) conducted a study on the effect of polyamine coating treatment on various physical properties of mango. The results demonstrated that the use of this coating during product warehousing delays the rate of water outflow from the fruit, reduces weight loss, and enhances firmness. Cangi *et al*. (2011), examined some physical and chemical properties of kiwifruit at the stage of physiological maturity. They measured the geometric mean diameter, sphericity, specific

gravity, the porosity, the area along the three axes Y, X, and Z, and the color properties.

The objective of this study was to examine the physical properties of persimmon fruit under different conditions, as this fruit is known for its sensitivity. In this study, using a quasi-static loading device, the loading force was applied to the product to change the internal tissues. As a result, the physical properties of this fruit, which significantly affect its marketability, undergo rapid changes when subjected to storage conditions. The use of proper packaging and coating for storage of this product increases the durability of this product. This study aimed to investigate the impact of force on persimmons and evaluate the influence of various packaging and coating methods on the rate of change in weight, volume, density, and firmness of persimmons under cold conditions.

Materials and Methods

Sample preparation

In this research, persimmons were obtained from a garden in the Hashemabad region near Gorgan City, Golestan province, Iran. The persimmons were then brought to the laboratory of the Department of Bio-System Mechanical Engineering, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran. Damaged persimmons were separated from the batch, and the remaining spotless persimmons were cleaned with a damp cloth. All persimmons were categorized based on their dimensions, ensuring equalization to minimize experimental errors. Persimmons with extreme dimensions and weight were excluded from the study. After sorting, the remaining persimmons were carefully coated. Subsequently, the coated persimmons were subjected to two levels of load: 150 N and 250 N. They were then packed in foam containers with polyolefin film, polyethylene terephthalate (PET), and ordinary boxes, and stored for 25 days. Then properties such as weight, volume, density, and firmness of persimmons were measured.

Coating

Polyamine putrescine was used for the coating. In the first type of coating, 1 ml of putrescine was applied, while in the second type of coating, 2 ml of putrescine was used. The third type of coating involved the use of distilled water as a control. Additionally, for a comprehensive study, uncoated samples of persimmons were considered as the control group in the experiment. All persimmons were immersed in the coating solution for 10 minutes and then placed on a flat surface in a laboratory at 20°C for drying. To ensure optimal immersion quality, eight persimmons were placed in containers at each stage.

Static loading

Samples of coated persimmons were loaded using a pressure-deformation device (the Santam Indestrone -STM5-Made in Iran) in two pressure load levels: 150 and 250 N. The compression test was conducted using two circular plates, applying a speed of 10 mm per minute. To ensure consistency, all persimmons were loaded in a single direction. Please refer to Fig. 1 for the placement of the samples (Vahedi Torshizi *et al*., 2020).

Packaging and storage

After loading, the samples were packaged using three foil container packs with polyolefin film (Fig. 2.A), polyethylene-terephthalate (Fig. 2.B), and ordinary box (Fig. 2.C). Four persimmons were placed in each pack with polyolefin and polyethylene terephthalate film packaging. After packing, the samples were taken to the cold storage of the Gorgan University of Agricultural Sciences and Natural Resources and placed in a refrigerator at a temperature of 5°C for 25 days.

Fig. 1- Loading of persimmons

Fig. 2- Types of packaging used

The physical properties

For physical properties, the weight of each sample was recorded individually and in packages. The package weight was subtracted from the total weight of the samples to obtain the net weight of the individual fruits. The volume of the samples was measured using the water displacement method before coating. The density of each sample was calculated using the appropriate density formula. To determine the package density, the densities of the four fruits placed in each package were summed. The samples' average weight and volume were measured. The packages were then stored in the warehouse for 25 days. After the storage period, the packages were returned to the laboratory, and the weight, volume, and density of each sample and package were re-evaluated. The percentage reduction in weight, volume, and density compared to the initial measurements was calculated. To assess firmness changes, persimmon samples from each package were tested using a penetrometer (EFFEGI model, made in Italy). To conduct this experiment, the skin of the persimmons was protected from damage by applying controlled pressure. Following the provided instructions, the penetrometer probe was positioned on the desired part of the persimmon. By applying the required pressure, the probe penetrated the fruit flesh, and the corresponding value displayed on the penetrometer represented the fruit's firmness (Azadbakht *et al.*, 2019) . The firmness was measured for each sample of persimmon and then an average of 4 persimmons was recorded for each package.

Statistical Analysis

The study focused on investigating the physical properties of persimmons. The independent parameters included loading force at two levels (150 and 250 N), three types of

foam container packaging with polyolefin film, polyethylene-terephthalate, and ordinary box, four types of coatings include polyamine with concentrations of 1 and 2 mM, distilled water and non-coated. The dependent factors examined were changes in firmness, weight loss, volume reduction, and density as a percentage during the pre- storage and post-storage stages. All experiments were replicated three times and the results were analyzed using factorial experiments and in a completely randomized design using SAS statistical software and finally, the comparison of means test was plotted on a graph.

Results and Discussion

The results of the analysis of variance for the effects of loading, coating, and packaging factors are shown in Table 1. All three factors showed significant effects on the percentage change in persimmon fruit volume at a statistical significance level of 1%. Additionally, the interaction effects of packaging and coating were found to be significant at a statistical significance level of 5%. Regarding the weight loss percentage, both coating and packaging factors exhibited statistical differences at probability levels of 5% and 1%, while the load force factor and the interaction effects of the factors were not found to be significant. The statistical study of the percentage of density changes indicated that the load force factor has a significant effect on this dependent factor at a 5% level of significance. Furthermore, the coating and packaging factors demonstrated significant effects at a 1% level of significance. No significant effect was observed on the interaction of independent factors regarding the percentage of density changes. Finally, the statistical study of persimmon firmness revealed that all three independent factors- loading force, coating, and packing- significantly affect fruit firmness at a 1% level of significance. Additionally, the interaction effect of loading and coating, as well as the interaction effects of packaging and coating, were found to be significant at a 5% level of significance.

Table 1- Analysis of loading, coating and packaging variance for the parameters of the percentage change in weight, volume, density and firmness of persimmons during storage

	Firmness		Weight loss percentage	
	Mean square	**F value**	**Mean square**	**F value**
Loading	13.43	67.70**	0.496	2.52ns
Coating	27.05	133.36**	0.559	2.85*
Packing	28.76	144.94**	13.93	70.78**
Loading × coating	0.677	3.41*	0.004	0.02ns
Loading × packing	0.22	1.12	0.007	0.04ns
Packing × coating	0.532	2.69*	0.044	0.23ns
CV	11.23		19.36	
	Volume loss percentage		Density loss percentage	
Loading	5.58	17.40**	2.75	6.10*
Coating	26.10	81.30**	19.05	42.24**
Packing	29.40	91.60**	6.05	13.41**
Loading × coating	0.078	0.25	0.115	0.25
Loading × packing	0.23	0.72	0.154	0.34
Packing × coating	0.817	2.55*	0.849	1.88
CV	13.61		25.89	

** Significant difference at the statistical level of 1%, * Significant difference at the statistical level of 5%, ns no significant difference

Firmness

The results of the interaction effect of loading force and coating are shown in Fig. 3. Based on the figure, it can be said that increasing the loading force has reduced the firmness of persimmon fruit and in fruits treated with putrescine coating, the amount of reduction has been less than other coatings and

the use of putrescine coating has improved the firmness of the samples. The reason for this can be explained by the fact that the use of coating has caused a decrease in the respiration rate of stored fruits, thereby delaying their ripening. When a film or layer of polyamine coating is applied to fruits, it reduces the exchange of ethylene and moisture. This reduction in gas exchange includes a decrease in the infiltration of oxygen and air into the fruit, as well as a decrease in ethylene emission. As a result, the synthesis of ethylene decreases, leading to a reduction in the respiration rate of the fruits and, consequently, a decrease in their ripening process. Meighani *et al.* (2015)on pomegranate (Meighani *et al*., 2014) and Mirdehghan et al.(2016) on grape (Mirdehghan *et al*., 2016) who used polyamine coating reported similar results. There was no significant difference in the loading power of 150 N between different concentrations of polyamine, but these concentrations created a significant difference with the coating of distilled and uncoated water. However, the treatment of coating with distilled water and uncoated samplesdid not differ significantly in this force. For the loading force of 250 N, there was a significant difference between all the applied coatings, and the treatment of polyamine with a concentration of 1 mM was able to make a significant difference for the firmness of persimmon fruit. The highest firmness for persimmon fruit was observed in the coating treatment with 1 mM putrescine and the loading force of 150 with a value of 5.91 N and the lowest value was observed in the uncoated state and a loading force of 250 N with a value of 2.36 N. The reason for the changes in the firmness of the fruit with increasing loading can be explained that the force causes changes in the texture and cell wall of the fruit and any change in internal pressure will change the mechanical properties of the cell wall and consequently the whole tissue. In other words, over time and during product storage, by reducing the internal pressure at the cellular level, the stresses caused by mechanical shocks in the product tissue are reduced and as a result, the vulnerability of the product is increased (García *et al*., 1995).

According to the results obtained for the firmness shown in Fig. 4, the highest amount of fruit firmness was in treatment which used 1 mM of putrescine and for all three packages, the highest value was observed in this coating. For packing foam foil with polyolefine film, polyethylene-terephthalate, and ordinary box, respectively, the highest amount was obtained in 1 mM coating with values of 6.5, 5.6 and 3.95 N. Also, the lowest values were obtained for all three packages in the uncoated state. In the foam container with polyolefin film, a significant difference was obtained between all four coating treatments and for the polyethylene-terephthalate container, no significant difference was observed between the putrescine concentrations. However, the coating of putrescine is significantly different from other coatings, and for the packaging mode with the ordinary box, there was no significant difference in the treatment of putrescine.

There was no significant difference between uncoated and distilled water. Also in the foam container with polyolefin film, persimmon has a limited space compared to other types of packaging, this in itself causes the ethylene content to be lower due to the limited space created and the same controlled space in this package has reduced the amount of ethylene, and this has caused the firmness of the samples in this type of packaging to be higher than other packages. Barmen *et al*. 2011 reported similar results, stated that polyamines maintain the strength of the fruit in their cross-links to the carboxyl group, this is attributed to the cell wall, which strengthens the firmness of the fruit. On the other hand, the use of polyamine binds it to pectin, which blocks and restricts the access of enzymes that destroy cell walls such as pec-methylesterase, pectinesterase, and polygalacturonase, thus lowering the softening speed during storage and Warehousing (Barman *et al*., 2011). Mirdehghan *et al*. 2007 reported a significant effect of polyamine on

pomegranate fruit firmness (Mirdehghan *et al.*, 2007).

Physiological Weight Loss

Fig. 5 shows a comparison of persimmon weight loss between packaging and coating. According to the results shown in Fig. 5-A, using foam container with polyolefin film led to lower percentage of weight loss than the other two types of packaging and had a significant difference with the other two packages. In samples containing coating, 1 mM coating resulted in a lower percentage of weight loss than other coatings, and no significant difference was observed between the water coatings and 2 mM polyamine. However, the weight loss percentage of uncoated persimmons differs significantly from other coatings as shown in Fig. 5-B. Fresh fruits lose 3 to 10 percent of their total weight after harvest, and this weight loss in crops may be due to loss of moisture, evaporation, and respiration, which in turn reduces fruit weight (Shiri *et al.*, 2013). Also, the loss of fruit weight during storage is due to the exchange of water between indoor and outdoor space, which accelerates the rate of transpiration by cellular degradation, which causes moisture to escape from the samples faster. On the other hand, the loss of water inside the fruit tissue occurs mainly through the cuticle or the physical properties of the fruit or both. For weight loss, the loss of carbon in each respiratory cycle can be considered an important reason for these changes. In fact, the purpose of using coating on the fruit is to prevent the internal moisture of the fruit from evaporating faster (Emamifar *et al.*, 2017).

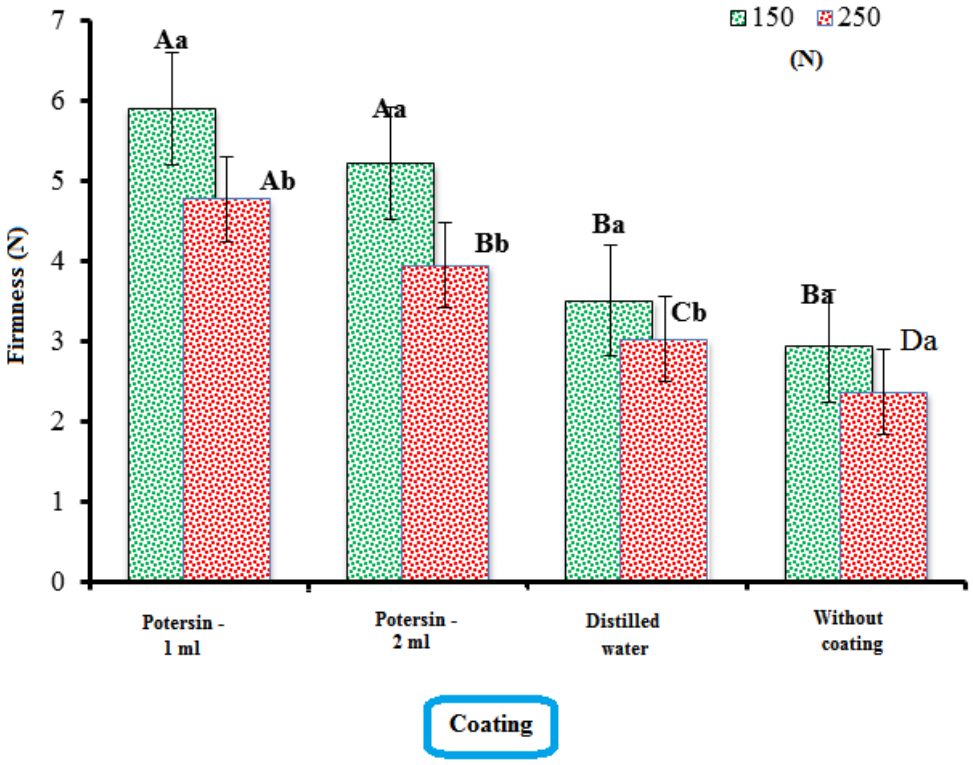


Fig. 3- Interaction of coating and loading on the firm content of persimmon fruit

Large letters similar to no significant difference in a fixed force and small letters similar to no significant difference in a fixed coating

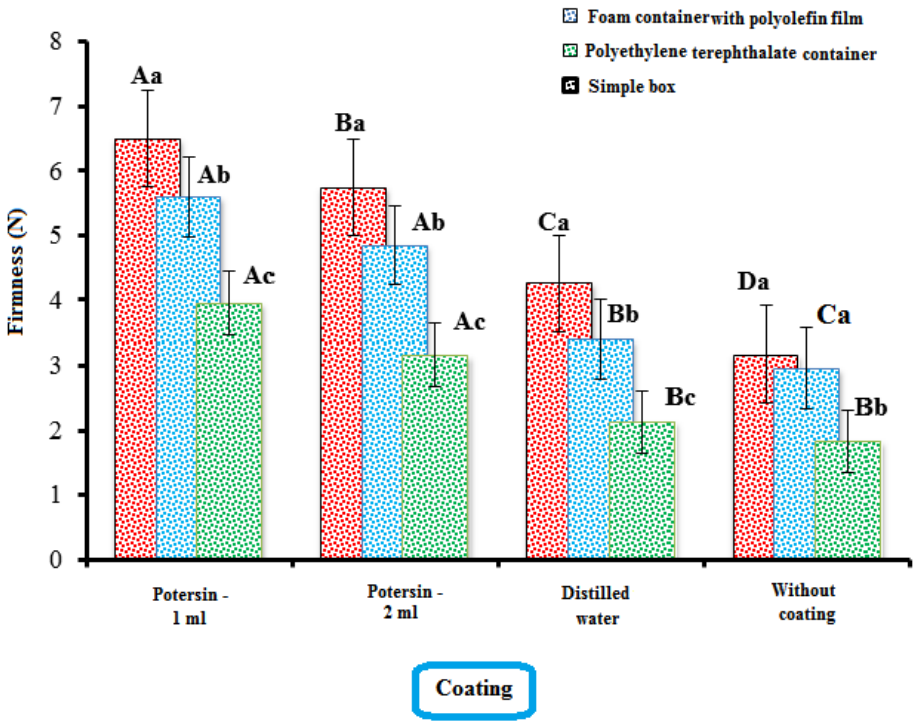


Fig. 4- Interaction of coating and packaging on the firm content of persimmon fruit
Large letters similar to no significant difference in a fixed package and small letters similar to no significant difference in a fixed coating

This is why a significant difference is seen on the use of polyamine coating compared to the control sample. The lowest percentage of weight loss was obtained from foam packaging with polyolefin film with 2.45% and in polyamine coating, 1 mM was obtained with a value of 2.098% and the highest amount was obtained in 4.95 and 4.94%, respectively, in ordinary and uncoated boxes. The results are similar to those of Patel *et al*. (2019) on the effect of coating and storage on pepper (Patel *et al*., 2019) and Champa *et al*. (2014) on grapes with polyamine coating (Champa *et al*., 2014).

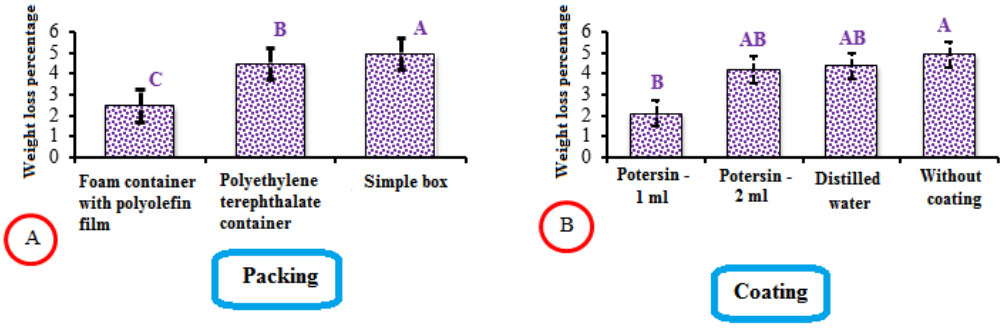


Fig. 5- Comparison of average
A: Packaging B: coating on the percentage of weight loss
The same letters in each part A and B indicate no significant difference

Volume changes

Fig. 6 shows the results of the effect of coating and type of packaging on the percentage change in persimmon fruit volume. Due to the shape of the 1 mM polyamine coating, there is a significant difference between all three boxes. This means that the use of 1 mM polyamine coating caused the number of percentage changes in volume in the type of packaging with "foam container and polyolefin film" to be less. There is no significant difference between this container and the polyethylene-terephthalate container in other coatings. However, the two packages show a significant difference from packing with an ordinary box for the percentage change in persimmon fruit volume. It can be stated that over time, due to environmental factors, moisture is removed and has a direct effect on reducing the volume during storage time (Strik *et al.*, 1998). The highest amount of volume changes was observed in the type of packaging of ordinary boxes and non-coated samples with a value of 6.954% and the lowest value was observed with a value of 1.824 in the packaging with foam container with polyolefin film in a 1 mM putrescine coating. Fig. 6-B showed that increasing the loading force had a significant effect on the number of changes in persimmon volume. The reason for this can be stated that by creating pressure and loading, the rate of destruction of the internal structure increases and causes the loss of interstitial water of the product, as a result the volume of the product decreases (Harker *et al.*, 1994).

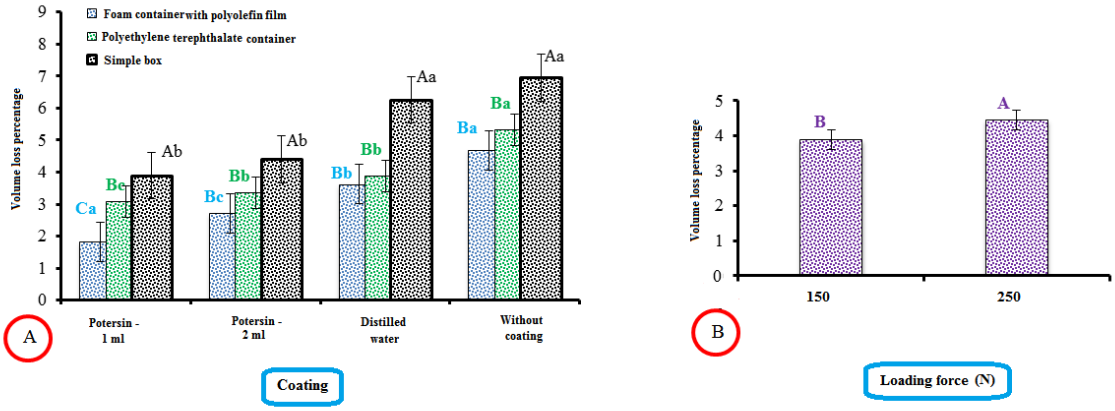


Fig. 6- A: The interaction effect of coating and packaging B: Comparison of the average load force on the percentage of volume changes

Large letters similar to no significant differences in a fixed coating and small letters similar to no significant differences in a fixed package

Density changes

The percentage change in density is shown in Fig. 7. Considering the results presented in Fig. 7-A, the use of polyamine putrescine caused a significant difference compared to other coatings. There was a significant difference between the putrescine concentrations used. The lowest density changes was by1 mM coating used (0.833%) and the highest percentage of density changes was 3.156% in non-coated samples. There was a significant difference between the four coatings applied. Bakhshi *et al.*, Who conducted a study on apples in 2011, found a similar result that storage reduced the density of fruits and the reason for this is the destruction of pectin between cells that have spread around after destruction and they are connected to smaller walls, which enlarges the space between the cells (Bakhshi Khaniki *et al.*, 2012). Fig. 7-B shows the difference in the type of packaging. The use of foam container

packaging with polyolefin and polyethylene films did not differ significantly from each other. However, the percentage of changes in the foam container with polyolefin film was less than the polyethylene-terephthalate container and this amount was 1.44%. However, using an ordinary box had the highest percentage of density changes with a value of 2.43%, which is statistically had significant difference with the other two types of packaging. This might due to the fact that in both types of foam packaging with polyolefin and polyethylene film, the difference in moisture does not exceed one value, and in such a way that persimmons can no longer loss more moisture. This reduces the weight of the specimens and due to the fact that the weight and density have a direct relationship with further weight loss, the density decreased. Fig. 7-C showed the percentage of density changes in different loading forces. Increase in loading force led to increase the rate of density changes with significant difference between the loading forces.

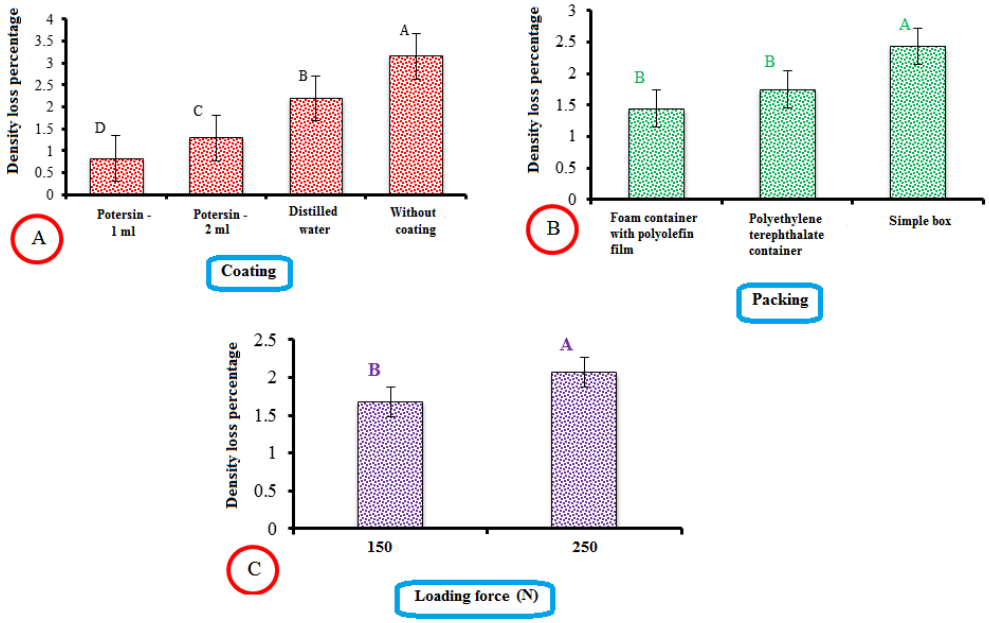


Fig. 7- A: Comparison of mean coating B: Comparison of mean packaging
C: Comparison of Average Load Force on Percentage of Density Changes
The same letters in each part A, B and C mean no significant difference

Conclusion

According to the results obtained from the study of three factors of loading, coating, and packing force, and their impact on the dependent variables of firmness, weight loss percentage, volume, and density, it can be concluded that packaging had a greater influence on firmness and percentage reduction in volume compared to the other two factors. After packaging, coating and loading showed an effect on these variables. In terms of the percentage of density reduction, coating had a more significant impact, followed by independent packing and loading force, which affected the density reduction percentage. Regarding fruit firmness, it was observed that using 1 mM putrescine coating and packaging the fruits with a foam container and polyolefin film resulted in up to twice the rigidity compared to uncoated fruits when using the same packaging. This indicates the effect of coating and the use of this type of packaging, which is observed in both loaded and control fruits. In terms of physiological weight loss, the utilization of equal concentration and packaging density resulted in a twofold

decrease in the percentage of changes compared to uncoated sample. For physiological weight loss, the most significant weight changes was observed with the same coating and package in putrescine coating. However, for the percentage decrease in volume, the use of putrescine coating with a concentration of 1 mM and packaging with foam container and polyolefin film resulted in a volume reduction in the samples that was three times lower than the non-coated samples. This highlights the significant impact of this particular packaging and coating. Finally, in terms of the percentage decrease in density, the use of 1 mM of putrescine coating resulted four times fewer density changes than uncoated sample and the use of packaging with foam and polyolefin film reduced the density changes twice compared to the ordinary box. Overall, the use of putrescine coating treatment at a concentration of 1 mM and packaging with foam and polyolefin film can be considered the best coating and packaging for persimmons, regardless of the loading forces.

References

1. Aidoo, R.P., Afoakwa, E.O., & Dewettinck, K. (2015). Rheological properties, melting behaviours and physical quality characteristics of sugar-free chocolates processed using inulin/polydextrose bulking mixtures sweetened with stevia and thaumatin extracts. *LWT- Food Science and Technology*, *62*(1), 592–597.
2. Ardakani, M.D., Mostofi, Y., & Hedayatnejad, R. (2010). Study on the effect of chitosan in preserving some qualitative factors of table grape (*Vitis vinifera*). *6th International Postharvest Symposium*, 8–12.
3. Azadbakht, M., Mahmoodi, M.J., & Vahedi Torshizi, M. (2019a). Determination of pear bruises due to impact loading via computed tomography scan method and relation them with physical properties pear. *Iranian Journal of Biosystems Engineering*, 50(2), 451-462. https://doi.org/10.22059/IJBSE.2019.255847.665052
4. Azadbakht, M., Vahedi Torshizi, M., & Mahmoodi, M.J. (2019b). The relation of pear volume and it's bruised volume by CT scan imaging. *Journal of Food Measurement and Characterization*, *13*(3), 1089–1099. https://doi.org/10.1007/s11694-018-00024-0
5. Azabakht, M.,Vahedi Torshizi, M., & Asghari, A. (2019c). Biological properties classification of pear fruit in dynamic and static loading using artificial neural network. *Innovative Food Technologies*, *6*(4), 507-520. https://doi.org/10,22104/JIFT.2018.3185.1764
6. Azadbakht, M., Moghbeli, H.R., & Torshizi, M.V. (2019d). Investigating the various effects of pear packaging box design factors subject to stress forces. *International Journal of Fruit Science*, *20*, 39-58 https://doi.org/10.1080/15538362.2019.1573161
7. Azadbakht, M., Mahmoodi, M.J., & Vahedi Torshizi, M. (2019e). Effects of different loading forces and storage periods on the percentage of bruising and its relation with the qualitative properties of pear fruit. *International Journal of Horticultural Science and Technology*, *6*, 177–188. https://doi.org/10,22059/ijhst.2019.280000.290
8. Azadbakht, M., Vehedi Torshizi, M., Ghajar jazi, E., & Ziaratban, A. (2016). Application of artificial neural network (ANN) in predicting mechanical properties of canola stem under shear loading. *Agricultural Engineering International*, *18*(2), 413–425.
9. Bakhshi Khaniki, G., Ghorbanli, M., & Mirbagheri, S. (2012). Biochemical changes of two cultivars of golab and Shafiabadi apple at harvesting time and after storing. *New Cellular and Molecular Biotechnology Journal*, *2*(5), 59–65.
10. Barman, K., Asrey, R., & Pal, R.K. (2011). Putrescine and carnauba wax pretreatments alleviate chilling injury, enhance shelf life and preserve pomegranate fruit quality during cold storage. *Scientia Horticulturae*, *130*(4), 795–800. https://doi.org/10.1016/j.scienta.2011.09.005

11. Cangi, R., Saracoglu, O., Altuntas, E., & Kaya, C. (2011). Some chemical and physical properties at physiological maturity and ripening period of kiwifruit ('Hayward'). *African Journal of Biotechnology*, *10*(27), 5304–5310. https://doi.org/10.5897/AJB11.192
12. Champa, H.A.H., Gill, M.I.S., Mahajan, B.V.C., & Arora, N.K. (2014). Postharvest treatment of polyamines maintains quality and extends shelf-life of table grapes (*Vitis vinifera* L.) cv. Flame Seedless. *Postharvest Biology and Technology*, *91*(May), 57–63. https://doi.org/10.1016/j.postharvbio.2013.12.014
13. Emamifar, A., & Bavaisi, S. (2017). Effect of mixed edible coatings containing gum tragacanth and *Aloe vera* on postharvest quality of strawberries during storage. *Iranian Journal of Food Science and Technology*, *13*(3), 39–54. https://doi.org/10.22067/ifstrj.v1396i3.62317
14. García, J.L., Ruiz-Altisent, M., & Barreiro, P. (1995). Factors Influencing Mechanical Properties and Bruise Susceptibility of Apples and Pears. *Journal of Agricultural Engineering Research*, *61*(1), 11–17. https://doi.org/10.1006/jaer.1995.1025
15. Harker, F.R., & Hallett, I.C. (1994). Physiological and mechanical properties of kiwifruit tissue associated with texture change during cool storage. *Journal of the American Society for Horticultural Science*, *119*(5), 987–993.
16. Jeong, S.T., Goto-Yamamoto, N., Kobayashi, S., & Esaka, M. (2004). Effects of plant hormones and shading on the accumulation of anthocyanins and the expression of anthocyanin biosynthetic genes in grape berry skins. *Plant Science*, *167*(2), 247–252. https://doi.org/10.1016/j.plantsci.2004.03.021
17. Masoudi, H., Tabatabaeefar, A., & Borghei, A. (2006). Mechanical properties of three varieties of apples after five months storage. *Food Engineering Research*, *27*(7), 61–74.
18. Meighani, H., Ghasemnezhad, M., & Bakhshi, D. (2014). Effect of different coatings on post-harvest quality and bioactive compounds of pomegranate (*Punica granatum* L.) fruits. *Journal of Food Science and Technology*, *52*(7), 4507–4514. https://doi.org/10.1007/s13197-014-1484-6
19. Mirdehghan, S.H., Rahemi, M., Castillo, S., Martínez-Romero, D., Serrano, M., & Valero, D. (2007). Pre-storage application of polyamines by pressure or immersion improves shelf-life of pomegranate stored at chilling temperature by increasing endogenous polyamine levels. *Postharvest Biology and Technology*, *44*(1), 26–33. https://doi.org/10.1016/j.postharvbio.2006.11.010
20. Mirdehghan, S.H., & Rahimi, S. (2016). Pre-harvest application of polyamines enhances antioxidants and table grape (*Vitis vinifera* L.) quality during postharvest period. *Food Chemistry*, *196*, 1040–1047. https://doi.org/10.1016/j.foodchem.2015.10.038
21. Opara, U.L., Atukuri, J., & Fawole, O.A. (2015). Application of physical and chemical postharvest treatments to enhance storage and shelf life of pomegranate fruit-A review. *Scientia Horticulturae*, *197*, 41–49. https://doi.org/10.1016/j.scienta.2015.10.046
22. Patel, N., Gantait, S., & Panigrahi, J. (2019). Extension of postharvest shelf-life in green bell pepper (*Capsicum annuum* L.) using exogenous application of polyamines (spermidine and putrescine). *Food Chemistry*, *275*, 681–687. https://doi.org/10.1016/j.foodchem.2018.09.154
23. Shiri, M.A., Ghasemnezhad, M., Bakhshi, D., & Sarikhani, H. (2013). Effect of postharvest putrescine application and chitosan coating on maintaining quality of table grape cv. "shahroudi" during long-term storage. *Journal of Food Processing and Preservation*, *37*(5), 999–1007. https://doi.org/10.1111/j.1745-4549.2012.00735.x
24. Strik, B.C., & Cahn, H. (1998). *Growing kiwifruit*.
25. Vahedi Torshizi, M., & Azadbakht, M. (2020). Study on Firmness and texture changes of pear fruit when loading different forces and stored at different periods using artificial neural network.

Iranian Food Science and Technology Research Journal, *15*(6), 113–132. https://doi.org/10.22067/ifstrj.v15i6.75049

26. Veberic, R., Jurhar, J., Mikulic-Petkovsek, M., Stampar, F., & Schmitzer, V. (2010). Comparative study of primary and secondary metabolites in 11 cultivars of persimmon fruit (*Diospyros kaki* L.). *Food Chemistry*, *119*(2), 477–483. https://doi.org/10.1016/j.foodchem.2009.06.044
27. Yurtlu, Y.B., & Erdoğan, D. (2005). Effect of storage time on some mechanical properties and bruise susceptibility of pears and apples. *Turkish Journal of Agriculture and Forestry*, *29*(6), 469–482.
28. Zahedi, S.M., Hosseini, M.S., Karimi, M., & Ebrahimzadeh, A. (2019). Effects of postharvest polyamine application and edible coating on maintaining quality of mango (*Mangifera indica* L.) cv. Langra during cold storage. *Food Science and Nutrition*, *7*(2), 433–441. https://doi.org/10.1002/fsn3.802

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد ۱۹، شماره ۳، مرداد- شهریور ۱۴۰۲ ص. ۱۴-۱

Iranian Food Science and Technology Research Journal
Vol. 19, No. 3, Aug.-Sep. 2023, p. 1-14

تأثیر انواع پوشش و بسته‌بندی بر روی خصوصیات فیزیکی میوه خرمالوی تحت بار

سجاد جعفرزاده[۱]- محسن آزادبخت[۲]*- فریال وارسته[۳]- محمد واحدی ترشیزی[۴]

تاریخ دریافت: ۱۴۰۱/۱۲/۲۶
تاریخ بازنگری۱۴۰۲/۰۳/۰۵
تاریخ پذیرش: ۱۴۰۲/۰۳/۰۶

چکیده

با توجه به اینکه خرمالو یک میوه‌ی حساس به فشار بوده و نگهداری این میوه در انبارها به سختی انجام می‌شود، در این تحقیق سعی شده به بررسی پارامترهای موثر بر کاهش تغییرات خصوصیات فیزیکی آن پرداخته شود. این پارامترها نیروی بارگذاری در ۲ سطح ۱۵۰ و ۲۵۰ نیوتن، سه نوع بسته‌بندی ظرف فومی با فیلم پلی‌اولفین، پلی‌اتیلن ترفتالات و جعبه‌ی معمولی و چهار نوع پوشش پلی آمین پوترسین با غلظت‌های ۱ و ۲ میلی‌مولار، آب مقطر و بدون پوشش بود. خصوصیاتی مانند درصد تغییرات وزن، حجم و چگالی میوه خرمالو و همچنین سفتی این میوه در مرحله‌ی قبل و بعد از انبارداری بررسی شد. با توجه به نتایج بدست آمده برای سفتی میوه‌ها، بیشترین مقدار سفتی در تیمار پوترسین با غلظت ۱ میلی‌مولار و پوشش ظرف فومی با فیلم پلی‌اولفین با مقدار ۶/۵ نیوتن بدست آمد که تقریبا سه برابر سفتی میوه‌های بدون پوشش بود. کمترین میزان درصد کاهش وزن، حجم و چگالی نیز در همین نوع پوشش و بسته‌بندی بدست آمد که مقادیر آن به ترتیب ۲/۴۵۸، ۱/۸۲ و ۰/۸۳۳ ٪ نسب به روز اول انبارداری می‌باشد. در مجموع استفاده از تیمار پلی‌آمین تاثیر مثبتی جهت تغییرات خصوصیات فیزیکی میوه‌ی خرمالو را نشان داد و بهترین ظرف نوع بسته‌بندی که کمترین تغییرات را ایجاد کرد ظروف فومی با فیلم پلی‌اولفین بود.

واژه‌های کلیدی: انبارداری، بارگذاری، بسته‌بندی، پلی‌آمین، خرمالو، خواص فیزیکی

۱، ۲ و ۳- به‌ترتیب دانشجوی کارشناسی ارشد، دانشیار و استادیار گروه مهندسی مکانیک بیوسیستم، دانشگاه علوم کشاورزی و منابع طبیعی گرگان
(* نویسنده مسئول: Email: azadbakht@gau.ac.ir)
۴- دانشجوی دکتری گروه مهندسی بیوسیستم، دانشگاه تربیت مدرس، تهران، ایران

DOI: 10.22067/ifstrj.2023.81571.1242

Iranian Food Science and Technology Research Journal
Vol. 19, No. 3, Aug.-Sep. 2023, p. 15-31

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد ۱۹، شماره ۳، مرداد- شهریور ۱۴۰۲ ص. ۳۱-۱۵

Research Article
Homepage: http://ifstrj.um.ac.ir

Encapsulation of Coenzyme Q_{10} by Gelatin–basil Seed Mucilage Using Complex Coacervation: Optimization, Physicochemical Characterizations and Milk Fortification

S. Ramezani[1], M. Shahedi[2], M. Fathi[3]*

Received: 2023.03.17
Revised: 2023.06.14
Accepted: 2023.06.28
Available Online: 2023.06.28

How to cite this article:
Ramezani, S., Shahedi, M., & Fathi, M. (2023). Encapsulation of coenzyme Q_{10} by gelatin–basil seed mucilage using complex coacervation: Optimization, physicochemical characterizations and milk fortification. *Iranian Food Science and Technology Research Journal, 19*(3), 15-31. https://doi.org/10.22067/ifstrj.2023.81438.1240

Abstract

Global concern about human health and the increase the prevalence of chronic diseases in recent years lead to growing appeals for nutritious and healthy compounds, such as coenzyme Q_{10}. Susceptibility to heat and lipophilic properties of coenzyme Q_{10} limit its utilization in food. Encapsulation is a technology that protects bioactive ingredients from harsh environmental conditions and extends shelf life. The purpose of this study was to encapsulate coenzyme Q_{10} using complex coacervation by gelatin–basil seed mucilage and characterize physical, thermal and chemical properties of produced microcapsules. Response surface methodology was applied to determine the optimum level of the four formulation variables for maximum encapsulation efficiency, loading capacity and turbidity and minimum supernatant absorption. The optimum microcapsules had encapsulation efficiency of 83.69%, encapsulation load of 16.32%, turbidity of 0.979 and supernatant absorption of 0.227. The microcapsules were assessed by scanning electron microscopy, Fourier transform infrared spectroscopy, and differential scanning calorimetry. The results of FTIR confirmed the formation of coacervates. The thermogram of Q_{10} loaded microcapsule melting point was not observed at its melting point (50°C) due to its solubility in the oil phase and appropriate entrapment. Release behavior of Q_{10} was studied by different mathematical models. Microencapsulated Q_{10} was used to fortify milk and the results showed that the developed protein-carbohydrate microcapsules can be applied for protection of hydrophobic compounds.

Keywords: Basil seed mucilage, Coenzyme Q_{10}, Encapsulation, Gelatin, Physicochemical characterizations

Introduction

Coenzyme Q_{10} was first discovered by Professor Fredricke L. Crane in 1957. It is a natural and efficient antioxidant that is found in the human body in both of reduction and oxidation form (Wang *et al.*, 2023). The Q_{10} is one of the main parts of electron transport chain in the mitochondria. It has an essential function in metabolism of mitochondrial energy and is responsible for converting energy from carbohydrate, protein, fatty acid to adenosine triphosphate (ATP). The Q_{10} is a reinforcer of immune function and it is an improver of muscular dystrophy, exercise capacity, heart function and overall quality of life (Li *et al.*, 2023).

Microencapsulation is a technology that protects various food ingredients from the environment and controls their release to target specific sites or meliorates their flow and

1, 2 and 3- M.Sc. Student, Professor and Associate Professor, Department of Food Science and Technology, College of Agriculture, Isfahan University of Technology, Isfahan, Iran, respectively.

(*- Corresponding Author Email: mfathi@iut.ac.ir)
DOI: 10.22067/ifstrj.2023.81438.1240

organoleptic properties. Various methods for preparation of microcapsules have been widely introduced, such as the phase separation, extrusion method, complex coacervation and polycondensation. Among these, complex coacervation is considered an excellent method for microencapsulation because of the simple experiment process and high loading capacity. Complex coacervation is used in food industry for encapsulation of bioactive ingredients. When two or more oppositely charged polyelectrolytes are combined under a reasonable condition, two phases are created, including polymer rich phase and polymer poor phase. The former phase is a dense soluble that participates in the process of encapsulating (Yang *et al.*, 2015). Many studies have been reported on complex coacervation of proteins and polysaccharides, such as gelatin/gum Arabic (Dong *et al.*, 2011), soybean protein isolate/pectin (Hu *et al.*, 2023), whey protein/gum Arabic (Sharifi *et al.*, 2021), canola protein isolate/chitosan (Chang *et al.*, 2016), gelatin/acacia gum (Nakagawa and Nagao, 2012) and gelatin/pectin (Byeon *et al.*, 2023).

Basil seed is obtained from the basil plant (*Ocimum basilicum*), which belongs to the Labiatae family. It has high mucilaginous components, which is classified as anion hydrocolloids. The mucilage of basil seed has been used in the food industry as a functional ingredient because of its potential applications as a thickening and stabilizing properties (Hosseini-Parvar *et al.*, 2010; Razavi *et al.*, 2010).

To the best of our knowledge, there is no work on complex coacervation involving basil seed mucilage and gelatin. Basil seed mucilage and gelatin are appropriate to be used to form coacervates as gelatin is positively charged below its isoelectric point, while basil seed mucilage is negatively charged in a wide pH range. Therefore the aims of this research were to encapsulate coenzyme Q_{10} by complex coacervation using basil seed mucilage and gelatin and characterize physicochemical properties of produced microcapsules. Effects of the gelatin percentage, basil seed mucilage percentage, oil phase percentage, and pH on the encapsulation efficiency (EE), encapsulation load (EL), turbidity, and supernatant absorbance were also assessed. Scanning electron microscopy, Fourier transform infrared (FTIR) spectroscopy, and differential scanning calorimetry (DSC) were used to characterize microcapsule properties.

Materials and Methods

Materials

Bovine gelatin was obtained from Global Capsule Company (Bangladesh). Basil seed was purchased from a local market (Esfahan, Iran). Coenzyme Q_{10} was obtained from Zhejiang Nhu Company (China). Sunflower oil was provided from Varamin Company (Iran). N-hexane was obtained from Sepahan Company (Iran). Methanol and n-hexane were HPLC grade and obtained from Merck Company (Germany).

Mucilage extraction

The extraction of basil seeds mucilage was carried out based on a method of Hosseini-Parvar, et al. (2010) with some modifications. The mucilage extraction was performed at 65 ^{0}C, pH=8 (0.1 M NaOH and HCl) and water to seed ratio of 50: 1 for 20 min. The seeds were passed through an extractor (Pars Khazar P700, Iran) with a rotating rough plate that scraped the mucilage layer on the seed surface to separate the mucilage from swollen seeds. Then the mucilage was collected and the impurities extracted in extraction mucilage were removed by filtering with cloth filter and centrifuging (Hermle Labortechnik GmbH Z 36 HK, Germany) at 12800 g for 20 min at 20 ^{0}C. It was then freeze-dried (Dena, Iran) for 24 h at -40 ^{0}C. Finally, the samples were kept in plastic bags at room temperature (Hosseini-Parvar *et al.*, 2010; Razavi *et al.*, 2010).

Optimization and statistical analysis

Formulation of samples was designed based on four independent variables (gelatin percentage of 1 to 3%, mucilage percentage of

0.5 to 1%, oil phase percentage of 0.4 to 0.8%, and pH of 3.3 to 4.2). Box-Behnken design was implemented to evaluate the effect of gelatin percentage, mucilage percentage, oil phase percentage, and pH on physicochemical properties of microcapsules. Twenty five different runs were proposed by Design Expert Software version 7.0.0 (Stat-Ease Inc., Minneapolis, MN) (Table 1). All the independent variables were kept within range. The analysis of variance (ANOVA) was used to identify significant parameters. Differences of $p<0.01$ were regarded to be significant.

The encapsulation method

Encapsulation was performed according to Silva, Favaro-Trindade, Rocha, and Thomazini (2012) method with some modifications. An oily dispersion, consisting of 20% Q_{10}, was prepared with sunflower oil. It was added to the aqueous solution of gelatin (based on the percentages provided in Table 1 at 40^0C and agitated in 14000 rpm for 3 min by a homogenizer (Ultra Turex T18, Germany). A 0.1 M NaOH was used to adjust the the pH at 8. Then an aqueous solution of basil seed mucilage (based on the percentages provided in Table 1 was added to the emulsion at 40 ^{0}C. The mixture was stirred using a magnetic stirrer (RH basic 2; IKA® Works) for 10 min and 0.1 M HCl was used to adjust the pH. The coacervate particles were formed by decreasing pH. The turbidity and supernatant absorption were determined by spectrophotometer (PG instruments, T60UV, United Kingdom) at 600 nm. The system was kept at 10 ^{0}C for 12 h. Then, the supernatant was separated and coacervate was freeze-dried (Dena, Iran) at 40 ^{0}C for 24 h. The freeze-dried material was transferred in a glass jar and aluminum foil was used to keep it away from light (Silva *et al.*, 2012).

Encapsulation efficiency

To determine encapsulation efficiency (EE) and encapsulation load (EL), hexane extractable surface and total oil in microcapsule were evaluated. To evaluate surface oil, 0.5 g microcapsule powder was added to 5 ml hexane and it was hand shaken for 5 min. The mixture was then centrifuged (SIGMA Laboratory Centrifuge 3-18, Germany) for 5 min at 2000 rpm. The supernatant was attentively accumulated, filtered through Watman 42 paper filter and transferred to a pre-weighted round-bottom flask. Solvent was eliminated by a rotary evaporator (Hei-VAP; Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) at 60 ^{0}C. Surface oil was prescribed gravimetrically (Ifeduba & Akoh, 2016).

To evaluate total oil, 2 ml of 5 M HCl was added to 0.5 g of microcapsule powder in order to release the inner oil and it was agitated using a magnetic stirrer (RH basic 2; IKA® Works) at 60 ^{0}C for 1 h. the mixture was allowed to react at room temperature and then it was transferred to a decanter and twice extracted with 5 ml hexane. The supernatant was filtered through Watman 42 paper filter and transferred to a pre-weighted round-bottom flask. The solvent was eliminated by a rotary evaporator (Hei-VAP; Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) at 60^0C. Total oil was measured gravimetrically (Ifeduba *et al.*, 2016). The encapsulation efficiency (%) and encapsulation load (%) were determined based on the following formula (Calderón-Oliver *et al.*, 2017).

$$\text{Encapsulation efficiency (\%)} = \frac{Wt - Ws}{Wt} \times 100 \quad (1)$$

$$\text{Encapsulation load (\%)} = \frac{Wt - Ws}{Wm} \times 100 \quad (2)$$

Where W_t and W_s are the total and surface oil of the microcapsules and W_m is the mass (g) of the microcapsules.

Characterization of microcapsules

To observe the morphology of microcapsules by scanning electron microscope (Philips XL30, Poland), a sample was spread on one side of the double-sided adhesive tape and the other side was glued to a special metal plate. The microcapsules were covered in a vacuum chamber with gold atoms (Peng *et al.*, 2014).

The chemical structures of the microcapsules, Q_{10}, gelatin and mucilage were

analyzed by infrared spectroscopy in the region from 4000 - 400 cm^{-1} by using a Fourier transform infrared (FTIR) spectrometer (JASCO FT/IR-680 PLUS, China). Samples were mixed with KBr in a ratio of 1:100 before analysis (Butstraen and Salaün, 2014).

Thermal characteristics of the samples were determined using thermal analyzer (SPA 440, Germany). Samples were transferred to the aluminum pan and heated from 25 to 550 °C at a rate of 10 °C/min under nitrogen atmosphere (Rocha-Selmi *et al*., 2013). The OrginPro (2016 64 Bit) software was used to plot the DSC curves.

Milk was chosen as a food matrix to be enriched with Q_{10} loaded microcapsules. A 0.750 g of microcapsules powder was added to 750 ml milk. Ascorbic acid was used in order to avoid possible oxidation. For saponification, 50 ml KOH 50% was added to 50 ml milk and the sample was kept at 80°C using water bath (WB 22; Memmert GmbH & Co. KG, Schwabach, Germany) for 30 minutes with continuous agitation. The solution was transferred to a decanter and twice extracted with 100 ml hexane. The solvent of the transparent layer was evaporated using a rotary vacuum (Hei-VAP; Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) at 50°C. Finally, a certain amount of n-hexane was added to the oil (Escriva *et al*., 2002; Zamarreño *et al*., 1992). A high-performance liquid chromatography (HPLC) system (Shimadzu, Japan) equipped with column (C18-ods) and UV detector (FPD-6AV) was used to measure Q_{10}. Detection of the CoQ_{10} was implemented at 275 nm. Methanol and n-hexane (72:28 v/v) were used as the mobile phase with a flow rate of 1 ml/min (Karpińska *et al*., 2006).

Results and Discussion

Encapsulation efficiency and load

This is the first study to figure out the possibility of encapsulating coenzyme Q_{10} using gelatin and basil seed mucilage as wall materials by complex coacervation. Microencapsulation by coacervation occurs by the phase separation of a homogeneous polymer solution into polymer-rich and polymer-poor phases (Silva *et al*., 2012).

Gelatin has a high emulsifying activity and can ionize $-NH_3^+$ and $-COO^-$ in the aqueous solution (Yang *et al*., 2015). When oil was added to the aqueous solution of gelatin, oil in water emulsion was created. By adding an aqueous solution of basil seed mucilage and lowering the pH to below the isoelectric point of gelatin, gelatin became positively charged whereas basil seed mucilage, due to containing carboxyl groups, was negatively charged. Thus, coacervates were formed based on the complexation occurring from the mixture of solutions of substances with opposite charges (Yang *et al*., 2015). In order to develop coacervation more efficiently and to create the greatest possible interaction between the polymers, four formulation variables (gelatin percentage, mucilage percentage, oil phase percentage, and pH) that affect the complex coacervation were assessed.

The values of encapsulation efficiency, encapsulation load, turbidity, and supernatant absorbance were given in Table 1. Analysis of variance indicated that the quadratic model with R^2 =0.96 was adequate for predicting encapsulation efficiency (Table 2). The selected model was significant and gelatin concentration, mucilage concentration and oil content had significant effects on encapsulation efficiency ($P<0.01$). Coefficient estimate is a yardstick for comparing the effect of corresponding term in relation to other terms in the model (Saeidy *et al*., 2014). The highest effect on encapsulation efficiency was related to gelatin percentage because it had the maximum coefficient estimate (Table 2). Equation (3) represents experimental model for predicting encapsulation efficiency. The positive coefficients show the increasing and the negative ones show the decreasing effects of model terms on encapsulation efficiency. Therefore, mucilage percentage had an increasing effect and oil phase percentage had a decreasing effect on encapsulation efficiency.

EE (%) = 9.3370+9.0433A+42.61347B+37.07401C–

$$47.82858D+1.93AB+1.13333AC+9.42375AD+0.31556BC-0.1100BD-5.55833CD-3.77616A^2-25.35653B^2-5.06374C^2+15.56792D^2 \quad (3)$$

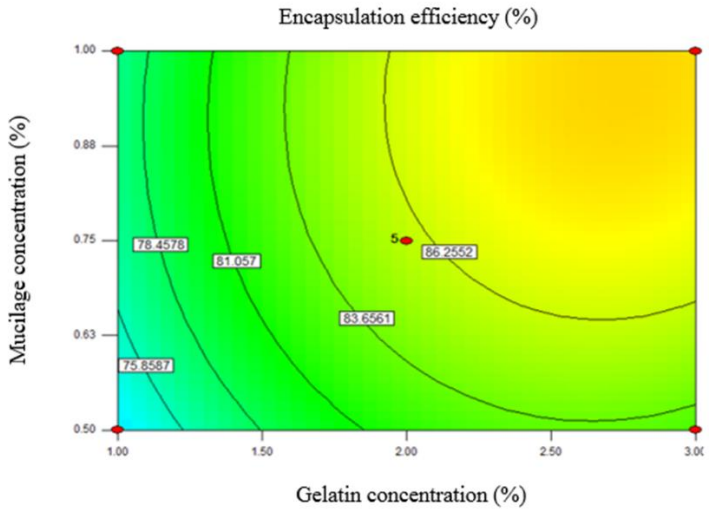
Where A, B, C and D are gelatin percentage, basil seed mucilage percentage, pH and oil phase percentage, respectively.

Table 1- The average values of encapsulation efficiency, encapsulation load, turbidity and supernatant absorbance for designed RSM

Run	Gelatin percentage	Mucilage percentage	Oil phase percentage	pH	Encapsulation efficiency (%)	Encapsulation load (%)	Turbidity	Supernatant absorbance
1	2	1	0.6	4.2	84.12	14.02	0.95	0.388
2	3	0.75	0.8	3.75	82.61	14.53	0.993	0.456
3	2	0.75	0.8	3.3	81.11	18.28	0.951	0.269
4	2	0.75	0.6	3.75	86.13	15.43	0.962	0.237
5	2	0.5	0.4	3.75	87.91	12.12	0.906	0.349
6	3	0.75	0.6	3.3	85.04	11.73	0.97	0.488
7	2	0.75	0.8	4.2	78.34	17.65	0.925	0.362
8	3	1	0.6	3.75	89.5	11.67	1.016	0.411
9	2	0.75	0.6	3.75	86.37	15.47	0.965	0.24
10	1	0.75	0.6	3.3	78.87	20.14	0.842	0.198
11	2	1	0.4	3.75	93.13	10.96	0.976	0.217
12	3	0.75	0.6	4.2	84.9	11.71	0.956	0.594
13	1	0.75	0.4	3.75	86.15	16.03	0.88	0.172
14	3	0.5	0.6	3.75	83.26	12.18	0.975	0.501
15	2	0.75	0.6	3.75	85.98	15.4	0.964	0.231
16	2	1	0.8	3.75	82.51	17.37	0.987	0.235
17	1	0.75	0.6	4.2	76.69	19.58	0.831	0.272
18	1	0.75	0.8	3.75	66.51	20.87	0.87	0.181
19	2	0.5	0.8	3.75	77.31	18.74	0.933	0.361
20	2	0.5	0.6	4.2	80.21	15.52	0.878	0.422
21	3	0.75	0.4	3.75	94.71	9.13	0.983	0.432
22	2	0.75	0.4	4.2	90.34	11.47	0.917	0.337
23	2	1	0.6	3.3	85.8	14.3	0.956	0.242
24	1	0.5	0.6	3.75	72	20.57	0.851	0.161
25	1	1	0.6	3.75	76.31	17.61	0.901	0.212
26	2	0.5	0.6	3.3	82.03	15.88	0.886	0.365
27	2	0.75	0.6	3.75	85.47	15.31	0.959	0.245
28	2	0.75	0.4	3.3	91.11	11.57	0.937	0.271
29	2	0.75	0.6	3.75	85.13	15.25	0.96	0.249
				Standard error	1.14	0.59	0.009	0.021

Wall to core ratio is an important factor in the final characteristics of the microcapsules (Hogan *et al*., 2001). Fig. 1 (A) shows the interaction of gelatin and mucilage concentrations on encapsulation efficiency. Increasing of wall materials led to enhance of encapsulation efficiency due to more available space for coenzyme Q10 to be entrapped. According to Fig. 1 (B), increasing of oil percentage from 0.4% to 0.8% resulted in a decrease in the encapsulation efficiency from 90.23% to 80.36%, which could be due to the shortage of wall materials for oil entrapment. Xiao *et al.* (2015) encapsulated styralyl acetate (SA) using gelatin and gum Arabic with complex coacervation method. They indicated that when the amount of SA remained 1.0 g and wall concentration increased from 0.5 to 1.3 %, encapsulation efficiency enhanced from 23 to 57 % (Xiao *et al*., 2015).

A



B

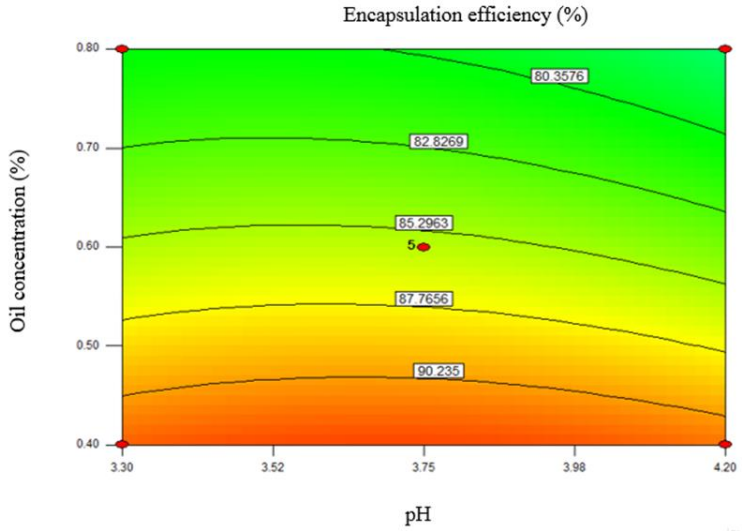


Fig. 1- Interaction of gelatin and mucilage concentrations (%) (A) and oil concentration (%) and pH (B) on encapsulation efficiency

Quadratic model with R^2=0.99 was appropriate to state encapsulation load (Table 2). Among the four independent variables, only pH had no significant effect on encapsulation load (P<0.01). According to Table 2, oil phase percentage had the highest coefficient in relation to other terms in the model.

EL (%) =8.51077-8.5565A-2.71435B+3.69399C+34.82916D+2.45061AB +0.29882AC+0.69747AD+0.16143BC-1.01307BD-1.46298CD+0.38153A^2-3.47631B^2-0.51904C^2-12.42667D^2 (4)

Where A, B, C and D are gelatin percentage, basil seed mucilage percentage, pH and oil phase percentage, respectively.

Table 2- Model parameters for the quadratic equation for prediction of encapsulation efficiency, encapsulation load, turbidity and supernatant absorbance

Source	Encapsulation efficiency (R^2=0.96)		Encapsulation load (R^2=0.99)		Turbidity (R^2=0.99)		Supernatant absorbance (R^2=0.97)	
	Coefficient estimate	P	Coefficient estimate	P	Coefficient estimate	P	Coefficient estimate	P
Model	85.82	< 0.0001	15.37	<0. 0001	0.96	< 0.0001	0.24	< 0.0001
A	5.29	< 0.0001	-3.65	< 0.0001	0.06	< 0.0001	0.14	<0.0001
B	2.39	0.0002	-0.76	< 0.0001	0.03	< 0.0001	-0.038	0.0002
C	-0.78	0.1253	-0.16	0.2366	0	0.0193	0.045	< 0.0001
D	-6.25	< 0.0001	3.01	< 0.0001	0	0.0973	0	0.3635
AB	0.48	0.5702	0.61	0.0167	0	0.6154	-0.035	0.0184
AC	0.51	0.5487	0.13	0.5607	0	0.9309	0	0.5546
AD	1.88	0.0394	0.14	0.5463	0	0.3045	0	0.7807
BC	0.036	0.9665	0.018	0.9370	0	0.9284	0.022	0.1144
BD	0	0.9948	-0.051	0.8256	0	0.4156	0	0.9112
CD	-0.5	0.5562	-0.13	0.5687	0	0.7534	0	0.6175
A2	-3.78	< 0.0001	0.38	0.0492	-0.025	<0.0001	0.061	<0.0001
B2	-1.58	0.029	-0.22	0.2403	0	0.0736	0.034	0.0057
C2	-1.03	0.1379	-0.11	0.5624	-0.034	< 0.0001	0.077	< 0.0001
D2	0.62	0.3555	-0.5	0.014	0	0.6294	0	0.5692

Fig. 2 (A) showed that in the constant concentration of oil, encapsulation load decreased from 19.09% to 13.21 % as wall materials concentration increased. Encapsulation load is the ratio of loaded CoQ_{10} to the weight of the final microcapsule; thus, the low EL can be ascribed to the thick wall of the capsules formed. By increasing the oil content, encapsulation load increased from 12.78% to 17.02 % due to the fact that wall materials were able to encapsulate more amount of oil (Fig. 2 (B). The result was similar from what was reported by Ahmadi *et al*. (2015) who applied β-Lactoglobulin/Arabic Gum complex coacervation. When the encapsulation load is low, the microcapsules have a larger wall thickness which ensures better protection of their core against environmental factors. However, high loading lead to placement of the core near the surface of the wall which results in a faster release to the surrounding environment (Calderón-Oliver *et al*., 2017).

Turbidity and supernatant absorbance

Turbidity and supernatant absorbance are reliable parameters to be taken into account when optimizing the complex coacervation process (Chang *et al*., 2016). Quadratic model with R^2=0.99 was suggested to explain turbidity (Table 2). Gelatin percentage and mucilage percentage had significant effects on turbidity (P<0.01). Equation (5) shows descriptive model for turbidity.

$$\text{Turbidity} = -1.81075+0.15696A+0.34321B+1.25882C+0.15102D-0.0096899AB-0.000925271AC+0.025128AD+0.00383722BC-0.079112BD-0.016786CD-0.025398A^2-0.11457B^2-0.16877C^2-0.045673D^2 \quad (5)$$

Where A, B, C and D are gelatin percentage, basil seed mucilage percentage, pH and oil phase percentage, respectively.

Turbidimetric assay enables visual evaluation of the behavior of polymers during complexation and simplifies the verification of complex formation (Prata and Grosso, 2015). The higher turbidity is desirable since it shows that more electrostatic complexes are formed. Fig. 3 (A) demonstrated that by decreasing the pH from 4.2 to 3.75, the turbidity increased that indicating the formation of dense microcapsules. A further decrease in the pH from about 3.75 to 3.3 led to lower turbidity that representing the low number of electrostatic complexes. The ionization degree of active groups dependents on solution's pH and in an effective pH, opposite charges are available which causes to their interaction (Jun-xia *et al*.,

2011). Similar results have been reported by Wang *et al.* for microencapsulation of tuna oil in gelatin- sodium hexametaphosphate using complex coacervation (Wang *et al.*, 2014).

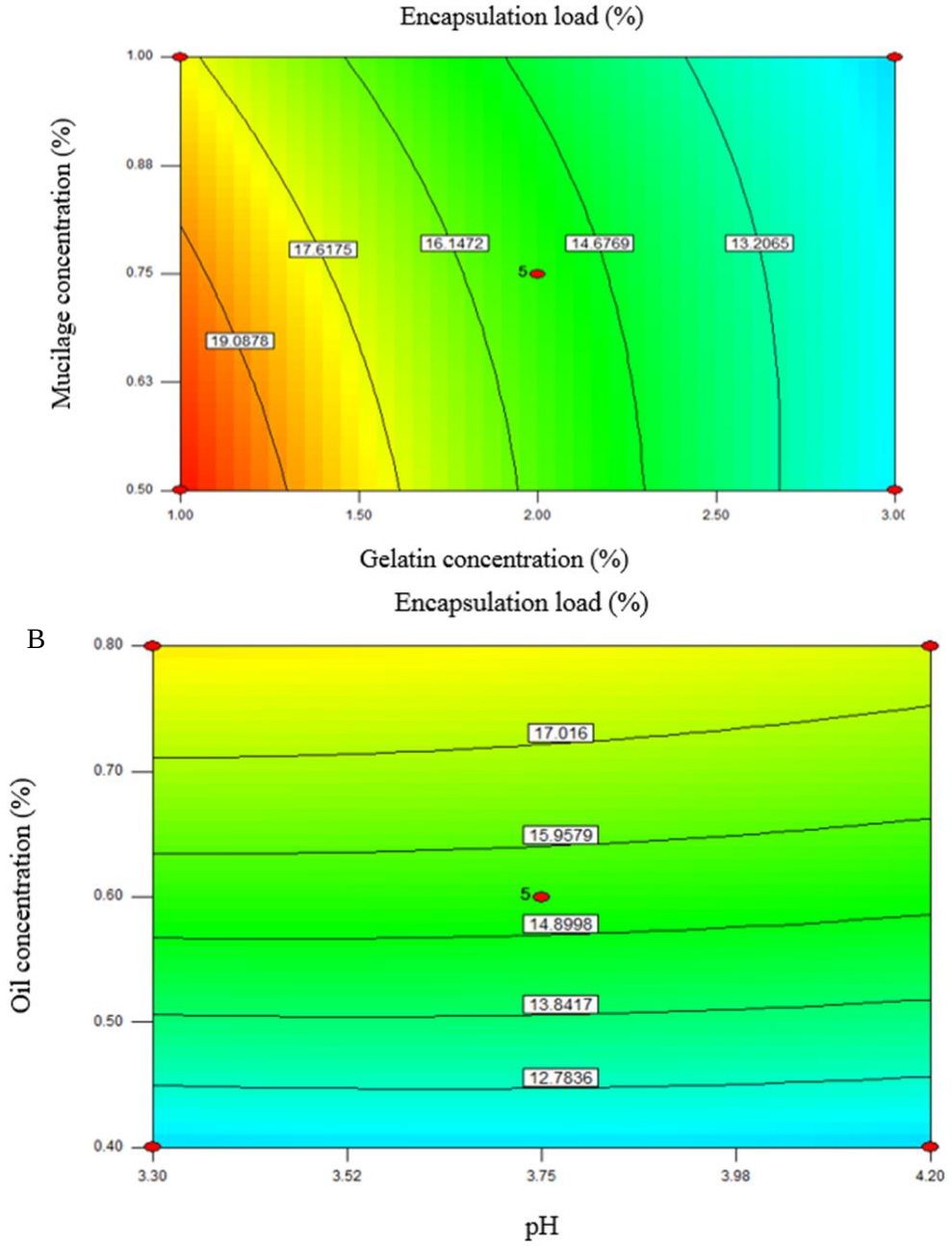


Fig. 2- Interaction of gelatin and mucilage concentrations (%) (A) and oil concentration (%) and pH (B) on encapsulation load

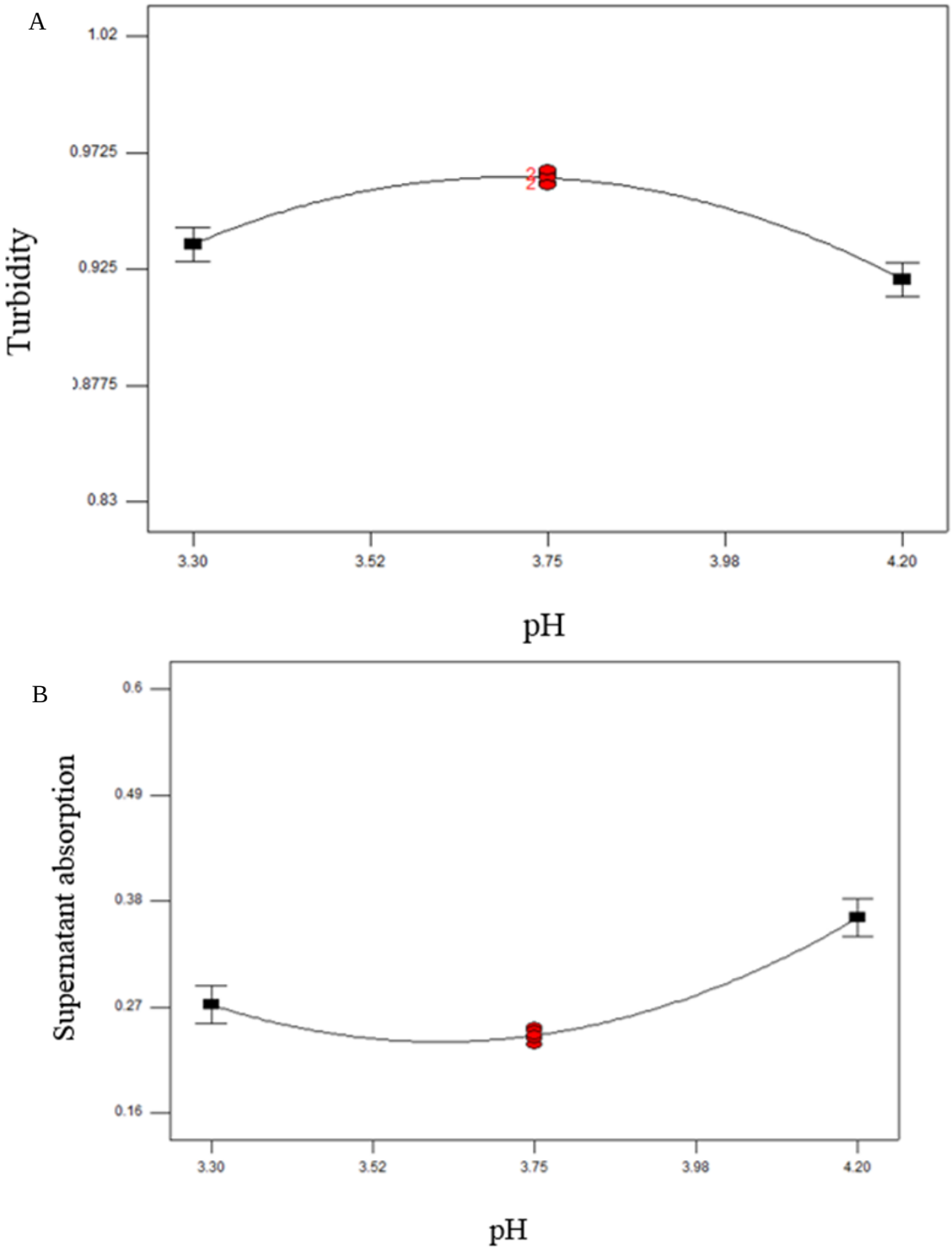


Fig. 3- Effect of pH on turbidity (A) and supernatant absorption (B) of solution

Analysis of variance indicated that the quadratic model with R^2 =0.97 was suitable to predict supernatant absorbance (Table 2). Gelatin percentage, mucilage percentage and pH had significant effects on supernatant absorbance ($P<0.01$). Equation (6) shows selected model for supernatant absorbance:

Supernatant absorbance =6.29203-0.073867A-1.44020B-2.97296C-0.48692D-0.14100AB+0.017778AC+0.018750 AD+0.19778BC+0.03BD+0.075CD+0.060550 A^2+0.54080B^2+0.37926C^2+0.15125D^2 (6)

where A, B, C and D are gelatin percentage, basil seed mucilage percentage, pH and oil phase percentage, respectively.

Lower the absorption is desirable since it represents that higher solid materials are in coacervate phase. Fig. 3 (B) demonstrated the effect of pH on supernatant absorption and indicated that by decreasing pH to 3.75, the absorption of the supernatant phase decreased, which showed more wall materials interacted and formed coacervate. A further decrease in pH range from about 3.75 to 3.3 led to higher absorption of the supernatant phase, indicating the low number of complexes in coacervate phase. The complex coacervation is significantly affected by pH of the solution. Jun-xia *et al*. (2011) used soy protein isolate and gum Arabic to encapsulate orange oil and reported that pH 4 is the electrical equivalence point. The charge densities of the two biopolymers were in stoichiometric equilibrium at pH 4 and therefore pH 4 had the highest coacervate yield.

Response surface optimization

The optimal formula was selected using the Design Expert software based on the highest percentage of encapsulation efficiency, encapsulation load, turbidity and lowest supernatant absorbance. The optimal formula with gelatin concentration of 2.02%, mucilage concentration of 0.91%, oil phase concentration of 0.71% and pH of 3.61 had encapsulation efficiency, encapsulation load, turbidity and supernatant absorbance of 83.69%, 16.32%, 0.979 and 0.227, respectively. Minor differences were observed between RSM data and our laboratory data. The optimum sample was selected to investigate the morphology, chemical structure and thermal behavior of microcapsules.

Characterization of microcapsules

Fig. 4 (A) shows a microscopic image of the Q_{10} loaded in the microcapsules after drying. The microcapsules were generally spherical and had rugged surfaces. These microcapsules stuck to each other because of the interaction of free oil and wall materials on the surface of the particles, which had not participated in complex coacervation process. The same result was reported by Yari *et al.* (2016). The presence of holes in the surface might due to the use of freeze dryer to dry the microcapsules. The removal of ice particles by sublimation leads to open holes (Fonte *et al*., 2012).

Fig. 4 (B) shows the IR spectra of gelatin, basil seed mucilage, sunflower oil, COQ_{10} and microcapsules. The IR spectrum of gelatin had a strong band at 3423 cm^{-1}, which was related to the N-H stretching vibrations. Weak peaks at 2921 cm^{-1} and 1450 cm^{-1} were attributed to the stretching and bending vibrations of C-H, respectively. Also, weak peaks at 1240 cm^{-1} and 1079 cm^{-1} were attributed to the C-N bending vibrations. The strong peak at 1638 cm^{-1} was related to the stretching vibrations of the carbonyl group (C=O) of amide I. The peak at 1546 cm^{-1} indicated the bending mods of N-H bond of amid II. The peak at 1202 cm^{-1} was attributed to the vibrations of N-H and C-N groups of amide III (Duhoranimana *et al*., 2017).

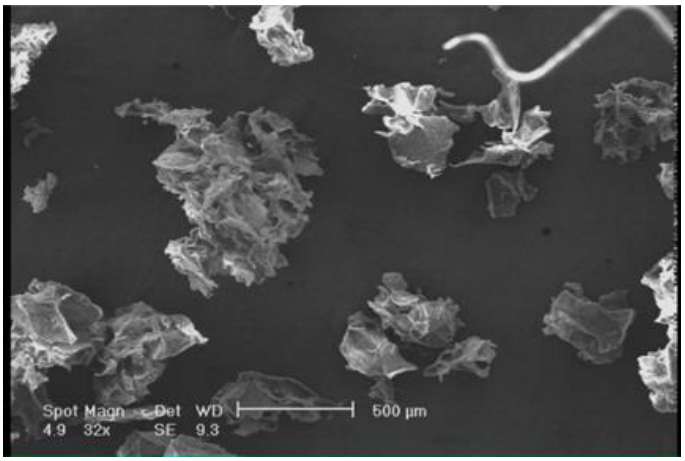
The IR spectrum of mucilage, showed different peaks at 3419 cm^{-1}, 2919 cm^{-1}, 1603 cm^{-1}, 1416 cm^{-1} and 1046 were assigned to OH, CH_2, C-OO asymmetric stretching, C-OO symmetric stretching and C-O-C stretching, respectively (Naji-Tabasi *et al*., 2016).

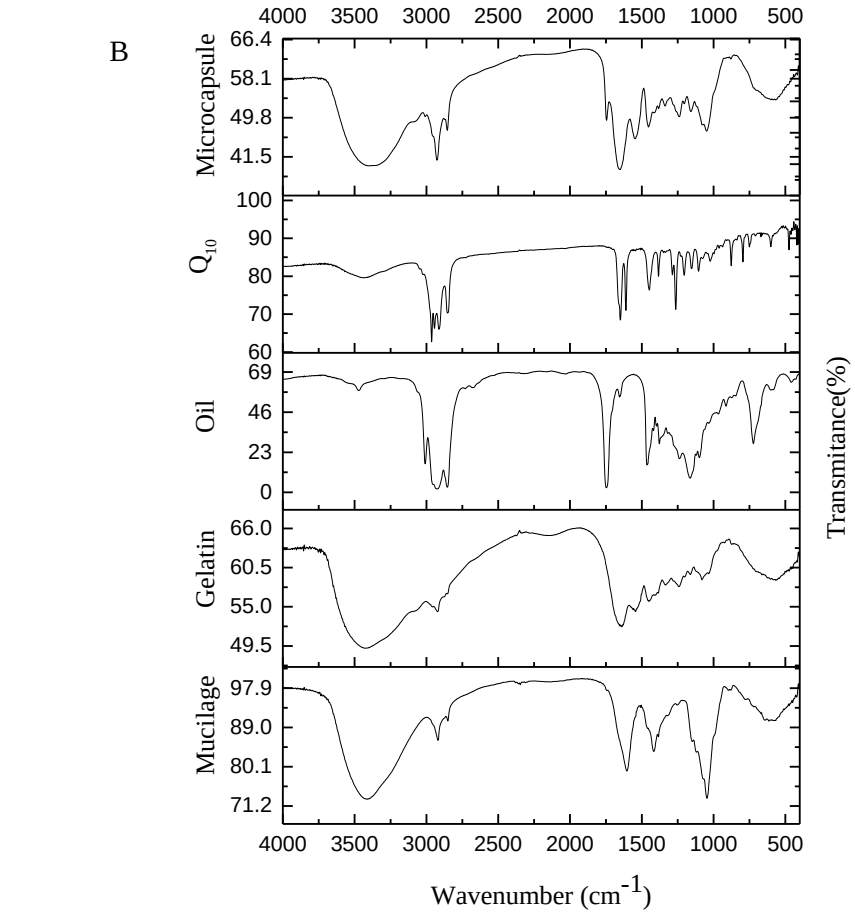
In the spectrum of sunflower oil, peaks at 2924 cm^{-1}, 2854 cm^{-1}, 1747 cm^{-1} ، 1654 cm^{-1} were attributed to the -CH asymmetric stretching, -CH symmetric stretching, -CO stretching vibrations and -C-C- stretching vibrations, respectively. The peak at 1163 cm^{-1} was assigned to the stretching and bending vibrations of -C-O and CH_2. Band at 723 cm^{-1} reflected the bending vibrations of -HC-CH- and - $(CH2)_n$-. The peak at 3471 cm^{-1} presented the overtone of -C-O ester. The -C-H stretching

vibrations at 3008 cm^{-1} and the bending vibrations of -C-H at 1463 cm^{-1} were other major peaks of the sunflower oil spectrum (Hamed and Allam, 2006).

In the spectrum of CoQ_{10}, the peaks at 2950 cm^{-1}, 2843 cm^{-1} and 1605 cm^{-1} were assigned to the stretching vibrations of =CH, -CH3 and carbonyl (-C=O), respectively. The stretching vibrations of the methoxy group at 1382 cm^{-1} and the ether group at 1266 cm^{-1} were other peaks of the CoQ_{10} spectrum (Akhter *et al.*, 2014).

The peaks of the microcapsule spectrum showed the main peaks of ingredients. While, peaks at 1546 cm^{-1} of gelatin spectrum and 1416 cm^{-1} of the basil mucilage spectrum were not detectable. A new peak appeared at 1490 cm^{-1}, indicating the formation of ionic interaction between the negative carboxyl group of the mucilage and the protonated amine group of gelatin. Presence of coenzyme Q_{10} bands was a reason supporting encapsulation of coenzyme Q_{10} by mucilage-gelatin microparticles. The sametrend was reported by Kavousi *et al.* (2017) for encapsulation of fish oil in hydrogels of cress seed mucilage and chitosan (Kavousi *et al.*, 2017).





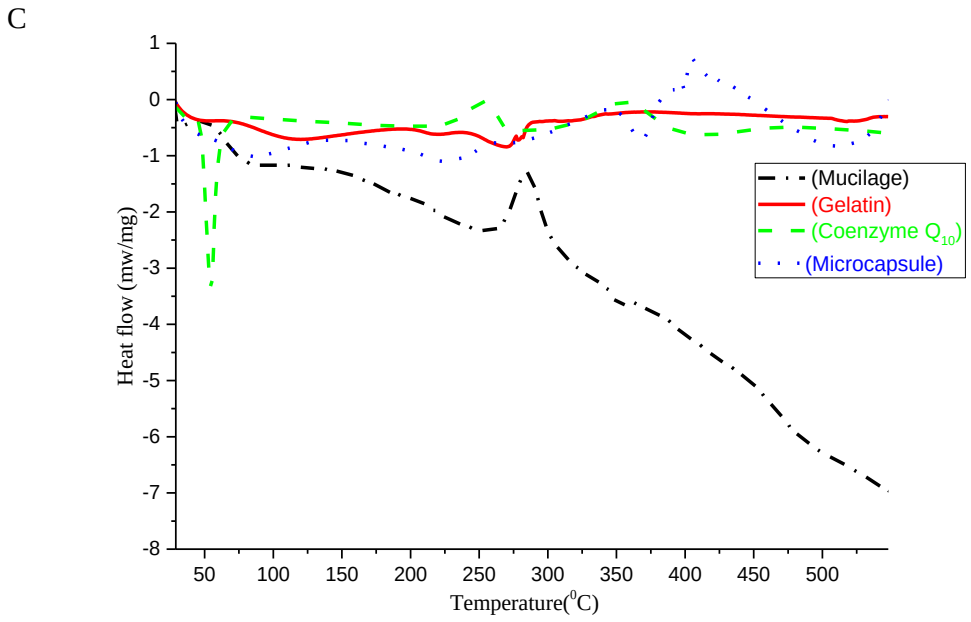


Fig. 4- SEM image of Q10 loaded microcapsules (A), FTIR spectra (B) and DSC curves (C) of ingredients and produced microcapsules

Fig. 4 (C) shows DSC curves of gelatin, mucilage, CoQ_{10}, and microcapsule. The glass transition (Tg) of gelatin was at 70°C. The peaks at 110 and 280°C represented melting point and decomposition of gelatin (Li *et al.*, 2006; Rahman *et al.*, 2010). The endothermic peaks at 47°C and 90°C represent glass transition temperature and water evaporation in basil mucilage, respectively (Khazaei *et al.*, 2014). The melting point of CoQ_{10} at 50°C indicated its crystalline nature (Swarnakar *et al.*, 2011). For the thermogram of Q_{10} loaded microcapsule, melting point was not observed at 50 °C due to its solubility in the oil phase and appropriate entrapment (Gokce *et al.*, 2012). The mucilage melting points and the glass transition point of gelatin were observed in the microcapsule's thermal curve. The peak at 350 °C could be attributed to the microcapsule decomposition temperature. The microcapsule had the higher thermal resistance in comparison to other ingredients due to interaction of wall materials. Kavousi *et al.* (2017) studied microencapsulation of fish oil in hydrogels of cress seed mucilage (CSM) and chitosan (CS) and reported that the thermal resistance of CSM/CS hydrogel loaded with fish oil was significantly higher than CSM and CS (Kavousi *et al.*, 2017).

Liposoluble vitamins in milk were taken up during the hexane-extraction process. CoQ_{10} shows its characteristic maxima of UV absorption at 275 nm. This analytical wavelength was selected for the HPLC-UV detection to enhance the selectivity and sensitivity of analysis (Karpińska *et al.*, 2006). In order to study the release of CoQ_{10} from microcapsules in milk, its amount was analyzed during storage at 4°C. About 4.2% and 12.4% of total added CoQ_{10} released in milk for the 1st and 5th days of storage, respectively. The release rate of CoQ_{10} from microcapsules was high at the beginning of storage because of CoQ_{10} distributed on the surface or surface layer which was easier to release. The first release followed with slower release because of CoQ_{10} in microcapsule mainly released through the microcapsules wall by swelling and diffusion. Jain *et al.* (2015) studied release of β-Carotene from whey protein isolates/gum acacia complex and reported the same issue (Jain *et al.*, 2015).

Conclusion

A new set of wall materials was applied to prepare microcapsules. Basil seed mucilage, an anionic polysaccharide was able to build complex and form coacervates with gelatin via electrostatic interaction under specific conditions. The efficiency of encapsulation increased as the higher wall materials were used under conditions where the core material in the system was kept constant. The optimal formula with gelatin concentration of 2.02%, mucilage concentration of 0.91%, oil phase concentration of 0.71% and pH of 3.61 was determined. The microcapsules were generally spherical and had rugged surfaces. FTIR spectra revealed that ionic interactions occurred between functional groups of gelatin and basil seed mucilage. The thermal stability of coacervate showed that basil seed mucilage- gelatin coacervate will be appropriate to be used for encapsulation of thermally sensitive materials. The results of this study indicated that the produced microcapsules can be used to encapsulate and control the release of bioactive ingredients. Further studies should be performed toward the stability of microcapsules in different food media and their large scale production.

References

1. Ahmadi, N., Nasirpour, A., Sheikhzeinodin, M., & Keramat, J. (2015). Microencapsulation of ubiquinone using complex coacervation for functional yoghurt. *Food Science and BiotechnologyBiochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 24*(3), 895-904. http://dx.doi.org/10.1007/s10068-015-0116-x

2. Akhter, M.H., Ahmad, A., Ali, J., & Mohan, G. (2014). Formulation and development of CoQ_{10}-loaded s-SNEDDS for enhancement of oral bioavailability. *Journal of Pharmaceutical Innovation*, *9*(2), 121-131. http://dx.doi.org/10.1007/s12247-014-9179-0
3. Butstraen, C., & Salaün, F. (2014). Preparation of microcapsules by complex coacervation of gum Arabic and chitosan. *Carbohydrate Polymers*, *99*, 608-616. https://doi.org/10.1016/j.carbpol.2013.09.006
4. Byeon, J.H., Kang, Y.-R., & Chang, Y.H. (2023). Physicochemical and in vitro digestion properties of gelatin/low-methoxyl pectin synbiotic microgels co-encapsulating Lacticaseibacillus casei and pectic oligosaccharides via double-crosslinking with transglutaminase and calcium ions. *Food Hydrocolloids*, *142*, 108757. https://doi.org/10.1016/j.foodhyd.2023.108757
5. Calderón-Oliver, M., Pedroza-Islas, R., Escalona-Buendía, H.B., Pedraza-Chaverri, J., & Ponce-Alquicira, E. (2017). Comparative study of the microencapsulation by complex coacervation of nisin in combination with an avocado antioxidant extract. *Food Hydrocolloids*, *62*, 49-57. https://doi.org/10.1016/j.foodhyd.2016.07.028
6. Chang, P.G., Gupta, R., Timilsena, Y.P., & Adhikari, B. (2016). Optimisation of the complex coacervation between canola protein isolate and chitosan. *Journal of Food Engineering*, *191*, 58-66. https://doi.org/10.1016/j.jfoodeng.2016.07.008
7. Dong, Z., Ma, Y., Hayat, K., Jia, C., Xia, S., & Zhang, X. (2011). Morphology and release profile of microcapsules encapsulating peppermint oil by complex coacervation. *Journal of Food Engineering*, *104*(3), 455-460. https://doi.org/10.1016/j.jfoodeng.2011.01.011
8. Duhoranimana, E., Karangwa, E., Lai, L., Xu, X., Yu, J., Xia, S., Zhang, X., Muhoza, B., & Habinshuti, I. (2017). Effect of sodium carboxymethyl cellulose on complex coacervates formation with gelatin: Coacervates characterization, stabilization and formation mechanism. *Food Hydrocolloids*, *69*, 111-120. https://doi.org/10.1016/j.foodhyd.2017.01.035
9. Escriva, A., Esteve, M., Farré, R., & Frıgola, A. (2002). Determination of liposoluble vitamins in cooked meals, milk and milk products by liquid chromatography. *Journal of Chromatography A*, *947*(2), 313-318. https://doi.org/10.1016/S0021-9673(01)01618-1
10. Fonte, P., Soares, S., Costa, A., Andrade, J. C., Seabra, V., Reis, S., & Sarmento, B. (2012). Effect of cryoprotectants on the porosity and stability of insulin-loaded PLGA nanoparticles after freeze-drying. *Biomatter*, *2*(4), 329-339. https://doi.org/10.4161/biom.23246
11. Gokce, E.H., Korkmaz, E., Tuncay-Tanrıverdi, S., Dellera, E., Sandri, G., Bonferoni, M.C., & Ozer, O. (2012). A comparative evaluation of coenzyme Q10-loaded liposomes and solid lipid nanoparticles as dermal antioxidant carriers. *International Journal of Nanomedicine*, *7*, 5109. https://doi.org/10.2147/ijn.s34921
12. Hamed, S., & Allam, M.A. (2006). Application of FTIR spectroscopy in the determination of antioxidant efficiency in sunflower oil. *Journal of Applied Sciences Research*, *2*(1), 27-33.
13. Hogan, S.A., McNamee, B.F., O'Riordan, E.D., & O'Sullivan, M. (2001). Microencapsulating properties of sodium caseinate. *Journal of Agricultural and Food Chemistry*, *49*(4), 1934-1938. https://doi.org/10.1021/jf000276q
14. Hosseini-Parvar, S., Matia-Merino, L., Goh, K., Razavi, S.M.A., & Mortazavi, S.A. (2010). Steady shear flow behavior of gum extracted from *Ocimum basilicum* L. seed: effect of concentration and temperature. *Journal of Food Engineering*, *101*(3), 236-243. https://doi.org/10.1016/j.jfoodeng.2010.06.025
15. Hu, R., Dong, D., Hu, J., & Liu, H. (2023). Improved viability of probiotics encapsulated in soybean protein isolate matrix microcapsules by coacervation and cross-linking modification. *Food Hydrocolloids*, *138*, 108457. https://doi.org/10.1016/j.foodhyd.2023.108457

16. Ifeduba, E.A., & Akoh, C.C. (2016). Microencapsulation of stearidonic acid soybean oil in Maillard reaction-modified complex coacervates. *Food Chemistry, 199*, 524-532. https://doi.org/10.1016/j.foodchem.2015.12.011
17. Jain, A., Thakur, D., Ghoshal, G., Katare, O., & Shivhare, U. (2015). Microencapsulation by complex coacervation using whey protein isolates and gum acacia: an approach to preserve the functionality and controlled release of β-carotene. *Food and Bioprocess Technology, 8*(8), 1635-1644. http://dx.doi.org/10.1007/s11947-015-1521-0
18. Jun-xia, X., Hai-yan, Y., & Jian, Y. (2011). Microencapsulation of sweet orange oil by complex coacervation with soybean protein isolate/gum Arabic. *Food Chemistry, 125*(4), 1267-1272. https://doi.org/10.1016/j.foodchem.2010.10.063
19. Karpińska, J., Mikołuć, B., Motkowski, R., & Piotrowska-Jastrzębska, J. (2006). HPLC method for simultaneous determination of retinol, α-tocopherol and coenzyme Q_{10} in human plasma. *Journal of Pharmaceutical and Biomedical Analysis, 42*(2), 232-236. https://doi.org/10.1016/j.jpba.2006.03.037
20. Kavousi, H. R., Fathi, M., & Goli, S. A. (2017). Stability enhancement of fish oil by its encapsulation using a novel hydrogel of cress seed mucilage/chitosan. *International Journal of Food Properties, 20*(sup2), 1890-1900. https://doi.org/10.1080/10942912.2017.1357042
21. Khazaei, N., Esmaiili, M., Djomeh, Z. E., Ghasemlou, M., & Jouki, M. (2014). Characterization of new biodegradable edible film made from basil seed (*Ocimum basilicum* L.) gum. *Carbohydrate Polymers, 102*, 199-206. https://doi.org/10.1016/j.carbpol.2013.10.062
22. Li, M., Guo, Y., Wei, Y., MacDiarmid, A.G., & Lelkes, P.I. (2006). Electrospinning polyaniline-contained gelatin nanofibers for tissue engineering applications. *Biomaterials, 27*(13), 2705-2715. https://doi.org/10.1016/j.biomaterials.2005.11.037
23. Li, X., Zhang, M., Zhou, L., Liu, J., & Marchioni, E. (2023). Construction of whey protein gels prepared by three methods to stabilize high internal phase Pickering emulsions loaded with CoQ_{10} under different pH. *Food Chemistry, 421*, 136192. https://doi.org/10.1016/j.foodchem.2023.136192
24. Naji-Tabasi, S., Razavi, S.M.A., Mohebbi, M., & Malaekeh-Nikouei, B. (2016). New studies on basil (Ocimum bacilicum L.) seed gum: Part I–Fractionation, physicochemical and surface activity characterization. *Food Hydrocolloids, 52*, 350-358. https://doi.org/10.1016/j.foodhyd.2015.07.011
25. Nakagawa, K., & Nagao, H. (2012). Microencapsulation of oil droplets using freezing-induced gelatin–acacia complex coacervation. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 411*, 129-139. https://doi.org/10.1016/j.colsurfa.2012.07.010
26. Peng, C., Zhao, S.-Q., Zhang, J., Huang, G.-Y., Chen, L.-Y., & Zhao, F.-Y. (2014). Chemical composition, antimicrobial property and microencapsulation of Mustard (Sinapis alba) seed essential oil by complex coacervation. *Food Chemistry, 165*, 560-568. https://doi.org/10.1016/j.foodchem.2014.05.126
27. Prata, A.S., & Grosso, C.R. (2015). Influence of the oil phase on the microencapsulation by complex coacervation. *Journal of the American Oil Chemists' Society, 92*(7), 1063-1072. http://dx.doi.org/10.1007/s11746-015-2670-z
28. Rahman, M. S., Al-Saidi, G., Guizani, N., & Abdullah, A. (2010). Development of state diagram of bovine gelatin by measuring thermal characteristics using differential scanning calorimetry (DSC) and cooling curve method. *Thermochimica Acta, 509*(1-2), 111-119. https://doi.org/10.1016/j.tca.2010.06.011.

29. Razavi, S.M., Bostan, A., & Rezaie, M. (2010). Image processing and physico-mechanical properties of basil seed (*Ocimum basilicum*). *Journal of Food Process Engineering, 33*(1), 51-64. https://doi.org/10.1111/j.1745-4530.2008.00259.x
30. Rocha-Selmi, G.A., Theodoro, A.C., Thomazini, M., Bolini, H.M., & Favaro-Trindade, C.S. (2013). Double emulsion stage prior to complex coacervation process for microencapsulation of sweetener sucralose. *Journal of Food Engineering, 119*(1), 28-32. https://doi.org/10.1016/j.jfoodeng.2013.05.002.
31. Saeidy, S., Keramat, J., & Nasirpour, A. (2014). Microencapsulation of calcium using water-in-oil-in-water double emulsion method. *Journal of Dispersion Science and Technology, 35*(3), 370-379. https://doi.org/10.1080/01932691.2013.788453
32. Sharifi, S., Rezazad-Bari, M., Alizadeh, M., Almasi, H., & Amiri, S. (2021). Use of whey protein isolate and gum Arabic for the co-encapsulation of probiotic Lactobacillus plantarum and phytosterols by complex coacervation: Enhanced viability of probiotic in Iranian white cheese. *Food Hydrocolloids, 113*, 106496. https://doi.org/10.1016/j.foodhyd.2020.106496.
33. Silva, D., Favaro-Trindade, C., Rocha, G., & Thomazini, M. (2012). Microencapsulation of lycopene by gelatin–pectin complex coacervation. *Journal of Food Processing and Preservation, 36*(2), 185-190. https://doi.org/10.1111/j.1745-4549.2011.00575.x.
34. Swarnakar, N.K., Jain, A.K., Singh, R.P., Godugu, C., Das, M., & Jain, S. (2011). Oral bioavailability, therapeutic efficacy and reactive oxygen species scavenging properties of coenzyme Q10-loaded polymeric nanoparticles. *Biomaterials, 32*(28), 6860-6874. https://doi.org/10.1016/j.biomaterials.2011.05.079.
35. Wang, B., Adhikari, B., & Barrow, C.J. (2014). Optimisation of the microencapsulation of tuna oil in gelatin–sodium hexametaphosphate using complex coacervation. *Food Chemistry, 158*, 358-365. https://doi.org/10.1016/j.foodchem.2014.02.135
36. Wang, C., Zhao, R., He, K., Zhang, S., Kemp, A.H., & Guo, M. (2023). Pharmacokinetic profile and sub-chronic toxicity of coenzyme Q10 loaded whey protein nanoparticles. *Food Bioscience, 52*, 102347. https://doi.org/10.1016/j.fbio.2022.102347
37. Xiao, Z., Li, W., & Zhu, G. (2015). Effect of wall materials and core oil on the formation and properties of styralyl acetate microcapsules prepared by complex coacervation. *Colloid and Polymer Science, 293*(5), 1339-1348.
38. Yang, X., Gao, N., Hu, L., Li, J., & Sun, Y. (2015). Development and evaluation of novel microcapsules containing poppy-seed oil using complex coacervation. *Journal of Food Engineering, 161*, 87-93. https://doi.org/10.1016/j.jfoodeng.2015.03.027
39. Yari, S., Nasirpour, A., & Fathi, M. (2016). Effect of polymer concentration and acidification time on olive oil microcapsules obtained by complex coacervation. *Applied Food Biotechnology, 3*(1), 53-58. http://dx.doi.org/10.22037/afb.v3i1.10226
40. Zamarreño, M.D., Pérez, A.S., Pérez, C.G., & Méndez, J.H. (1992). High-performance liquid chromatography with electrochemical detection for the simultaneous determination of vitamin A, D3 and E in milk. *Journal of Chromatography A, 623*(1), 69-74. https://doi.org/10.1016/0021-9673(92)85300-I

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد ۱۹، شماره ۳، مرداد- شهریور ۱۴۰۲ ص. ۳۱-۱۵

Iranian Food Science and Technology Research Journal
Vol. 19, No. 3, Aug.-Sep. 2023, p. 15-31

ریزپوشینه کردن کوآنزیم Q_{10} به روش توده‌سازی مرکب ژلاتین و موسیلاژ دانه ریحان: بهینه‌سازی، ویژگی‌های فیزیکوشیمیایی و غنی‌سازی شیر

ستاره رمضانی[۱]- محمد شاهدی[۲]- میلاد فتحی[۳*]

تاریخ دریافت: ۱۴۰۱/۱۲/۲۶
تاریخ بازنگری۱۴۰۲/۰۳/۲۴
تاریخ پذیرش: ۱۴۰۲/۰۴/۰۷

چکیده

نگرانی جهانی در مورد سلامت انسان و افزایش شیوع بیماری‌های مزمن در سال‌های اخیر منجر به افزایش تقاضا برای ترکیبات مغذی مانند کوآنزیم Q_{10} شده است. حساسیت به گرما و خواص چربی دوست کوآنزیم Q_{10} استفاده از آن را در غذا محدود می‌کند. کپسولاسیون فناوری است که از مواد زیست فعال در برابر شرایط محیطی نامناسب محافظت می‌کند و عمر مفید را افزایش می‌دهد. هدف از این مطالعه کپسوله‌سازی کوآنزیم Q_{10} با استفاده از کواسرواسیون مرکب توسط موسیلاژ دانه ژلاتین-ریحان و مشخص کردن خواص فیزیکی، حرارتی و شیمیایی میکروکپسول‌های تولید شده بود. روش سطح پاسخ برای تعیین سطح بهینه چهار متغیر فرمولاسیون برای حداکثر راندمان کپسولاسیون، ظرفیت بارگذاری و کدورت و حداقل جذب مایع رویی استفاده شد. میکروکپسول‌های بهینه دارای راندمان کپسولاسیون ۸۳/۶۹٪، ظرفیت بارگذاری ۱۶/۳۲٪، کدورت ۰/۹۷۹ و جذب مایع رویی ۰/۲۲۷ بودند. میکروکپسول‌ها با میکروسکوپ الکترونی روبشی، طیف‌سنجی فروسرخ تبدیل فوریه و کالری‌سنجی روبشی تفاضلی ارزیابی شدند. نتایج FTIR تشکیل کواسروات‌ها را تایید کرد. ترموگرام نقطه ذوب میکروکپسول بارگذاری شده Q_{10} در نقطه ذوب آن (۵۰ درجه سلسیوس) به دلیل حلالیت آن در فاز روغن و به تله افتادن مناسب حین کپسولاسیون مشاهده نشد. رفتار رهایش Q_{10} توسط مدل‌های مختلف ریاضی مورد بررسی قرار گرفت. ریزپوشینه‌های Q_{10} برای غنی‌سازی شیر مورد استفاده قرار گرفت و نتایج نشان داد که میکروکپسول‌های پروتئین-کربوهیدرات توسعه یافته را می‌توان برای محافظت از ترکیبات آبگریز استفاده کرد.

واژه‌های کلیدی: انکپسولاسیون، ژلاتین، کوآنزیم Q_{10}، موسیلاژ دانه ریحان، ویژگی‌های فیزیکوشیمیایی

۱، ۲ و ۳- به‌ترتیب دانشجوی کارشناسی ارشد، استاد و دانشیار گروه علوم و صنایع غذایی، دانشکده کشاورزی، دانشگاه صنعتی اصفهان
(*- نویسنده مسؤل: Email: mfathi@iut.ac.ir)

DOI: 10.22067/ifstrj.2023.81438.1240

Iranian Food Science and Technology Research Journal
Vol. 19, No. 3, Aug.-Sep. 2023, p. 33-47

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد ۱۹، شماره ۳، مرداد- شهریور ۱۴۰۲ ص. ۴۷-۳۳

Research Article
Homepage: http://ifstrj.um.ac.ir

Investigating the Quality and Safety Characteristics of Skin Collagen of Bighead (*Hypophthalmichthys nobilis*), Silver Carp (*Hypophthalmichthys molitrix*), Grass Carp (*Ctenopharyngodon idella*), and Common Carp (*Cyprinus carpio*) Cultured

M. Seifzadeh[1]*

Received: 2023.04.10
Revised: 2023.06.03
Accepted: 2023.07.01
Available Online: 2023.07.04

How to cite this article:
Seifzadeh, M. (2023). Investigating the quality and safety characteristics of skin collagen of Bighead (*Hypophthalmichthys nobilis*), Silver carp (*Hypophthalmichthys molitrix*), Grass carp (*Ctenopharyngodon idella*), and Common carp (*Cyprinus carpio*) cultured. *Iranian Food Science and Technology Research Journal, 19*(3), 33-47. https://doi.org/10.22067/ifstrj.2023.81732.1245

Abstract

Collagen has diverse general and biomedical applications and its important role in the future of society have made it a key biopolymer for human health and well-being. Therefore, the present study was conducted with the objectives of extracting collagen from the skin of farmed carp, determining the quality characteristics of collagen, and comparing them. Collagen was extracted from the skin of carp fishes by acidic enzymatic method using 0.5 M acetic acid and pepsin in 48 hours. Collagen treatments (5 treatments) included collagen prepared from the skin of common species, grass carp, bighead, silver, and cow (control). Collagen treatments were not capable of hemolysis and did not show toxic effects on human fibroblast cells. Heavy metals (0.01-0.18 ppm) in collagen extracted from cultured carp species were within the standard range. The color (brightness) of experimental collagen (92.74-93.68) and control (92.38) showed no significant difference ($p>0.05$). Amino acids cysteine and tryptophan were not observed in collagen. Glycine and hydroxylysine amino acids (352 and 3 residues 1000g-1, respectively) had the highest and lowest amounts in collagen. Amino acids profile and collagen production efficiency (10.51-10.59%) did not show significant differences in carp fish species ($p>0.05$). Based on the results of the present study, production efficiency, safety and quality characteristics of collagen in cultured carp species did not show any significant difference ($p>0.05$), and no significant difference was observed between these characteristics and the control ($p>0.05$). Therefore, the skin of these species can be used to produce collagen and introduce it to the industry as a substitute for mammalian collagen.

Keywords: Acid hydrolysis, Cultured carp, Collagen, Pepsin enzyme, Safety

Introduction

The oceans are home to billions of plants, animals, and microorganisms, which are a huge source of natural compounds. The discovery of the ocean as an abundant source of biological compounds has a progressive effect on the research of marine biomolecules. Collagen is derived from the kola Greek word, which means glue and the French term collagen was initially described as a component of connective tissue that provides gelatin during cooking (Carvalho *et al*., 2018). Also, collagen

1- Assistant Professor, National Aquatic Processing Research Center, Inland Aquaculture Research Institute, Fisheries Science Research Institute, Agricultural Education and Extension Research Organization, Anzali, Iran
(*- Corresponding Author Email: m.seifzadeh@areeo.ac.ir)
DOI: 10.22067/ifstrj.2023.81732.1245

is defined according to the connection with specific groups, which are defined based on the complexity and structural diversity, the presence of non-helical parts, the functions, and the ability to connect the supramolecular structures that are assigned to them. About a quarter of the total protein in mammals is collagen, which is the most abundant protein in the body and a key component of connective tissue. Type 1 collagen constitutes 90% of the collagen in the human body, and accounts for approximately 25-35% of the total protein in the fish body (Song *et al*., 2019). Marine collagen is obtained from skin, cartilage, bone, and fish scales. Collagen comprises 50-70% of cartilage and skin and 80-90% of tendons and bones without minerals. In addition, it is possible to produce it from marine vertebrate and invertebrate sources. Marine collagens have attracted much attention during the past decade, and the search for exploitation of marine collagen has increased since 2014 (Xu *et al*., 2021).

Collagen has a wide range of applications and is widely used in cosmetic and hygiene industries, ophthalmology (corneal filling), pharmaceutical, medicine (wound healing, biomedicine, preparation of medical supplies and regenerative medicine, orthopedics, surgery to make artificial skin and treatment of severe burns), prolotherapy as a strengthening of loose ligaments, blood coagulant cotton textiles, injected for the treatment of soft tissue abscess, absorbable wound dressing, (a barrier to prevent epithelial migration, helps cells to regenerate the damaged area and to build ligaments and tendons), dentistry (dental bone fillers, implants, making a permeable membrane for rebuilding the soft tissue around the periodontal gum (gum disease) and dressing pulled teeth), beauty (face mask, etc), biotechnology, tissue repair, cell migration (necessary for tissue maintenance), tissue engineering (biopolymer) and food (production of functional products). Collagen also plays important roles in cellular processes, including immune response and cell communication (Mullen *et al*., 2019; Govindharaj *et al*., 2019; Raman *et al*., 2018).

The molecular weight of collagen is 300 kilodaltons, whose length, width, and thickness are 300 nm, and the total number of amino acids in this molecule is about 3000 units. Collagen is the most abundant structural protein in animals. About 28–30 types of collagen are known, all of which are composed of three α-helices (tropocollagen) that, once secreted into the extracellular environment, can assemble into their final supermolecular organization (Cumming *et al*., 2019). Collagen molecules are naturally composed of three long helical chains of amino acid residues with non-helical ends at both ends and make different collagen structures. The basic structure of collagen consists of amino acids, mainly glycine (33%), proline, and hydroxyproline (22%). The secondary structure consists of amino acid chains that are connected in a spiral with three amino acids. They twist around each other and form a strong third structure. The basic structure of collagen is the quaternary structure related to the superhelix. The variety of collagen types is also due to the difference in the expression of genes involved in protein biosynthesis. In addition, post-translational modifications of collagens also have a significant effect on collagen diversity (Lupu *et al*., 2019; Lin *et al*., 2019). Differentiating the nature of collagen is determined by the presence of different chains, isoforms, and molecular structures of each type of collagen. At least 46 unique polypeptide chains have been found in collagen from different animals. Collagen chains are usually composed of the repeating motif Gly-X-Y, where Gly is the amino acid glycine, while X and Y are the amino acids proline and 4-hydroxyproline, respectively (Wu *et al*., 2019). This motif is distinct from other extracellular matrix components. The chains of different types of collagens differ in composition, depending on the number of repeats and the length of the fragment containing the Gly-X-Y

motif, and the amino acid residues present at the X and Y positions (Sousa *et al*., 2020).

The qualitative characteristics of collagen obtained from marine sources compared to collagen obtained from cow and pig sources include the same composition of amino acids of higher heat resistance, non-transmission of zoonotic diseases such as spongiform encephalopathy, being made of smaller peptide units compared to other collagens, easy digestion, absorption and distribution in the human body, having a large amount of amino acids lysine, glycine, proline and hydroxyproline, the essential role of lysine in the production of this protein, high biocompatibility, solubility in water, antimicrobial properties, preventing the penetration of microorganisms into wounded and damaged tissue, skin enlightenment and moisturizing, high tensile strength, high and adjustable mechanical properties, resistance to acid treatments, natural, non-allergenic, very high capacity to maintain the moisture of the skin, does not irritate the human skin, suitable for skin applications, repeatability of its effects, anti-aging and wrinkles of the skin of the face and around the eyes, non-toxicity and biodegradability (Chinh *et al*., 2019; Sukhikh *et al*., 2021). Therefore, the present study was conducted with the objectives of extracting collagen from the skin of farmed carp, determining the quality characteristics of collagen based on the hydrolysis time, and comparing them.

Materials and Methods

The waste materials needed for the preparation of collagen were obtained from the model aquatic market located in Rasht. The sample was transferred to the laboratory using Unilith-containing ice at a ratio of 2 times the weight of the skin. All extraction steps were performed at 0-4 ^{0}C. The fish skin was washed with cold water and divided into small pieces of 0.5 x 0.5 cm. For pretreatment, 0.1 m sodium hydroxide was used, which is the ratio of sample to alkali in the ratio of 1 to 10 (volume/weight). The mixture was continuously stirred for 6 hours. The alkaline solution was changed every two hours. Then, the alkali-treated skin was washed with cold water until a neutral pH. The treated skin was placed in 10% butyl alcohol, where the ratio of solid to solvent was 1:10, for 18 hours. The solvent was changed every 6 hours. The defatted skin was washed with cold water before lyophilization until a neutral pH. Collagen was extracted from the skin of cultured carp fishes by an acid-enzymatic method based on the method of Wei *et al.* (2019). To extract collagen, the lyophilized skin was placed in 0.5 M acetic acid, which has a 1:10 ratio of sample to solvent and contains pepsin (10 g per 100 g of lyophilized skin), for 48 hours. Five treatments were considered for this study. The treatments include collagen prepared from species of carp including grass carp, bighead, common and silver carp. Bovine collagen (imported) was used as a control treatment. The treatments were carried out in 3 repetitions. The quality of collagen treatments obtained through colorimetric tests using Hunter Lab (Gilbert, 2014), blood hemolysis by culture on blood agar medium (Momtahan *et al*., 2016), investigation of toxicity through culture on human fibroblasts cells (Chotphruethipong *et al*., 2022), amino acids profile using HPLC (Iranian National Standard No. 10699. 2015) and the heavy metals including arsenic, lead, cadmium, mercury, bismuth, antimony, tin, molybdenum, copper, chromium, cobalt and nickel were evaluated by acid chemical digestion method (Iranian National Standard No. 12014. 1999; AOAC, 2000). Collagen production efficiency was also determined by the hydroxyproline calculation method (Qiu *et al*., 2014). The culture media and required chemicals were obtained from Merck.

Cell culture

Human dermal fibroblast cells were cultured in T-75 flasks using Dulbecco's complete modified Eagle's medium (DMEM) containing 10% fetal bovine serum (100 units/ml), penicillin, and streptomycin at 100 µg ml^{-1} and 2 ml of L-glutamine. The cells were incubated in a humidified 5% carbon dioxide incubator (Binder Model C 170, Binder Inc, Bohemia, NY, USA) at a temperature of 37°C. Then they were cultured using a trypsin-EDTA solution of 0.25% at a concentration of 80-90%. Cell proliferation assay of collagen at concentrations of 50, 100, 200, 400, and 800 µg ml-1 were tested for cytotoxicity on human skin fibroblast cells. A culture medium without collagen was used as a control. These concentrations were tested using the MTT method. The result was reported as the percentage of cell proliferation compared to the control (Andini *et al*., 2020).

MTT method (methyl thiazolyldiphenyl-tetrazolium bromide)

After removing the supernatant from the cell culture medium, 100 µl of MTT solution was added to the cultures and kept warm for 2 hours. The cells were dissolved using 200 µl of DMSO and 100 µl of the gel was then transferred to a 96-well plate. The color expansion was determined photometrically at 540 nm. WEST-1 reagent was mixed in 5 ml of electrolyte solution and 10 µl of it was pipetted on the gels along with 90 µl of the culture medium (after removing the original culture medium). In the next step, it was incubated for 2 hours. Then the cultures were agitated for 5 minutes (100 rpm) and 100 µl of the supernatant was transferred to a 96-well plate. Photometric measurement was done at 450 nm. The initial cell density planted in collagen hydrogels on polyamide meshes was evaluated microscopically. The criterion for evaluating the best cell density was the smaller cell number and no empty cells (cell layer) between the mesh threads (ISO 10993-5).

Amino acid profile

Amino acids were measured by the electrospray ionization method. In this way, 0.5 g of the sample was acid hydrolyzed with 4 ml of hydrochloric acid solution for 24 hours at 110°C. When the sample temperature reached 24°C, it was centrifuged (Hettich Universal 320, Germany) at 4000 rpm (power gram: 3756/48) for 5 minutes. In the next step, 10 µl of the supernatant along with 1 ml of distilled water was transferred to the sample. Except for isoleucine and histidine, which included the standard leucine and 3-methylhistidine, stable isotope mixtures of each amino acid were considered as standards for calibration. After hydrolysis, calibration standards and samples were prepared by transferring 50 µl of the hydrolyzed or diluted standard to the sample. Then 50 µl of labeled stable isotope mixture as standard and 700 µl of non-centrifuged reagent were added to the sample for 5 seconds. Then, 3 µl of the prepared sample was kept at 30 ^{0}C, for amino acid analysis, and was injected into the C18 column of HPLC (- HP 1100, Germany). Mobile phases at a flow rate of 0.7 ml/min were used for separation through high-pressure liquid chromatography, and amino acids were determined at a wavelength of 254 nm (Iranian National Standard No. 10699. 2015).

Determination of yield

To determine the yield, the ratio of collagen hydroxyproline to hydroxyproline of raw skin was evaluated. The hydroxyproline content of extracted collagens was determined using a slightly modified version of the method described by Nalinanon *et al*. (2007). The samples were hydrolyzed with 6 M hydrochloric acid for 24 hours at 110° in an oven (Memmert U10, Schwabach, Germany). The hydrolyzate was clarified before filtering through Whatman No. 4 filter paper using activated carbon. To reach a pH equal to 6- 6.5, the filtered liquid was neutralized with sodium

hydroxide 10 M and 1 M. The neutralized sample (0.1 ml) was transferred to a test tube and isopropanol (0.2 ml) was added and mixed thoroughly.To the mixture 0.1 ml of an oxidant solution (a mixture of 7% (w/v) chloroamine T (w/v) and acetate/citrate buffer, pH 6, at a ratio of 1:4 (v/v)) was added and mixed thoroughly Subsequently, 1.3 ml of Ehrlich's reagent (mixture of solution A and B) was added and mixed thoroughly. The mixture was stirred and heated in a water bath (Memmert GmbH, Schwabach, Germany) at 60°C for 25 min before cooling with running water for 2-3 min. The solution was diluted to 5 ml with isopropanol. The absorbance was measured At a wavelength of 558 nm. Also, the standard curve of hydroxyproline with different concentrations from 10-60 ppm was prepared. Hydroxyproline content was expressed as mg g^{-1} of the sample (Qiu *et al*., 2014). Distilled water was used as a blank.

Determination of heavy metals

The acid chemical digestion determined the amounts of heavy metals arsenic, lead, cadmium, mercury, bismuth, antimony, tin, molybdenum, copper, chromium, cobalt, and nickel. To the ash (20 g) was added 50 ml of 6 M hydrochloric acid. To evaporate the acid the flask was placed in a water bath. Then 30 ml of 0.1 M nitric acid was added to this. Croze was placed in a water bath for 15 minutes. After this step, the container containing the sample was covered with aluminum foil and was placed in the environment temperature for 2 hours. Then, the contents of the flask were mixed using a glass rod. The cooled sample was filtered. The sample was transferred to a flask and filled with deionized 2 times distilled water. It was shaken to homogenize. Then, an optical atomic absorption spectrometer with a graphite furnace (Japan/ZA3700) was used to measure the light absorption of heavy metals (Wavelength of 390-410 nm) based on the calibration curve. The measuring range of the device for heavy metals is 0.15-1-30 ppm and the recycling percentage is 80-107 percent. The limit of Quantitation values is 0.02-0.01 mg kg-1 (Iranian National Standard No. 12014. 1999; AOAC, 2000).

Colorimetry (amount of color substance)

The color of the samples was determined by the Hunterlab device (color flex model USA). Color intensity was expressed using Hunter's parameters in terms of brightness (L), red-green (a), and blue-yellow (b) (Gilbert, 2014).

Blood hemolysis

A suspension was prepared from collagen in concentrations of 50, 100, 200, 400, and 800 µg ml^{-1} in distilled water. Wells were created on the blood agar culture medium. 0.5 ml of collagen solution was poured into the well. The plate was incubated at 37°C for 48 hours. The formation of a clear halo around the well-containing collagen indicates hemolysis and a positive test.

Statistic analysis

In the present study, the obtained results were analyzed using SPSS version 25 software. The results were analyzed at a significant level of 95%. One-way analysis of variance was used to compare the results of color and heavy metal tests between experimental and control treatments at a significant level of 5%. The results were expressed as mean with standard deviation.

Results and Discussion

As shown in Table 1, the amount of heavy metals in the treatments of collagen extracted from different species of carp showed no significant difference ($p>0.05$). The amount of heavy metals in the collagen of cultured carp was within the acceptable range according to the Food and Drug Administration. Although the proximity of fish breeding ponds to areas with a lot of agricultural activity or rural residential areas, the infiltration of agricultural effluents containing chemical fertilizers used in

agriculture and rice paddies, including phosphate fertilizers containing cadmium, into underground water and the entry of untreated rural sewage into the underground water, they cause an increase in the concentration of heavy metals in water and subsequently in fish.

Table 1- The results of heavy metals in collagen extracted from the skin of cultured and control carp (ppm)

Treatment Index	Bighead collagen	Common carp collagen	Grass carp collagen	Silver Carp collagen	Control	FDA limit (ppm)
Arsenic	0.07 A	0.09 A	0.21 A	0.2 A	0.06 A	3
Lead	0.02A	0.11 A	0.08 A	0.06 A	0.01 A	10
Cadmium	0.05 A	0.01 A	0.02 A	0.19 A	0.02 A	0.5
Mercury	0.12 A	0.20 A	0.05 A	0.10 A	0.05 A	1
Bismuth	0.13 A	0.02 A	0.09 A	0.02 A	0.07 A	Not determined
Antimony	0.12 A	0.02 A	0.07 A	0.04 A	0.05 A	Not determined
Tin	0.05 A	0.05 A	0.01 A	0.02 A	0.15 A	Not determined
Molybdenum	0.01 A	0.06 A	0.01 A	0.13 A	0.18 A	Not determined
Copper	0.07 A	0.02 A	0.11A	0.15 A	0.16 A	Not determined
Chrome	0.05 A	0.08 A	0.14 A	0.21 A	0.14 A	50
Cobalt	0.04 A	0.03 A	0.17 A	0.19 A	0.12 A	5
Nickel	0.09 A	0.01 A	0.02 A	0.18 A	0.13 A	0.2

Dissimilar uppercase letters in a row indicate a significant difference at the 5% level ($p<0.05$).

However, much data showed that environmental conditions are effective in the absorption and accumulation of heavy metals by fish. Heavy metals exist in surface waters in colloidal, particulate, and dissolved forms, whose behavior in natural waters depends on water chemistry such as pH, hardness, temperature, and other factors. During transport in water, these metals undergo many changes, such as dissolution, river flow, metal concentration and oxidation potential and regeneration of the river environment, sedimentation, surface absorption, and complex formation with water or soil compounds, which affect their behavior and bioavailability. In addition, the skin of farmed carp contains a lot of mucous material and prevents the binding of metals to the fish skin (Seifzadeh *et al*., 2018). Carvalho *et al.* (2018) reported the levels of mercury, lead, cadmium, and arsenic in the collagen extracted from cod fish skin were in the acceptable range. These researchers stated that the amount of lead, cadmium, mercury, and arsenic were less than 2.5 ppm, 0.25 ppm, 0.50 ppm, and 0.35 ppm, respectively. Dervan and Gulay (2023) randomly purchased 25 samples of commercial fish collagen and measured the amount of zinc, lead, arsenic, cadmium, and mercury. They did not find lead, arsenic, and mercury in the samples. The amount of cadmium in the samples was between 0.152-0.288 mg kg^{-1} and the amount of zinc was between 1.368-2673 mg kg^{-1}. The results of these researchers were higher compared to the results obtained from the measurement of heavy metals in collagen (0.01-0.18 ppm) of the present study. This difference can be due to the pollution of the primary source used for collagen production (fish skin), which may originate from the pollution of the fish habitat with sewage. In addition, cadmium is a metal that occurs naturally in the earth's crust, which may be another reason for fish contamination.

The collagen of the common, bighead, grass carp, and silver species and control did not show toxic effects on human fibroblast cells and did not cause proliferation of fibroblast cells (Table 2). Based on the obtained results, the collagen of the common, bighead, grass carp and silver species and control species did not have the ability of hemolysis.

Table 2- Examination of the toxicity (Cell Proliferation) of collagen extracted from the skin of cultured carp species and control samples

Index	Cell Proliferation					
	Concentration (µl)					
Treatment	0	50	100	200	400	800
Common carp collagen	0.07±0.12aA	0.09±0.13aA	0.16±0.18aA	0.02±0.15aA	0.01±0.13aA	0.11±0.19aA
Grass carp collagen	0.06±0.17aA	0.02±0.11aA	0.21±0.10aA	0.01±0.14aA	0.02±0.16aA	0.09±0.23aA
Bighead collagen	0.09±0.10aA	0.05±0.20aA	0.12±0.19aA	0.03±0.17aA	0.09±0.19aA	0.08±0.24aA
Silver Carp collagen	0.05±0.22aA	0.14±0.15aA	0.23±0.17aA	0.05±0.12aA	0.01±0.08aA	0.04±0.16aA
Control	0.03±0.09aA	0.12±0.17aA	0.18±0.25aA	0.07±0.11aA	0.04±0.03aA	0.11±0.14aA

The results are presented as mean ± standard deviation.

Dissimilar uppercase letters in a row and dissimilar lowercase letters in a column indicate a significant difference at the 5% level ($p<0.05$).

Safety is one of the basic features that can be mentioned to check the quality of collagen. Since no toxic compounds are used during the various stages of carp breeding, and the compounds used to prepare collagen do not have toxic effects, therefore these features were not observed in collagen. Andini *et al.* (2020) investigated collagen prepared from the skin and scales of snakehead fish (*Channidae*) and did not observe toxicity effects. Chotphruethipong *et al.* (2022) investigated the toxic effects of salmon skin collagen on human fibroblast cells and did not determine the toxic effects. Ferreira *et al.* (2022) investigated the toxicity effects of collagen prepared from Guriguba (*Hexanematichthys parkeri*) swimming bladder on fibroblast cells and did not determine toxicity effects. Wang *et al.* (2020) evaluated the toxicity effects of collagen sponge on L929 cells of mouse fibroblast cells and observed that the sponge has no toxicity effects on the skin. Carvalho *et al.* (2018) investigated the toxic effects of collagen extracted from Codfish skin on MRC-5 lung fibroblast cells and reported the collagen to be safe for biological applications. Song *et al.* (2019) investigated the toxicity of collagen prepared from tilapia fish skin on mouse fibroblast L929 cells and human umbilical vein endothelial cells (HUVEC) and did not observe toxicity effects. The compatibility of biological materials in contact with blood is one of the most important criteria for their successful application in the body. Hemolysis refers to the release of hemoglobin from red blood cells due to their rupture. A low rate of hemolysis indicates that fewer red blood cells have been destroyed, which indicates that the biological product (collagen) is a material with favorable blood compatibility. Therefore, blood lysis is considered a new method to evaluate the toxicity of biological compounds (Momtahan *et al.*, 2016; Weber *et al.*, 2018). In the present study, collagen does not cause blood hemolysis. Therefore, Marine collagen is a safe and generally well-tolerated supplement. Wang *et al.* (2020) used a sponge prepared using tilapia fish skin collagen to investigate human wounds and found that the collagen sponge does not have hemolytic properties, and can be used as a blood coagulant. Li *et al.* (2017) showed that hemostatic effects were improved in collagen enriched with oxidized microcrystalline cellulose. Considering that no toxicity effects were observed in the present study, the results of these researchers are consistent with the results of the present study.

Table 3- Colorimetric study of collagen extracted from the skin of cultured carp species and control samples

Treatment Index	Bighead collagen	Common carp collagen	Grass carp collagen	Silver Carp collagen	Control
b (yellow bluish-)	1.86±0.93 A	1.82±0.95 A	1.85±0.42 A	1.81±0.24 A	1.47±0.73 A
a (red yellowish)	1.98±0.79 A	1.73±0.90 A	1.95±0.78 A	1.71±0.42 A	1.52±0.84A
Light	93.68±0.89A	92.76±0.98A	93.66±0.78 A	92.74±0.26A	92.38±0.71A

The results are presented as mean ± standard deviation.

Dissimilar uppercase letters in a row indicate a significant difference at the 5% level ($p<0.05$).

In terms of color, the quality of collagen can be measured. There are three values in the color scale including L (lightness), a (green-red), and b (blue-yellow), which were considered in the present study. As can be seen in Table 3, the transparency of collagen in the experimental treatments (92.74-93.68) and the control (92.38) did not show a significant difference ($p>0.05$). According to studies conducted by other researchers, there is a correlation between collagen microstructure and its transparency. Changes in collagen transparency indicate slight differences in the concentration of collagen compounds. Since the collagen was extracted from different types of carp fishes, and the concentration of amino acids detected in different collagen treatments did not differ significantly, hence the transparency did not show a significant difference in these treatments. Ramle *et al.* (2022) investigated the color characteristics of collagen prepared using acetic acid from the skin of Needlefish (*Tylosurus acus melanotus*) and reported L, a, and b as 69.77, 0.73, and 4.69 respectively. In the present study, the values of L, a, and b in carp skin collagen were determined as 92.74-93.68, 1.71-1.98, and 1.81-1.86, respectively (Table 3). The characteristics of color in the present study were evaluated better compared to other studies. The difference in the results of this study with the results of the other studies may be due to differences in the extraction method, fish species, and genetic diversity.

Table 4- Collagen production efficiency from the skin of farmed carp species and control samples%

Species	Common carp	Silver Carp	Bighead	Grass carp
Efficiency	10.59 A	10.56 A	10.57 A	10.51 A

Dissimilar letters in a row indicate the existence of a significant difference at the 5% level ($p<0.05$).

As shown in Table 4, collagen yield did not show any significant difference between experimental treatments ($p>0.05$). Hydroxyproline is unique to collagen as an amino acid, and its amount is not high in other proteins. It was considered to check collagen efficiency (Silva *et al.*, 2014). Ahmed *et al.* (2019) reported that collagen production efficiency from big eye tuna skin extracted with 0.5 M acetic acid at 4 C° for 48 hours and a pepsin concentration of 0.2 g per gram of skin was 16.7%. They also expressed 13.5% collagen production efficiency from tuna skin extracted with 0.5M acetic acid at 4°C for 72 hours. Tan *et al.* (2018) found the production efficiency of collagen from Catfish skin extracted with hydrochloric acid at 0.118 -23.6 IU g-1 pepsin concentration and 4°C to be 59.03%, and the yield of collagen production from Catfish skin extracted with hydrochloric acid at 4°C and 60 hours was found to be 5.00 -

42.36%. Junianto *et al.* (2018) found the extraction efficiency of collagen from Nilem fish skin extracted with 0.5, 0.7, and 0.9 M acetic acid in concentrations of 0.5, 1, and 1.5% pepsin and under 4°C 5.25-6.18%. Zhang *et al.* (2019) determined the production efficiency of collagen from golden pompano skin extracted with 0.5 M acetic acid at 4 °C and within 48 hours 21.81%. Arumugam *et al.* (2018) expressed the production efficiency of collagen from sole fish skin using 0.5 M acetic acid at 25°C and 32 hours 19.9%. Oslan *et al.* (2022) reported the yield of acid-soluble collagen from the skin of Purple-Spotted Bigeye Snapper to be 5.79% and pepsin-soluble collagen to be 6.65%. In the present study, 48 hours were spent for collagen extraction, and the collagen extraction efficiency (10.51-10.59%) did not show any significant difference between different species of carp. Researchers have expressed the difference in the obtained results under the influence of various parameters such as temperature, stirring, time, solvent-to-solid ratio, and solvent concentration, which affect collagen extraction efficiency (Menezes *et al.*, 2020).

Table 5- The profile of collagen amino acids extracted from the skin of farmed carp species (Residues 1000g^{-1})

Samples Amino acids	Bighead collagen	Common carp collagen	Silver Carp collagen	Bighead collagen	Grass carp collagen
		Non–essential amino acids			
Aspartic acid	45A	43B	43B	43B	43B
Tryptophan	20A	0B	0B	0B	0B
Serine	33A	28B	28B	29B	29B
Glutamic acid	75A	74A	74A	74A	74A
Glycine	330B	352A	352A	352A	352A
Cysteine	1.24A	0B	0B	0B	0B
Arginine	50B	52A	52A	52A	52A
Proline	121B	125A	125A	125A	125A
Alanine	119B	129A	129A	129A	129A
Tyrosine	3A	4A	4A	4A	4A
		Essential amino acids			
Valine	21A	20A	20A	20A	20A
Isoleucine	11A	9B	9B	9B	9B
Histidine	5B	9A	9A	9A	9A
Lysine	26B	32A	32A	32A	32A
Leucine	23B	27A	27A	27A	27A
Methionine	6B	12A	12A	12A	12A
Phenylalanine	3B	7A	7A	7A	7A
Threonine	18B	20A	20A	20A	20A
Hydroxyproline	94A	78B	78B	78B	78B
Hydroxylysine	7A	3B	3B	3B	3B
Imino acids	215A	193B	193B	193B	193B

Dissimilar uppercase letters in a row indicate the existence of a significant difference at the 5% level ($p<0.05$).

As can be seen in Table 5, the amino acids in the collagen extracted from the skin of farmed carp did not show any significant difference with each other ($p>0.05$). Tryptophan and cysteine were not observed in carp collagen. Amino acids glycine, alanine, proline, and hydroxyproline had the highest amounts. Hydroxylysine had the lowest amount. In general, collagen and proteins absorb a lot of ultraviolet rays due to peptide connections and side chains. Protein absorbs light at a wavelength of 280 nm in the best way. Also, amino acids such as histidine, tryptophan, phenylalanine, and tyrosine have absorption bands between 250 and 288 nm. While the collagen extracted from the skin has a lower absorption wavelength because the amino acids

glycine, proline, and hydroxyproline form the triple helix of collagen, the maximum absorption of which occurs at a wavelength of 230 nm (Rosmawati *et al.*, 2018). In the collagen samples of the present study, the maximum absorption peak was observed between 230 and 240 nm, which was influenced by the simultaneous use of acetic acid and pepsin in the extraction of collagen from fish skin. In addition, absorption values between 200 and 220 nm were attributed to structural materials such as ester or carboxylic compounds. Fish collagen varies widely in amino acid composition. According to studies conducted by other researchers, the living environment has a strong effect on the composition of amino acids in collagen extracted from fish species (García-Sifuentes *et al.*, 2016). However, since the species under study were cultivated species of the same fish that were raised in similar water environments, therefore, the composition of collagen amino acids extracted from their skin did not show any difference. Data on the amino acid composition of pure collagen showed that collagen contains tryptophan, which is a rare amino acid in collagen extracted from carp fish scales. Since the skin of the fish contains scales, This amino acid along with cysteine should have been determined in the collagen amino acid profile, but because the pre-treatment stage and successive washes caused the scales to be separated from the skin, therefore these amino acids were not included in the collagen amino acid profile in the present study (Gauza Włodarczyk *et al.*, 2017). Chinh *et al.* (2019) showed the amino acid sequence in the collagen obtained by acid method from freshwater carp scales as threonine (21), proline (110), glutamic acid (77), arginine (50), serine (38), alanine (117), glycine (306), aspartic acid (49), cysteine (32), histidine (6), lysine (26), leucine (24), phenylalanine (15), valine (19), identified isoleucine (12), methionine (12), tyrosine (21) and hydroxyproline (89) residues $1000g^{-1}$ in the collagen of carp scales. In the present study, cysteine was not observed, aspartic acid (43), glutamic acid (74), tyrosine (4), isoleucine (9), threonine (20), and hydroxyproline (78 residues per $1000g^{-1}$ were observed in smaller amounts. Hydroxylysine (3) and leucine (27) were also determined. Serine (28) and methionine (12) residues $1000g^{-1}$ were reported in the same amount in different treatments. Glycine (352), arginine (52), proline (125), alanine (129), valine (20), histidine (9), and lysine (32) residues $1000g^{-1}$ were observed in more quantity. Moses *et al.* (2018) analyzed the amino acid composition of tilapia collagen and showed that glutamic acid (11.96–15.14 g $100g^{-1}$) and aspartic acid (7.69-9.427.69 g $100g^{-1}$) are the most amino acids of collagen. In the present study, glycine and alanine accounted for the highest amounts of amino acids with 352 and 129 residues $1000g^{-1}$, respectively. Carvalho *et al.* (2018) reported the amino acid sequence in collagen extracted from cod fish including alanine (91.48), arginine (30.45), aspartic acid (38.82), cysteine (1.28), glutamic acid (56.08), glycine (266.12), histidine (5/01), hydroxylysine (6.65), hydroxyproline (39.60), isoleucine (5.61), leucine (16.51), lysine (19.62), methionine (15.04), valine (12.02), tyrosine (2.25), threonine (16.89), serine (53.87), proline (62.69), phenylalanine (12.70), and n-isobutyl glycine (13.75) residues $1000g^{-1}$. In the results of this study compared to other studies isobutyl glycine and cysteine were observed in collagen. The amount of amino acids alanine, aspartic acid, glycine, hydroxyproline, isoleucine, leucine, lysine, valine, threonine, and proline were higher in collagen. However, the amount of amino acids arsenine, glutamic acid, histidine, hydroxylysine, methionine, tyrosine, serine, and phenylalanine was lower. The difference in the amino acid profile of the present study compared to the results of other studies can be might be due to the difference in the primary source of collagen production, species, tissue, and genetic diversity. Chuaychan *et al.* (2016)

reported that collagen extracted using acid and pepsin from spotted golden goatfish scales had a high glycine content (196 and 198 (residues $1000g^{-1}$, respectively). In the present study, collagen had a high amount of glycine (352 residues $1000g^{-1}$). The difference in the results of the present study with the aforementioned study was due to the use of a combination of acid and pepsin for collagen extraction.

Conclusion

The safety features and other quality features such as transparency and aggregation were not significantly different between carp species and were within the acceptable range. Also, the yield of collagen extraction did not show any significant difference between different species of carp. A time of 48 hours is suggested for extracting collagen from cultured carp and replacing it with mammalian collagen.

Acknowledgments

From Dr. Bahmani, the Honorable Director of the Iranian Fisheries Science Research Institute, Dr. Borani, the Honorable Director of the Inland Water Aquaculture Research Institute, Dr. Mirbakhsh, the Honorable Director of the Biotechnology Department of the Iranian Fisheries Science Research Institute, Dr. Jalili, the Director and colleagues of the National Aquaculture Research Center thanks and appreciation is given.

References

1. Ahmed, R., Haq, M., & Chun, B.S. (2019). Characterization of marine derived collagen extracted from the by-products of bigeye tuna (*Thunnus obesus*). *International Journal Biological Macromoleculs, 135(1),* 668–676. https://doi.org/10.1016/j.ijbiomac.2019.05.213
2. Andini, A., Prayekti, E., Wulandari, D.D., & Nidianti, E. (2020). Cytotoxicity assay using brine shrimp lethality test on collagen chitosan wound dressing sterilized by ultraviolet Light. *Indonesian Journal of Medical Laboratory Science and Technology, 2(1),* 21-26. https://doi.org/10.33086/ijmlst.v2i1.1467
3. Association of Official Analytical Chemists (AOAC). (2000). Fish and Marine Products Treatment and Preparation of Sample. *AOAC Standard No. 937.07*. Washington, DC: U.S. Patent and Trademark Office.
4. Arumugam, G.K.S., Sharma, D., Balakrishnan, R.M., Babu, J., & Ettiyappan, P. (2018). Extraction, optimization, and characterization of collagen from sole fish skin. *Sustainable Chemistry and Pharmacy, 9(2018),* 19–26. https://idr.nitk.ac.in/jspui/handle/123456789/11243
5. Blanco, M., Vázquez, J.A., Pérez-Martín, R.I., & Sotelo, C.G. (2017). Hydrolysates of fish skin collagen: an opportunity for valorizing fish industry byproducts. *Marine Drugs, 15(5),* E131. https://doi.org/10.3390/md15050131
6. Carvalho, A.M., Marques, A.P., Silva, T.H., & Reis, R.L. (2018). Evaluation of the potential of collagen from Codfish skin as a biomaterial for biomedical applications. *Marine Drugs, 16(12),* 495. https://doi.org/10.3390/md16120495
7. Cao, J., Duan, Q., Liu, X., Shen, X., & Li, C. (2019). Extraction and physicochemical characterization of pepsin soluble collagens from golden Pompano (*Trachinotus blochii*) skin and bone. *Journal Aquatic Food Product Technology, 28(3),* 837–847. https://doi.org/10.1080/10498850.2019.1652216
8. Chinh, N.T., Manh, V.Q., Trung, V.Q., Lam, T.D., Huynh, M.D., Tung, N.Q., Trinh, N.D., & Hoang, T. (2019). Characterization of collagen derived from tropical freshwater Carp fish scale Wastes and its amino acid sequence. *Natural Product Communications, 14(7),* 1–12. https://doi.org/10.1177/1934578X19866288
9. Chotphruethipong, L., Hutamekalin, P., Nilsuwan, K., Sukketsiri, W., Aluko, R.E., Abdul, N.R., & Benjakul, S. (2022). Combined effects of defatted hydrolyzed collagen from salmon skin and

vitamin C on proliferation and migration of human fibroblast cell. *Fishes, 7(5)*, 265. https://doi.org/10.3390/ fishes7050265
10. Chuaychan, S. (2016). *Production and characterization of collagen, gelatin, and gelatin hydrolysate powder from scales of spotted golden Goatfish*. Feculty of Food Science and Technology, Prince of Songkla University, Songkhla, Thailand.
11. Cumming, M.H., Hall, B., & Hofman, K. (2019). Isolation and characterisation of major and minor collagens from Hyaline Cartilage of Hoki (*Macruronus novaezelandiae*). *Marine Drugs, 17(4)*, 223. https://doi.org/10.3390/md17040223
12. Devran, D., & Gulay, O.Y. (2023). Investigation of possible heavy metals and antibiotic residues in commercial collagen. *Mehmet Akif Ersoy Üniversitesi Veteriner Fakültesi Dergisi, 8(1)*, 31–36. https://doi.org/10.24880/maeuvfd.1218939
13. Junianto, J., Iskandar, I., Rizal, A., & Damayanti, W. (2018). The influence of concentration of acetic acid and pepsin enzyme in Nilem fish skin collagen extractionto the amount of rendement produced. *World News Natural Science, 21(2018)*, 164–170.
14. Ferreira, A.C., Bomfim, M.R.Q., & da Costa Sobrinho, C.H.d.B. (2022). Characterization, antimicrobial and cytotoxic activity of polymer blends based on chitosan and fish collagen. *AMB Express, 12(1)*, 102. https://doi.org/10.1186/s13568-022-01433-7
15. García-Sifuentes, C.O., Lugo-Sánchez, M.E., Scheuren-Acevedo, S., Martínez-Porchas, M., & Peralta-Martínez, V. (2016). Amino acid profile of collagen fractions extracted from by-products of Ophistonemalibertate and Scomberjaponicus, CyTA. *Journal of Food, 14(2)*, 296-301. https://doi.org/10.1080/19476337.2015.1101711
16. Gauza-Włodarczyk, M., Kubisz, L., & Włodarczyk, D. (2017). Amino acid composition in determination of collagen origin and assessment of physical factors effects. *International Journal Biology Macromolecule, 104(PtA)*, 987–991. https://doi.org/10.1016/j.ijbiomac.2017.07.013
17. Gilbert, S.W. (2014). *Applying the hedonic method*. National Institute of Standards and Technology Technical Note 1811. https://doi.org/10.6028/nist.tn.1811
18. Iranian National Standard No. 12014. (1999). Heavy metal measurement of cosmetic products. Iranian Standard and Industrial Research Institute.
19. Iranian National Standard No. 10699. (2015). Livestock, poultry, and aquatic feed - measuring the amount of amino acids. Iranian Standard and Industrial Research Institute.
20. Gómez-Limia, L., Carballo, J., Rodríguez-González, M., & Martínez, S. (2022). Proximate composition and amino acid profle of European eel skin: infuence of body weight. *European Food Research and Technology, 248*(1053–1063), 1437–1446. https://doi.org/10.1007/s00217-022-03978-0
21. Govindharaj, M., Roopavath, U.K., & Rath, S.N. (2019). Valorization of discarded Marine Eel fish skin for collagen extraction as a 3D printable blue biomaterial for tissue engineering. *Journal of Cleaner Production, 230(2)*, 412–419. https://doi.org/10.1016/j.jclepro.2019.05.082
22. ISO 10993-6. (2016). *Biological evaluation of medical devices*. Part 6: tests for local effects after implantation.
23. Li, H., Cheng, W., & Liu, K. (2017). Reinforced collagen with oxidized microcrystalline cellulose shows improved hemostatic effects. *Carbohydr Polym, 165(2)*, 30–38. https://doi.org/10.1016/j.carbpol.2017.02.023
24. Lin, X., Chen, Y., Jin, H., Zhao, Q., Liu, C., Li, R., Yu, F., Chen, Y., Huang, F., Yang, Z., Ding, G., & Tang, Y. (2019). Collagen extracted from bigeye Tuna skin by isoelectric precipitation: physicochemical properties, proliferation, and migration activities. *Marine Drugs*, 17(5), 261. https://doi.org/10.3390/md17050261
25. Menezes, M.D.L.L.R., Ribeiro, H.L., Flávia de Oliveira, M., & de Andrade Feitosa, J.P. (2020). Optimization of the collagen extraction from Nile tilapia skin (*Oreochromis niloticus*) and its

hydrogel with hyaluronic acid. *Colloids and Surfaces B: Biointerfaces, 189(2),* 110852. https://doi.org/10.1016/j.colsurfb.2020.110852

26. Momtahan, N., Panahi, T., Poornejad, N., Stewart, M.G., Vance, B.R., Struk, J.A., Castleton, A.A., Roeder, B.L., Sukavaneshvar, S., & Cook, A.D. (2016). Using hemolysis as a novel method for assessment of cytotoxicity and blood compatibility of decellularized heart tissues. *ASAIO Journal, 62(3*), 340-8. https://doi.org/10.1097/MAT.0000000000000373
27. Moses, S., Agbaji, E.B., Ajibola, V.O., & Gimba, C.E. (2018). Amino acid composition and proximate analysis in Tilapia (*Oreochromis mossambicus*) Fish from dams and rivers in Zamfara state, Nigeria. *Journal Applied Science Environment Management, 22(6),* 8899–905. https://doi.org/10.4314/jasem.v22i6.10
28. Mullen, A., Bayon, Y., Pandit, A., & Raghunath, M. (2019). The collagen suprafamiliy: From biosynthesis to advanced biomaterial development. *Advanced Materials, 31(1),* 6–11. https://doi.org/10.1002/adma.201801651
29. Nalinanon, S., Benjakul, S., Visessanguan, W., & Kishimura, H. (2007). Use of pepsin for collagen extraction from the skin of bigeye snapper (*Priacanthus tayenus*). *Food Chemistry, 104(2),* 593-601. https://doi.org/10.1016/j.foodchem.2006.12.035.
30. Oslan, S.N.H., Shapawi, R., Mokhtar, R.A.M., Noordin,W.N.M., & Huda, N. (2022). Characterization of acid and pepsin-soluble collagen extracted from the skin of purple-Spotted Bigeye Snapper. *Gels, 8(10),* 665. https://doi.org/10.3390/gels8100665
31. Qiu, B., Wei, F., Sun, X., Wang, X., Duan, B., Shi, C., Zhang, J., Zhang, J., Qiu, W., & Mu, W. (2014). Measurement of hydroxyproline in collagen with three different methods. *Molecular Medicine Reports, 10(2),* 1157–1163. https://doi.org/10.3892/mmr.2014.2267
32. Raman, M., & Gopakumar, K. (2018). Fish collagen and its applications in food and pharmaceutical industry: A review. *EC Nutrition, 13(2018),* 752–767. https://api.semanticscholar.org/CorpusID:54176016
33. Rosmawati, Abustam, E., Tawali, A., & Said, M.I. (2018). Amino acid and collagen content of Snakehead (*Channa striata*) fish skin and bone. *Scientific Research Journal, 6(1),* https://api.semanticscholar.org/CorpusID:212409859
34. Ramle, S.Z., Oslan, S.N.H., Shapawi, R., Mokhtar, R.A.M., Noordin, W.N.M., & Huda, N. (2022). Biochemical characteristics of acid-soluble collagen from food processing by-products of Needlefish Skin (*Tylosurus acus melanotus*). *Appliued Science, 12(24*), 12695. https://doi.org/10.3390/app122412695
35. Seifzadeh, M., Golashahi, E., & Safiyari, Sh. (2018). Study the concentrations of lead and cadmium in farmed rainbow trout in Talesh of Guilan. *Animal Science Research Journal, 28(2),* 65–79. https://sid.ir/paper/365590/en
36. Song, W.K., Liu, D., Sun, L.L., Li, B.F., & Hou, H. (2019). Physicochemical and biocompatibility properties of type I collagen from the skin of Nile Tilapia (*Oreochromis niloticus*) for biomedical applications. *Marine Drugs, 17(3),* 137. https://doi.org/10.3390/md17030137
37. Sorushanova, A., Delgado, L., Zhuning, W., Shologu, N., Kshirsagar, A., Raghunath, R., Sousa, R.O., Alves, A. L., Carvalho, D.N., Martins, E., Oliveira, C., Silva, T.H., & Reis, R.L. (2020). Acid and enzymatic extraction of collagen from Atlantic cod (*Gadus morhua*) swim bladders envisaging health-related applications. *Journal of Biomaterials Science, Polymer Edition, 31(3),* 20–37. https://doi.org/10.1080/09205063.2019.1669313
38. Sukhikh, S., Noskova, S., Ivanova, S., Ulrikh, E., Izgaryshev, A., Larichev, T., Kozlova, O., Prosekov, A., & Babich, O. (2021). Comparative analysis of collagen-containing waste biodegradation, amino acid, peptide and carbohydrate composition of hydrolysis products. *Applied Science, 11(23),* 11511. https://doi.org/10.3390/app112311511

39. Tan, Y., & Chang, S.K.C. (2018). Isolation and characterization of collagen extracted from channel catfish (*Ictalurus punctatus*) skin. *Food Chemistry, 242(1)*, 147–155. https://doi.org/10.1016/j.foodchem.2017.09.013
40. Xu, N., Peng, X.L, Li, H.R., Liu, J.X., Cheng, J.S.Y., Qi, X,Y., Ye, S.J, Gong, H.L, Zhao, X.H., Yu, J., Xu, G., & Wei, D.X. (2021). Marine-derived collagen as biomaterials for human health. *Frontires Nutrition, 8(3)*, 702108. https://doi.org/10.3389/fnut.2021.702108
41. Zhang, W., Zheng, J., Tian, X., Tang, Y., Ding, G., Yang, Z., & Jin, H. (2019). Pepsin-soluble collagen from the skin of Lophius litulo: A preliminary study evaluating physicochemical, antioxidant, and wound healing properties. *Marine Drugs, 17(12)*, 708. https://doi.org/10.3390/md17120708
42. Wang, T., Yang, L., Wang, G., Han, L., Chen, K., Liu, P., Xu, S., Li, D., Xie, Z., Mo, X., Wang, L., Liang, H., Liu, X., Zhang, S., & Gao, Y. (2021). Biocompatibility, hemostatic properties, and wound healing evaluation of tilapia skin collagen sponges. *Journal of Bioactive and Compatible Polymers, 36(1)*, 44–58. https://doi.org/10.1177/0883911520981705
43. Weber, M., Steinle, H., Golombek, S., Hann, L., Schlensak, C., Wendel, H. P., & Avci-Adali, M. (2018). Blood-Contacting biomaterials: in vitro evaluation of the Hemocompatibility. *Frontires Bioenginering Biotechnology, 6(3)*, 99. https://doi.org/10.3389/fbioe.2018.00099
44. Wei, P., Zheng, H., Shi, Z., Li, D., & Xiang, Y. (2019). Isolation and characterization of acid-soluble collagen and pepsin-soluble collagen from the skin of hybrid sturgeon. *Journal of Wuhan University of Technology-Mater Science Edition, 34(2019)*, 950–959. https://doi.org/10.1007/s11595-019-2143-6
45. Wu, J., Kong, L., Zhang, J., & Chen,W. (2019). Extraction and properties of acid-soluble collagen and pepsin-soluble collagen from silver carp (*Hypophthalmichthys molitrix*) scales: Prerequisite information for fishery processing waste reuse. *Polish Journal Environmental Studies, 28(4)*, 2923–2930. https://doi.org/10.15244/pjoes/93742

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد ۱۹، شماره ۳، مرداد- شهریور ۱۴۰۲ ص. ۴۷-۳۳

Iranian Food Science and Technology Research Journal
Vol. 19, No. 3, Aug.-Sep. 2023, p. 33-47

بررسی ویژگی‌های کیفی و ایمنی کلاژن پوست گونه‌های سرگنده (*Hypophthalmichthys nobilis*)، معمولی (*Cyprinus carpio*)، علف‌خوار (*Ctenopharyngodon idella*) و نقره‌ای (*Hypophthalmichthys molitrix*) کپور ماهیان پرورشی

مینا سیف زاده[1*]

تاریخ دریافت: ۱۴۰۲/۰۱/۲۱
تاریخ بازنگری۱۴۰۲/۰۳/۱۳
تاریخ پذیرش: ۱۴۰۲/۰۴/۱۰

چکیده

تنوع زیاد کاربردهای کلاژن و نقش مهم آن در آینده جامعه، سبب شد که به پلیمر زیستی کلیدی برای سلامت و رفاه انسان تبدیل شود. بنابراین مطالعه حاضر با هدف استخراج کلاژن از پوست ماهی کپور پرورشی، تعیین خصوصیات کیفی کلاژن و مقایسه آنها انجام شد. کلاژن از پوست ماهی کپور به روش آنزیمی اسیدی با استفاده از اسید استیک ۰/۵مولار و پپسین طی مدت زمان ۴۸ ساعت استخراج شد. تیمارهای کلاژن (۵ تیمار) شامل کلاژن تهیه شده از پوست گونه‌های معمولی، علف‌خوار، سر گنده و نقره‌ای کپور ماهیان و گاو (شاهد) هستند. تیمارهای کلاژن قادر به همولیز خون نبودند و اثرات سمی روی سلول‌های فیبروبلاست انسانی نشان ندادند. فلزات سنگین (۰/۱۸–۰/۰۱ ppm) در کلاژن استخراج شده از گونه‌های کپور پرورشی در محدوده استاندارد قرار داشتند. رنگ (روشنایی) کلاژن آزمایشی (۹۳/۶۸–۹۲/۷۴) و شاهد (۹۲/۳۸) تفاوت معنی‌داری نشان ندادند ($P> ۰/۰۵$). اسیدهای آمینه سیستئین و تریپتوفان در کلاژن مشاهده نشد. اسیدهای آمینه گلیسین و هیدروکسی لیزین (به‌ترتیب ۳۵۲ و ۳ residues $1000g^{-1}$) بیشترین و کمترین میزان کلاژن را داشتند. پروفایل اسیدهای آمینه و راندمان تولید کلاژن (۱۰/۵۹–۱۰/۵۱ درصد) تفاوت معنی‌داری را در گونه‌های ماهی کپور نشان ندادند ($P> ۰/۰۵$). بر اساس نتایج مطالعه حاضر، بین راندمان تولید، ایمنی و ویژگی‌های کیفی کلاژن در گونه‌های کپور پرورشی تفاوت معنی‌داری مشاهده نشد ($P> ۰/۰۵$). این ویژگی‌ها بین کلاژن آزمایشی و شاهد تفاوت معنی‌داری نشان ندادند ($P> ۰/۰۵$). از این رو پوست این گونه‌ها را می‌توان برای تولید کلاژن به کار گرفت و جایگزین کلاژن پستانداران کرد.

واژه‌های کلیدی: آنزیم پپسین، ایمنی، کپور ماهیان پرورشی، کلاژن، هیدرولیز اسیدی

۱- استادیار مرکز ملی تحقیقات فرآوری آبزیان، پژوهشکده آبزی‌پروری آب‌های داخلی، موسسه تحقیقات علوم شیلاتی کشور، سازمان تحقیقات آموزش و ترویج کشاورزی، انزلی، ایران
(*- نویسنده مسئول: Email: m.seifzadeh@areeo.ac.ir)

DOI: 10.22067/ifstrj.2023.81732.1245

Iranian Food Science and Technology Research Journal
Vol. 19, No. 3, Aug.-Sep. 2023, p. 49-63

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد ۱۹، شماره ۳، مرداد- شهریور ۱۴۰۲ ص. ۶۳-۴۹

Research Article
Homepage: http://ifstrj.um.ac.ir

Evaluation of Antioxidant and Antibacterial Activities of *Apis florea* Fabricius (Hymenoptera: Apidae) Honey on *Helicobacter pylori*

Sh. Parichehreh[1]*, Gh. Tahmasbi[2], M. Eslampanah[3], P. Khaki[4]

Received: 2023.04.22
Revised: 2023.07.08
Accepted: 2023.07.09
Available Online: 2023.07.09

How to cite this article:
Parichehreh, Sh., Tahmasbi, Gh., Eslampanah, M., & Khaki, P. (2023). Evaluation of antioxidant and antibacterial activities of *Apis florea* Fabricius (Hymenoptera: Apidae) honey on *Helicobacter pylori*. *Iranian Food Science and Technology Research Journal*, *19*(3), 49-63. https://doi.org/10.22067/ifstrj.2023.81933.1248

Abstract

Iranian men are at risk of developing gastrointestinal cancer caused by *H. pylori*. It is very imperative to find effective methods to control this bacterium as there are currently no very effective treatments for it. Honey has been shown to have antimicrobial properties against various pathogens. This study analyzed 15 honey samples from *A. florea* bees, collected from different floral and geographical origins, for their antimicrobial efficacy against *H. pylori*. Using atomic absorption measurements, the honey samples were also tested for their phenolic and flavonoid content, protein concentration, and mineral content. Antioxidant activity was determined using the FRAP, DPPH, and ABTS methods. The antibacterial activity of honey samples was investigated both *in-vitro* and *in-vivo* in the gastrointestinal tract of mice. Statistical analysis revealed a significant positive correlation between antioxidant activity and antibacterial activity. All honey samples showed antimicrobial activity *in-vitro*, among which jujube honey from Bushehr exhibiting the highest activity. Differences in antioxidant and antimicrobial activities were likely due to the flora of the plants and the geographic region from which the honey was harvested. Based on these results, *A. florea* honey may be used in the prevention and treatment of *H. pylori*-associated infections and inflammation of the gastrointestinal tract. This feature can be applied to the control of *Helicobacter pylori* along with other available measures.

Keywords: Antioxidant activity, Antimicrobial activities, Honey, *H. pylori*

Introduction

H. pylori is a gram-negative bacterium that is resistant to gastric acid and colonizes the gastrointestinal tract (Khatun *et al.*, 2013). It is a known carcinogen and has been classified as a Class I carcinogen by the World Health Organization. Chronic inflammation caused by *H. pylori* infection increases the risk of gastric and duodenal ulcers, which can lead to gastric cancer (Graham, 2015). It is a known risk factor for gastric cancer development and its pathogenesis is associated with oxidative stress. *H. pylori* infection induces the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the host, leading to oxidative damage in gastric epithelial cells. Host antioxidant systems are activated to counteract this damage, including enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx). However, *H.*

1 and 2- Assistant Professor and Professor Department of Honeybee, Animal Science Research Institute of Iran, Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran, respectively.
(*- Corresponding Author Email: Sh.pariichehreh@gmail.com)

3 and 4- Ph.D. Graduate and Associate Professor, Department of Microbiology, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran, respectively.
DOI: 10.22067/ifstrj.2023.81933.1248

pylori have evolved strategies to evade host immune responses and antioxidant defense systems, resulting in chronic inflammation and increased cancer risk. Despite its prevalence, most infected people (80%) are asymptomatic. However, long-term infection significantly increases the risk of cancer (Saha *et al.*, 2010; Wroblewski *et al.*, 2010). Although many different drugs have been tested to treat *H. pylori*, none have been shown to be effective (Take *et al.*, 2015). Due to the side effects and antibiotic resistance associated with antibiotic treatment, researchers are exploring natural compounds with anti-inflammatory and antimicrobial properties (Bonacorsi *et al.*, 2009). Honey is gaining attention for its antibacterial and antioxidant properties, and its clinical use is increasing (Selahvarzian *et al.*, 2015).

Honey is believed to be the first product discovered by prehistoric people, compared to other products produced by honey bees. Honey is produced by two species of honey bees: the European honey bee (*Apis mellifera*) and the Asian dwarf honey bee (*A. florea*), and is stored in honeycombs after a series of interactions in their digestive tracts. *A. florea* is found in Iran from the South West (Khuzestan Province) to the South (Boushehr Province) and the South East (Sistan and Balouchestan Provinces) (Parichehreh *et al.*, 2020). The high osmolality and antibacterial properties of honey make it a useful substance to promote human health. The effectiveness of honey in this regard is influenced by the species of honey bees, the plants they feed on, and the local climate (Aliyazicioglu and Boukraa, 2015). The antibacterial effect of honey is mainly due to the presence of hydrogen peroxide, which depends on the levels of glucose oxidase and catalase (Weston, 2000). These levels can vary between different types of honey and contribute to differences in their antimicrobial properties. The antibacterial and antioxidant properties of honey are due to the presence of lysozyme, phenolic acids and flavonoids (Snowdon and Cliver, 1995). Honey is a unique wound dressing as it can clear the infection, promote rapid wound healing, inhibit inflammation, minimize scarring, stimulate angiogenesis, and expand epithelial tissue in a short time (Molan, 2002). Numerous studies have investigated the antimicrobial activity of honey from different botanical and geographical origins. For example, the antibacterial activity of honey from *A. mellifera* was studied by Selahvarzian *et al.* (2015), who found that honey from bees fed on licorice extract showed the highest antibacterial activity. Another study by Boyanova *et al.* (2015) found that honey of *A. mellifera* reduced the risk of *H. pylori* infection in 150 Bulgarian patients. Patients who consumed honey more than once a week had a lower rate of *H. pylori* infection than those who did not consume honey. Research conducted by Grego *et al.* (2016) on Italian honey highlighted that the antimicrobial activity of honeydew, polyfloral, and chestnut honey against *S. aureus* was similar to that of manuka honey. A study by Gośliński *et al.* (2020) compared the antioxidant and antimicrobial properties of manuka honey and Polish honey. The results showed that manuka honey had higher antioxidant capacity and stronger antimicrobial activity than Polish honey, suggesting that it may be a more effective natural remedy in the prevention or treatment bacterial infections and oxidative stress-related conditions. Kolayli *et al.* (2020) reported strong antimicrobial activity against *S. aureus* in buckwheat honey (*Fagopyrum eculentum*), heather honey (*Calluna vulgaris*), nettle or urtica honey (*Urtica dioica*), thistle honey (*Silybium marianum*), caltrop honey (*Eryngium campestre*), coriander honey (*Coriandrum sativum*), thyme honey (*Thymus vulgaris*), and honeydew. They also observed moderate antimicrobial activity in heather honey (*Calluna vulgaris*) and honeydew against *E. coli*, and heather honey (*Calluna vulgaris*) against *C. albicans*.

While *A. mellifera* honey has been extensively studied for its antioxidant and antibacterial activity, there is not sufficient research on the case of *A. florea* honey. Therefore, the study aimed at assessing the

antioxidant and antibacterial properties of *A. florea* honey collected from various regions in Iran including Bushehr, Dezful, Iranshahr, Jahrom, and Jiroft, which are characterized by different vegetation types. In this investigation, we tried to clarify the potential health benefits of *A. florea* honey and how it could be used as a natural remedy for various illnesses.

Materials and Methods

Preparation of samples

In August and September 2019, 15 samples of *A. florea* honey (1-2 kg per region) were collected from the southern region of Iran including Bushehr, Dezful, Iranshahr, Jahrom and Jiroft.

Identification of mineral compounds

Mineral content in honey samples was measured using an atomic absorption spectrophotometer (Tosic *et al.*, 2017).

Determination of total phenolic compounds

Total phenolic compounds were determined using the Folin-Ciocalteu colorimetric method using gallic acid as a standard at 760 nm (Singleton *et al*., 1999).

Identification of phenolic and flavonoid compounds

Analysis of phenolic and flavonoid compounds of honey samples was performed by high-performance liquid chromatography (HPLC) (Agilent 1200-Germany) detector of Diode Array at 260 nm. For this purpose, 300 µl of the solution was injected into the instrument. The mobile phase consisted of water/acetic acid (ratio 1.19 v/v) (solvent A) and methanol (solvent B) at a constant flow rate of 1 mL/min. The column temperature was kept constant at 30°C and the chromatograms were processed with Chemstation chromatography software (Mello *et al.*, 2010). A C18 reversed-phase Acquity column (1.7 µm, 150 mm, 4.6 mm) protected by a guard column was used in this study.

Investigation of antioxidant activity of honey samples

The antioxidant activity of honey samples was measured using ABTS, FRAP and DPPH methods.

Trolox equivalent antioxidant activity method (ABTS)

The antioxidant activity of honey was determined according to the method of Re *et al.* (1999) using the Trolox equivalents (TEAC) as a measure of antioxidant activity. ABTS was obtained by reacting the prepared 7 mM ABTS aqueous solution with 2.4 mM potassium persulfate ($K_2S_2O_8$). Samples were stored in the dark at room temperature for 12-16 h. 160 µL of ABTS+ solution was added to 40 µL of the sample at different concentrations. Absorbance at 734 nm was measured after incubation for 10 min at room temperature using a 96-well microplate reader.

FRAP method

The ferric ion regenerative antioxidant activity (FRAP) method was measured following the method of Benzie and Strain (1996) with some modifications. The honey sample was first dissolved in 10 mL of n-hexane-acetone mixture (6:4) and then filtered through the Whatman number 4 filter paper. The honey solution was mixed with 1.8 mL of FRAP reagent, and the absorbance of the reagent mixture was measured spectrophotometrically at 593 nm after incubation for 10 min. The calibration curve was constructed using Trolox, and the results were expressed as mg of Trolox equivalent (TE) per 100 gr of honey.

DPPH method

The antioxidant activity of honey samples was measured by diphenyl picrylhydrazyl (DPPH) at 517 nm according to the method of Von Gadow *et al.* (1997). Honey samples were dissolved in 5 mL of methanol and filtered through Whatman #4. Subsequently, 100 mL of honey, butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA) in methanol

were mixed with 2.9 mL of a $6 \times 10{-}5$ M solution of DPPH in methanol. The mixture was shaken vigorously and left in the dark at 25°C for 60 min. The absorbance of the solution was measured at 517 nm against a methanol blank using a spectrophotometer (Hitachi U-1900, Japan).

Measuring protein levels

Protein concentration was measured according to the method described by Bradford (1976).

***In-vitro* antibacterial activities of different honey samples**

Testing was performed using the agar well diffusion method (de Queiroz Pimentel *et al*., 2013). After preparing Muller-Hinton agar according to the manufacturer's instruction, 0.5 McFarland bacterial suspension was plated in four wells of each plate using a sterile Pasteur pipette. After the Hinton agar was thawed and sealed, 80 µL of each honey sample was poured into each well. After 37 h of incubation at 37°C, the size of the zone of growth inhibition was measured with a ruler. Each test was repeated three times and recorded.

***In-vivo* antibacterial activities of different honey samples**

Honey samples were tested for antimicrobial activity against *H. pylori* provided by the Iranian National Center for Genetic and Biological Resources.

BALB/c mice (6-7 weeks old) were provided by Razi Vaccine and Serum Research Institute, Tehran and were kept at 25°C and 12:12 L:D under pathogen-free conditions. The mice were fed autoclaved food and water every 2 days. After the bacterial strain was cultured on Nutrient Agar (NA) and placed under anaerobic conditions at 24°C for 24 h, the cells were washed twice with distilled water and a suspension of 10^9 CFU/mL was administered to the mice. In this study, an orogastric tube was inserted into the stomach of mice to observe the occurrence of infection and changes (ulcers, decay, and abnormal tissue growth) (Shamala *et al*., 2002).

Evaluation of gastritis and status of *H. pylori* infection after treatments

A total of sixty healthy female BALB/c mice were prepared and kept at the above-mentioned conditions. There were six groups of 10 mice, and two were considered control groups (one infected with the bacteria but not eating honey, and 1 without the infection). After introducing *H. pylori* into the gastrointestinal tract of mice by gavage, several mice were randomly selected and their stomachs were sampled. After inoculation with *H. pylori*, the mice were fed honey at a concentration of 6:1 (85.7% water: 14.3% honey) every other day, mice stomachs were removed after 75 days and placed in a Styrofoam tray covered with drawing paper. They were then fixed in 10% formaldehyde for 30 days, divided into four equal parts and embedded in paraffin. These four pieces (5 µm thick) were cut horizontally, immersed in albumin solution overnight at 40°C before staining with hematoxylin and eosin (Boldt *et al*., 2015). Stained sections were observed under an optical microscope.

Scoring gastric inflammation

The Sydney system was also used to assess the degree of inflammation in addition to detecting *H. pylori* contamination (Jones *et al*., 2002; Nakamura *et al*., 2002). Grading and scoring are based on endoscopic and histopathological criteria, which are more concerned with topography, morphology, and degree of inflammation (zero = absent, + (mild), ++ (moderate), +++ (severe)). *H. pylori* can also be treated by scoring: zero = absence of *H. pylori*, one = low presence of bacteria up to 5 glands, 2 = moderate presence of bacteria from 6 to 10 glands, 3 = high presence of *H. pylori* from 11 glands upwards.

Statistical analysis

Physicochemical results were reported as mean ± SD (standard deviation) of triplicate samples and statistical differences were tested

using one-way analysis of variance (ANOVA). Differences on the histology score were tested by the Mann-Whitney U test. Both tests considered the results as statistically significant when $p < 0.05$.

Results

Inorganic compounds

The mineral compounds identified in the honey samples are listed in Table 1. The highest and lowest calcium levels were recorded for Bushehr and Jahrom honey samples with 187.70 and 86.08 ppm, respectively. The highest and lowest magnesium levels were measured in Iranshahr (460.09 ppm) and Jiroft (55/43 ppm). In addition, the highest and lowest levels of phosphorus were 7.57 and 0.36 ppm for Iranshahr and Jahrom, respectively. The highest and lowest zinc was recorded for Jiroft (10.03 ppm) and Iranshahr (7.63 ppm), and the highest and lowest percentages of potassium were for Bushehr (846.21 ppm) and Iranshahr (412.15 ppm). Iranshahr honey samples had the highest iron concentration, while Jiroft honey samples had the lowest iron concentration (Table 1).

Table 1- Assessment of mineral compounds in different honey samples

Sample	Ca (ppm)	Mg (ppm)	P (ppm)	Zn (ppm)	K (ppm)	Fe (ppm)
Dezful	187.17	371.07	0.39	9.38	843.82	144.28
Iranshahr	83.80	460.09	5.24	7.63	412.15	219.99
Bushehr	187.70	171.75	0.39	8.51	846.21	117.32
Jahrom	86.08	288.79	0.36	9.50	776.10	114.77
Jiroft	136.32	55.43	7.57	10.03	819.39	30.29

Protein content

The protein content of honey samples was calculated 94.23 ± 0.85, 102.11 ± 0.55, 137.92 ± 0.25, 138.75 ± 0.27, and 133.44 ± 0.12 ppm for Dezful, Iranshahr, Bushehr, Jahrom, and Jiroft respectively (Fig. 1). Statistical analysis of the results showed that the samples differed significantly in protein content (F: 4873.09, df: 14, P: 0.000).

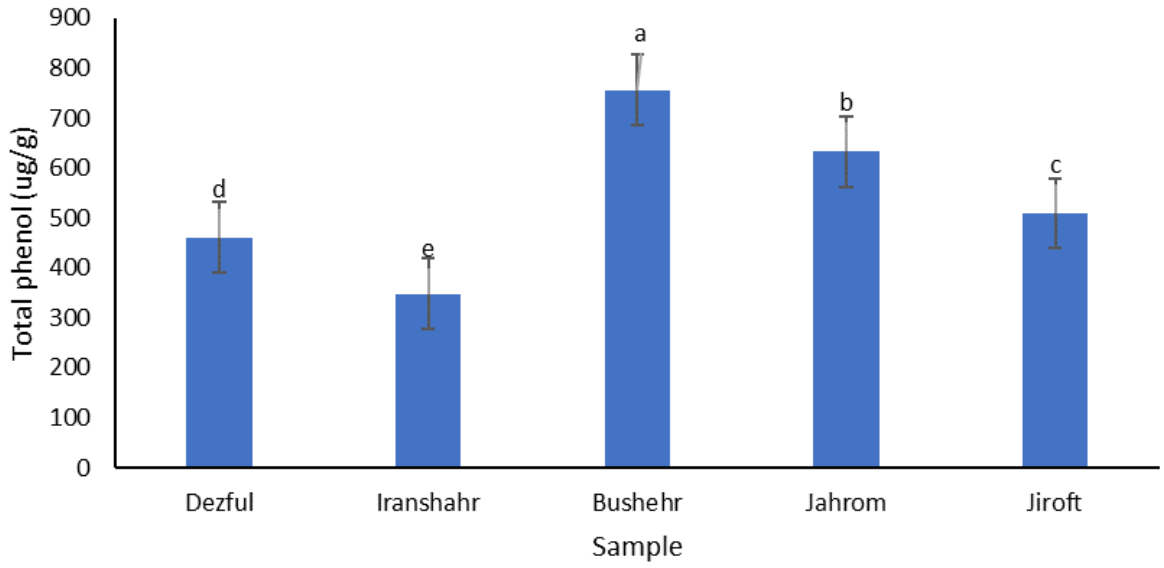


Fig. 1- The amount of total phenol in honey samples
Different letters denote significantly different values from one another (LSD) at $P \leq 0.01$ level.

Phenol and flavonoids

Phenolic and flavonoid compounds of honey samples were identified by HPLC chromatography. Isolated compounds identified in honey samples are shown in Table 2. The compounds included gallic acid, chlorogenic acid, caffeic acid, p-coumaric acid, quercetin, kampefrol, hespertin, and apegenin (Table 2). It was found that the most extracted compounds in the extracts of Jiroft, Bushehr, Iranshahr, Jahrom, and Dezful honey were apigenin (54.16%), apigenin (94.51%), kaempferol (66.95%), apigenin (46.90%), and kaempferol (47.15%), respectively.

Table 2- Analysis of phenolic and flavonoid compounds of honey samples by HPLC

Compounds	Dezful	Iranshahr	Bushehr	Jahrom	Jiroft
Gallic acid	0.13*	0.30	0.16	0	0
Chlorogenic acid	0	0	2.64	33.40	0
Caffeic acid	0.69	0.07	0.05	0.10	0.07
p-Coumaric acid	0	0	0	8.04	5.11
Quercetin	0.1	0.14	0.02	0.03	0.05
Kampefrol	2.81	2.35	5.75	11.17	2.78
hespertin	0.31	0.31	0.38	0.62	0.31
Apegenin	1.92	0.34	154.98	47.14	9.83

*Retention time

Total phenol content

The highest and lowest phenol contents were observed in Bushehr (755.83 g/g) and Iranshahr (348.83 g/g), respectively (Fig. 2). Based on statistical analysis, there were significant differences in total phenol content among the collected samples (F: 38.03, df: 14, P: 0.000).

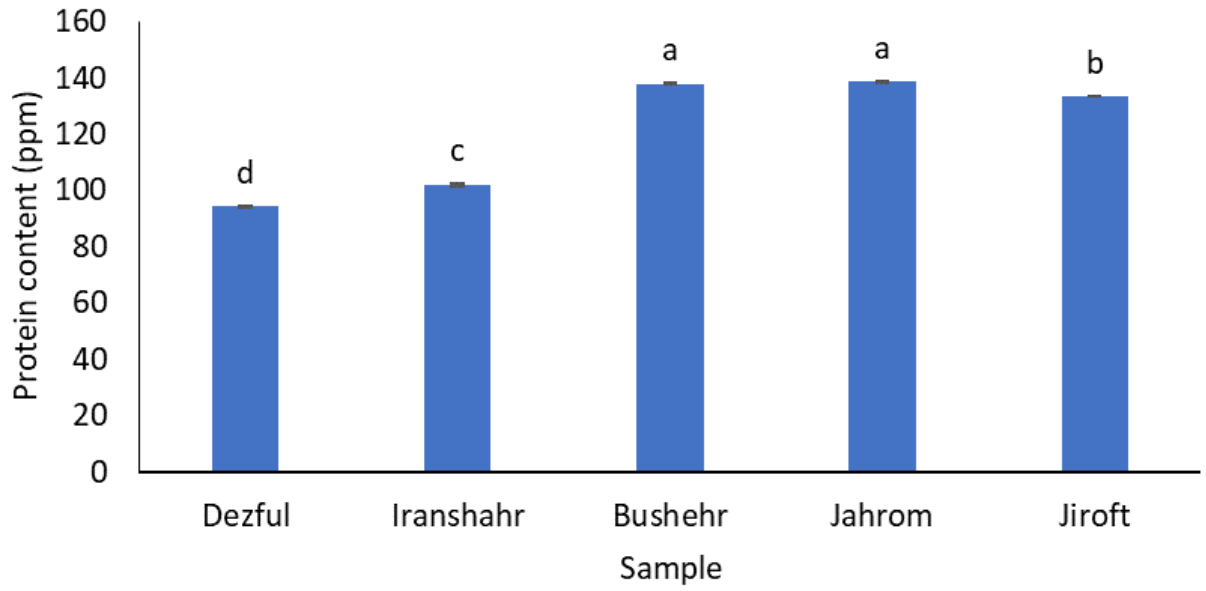


Fig. 2- Measurement of protein content in different honey samples
Different letters denote significantly different values from one another (LSD) at P ≤ 0.01 level.

Antioxidant activity

The antioxidant activity of different honey samples was measured by three methods: ABTS, FRAP, and DPPH. According to ABTS, the highest and lowest levels of antioxidant activity were found in Bushehr (786.40 ± 29.73 µg/g) and Iranshahr (324.90 ± 4.90 µg/g samples (Table 3). Based on statistical analysis,

significant differences were found among honey samples in terms of antioxidant activity (F: 64.40, df: 14, P: 0.000). There were also significant differences in antioxidant activity between different samples according to the FRAP method (F: 14.32, df: 14, P: 0.000). Dezful, Iranshahr, Bushehr Jahrom, and Jiroft honey samples were tested by FRAP method for antioxidant activity, among which Bushehr and Iranshahr samples showing the maximum and minimum antioxidant activity, respectively (Table 3). The DPPH method confirmed the results of the ABTS and FRAP methods for honey antioxidant activity (F: 41.77, df: 14, P: 0.000). According to Table 3, Bushehr honey samples (625.76 ± 26.17 µg/g) and Iranshahr honey samples (393.03 ± 11.98 µg/g) had the maximum and minimum antioxidant activities, respectively (Table 3).

Table 3- Antioxidant power of honey samples collected from different areas
Different letters denote significantly different values from one another (LSD).

Sample	Antioxidant capacity (µgr/gr)		
	ABTS (Mean±SE)	FRAP (Mean±SE)	DPPH (Mean±SE)
Dezful	533.44±19.9 ^{c}	23.28±0.80 ^{ab}	424.64±4.63 ^{d}
Iranshahr	324.90±4.90 ^{d}	14.41±0.95 ^{d}	393.03±11.98 ^{d}
Bushehr	786.40±29.73 ^{a}	26.98±0.92 ^{a}	625.20±26.17 ^{a}
Jahrom	691.96±32.38 ^{b}	23.37±1.81 ^{ab}	570.62±16.58 ^{b}
Jiroft	626.19±6.84 ^{b}	19.97±1.45 ^{c}	472.73±5.59 ^{c}

Different letters indicate significant differences

Antibacterial activities

Table 4 shows the antibacterial activities of *A. florea* honey samples. A 50% decrease in honey concentration *in-vitro* resulted in a decrease in the diameter of the zone of growth inhibition in all samples. This indicates a strong relationship between honey concentration and antimicrobial activity. The antimicrobial activities of honey samples were significantly different based on statistical analysis. Bushehr and Iranshahr honey samples had the highest and lowest antimicrobial activities, respectively, with growth halo diameters of 19.17 ± 0.12, and 13.14 ± 13.09 (mm), respectively.

Based on histopathological observations and the classification scale, inflammation (no = 0, + mild, ++ moderate, +++ severe) was rated (Table 5). A mild inflammatory response and severe mucosal degeneration have been observed in Dezful honey (Fig. 3-a). In the Bushehr honey sample, gastric mucosa showed mild inflammation and mild degeneration, along with severe eosinophilia (Fig. 3-b). Fig. 3c shows gastric mucosa with mild inflammation and moderate degeneration and moderate to severe eosinophilia in the Jahrom honey sample. Furthermore, Jiroft honey samples showed mild inflammatory processes, moderate to severe degeneration, and severe eosinophilia in the gastric mucosa (Fig. 3-e). In the Iranshahr sample, mild inflammatory and moderate to severe degeneration and moderate presence of eosinophils in gastric mucosa were observed (Fig. 3-g). The positive control group showed moderate inflammatory reactions and severe degeneration (Fig. 3-d). According to the results, Bushehr honey and Jahrom honey had less inflammation and degeneration and had higher eosinophil content, so they had higher control against *H. pylori* than other honeys, which is similar to the results of *in-vitro* analysis.

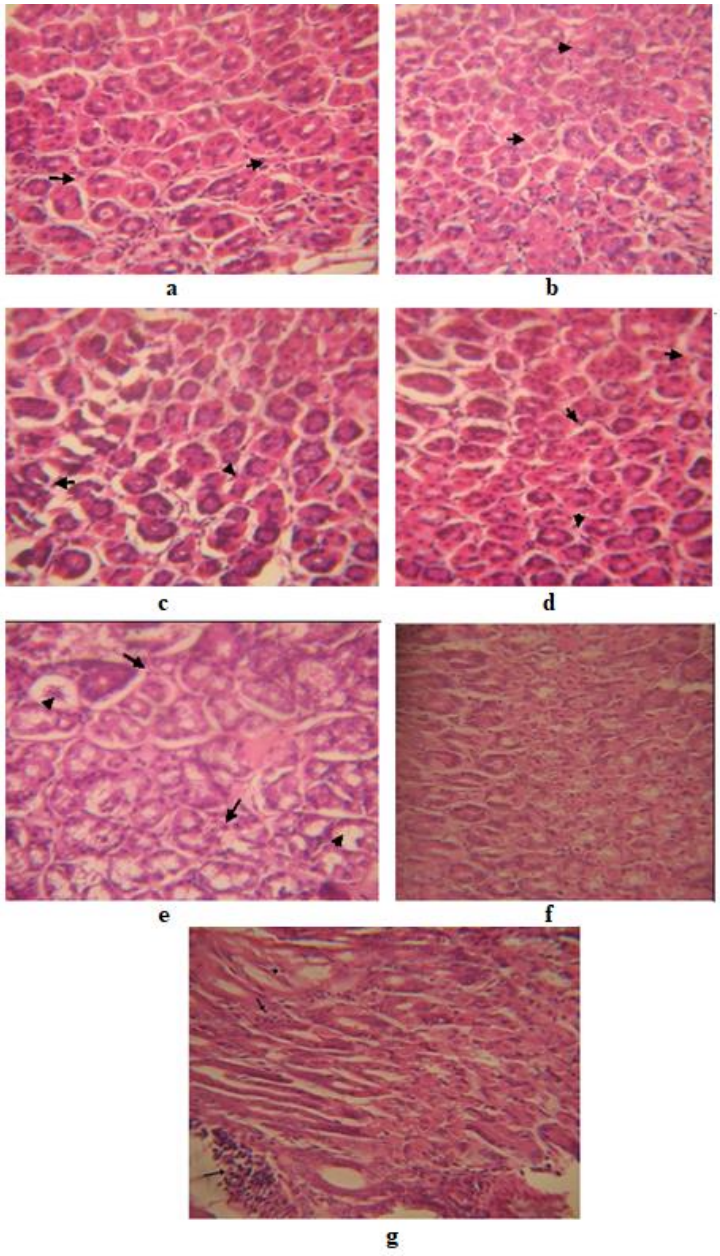


Fig. 3- (a-g). Stomach. Gastric mucosa of mice infected with *Helicobacter pylori* after different honey treatment groups

Stomach. Dezful. Mild inflammatory process and severe degeneration in the gastric mucosa were observed. Haematoxylin and eosin× 400.

Stomach. Bushehr. Mild inflammatory process, mild degeneration and severe presence of eosinophils in the gastric mucosa were observed. Haematoxylin and eosin× 400.

Stomach. Jahrom. Mild inflammatory process, relative degeneration and moderate to severe presence of eosinophils in gastric mucosa were observed. Haematoxylin and eosin× 400.

Stomach. Positive control. Moderate inflammatory process, mild degeneration was observed. Haematoxylin and eosin× 400.

Stomach. Jiroft. Mild inflammatory process, moderate to severe degeneration and severe presence of eosinophils in gastric mucosa were observed. Haematoxylin and eosin× 400.

Stomach. Negative control. Haematoxylin and eosin× 400.

Stomach. Iranshahr. Mild inflammatory process, moderate to severe degeneration and moderate presence of eosinophils in gastric mucosa were observed. Haematoxylin and eosin× 400.

Table 4- Diameter of growth inhibition zone of *Heliobacter pylori* in different concentrations of different honey samples

Different letters denote significantly different values from one another (LSD).

Sample	Diameter of growth inhibition zone (mm)	
	100% solution	50% solution
Dezful	14.83±0.15 ^{c}	12.74±0.05 ^{C}
Iranshahr	13.09±0.04 ^{d}	11.32±0.07 ^{D}
Bushehr	19.17±0.12 ^{a}	17.53±0.13 ^{A}
Jahrom	18.83±0.06 ^{ab}	11.65±0.13 ^{D}
Jiroft	18.36±0.15 ^{b}	14.22±0.07 ^{B}

Table 5– Presence of *Helicobacter pylori*, inflammation, degeneration, and eosinophil in the gastrointestinal tract of mice, according to the Sydney System classification scale (0 = Absent, + = Mild, ++ = Moderate, +++ = Severe)

Sample	Inflammation	Degeneration	Eosinophil
Dezful	1	3	1.5
Iranshahr	0.5	2	1.5
Bushehr	0.5	1	3
Jahrom			
Jiroft	1.5	1	1.5
Negative control			
Positive control			

Correlation between antioxidant and antibacterial properties

As shown in Table 6, a Pearson correlation analysis was conducted between the antioxidant and antibacterial activities of honey samples. The results showed that antibacterial activity against *H. pylori* was positively correlated with honey samples' antioxidant content.

Table 6- Correlation between antioxidant and antibacterial activities of honey samples against *Helicobacter pylori*

		ABTS	DPPH	FRAP
Antibacterial peroperties	Pearson correlation	0.929	0.851	0.672
	Sig	0.000	0.000	0.000
	N	15	15	15

Discussion

Honey is a popular natural food that contains both organic and inorganic compounds. Its composition is influenced by natural and anthropogenic factors such as vegetation diversity and geography. Honey contains small amounts of protein and lipids, but its composition is mostly composed of fructose and glucose (65%), water (18%), and trace amounts of protein (Khalil *et al.*, 2001; Silva *et al.*, 2009). The presence of minerals and heavy metals in honey can affect its quality, with light-colored honey containing 0.04% minerals and dark-colored honey containing 0.2% minerals (Bogdanov *et al.*, 2007). Minerals derived from organic or plant sources have been reported to have beneficial effects on human health, whereas minerals from inorganic or heavy metal sources can be toxic (Hernández *et al.*, 2005; Pohl *et al.*, 2009). In this study, we found that 0.04-0.2% of inorganic compounds were present in *A. florea* honey collected from different regions of Iran, which varied in vegetation types, including alfalfa (*Medicago sativa*), *Astragalus* spp., *Citrus*, mesquites (*Prosopis* spp.), and jujube (*Ziziphus spina-christi*).

The phenol and flavonoid profiles of *A. florea* honey have not yet been determined. However, studies of *A. mellifera* honey have shown that the profile of these compounds depends on the dominant flora and

geographical region. Gallic acid has been identified as the most abundant phenolic compound in numerous honey samples (Cheung *et al.*, 2019). HPLC analysis of 40 samples of *A. mellifera* honey revealed 16 phenolic and 14 flavonoid compounds, with gallic acid and chrysin being the most abundant (Cheung *et al.*, 2019). The gallic acid content of honey collected in Australia ranged from 13.9 to 45.2 µg/g, with differences attributed to geographical variations and the types of plants the honeybees were feeding on (Yaoa *et al.*, 2005). In this study, we found that *A. florea* honey samples contained chlorogenic and caffeic acids, which have antibacterial properties (Estevinho *et al.*, 2008). Apigenin was the predominant phenolic compound identified in the Jiroft, Bushehr and Jahrom honey samples, and kampefrol was the predominant compound identified in the Iranshahr and Dezful honey samples. Phenols and flavonoids are naturally occurring compounds found in honey and have been shown to have a positive correlation with the antioxidant effect of honey samples. These compounds also exhibit antimicrobial properties. In this study, the antioxidant activity of honey samples was evaluated using three methods: ABTS, FRAP, and DPPH. The results indicated that the honey samples collected from Bushehr and Iranshahr had the highest and lowest antioxidant activity, respectively, by all three methods. The antioxidant activity of natural honey is attributed to the presence of various compounds such as enzymes, organic acids, phenolic acids, flavonoids, carotenoids, amino acids, and ascorbic acid (Hussein *et al.*, 2011). Different types of honey contain different amounts of antioxidants, especially phenolic compounds, and exhibit different antioxidant activity. These antioxidants are highly dependent on the number of hydroxyl groups attached to the benzene ring of these compounds. . In this study, the total concentrations of phenolic and flavonoid compounds identified in honey samples from Dezful, Iranshahr, Bushehr, Jahrom, and Jiroft honey samples were 5.96, 3.51, 163.98, 100.5, and 18.15 µg/g, respectively. The Iranshahr honey sample had the lowest levels of phenols and flavonoids among the other honey samples, while the Bushehr honey sample had the highest levels. The lower antioxidant activity of Iranshahr honey compared to other honey samples is likely due to the lower concentration of phenolic and flavonoid compounds. . The results of the total phenol content of honey samples also showed that the highest and lowest levels of total phenol were related to Bushehr and Iranshahr honey, respectively. The antioxidant activity of honey was directly related to its phenolic and flavonoid content. Amaral *et al.*, 2017 indicated a positive correlation between phenolic and flavonoid content and antioxidant activity of honey. Some polyphenols have been reported to exhibit antimicrobial activity (Marín *et al.*, 2015). Stagos *et al.* (2018) showed there was a significant moderate positive correlation between the total polyphenolic content and antioxidant activity of honey.

Conclusion

With the rise of antibiotic-resistant strains, there is an urgent need for new effective, low-risk antimicrobial compounds. The search for new antibacterial compounds is therefore one of the interesting topics in the fields of health and medicine (Selahvarzian *et al.*, 2015). Numerous studies have shown that *A. mellifera* honey inhibits the growth of bacteria that are resistant to common antibiotics (Molan, 1992; Al Somal *et al.*, 1994). In laboratory tests, honey was able to effectively control and suppress many pathogens. . Several factors are responsible for the antibacterial activity of honey, including osmotic pressure, acidity, hydrogen peroxide, phenols, flavonoids, and lysozyme (Manyi-Loh *et al.*, 2010). *H. pylori* infection in mice was treated with *A. florea* honey. A positive control group of mice that were treated with *H. pylori* had a higher rate of inflammation than the treatment group. A low rate of inflammation in the stomach of honey-treated mice indicates that honey reduces inflammation severity and has a therapeutic

effect. Degeneration and eosinophils also contribute to this process. In the treatment groups, high levels of eosinophils indicate that honey enhanced mice's immunity against pathogens, including bacteria. There are no studies on the antimicrobial properties of *A. florea* honey. According to this study, *A. florea* honey has been found to have antibacterial effects. Some of the commercial honey samples collected from different regions of Urmia, Iran have been shown to have higher antibacterial activity than other honey samples against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. . This might be attributed to phytochemicals in plants distributed in those areas (Tajik *et al.*, 2007). In Brazil, Amaral *et al*. (2017) investigated the effect of *A. mellifera* honey on *H. pylori* both *in-vivo* and *in-vitro*. Omeprazole, amoxicillin, clarithromycin, and honey were administered to mice after they were infected by *H. pylori*. All treatments were effective in controlling infection, but treatment with honey reduced inflammation and treatment with antibiotics increased eosinophil levels. A study by Rahimifard *et al.* (2019) showed that Thyme honey, royal jelly, and their mixtures exhibited antimicrobial activity against *H. pylori*. They showed that honey due to high osmolality, low acidity, and hydrogen peroxide and non-peroxide content, and royal jelly due to Royalisin protein, fatty acids 10-Hydroxy-2-Decenoic acid (10-HAD), and Jelleins peptides; have a wide spectrum of antibacterial properties. In this study, *A. florea* honey was shown to be effective in treating H. pylori infections. This study found similar results to those obtained by Amaral *et al*. (2017). In particular, jujube honey from Bushehr showed antibacterial and antioxidant properties in this study. This, in combination with other treatments, can reduce infections and inflammation caused by *Helicobacter pylori*.

Acknowledgments

The authors thank the Razi Vaccine and Serum Research Institute and Animal Science Research Institute of Iran for the provision of necessary facilities to carry out experiments. This work was supported by the Iran National Science Foundation (INSF). Project No.: 97018902.

Conflicts of interest

The authors have declared that no competing interests exist.

Ethics approval

All experiments with animals were performed according to the recommendations in the Guide for the Care and Use of Laboratory Animals of the Research Station of Department of Animal Science, Razi Vaccine and Serum Research Institute, Iran**.**

References

1. Al Somal, N., Coley, K.E., Molan, P.C., & Hancock, B.M. (1994). Susceptibility of *Helicobacter pylori* to the antibacterial activity of manuka honey. *Journal of the Royal Society of Medicine*, *87*(1), 9. https://doi.org/10.1177/014107689408700106
2. Aliyazicioglu, R., & Boukraa, L. (2015). Honey: the natural inhibine". *Anti-Infective Agents*, *13*(1), 42-49.
3. Amaral, T.Y., Padilha, I.G., Presídio, G.A., Silveria, E.A.A.S.D., Duarte, A.W.F., Barbosa, A.P.F., & López, A.M. Q. (2017). Antimicrobial and anti-inflammatory activities of *Apis mellifera* honey on the *Helicobacter pylori* infection of Wistar rats gastric mucosa. *Food Science and Technology*, *37*, 34-41. https://doi.org/10.1590/1678-457X.31016
4. Benzie, I.F., & Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*, *239*(1), 70-76. https://doi.org/10.1006/abio.1996.0292

5. Bogdanov, S., Haldimann, M., Luginbühl, W., & Gallmann, P. (2007). Minerals in honey: environmental, geographical and botanical aspects. *Journal of Apicultural Research*, *46*(4), 269-275. https://doi.org/10.1080/00218839.2007.11101407
6. .Boldt, M.S., Pereira, R.D., & Barbosa, A.J. (2015). Histological identification of *H. pylori* stained by hematoxylin-eosin and Giemsa: review for quality control. *Jornal Brasileiro de Patologia e Medicina Laboratorial*, *51*(2), 108-112.
7. Bonacorsi, C., Raddi, M.S.G., Carlos, I.Z., Sannomiya, M., & Vilegas, W. (2009). Anti-*Helicobacter pylori* activity and immunostimulatory effect of extracts from *Byrsonima crassa* Nied. (Malpighiaceae). *BMC Complementary and Alternative Medicine*, *9*(2), 2-7. https://doi.org/10.1186/1472-6882-9-2
8. Boyanova, L., Ilieva, J., Gergova, G., Vladimirov, B., Nikolov, R., & Mitov, I., 2015. Honey and green/black tea consumption may reduce the risk of *Helicobacter pylori* infection. *Diagnostic Microbiology and Infectious Disease*, *82*(1), 85-86. http://dx.doi.org/10.1016/j.diagmicrobio.2015.03.001
9. Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1-2), 248-254. https://doi.org/10.1016/0003-2697(76)90527-3
10. Cheung, Y., Meenu, M., Yu, X., & Xu, B. (2019). Phenolic acids and flavonoids profiles of commercial honey from different floral sources and geographic sources. *International Journal of Food Properties*, *22*(1), 290-308. https://doi.org/10.1080/10942912.2019.1579835
11. de Queiroz Pimentel, R.B., da Costa, C.A., Albuquerque, P.M., & Junior, S.D. (2013). Antimicrobial activity and rutin identification of honey produced by the stingless bee *Melipona compressipes* manaosensis and commercial honey. *BMC Complementary and Alternative Medicine*, *13*(1), 1-14.
12. Estevinho, L., Pereira, A.P., Moreira, L., Dias, L.G., & Pereira, E. (2008). Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. *Food and Chemical Toxicology*, *46*(12), 3774-3779. https://doi.org/10.1016/j.fct.2008.09.062
13. Gośliński, M., Nowak, D., & Kłębukowska, L. (2020). Antioxidant properties and antimicrobial activity of manuka honey versus Polish honeys. *Journal of Food Science and Technology, 57*, 1269-1277.
14. Graham, D.Y. (2015). *Helicobacter pylori* update: gastric cancer, reliable therapy, and possible benefits. *Gastroenterology*, *148*(4), 719-731. https://doi.org/10.1053/j.gastro.2015.01.040
15. Grego, E., Robino, P., Tramuta, C., Giusto, G., Boi, M., Colombo, R., Serra, G., Chiadò-Cutin, S., Gandini, M., & Nebbia, P. (2016). Evaluation of antimicrobial activity of Italian honey for wound healing application in veterinary medicine. *Band*, *158*, 521–527. http://hdl.handle.net/2318/1611345
16. Hernández, O.M., Fraga, J.M.G., Jiménez, A.I., Jiménez, F., & Arias, J.J. (2005). Characterization of honey from the Canary Islands: determination of the mineral content by atomic absorption spectrophotometry. *Food Chemistry*, *93*(3), 449-458. https://doi.org/10.1016/j.foodchem.2004.10.036
17. Hussein, S.Z., Yusoff, K.M., Makpol, S., & Yusof, Y.A.M. (2011). Antioxidant capacities and total phenolic contents increase with gamma irradiation in two types of Malaysian honey. *Molecules*, *16*(8), 6378-6395. https://doi.org/10.3390/molecules16086378
18. Jones, N.L., Day, A.S., Jennings, H., Shannon, P.T., Galindo-Mata, E., & Sherman, P.M. (2002). Enhanced disease severity in *Helicobacter pylori*-infected mice deficient in Fas signaling. *Infection and Immunity*, *70*(5), 2591-2597. https://doi.org/10.1128/iai.70.5.2591-2597.2002

19. Khalil, M.I., Motallib, M.A., Anisuzzaman, A.S.M., Sathi, Z.S., Hye, M.A., & Shahjahan, M. (2001). Biochemical analysis of different brands of unifloral honey available at the northern region of Bangladesh. *Sciences*, *1*(6), 385-338.
20. *Khatun, S. (2014). Evaluation of helicobacter pyloristool antigen detection test* (Doctoral dissertation, BRAC University).
21. Kolayli, S., Palabiyik, I., Atik, D.S., Keskin, M., Bozdeveci, A., Karaologu, S.A. (2020). Comparison of antibacterial and antifungal effects of different varieties of honey and propolis samples. *Acta Aliment*, *49*, 515–523. https://doi.org/10.1556/066.2020.49.4.18
22. Manyi-Loh, C.E., Clarke, A.M., Munzhelele, T., Green, E., Mkwetshana, N.F., & Ndip, R.N. (2010). Selected South African honeys and their extracts possess *in-vitro* anti-*Helicobacter pylori* activity. *Archives of Medical Research*, *41*(5), 324-331. https://doi.org/10.1016/j.arcmed.2010.08.002
23. Marín, L., Miguélez, E.M., Villar, C.J., & Lombó, F. (2015). Bioavailability of dietary polyphenols and gut microbiota metabolism: antimicrobial properties. *BioMed Research International*, *2*, 12-25. https://doi.org/10.1155/2015/905215
24. Mello, B.C.B.S., Petrus, J.C.C., & Hubinger, M.D. (2010). Concentration of flavonoids and phenolic compounds in aqueous and ethanolic propolis extracts through nanofiltration. *Journal of Food Engineering*, *96*(4), 533-539. https://doi.org/10.1016/j.jfoodeng.2009.08.040
25. Molan, P.C. (1992). The antibacterial activity of honey: 1. The nature of the antibacterial activity. *Bee World*, *73*(1), 5-28. https://doi.org/10.1080/0005772X.1992.11099109
26. Molan, P.C. (2002) Re-introducing honey in the management of wounds and ulcers theory and practice. *Ostomy/Wound Management 4811*, 28-40.
27. Nakamura, M., Haruma, K., Kamada, T., Mihara, M., Yoshihara, M., Sumioka, M., & Chayama, K. (2002). Cigarette smoking promotes atrophic gastritis in *Helicobacter pylori*-positive subjects. *Digestive Diseases and Sciences*, *47*(3), 675-681.
28. Parichehreh, S., Tahmasbi, G., Khaki, P., & Babaei, M. (2021). Evaluation of physicochemical characteristics of *Apis florea* honey in some southern provinces of Iran. *Veterinary Researches and Biological Products*, *34*(2), 135-121. https://doi.org/10.22092/VJ.2020.128700.1668
29. Parichehreh, S., Tahmasbi, G., Sarafrazi, A., Tajabadi, N., & Solhjouy-Fard, S. (2020). Distribution modeling of *Apis florea* Fabricius (Hymenoptera, Apidae) in different climates of Iran. *Journal of Apicultural Research*, 1-12. https://doi.org/10.1080/00218839.2020.1775962
30. Pohl, P. (2009). Determination of metal content in honey by atomic absorption and emission spectrometries. *TrAC Trends in Analytical Chemistry*, *28*(1), 117-128. https://doi.org/10.1016/j.trac.2008.09.015
31. Rahimifard, N., Hosseinzadeh Shanjani, B., & Shoeibi, S. (2019). The comparative study of antibacterial effects of Royal Jelly and Honey and the combination of them against standard strain of *Helicobacter pylori*. *Journal of Food Microbiology*, *6*(2), 1-10.
32. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*(9-10), 1231-1237. https://doi.org/10.1016/S0891-5849(98)00315-3
33. Saha, A., Hammond, C.E., Beeson, C., Peek, R.M., & Smolka, A.J. (2010). *Helicobacter pylori* represses proton pump expression and inhibits acid secretion in human gastric mucosa. *Gut*, *59*(7), 874-881. http://dx.doi.org/10.1136/gut.2009.194795
34. Selahvarzian, A., Abdolahpour, F., Sepahvand. F., & Azadpour, M. (2015). Anti-oxidant and anti-microbial activities of two types of honey by change in bees, diet in comparison with other honey products in Abestan region of Khorramabad province. *Yafte*, *17*(3): 115-125.

35. Shamala, T.R., Shri Jyothi, Y.P., & Saibaba, P. (2002). Antibacterial effect of honey on the *in-vitro* and *in-vivo* growth of *Escherichia coli*. *World Journal of Microbiology and Biotechnology*, *18*(9), 863-865.
36. Silva, L.R., Videira, R., Monteiro, A.P., Valentão, P., & Andrade, P.B. (2009). Honey from Luso region (Portugal): Physicochemical characteristics and mineral contents. *Microchemical Journal*, *93*(1), 73-77. https://doi.org/10.1016/j.microc.2009.05.005
37. Singleton, V.L., Orthofer, R., & Lamuela- Raventos, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymology*, *299*, 152–178.
38. Snowdon, J.A., & Cliver, D.O. (1996). Microorganisms in honey. *International Journal of Food Microbiology*, *31*(1-3), 1-26. https://doi.org/10.1016/0168-1605(96)00970-1
39. Stagos, D., Soulitsiotis, N., Tsadila, C., Papaeconomou, S., Arvanitis, C., Ntontos, A., & Mossialos, D. (2018). Antibacterial and antioxidant activity of different types of honey derived from Mount Olympus in Greece. *International Journal of Molecular Medicine*, *42*(2), 726-734. https://doi.org/10.3892/ijmm.2018.3656
40. Tajik, H., Shokouhi, F., & Valehi, S. (2007). Evaluation of antimicrobial potential of commercial honey produced in Urmia. *Journal of Food, Science, and Technology, 4*(2), 39-45. https://doi.org/10.22067/ifstrj.2023.81933.1248
41. Take, S., Mizuno, M., Ishiki, K., Hamada, F., Yoshida, T., Yokota, K., & Yamamoto, K. (2015). Seventeen-year effects of eradicating *Helicobacter pylori* on the prevention of gastric cancer in patients with peptic ulcer; a prospective cohort study. *Journal of Gastroenterology*, *50*(6), 638-644.
42. Tosic, S., Stojanovic, G., Mitic, S., Pavlovic, A., & Alagic, S. (2017). Mineral composition of selected Serbian propolis samples. *Journal of Apicultural Science*, *61*(1), 5-15.
43. Von Gadow, A., Joubert, E., & Hansmann, CF. (1997). Comparison of antioxidant activity of aspalathin with that of other plant phenols of Rooibosed tea (*Aspalathon linearis*), atocopherol, BHT, and BHA. *Journal of Agricultural and Food Chemistry, 45*, 632–638. https://doi.org/10.22067/ifstrj.2023.81933.1248
44. Weston, R.J. (2000). The contribution of catalase and other natural products to the antibacterial activity of honey: a review. *Food Chemistry*, *71*(2), 235-239. https://doi.org/10.1016/S0308-8146(00)00162-X
45. Wroblewski, L.E., Peek Jr, R.M., & Wilson, K.T. (2010). *Helicobacter pylori* and gastric cancer: factors that modulate disease risk. *Clinical Microbiology Reviews*, *23*(4), 713-739. https://doi.org/10.1128/cmr.00011-10
46. Yaoa, L., Jiang, Y., Singanusong, R., Datta, N., & Raymont, K. (2005). Phenolic acids in Australian Melaleuca, Guioa, Lophostemon, Banksia and Helianthus honeys and their potential for floral authentication. *Food Research International*, *38*(6), 651-658. https://doi.org/10.1016/j.foodres.2005.01.002

Iranian Food Science and Technology Research Journal
Vol. 19, No. 3, Aug.-Sep. 2023, p. 49-63

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد ۱۹، شماره ۳، مرداد- شهریور ۱۴۰۲ ص. ۶۳-۴۹

بررسی خواص آنتی‌اکسیدانی و آنتی‌باکتریالی عسل زنبور عسل کوچک *Apis florea* Fabricius (Hymenoptera: Apidae) علیه *Helicobacter pylori*

شبنم پری چهره[1*]- غلامحسین طهماسبی[2]- محمد اسلام پناه[3]- پژواک خاکی[4]

تاریخ دریافت: ۱۴۰۲/۰۲/۰۲
تاریخ بازنگری: ۱۴۰۲/۰۴/۱۷
تاریخ پذیرش: ۱۴۰۲/۰۴/۱۸

چکیده

هلیکوباکتر پیلوری با آلوده‌سازی دستگاه گوارش فوقانی، منجر به آسیب‌دیدگی در مخاط شده و امکان ابتلا به سرطان لوله گوارش را بویژه در مردان افزایش می‌دهد. با توجه به اینکه امکان درمان آن با روش‌های سنتی همیشه به طور موثری امکان‌پذیر نیست، بنابراین تا زمان تهیه واکسن علیه آن، یافتن روش‌های ایمن تر مبارزه با این باکتری بسیار حائز اهمیت است. عسل یک مکمل غذایی با محتوای کربوهیدرات بالا و فعالیت آنتی‌اکسیدانی و همچنین طیف وسیع ضد میکروبی است که در سال‌های اخیر به عنوان یکی از روش‌های مقابله با طیف گسترده‌ای از عوامل میکروبی از جمله *H. pylori* مطرح بوده است. در این پژوهش برای نخستین بار خواص آنتی‌اکسیدانی و آنتی‌باکتریالی ۱۵ نمونه عسل زنبور عسل کوچک *A. florea* جمع‌آوری شده از بوشهر، دزفول، ایرانشهر، چابهار، رودان، جهرم و جیرفت مورد بررسی قرار گرفت. ترکیبات فنلی، فلاونوئیدی، پروتئین و ترکیبات معدنی عسل‌های جمع‌آوری شده مورد بررسی قرار گرفت. همچنین خواص آنتی اکسیدانی عسل‌ها با استفاده از سه روش DPPH، FRAP و ABTS و خواص ضدباکتریایی در شرایط درون‌تنی و برون‌تنی ارزیابی شد. نتایج نشان داد که همبستگی مثبت و معنی‌داری بین خواص آنتی‌اکسیدانی و آنتی‌باکتریالی عسل‌ها وجود دارد. همچنین براساس نتایج بدست آمده بیشترین خواص آنتی‌باکتریالی علیه هلیکوباکتر پیلوری مربوط به عسل کنار بوشهر بود. طبق نتایج بدست آمده علت تفاوت قدرت آنتی‌اکسیدانی و ضد میکروبی مشاهده شده در بین نمونه‌های عسل به دلیل تفاوت در تنوع فلور گیاهی منطقه و تفاوت جغرافیایی مناطق جمع‌آوری عسل بوده است. بررسی قدرت ضد میکروبی در این پژوهش نشان داد، عسل توانایی بالایی در جلوگیری از آلودگی و نیز درمان آلودگی و التهاب ایجاد شده در دستگاه گوارش به وسیله باکتری هلیکوباکتر پیلوری را داشته است و می‌تواند در کنار سایر روش‌های موجود، در درمان آلودگی به این باکتری مورد استفاده قرار گیرد.

واژه‌های کلیدی: خواص آنتی اکسیدانی، خواص ضد باکتریایی، عسل، *H. pylori*

۱ و ۲- استادیار پژوهشی و استاد پژوهشی، بخش زنبور عسل، موسسه تحقیقات علوم دامی کشور، سازمان تحقیقات، آموزش و ترویج کشاورزی، کرج، ایران
(*- نویسنده مسئول: Email: Sh.pariichehreh@gmail.com)
۳ و ۴- فارغ التحصیل دکتری و دانشیار پژوهشی، بخش میکروب‌شناسی، موسسه تحقیقات واکسن و سرم‌سازی رازی، سازمان تحقیقات، آموزش و ترویج کشاورزی، کرج، ایران
DOI: 10.22067/ifstrj.2023.81933.1248

Iranian Food Science and Technology Research Journal
Vol. 19, No. 3, Aug.-Sep. 2023, p. 65-77

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد ۱۹، شماره ۳، مرداد- شهریور ۱۴۰۲ ص. ۷۷-۶۵

Research Article
Homepage: http://ifstrj.um.ac.ir

Oliveria decumbens and *Pistacia atlantica* Gum's Essential Oils: Assessment of Antimicrobial and Chemical Properties During Thermal Process

S.J. Hosseini[1], M. Shahidi-Noghabi[2*], H. Zamani[3], Gh. Zohuri[4], M. Sarabi-Jamab[5]

Received: 2023.04.26
Revised: 2023.07.18
Accepted: 2023.07.19
Available Online: 2023.07.19

How to cite this article:
Hosseini, S.J., Shahidi-Noghabi, M., Zamani, H., Zohuri, Gh., & Sarabi-Jamab, M. (2023). *Oliveria decumbens* and *Pistacia atlantica* gum's essential oils: Assessment of antimicrobial and chemical properties during thermal process. *Iranian Food Science and Technology Research Journal*, *19*(3), 65-77. https://doi.org/10.22067/ifstrj.2023.82016.1252

Abstract

The essential oils usually have a good effect against undesirable microorganisms; therefore, they can be utilized as natural antimicrobial agents in food or their packaging. In this research, the antimicrobial attributes of two essential oils (*Oliveria decumbens and Pistacia atlantica* gum), have been investigated before and after thermal process (200°C - 10 minutes) against bacterial and mold spoilage in bread. Also, the compounds of essential oils were detected by gas chromatography-mass spectrometry. The main compounds of the essential oil of *O. decumbens* were carvacrol, thymol, and elemicin before and after thermal treatment. In the case of *P. atlantica* gum, only one prominent peak was observed in the chromatogram, which was related to the α-pinene. For both essential oils, the MIC and MFC against *Aspergillus niger* were 4000 and 8000 µL/ml, respectively. In comparison, the antimicrobial effect of both essential oils against *Bacillus subtilis* was higher than the mold. The amount of MIC and MBC were 125 and 250 µL/ml for *Oliveria decumbens* and 62.5 and 125 µL/ml for *Pistacia atlantica* gum, respectively. The results showed that these two essential oils have a promising effect against the main microorganisms of bread spoilage. The thermal process did not significantly affect the antimicrobial activity of *Pistacia atlantica* gum essential oil against *A. niger* but significantly decreased the antimicrobial activity against *B. subtilis*, while in the case of antimicrobial activity *of Oliveria decumbens* essential oil, the results were the opposite. Considering the fact that the most spoilage agents of the bread are molds so the use of *Pistacia atlantica* gum essential oil is recommended as natural preservatives in products that tolerate high heat treatment, such as bread and bakery products.

Keywords: GC-Mass, MBC/MFC, *Oliveria decumbens*, *Pistacia atlantica* gum, Thermal stability

Introduction

The most common molds detected in bakery products are genera of *Penicillium*, *Aspergillus*, *Monilia*, *Mucor*, *Endomyces*, *Cladosporium*, *Fusarium*, *Alternaria*, and *Rhizopus* (Alhendi and Choudhary, 2013; Cioban *et al.*, 2010;

1- Ph.D. Student, Department of Food Technology, Research Institute of Food Science and Technology, Mashhad, Iran
2- Associate Professor, Department of Food Chemistry, Research Institute of Food Science and Technology, Mashhad, Iran
(*- Corresponding Author Email: m.shahidi@rifst.ac.ir)
3- Assistant Professor, Department of Food Industry Machineries Research Institute of Food Science and Technology, Mashhad, Iran
4- Professor, Department of Chemistry, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran
5- Associate Professor, Department of Food Biotechnology, Research Institute of Food Science and Technology, Mashhad, Iran
DOI: 10.22067/ifstrj.2023.82016.1252

Gerez *et al.*, 2009; Rodriguez *et al.*, 2008). According to recent scientists' findings, about 60% of the dough spoilage is due to the presence of *Penicillium* genus and *Aspergillus niger* (Alhendi and Choudhary, 2013). Faparusi and Adewole, 2019 reported that *A. niger* is the most common bread mold spoilage agent. On the other hand, ropiness is the utmost bacterial spoilage of bread, usually caused by *Bacillus* spp., especially *Bacillus subtilis* (Faparusi and Adewole, 2019). This spoilage often occurs when the bacterial population reaches 10^5 CFU per gram of sourdough (Sadeghi *et al.*, 2011).

The use of a limited number of synthetic preservative compounds, in trace amounts, is allowed in baking products., However, due to consumers' awareness of the dangers of chemical preservatives, in recent years researchers have focused on finding a substitution natural ingredients such as plant essential oils for preventing spoilage in food products (Borghei *et al.*, 2010; Jideani and Vogt, 2016). The antimicrobial, antioxidant and anti-carcinogenic properties of essential oils, which is mainly due to the presence of phenolic compounds, have been proven (Bagamboula *et al.*, 2004; Neffati *et al.*, 2017).

Pistacia atlantica (wild pistachio) gum (Fig. 1), called "Saqqez" or "Banneh" in Iran, is secreted from the outer layer of the plant's inner skin. The predominant composition of this gum's essential oil is α- pinene (Rahimi *et al.*, 2016). Antimicrobial activity of the essential oils and extract of this plant has been proven by many researchers (Azeez and Gaphor, 2019; Ellahi *et al.*, 2019; Mahjoub *et al.*, 2018). Ahmed *et al.* (2020) reviewed ethnobotany, phytochemistry, and pharmacology properties of some subspecies of *Pistacia*, including *atlantica*, *cabulica*, *kurdica*, and *mutica*. They reported gum antimicrobial properties against various Gram-positive (*Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Acenitobacter boumanii*) and emphasized its antifungal activities against *Aspergillus* species (*A. niger*, *A. flavus*, *A. fumigates*) (Ahmed *et al.*, 2021).

Fig. 1- *Pistacia atlantica* gum

Oliveria decumbens (Fig. 1) is a medicinal plant locally called "Dan" or "Danak" (Zali and Tahmasb, 2016). Thymol and carvacrol are the major constituents of *O. decumbens* essential oil (Amin *et al.*, 2005). Some researchers, such as Behrooz (2016), demonstrated that the antifungal activity of the *O. decumbens* essential oil was higher than *Thymus vulgaris*, *Zataria multiflora*, rosemary, and cinnamon. The essential oil was able to prevent the growth of *A. niger* and *A. fumigatus* as well as two species of *Penicillium* (Behrooz, 2016).

Fig. 2- *O. decumbens* plant

There are two main challenges when adding essential oils in bread. Firstly, they might change bread`s taste and secondly, the high temperature during baking can alter the composition of the essential oils. Using natural antimicrobial compounds in bread and baking products packaging can reduce the problems mentioned above to some extent. However, high temperature is also used in the production of packaging film; therefore, in addition to investigating the antimicrobial properties of essential oils against the main bread spoilage microorganisms, their thermal resistance should also be carefully studied.

This study aimed to investigate the antimicrobial properties of *P. atlantica* gum and *O. decumbens* essential oils (before and after thermal treatment) against the most common mold and bacterial spoilage agents of bread (*A. niger* and *B. subtilis*). Moreover, the effect of heat treatment on the type and amount of constituents of essential oils was evaluated.

Materials and Methods

A. niger isolated from raisins and identified by the molecular method was prepared from the microbial bank of the Research Institute of Food Science and Technology, Iran. Lyophilized powder of *B. subtilis* (UBBS-14) was bought from Unique Biotech Company (India). Microbial culture mediums, PDB (Potato Dextrose Broth), PDA (Potato Dextrose Agar), MHB (Mueller Hinton Broth), and MHA (Mueller Hinton Agar) as well as ringer tablets and N–hexane (HPLC Grade) were purchased from the Merck Company (German). Antibiotic discs such as gentamicin, ampicillin, and erythromycin were purchased from a pharmacy and Tajhiz Avaran-e Shargh Company in Iran.

Essential oils extraction

Clevenger was used for extracting essential oils. Fresh *P. atlantica* gum and the flower and stem of *O. decumbens* were purchased from local markets in Mashhad, Iran. The samples were air-dried to a constant mass at room temperature (in the shade) and then were grounded with a miller (Model 8300- Toos Shekan-e Khorasan Company, Iran) for about 1 min and sieved with the size of 35 mesh. After that 40–50 g of milled powder were poured into a volumetric balloon (1000 cc), and 70% of the balloon was filled with distilled water. After heating (100˚C) for 6 hours, the essential oil was collected and stored in sealed vials in a dark place at 4°C, until the subsequent experiments (Elyemni *et al.*, 2019). It should be noted that the sterilization of extracted essential oils for antimicrobial tests was done using 0.45 µm filters.

The yield of essential oils extraction

The extraction yield was expressed in g in 100 g of dry plant powder and was calculated according to Equation (1) (Elyemni *et al.*, 2019):

$$\text{Extraction yield (\%)} = \frac{\text{Amount of extracted essential oil (g)}}{\text{Amount of dry plant powder (g)}} \times 100 \quad (1)$$

Thermal processing

In order to investigate the effects of thermal processing on the essential oils and their active compounds, they were kept in oven (Memmert-Germany) at 200°C for about 10 min (Krepker *et al*., 2018).

Determination of the chemical composition of Essential oils by GC-MS

The chemical composition of Essential oils before and after thermal processing of essential oils was resolved using gas chromatography. Five microliters of each essential oil was dissolved in n–hexane solvent (HPLC Grade), and one microliter was injected into a gas chromatography with a mass spectrometry detector (GC-MS 5977 A- Agilent Technologies, USA) equipped with an HP-5MS capillary column (30 m, 0.25 mm i.d., 0.25 µm o.d– Agilent). The injection temperature was 279 °C. The oven temperature was programmed from 60 °C to 200 °C with an increase of 5°C/min. The helium carrier gas was injected at a volume of 1 ml, and the flow rate of helium gas was maintained at 1 ml/min. The mass spectrometer was also fixed to EI mode at 70 eV. The interface temperature and the mass spectrum were set at 280°C and 35 to 700 m/z, respectively (Paventi *et al*., 2020).

Disk diffusion method for determination of inhibition zone

Antimicrobial activity of the stock essential oil (32000 µL/ml) against *A. niger* and *B. subtilis* was investigated by the disk diffusion technique. The lyophilized *B. subtilis* were grown in Mueller-Hinton Broth at 37 °C for 18-24 hours. Then, the turbidity equivalent to 0.5 McFarland standard was prepared and diluted to 10^6 CFU/ml. For *A. niger*, the hemocytometer chamber was used to reach 10^6 spores per ml of fungal suspension. After that, 100 µl of microbial suspensions was spread on the surface of the plate containing MHA medium. Paper disks (6 mm) were impregnated with 30 µl of essential oils and alcohol (70 %) was used as negative control. Moreover, the antibiotic standard disks, including Erythromycin, Ampicillin, and Gentamicin were applied as positive controls (10 µl per disc). The plates were incubated at 37 °C for 18 h and 25 °C for 3-5 days for bacteria and fungi, respectively. Finally, inhibition zone diameters (mm) were measured (Aljeldah, 2022).

Determination of the MIC, MBC and MFC of essential oils by Microdilution Method

The microdilution method was used to determine the MIC of essential oils. Aliquots (100 µl) of different concentrations (32000, 16000, 8000, 4000, 2000, 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7. 8125, and 3.9062 µl /ml) of two essential oils were added to each well of 96 well-plate, which contained 95 µl Mueller-Hinton Broth and Potato Dextrose Broth for bacteria and mold, respectively. Then, five microliters of 10^5 CFU/ml suspensions of microorganisms were added to each well. The negative controls were prepared with MHB and PDB media containing the essential oils without the microorganisms. The positive control was prepared with the MHB and PDB media containing the examined microorganisms without adding essential oils. The 96 well-plates were incubated at 37 °C for 24 h for bacteria and 25 °C for 48 h for molds. The turbidity of the microorganism's growth was detected by an ELISA reader (ELX 808, Biotech USA). After the incubation period, the lower concentration of the essential oils with turbidity similar to negative wells was defined as MIC. To detect MBC/MFC, 100 µl of each well with turbidity similar to the negative controls was spread on the surface of MHA and BDA media and incubated at a suitable temperature and time for the microorganisms. The minimum bactericidal / fungicidal concentration was the lowest concentration of essential oils that caused the death of microorganisms in a way that no colony of microorganisms are observed (Behrooz, 2016).

Results and Discussion

Essential oils extraction yield

The extraction yield was 1/5 % for *O. decumbens* and 3% for *P. atlantica*.

GC-MS results

GC-MS measured the most important compounds of essential oils before and after thermal treatment. The results are as follows

GC-MS chromatogram of essential oils (before and after heat treatment)

GC-MS chromatogram of *O. decumbens* essential oil, before and after thermal treatment are presented in Fig. 3. Also the main compounds of this plant is available in Table 1.

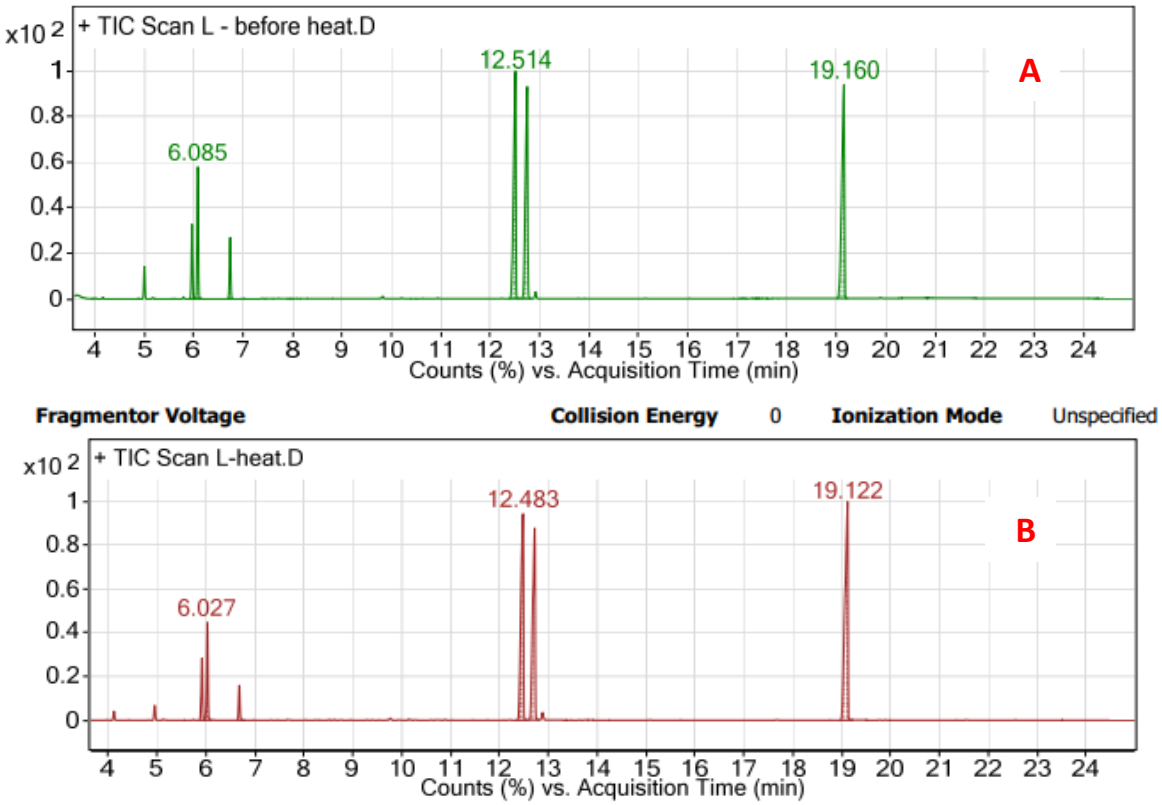


Fig. 3- GC-MS chromatogram of Oliveria decumbens (A= Before heating, B= After heating treatment)

As shown in Table 1, the main compounds of *O. decumbens* before heat treatment were elemicin (28.71 %), carvacrol (27.91 %), and thymol (24.76 %), that totally account for about 80% of *O. decumbens* composition. By comparing the peak of main compounds of *O. decumbens* essential oil before and after heat treatment, it is clear that after heating the retention time of all compounds was slightly decreased although the main compounds remained unchanged. In addition, the amount of minor compounds decreased 6% in total, and the same amount was added to the elemicin (34.58 %); possibly due to the chemical interactions and material decomposition/ composition.

Table 1- The main compounds and retention time of *Oliveria decumbens* (before and after heating treatment)

Number	Treatment	Name of compound	Retention time	Area
1	Before heating	o-Cymene	5.97	5.03
	After heating	o-Cymene	5.918	4.44
2	Before heating	D-Limonene	6.085	9.26
	After heating	D-Limonene	6.027	6.82
3	Before heating	γ-Terpinene	6.744	4.33
	After heating	γ-Terpinene	6.679	2.56
4	Before heating	Carvacrol	12.514	27.91
	After heating	Carvacrol	12.483	27.27
5	Before heating	Thymol	12.752	24.76
	After heating	Thymol	12.720	24.33
6	Before heating	Elemicin	19.16	28.71
	After heating	Elemicin	19.122	34.58

Sereshti *et al*. (2011) declared that the main components of the essential oil of *O. decumbens* vent are: thymol (47.06%), carvacrol (23.31%), gamma-terpinene (18.94%), p-cymene (8.71%), (Sereshti *et al*., 2011). Esmaeili *et al*. (2018) studied the aerial parts of *O. decumbens* and reported that, according to the GC and GC-MS results, γ-terpinene (33.6%), carvacrol (16.9%), and thymol (16%) in the vegetative stage were the main components; whereas during the flowering stage thymol (37.8%) and carvacrol (29.38%) were dominated components (Esmaeili *et al*., 2018). Karami *et al*. (2020) studied the variation in the content of essential oil and composition of 12 types of *O. decumbens* populations in several habitats of Iran. They found that, the highest essential oil content was obtained from Behbahan (8.52%) and it was significantly higher than those previously reported for other areas in Iran and elsewhere. They announced that carvacrol (18.8–51.8%), thymol (20.3–38.7%), γ-terpinene (0.9–28.8%) and p-cymene (1.6–21.3%) were the major volatile compounds of *O. decumbens* based on GC-FID and GC-MS analysis (Karami *et al*., 2020). The differences observed in this regard are associated with the plant origin, the parts of the plants selected or gathered for extraction (Karami *et al*., 2020; Sereshti *et al*., 2011) different phenological stages and also the extraction methods (Esmaeili *et al*., 2018).

GC-MS chromatogram of *P. atlantica* gum's essential oil (post and pre-heating) is shown in Fig. 4. As seen in Fig. 4, before the heat treatment, only one sharp peak appears in the chromatogram (4.12 min), related to a-pinene (3-carene), the main composition of *P. atlantica* gum (67 %). It should be noted that there were other minor compounds, but they could be ignored. After heat treatment, it was observed that the amount of α- pinene decreased from 67% to 60.5% in comparison to pre-heat treatment; however the amount of some trace compounds such as trans-verbenol rose increasedafter heat treatment. Hasheminya and Dehghannya (2020) also declared that α-pinene is the main compound found in essential oil of hull of *P. kurdica* subsp. (Hasheminya and Dehghannya, 2020). Elahi *et al*. (2019) reported that α-pinene (92.08%) is the main ingredient of *P. atlantica* gum essential oil (Ellahi *et al*., 2019). In another study carried out by Azeez and Gaphor (2019), it was reported that α-pinene made up 79.76% of the gum's essential oil of *P. atlantica Kurdica* (Kurdistan, Iraq) (Azeez and Gaphor, 2019). According to the research of Rahimi *et al*. (2013), it is worth mentioning that in both males and females of *P. atlantica* subsp. *Kurdica*, α-pinene (92.42%-84.10%), and Limonene (5.23%-1.26%) were the major compounds of the gum, (Rahimi *et al*., 2013). Jaradat *et al*. (2022) also found that Limonene and α-pinene were characterized as the major *Pistacia lentiscus* essential oil components (Jaradat *et al*., 2022). Sharifi *et al*. (2011) determined the gum's composition of *P. atlantica* subsp *kurdica* and showed that α-pinene contents (97.18) giving a unique

characteristic to this species. Overall, in the present research in line with other relevant studies working on all other parts of this plant, such as fruit (71.9%), (Fathollahi *et al.*, 2019) leaves (5.54-66.1% from 34 samples), (Gourine *et al.*, 2010) hull (20.80%)(Rezaie *et al.*, 2015), industrial essential oil (Saqez Company of Kurdistan– 91.47%) (Hesami *et al.*, 2014); the main component of the essential oils of *P. atlantica* was α-pinene (Memariani *et al.*, 2017; Rahimi *et al.*, 2013; Sharifi and Hazell, 2011).

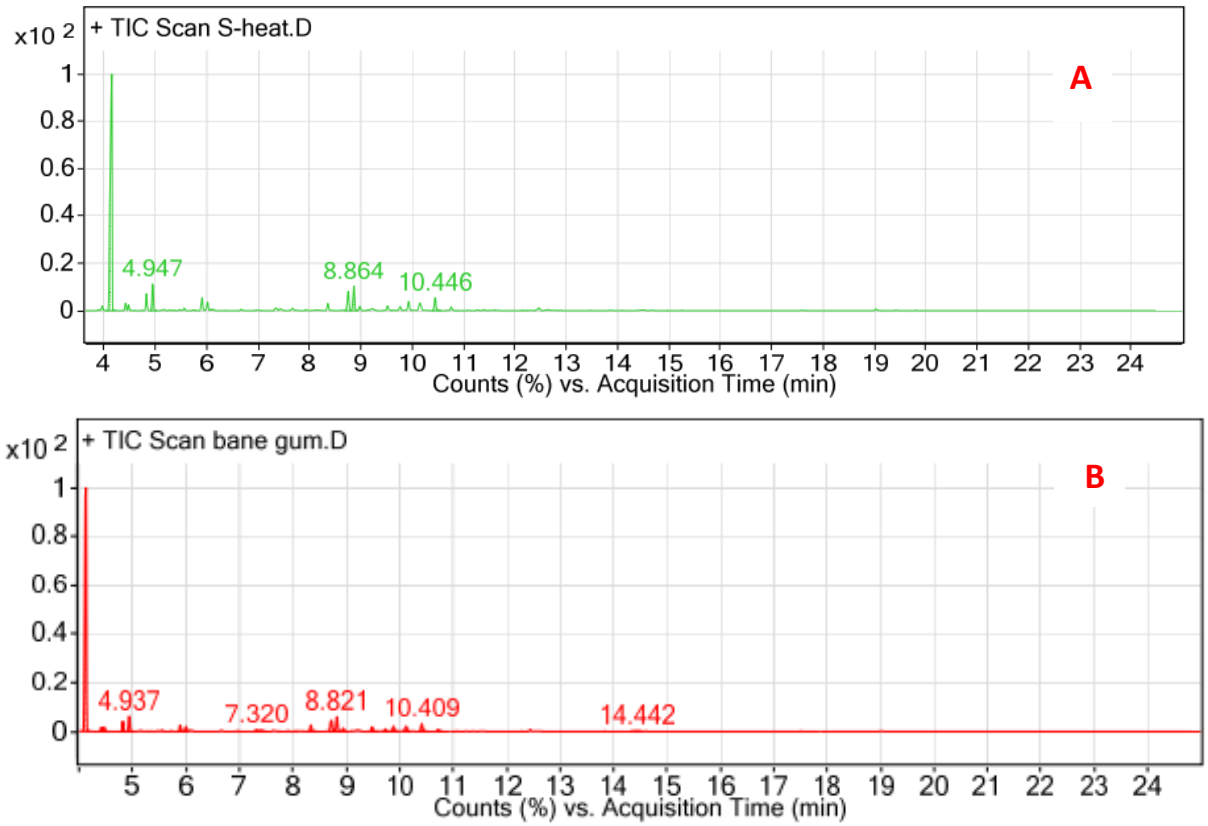


Fig. 4- GC-MS chromatogram of *Pistacia atlantica* (A=before heating, B=after heating)

Microbial tests

Molds

According to the results of the disk diffusion test, the inhibition zone of *O. decumbens* essential oil against *A. niger* was 25.50±0.08 mm before heat treatment and 20.87±0.43 mm after heat treatment, which showed the antimicrobial activity after heat treatment decreased significantly, although due to the high antimicrobial activity of the essential oil based on inhibition zone, after the heat treatment, it can be used as an antifungal compound. On the other hand, no significant difference was observed before (11.00±0.16) and after (10.85±0.04) the heat treatment in inhibition zone of *P. atlantica* essential oil showing entire resistant to the heat treatment (200°C- 10 minutes). Although Erythromycin and Gentamicin showed the biggest inhibition zone (40.61±0.27 mm and 40.50±0.41 respectively), Ampicillin inhibition zone was lower (20.15±0.28 mm).

Bacteria

The inhibition zone of *P. atlantica and O. decumbens* essential oil with a concentration of 32000 µL/ml against *B. subtilis* was 28.28±0.39 and 42.75±0.20 mm before heat treatment and 13.86±1.29 and 42.77±0.22 mm after heat treatment respectively; whereas inhibition zone of Erythromycin, Ampicillin and Gentamicin were 28.40±0.33, 25.94±0.11 and 24.88±0.68

mm respectively. Based on the results, heat treatment reduced the antimicrobial properties of *P. atlantica* essential oil against *B. subtilis* significantly, while the inhibition zone of the heated *O. decumbens* essential oil on the mentioned bacteria was not different from the unheated essential oil, which indicates the stability of *O. decumbens* essential oil to heat treatment.

Ghalem and Mohamed (2009) evaluated the antimicrobial activity of *P. atlantica Desf* essential oil (PEO) against *S. aureus*, *Streptococcus pyogenes*, and *E. coli*. The PEO concentration was 10^{-1} dilution of the extracted essential oil. They declared that the largest inhibition zone was 9 mm obtained against *E. coli* (10^3 CFU/ml), and the lowest was against *S. pyogenes* (10^2 CFU/ml) with no inhibition zone. Moreover, the PEO at 10^{-2} and 10^{-3} µg/ml showed moderate antimicrobial activity. (Ghalem and Mohamed, 2009). Hama Amin *et al*. (2022) investigated antifungal activity of *Pistacia atlantica subsp. kurdica* oil gum extract (100, 50, 25 µl/ml) against *Aspergillus brasiliensis*. According to their results, oil gum extract showed strong antifungal activity because no growth was observed at different concentrations of the extract added to the inoculated PDA medium (HamaAmin *et al*., 2022).

The MIC and MBC of both essential oils (*P. atlantica* and *O. decumbens*) against *A.niger* and *B. subtilis* were measured using microdilution test. The results are shown in Table 2.

Table 2- The MIC and MBC of both tested essential oils (*P. atlantica* and *O. decumbens*) against the tested microorganism (*A. niger* and *B. subtilis*) before heat treatment

Type of Microorganism	Test	*Pistacia atlantica*	*Oliveria decumbens*
niger	MIC	4000 µL/ml	4000 µL/ml
	MBC	8000 µL/ml	8000 µL/ml
subtilis	MIC	62.5 µL/ml	125 µL/ml
	MBC	125 µL/ml	250 µL/ml

Azeez and Gaphor (2019) reported that both MIC and MBC of the essential oils of *P. atlantica* gum against *Porphyromonas gingivalis* ($5*10^5$ CFU/ml) were 12.5 µL/ml (Azeez and Gaphor, 2019). In another study, Sharifi *et al*. (2011) evaluated the antimicrobial activity of essential oil of *P. atlantica kurdica* crude gum against nine strains of *Helicobacter pylori* ($1.5*10^8$ CFU/ml) and some other Gram-positive and negative bacteria. They reported that the MIC values ranged from 500-1000 mg/mL (Sharifi and Hazell, 2011). Ghalem and Mohamed (2009) determined the MIC of the resin oil of *P. atlantica* in different concentrations (0.1, 0.01, and 0.001 µg/ml). They proved that its values ranged from 3- 11 µg/ml against *E. coli*, 1- 10 µg/ml against *S. aureus*, and 0– 8 µg/ml against *S. pyogenes* (Ghalem and Mohamed, 2009). In addition, Doosti (2019) investigated the antimicrobial effects of *P. atlantica*'s gum against *S. aureus*, *P. aeruginosa*, *E. coli*, *Candida albicans* and *Candida glaberata* by disc diffusion, and then MIC, MBC, MFC was determined. The results revealed that *S. aureus* had the greatest inhibition zone diameter at a concentration of 5 mg/ml while *P. aeruginosa* showed the least diameter of the inhibition zone at a concentration of 0.156 mg/ml. The lowest MIC and MBC for *S. aureus* were 5.312 and 625 µg/ml, respectively. Among the fungi, the biggest diameter of the inhibition zone at a concentration of 5 mg/ml was related to *C. albicans*, and the smallest diameter at a concentration of 0.156 mg/ml was related to *C. glaberata*. *C. albicans* had the lowest MIC and MFC, 625 and 1250 µg/ml, respectively (Doosti, 2019). In another study, Mokhtari *et al*. (2021) compared the effects of the leaf extract and gum of *p. atlantica* (0/2 g/ml) with 0.2 % chlorhexidine (CHX) against the growth of *Streptococcus mutans*. The results demonstrated a 24-mm inhibition zone around CHX, but no inhibition zones were observed

neither of the extract nor the gum of *P. atlantica* against *S. mutans*. Furthermore, they declared that the MIC values for CHX, the leaf and gum extract of *P. atlantica* were 1.256, 1.8, and 1 mm, respectively (Mokhtari *et al*., 2021).

To the best of our knowledge, few articles have been published about the heat stability of essential oils. Pina-Pérez *et al.* (2018) investigated the antimicrobial potential of Açaí (*Euterpe oleracea*), Ginseng (*Panax quinquefolius* L.), and Stevia (*Stevia rebaudiana* Bertoni) extracts treated with microwave, pulsed electric field, and conventional thermal treatment (90 °C, 60 s) against sporeforming/ non-sporeforming foodborne pathogens. The results pointed out that both microwave and pulsed electric field increased the antimicrobial potential of the extracts against vegetative and sporulated microorganisms; while the bioactive compounds of Açaí extract were sensitive to the conventional heating method and caused its antimicrobial activity to decrease. They declared that new lines of research should be opened regarding validation of the antimicrobial potential of these ingredients when integrated into real food matrices (Pina-Pérez *et al*., 2018).

However, some articles investigate the thermal stability of encapsulated essential oils. For example, Garcia-Sotelo *et al.* (2019) encapsulated rosemary essential oil within β-cyclodextrin and proved that thermal stability and antimicrobial capacity of the encapsulated essential oil (Garcia-Sotelo *et al*., 2019). Fonseca *et al*. (2020) encapsulated the thyme essential oil with starch by electrospinning method and found that nanofibers with 5% Thyme essential oil retained up to 50% of the phenolic compounds after thermal treatment (Fonseca *et al*., 2020). Based on the mentioned articles, it seems that the encapsulation process is an effective solution in preserving the bioactive compounds of essential oils during thermal processing.

Conclusion

The results showed that both essential oils of *O. decumbens* and *P. atlantica*'s gum have antimicrobial activity against examined microorganisms that are the leading cause of bread spoilage according to the MIC, MBC, MFC, and also disk diffusion test. Overall, the essential oil's content does not change significantly during the thermal process, so the authors declared that both oils were resistant to thermal processing (200°C- 10 minutes). These essential oils have the potential to be employed at active film to preserve food products. The authors suggested that the essential oils of *O. decumbens* and *P. atlantica* gum could be used in food packaging, such as bread, and bakery products, and also in the formulation of bakery products to increase food safety and reduce food pathogens risks.

Conflict of interest

The authors declare that there is not any conflict of interest between them.

Acknowledgement

The authors would like to thank the Research Institute of Food Science and Technology Fund for financially supporting of the research project.

References

1. Ahmed, Z.B., Yousfi, M., Viaene, J., Dejaegher, B., Demeyer, K., & Vander Heyden, Y. (2021). Four *Pistacia atlantica* subspecies (*atlantica, cabulica, kurdica* and *mutica*): A review of their botany, ethnobotany, phytochemistry and pharmacology. *Journal of Ethnopharmacology*, *265*, 113329. https://doi.org/10.1016/j.jep.2020.113329
2. Alhendi, A., & Choudhary, R. (2013). Current practices in bread packaging and possibility of improving bread shelf-life by nano-technology. *INT Journal Food Science Nutrient*, *3*(4), 55-60. https://doi.org/10.5923/j.food.20130304.02

3. Aljeldah, M.M. (2022). Antioxidant and antimicrobial potencies of chemically-profiled essential oil from *Asteriscus graveolens* against clinically-important pathogenic microbial strains. *Molecules*, *27*(11), 3539. https://doi.org/10.3390/molecules27113539 .
4. Amin, G., Sourmaghi, M.S., Zahedi, M., Khanavi, M., & Samadi, N. (2005). Essential oil composition and antimicrobial activity of *Oliveria decumbens*. *Fitoterapia*, *76*(7-8), 704-707. https://doi.org/10.1016/j.fitote.2005.06.009
5. Azeez, S.H., & Gaphor, S.M. (2019). Evaluation of antibacterial effect against *Porphyromonas gingivalis* and biocompatibility of essential oil extracted from the gum of *Pistacia atlantica kurdica*. *BioMed Research International*, *2019*. https://doi.org/10.1155/2019/9195361
6. Bagamboula, C., Uyttendaele, M., & Debevere, J. (2004). Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards *Shigella sonnei* and *S. flexneri*. *Food Microbiology*, *21*(1), 33-42. https://doi.org/10.1016/S0740-0020(03)00046-7
7. Behrooz, M. (2016). *Antimicrobial and antioxidant activity of the essential oil and different extracts of Oliveria decumbens* Shiraz]. Faculty of agriculture. (In Persian)
8. Borghei, M., Karbassi, A., Oromiehie, A., & Javid, A. (2010). Microbial biodegradable potato starch based low density polyethylene. *African Journal of Biotechnology*, *9*(26), 4075-4080.
9. Cioban, C., ALEXA, E., Sumalan, R,. & Merce, I. (2010). Impact of packaging on Bread physical and chemical properties. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Agriculture*, *67*(2).
10. Doosti, B. (2019). The comparison of antibacterial and antifungal effects of *Pistacia atlantica* gum with some inuse antibiotics. *Journal of Cellular and Molecular Research (Iranian Journal of Biology)*, *32*(1), 32-43.
11. Ellahi, H., Khalili Sadrabad, E., Hekmatimoghaddam, S., Jebali, A., Sadeghizadeh-yazdi, J., Rastiani, F., & Akrami Mohajeri, F. (2019). Antimicrobial activity and chemical composition of *Pistachia atlantica* gum subsp. *kurdica*. essential oil. *Journal of Nutrition and Food Security*, *4*(3), 186-190. https://doi.org/10.18502/jnfs.v4i3.1310
12. Elyemni, M., Louaste, B., Nechad, I., Elkamli, T., Bouia, A., Taleb, M., & Eloutassi, N. (2019). Extraction of essential oils of R*osmarinus officinalis* L. by two different methods: hydrodistillation and microwave assisted hydrodistillation. *The Scientific World Journal*, *13*(1), 426-433. https://doi.org/10.1155/2019/3659432
13. Esmaeili, H., Karami, A., & Maggi, F. (2018). Essential oil composition, total phenolic and flavonoids contents, and antioxidant activity of *Oliveria decumbens* Vent). Apiaceae) at different phenological stages. *Journal of Cleaner Production*, *198*, 91-95. https://doi.org/10.1016/j.jclepro.2018.07.029
14. Faparusi, F., & Adewole, A. (2019). Characterization of moulds associated with spoilage of bread sold in Ilaro, Yewa-South, Nigeria. *International Journal of Biological and Chemical Sciences*, *13*(1), 426-433. https://doi.org/10.4314/ijbcs.v13i1.33
15. Fathollahi, M., Aminzare, M., Mohseni, M., & Hassanzadazar, H. (2019). Antioxidant capacity, antimicrobial activities and chemical composition of *Pistacia atlantica* subsp. *kurdica* essential oil. *Veterinary Research Forum*.
16. Fonseca, L.M., Radünz, M., dos Santos Hackbart, H.C., da Silva, F.T., Camargo, T.M., Bruni, G.P.,& Dias, A.R.G. (2022). Electrospun potato starch nanofibers for thyme essential oil encapsulation: antioxidant activity and thermal resistance. *Journal of the Science of Food and Agriculture*, *100*(11), 4263-4271. https://doi.org/10.1002/jsfa.10468
17. Garcia-Sotelo, D., Silva-Espinoza, B., Perez-Tello, M., Olivas, I., Alvarez-Parrilla, E., González-Aguilar, G. A., & Ayala-Zavala, J.F. (2019). Antimicrobial activity and thermal stability of rosemary essential oil:β−cyclodextrin capsules applied in tomato juice .*LWT*, *111*, 837-845. https://doi.org/10.1016/j.lwt.2019.05.061

18. Gerez, C.L., Torino, M.I., Rollán, G., & De Valdez, G.F. (2009). Prevention of bread mould spoilage by using lactic acid bacteria with antifungal properties. *Food Control*, *20*(2), 144-148 . https://doi.org/10.1016/j.foodcont.2008.03.005
19. Ghalem, B., & Mohamed, B. (2009). Essential oil from gum of *Pistacia atlantica* Desf.: screening of antimicrobial activity. *African Journal of Pharmacy and Pharmacology*, *3*(3), 87-91.
20. Gourine, N., Yousfi, M., Bombarda, I., Nadjemi, B., Stocker, P., & Gaydou, E. (2010). Antioxidant activities and chemical composition of essential oil of *Pistacia atlantica* from Algeria. *Industrial Crops and Products*, *31*(2), 203-208. https://doi.org/10.1016/j.indcrop.2009.10.003
21. HamaAmin, H.H., Aziz, P.Y., & Azeez, S.H. (2022). Evaluation of antibacterial and antifungal activity of *Pistacia atlantica* Subsp. *kurdica* oil gum extract from Halabja Province/Kurdistan region of Iraq. *Polytechnic Journal*, *12*(1), 166-170. https://doi.org/10.25156/ptj.v12n1y2022.pp166-170
22. Hasheminya, S.-M., & Dehghannya, J. (2020). Composition, phenolic content, antioxidant and antimicrobial activity of *Pistacia atlantica* subsp. *kurdica* hulls' essential oil. *Food Bioscience*, *34*, 100510. https://doi.org/10.1016/j.fbio.2019.100510
23. Hesami, G., Bahramian, S., Fatemi, A., & Hesami, S. (2014). Effect of *Pistacia atlantica* subsp. *kurdica* essential oil and acetic acid on *Botrytis cinerea* growth in culture media and strawberry fruits. *Bulletin of Environment, Pharmacology and Life Sciences*, *3*(2), 100-106.
24. Jaradat, N., Al-Maharik, N., Hawash, M., Abualhasan, M.N., Qadi, M., Ayesh, O., & Arar, M. (2022). Essential oil composition, antimicrobial, cytotoxic, and cyclooxygenase inhibitory areas of activity of *Pistacia lentiscus* from Palestine. *Arabian Journal for Science and Engineering*, *47*, 6869–6879. https://doi.org/10.1007/s13369-021-06555-x
25. Jideani, V.A., & Vogt, K. (2016). Antimicrobial packaging for extending the shelf life of bread—A review. *Critical Reviews in Food Science and Nutrition*, *56*(8), 1313-1324. https://doi.org/10.1080/10408398.2013.768198
26. Karami, A., Khoshbakht, T., Esmaeili, H., & Maggi, F. (2020). Essential oil chemical variability in *Oliveria decumbens* (Apiaceae) from different regions of Iran and its relationship with environmental factors. *Plants*, *9*(6), 680. https://doi.org/10.3390/plants9060680
27. Krepker, M., Zhang, C., Nitzan, N., Prinz-Setter, O., Massad-Ivanir, N., Olah, A., & Segal, E. (2018). Antimicrobial LDPE/EVOH layered films containing carvacrol fabricated by multiplication extrusion. *Polymers*, *10*(8).
28. Mahjoub, F., Rezayat, K.A., Yousefi, M., Mohebbi, M., & Salari, R. (2018). *Pistacia atlantica* Desf. A review of its traditional uses, phytochemicals and pharmacology. *Journal of Medicine and Life*, *11*(3), 180. https://doi.org/10.25122/jml-2017-0055
29. Memariani, Z., Sharifzadeh, M., Bozorgi, M., Hajimahmoodi, M., Farzaei, M.H., Gholami, M., & Saniee, P. (2017). Protective effect of essential oil of *Pistacia atlantica* Desf. On peptic ulcer: role of α-pinene. *Journal of Traditional Chinese Medicine*, *37*(1), 57-63. https://doi.org/10.1016/S0254-6272(17)30027-4
30. Mokhtari, S., Ahrari, A., & Hosseini, Z. (2021). Comparison of the effects of the leaf extract and gum of *Pistacia atlantica* and chlorhexidine on the growth initiation of *S. mutans*: an in vitro study. *European Journal of Molecular and Clinical Medicine*, *8*(3), 2688-2697. https://ejmcm.com/article_10726_54619c45ff94fe1d0b1df27022780590.pdf
31. Neffati, N., Aloui, Z., Karoui, H., Guizani, I., Boussaid, M., & Zaouali, Y. (2017). Phytochemical composition and antioxidant activity of medicinal plants collected from the Tunisian flora. *Natural Product Research*, *31*(13), 1583-1588. https://doi.org/10.1080/14786419.2017.1280490

32. Paventi, G., de Acutis, L., De Cristofaro, A., Pistillo, M., Germinara, G.S., & Rotundo, G. (2020). Biological activity of *Humulus lupulus* (L.) essential oil and its main components against *Sitophilus granarius* (L.). *Biomolecules*, *10*(8), 1108. https://doi.org/10.3390/biom10081108
33. Pina-Pérez, M.C., Rivas, A., Martínez, A., & Rodrigo, D. (2018). Effect of thermal treatment, microwave, and pulsed electric field processing on the antimicrobial potential of açaí (*Euterpe oleracea*), stevia (*Stevia rebaudiana* Bertoni), and ginseng (*Panax quinquefolius* L.) extracts. *Food Control*, *90*, 98-104. https://doi.org/10.1016/j.foodcont.2018.02.022
34. Rahimi, A.R., Hadian, J., Azizi, M., Abdosi, V., & Larijani, K. (2016). Quantity and quality of essential oil of *Pistacia atlantica* subsp. *kurdica* in response to gradual harvest of oleoresin. *Journal of Essential Oil Bearing Plants*, *19*(3), 616-623. https://doi.org/10.1080/0972060X.2014.958571
35. Rahimi, A.R., Hadyan, J., Azizi, M., Abdosi, V., & Larijani, K. (2013). Comparison of chemical compounds found in the gum essential oil of male and female *Pistacia atlantica* subsp. *kurdica*. *Journal of Biodiversity and Environmental Sciences (JBES)*, *3*(12), 174-178 .
36. Rezaie, M., Farhoosh, R., Sharif, A., Asili, J., & Iranshahi, M. (2015). Chemical composition, antioxidant and antibacterial properties of Bene (*Pistacia atlantica* subsp. *mutica*) hull essential oil. *Journal of Food Science and Technology*, *52*(10), 6784-6790. https://doi.org/10.1007/s13197-015-1789-0
37. Rodriguez, A., Nerin, C., & Batlle, R. (2008). New cinnamon-based active paper packaging against *Rhizopus stolonifer* food spoilage. *Journal of Agricultural and Food Chemistry*, *56*(15), 6364-6369. https://doi.org/10.1021/jf800699q
38. Sadeghi, A., Mortazavi, S.A., & Bahrami, A. (2011). *Detection of rupins born factors in bread dough using polymerase chain reaction*. Second National Conference on Applied Microbiology of Iran (In Persian) ,
39. Sereshti, H., Izadmanesh, Y., & Samadi, S. (2011). Optimized ultrasonic assisted extraction–dispersive liquid–liquid microextraction coupled with gas chromatography for determination of essential oil of *Oliveria decumbens* Vent. *Journal of Chromatography A*, *1218*(29), 4593-4598. https://doi.org/https://doi.org/10.1016/j.chroma.2011.05.037
40. Sharifi, M.S., & Hazell, S.L. (2011). GC-MS analysis and antimicrobial activity of the essential oil of the trunk exudates from *Pistacia atlantica kurdica*. *Journal of Pharmaceutical Sciences and Research*, *3*(8), 1364.
41. Zali, S.H., & Tahmasb, R. (2016). Medicinal plants of Farashband tribe's winter pastures and their traditional uses. *Journal Advances Health Medical Sciences*, *2*(1), 18-27. https://doi.org/10.20474/jahms-2.1.3

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد ۱۹، شماره ۳، مرداد- شهریور ۱۴۰۲ ص. ۷۷-۶۵

Iranian Food Science and Technology Research Journal
Vol. 19, No. 3, Aug.-Sep. 2023, p. 65-77

اسانس لعل کوهستان و صمغ بنه: ارزیابی خواص ضد میکروبی و شیمیایی در طی فرآیند حرارتی

سید جواد حسینی[۱]- مصطفی شهیدی نوقابی[۲*]- حسین زمانی[۳]- غلامحسین ظهوری[۴]- محبوبه سرابی جماب[۵]

تاریخ دریافت: ۱۴۰۲/۰۲/۰۶
تاریخ بازنگری۱۴۰۲/۰۴/۲۷
تاریخ پذیرش: ۱۴۰۲/۰۴/۲۸

چکیده

اسانس‌های ضروری معمولاً در برابر میکروارگانیسم‌های نامطلوب تاثیر خوب و بسزایی دارند. بنابراین می‌توان از آن‌ها به عنوان عوامل ضد میکروبی طبیعی در مواد غذایی یا بسته‌بندی آن‌ها استفاده کرد. در این تحقیق خصوصیات ضد میکروبی دو اسانس (صمغ بنه و لعل کوهستان) قبل و بعد از فرآیند حرارتی (۱۰ دقیقه– ۲۰۰ درجه سانتی‌گراد) در برابر فساد باکتری و کپک در نان بررسی شده است. همچنین ترکیبات اسانس‌ها با کروماتوگرافی گازی- طیف‌سنجی جرمی شناسایی شدند. ترکیبات اصلی اسانس لعل کوهستان کارواکرول، تیمول و المیسین قبل و بعد از عملیات حرارتی بودند. در مورد صمغ بنه، تنها یک پیک شاخص در کروماتوگرام مشاهده شد که مربوط به آلفا-پینن بود. برای هر دو اسانس، کمترین غلظت ممانعت کنندگی (MIC) و کمترین غلظت قارچ‌کشی (MFC) در برابر *آسپرژیلوس نایجر* به ترتیب ۴۰۰۰ و ۸۰۰۰ میکرولیتر بر میلی‌لیتر بود. اثر ضد میکروبی هر دو اسانس در برابر *باسیلوس سوبتیلیس* بیشتر از کپک بود. مقدار MIC و MBC برای لعل کوهستان به‌ترتیب ۱۲۵ و ۲۵۰ میکرولیتر در میلی‌لیتر و برای صمغ بنه ۶۲/۵ و ۱۲۵ میکرولیتر بر میلی‌لیتر بود. نتایج نشان داد که این دو اسانس اثر امیدوارکننده‌ای بر میکروارگانیسم‌های اصلی فساد نان دارند. فرآیند حرارتی تأثیر معنی‌داری بر فعالیت ضدمیکروبی اسانس صمغ بنه در برابر آسپرژیلوس نایجر نداشت، اما به طور قابل‌توجهی فعالیت ضدمیکروبی علیه باسیلوس سوبتیلیس را کاهش داد، در حالی که در مورد فعالیت ضد میکروبی اسانس لعل کوهستان، نتایج برعکس بود. با توجه به اینکه کپک‌ها بیشترین عامل فساد نان هستند، استفاده از اسانس صمغ بنه به عنوان نگهدارنده طبیعی در محصولاتی که عملیات حرارتی بالایی را تحمل می‌کنند مانند نان و فرآورده‌های نانوایی توصیه می‌شود.

واژه‌های کلیدی: پایداری حرارتی، صمغ بنه، کروماتوگرافی‌گازی، لعل کوهستان، MBC/MFC

۱- دانشجوی دکتری، گروه صنایع غذایی، موسسه پژوهشی علوم و صنایع غذایی، مشهد، ایران
۲- دانشیار، گروه شیمی مواد غذایی، موسسه پژوهشی علوم و صنایع غذایی، مشهد، ایران
(*-نویسنده مسئول: Email: m.shahidi@rifst.ac.ir)
۳- استادیار گروه ماشین آلات صنایع غذایی موسسه پژوهشی علوم و صنایع غذایی، مشهد، ایران
۴- استاد، گروه شیمی، دانشکده علوم، دانشگاه فردوسی مشهد، مشهد، ایران
۵- دانشیار، گروه بیوتکنولوژی مواد غذایی، موسسه پژوهشی علوم و صنایع غذایی، مشهد، ایران

DOI: 10.22067/ifstrj.2023.82016.1252

Iranian Food Science and Technology Research Journal
Vol. 19, No. 3, Aug.-Sep. 2023, p. 79-94

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد ۱۹، شماره ۳، مرداد- شهریور ۱۴۰۲ ص. ۹۴-۷۹

Research Article
Homepage: http://ifstrj.um.ac.ir

The Effect of Persian Gum Coating Enriched with Pomegranate Seed Oil on the Quality of Mexican Lime (*Citrus aurantifolia*)

M. Mohammadi[1], S. Aghaei Dargiri[2], S. Rastegar[3]*

Received: 2023.05.17
Revised: 2023.06.25
Accepted: 2023.07.01
Available Online: 2023.07.10

How to cite this article:
Mohammadi, M., Aghaei Dargiri, S., & Rastegar, S. (2023). The effect of Persian gum coating enriched with pomegranate seed oil on the quality of Mexican lime (*Citrus aurantifolia*). *Iranian Food Science and Technology Research Journal, 19*(3), 79-94. https://doi.org/10.22067/ifstrj.2023.82418.1257

Abstract

The use of edible coatings has been considered as an effective solution to improve the shelf life and quality of fruits. In this research, increase in the shelf life of citrus fruits (*Citrus aurantifolia* cv. Mexican lime) coated with Persian gum and pomegranate seed oil was investigated. Different treatments of lemon fruit coated with Persian gum and pomegranate seed oil with concentrations (zero (control), 0.5% and 1% gum, combination of 0.5% and 1% gum and pomegranate seed oil, 0.05% and pomegranate seed oil 0.05 percent) were prepared and after 24 days of storage at ambient temperature (20 ± 2 °C and relative humidity of 50-60 percent) were statistically evaluated in the form of a completely random design with three replications. The results of this research showed that the treatments used had an effective role in controlling the weight loss of fruit during storage. Thus, the lowest percentage of weight loss was observed in the pomegranate seed oil treatment. Except pomegranate seed oil treatment, other treatments showed less TSS than the control. In most of the treatments, the content of phenol, flavonoid and antioxidant was at a higher level than the control. The average comparison results showed that the fruits coated with 1% gum (85.36 units/ml) showed significantly more peroxidase activity than the control (60.35 U/ml). Persian gum edible coating 1% and 0.5% as well as Persian gum 1% in combination with pomegranate seed oil significantly controlled the activity of polyphenol oxidase enzyme. The treated samples showed less yellowness (b*) than the control. In general, the best marketability was observed in fruits coated with 1% gum. Therefore, it is recommended to use this coating to preserve the freshness and quality of the Mexican lime fruit during storage in the environment.

Keywords: Antioxidant, Edible coatings, Mexican lime, Postharvest

Introduction

Lime (*Citrus aurantifolia* cv. Mexican lime) is an acidic citrus fruit of great economic importance, grown in tropical and subtropical regions, accounting for 5% of worldwide citrus production (Dunkersley *et al.*, 2018). According to data from the World Food and Agriculture Organization, the cultivation area of citrus fruits is 9.3 million hectares and yields an annual production of 132 million tons. Iran is ranked ninth in the world for lime production, while global lime production is estimated at approximately 21 million tons per year (FAO, 2019). The color of the fruit is a crucial determinant of the quality and marketability of citrus fruits (Ma *et al.*, 2023). Given the lime's economic importance, it's crucial to preserve its quality using environmentally-friendly techniques that benefit both people and the

1, 2 and 3- Ph.D. Student, Former 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)
DOI: 10.22067/ifstrj.2023.82418.1257

planet, especially during long-term storage (Champa *et al*., 2020).

Using edible coatings is a vital approach to reducing food waste and maintaining the quality of agricultural products (Ezati *et al*., 2022; Otoni *et al*., 2017; Galus *et al*., 2020; Hoffmann *et al*., 2019). Immersing fruits in an edible coating form a thin layer on the fruit, which can enhance tissue quality, preserve color and volatile compounds, and inhibit microbial growth by reducing respiration and transpiration (Mahfoudhi *et al*., 2014). Natural coatings with antimicrobial agents, antioxidants, nutrients, flavorings, enzymes, and colors have been explored in various foods (Mohammadi *et al*., 2016; Motallebi *et al*., 2017). To produce edible coatings, natural gums are a highly promising alternative due to their biocompatibility, cost-effectiveness, non-toxicity, and wide availability (Salehi, 2020; Yun *et al*., 2022). A study by Shahbazi and Shavisi (2020) found that gum-based coatings and films significantly decreased microbial proliferation in bananas during storage. Guar gum coating enhanced the preservation quality of green mangoes and delayed their ripening (Naeem *et al*., 2018). However, limited research has been conducted on Mexican lime during storage, despite its increasing socioeconomic significance over time. Thus, this research aims to assess the viability of using an edible coating derived from Farsi gum, either alone or in combination with pomegranate seed oil, to enhance the storage quality of lime.

Materials and Methods

Lime (*Citrus aurantifolia* cv. Mexican lime) fruit in the mature green stage (when the fruit is juicier) was harvested from a commercial orchard in Hormozgan province, Rodan City, River section at geographical coordinates (57°29°E and 27°59°N) according to technical criteria. Only fruits with uniform size, absence of defects and mechanical damage were chosen for the experiment. The fruits were washed and then treated with a one-minute wash of 0.05% sodium hypochlorite solution to disinfect them.

Preparation of Coatings

Preparation of coating using Persian gum (acquired from Rihan Gam Persian Company) involved gradually dissolving the gum in water and stirring for 30 minutes at room temperature. The resulting solution was stored in a refrigerator for 24 hours. Two concentrations of Persian gum coating, 0.5% and 1%, were prepared. To create different coating treatments, glycerol (0.5% w/v), pomegranate seed oil (0.05% w/v), and Tween 80 were sequentially added to the prepared gum solution at room temperature. The treatments included a control, gum 0.5%, gum 1%, gum 0.5% and 1% with pomegranate seed oil 0.05%, and pomegranate seed oil 0.05%. Lime fruits were coated by immersion in coating solution at room temperature for 5 minutes, and after the surface coating dried, they were stored in disposable plastic containers for 24 days at a temperature of 20±2 °C and a relative humidity of 50-60% (Khaledian *et al*., 2021). Finally, the weight loss factor, soluble solids, phenol content, antioxidant, total flavonoid, peroxidase and polyphenol oxidase activity, peel color (a*, b* and L*), and marketability of lime fruits were evaluated.

Weight Loss

The fruits were weighed using a digital scale with an accuracy of 0.1 grams, and the percentage of weight loss was calculated using following formula (Dong and Wang, 2018):

$$WL\ (\%) = (w_1-w_2) \times 100/w_1 \quad (1)$$

(WL: Weight loss percentage, W_1: Primary weight in grams, W_2: Secondary weight in grams).

Total Soluble Solids

The Brix degree of the fruit juice was measured using a digital refractometer (DBR95, Taiwan) (Barry *et al*., 2004).

Phenol Content

To measure the phenolic content of the fruit juice, 0.5 mL of lime juice was mixed with 3 milliliters of 85% methanol and kept in the refrigerator for 24 hours. Then, 150 µL of the

methanolic extract and 750 µL of Folin's reagent (10%) were added to the mixture and left for 5 minutes. Next, 600 µL of 7.5% sodium carbonate solution was added to the mixture, and the sample was placed on a shaker in the dark for 2 hours and then the absorbance was read using a spectrophotometer at 750 nm (Ordonez *et al*., 2006).

Antioxidants activity

The antioxidant activity of the fruit juice was measured using the 2,2-dipheny-1-picrylhydrazyl (DPPH) free radical inhibition method. To do so, 30 µL of 85% methanol extract from the fruit and 1170 µL of DPPH (150 µmol) were combined and shaken for 1 minute. After keeping the samples in the dark for 40 minutes, the absorbance was read with a spectrophotometer at 515 nm and the antioxidant activity was calculated using the following formula (Bourtoom, 2008).

DPPH % = (Ablank- Asample / Ablank) × 100 (2)

Total Flavonoids

To measure flavonoids, 200 µL of methanolic extract was mixed with 600 µL of 85% methanol, 40 µL of 10% aluminum chloride, 40 µL of potassium acetate, and 1120 µL of distilled water and incubated at room temperature for 30 minutes. The absorbance was then measured using a spectrophotometer at 415 nm (Chang *et al*., 2002).

Peroxidase and Polyphenol Oxidase Activity

To prepare the extract, 500 µL of lime juice was mixed with a potassium buffer (pH: 7.4) containing 1 M EDTA and 1% PVP at 4 °C. The resulting mixture was centrifuged at 13,000 rpm at 4° C for 20 minutes, and the supernatant was used to investigate the activities of peroxidase and polyphenol oxidase.

The peroxidase enzyme activity of the fruit juice was measured using the method of Maehly and Chance (1954) with some modifications. In this method, 30 µL of the extracted sample was mixed with 1 mL of peroxidase solution containing 10 µmol of guaiacol, 5 µmol of hydrogen peroxide, and 50 µmol of potassium phosphate buffer, and the absorbance was read at 470 nm for 1 minute. The extinction coefficient used to calculate the enzyme unit was equivalent to 26.6 $mM^{-1}Cm^{-1}$, and the peroxidase enzyme activity was expressed in U/ mL FW.

For the measurement of polyphenol oxidase activity, pyrogallol was used as an enzyme precursor. The reaction mixture included 2.5 ml of potassium buffer (50 mM and pH 7), 200 µl of 0.02 M pyrogallol, and 100 µl of enzyme extract. The absorbance of the samples was measured at 420 nm after 3 minutes. The extinction coefficient used to calculate the enzyme unit was 6.2 $mM^{-1}Cm^{-1}$, and the polyphenol oxidase enzyme activity was expressed in U/ mL FW.

Peel color and Marketability

The peel color of the fruit was measured using a colorimeter (Minolta CR400, Japan) based on the color characteristics L*, a*, and b* (Zhou *et al*., 2010). The evaluation of the marketability of fruits was done according to the appearance characteristics of the fruits and the scoring method. The grading criteria included aroma, color uniformity, dryness, rotting, and the appearance of the fruit, and scores from 1 to 5 were assigned to them. 1- poor, 2- average, 3- good, 4- very good, 5- excellent. Asghari and Aghdam, 2010).

Statistical Analysis

The experiment was conducted using a completely randomized factorial design with three repetitions, and each repetition included 9 fruits. The data were analyzed using SAS software version 9.4, and the means were compared using the LSD test at a significance level of 5%.

Results and Discussion

Fruit Weight Loss

The results of the analysis of variance showed that the treatments had a significant effect on preventing weight loss of lime fruit during storage (24 days) at a probability level

of 1%. The pomegranate seed oil treatment (12.5%) had the greatest effect (Fig. 1). The reduction in fruit mass during storage can be attributed to the loss of water content resulting from alterations in surface transfer resistance towards water vapor, alongside variations in respiratory activity and the emergence of minute apertures that facilitate the exchange of atmospheric gases between the interior and exterior environments (Shahid and Abbasi, 2011). To prevent post-harvest weight loss, regulating the factors that trigger metabolic activity in fruit tissue is imperative. Coatings can be an efficient barrier against carbon dioxide, oxygen, and moisture, curtail respiration, and minimize water loss and oxidation reactions (Dong and Wang, 2018). Studies have shown that the use of gum Arabic coatings on guava (Murmu and Mishra, 2017) and banana (Maqbool *et al*., 2011) fruits at an ambient temperature effectively maintained fruit weight, which is consistent with our results.

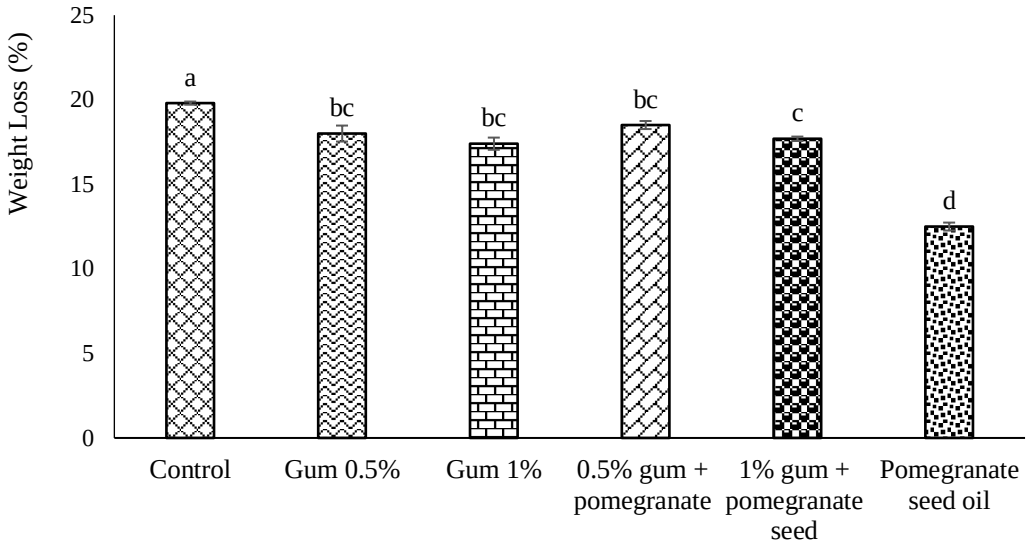


Fig. 1- The effect of Persian gum edible coatings, Persian gum in combination with pomegranate seed oil and pomegranate seed oil on the weight loss of lime fruit stored at ambient temperature
The same letters have no significant difference with each other at the probability level of $p \leq 0.05$.

Total Soluble Solids (TSS)

The analysis of variance showed that the treatments had a significant effect on the dissolved solids at a 1% probability level. The gum treatment of 0.5% (6.5%) had the lowest soluble solids compared to the control (7.9%) (Fig. 2). The quantity of dissolved substances in fruit is a key marker of fruit maturation (Kazemi *et al*., 2011). The increase in soluble substances during storage is due to hydrolysis of starch to sugar (Wang *et al*., 2013). Storage leads to increased concentration of dry matter and destruction of the cell wall, resulting in a rise in soluble solids and a decrease in fruit juice content (Khorram *et al*., 2017; Dhall, 2013). Edible coatings can prevent the escalation of soluble solids in fruit by reducing respiration, altering the internal fruit atmosphere, and increasing carbon dioxide while decreasing oxygen and ethylene (Dong and Wang, 2018). During storage, organic acids degrade more rapidly than sugars, resulting in slight sweetness in the fruit (Razzaq *et al*., 2014). Blood oranges (Habibi and Ramezanian, 2017), oranges (Rasouli *et al*., 2019), and limes (Atrash *et al*., 2018) have shown an increase in the soluble solids.

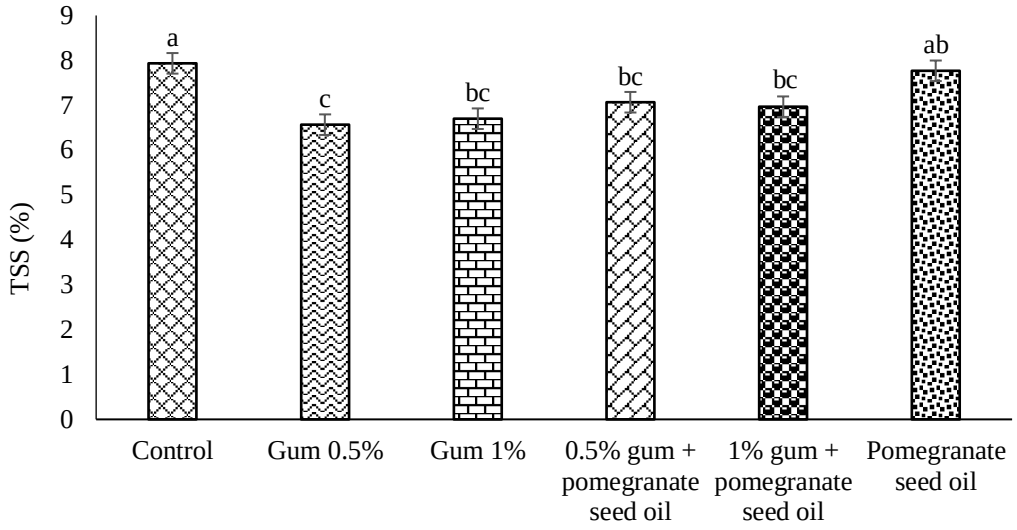


Fig. 2- The effect of Persian gum edible coatings, Persian gum in combination with pomegranate seed oil and pomegranate seed oil on soluble solids of lime fruit stored at ambient temperature
The same letters have no significant difference with each other at the probability level of p≤0.05.

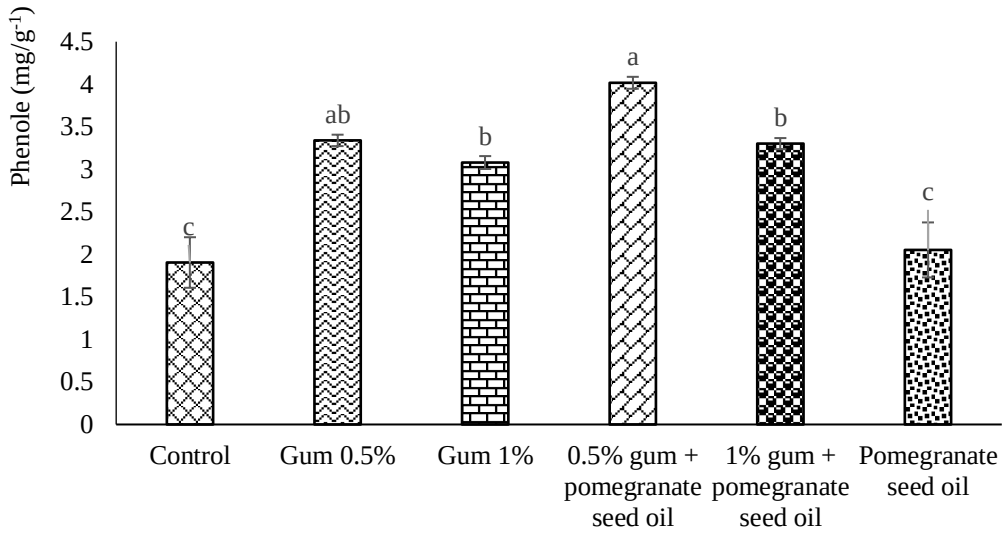
Phenol

The variance analysis of data showed that all treatments had a significant effect on lime phenol at a 1% probability level. The 0.5% gum treatment with pomegranate seed oil (4.01 mg/g) had significantly higher phenol content than the control (1.9 mg/g) (Fig. 3a). Previous research on tomato (Uckoo *et al*., 2015) and papaya (Addai *et al*., 2013) showed that the use of gum Arabic retained phenolic compounds in the plants, which is consistent with our results. Similarly, previous research on blood orange (Habibi and Ramezanian, 2017), orange (Rasouli *et al*., 2019; Khorram *et al*., 2017), and guava (Nair *et al*., 2018) were also observed the effects of using food coatings in maintaining phenol content.

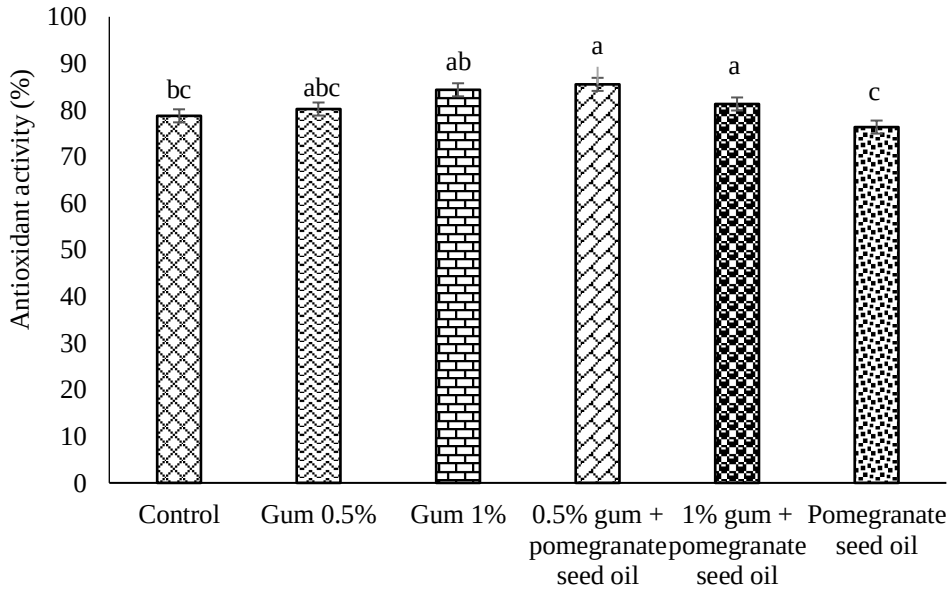
Antioxidants activity

The variance analysis of the data showed that the interaction effect of the treatments on the lime antioxidant activities was significant at a 1% probability level. The 0.5% and 1% lime gum treatments with 8.57% and 3.22% pomegranate seed oil had a higher content of antioxidants compared to the control (Fig. 3b). The increased antioxidant activity in these fruits can be attributed to their higher total phenol content, as there is a positive correlation between the quantity of phenol and antioxidant activity (Khaliq *et al*., 2016). Habibi and Ramezanian (2017) showed that antioxidant activity and phenol levels shift in parallel over the course of storage, as the generation of free radicals in fruits due to aging and stress prompts the deployment of antioxidants by fruit cells to counteract their deleterious effects. Thus, interventions that reduce respiration, alleviate stress, mitigate aging, and preserve the cellular concentration of antioxidants may be effective (Asghari and Aghdam, 2010). Nair *et al*. (2018) and Shah *et al*. (2015) demonstrated that the implementation of alginate coating on guava and tangerine, preserved antioxidant activity, which is consistent with the outcomes presented in this research.

a


Phenole (mg/g-1)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
c
ab
b
a
b
c
Control
Gum 0.5%
Gum 1%
0.5% gum + pomegranate seed oil
1% gum + pomegranate seed oil
Pomegranate seed oil

b


Antioxidant activity (%)
100
90
80
70
60
50
40
30
20
10
0
bc
abc
ab
a
a
c
Control
Gum 0.5%
Gum 1%
0.5% gum + pomegranate seed oil
1% gum + pomegranate seed oil
Pomegranate seed oil

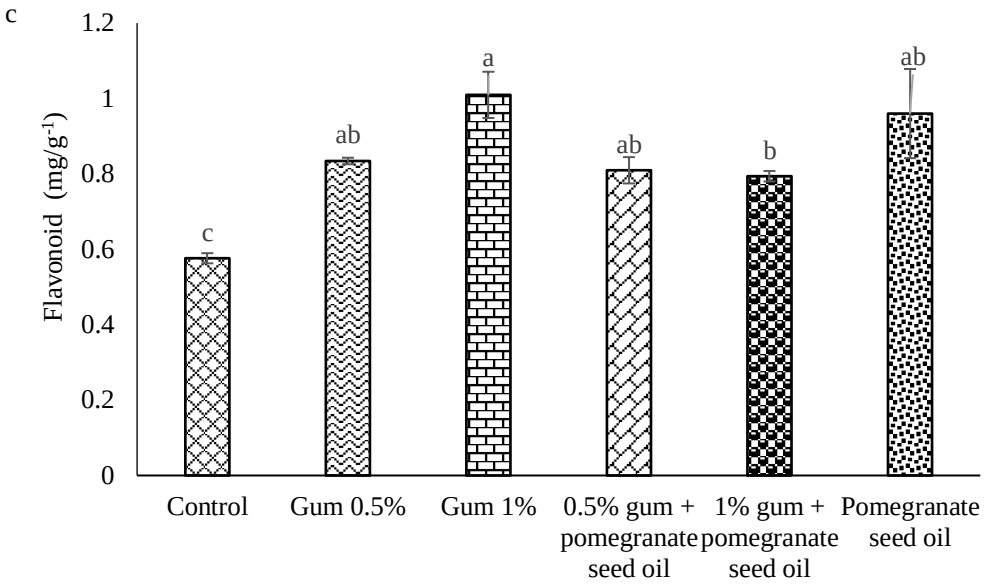


Fig. 3- The effect of Persian gum edible coatings, Persian gum in combination with pomegranate seed oil and pomegranate seed oil on a) phenol, b) antioxidant and c) flavonoid of lime fruit stored at ambient temperature
The same letters have no significant difference with each other at the probability level of p≤0.05.

Flavonoid

The analysis of variance showed that the effect of treatments on the amount of lime flavonoids was significant at a 1% probability level. The 1% gum treatment had a higher flavonoid content in lime fruit (1 mg/g) compared to the control (0.57) (Fig. 3c). Flavonoids and other phenolic compounds play a crucial role in the non-enzymatic antioxidant network, protecting against the harmful effects of free radicals and reducing oxidative stress (Shamloo *et al*., 2015). Lime contains a considerable of flavonoids, including naringenin, diosmin, and hesperidin, primarily present in its juice. Flavonoids, which are polyphenolic compounds, are released as part of the plant's defense mechanism, and hesperidin plays a significant role in this process (Uckoo *et al*., 2015).

Measurement of Peroxidase and Polyphenol Oxidase Activity

The analysis of variance showed that the activity of peroxidase, a polyphenol oxidizing enzyme in lime, was significant at a 5% probability level. The highest peroxidase activity was observed in the 1% gum treatment (85.3 units/ml), and the lowest was observed in the control treatment (60.3 units/ml) (Fig. 4a). No significant difference was observed between the other treatments. The lowest polyphenol oxidizing enzyme activity was observed in the 1% gum treatment (61.5 units/ml), while the highest was observed in the control treatment (101 units/ml) (Fig. 4b). Phenolic compounds are synthesized at an increased rate during the ripening process, but their levels decreased as the fruit approaches senescence. The contribution of phenolic compounds to fruit quality and phytochemical levels is significant (Shamloo *et al*., 2015), and their overall variation is influenced by genetics, temperature, and environmental conditions during post-harvest (Rasouli *et al*., 2019). Phenolic compounds in fruits directly correlate with their antioxidant capacity, and a decrease in phenolic content results in a decrease in antioxidant activity (Shiri *et al*., 2011). Edible coatings alter the fruit's atmosphere, increasing the concentration of carbon dioxide and reducing respiration and the rate of phenol oxidation reactions and polyphenol oxidase enzyme activity (Asghari and Aghdam, 2010). Polyphenol oxidase and peroxidase are crucial

enzymes in plant defense mechanisms that contribute significantly to their ability to resist pathogenic attacks (Babu *et al.*, 2015). These defense mechanisms play a critical role in preserving fruit quality after harvesting (Zheng *et al.*, 2011). Peroxidase, the primary antioxidant enzyme in plants, impedes the accumulation of free radicals and works synergistically to inhibit their formation, promoting overall plant health (Kou *et al.*, 2014).

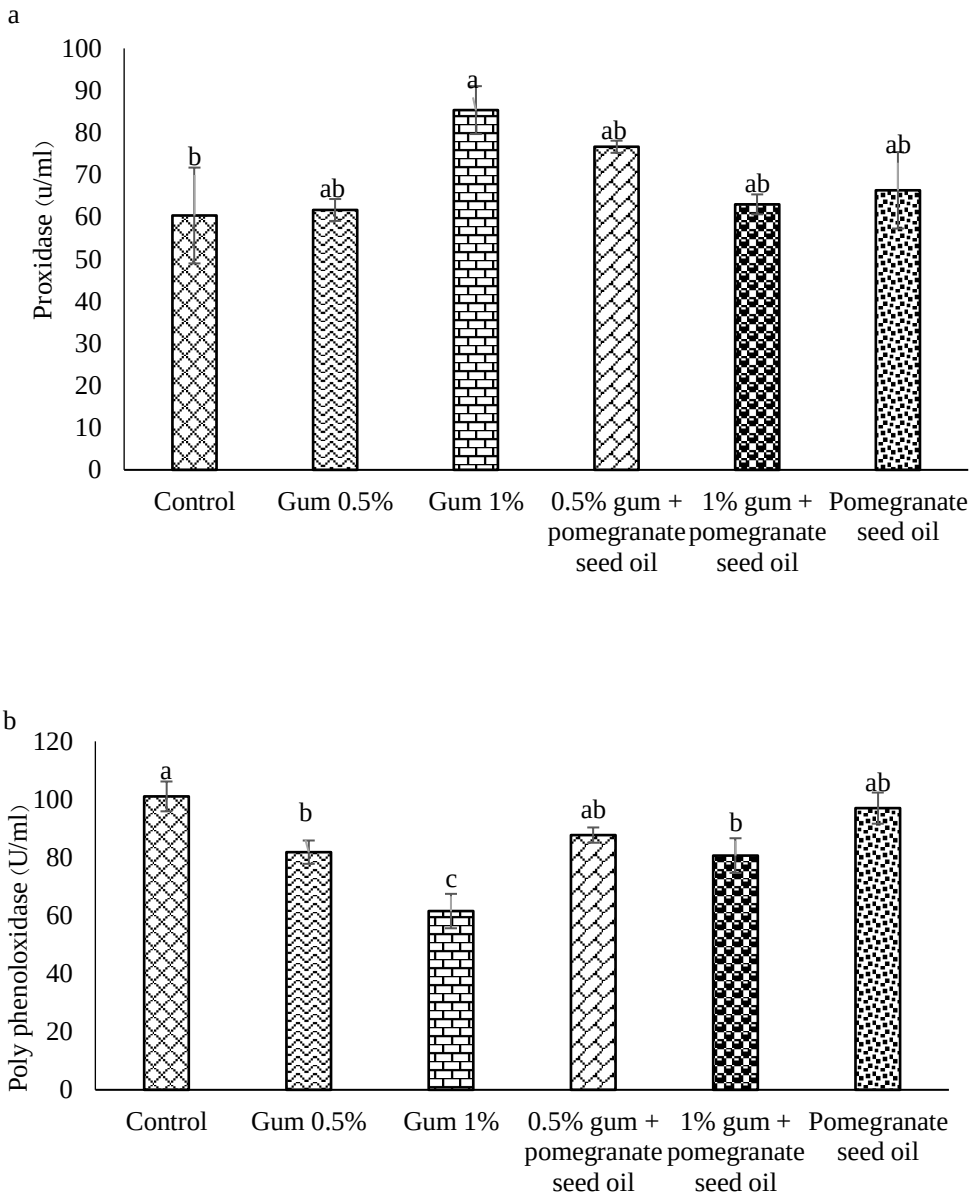
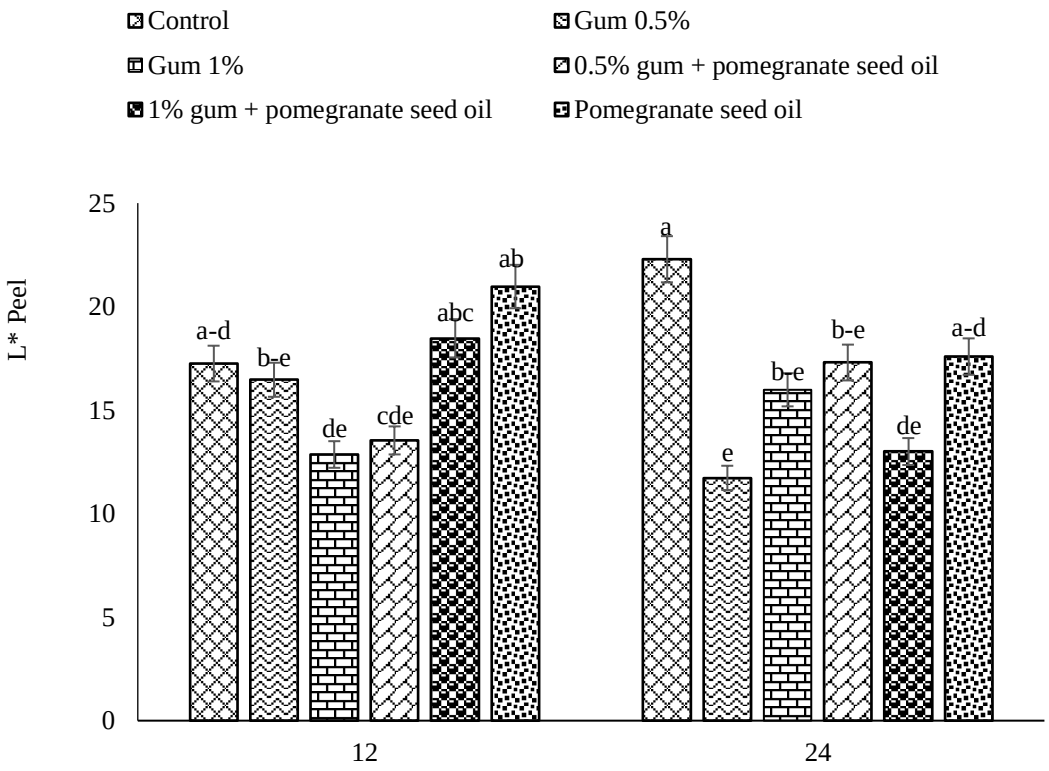


Fig. 4- The effect of Persian gum edible coatings, Persian gum in combination with pomegranate seed oil and pomegranate seed oil on a) peroxidase, and b) polyphenol oxidase of lime fruit stored at ambient temperature

The same letters have no significant difference with each other at the probability level of $p \leq 0.05$.

Peel Color Index

The analysis of variance showed that the lime peel color index was significant at a 1% probability level. Immersion of lime fruit in the treatments decreased the L* index of lime peel color during the storage period (24 days). The combined treatment of 1% gum and pomegranate seed oil decreased the L* index of lime fruit compared to the control (Fig. 5a). The a* index increased with the storage period, indicating that the fruit became yellower (more positive a* value) as it ripened. After 24 days of storage, the highest amount of a* index was positive in all treatments except for pomegranate seed oil (Fig. 5b). The treatment of 0.5% gum and the combination of 1% gum with pomegranate seed oil decreased the b* index compared to the control (Fig. 5c). The b* index (yellow color) of the fruit peel increased during the storage time due to the decrease in chlorophyll and the increase in carotenoid as the fruit ripened. This is consistent with the results of a study that used guar gum and chickpea starch coating to increase the shelf life of Valencia oranges (Saberi *et al*., 2018). The peel color of lime fruit is an important quality parameter that affects consumer choice, and the L*, a*, and b* indexes are used to evaluate fruit color (Nair *et al*., 2018). The polyphenol oxidase enzyme affects the fruit color by increasing the amount of brown color (L*) in the fruit (Nunes *et al*., 2005). The increase in a* during storage is the result of fruit ripening and aging and the speed of the non-enzymatic process of browning in the fruit (Etemadipoor *et al*., 2019). Visual appearance and freshness are key factors in determining the external quality of limes (Asencio *et al*., 2018).



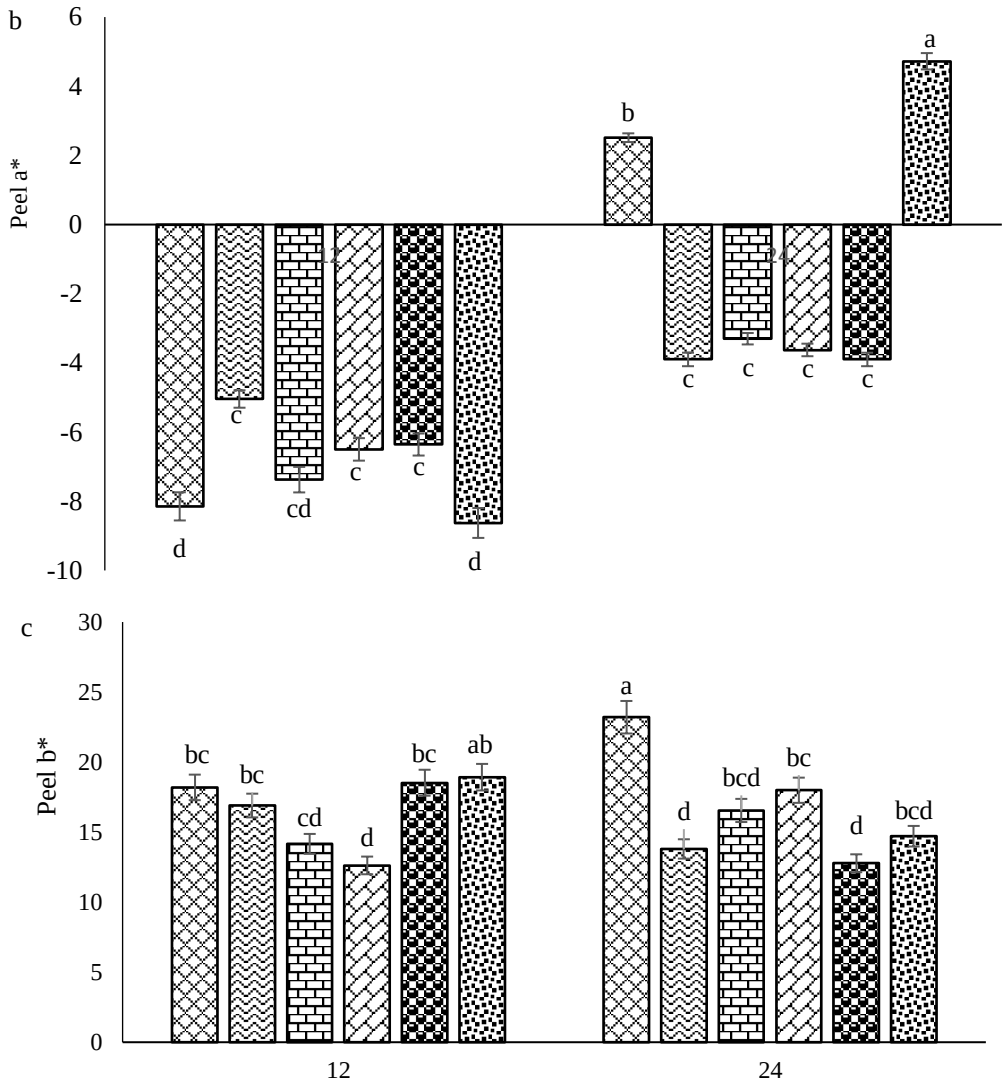


Fig. 5- The effect of Persian gum edible coatings, Persian gum in combination with pomegranate seed oil and pomegranate seed oil on a) L *, b) a * and c) b * lime fruit stored at ambient temperature

The same letters have no significant difference with each other at the probability level of $p \leq 0.05$.

Marketability

Appearance is the most crucial factor in determining the evaluation and marketability of a product. The 1% gum treatment was the most marketable among the various treatments. Although pomegranate seed oil effectively controlled fruit weight loss, it was not marketable due to the effect on browning of the fruit peel. The control fruits exhibited the lowest level of marketability. The quality of fruits, such as color uniformity, firmness, and absence of decay, is crucial in maintaining quality and facilitating export to distant markets. The impact of treatments on 24 days of lime fruit storage can beobserved in Fig. 6.

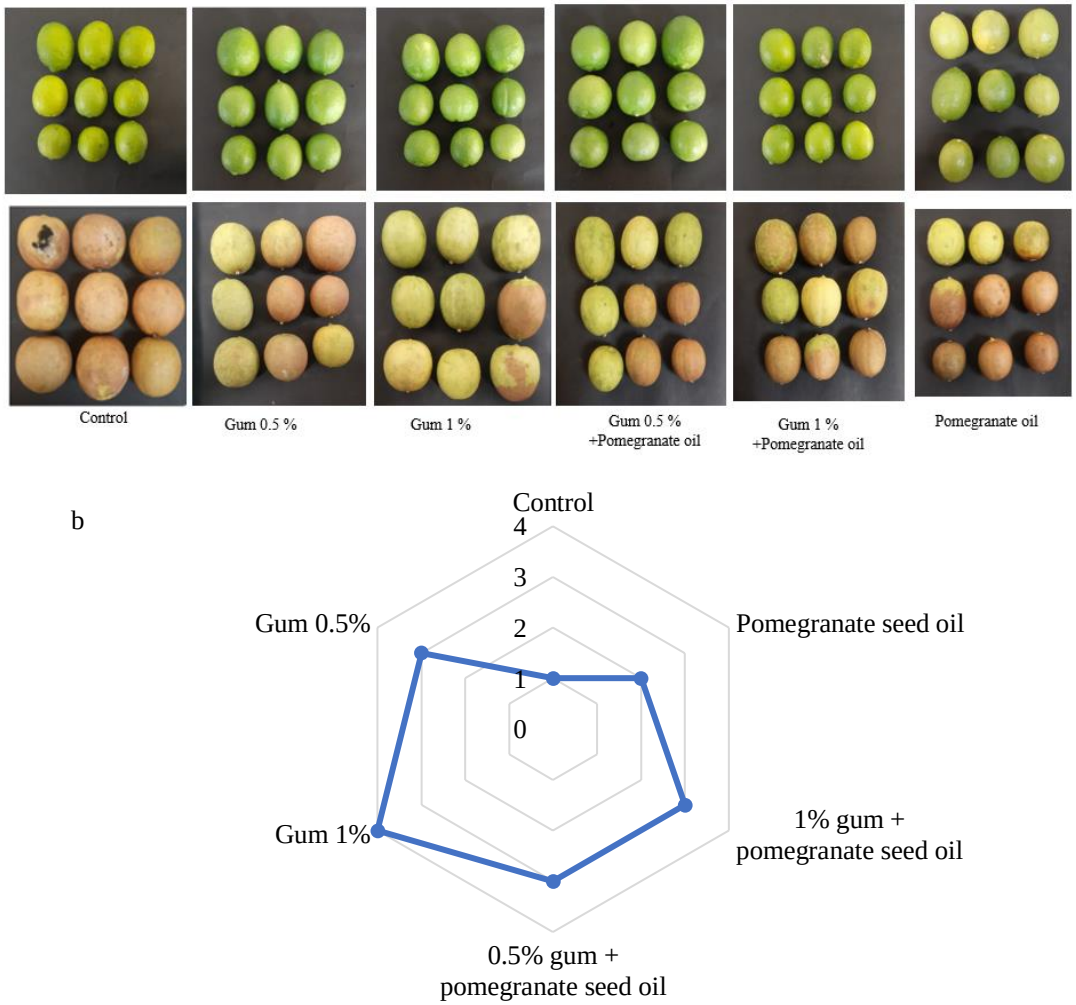


Fig. 6- a) Treated fruits after 24 days of storage b) Comparison of treatment combinations (control, 0.5% gum, 1% gum, 0.5% gum with pomegranate seed oil, 1% gum with pomegranate seed oil, pomegranate seed oil) on the marketability of Mexican lime

Conclusion

Persian gum and pomegranate seed oil coatings can retain the peel color, marketability, fruit phenol content, and soluble solids of Mexican lime at ambient temperature. These coatings are recommended as a substitute for chemical compounds to ensure storage quality. The addition of 1% gum in conjunction with pomegranate seed oil resulted in a significant increase in peroxidase and flavonoid levels compared to the control group. The 0.5% gum treatment, which incorporated pomegranate seed oil, showed a decrease in the concentration of soluble solids and polyphenol oxidase compared to the control group. As gum-based coatings and films for consumption are still in the developmental phase, future investigations should focus on creating prototypes. It is predicted that researchers will be able to overcome fundamental obstacles and develop appropriate skills to enhance edible films and coatings for food products.

Reference

1. Addai, Z.R., Abdullah, A., Mutalib, S.A., & Musa, K.H. (2013). Effect of gum Arabic on quality and antioxidant properties of papaya fruit during cold storage. *International Journal of ChemTech Research*, *5*(6), 2854-2862.
2. Ali, A., Maqbool, M., Alderson, P.G., & Zahid, N. (2013). Effect of gum Arabic as an edible coating on antioxidant capacity of tomato (*Solanum lycopersicum* L.) fruit during storage. *Postharvest Biology and Technology*, *76*, 119-124. https://doi.org/10.1016/j.postharvbio.2012.09.011
3. Asencio, A.D., Serrano, M., García-Martínez, S., & Pretel, M.T. (2018). Organic acids, sugars, antioxidant activity, sensorial and other fruit characteristics of nine traditional Spanish Citrus fruits. *European Food Research and Technology*, *244*, 1497-1508. https://doi.org/10.1007/s00217-018-3064-x
4. Asghari, M., & Aghdam, M.S. (2010). Impact of salicylic acid on post-harvest physiology of horticultural crops. *Trends in Food Science & Technology*, *21*(10), 502-509. https://doi.org/10.1016/j.tifs.2010.07.009
5. Atrash, S., Ramezanian, A., Rahemi, M., Ghalamfarsa, R.M., & Yahia, E. (2018). Antifungal effects of savory essential oil, gum Arabic, and hot water in Mexican lime fruits. *HortScience*, *53*(4), 524-530.\
6. http://dx.doi.org/10.21273/hortsci12736-17
7. Bonazzi, C., & Dumoulin, E. (2011). Quality changes in food materials as influenced by drying processes. *Modern Drying Technology*, *3*, 1-20. http://dx.doi.org/10.1002/9783527631728.ch
8. Bourtoom, T. (2008). Edible films and coatings: characteristics and properties. *International Food Research Journal*, *15*(3), 237-248. http://dx.doi.org/10.5937/FFR1501011S
9. Champa, W.A.H., & Gamage, K.G.N.M. (2020). Postharvest dip application of putrescine and salicylic acid delayed postharvest quality deterioration and extended the storage life of lime (*Citrus aurantifolia* Swingle) fruit. *International Journal of Fruit Science*, *20*(sup3), S1629-S1638. https://doi.org/10.1080/15538362.2020.1822268
10. Chang, C.C., Yang, M.H., Wen, H.M., & Chern, J.C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, *10*(3). http://dx.doi.org/10.38212/2224-6614.2748
11. Dhall, R.K. (2013). Advances in edible coatings for fresh fruits and vegetables: a review. *Critical Reviews in Food Science and Nutrition*, *53*(5), 435-450. http://dx.doi.org/10.1080/10408398.2010.541568
12. 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
13. Donkersley, P., Silva, F.W., Carvalho, C.M., Al-Sadi, A.M., & Elliot, S.L. (2018). Biological, environmental and socioeconomic threats to citrus lime production. *Journal of Plant Diseases and Protection*, *125*, 339-356. https://doi.org/10.1007/s41348-018-0160-x
14. Etemadipoor, R., Ramezanian, A., Dastjerdi, A.M., & Shamili, M. (2019). The potential of gum Arabic enriched with cinnamon essential oil for improving the qualitative characteristics and storability of guava (*Psidium guajava* L.) fruit. *Scientia Horticulturae*, *251*, 101-107. https://doi.org/10.1016/j.scienta.2019.03.021
15. Ezati, P., Roy, S., & Rhim, J.W. (2022). Effect of saffron on the functional property of edible films for active packaging applications. *ACS Food Science & Technology*, *2*(8), 1318-1325. http://dx.doi.org/10.1021/acsfoodscitech.2c00151
16. FAOSTAT. (2019). FAOSTAT crops. Available at http://faostat3.fao.org/faostatgateway/go/to/download/Q/QC/E

17. Galus, S., Arik Kibar, E.A., Gniewosz, M., & Kraśniewska, K. (2020). Novel materials in the preparation of edible films and coatings—A review. *Coatings*, *10*(7), 674. https://doi.org/10.3390/coatings10070674
18. Gao, Y., Kan, C., Wan, C., Chen, C., Chen, M., & Chen, J. (2018). Quality and biochemical changes of navel orange fruits during storage as affected by cinnamaldehyde-chitosan coating. *Scientia Horticulturae*, *239*, 80-86. http://dx.doi.org/10.1016/j.scienta.2018.05.012
19. Gupta, V., Biswas, D. and Roy, S. (2022). A comprehensive review of biodegradable polymer-based films and coatings and their food packaging applications. *Materials*, *15*(17), 5899. https://doi.org/10.3390/ma15175899
20. Habibi, F., & Ramezanian, A. (2017). Vacuum infiltration of putrescine enhances bioactive compounds and maintains quality of blood orange during cold storage. *Food Chemistry*, *227*, 1-8. http://dx.doi.org/10.1016/j.foodchem.2017.01.057
21. Hoffmann, T., Peters, D., Angioletti, B., Bertoli, S., Péres, L., Reiter, M., & De Souza, C. (2019). Potentials nanocomposites in food packaging. *Chem. Eng. Trans*, *75*. http://dx.doi.org/10.22034/jchr.2022.1948882.1482
22. Kaewsuksaeng, S., Urano, Y., Aiamla-or, S., Shigyo, M., & Yamauchi, N. (2011). Effect of UV-B irradiation on chlorophyll-degrading enzyme activities and postharvest quality in stored lime (*Citrus latifolia* Tan.) fruit. *Postharvest Biology and Technology*, *61*(2-3), 124-130. https://doi.org/10.1016/j.postharvbio.2011.02.014
23. Kar, M., & Mishra, D. (1976). Catalase, peroxidase, and polyphenol oxidase activities during rice leaf senescence. *Plant physiology*, *57*(2), 315-319. https://doi.org/10.1104%2Fpp.57.2.315
24. Karimi, R., Mirzaei, F., & Rasouli, M. (2017). Phenolic acids, flavonoids, antioxidant capacity and minerals content in fruit of five grapevine cultivars. *Iranian Journal Horticultural Science Technology 18*(1), 89-102. http://journal-irshs.ir/article-1-228-en.html
25. Kazemi, M., Aran, M., & Zamani, S. (2011). Effect of salicylic acid treatments on quality characteristics of apple fruits during storage. *American Journal of Plant Physiology*, *6*(2), 113-119. https://doi.org/10.3923/ajpp.2011.113.119
26. Khaliq, G., Mohamed, M.T.M., Ghazali, H.M., Ding, P., & Ali, A. (2016). Influence of gum arabic coating enriched with calcium chloride on physiological, biochemical and quality responses of mango (*Mangifera indica* L.) fruit stored under low temperature stress. *Postharvest Biology and Technology*, *111*, 362-369. https://doi.org/10.1016/j.postharvbio.2015.09.029
27. Khorram, F., Ramezanian, A., & Hosseini, S. M. H. (2017). Effect of different edible coatings on postharvest quality of 'Kinnow'mandarin. *Journal of Food Measurement and Characterization*, *11*, 1827-1833.
28. https://doi.org/10.1007/s11694-017-9564-8
29. Kou, X. H., Guo, W. L., Guo, R. Z., Li, X. Y., & Xue, Z. H. (2014). Effects of chitosan, calcium chloride, and pullulan coating treatments on antioxidant activity in pear cv."Huang guan" during storage. *Food and Bioprocess Technology*, *7*, 671-681. https://doi.org/10.1007/s11947-013-1085-9
30. Ma, G., Zhang, L., & Kato, M. (2023). Molecular regulation of carotenoid biosynthesis in citrus juice sacs: new advances. *Scientia Horticulturae*, *309*, 111629. https://doi.org/10.1016/j.scienta.2022.111629
31. Maehly, A., & Chance, B. (1954). Catalases and peroxidases. *Methods Biochem Anal*, *1*, 357-424.
32. Mahfoudhi, N., Chouaibi, M., & Hamdi, S. (2014). Effectiveness of almond gum trees exudate as a novel edible coating for improving postharvest quality of tomato (*Solanum lycopersicum* L.) fruits. *Food Science and Technology International*, *20*(1), 33-43. https://doi.org/10.1177/1082013212469617

33. Maqbool, M., Ali, A., Alderson, P. G., Zahid, N., & Siddiqui, Y. (2011). Effect of a novel edible composite coating based on gum Arabic and chitosan on biochemical and physiological responses of banana fruits during cold storage. *Journal of Agricultural and Food Chemistry*, *59*(10), 5474-5482. https://doi.org/10.1021/jf200623m
34. Mohammadi, M., Bahramian, S., & Rokhzadi, A. (2016). The effect of chitosan coating and essential oil of *Pistacia atlantica* subsp. kurdica on rainbow trout's fat oxidation process during storage. http://isfj.ir/article-1-1555-en.html
35. Motallebi, Y., Ojagh, M., Motallebi, A.A., & Safari, R. (2017). The effects of chitosan coating combined with natamycin on quality of salted kutum (*Rutilus frisii kutum*) roe during refrigerated storage. *ISFJ*, *25*(4), 109-120. http://isfj.ir/article-1-1634-en.html
36. Murmu, S.B., & Mishra, H.N. (2017). Optimization of the Arabic gum based edible coating formulations with sodium caseinate and Tulsi extract for guava. *LWT*, *80*, 271-279. https://doi.org/10.1016/j.lwt.2017.02.018
37. Naeem, A., Abbas, T., Ali, T.M., & Hasnain, A. (2018). Effect of guar gum coatings containing essential oils on shelf life and nutritional quality of green-unripe mangoes during low temperature storage. *International Journal of Biological Macromolecules*, *113*, 403-410. http://dx.doi.org/10.1016/j.ijbiomac.2018.01.224
38. Nair, M.S., Saxena, A., & Kaur, C. (2018). Effect of chitosan and alginate-based coatings enriched with pomegranate peel extract to extend the postharvest quality of guava (*Psidium guajava* L.). *Food chemistry*, *240*, 245-252. https://doi.org/10.1016/j.foodchem.2017.07.122
39. 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
40. Otoni, C.G., Avena-Bustillos, R.J., Azeredo, H.M., Lorevice, M.V., Moura, M.R., Mattoso, L.H., & McHugh, T.H. (2017). Recent advances on edible films based on fruits and vegetables—a review. *Comprehensive Reviews in Food Science and Food Safety*, *16*(5), 1151-1169.
41. Pak, E.S., Ghaghelestani, S.N., & Najafi, M.A. (2020). Preparation and characterization of a new edible film based on Persian gum with glycerol plasticizer. *Journal of Food Science and Technology*, *57*, 3284-3294. https://doi.org/10.1007%2Fs13197-020-04361-1
42. Rasouli, M., Saba, M.K., & Ramezanian, A. (2019). Inhibitory effect of salicylic acid and Aloe vera gel edible coating on microbial load and chilling injury of orange fruit. *Scientia Horticulturae*, *247*, 27-34. https://doi.org/10.1016/j.scienta.2018.12.004
43. Razzaq, K., Khan, A.S., Malik, A.U., Shahid, M., & Ullah, S. (2014). Role of putrescine in regulating fruit softening and antioxidative enzyme systems in 'Samar Bahisht Chaunsa'mango. *Postharvest Biology and Technology*, *96*, 23-32. https://doi.org/10.1016/j.postharvbio.2014.05.003
44. Saberi, B., Golding, J.B., Marques, J.R., Pristijono, P., Chockchaisawasdee, S., Scarlett, C.J., & Stathopoulos, C.E. (2018). Application of bio composite edible coatings based on pea starch and guar gum on quality, storability and shelf life of 'Valencia 'oranges. *Postharvest biology and technology*, *137*, 9-20. https://doi.org/10.1016/j.postharvbio.2017.11.003
45. http://dx.doi.org/10.1080/07388551.2017.1286449
46. 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
47. Shah, S.W.A., Jahangir, M., Qaisar, M., Khan, S.A., Mahmood, T., Saeed, M., & Liaquat, M. (2015). Storage stability of kinnow fruit (*Citrus reticulata*) as affected by CMC and guar gum-based silver nanoparticle coatings. *Molecules*, *20*(12), 22645-22661. https://doi.org/10.3390/molecules201219870

48. Shahbazi, Y., & Shavisi, N. (2020). Application of active Kurdi gum and Farsi gum-based coatings in banana fruits. *Journal of Food Science and Technology*, *57*(11), 4236-4246. https://doi.org/10.1007%2Fs13197-020-04462-x
49. 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.
50. 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
51. Shiri, M.A., Ghasemnezhad, M., Bakhshi, D., & Dadi, M. (2011). Changes in phenolic compounds and antioxidant capacity of fresh-cut table grape (*'Vitis vinifera*') cultivar'Shahaneh'as influence by fruit preparation methods and packagings. *Australian Journal of Crop Science*, *5*(12), 1515-1520.
52. 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. http://dx.doi.org/10.1016/j.jfca.2015.01.009
53. 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
54. Yun, D., He, Y., Zhu, H., Hui, Y., Li, C., Chen, D., & Liu, J. (2022). Smart packaging films based on locust bean gum, polyvinyl alcohol, the crude extract of *Loropetalum chinense* var. rubrum petals and its purified fractions. *International Journal of Biological Macromolecules*, *205*, 141-153. https://doi.org/10.1016/j.ijbiomac.2022.02.068

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد ۱۹، شماره ۳، مرداد- شهریور ۱۴۰۲ ص. ۹۴-۷۹

Iranian Food Science and Technology Research Journal
Vol. 19, No. 3, Aug.-Sep. 2023, p. 79-94

اثر پوشش خوراکی صمغ فارسی غنی‌شده با روغن دانه انار بر کیفیت لیموترش مکزیکن لایم (*Citrus aurantifolia*)

محبوبه محمدی[۱]- سهیلا آقایی درگیری[۲]- سمیه رستگار[۳*]

تاریخ دریافت: ۱۴۰۲/۰۲/۲۷
تاریخ بازنگری۱۴۰۲/۰۴/۰۴
تاریخ پذیرش: ۱۴۰۲/۰۴/۱۰

چکیده

استفاده از پوشش‌ها به عنوان راهکاری موثر برای بهبود عمر میوه و کیفیت آنها مورد توجه قرار گرفته است. در این تحقیق، افزایش عمر ماندگاری میوه‌های لیمو ترش مکزیکن لایم (*Citrus aurantifolia* cv. Mexican lime) با پوشش صمغ فارسی و روغن دانه انار بررسی شد. ترکیبات مختلف لیموترش با پوشش صمغ فارسی و روغن دانه انار با غلظت‌های مختلف (صفر (کنترل)، ۰/۵ درصد و ۱ درصد صمغ فارسی، ترکیب ۰/۵ درصد و ۱ درصد صمغ فارسی با روغن دانه انار، و روغن دانه انار به تنهایی ۰/۰۵ درصد) تهیه شده و پس از ۲۴ روز نگهداری در دمای محیطی (۲۰ ± ۲ درجه سانتی‌گراد و رطوبت نسبی ۵۰-۶۰ درصد) با تکرار سه بار به صورت طرح کاملاً تصادفی مورد ارزیابی قرار گرفتند. نتایج این تحقیق نشان داد که تیمارهای استفاده شده نقش مؤثری در کنترل افت وزن میوه در طول نگهداری داشتند. بنابراین، کمترین درصد افت وزن در تیمار روغن دانه انار مشاهده شد. به جز تیمار روغن دانه انار، سایر تیمارها مقدار کمتری از میزان محلول جامد قابل اندازه‌گیری (TSS) نسبت به گروه کنترل نشان دادند. در بیشتر تیمارها، محتوای فنل، فلاونوئید و آنتی‌اکسیدان در سطح بالاتری نسبت به گروه کنترل بود. نتایج مقایسه میانگین نشان داد که میوه‌های پوشش داده شده با ۱ درصد صمغ فارسی (۸۵/۳۶ واحد/میلی‌لیتر) فعالیت پراکسیداز را به طور قابل توجهی بیشتر از گروه کنترل (۶۰/۳۵ واحد/میلی‌لیتر) نشان دادند. پوشش صمغ فارسی ۱ درصد و ۰/۵درصد به همراه روغن دانه انار به طور قابل توجهی فعالیت آنزیم پلی فنول اکسیداز را کنترل کردند. نمونه‌های تیمار شده میزان زردی (b*) کمتری نسبت به گروه کنترل نشان دادند. به طور کلی، بهترین قابلیت بازارپذیری در میوه‌های پوشش داده شده با صمغ فارسی ۱ درصد مشاهده شد. بنابراین استفاده از این پوشش برای حفظ تازگی و کیفیت میوه لیمو ترش مکزیکی در طول نگهداری در محیط توصیه می‌شود.

واژه‌های کلیدی: آنتی‌اکسیدان، پس از برداشت، پوشش خوراکی، مکزیکن لایم

۱، ۲ و ۳- به‌ترتیب دانشجوی دکتری، دانش آموخته دکتری و دانشیار گروه باغبانی، دانشگاه هرمزگان، بندرعباس، ایران
(*- نویسنده مسئول: Email: rastegarhort@gmail.com)

DOI: 10.22067/ifstrj.2023.82418.1257

بسم الله الرحمن الرحیم

مندرجات

نشریه پژوهشهای علوم و صنایع غذایی ایران

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

جلد ۱۹ شماره ۳ مرداد- شهریور ۱۴۰۲

درجه علمی- پژوهشی این نشریه طی نامه ۳/۱۱/۴۷۶۷۳ / ۹۰/۴/۱۴ از وزارت علوم، تحقیقات و فناوری تا سال ۱۳۹۳ تمدید شده است.

ناشر: دانشگاه فردوسی مشهد **چاپ:** چاپخانه دانشگاه فردوسی مشهد

نشانی: مشهد- کد پستی ۹۱۷۷۵ صندوق پستی ۱۱۶۳، دانشگاه فردوسی مشهد، دانشکده کشاورزی - گروه علوم وصنایع غذایی- دفتر نشریه پژوهشهای علوم و صنایع غذایی ایران. تلفن: ۲۰-۸۷۹۵۶۱۸ داخلی ۳۲۱ نمابر: ۸۷۸۷۴۳۰

این نشریه درپایگاههای زیر نمایه شده است:

پایگاه استنادی علوم ایران (ISC)، پایگاه اطلاعات علمی جهاد دانشگاهی (SID)، بانک اطلاعات نشریات کشور (MAGIRAN)

پست الکترونیکی: ifstrj@um.ac.ir

نشریه علمی

پژوهشهای علوم و صنایع غذایی ایران

جلد ۱۹ شماره ۳

سال ۱۴۰۲

شماره پیاپی ۸۱

شاپا: ۴۱۶۱-۱۷۳۵

عنوان مقالات

نشریه علمی پژوهشهای علوم و صنایع غذایی ایران جلد ۱۹ شماره ۳ سال ۱۴۰۲ 2023 No.3 Vol.19 Iranian Food Science and Technology Research Journal