Ferdowsi University of Mashhad

Iranian Food Science and Technology Research Journal

Iranian food science and technology Association

Vol.18 No.3

2022

ISSN:1735-4161

نشریه علمی پژوهشهای علوم و صنایع غذایی ایران

جلد ۱۸ شماره ۳ سال ۱۴۰۱ 2022 No.3 Vol.18 Iranian Food Science and Technology Research Journal

Contents

Iranian Food Science and Technology Research Journal

Vol. 18 No. 3 2022

Published by: Ferdowsi University of Mashhad

Executive Manager: Shahnoushi, N.

Editor-in-Chief: Tabatabaei yazdi, F

Editorial Board:

Mortazavi, Seyed A.	Prof. in Food Microbiology and
Shahidi, F.	Prof. in Food Microbiology
Habibi najafi, M.	Prof. in Food Microbiology
Razavi, Seyed M. A.	Prof. in Food Engineering
Kashaninejad, M.	Prof. in Food Engineering
Khomeiri, M.	Assoc. Prof. in Food Microbiology
Farhoosh, R.	Prof. in Food Chemistry
Fazli Bazzaz, S.	Prof. in Food Microbiology
Koocheki, A.	Prof. in Food Technology
Mohebbi, M.	Prof. in Food Engineering
Ghanbarzadeh, B.	Prof. in Food Engineering
Alemzadeh, I.	Prof. in Food Biotechnology
Rajabzadeh, GH.	Assoc. Prof. in Nanotechnology
Heydarpour, M.	Assoc. Prof. in Food Microbiology
Ghoddusi, H. B.	Assoc. Prof. in Food Microbiology
Khosravidarani, K.	Prof. in Food Biotechnology
Abbaszadegan, M.	Prof. in Food Microbiology
Mohammadifar, M. A.	Assoc. Prof. in Food Engineering
Vosoughi, M.	Prof. in Food Biotechnology
Almasi, H.	Assoc. Prof. in Nanotechnology
Fathi, M.	Assoc. Prof. in Nanotechnology
Abbasi, S.	Prof. in Food Engineering
Borges, N.	Prof. in Food Biotechnology

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. 18, No. 3, Aug. Sep. 2022, p. 1-14

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد ۱۸، شماره ۳، مرداد - شهریور ۱۴۰۱، ص. ۱۴-۱

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

Full Research Paper

Effects of various coatings and packing materials on persimmon fruit color indexes during quasi-static loading

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

Received: 2021.10.07
Accepted: 2021.12.11

How to cite this article:
Jafarzadeh, S., Azadbakht, M., Varasteh, F., Vahedi Torshizi, M. (2022). Effects of various coatings and packing materials on persimmon fruit color indexes during quasi-static loading. *Iranian Food Science and Technology Research Journal*. 18 (3), 1-14.

Abstract

Nowadays, the quality of processed fruits or products is defined by a set of physical and chemical properties. In this study, due to the sensitivity of persimmon fruit to pressure, the parameters affecting the color changes of this fruit after pressure have been investigated. Three different coatings and packing materials and two loading were applied to study the color changes of the samples. Samples were stored in the refrigerator for 25 days. According to the results obtained for the value of L*, b*, Chroma index, Hue index and color changes, the use of 1 mM polyamine coating had a significant effect and caused less change than other coatings. Foil container packaging with polyolefin film has also been better packaged. The lowest percentage reduction for L*, a*, b* , Chroma index and Hue index values was obtained in the 1 mM polyamine with a value of 8.26%, -26.43%, 12.35%, 1.31% and 120.995% respectively, Also the highest value was obtained in the uncoated state with a value of 18.49%, 73.32%, 19.84%, 15.95%, 152.36%. Finally, polyamine coating treatment has a positive effect to prevent the percentage reduction of color parameters of samples. The best coating treatment was polyamine with a concentration of 1 mM.

Keywords: Persimmon fruit, Color changes, Putrescine, Foam containers, Loading.

Introduction

About the appearance of persimmon fruits, it can be said that their color varies from orange and light yellow to dark orange and red and their diameter, depending on, their variety is between 2 to 8 cm. Usually the flower bowl remains with the fruit after picking. The shape of the persimmon fruit, based on its variety, may be spherical or oak-like (Jing et al., 2013). There are more than 400 varieties of persimmon fruits in various colors and shapes. Due to the fact that there are many varieties of this fruit, it is divided into two categories: astringent and non-astringent (Telis et al., 2002). On the other hand, the quality of fruits or processed fruit products is defined by a set of physical and chemical properties and makes its use more attractive to the consumer, such as size, weight, shape, color, etc. Therefore, it is necessary to pay more attention to the quality of the fruits. Fresh fruits and vegetables that are mechanically damaged during harvesting, transportation, sorting, grading, and packaging need to be investigated. There are also some bruises on fruits that the size of the bruises depends on several factors such as maturity,

1, 2 and 4. MSc Student, Associate Professor and Former MSc Student, Department of Bio-System Mechanical Engineering, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

3. Assistant Professor of Department of Horticulture, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.
(Corresponding Author Email: azadbakht@gau.ac.ir)
DOI: 10.22067/IFSTRJ.2021.72937.1098

harvest date, temperature, irrigation, and climatic conditions (Stropek and Gołacki, 2015). Due to mechanical damage and the transportation of products and fruits in recent years, there has been a great interest in developing active food packaging. Active packaging can improve food safety and maintain food quality by controlling the environment in the packaging (Wang et al., 2015). Various researchers have done research on the effect of packaging and coating on color changes in agricultural products. Wijewardane et al. investigated the effect of pre-cooling, fruit coating and packaging on post-harvest quality of apple fruit. Their results showed that a concentration of 1.5 to 2% of neem oil as a surface coating along with pre-cooling, in addition to maintaining better physiological properties, significantly reduced the pathogenic microorganisms level on the fruit (Wijewardane and Guleria, 2013). Hazrati et al. evaluated the aloe vera gel as an alternative edible coating for peach fruits during cold storage period. The result showed that the amount of weight loss, color change, total soluble solids (TSS) and titratable acidity (TA) in coated fruit was lower than control (Hazrati et al., 2007). Abebe et al. studied the effects of edible coating materials and stage of maturity at harvest time on storage life and quality of tomato (*lycopersicon esculentum* mill.) fruits. They reported that color is a very important indicator of ripening and determinant of quality and consumer acceptability. The total color difference (ΔE) extensively used to determine ripening due to chlorophyll degradation and formation of lycopene, also coated fruits showed significant delay on change of color as compared to uncoated ones (Abebe et al., 2017).

One of the most important problems of persimmon fruit is its maintenance and sensitivity to compressive strength. It may suffer internal damage during transportation and storage, and its storage life and quality properties may be reduced. Therefore, the aim of this study was to investigate the effect of compressive loading forces and also the type of packaging and coating to increase the durability of this product, in order to provide suitable conditions which increase the quality of persimmon fruit after natural damage and keep it in the best possible condition.

Materials and methods

Sample preparation

Persimmon fruits were obtained from a garden in the Hashemabad region near Gorgan city, Golestan province, Iran. All the required persimmon fruits were then brought to the laboratory of the Department of bio-system mechanical engineering, Gorgan, Iran. At this stage, the persimmon fruits that were exposed to external damage were separated. After separating the flawless persimmon fruits in appearance and cleaning them with a damp cloth, all persimmon fruits were categorized in terms of dimensions so that by equalizing the persimmon fruits in terms of dimensions and weight, the error rate of the experiment could be reduced. The persimmon fruits, which were very large and very small, were removed from the samples to be examined. After sorting the persimmon fruits, they were all covered, and then the persimmon fruits covered at two levels of 150 and 250 N were placed under the load. They were then packed in foam containers with polyolefin film, polyethylene terephthalate, and an ordinary box and stored for 25 days. Then, the color parameters of persimmon fruit including L*, a* and b*, browning index, Chroma index, Hue index and color change index were examined as a percentage of changes in the pre- and post-storage stage as a dependent factor. The persimmon fruit moisture content was 75.21% wb.

Coating

Four types of coating were used in this study, first and second coatings were Polyamine putrescine in different concentrations and third coating was distilled water, then the fruit without any coating that called zero coating was used as control. For the first type of coating, 1 ml of putrescine was used and in the second

type of coating, 2 ml of putrescine was used and the third type of coating was distilled water. Also, for better study, the samples of persimmon fruits that were uncoated were considered as control. All persimmon fruits were immersed for 10 minutes and then placed on a flat surface in a laboratory at 20°C to drying. At each stage, 8 persimmon fruits were placed in buckets to achieve the best quality in terms of immersion.

Static loading

Samples of coated persimmon fruits were loaded using the Instron device in two load values of 150 and 250 N. Two circular plates were used for the compression test and the test was performed at a speed of 10 mm per minute. To reduce the error, all loadings were done in one direction for all persimmon fruits. Figure 1-d shows the placement of the samples (Vahedi Torshizi and Azadbakht, 2020).

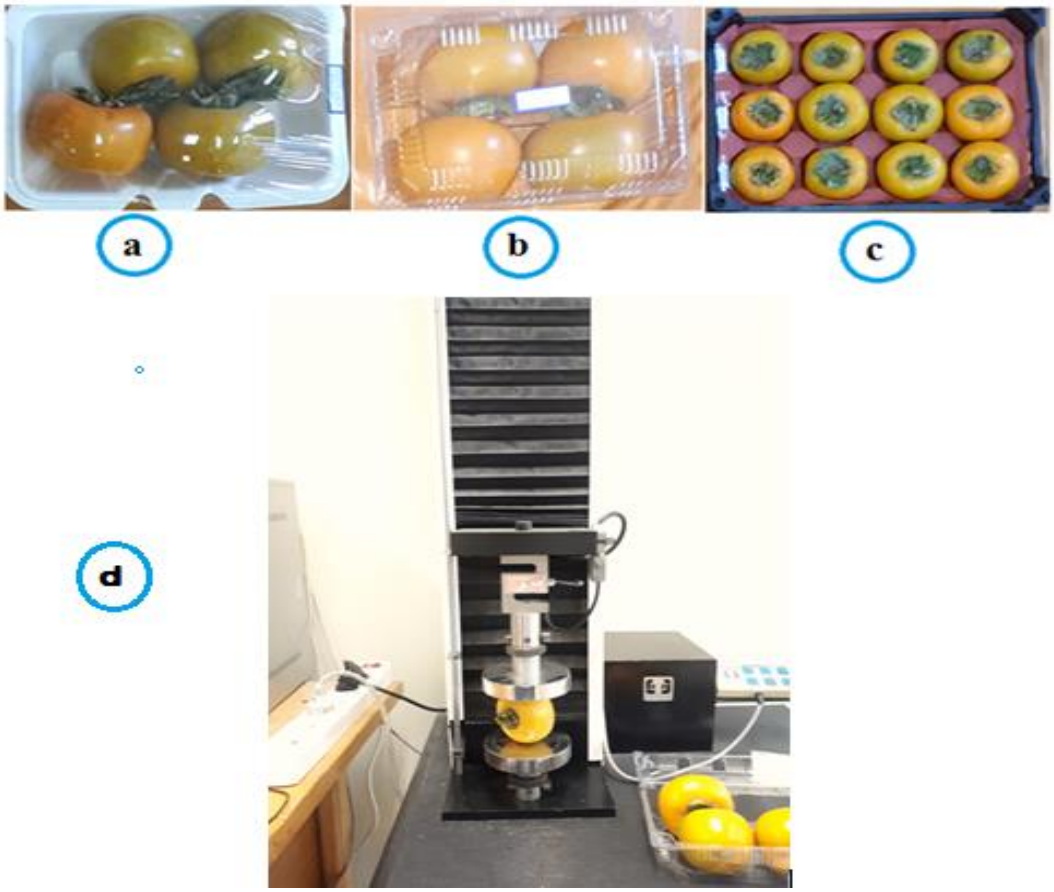


Fig. 1. Types of packaging used a) Foil container packs with polyolefin film, b) Polyethylene terephthalate, c) Ordinary box, d) Loading of persimmon fruits

Packaging and storage

After loading, the samples were packed using three foil container packs with polyolefin film (Fig.1.a), polyethylene terephthalate (Fig.1.b), and ordinary box (Fig.1.c). Four persimmon fruits were placed in 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.

Image processing

Images of all persimmon fruits were first taken after coating and before loading and storage, using Image J software, which is a powerful image processing software. To do this, images of each persimmon in the intended packaging were transferred to image J software.

Considering that 4 persimmon fruits were considered for each package, all four persimmon fruits were analyzed and an average of 4 persimmon were considered for L* a* b*. Also, in this study, all cases are expressed as a percentage so that the errors created can be reduced. In order to study the color indicators, the color space L* a* b* was used. In this space, the L* component indicates the brightness of the persimmon samples, which varies from 0 to 100. If the index goes to zero, the persimmon samples will be darker, and if it goes to 100, the persimmon sample will be lighter. The a* component is also composed of two colors, red and green, and is between 120 and 120+, with positive values indicating more redness and negative values indicating more green in the samples. The values of b* are the same as the components of a*, and the negative values indicate more blue and the positive values are equivalent to yellow.

Samples were photographed in a photo box using the Canon Ixus 132. All photos were saved in high quality as JPEG format. Then, the initial corrections were made to the images, and the images were converted to L* a* b* using the Image J software and the program under Image J, called Convert Color-Space, which is called the program add-on. In the analysis of color values, the values of L, a, and b were used and the reason for this was the independence of this analysis from the device and it covered a wider range than RGB and CMYK. First, pre-processing was performed to improve the images and eliminate unnecessary components in the image for all images. In image processing, the overall goal at this stage is to identify features of the image that can be used for their intended use. Images were converted from RGB color space to XYZ and then to L*, a* and b* using two steps. Using Equations 1, images can be converted from RGB color space to XYZ color space. Using equations 1 to 4, XYZ images can also be converted to L*, a* and b* values (Cheng et al., 2001).

$$\begin{bmatrix}\hat{X}\\ \hat{Y}\\ \hat{Z}\end{bmatrix} = \begin{pmatrix}0412456 & 0.257580 & 0.180423\\ 0212671 & 0.715160 & 0.072169\\ 0.019334 & 0.119194 & 0.950227\end{pmatrix}\begin{bmatrix}\hat{R}\\ \hat{G}\\ \hat{B}\end{bmatrix} \quad (1)$$

$$\hat{L} = \begin{bmatrix}116 \times \left(\frac{\hat{Y}}{Y'}\right)^{\frac{1}{3}} - 16\\ 903.3 \times \left(\frac{\hat{Y}}{Y'}\right) \text{ELSE}\end{bmatrix} \quad (2)$$

$$\hat{a} = 500 \times \left[\left(\frac{\hat{X}}{X'}\right)^{\frac{1}{3}} - \left(\frac{\hat{Y}}{Y'}\right)^{\frac{1}{3}}\right] \quad (3)$$

$$\hat{b} = 200 \times \left[\left(\frac{\hat{Z}}{Z'}\right)^{\frac{1}{3}} - \left(\frac{\hat{Y}}{Y'}\right)^{\frac{1}{3}}\right] \quad (4)$$

R^*= The amount of redness.
G^*= The amount of greenness.
B^*= The amount of blueness.
L*= Indicates the intensity of the light.
a^*= The position is between green and red.
b^*= The position is between blue and yellow.

There are a number of CIE spaces that can be created once the X> Z tristimulus coordinates are known. CIE ($L^*a^*b^*$) space and CIE ($L^*u^*v^*$) space are two typical examples. They can all be obtained through nonlinear transformations of X, Y, and Z values.

Where, frac Y/ Y_0> 0.01, X/ X_0> 0.01, and Z/ Z_0> 0.01. (X_0, Y_0, Z_0) are X, Y, Z values for the standard white.

Where the values of x ', Y' and Z 'are XYZ values for standard D65.

$$\begin{bmatrix}\hat{X}\\ \hat{Y}\\ \hat{Z}\end{bmatrix} = \begin{pmatrix}95.047\\ 100\\ 108.883\end{pmatrix} \quad (5)$$

Also, the browning index (BI) was obtained based on the color components and was calculated using Equations 6 and 7 (Moreno et al., 2016):

$$x = \frac{a* + 1.75 \times\times L*}{5.645L* + a* - 3.012b*} \quad (6)$$

$$BI = \frac{(100(x - 0.33))}{0.17} \quad (7)$$

The measurement of Chroma index and total color difference to describe color changes during persimmon storage under different conditions are shown in equations 8 and 9

(Abdelmotaleb et al., 2009; Montazer and Niakousari, 2012).

$$C = \sqrt{a^{*2} + b^{*2}} \quad (8)$$

$$TCD = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \quad (9)$$

TCD= Colour difference

Zero-sub-indexes are related to the values read from a fresh and non-processed persimmon sample.

The Hue Index is a food color indicator that represents 0 or 360 degrees, red, and 90, 180, and 270, respectively, indicating yellow, green, and blue.

$$HueAngel = \tan^{-1}(\frac{b}{a}) \quad (10)$$

Table 1- Analysis of loading, coating and packaging variance for persimmon fruit color parameters including L*, a* and b*, browning index, Chroma index, Hue index, color change index, and weight loss percentage.

	L*		a*		b*	
	Mean Square	**F value**	**Mean Square**	**F value**	**Mean Square**	**F value**
Loading	605.63	18**	808.61	1.11^{ns}	329.19	5.26*
Coating	415.106	12.37**	1500.36	2.05*	228.89	3.66*
Packing	315.53	9.38**	13199.57	0.07**	341.84	5.46*
Loading ×Coating	8.10	0.24^{ns}	138.75	0.19^{ns}	2.51	0.04^{ns}
Loading× Packing	75.107	3.20*	89.26	0.12^{ns}	2.18	0.31^{ns}
Coating× Packing	3.94	0.12^{ns}	1236.06	1.69^{ns}	14.85	0.24^{ns}
C.V.	14.90	33.64	35.97	730.61	19.92	62.57
	Browning index		**Chroma index**		**Hugh's Index**	
Loading	612.50	1.47^{ns}	525.20	9.96**	2267.66	4.69*
Coating	679.66	1.63^{ns}	188.04	3.57*	1989.87	4.12*
Packing	11.70	0.03^{ns}	1571.42	80.29**	12639.24	26.16**
Loading ×Coating	265.25	0.64^{ns}	7.53	0.14 ^{ns}	33.97	0.07^{ns}
Loading ×Packing	1611.99	1.04^{ns}	38.74	0.73 ^{ns}	30.91	0.06^{ns}
Coating× Packing	85.70	0.21^{ns}	11.25	0.21 ^{ns}	809.24	1.67^{ns}
C.V.	27.44	416.95	10.81	52.73	16.11	483.46
	Total color change index					
Loading	91.83	3.01^{ns}				
Coating	100.06	3.28*				
Packing	2.004	0.07^{ns}				
Loading ×Coating	2.42	0.08^{ns}				
Loading ×Packing	34.84	1.14^{ns}				
Coating× Packing	13.41	0.44^{ns}				
C.V.	29.82	30.54				

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

Statistical analysis

Independent parameters in this study include loading force at 2 levels of 150 and 250 N, three types of foam container packaging with polyolefin film, polyethylene terephthalate and ordinary box and four types of polyamine coating with concentrations of 1 and 2 mM, distilled water and without coating. The color parameters of persimmon fruit, including L*, a* and b*, browning index, Chroma index, Hue

index and color change index, were examined as a percentage of changes in the pre- and post-storage period as a dependent factor. All experiments were performed in three replications, and the results were analyzed using a factorial experiment and in a completely randomized design using SAS statistical software.

Results and discussion

In Table 1, the results of variance analysis of loading, coating and packaging for persimmon fruit color parameters including L*, a* and b*, browning index, Chroma index, Hue index, color change index, and weight loss percentage are shown.

Comparison of the average percentage of decrease in the amount of L *

Figure 2-A shows a comparison of the average percentage of L*. According to the figure, it is observed that there is no difference between distilled and uncoated water and both the concentrations of polyamine are significantly different from each other and compared to the other two coatings. The use of polyolefin coating has reduced the amount of L* value.

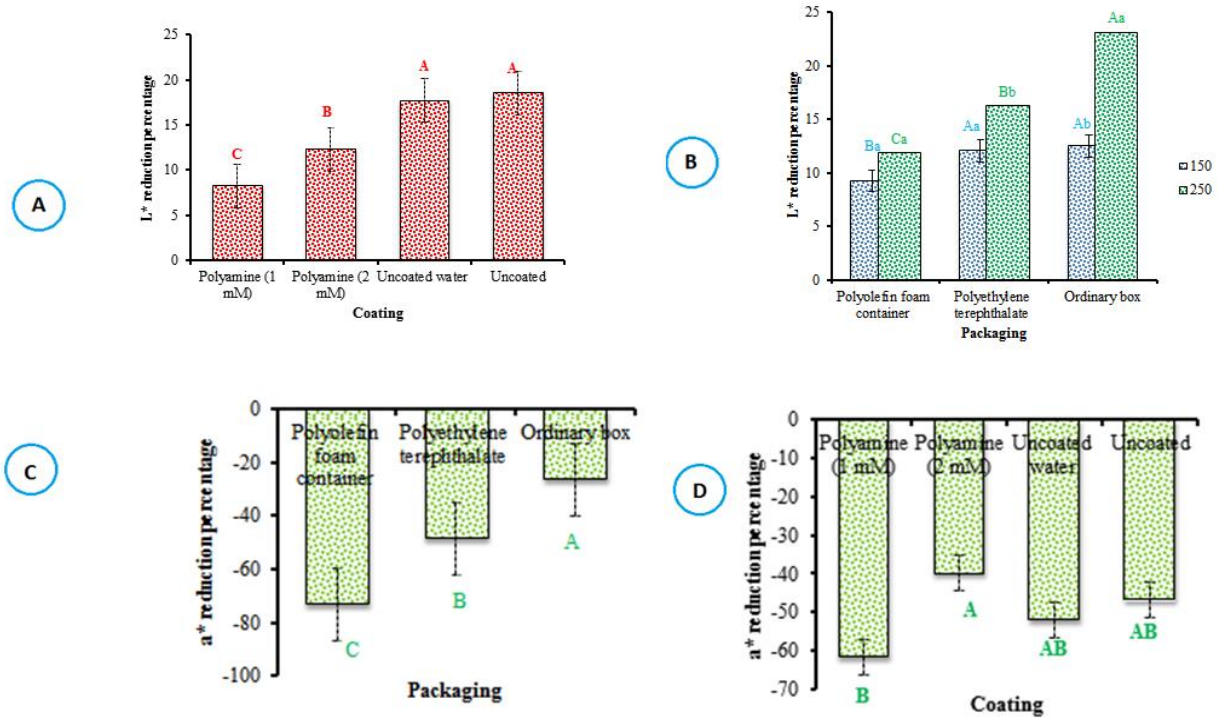


Fig. 2.Comparing the average for A) amount L* in coating, B) Effect of loading and packing on the amount of L *, C) Amount a* in packing, D) Amount a* in coating,

The same letters indicate no significant difference.

Large similar letters indicate a significant difference between different packing levels and small similar letters indicate a significant difference between different levels of loading force.

The lowest p reduction ercentage in L* value was obtained in the 1 mM polyamine with a value of 8.26% and the highest value was obtained in the uncoated sample with a value of 18.49%. Due to the stabilization of the membrane, polyamines can maintain the appearance of product and delay their aging during storage. As fruits get closer to aging, they increase the amount of wrinkles and weight loss. Polyamines, on the other hand, are known as anti-aging compounds and have the ability to maintain the integrity of the membrane, so it can be said that fruits treated with polyamines will have less wrinkles and weight loss. Ultimately, this will keep the product looking good and shiny. The low

percentage of shrinkage index reduction with the application of coating on the fruit surface in this study is consistent with the results of other study on strawberries (Asghari et al., 2009; Hernandez-Munoz et al., 2008) .

The interaction effect of loading and packaging on the percentage reduction of L* value

Figure 2-B shows the results of the interaction of packing materials and loading force on the percentage reduction of L* value. Using the results at 150 N, there was no significant difference between the ordinary box and the polyethylene terephthalate but the polyolefin foam container was significantly different from the other two packages. For the loading force of 250 N, there was a significant difference between all three packages. Also in the ordinary box and polyethylene terephthalate, there was a significant difference between 150 and 250 N, because the more loading force created more damage into fruit texture and this is the reason for the significant difference between the amount of L* in 150 and 250 N. No significant difference was observed for polyolefin film. These results are due to the fact that foam containers, with polyolefin and polyethylene terephthalate films, inhibit the activity of decomposing enzymes and are good barriers to prevent the dehydration of the samples. As a result, due to the lower dehydration in the samples in the mentioned packages, the low level of L* value or fruit brightness was also found. This result is similar to the other results (Hernandez-Munoz et al., 2008; Serrano et al., 2005). Other researchers, by adding different coatings of thymol, menthol and eugenol in the packaging of cherry fruit, delayed the change in skin color and fruit tail relative to the control samples and obtained similar results (Serrano et al., 2005).

Comparing the average percentage of decrease in the amount of a*

Figure 3-C shows the average comparison for a* coating and packaging. According to Figure 3-C, it can be seen that the value of a* has increased compared to the value of a* on the first day of the samples, and foam containers with polyolefin film have shown the highest decrease value for the value of a*. There is a significant difference between all three types of packaging used.

For coating, the highest percentage increase of a* was 1 mM, and no significant difference was found for coating between distilled and uncoated. But 1 mL of coating was significantly different from other coatings. The lowest percentage increase of a* was obtained in foam packaging with polyolefin film with a value of -26.43% and in polyamine coating of 1 mM with a value of -39.93%. Negativity indicates an increase in the amount of a* compared to the first day, and the highest value is 73.32% and 61.62%, respectively, in foam packaging with polyolefin film and 1 mM polyamine concentration. The reason for this observation is that by increasing storage time the pH of persimmon specimens increases and thus destroys the anthocyanin pigments responsible for the red dyes. This reduces the color of the fruit. Increasing the concentration of the coating also prevents respiration and maintains the levels of organic acids and reduces the pH. Therefore, lowering the pH will prevent the reduction of a*. Also, the use of polyamine has reduced the aging period, and therefore the use of this coating with higher concentration has caused less color changes. This is similar to the results of Jiang and Li (2001).

Comparison of average percentage reduction of b* value

The results of the average value of b* for packages, coatings and loading forces are shown in Figure 3. According to Figure 3-a, it can be said that the use of ordinary boxes has increased the amount of b* of persimmon fruits, and of course, there was no significant difference between the packaging of polyethylene terephthalate compared to the other two boxes. However, the foam container covered with polyolefin film makes a significant difference in the b* value compared to the ordinary box. Figure 3-b, which is to

compare the average effect of the load force, shows that the increase in load force has made a significant difference for the value of b* and a significant increase for this factor was obtained with increasing load force. Figure 3-c also shows that the use of polyamine coating causes significant changes in the amount of b* compared to other coatings and the use of polyamine coating with a concentration of 1 mM has caused less changes in the amount of b* during storage. There was also no statistically significant difference between distilled water coating and non-coated treatments. The lowest percentage of b* reduction was obtained in foam packaging covered with polyolefin film with 12.35% and in loading force of 150 N with 13.7% and in polyamine coating of 1 mM with 11.23%. The highest values were 19.84%, 17.98 and 19.86, respectively, in the ordinary box, 250 N and uncoated. The reason for these results is that after harvest time and during storage, the color of the fruit changes. An increase in the percentage of reduction the amount of b* in the samples packed in ordinary box can also be due to further dehydration in the samples in this type of packaging due to the lack of inhibitory activity of hydrolyzing enzymes. These results are similar to the results of Hernandez-Munoz et al. (2008). In similar experiments, the addition of a coating to the cherry fruit delayed the discoloration of the skin and tail of the fruit compared to the control samples (Hernandez-Munoz et al., 2008; Serrano et al., 2005).

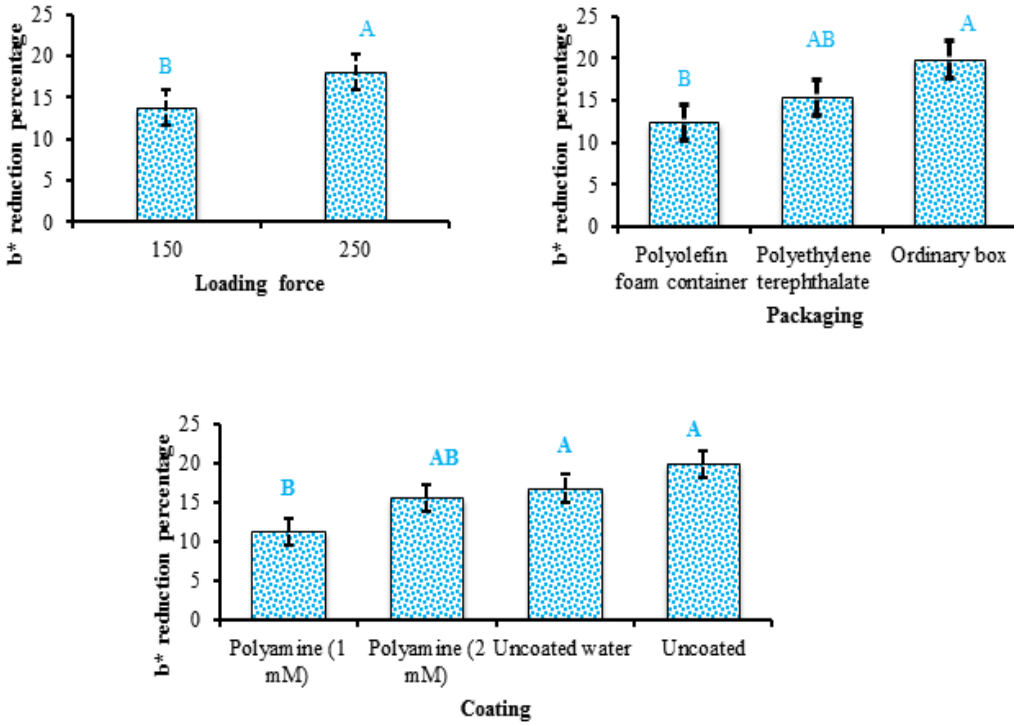


Fig. 3. Average comparison on b *, a) Packing, b) Loading, c) Coating.
The same letters indicate no significant difference.

Comparison of the average percentage reduction of Chroma index

The results of the mean comparison for the Chroma index for loading force, packaging and coating are shown in Figure 4. According to the results, for the packaging factor, the use of ordinary boxes increases the Chroma index, and there was no significant difference between the packaging of foam containers coated with polyolefin film and polyethylene terephthalate. Figure 4-B shows that an increase in the loading force increases the b* reduction percentage and a significant difference was observed between the loading forces. For coatings, only the use of

1 mM coating has caused a significant difference between the percentage reduction of Chroma index for persimmon fruits and the other three coatings were not significantly different. The lowest percentage reduction of Chroma index was obtained in foam packaging covered with polyolefin film with a value of 1.31% and in loading force of 150 N with a value of 4.22% and in polyamine coating of 1 mM with a value of 2.34%. The highest values were 15.95%, 9.62 and 9.93, respectively, in the normal box, 250 N, and uncoated. The reason for these observations is that the color of the fruit changes over time after harvest and during the storage period. The fruits are darkened, the brightness and clarity of the color of the surface of the fruit decreases. Eventually browning of the fruit surface occurs. The reason for the low percentage of Chroma index reduction in vegetation samples can be due to the prevention of fruit dehydration. Dehydration causes browning of the fruit. In a similar study, the addition of thymol, menthol, and eugenol coatings in cherry delayed skin and fruit color variability compared to the control (Hernandez-Munoz et al., 2008; Serrano et al., 2005). The increase in this index is consistent with the results of research performed on strawberries by applying the coating on the fruit surface (Lester, 2000; Asghari et al., 2009).

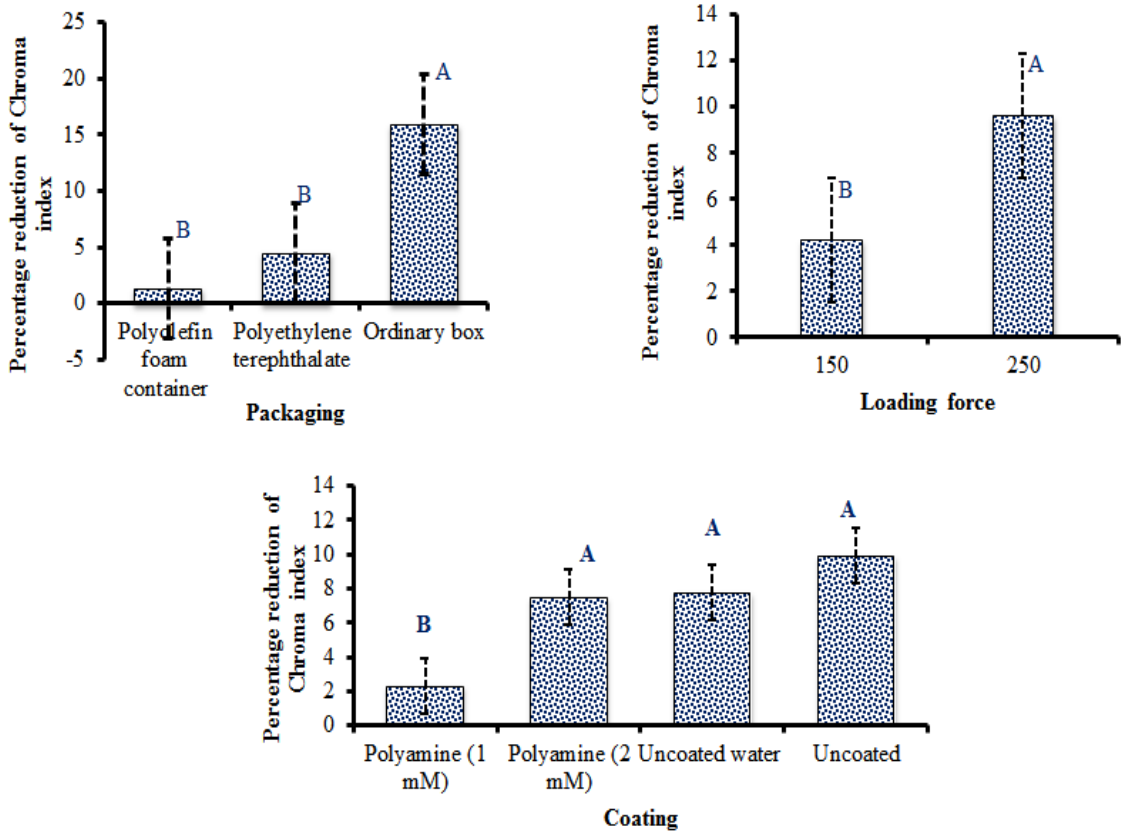


Fig. 4. Comparison of average on Chroma index value, a) Packaging, b) Loading, c) Coating.
The same letters indicate no significant difference.

Comparison of average percentage decrease of Hue index

Figure 5 shows a comparison of the average percentage of reduction in the Hue index. Section A shows that the foam packages covered with polyolefin and polyethylene terephthalate did not differ significantly. However, the ordinary box with these two types of packaging has shown a significant difference and a significant increase for the percentage reduction of the Hue index. In Figure 5-B, the increase in load force also causes a significant increase in the percentage reduction of the Hue

index, and a significant difference was obtained between the two loading forces. It can be seen in Figure 5-C, that the use of 1 mM polyamine treatment has caused a significant difference compared to other coatings for the percentage reduction of Hue index and no significant difference was observed between other coatings. Minimum percentage reduction of Hue index was obtained in foam packaging covered with polyolefin film with 120.995% and in the loading force of 150 N with a value of 130.8% and in the polyamine coating of 1 mM with a value of 120.84%. The highest values were obtained in 144.03, 152.36 and 143.33%, respectively, in a normal box, 250 N and uncoated. The reason for these observations is that coating polyamines on the fruit surface reduces the hydrolytic activity of thylakoid membrane enzymes, thus delaying the decomposition of chlorophyll and the production of carotenoids, as well as reducing discoloration. The color changes and the amount of Hue index in the skin of the fruit during storage were also reduced. Many researchers have also cited this phenomenon as evidence for their laboratory observations (Lester, 2000; Malik and Zora, 2005; Martinez-Romero et al., 2002). In a similar study, Valero et al. (2013) investigated the effect of edible coatings on maintaining fruit quality in four types of plums during storage after harvest and they concluded that discoloration was delayed by using both edible coatings (Valero et al., 2013).

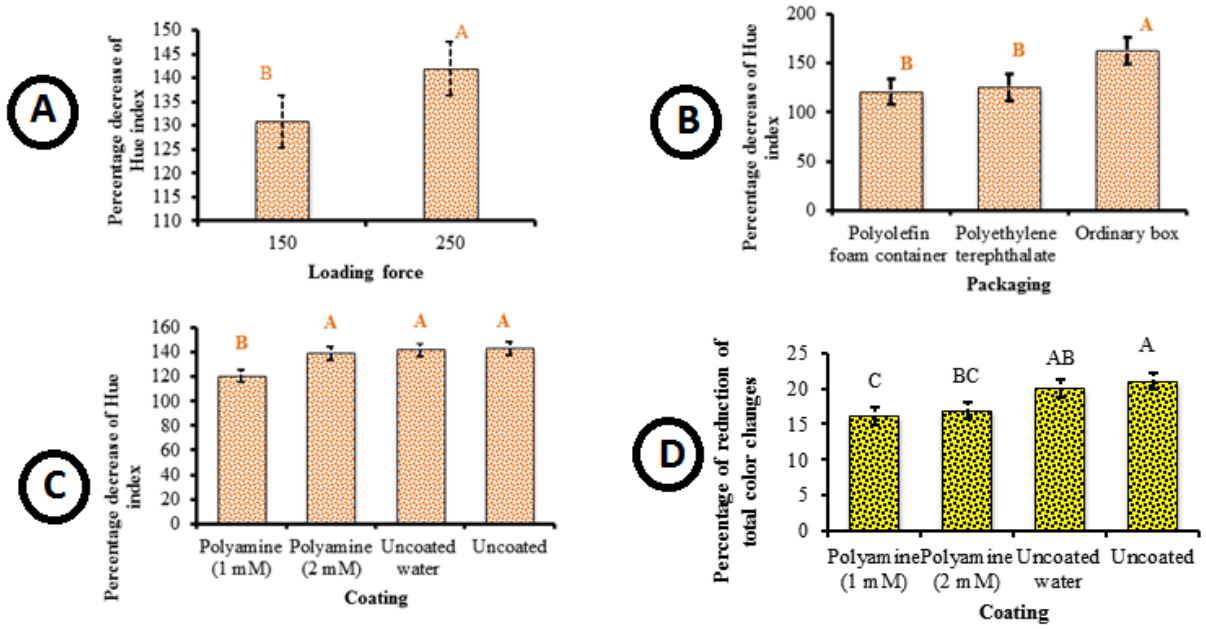


Fig. 5. Comparison of average on Hue index and total color changes rate, a) Loading, b) Packing, c) Coating, d) Coating.

The same letters indicate no significant difference.

Comparing the average percentage of reduction of total color changes

Figure 5-D shows an average comparison for the percentage reduction in the color of the whole persimmon fruit. According to Figure 5-D, the use of polyamine has caused the percentage reduction in color changes to be significantly different from that of distilled and uncoated samples. The lowest amount was in the 1 mM polyamine coating with a value of 16.33% and the highest amount of color changes was for the un-coated treatment with a value of 21.029%. One of the indicators of fruit ripening is the change in skin color. The color

of a fruit is a very important factor in evaluating its quality. The reason for this result is that increasing the amount of CO_2 in the fruit and also reducing the production of ethylene due to coating reduced the rate of respiration and discoloration of the fruit. In similar experiments, delays in discoloration due to the use of coatings have demonstrated in many fruits, including mangoes (Valero et al., 2013), guava (Hong et al., 2012), and plums (Liu et al., 2014). Researchers in a similar study reported that increasing the amount of CO_2 in the fruit, as well as reducing ethylene by coating the fruit with chitosan, reduced respiration and discoloration of the fruit (Martínez- Romero et al., 2006).

Conclusion

Based on the results obtained in general, it can be stated that the use of foam packaging covered with polyolefin film has caused the percentage reduction of L*, b* parameters, Chroma index, and Hue index to be less than other packages. Also, after this packaging, polyethylene terephthalate had the best values. The loading factor for the parameters also showed that when the load force increases, the amount of change of all parameters will be higher than the samples with less pressure. The lowest percentage reduction for L*, a*, b*, Chroma index and Hue index values were obtained in the 1 mM polyamine with a value of 8.26%, -26.43%, 12.35%, 1.31% and 120.995%, respectively, Also the highest value was obtained in the uncoated tratment with a value of 18.49%, 73.32%, 19.84%, 15.95%, 152.36%. Finally, polyamine coating treatment has a positive effect to prevent the percentage reduction of color parameters of samples. The best coating treatment was polyamine with a concentration of 1 mM.

Acknowledgments

We are grateful to the Gorgan University of Agricultural Sciences and Natural Resources for their support.

References

1. Abdelmotaleb, A., El-Kholy, M.M., Abou-El-Hana, N. H., Younis, M. A. (2009). Thin layer drying of garlic slices using convection and combined (convection- infrared) heating modes. Misr J*ournal of Agricultural Engineering*, 26, 251–281. DOI:10.21608/MJAE.2009.109883
2. Abebe, Z., Tola, Y. B. and Mohammed, A. (2017). Effects of edible coating materials and stages of maturity at harvest on storage life and quality of tomato (*Lycopersicon Esculentum Mill*.) fruits. *African journal of agricultural research*, 12(8), pp.550- 565.
3. Asghari, M. A. A. F., Mostoufi, Y., Shoeybi, S. H., Fatahi, M. (2009). Effect of cumin essential oil on postharvest decay and some quality factors of strawberry.
4. Cheng, H. D., Jiang, X. H., Sun, Y., Wang, J. L., (2001). Color Image Segmentation : Advances & Prospects 1 Introduction. Pattern Recognition, 34(12), 2259-2281. https://doi.org/10.1016/S0031-3203(00)00149-7
5. Hazrati, S., Kashkooli, A.B., Habibzadeh, F., Tahmasebi- Sarvestani, Z. and Sadeghi, A. R. (2017). Evaluation of Aloe vera gel as an alternative edible coating for peach fruits during cold storage period. Gesunde Pflanzen, 69(3), pp.131-137. https://doi.org/10.1016/S0031-3203(00)00149-7
6. Hernandez- Munoz, P., Almenar, E., Del Valle, V., Velez, D., Gavara, R. (2008). Effect of chitosan coating combined with postharvest calcium treatment on strawberry (Fragaria× ananassa) quality during refrigerated storage. Food Chemistry 110, 428–435. https://doi.org/10.1016/j.foodchem.2008.02.020
7. Hong, K., Xie, J., Zhang, L., Sun, D., Gong, D. (2012). Effects of chitosan coating on postharvest life and quality of guava (*Psidium guajava* L.) fruit during cold storage. Scientia Horticulturae 144, 172–178. https://doi.org/10.1016/j.scienta.2012.07.002
8. Jiang, Y., Li, Y. (2001). Effects of chitosan coating on postharvest life and quality of longan fruit.

Food Chemistry 73, 139–143. https://doi.org/10.1016/S0308-8146(00)00246-6
9. Jing, Z., Ruan, X., Wang, R., Yang, Y.(2013). Genetic diversity and relationships between and within persimmon (*Diospyros* L.) wild species and cultivated varieties by SRAP markers. Plant Systematics and Evolution 299, 1485–1492. https://doi.org/10.1007/s00606-013-0810-1
10. Lester,G.E.(2000). Polyamines and their cellular anti-senescence properties in honey dew muskmelon fruit. Plant Science 160, 105–112. https://doi.org/10.1016/S0168-9452(00)00369-1
11. Liu, K., Yuan, C., Chen, Y., Li, H., Liu, J. (2014). Combined effects of ascorbic acid and chitosan on the quality maintenance and shelf life of plums. *Scientia Horticulturae* 176, 45–53. https://doi.org/10.1016/j.scienta.2014.06.027
12. Malik, A. U., Zora, S. (2005). Pre-storage application of polyamines improves shelf-life and fruit quality of mango. *The Journal of Horticultural Science and Biotechnology*, 80, 363–369. https://doi.org/10.1080/14620316.2005.11511945
13. Martínez Romero, D., Alburquerque, N., Valverde, J. M., Guillén, F., Castillo, S., Valero, D., Serrano, M. (2006). Postharvest sweet cherry quality and safety maintenance by Aloe vera treatment: a new edible coating. *Postharvest Biology and Technology*, 39, 93–100. https://doi.org/10.1016/j.postharvbio.2005.09.006
14. Martinez- Romero, D., Serrano, M., Carbonell, A., Burgos, L., Riquelme, F., Valero, D. (2002). Effects of postharvest putrescine treatment on extending shelf life and reducing mechanical damage in apricot. *Journal of Food Science*, 67, 1706–1712. https://doi.org/10.1111/j.1365-2621.2002.tb08710.x
15. Montazer, Z., Niakousari, M. (2012). Evaluation of color change of sour orange juice (from different stages of processing line) during storage, 109– 121.
16. Moreno, J., Espinoza, C., Simpson, R., Petzold, G., Nuñez, H., Gianelli, M. P. (2016). Application of ohmic heating/ vacuum impregnation treatments and air drying to develop an apple snack enriched in folic acid. *Innovative Food Science and Emerging Technologies*, 33, 381–386. https://doi.org/10.1016/j.ifset.2015.12.014
17. Serrano, M., Martinez- Romero, D., Castillo, S., Guillén, F., Valero, D. (2005). The use of natural antifungal compounds improves the beneficial effect of MAP in sweet cherry storage. *Innovative food science & emerging technologies*, 6, 115–123. https://doi.org/10.1016/j.ifset.2004.09.001
18. Stropek, Z., Gołacki, K. (2015). A new method for measuring impact related bruises in fruits. *Postharvest Biology and Technology* 110, 131– 139. https://doi.org/10.1016/j.postharvbio.2015.07.005
19. Telis, V. R., Gabas, A., Menegalli, F., Telis- Romero, J. (2002). Water sorption thermodynamic properties applied to persimmon skin and pulp. *Thermochimica Acta*, 343, 49–56. https://doi.org/10.1016/s0040-6031(99)00379-2
20. 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, 113–132. https://doi.org/10.22067/ifstrj.v15i6.75049
21. Valero, D., Díaz- Mula, H. M., Zapata, P. J., Guillén, F., Martínez- Romero, D., Castillo, S., Serrano, M., (2013). Effects of alginate edible coating on preserving fruit quality in four plum cultivars during postharvest storage. *Postharvest Biology and Technology* 77, 1–6. https://doi.org/10.1016/j.postharvbio.2012.10.011
22. Wang, Q., Tian, F., Feng, Z., Fan, X., Pan, Z., Zhou, J. (2015). Antioxidant activity and physicochemical properties of chitosan films incorporated with *Lycium barbarum* fruit extract for active food packaging. *International Journal of Food Science & Technology*, 50, 458–464. https://doi.org/10.1111/ijfs.12623

23. Wijewardane, R. M. N. A., Guleria, S. P. S. (2013). Effect of pre- cooling, fruit coating and packaging on postharvest quality of apple. *Journal of Food Science and Technology* 50, 325–331. https://doi.org/10.1007/s13197-011-0322-3

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد ۱۸، شماره ۳، مرداد - شهریور ۱۴۰۱، ص. ۱۴-۱

Iranian Food Science and Technology Research Journal
Vol. 18, No. 3, Aug. Sep. 2022, p. 1-14

تاثیر پوشش و بسته‌بندی مختلف بر روی تغییرات شاخص رنگ میوه خرمالو در بارگذاری شبیه استاتیکی

سجاد جعفرزاده[۱]- محسن آزادبخت[۲*]- فریال وارسته[۳]- محمد واحدی ترشیزی[٤]

تاریخ دریافت: ۱۴۰۰/۰۷/۱۵
تاریخ پذیرش: ۱۴۰۰/۰۹/۲۰

چکیده

امروزه، کیفیت میوه‌ها و یا محصولات فراوری شده توسط مجموعه‌ای از خصوصیات فیزیکی و شیمیایی تعریف می‌شود. در این تحقیق، با توجه به حساسیت میوه خرمالو به فشار، به بررسی پارامترهای موثر بر تغییرات رنگی میوه پرداخته شده است. بر روی نمونه‌ها سه پوشش مختلف شامل پوترسین با غلظت ۱ میلی مولار، پوترسین با غلظت ۲ میلی‌مولار و آب مقطر بر روی آن‌ها قرار گرفت. سپس نمونه‌ها تحت نیروی بارگذاری ۱۵۰ و ۲۵۰ نیوتن قرار گرفته و در ظروف فومی با فیلم پلی‌اولفین، پلی‌اتیلن ترفتالات و جعبه معمولی بسته‌بندی شدند. نمونه‌ها به مدت ۲۵ روز در سردخانه انبار شدند. پس از اتمام دوره انبارمانی، خواص کیفی نمونه‌ها شامل مقادیر *L، *a، *b، شاخص قهوه‌ای شدن، شاخص کروما، شاخص هیو و شاخص تغییرات رنگ کل اندازه‌گیری شد. با توجه به نتایج به‌دست آمده برای مقدار *L، *b، شاخص کروما، شاخص هیو و تغییرات رنگ استفاده از پوشش ۱ میلی‌مولار پلی‌آمین تاثیر معنی‌داری داشته است و سبب تغییرات کمتر نسبت به پوشش‌های دیگر شده است. بسته‌بندی ظرف فومی با فیلم پلی‌اولفین نیز بهتر بسته‌بندی بوده است. کمترین درصد کاهش برای مقادیر *L، *a، *b، شاخص کروما و شاخص Hue در پلی‌آمین ۱ میلی‌مولار به‌ترتیب با مقادیر ۸/۲۶٪، ۲۶/۴۳-، ۱۲/۳۵، ۱/۳۱٪ و ۱۲۰/۹۹۵٪ به‌دست آمد. بیشترین مقدار در حالت بدون پوشش با مقادیر ۱۸/۴۹٪، ۷۳/۳۲٪، ۱۹/۸۴٪، ۱۵/۹۵٪، ۱۵۲/۳۶٪ به‌دست آمد. در نهایت تیمار پوشش پلی‌آمین تاثیر مثبتی در جلوگیری از درصد کاهش پارامترهای رنگی نمونه‌ها دارد. بهترین تیمار پوشش پلی‌آمین با غلظت ۱ میلی‌مولار بود.

واژه‌های کلیدی: خرمالو، تغییرات رنگ، پوشش‌دهی، بسته‌بندی، بارگذاری.

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

Iranian Food Science and Technology Research Journal
Vol. 18, No. 3, Aug. Sep. 2022, p. 15-29

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد ۱۸، شماره ۳، مرداد - شهریور ۱۴۰۱، ص. ۲۹-۱۵

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

Full Research Paper

Finding effective plasma process factors on yeast deactivation by numerical simulation and RSM

A. Ranjbar Nedamani*

Received: 2022.02.27
Accepted: 2022.04.30

How to cite this article:
Ranjbar Nedamani, A. (2022). Finding effective plasma process factors on yeast deactivation by numerical simulation and RSM. *Iranian Food Science and Technology Research Journal*. 18 (3), 15-29.

Abstract

In recent years, there has been an increasing interest in the application of plasma technology in food preservation technologies. Plasma is nonthermal physical processing that has a high potential in the field of food processing. In this study, a mathematical model was investigated for yeast deactivation during plasma treatment. The definitive screen design was used to investigate the factors that affect yeast deactivation by plasma. Four factors of voltage (A: 20- 30 kV), Vessel diameter (B: 40- 60 mm), process temperature (C: 20-40ºC), and type of plasma media (air or water) were selected. Then the treatment was simulated by COMSOL software. The responses of reaction kinetics coefficient, the ozone concentration, and final deactivation time were analyzed by definitive screen design expert to find the effective model parameters and process optimization. The results show that plasma treatment in water can have the strongest effect than air plasma. The changes in the number of microorganisms have a linear relationship with process time at different voltage-temperature conditions, but the ozone concentration dramatically changes at different combinations of voltage and temperature. The analyzed data show the k_{reac} is affected significantly by the diameter of the vessel and the 221 types of process media (water or air). The ozone concentration only depends on the type of plasma media and the final 223 process time significantly depends on vessel diameter and type of media. Also, in plasma treatment, media type had a significant effect on all 3 responses, while the effect of temperature was only on final process time. For example, at temperature 20ºC the ozone concentration decreased at the first time of treatment and then stay constant, but at 30ºC, the ozone production increased with treatment time. This study showed when an RSM design was applied for designing the experiment which considers different process factors, the results can significantly differ from the study on only one-factor. In plasma treatment, media type had a significant effect on all 3 responses, while the temperature shows its effect only on final process time. Thus it can be concluded that with proper selecting of plasma media, this technology can be used for deactivation of food microorganisms.

Keywords: Plasma treatment, Microorganism deactivation, CFD simulation, RSM design

Introduction

Increasing the shelf- life of raw food materials is an important aspect of food processing. Traditional technologies such as thermal processing can reduce the deterioration of food materials. However, they also have some disadvantages on nutritional, color, taste, and texture characteristics of food products. In

* Assistant Professor, Department of Biosystem Engineering, Faculty of Agricultural Engineering, Sari Agricultural Sciences and Natural Resources University, Sari, Mazandaran, Iran.

(Corresponding Author Email: a.ranjbar@sanru.ac.ir)
DOI: 10.22067/IFSTRJ.2022.75558.1152

recent years, there has been an increasing interest in the application of plasma technology in food preservation technologies. Plasma is nonthermal physical processing that has a high potential in the field of food processing (Xiang et al., 2019). Plasma is the fourth state of material and consists of gas molecules and charged particles. This technology is categorized into equilibrium and non-equilibrium plasma (Tabibian et al., 2020). In the recent decade, the atmospheric pressure of nonthermal plasma has gained significant attention for use in food processing (Perinban et al., 2019). The plasma technology has been used for inactivating the bacteria (Pankaj et al., 2018; Perinban et al., 2019; Xiang et al., 2018), viruses (Bourke et al., 2018; Guo et al., 2018), enzymes (Misra et al., 2016; Surowsky et al., 2013), removing the pesticide and antibiotic residues (Bourke et al., 2018; Chizoba Ekezie et al., 2017; Perinban et al., 2019), and altering the functional properties of food (Chizoba Ekezie et al., 2017; Muhammad et al., 2018) and packaging materials (Pankaj et al., 2014; Perinban et al., 2019). One of the most significant current research on plasma application in food preservation is the non-thermal effect on microorganisms' deactivation. So far, there has been very little research to simulate the effect of the parameters on the plasma deactivation process. Wang et al. (2020) simulate the gas-phase surface discharge plasma on the sterility of the water containing *Z. rouxii* LB (B- WHX- 12- 54). They found that yeast concentration slowing down its inactivation and the reactor diameter does not affect the inactivation process (Wang et al., 2020).

The advantage of using COMSOL simulation is that it is helpful to study more detailed process conditions (Chilka & Ranade, 2019). However, when we consider multiple process factors during simulations, the application of computational fluid dynamics (CFD) modeling leads to having a high number of runs that make them hard to analyze. Thus, using RSM (response surface methodology) techniques for reducing the CFD simulation can be helpful to minimize the CFD runs and data analysis time. When a certain response is dependent on several factors, RSM can be used as a collection of statistical and mathematical techniques that are used to improve, optimize and develop such processes (Sumic et al., 2016). The response surface methodology was used to determine factors affecting bacterial deactivation during plasma treatment through setting up a mathematical model. A key advantage of using the response surface methodology (RSM) is that it has the statistical ability to reduce the total treatments in a multi-independent factors study (Misra et al., 2013), it can be a suitable choice before numerical calculations by CFD methods to reduce the final runs behind considering all possible factors including a unit food process. The objective of this study was to investigate a mathematical model consisting of temperature, voltage, vessel diameter, and the type of plasma medium for yeast deactivation during plasma treatment.

Materials and methods

Microbial species

The strain *Z. rouxii* LB (B- WHX- 12- 54), was selected to investigate the deactivation effect of plasma treatment conditions. Data were obtained from Wang et al. (2020) to simulate the deactivation process.

The experimental design

At first, we studied the effect of temperature (20 and 30°C) and voltage (15 and 20kV) to validate the data obtained from Wang et al. (2020) for simulation. After validation and simulation set-up, the design was created by Design- Expert software v.11 to further simulation in COMSOL. The definitive screen design was used to investigate the factors that affect plasma deactivation. Four factors of voltage (A: 20- 30 kV), Vessel diameter (B: 40- 60 mm), the process temperature (C: 20- 40°C), and the type of plasma media (air or water) were selected. Table 1 shows 14 combinations of four factors that were simulated in COMSOL

for investigating the factors affecting the bacterial inactivation.

Table 1- Definitive screen design in response surface methodology

Run	Voltage (kV)	Diameter (mm)	Temperature (°C)	Media
1	30	50	20	air
2	20	40	40	air
3	25	50	30	water
4	30	40	40	water
5	30	60	20	water
6	20	50	40	water
7	30	40	30	air
8	30	60	40	air
9	20	60	20	air
10	20	40	20	water
11	20	60	30	water
12	25	60	40	air
13	25	40	20	water
14	25	50	30	air

Experimental setup

A cylinder reactor with 40 mm diameter and 300 mm length was selected. A quartz tube was used at the center of the reactor (d= 10 mm, h= 270). A magnetic field was established through two inner stainless electrodes (15 kV, 50 Hz) inside the quartz tube. The plasma media between the quartz tube and the reactor was selected as the ground electrode. The gas flow inside the tube had a rate of 0.003 m/s. since the gas phase can produce chemical components such as ozone (Wang et al., 2020), in this study the ozone was selected as an indicator for simulation. The high-energy electrons can conduct the reaction between oxygen radicals and oxygen. This leads to ozone generating which presents in most plasmas and can have a deactivating effect on microbial population of the food surfaces. Wang et al. proposed that voltage and reactor dimensions can affect ozone concentration (Wang et al., 2020). Thus these factors were selected for this study. Also, we find in our pretreatment test that the temperature and the media inside the reactor can have an effective role in ozone production. Thus these four factors were selected for investigating their effect on ozone production and microbial deactivation rate.

Model definition in COMSOL

The simulation in COMSOL multiphysics 5.3a software was run for a 2D geometry (Fig. 1) in four modules of bubbly flow k-ε, transport of diluted species (for water/ air as the media), transport of diluted species (for microbial removal from the studied media), and the magnetic field.

Fig. 1. The 2D geometry of reactor simulated in COMSOL

Bubbly flow k-ε

This module was used to simulate the ozone bubble rise in the reactor. The gas density is assumed negligible in comparison with the liquid density. The RANS k-ε equations were

solved for liquid and ozone bubbles. The density and diameter of ozone bubbles were set as 2.14 kg/m^3 and 3.21 mm, respectively. The ozone diffusion coefficient was 1.74× 10^{-9} m^2/s.

The l and g are subscribed related to the liquid and gas, respectively.

$$\emptyset_l\rho_l\frac{\partial u_l}{\partial t}+\emptyset_l\rho_l(u_l.\nabla)u_l=\nabla.[-pI+\emptyset_l(\mu_l+\mu_T)(\nabla u_l+(\nabla u_l)^T)]+\emptyset_l\rho_l g+F \quad (1)$$

$$\rho_1\nabla.(u_l)=0, \qquad u_l=u \quad (2)$$

$$\frac{\partial\emptyset_g\rho_g}{\partial t}+\nabla.N_{\rho_g\emptyset_g}=-m_{gl}, \qquad \emptyset_g\rho_g=rhogeff \quad (3)$$

$$N_{\rho_g\emptyset_g}=\emptyset_g\rho_g u_g, \qquad u_g=u_l+u_{slip}-\mu_T\frac{\nabla\emptyset_g}{\rho_l\emptyset_g} \quad (4)$$

The transport of diluted species

The deactivation of microbial cells had a dependency on ozone concentration. The reaction rate was calculated based on Fick's law as:

$$\frac{\partial c_i}{\partial t}+\nabla.(-D_i\nabla c_i)+u.\nabla c_i=R_i \quad (5)$$

$$N_i=-D_i\nabla c_i+uc_i \quad (6)$$

During simulation, the R_i was the reaction rate of ozone and microbial cells which defied as:

$$R_i=-k_{reac}c_{o_3} \quad (7)$$

Which the k_{reac} is the death rate constant. The death rate of a microorganism in a determined condition follows the first- order kinetics. Thus, we can show the death rate by Ibarz & Barbosa-Cánovas, (2002); Valentas, Rotstein, & Singh, 1997):

$$N=N_0\exp(-k_{reac}t) \quad (8)$$

When this equation is plotted in semilogarithmic coordinates, a straight line with –k as the slope is obtained which is called the thermal death curve (Ibarz & Barbosa-Cánovas, 2002).

Initial and boundary conditions

The outlet boundary was selected at the top of the reactor as a free surface and the motions on the surface were ignored. The ozone inlet boundary was at the bottom of the reactor. the ozone flux rate was as:

$$n.N_1=n.(uc_{0.j}) \quad (9)$$

The pressure point constraint was added on the outlet boundary (p= 0). The microorganism concentration in the reactor was set at 1.5× 10^4 CFU/ml. the initial temperature of plasma was set as the T based on the RSM design.

Problem-solving

The COMSOL multiphysics 5.3a was used to solve four modules based on turbulent RANS k-ε. The processor was a surface desktop Intel® Core™ i5-4300U, 2.50 GHz, RAM 4 GB, and Windows 10 64-bit operating system. The relative tolerance was 0.01. the data were recorded every 1 min. The inactivation was simulated for 20 min.

Simulation validation and statistics procedures

The simulated data for k_{reac} were used for validation with experimental data. After validation, the RSM design factors were set for each run in COMSOL and the deactivation time, the ozone concentration at the end of deactivation time, and the final k_{reac} at the deactivation time were recorded as the results. The response variables were fitted to a second-order polynomial model (Equation (10)) which is generally able to describe the relationship between the responses and the independent variables.

$$Y=\beta_0+\sum_{i=1}^{2}\beta_i X_i+\sum_{i=1}^{2}\beta_{ii}X_i^2+\sum_{i<i=1}^{2}\beta_{ij}X_{ij} \quad (10)$$

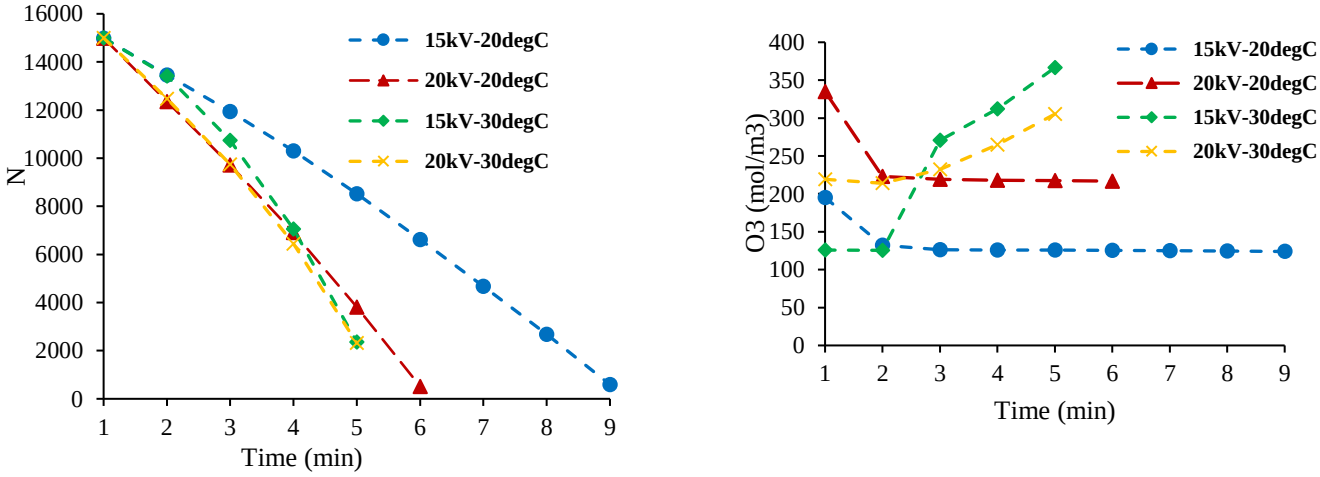
where Y is the response, X_i and X_j are the independent variables affecting the response,

and β_0, β_i, β_{ii}, and β_{ij}, are the regression coefficients for the intercept, linear, quadratic, and interaction terms, respectively. To evaluate model adequacy and determine regression coefficients and statistical significance, the analysis of variance (ANOVA) was used. The Design- Expert v.11 was used for RSM statistical analysis. The results were statistically tested at the significance level of p= 0.05. The adequacy of the model was evaluated by the coefficient of determination (R^2), model p-value, and lack of fit testing (Aliakbarian et al., 2018; Lisboa et al., 2018; Majeed et al., 2016) and the coefficient of variation (CV). The CV is a measure of deviation from the mean values, which shows the reliability of the experiment. In general, CV< 10% indicates better reliability (Islam Shishir et al., 2016). The final optimum parameters proposed by RSM were selected to simulate the optimum conditions with the CFD method. The proposed and the simulated data were compared to study the accuracy of final optimum conditions proposed by RSM.

Results and discussion

Deactivation kinetics and Simulation validation

In microbial deactivation, if equation 8 is plotted as a semi-logarithmic coordinate graph named "The thermal death curve", we can consider the slope as k_{reac} (Ibarz & Barbosa-Cánovas, 2002). The temperature of deactivation can change the slope of this curve. However, the effect of the ozone concentration during the process should be considered. Especially when it is obvious that the temperature changes can affect the ozone concentration produced by the plasma.



(a) **(b)**

Fig. 2. The changes of number of microorganisms (a) and ozone concentration at different combination of voltage and temperature.

Figure 2 shows the results of pretreatment to find the role of temperature and voltage on deactivation time. According to Figure 2, any changes in the number of microorganisms have a linear relationship with the process time at different voltage- temperature conditions. However, the ozone concentration dramatically changes at different combinations of voltage and temperature. Our finding is in agreement with Tabibian et al. (2020), Wang et al. (2020) and Yuan et al. (2019). Figure 2a shows that at 15 kV and 20°C the time required for microorganism reduction is significantly longer than the 15 and 20kV at 30°C. This shows that temperature can have a significant effect on deactivation by plasma. Figure 2b, also shows that changes in ozone concentration during plasma treatment have a direct relation with temperature. At temperature 20°C, the ozone concentration decreased at the beginning of

treatment and then stayed constant, but at 30°C, the ozone production increased with treatment time. With respect to Figure 2a, the deactivation kinetics can be calculated.

We know the units of rate constants are the cycles per time units (t^{-1}) (Heldman & Lund, 2007; Valentas et al., 1997) and k_{reac} depends on the process temperature, food, and microorganism type and growth state (vegetative, or spore form) (Ibarz & Barbosa-Cánovas, 2002). The k_{reac} (m^3/ mol.s) as a function of time at 20°C for initial concentration of microorganism (N_0) was 1.5×10^4 CFU/ml which is shown in Figure 3.

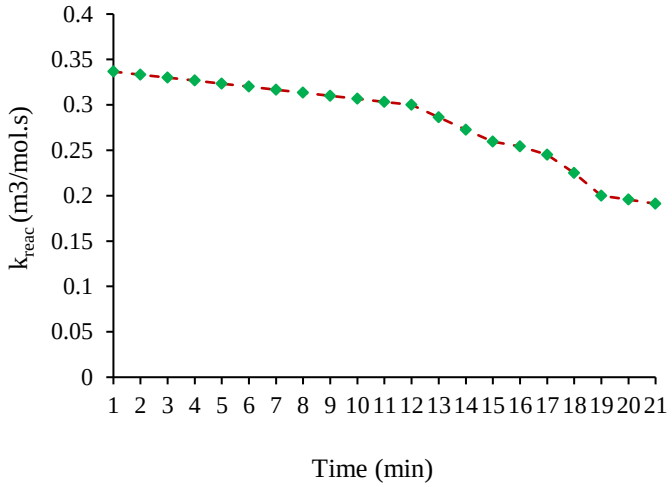


Fig. 3. The k_{reac} (m^3/mol.s) as a function of time at 20°C for initial concentration of *Z. rouxii* (N_0) was 1.5×10^4 CFU/ml

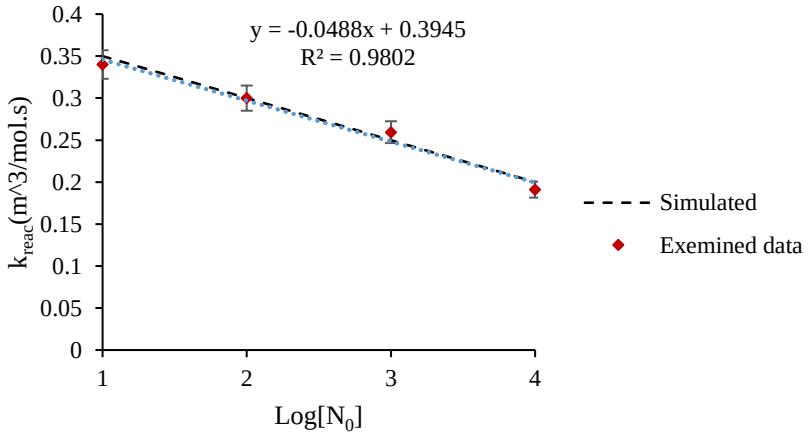
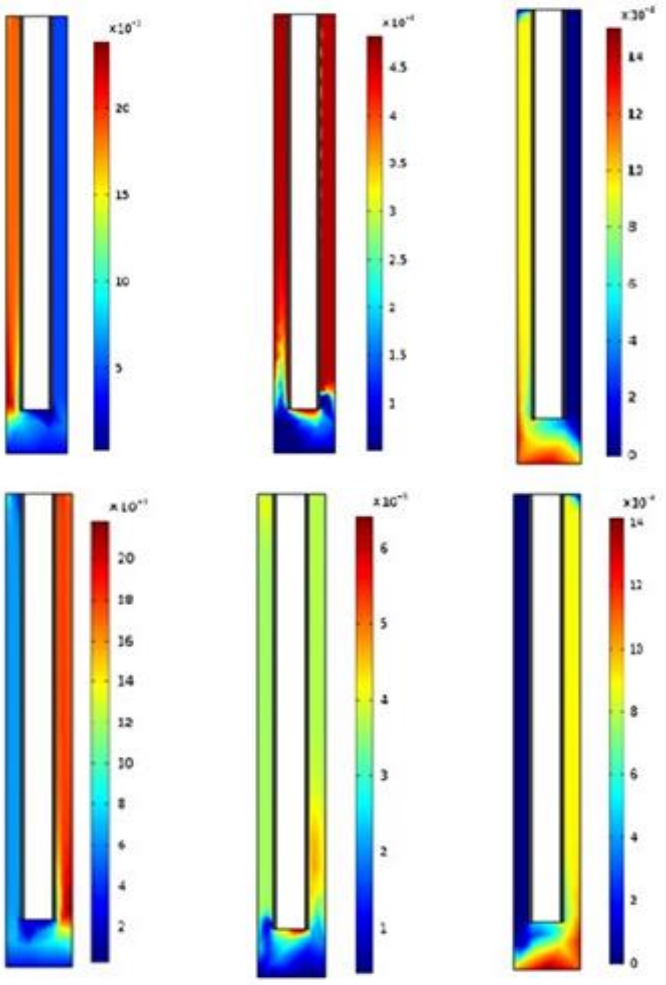


Fig. 4. The simulated and experimental data of k_{reac} (m^3/mol.s) as a function of time at 20°C for initial concentration of *Z. rouxii* (N_0) was 1.5×10^4 CFU/ml

Finally, Figure 4 shows the simulated data compared with experimental data for k_{reac} obtained from Wang et al. (2020). The regression coefficient R^2= 0.9802 shows a good agreement between experimental and simulated data. When the initial concentration is increased, the k_{reac} (or death rate constant) decreased.

The simulation results in Figure 5, also validate that the temperature can have a significant effect on the velocity magnitude of liquid phase (m/s) and ozone phase dispersion coefficient (m^2/s) in a plasma treatment in water. The minimum velocity of liquid phase displacement due to ozone bubbles (Wang et al., 2020), was 5 m/s at 20°C, but with 10°C increase in treatment temperature leads to a 1.5

fold decrease in minimum velocity of the liquid phase.



(a) (b) (c)

Fig. 5. Velocity magnitude of liquid phase (m/s) (a), gas phase dispersion coefficient (m^2/s) (b), volume fraction of gas phase (c) during plasma treatment

We know that plasma can generate reactive oxygen species (ROS) (Zhang et al., 2013) such as atomic oxygen and hydroxyl radicals in water (Surowsky et al., 2014). The ROS produced in plasma- activated water (PAW), has an important role in bacterial inactivation. Especially atomic oxygen which reacts with hydrogen compounds and leads to protein oxidation and etching processes (Sakudo et al., 2019; Surowsky et al., 2014). The etching effects such as bacterial spore shrinkage contributes especially in oxygen gas plasma but not in nitrogen plasma (Sakudo et al., 2019). The ROS can initiate the breaking of single-stranded DNA, oxidation in amino acid and unsaturated fatty acids, protein cross- links, and cleavage in peptide bond (Surowsky et al., 2014). Ozone is formed as a consequence of combining atomic oxygen in presence of water (Surowsky et al., 2014). Thus it can be explained that when we use the gas as the plasma medium, the mechanism of plasma deactivation depends on the type of gas (Sakudo et al., 2019). The reactive species produced by gas, the UV radiated from the magnetic field, the electric field are involved in plasma deactivation in plasma with the medium of gas (Sakudo et al., 2019). Reactive nitrogen species (RNS) are produced when the gas is nitrogen or ambient air. One of them is nitric

oxide which then contributes to oxidation reactions with ozone and makes some products such as nitric oxide, nitrite, and oxygen (Surowsky et al., 2014). Nitric oxide and nitrite can play as a strong anti- microbial agent, especially for gram-positive microorganisms. Since conductivity, temperature, and pH have the same role as electrode material, electric field characteristics, and gas composition or medium type, the result of this study about the effect of temperature on ozone concentration and plasma deactivation is in agree with Bruggeman & Leys (2009), Locke et al. (2006) and Thagard et al. (2009). This complexity of the role of different factors on microorganism deactivation by plasma leads to the investigation of other factors that affect the ozone concentration during plasma deactivation of microorganisms.

Data analyzing and model definition

After validating the results, we simulate the 14 treatments of RSM to find models for responses. The CFD simulation of plasma treatment showed a better efficiency for studying the plasma treatment conditions. When the RSM was used for investigating the most effective factors in plasma treatment, it was possible to study more than one factor in the process. the simulation was based on data obtained from Wang et al. (2020). The results for yeast deactivation under different levels of factors shown in Table 2, analyzed for finding the significant factors. Responses were k_{reac}, ozone concentration, at the end of deactivation, and final treatment time (min) for reaching the minimum amount of microorganism concentration.

Table 2- The Responses of Definitive screen design in RSM

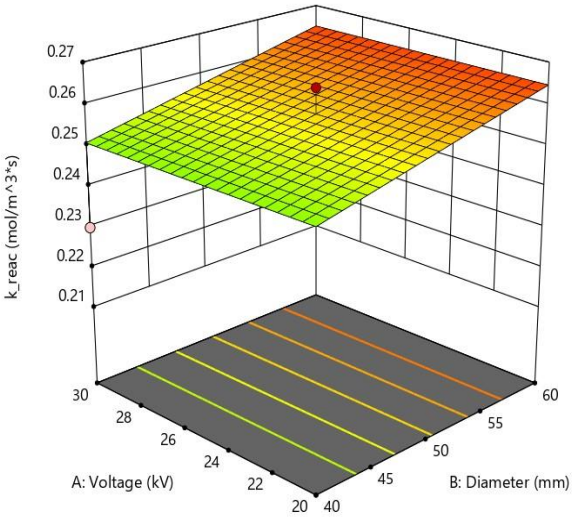
Run	Voltage (kV)	Diameter (mm)	Temperature (°C)	Media	O_3 (mol/m^3)	Deactivation time (min)
1	30	50	20	air	50	6
2	20	40	40	air	47.5	5.1
3	25	50	30	water	125	3
4	30	40	40	water	125	2.9
5	30	60	20	water	125	3.05
6	20	50	40	water	125	3
7	30	40	30	air	75	3.25
8	30	60	40	air	55	6.55
9	20	60	20	air	55	6.95
10	20	40	20	water	125	2.95
11	20	60	30	water	125	3.1
12	25	60	40	air	52.5	6.65
13	25	40	20	water	125	3.05
14	25	50	30	Air	50	6.2

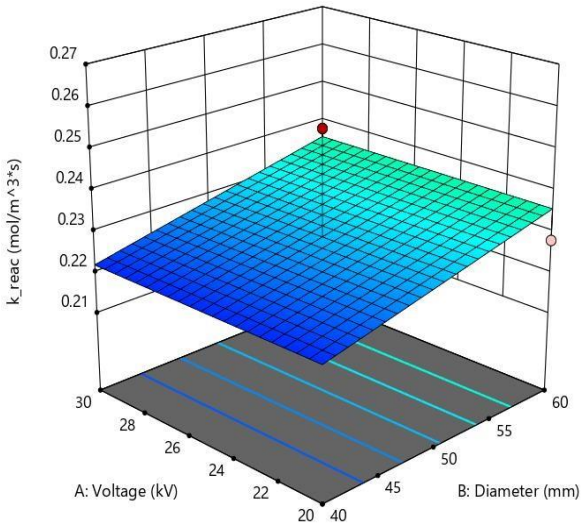
The analyzed data show that the k_{reac} is affected significantly by the diameter of the vessel and the types of process media (water or air). The effect of media in plasma treatment on all 3 responses was highly significant. The ozone concentration only depends on the type of plasma media and the final process time significantly depends on vessel diameter and type of media. The models obtained for each response are as bellow:

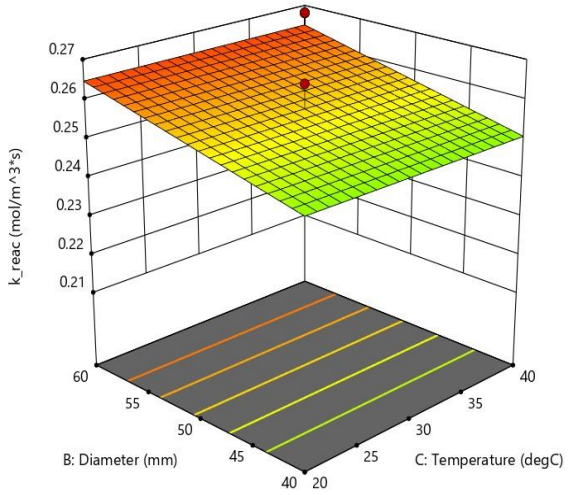
$$k_{reac} = 0.2433 + 0.0069B + 0.0144D \quad R^2 = 0.9396\ P = 0.0011 \quad (11)$$

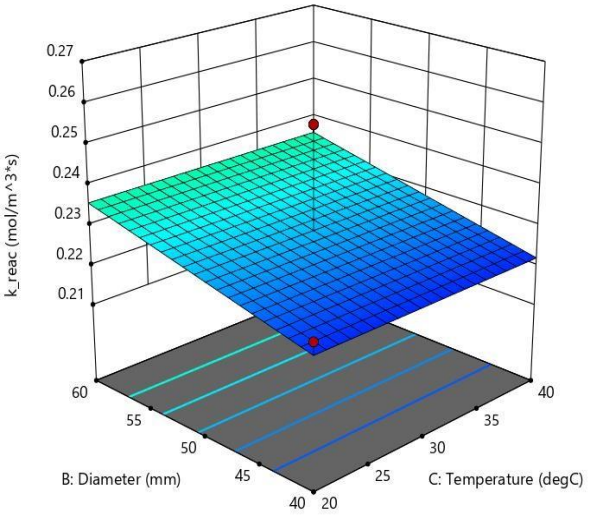
$$Ozone = 87.5 - 35.04D \quad R^2 = 0.9898\ P = 0.0001 \quad (12)$$

$$Final\ process\ time\ (water/air) = 4.37 + 0.6351B + 1.34D + 1.02C^2 \quad R^2 = 0.9396\ P = 0.0028 \quad (13)$$

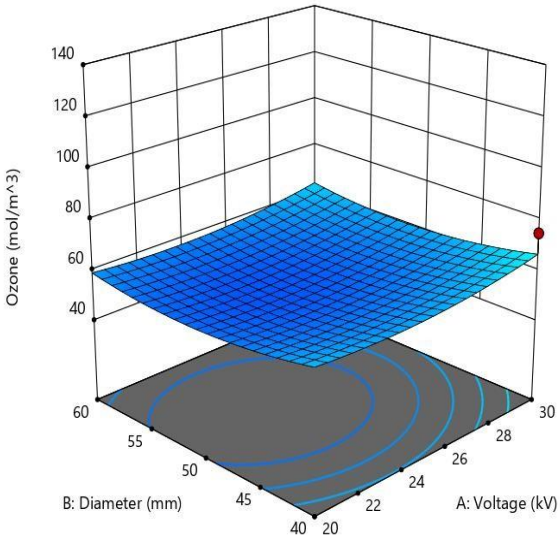

k_reac (mol/m^3*s)
0.27
0.26
0.25
0.24
0.23
0.22
0.21
A: Voltage (kV)
30
28
26
24
22
20
B: Diameter (mm)
40
45
50
55
60

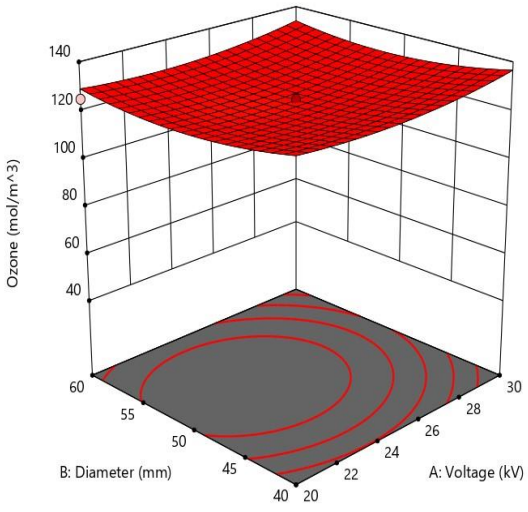

k_reac (mol/m^3*s)
0.27
0.26
0.25
0.24
0.23
0.22
0.21
A: Voltage (kV)
30
28
26
24
22
20
B: Diameter (mm)
40
45
50
55
60

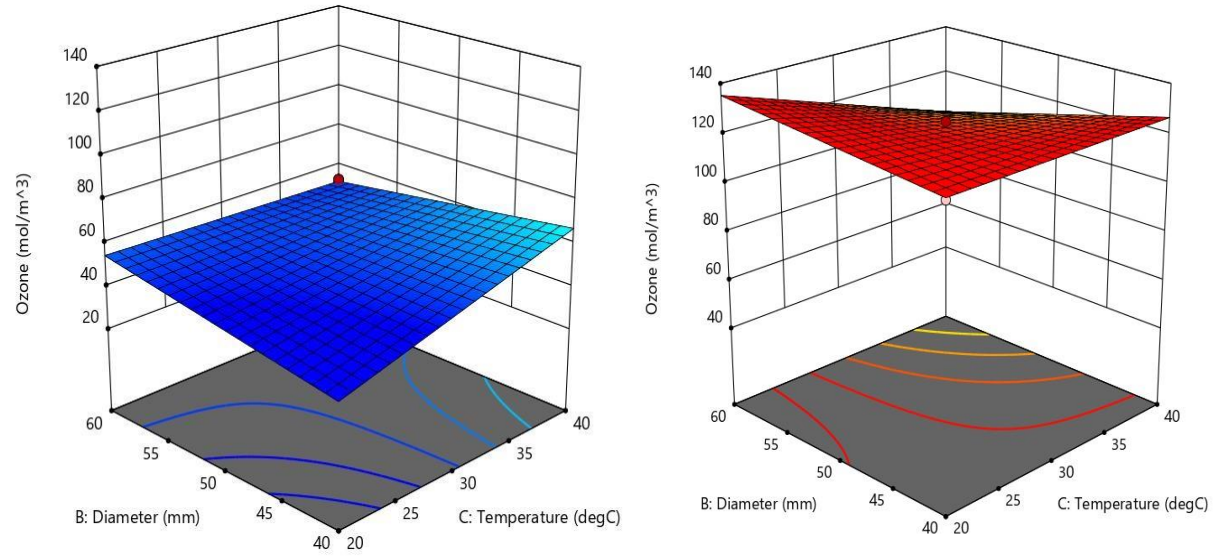

k_reac (mol/m^3*s)
0.27
0.26
0.25
0.24
0.23
0.22
0.21
B: Diameter (mm)
60
55
50
45
40
C: Temperature (degC)
20
25
30
35
40


k_reac (mol/m^3*s)
0.27
0.26
0.25
0.24
0.23
0.22
0.21
B: Diameter (mm)
60
55
50
45
40
C: Temperature (degC)
20
25
30
35
40

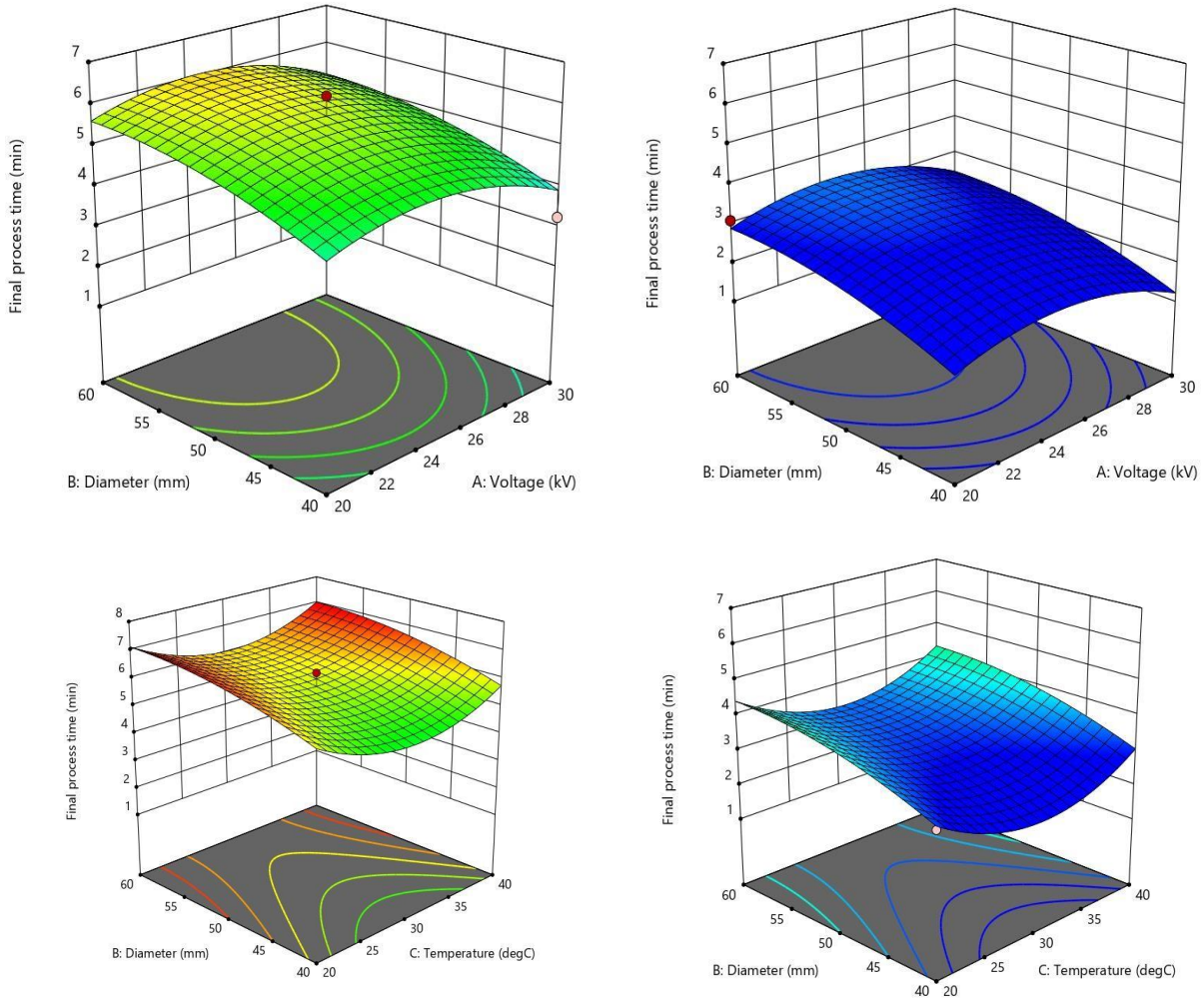
Ozone


Ozone (mol/m^3)
140
120
100
80
60
40
B: Diameter (mm)
60
55
50
45
40
A: Voltage (kV)
20
22
24
26
28
30


Ozone (mol/m^3)
140
120
100
80
60
40
B: Diameter (mm)
60
55
50
45
40
A: Voltage (kV)
20
22
24
26
28
30



Final process time



Air **Water**

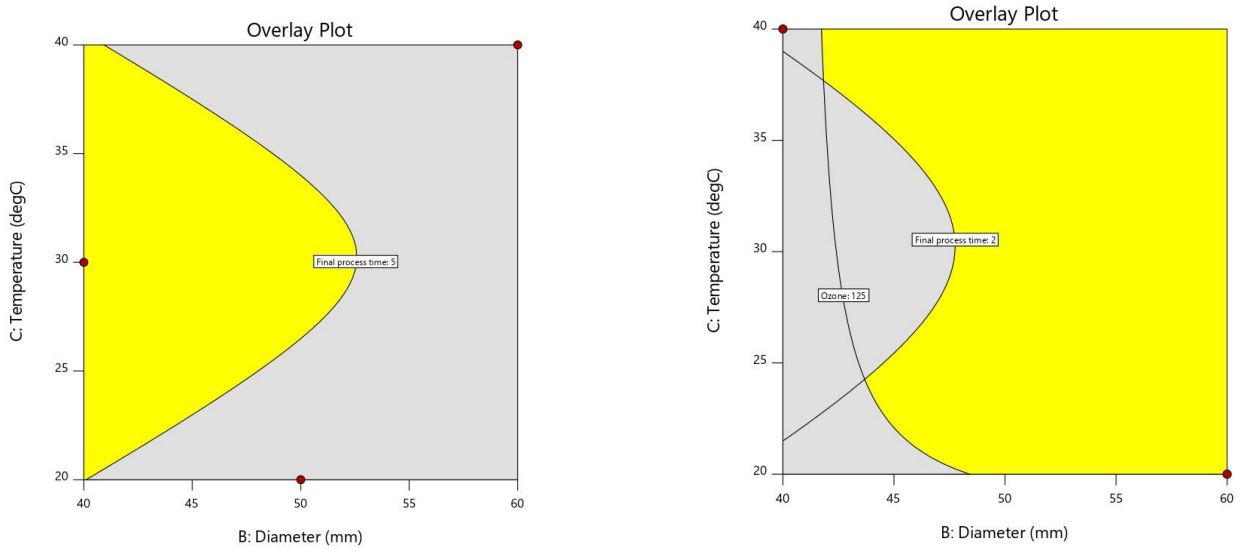
Fig. 6. Responses for k_{reac}, ozone concentration, at the end of deactivation, and final treatment time (min) at 30℃

During data analysis, it was demonstrated that the medium had a highly significant effect ($p< 230 0.0001$) on k_{reac} and ozone concentration responses. The final process time was affected by the diameter of the reactor vessel and the square of temperature. The variation of response by studied factors is shown in Figure 6. It can be seen that the responses are significantly different at two different reaction mediums (air and water). As can be seen in Figure 6, the ozone production in air plasma is lower than the water plasma. Meanwhile, the processing time is higher at air plasma which is related to lower ozone production during air plasma treatment. This result is according to the basis of production of second plasma species from ozone in water (such as hydrogen peroxide) (Julák et al., 2018; Perinban et al., 2019).

Liao et al. reviewed the influence of non-thermal plasma on microbial inactivation. They explained when researchers added the oxygen to the dielectric barrier discharge (DBD) plasma for deactivation of *E.coli* and Staphylococcus in cheese slices, more oxygen-based radicals were produced and lead to a strong antibacterial effect (Basaran et al., 2008; Liao et al., 2017). Thus the results from RSM are in agreement with other results from literature (Liao et al., 2018; Xiang et al., 2019).

Process optimization

Optimization aimed to reduce the processing time. The k_{reac} was set at the lowest and the ozone was set at the highest amount of 0.22 and 125, respectively. The final process time was set for 1 to 5 minutes. Figure 7 shows the graphical results of optimization. In this study, the optimium conditions were 29.79V of voltage, 46.34 mm of vessel diameter, and 4.86 min of process temperature with water as the media for plasma discharge.



Air **Water**

Fig. 7. The graphical optimization of final deactivation time for plasma treatment

Conclusion

The results of this study showed that for the deactivation of microorganisms, the plasma treatment in water can have the strongest effect than air plasma. Also, during studying the effect on plasma, a combination of factors should be considered. It has demonstrated that study one factor such as temperature on process responses such as ozone concentration, the results will be restricted by other factors that are ignored. This study showed when an RSM design was applied for designing the experiment which considers different process factors, the results can significantly differ from the only one-factor study. In plasma treatment, media type had a significant effect on all 3 responses, while the

temperature shows its effect only on final process time. Thus it is recommended to use RSM or other statistical methods for studying various factors during plasma treatments.

Conflict of Interest

We have no conflicts of interest.

Nomenclature

φ	Phase volume fraction [m/m^3]
ρ	Density [kg/m^3]
u	Velocity vector [m/s]
μ	Viscosity [Pa.s]
g	Gravity vector [m/s^2]
$N_{\rho_g \phi_g}$	Flux vector [$mol/m^3.s$]
c	Concentration of the species [mol/m^3]
D	Diffusion coefficient [m^2/s]
R	Reaction rate expression for the species [$mol/m^3.s$]
N	Flux vector [$mol/m^3.s$]
k_{reac}	Reaction rate coefficient [$m^3/mol.s$]

References

1. Aliakbarian, B., Sampaio, F. C., de Faria, J. T., Pitangui, C. G., Lovaglio, F., Casazza, A. A., Perego, P. (2018). Optimization of spray drying microencapsulation of olive pomace polyphenols using Response Surface Methodology and Artificial Neural Network. *LWT*, 93, 220-228. https://doi.org/10.1016/j.lwt.2018.03.048
2. Basaran, P., Basaran-Akgul, N., & Oksuz, L. (2008). Elimination of *Aspergillus parasiticus* from nut surface with low pressure cold plasma (LPCP) treatment. *Food Microbiology*, 25(4), 626-632. https://doi.org/10.1016/j.fm.2007.12.005
3. Bourke, P., Ziuzina, D., Boehm, D., Cullen, P. J., & Keener, K. (2018). The Potential of Cold Plasma for Safe and Sustainable Food Production. *Trends Biotechnol*, 36(6), 615-626. https://doi.org/10.1016/j.tibtech.2017.11.001
4. Bruggeman, P., & Leys, C. (2009). Non-thermal plasmas in and in contact with liquids. *Journal of Physics D: Applied Physics*, 42(5), 053001.
5. Chilka, A. G., & Ranade, V. V. (2019). CFD modelling of almond drying in a tray dryer. *The Canadian Journal of Chemical Engineering*, 97(2), 560-572. doi:10.1002/cjce.23357
6. Chizoba Ekezie, F. G., Sun, D. W., & Cheng, J. H. (2017). A review on recent advances in cold plasma technology for the food industry: Current applications and future trends. *Trends in Food Science & Technology*, 69, 46-58. doi:10.1016/j.tifs.2017.08.007
7. Guo, L., Xu, R., Gou, L., Liu, Z., Zhao, Y., Liu, D.,. . . Kong, M. G. (2018). Mechanism of Virus Inactivation by Cold Atmospheric-Pressure Plasma and Plasma-Activated Water. *Applied and*
8. *Environmental Microbiology*, 84(17), e00726-00718. doi:10.1128/aem.00726-18
9. Heldman, D. R., & Lund, D. B. (2007). Handbook of food engineering.
10. Ibarz, A., & Barbosa-Cánovas, G. V. (2002). *Unit operations in food engineering*: CRC press.
11. Islam Shishir, M. R., Taip, F. S., Aziz, N. A., Talib, R. A., & Hossain Sarker, M. S. (2016). Optimization of spray drying parameters for pink guava powder using RSM. *Food Sci Biotechnol*, *25*(2), 461468. doi:10.1007/s10068-016-0064-0
12. Julák, J., Hujacová, A., Scholtz, V., Khun, J., & Holada, K. (2018). Contribution to the Chemistry of Plasma-Activated Water. *Plasma Physics Reports*, 44(1), 125-136. doi:10.1134/S1063780X18010075

13. Liao, X., Liu, D., Xiang, Q., Ahn, J., Chen, S., Ye, X., & Ding, T. (2017). Inactivation mechanisms of non-thermal plasma on microbes: A review. *Food Control,* 75, 83-91. doi:10.1016/j.foodcont.2016.12.021
14. Liao, X., Su, Y., Liu, D., Chen, S., Hu, Y., Ye, X., . . . Ding, T. (2018). Application of atmospheric cold plasma-activated water (PAW) ice for preservation of shrimps (*Metapenaeus ensis*). *Food*
15. *Control, 94*, 307-314. doi:10.1016/j.foodcont.2018.07.026
16. Lisboa, H. M., Duarte, M. E., & Cavalcanti-Mata, M. E. (2018). Modeling of food drying processes in industrial spray dryers. *Food and Bioproducts Processing, 107*, 49-60. doi:10.1016/j.fbp.2017.09.006
17. Locke, B., Sato, M., Sunka, P., Hoffmann, M., & Chang, J. S. (2006). Electrohydraulic discharge and nonthermal plasma for water treatment. *Industrial & engineering chemistry research,* 45(3), 882905. https://doi.org/10.1021/ie050981u
18. Majeed, M., Hussain, A. I., Chatha, S. A., Khosa, M. K., Kamal, G. M., Kamal, M. A., . . . Liu, M. (2016). Optimization protocol for the extraction of antioxidant components from *Origanum vulgare* leaves using response surface methodology. *Saudi J Biol Sci,* 23(3), 389-396. doi:10.1016/j.sjbs.2015.04.010
19. Misra, N. N., Pankaj, S. K., Segat, A., & Ishikawa, K. (2016). Cold plasma interactions with enzymes in foods and model systems. *Trends in Food Science & Technology,* 55, 39-47. doi:10.1016/j.tifs.2016.07.001
20. Misra, S., Raghuwanshi, S., & Saxena, R. K. (2013). Statistical approach to study the interactive effects of process parameters for enhanced xylitol production by *Candida tropicalis* and its potential for the synthesis of xylitol monoesters. *Food Science and Technology International,* 19(6), 535-548. doi:10.1177/1082013212462230
21. Muhammad, A. I., Liao, X., Cullen, P. J., Liu, D., Xiang, Q., Wang, J., . . . Ding, T. (2018). Effects of Nonthermal Plasma Technology on Functional Food Components. *Comprehensive Reviews in Food Science and Food Safety*, 17(5), 1379-1394. doi:10.1111/1541-4337.12379
22. Pankaj, S. K., Bueno-Ferrer, C., Misra, N. N., Milosavljević, V., O'Donnell, C. P., Bourke, P., Cullen, P. J. (2014). Applications of cold plasma technology in food packaging. *Trends in Food Science & Technology*, 35(1), 5-17. doi:10.1016/j.tifs.2013.10.009
23. Pankaj, S. K., Wan, Z., & Keener, K. M. (2018). Effects of Cold Plasma on Food Quality: A Review. *Foods,* 7(1). doi:10.3390/foods7010004
24. Perinban, S., Orsat, V., & Raghavan, V. (2019). Nonthermal Plasma–Liquid Interactions in Food Processing: A Review. *Comprehensive Reviews in Food Science and Food Safety,* 18(6), 19852008. doi:10.1111/1541-4337.12503
25. Sakudo, A., Yagyu, Y., & Onodera, T. (2019). Disinfection and sterilization using plasma technology: Fundamentals and future perspectives for biological applications. *International journal of molecular sciences,* 20(20), 5216. **https://doi.org/10.3390/ijms20205216**
26. Sumic, Z., Vakula, A., Tepic, A., Cakarevic, J., Vitas, J., & Pavlic, B. (2016). Modeling and optimization of red currants vacuum drying process by response surface methodology (RSM). *Food Chem,* 203, 465-475. doi:10.1016/j.foodchem.2016.02.109
27. Surowsky, B., Fischer, A., Schlueter, O., & Knorr, D. (2013). Cold plasma effects on enzyme activity in a model food system. *Innovative Food Science & Emerging Technologies,* 19, 146-152. doi:10.1016/j.ifset.2013.04.002
28. Surowsky, B., Schlüter, O., & Knorr, D. (2014). Interactions of Non-Thermal Atmospheric Pressure Plasma with Solid and Liquid Food Systems: A Review. *Food Engineering Reviews,* 7(2), 82108. doi:10.1007/s12393-014-9088-5
29. Tabibian, S., Labbafi, M., Askari, G., Rezaeinezhad, A., & Ghomi, H. (2020). Effect of gliding arc discharge plasma pretreatment on drying kinetic, energy consumption and physico-chemical

properties of saffron (*Crocus sativus* L.). *Journal of Food Engineering*, 270, 109766. https://doi.org/10.1016/j.jfoodeng.2019.109766

30. Thagard, S. M., Takashima, K., & Mizuno, A. (2009). Chemistry of the positive and negative electrical discharges formed in liquid water and above a gas–liquid surface. *Plasma Chemistry and Plasma Processing*, 29(6), 455-473. https://doi.org/10.1007/s11090-009-9195-x
31. Valentas, K. J., Rotstein, E., & Singh, R. P. (1997). *Handbook of food engineering practice*: CRC press.
32. Wang, Y., Wang, Z., Yang, H., & Zhu, X. (2020). Gas phase surface discharge plasma model for yeast inactivation in water. *Journal of Food Engineering*, *286*, 110117. https://doi.org/10.1016/j.jfoodeng.2020.110117
33. Xiang, Q., Kang, C., Niu, L., Zhao, D., Li, K., & Bai, Y. (2018). Antibacterial activity and a membrane damage mechanism of plasma-activated water against Pseudomonas deceptionensis CM2. *LWT*, 96, 395-401. doi:10.1016/j.lwt.2018.05.059
34. Xiang, Q., Liu, X., Liu, S., Ma, Y., Xu, C., & Bai, Y. (2019). Effect of plasma-activated water on microbial quality and physicochemical characteristics of mung bean sprouts. *Innovative Food*
35. *Science & Emerging Technologies*, 52, 49-56. doi:10.1016/j.ifset.2018.11.012
36. Yuan, Y., Tan, L., Xu, Y., Yuan, Y., & Dong, J. (2019). Numerical and experimental study on drying shrinkage-deformation of apple slices during process of heat-mass transfer. *International Journal of Thermal Sciences*, 136, 539-548. https://doi.org/10.1016/j.ijthermalsci.2018.10.042
37. Zhang, Q., Liang, Y., Feng, H., Ma, R., Tian, Y., Zhang, J., & Fang, J. (2013). A study of oxidative stress induced by non-thermal plasma-activated water for bacterial damage. *Applied physics letters*, 102(20). doi:10.1063/1.4807133

Iranian Food Science and Technology Research Journal
Vol. 18, No. 3, Aug. Sep. 2022, p. 15-29

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد ۱۸، شماره ۳، مرداد - شهریور ۱۴۰۱، ص. ۲۹-۱۵

یافتن فاکتورهای موثر فرآیند پلاسما بر فعالیت مخمراز طریق شبیه‌سازی و روش سطح پاسخ

آزاده رنجبر ندامانی*

تاریخ دریافت: ۱۴۰۰/۱۲/۰۸
تاریخ پذیرش: ۱۴۰۱/۰۲/۱۰

چکیده

در این مطالعه، یک مدل ریاضیاتی برای غیرفعال کردن مخمر از طریق تیمار پلاسما مورد بررسی قرار گرفت. از طرح غربالگری تشخیصی برای جستجوی فاکتورهایی که در غیرفعال کردن با پلاسما موثر هستند، استفاده شد. چهار فاکتور ولتاژ (۲۰- ۳۰ کیلوولت) (A)، قطر ظرف (۴۰- ۶۰ میلی‌متر) (B)، درجه حرارت فرآیند (۲۰- ۴۰ درجه سانتی‌گراد) (C) و نوع محیط پلاسما (هوا یا آب) در این مطالعه بررسی شدند. سپس تیمارها با نرم‌افزار COMSOL شبیه‌سازی شدند. پاسخ‌های ضریب سنتیک واکنش، غلظت ازون و زمان غیرفعال شدن نهایی توسط طرح غربالگری فاکتورها در نرم‌افزار دیزاین اکسپرت تحلیل شدند تا پارامترهای موثر مدل ریاضیاتی و شرایط بهینه تعیین شوند. نتایج نشان دادند که تیمار با پلاسما در محیط آبی می‌تواند اثر قوی‌تری نسبت به هوا داشته باشد. همچنین در تیمار با پلاسما، نوع محیط اثر بارزی بر هر سه پاسخ داشت، در حالیکه درجه حرارت تنها بر زمان فرآیند موثر بود. بنابراین می‌توان نتیجه گرفت که با بررسی و انتخاب مناسب محیط، می‌توان از تکنولوژی پلاسما برای غیرفعال‌سازی میکروارگانیسم‌ها در مواد غذایی استفاده کرد.

واژه‌های کلیدی: تیمار پلاسما، غیرفعال‌سازی میکروارگانیسم، شبیه‌سازی عددی، روش سطح پاسخ.

* استادیار، گروه مهندسی بیوسیستم، دانشکده مهندسی کشاورزی، دانشگاه علوم کشاورزی و منابع طبیعی ساری، ساری، مازندران، ایران.
(*- نویسنده مسئول: Email: a.ranjbar@sanru.ac.ir)

Iranian Food Science and Technology Research Journal
Vol. 18, No. 3, Aug. Sep. 2022, p. 31-51

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد ۱۸، شماره ۳، مرداد - شهریور ۱۴۰۱، ص. ۵۱-۳۱

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

Full Research Paper

Detection of adulterants and contaminants in black pepper, cumin, fennel, coriander and turmeric using GC-MS technique for forensic investigation

P. Choudhary[1], A. Seth[2], K. Lata Verma[3*]

Received: 2022.03.03
Accepted: 2022.04.18

How to cite this article:
Choudhary, P., Seth, A., Lata Verma, K. (2022). Detection of adulterants and contaminants in black pepper, cumin, fennel, coriander and turmeric using GC-MS technique for forensic investigation. *Iranian Food Science and Technology Research Journal*. 18 (3), 31-51.

Abstract

Forensic investigation is very crucial for identifying quality and safety issues related to food and its product. GC- MS has been used extensively in food analysis, and in the present study, it was used to identify the active constituents of some household spices as well as to detect the adulterants and contaminants which might be present in the samples. Spices used in the present study are black pepper, cumin, fennel, coriander and turmeric. The spice extracts were found to be useful in authenticating the spices by identifying various active constituents of the spices, like piperine, caryophyllene and 3- carene in black pepper; cuminaldehyde and 1, 3- methadien-7- al in cumin; anethole and fenchone in fennel; linalool, and geranyl vinyl ether in coriander and turmerone and zingiberene in turmeric. The adulterants detected qualitatively were plant- based adulterants. GC- MS is proved to be an effective tool in detecting plant- based adulterants, microbial contaminants, and agro chemical residues as well as industrial and manufacturing waste.

Keywords: GC-MS, Spice adulteration, Spice authentication, Spice contaminants, Qualitative.

Introduction

Spices are dried plant materials, which are usually used as a flavoring, coloring, or preservative agents in the foodstuff. They are used extensively all over the world for their culinary and medicinal properties (Attokaran, 2011). According to Food and Agriculture Organization (FAO), 28, 94,248 tons of spices were produced worldwide in the year 2019 and the major producer countries are India, China, Ethiopia, Indonesia, and Turkey (FAOSTAT, 2019). India is the spice bowl of the world, spices are used extensively in various Indian recipes and household remedies (Raghavan, 2006). India is the world's largest producer, consumer, and exporter of spices as it produces 75 of 109 varieties listed by International Organization for Standardization (ISO) and accounts for half of the global trading in spices (Indian trade portal, 2021). Spices are a very essential commodity; they are very important not only as food condiments but also have valuable medicinal properties (Attokaran, 2011; Raghavan, 2006). The global spice market was valued at 13.77 billion USD, in the year 2019 and will continue to grow in the future (Seasonings and Spices Market Size, 2020). As the global trade of spices is growing rapidly, spices are becoming very prone to fraudulence. Food fraud refers to the illegal tampering of

1 and 3. Regional Forensic Science Laboratory, Government of NCT of Delhi, Home Department, Chanakyapuri, New Delhi, 110021, India.

2. Department of Anthropology, University of Delhi, Delhi, 110007, India.
(Corresponding Author Email: kanak.dfsl@gmail.com)
DOI: 10.22067/IFSTRJ.2022.75621.1155

foodstuffs and materials for monetary gain. Food stuffs are considered fraudulent if it contains– (a) any foreign material which is responsible for health hazard, (b) does not have any useful ingredients, (c) inferior ingredients, substances which resemble the product but does not contains any essential compounds, (d) any decomposed or spoiled compounds which can be incorporated during production or processing or storage and (e) mislabeling i.e., providing false claims about the food product such as the origin of production, additives used, etc (Msagat, 2018). Economically motivated adulteration (EMA) is considered the most common practice of food fraud in the food industry. It is the fraudulent and intentional adulteration of food products to increase their market value or to reduce their production cost for financial purposes (Braden, 2014). Spices and their products are often sold in bulk and loose form in the market which can be easily adulterated. Detection becomes more difficult as the final product had gone through various incorporations during the manufacturing and production phase. Dyes may be added to make a spice look fresher, older spices may be added to freshly ground ones to increase weight, non-spice material may be added as an extender, or spent spices with valuable constituents removed may be sold as whole spices (Everstine, 2013). The cases related to food fraud in India are significantly increasing according to the Food Safety & Standards Authority of India (FSSAI) enforcement report; 18,550 civil cases and 2,819 criminal cases were reported from 2018- 19 (FSSAI enforcement report, 2021). Food fraud is a growing concern that is affecting the health as well as the economy of society. Fifty seven percent of people developed health problems like stomach disorder, heart problem, anemia, liver disorder, indigestion, nausea, vomiting, diarrhea, dysentery, acidity, ulcer, cancer, kidney malfunction, metabolic dysfunctions, food poisoning, skin disorders, etc, due to consumption of adulterated and contaminated food worldwide (Pal and Mahinder, 2020). Food fraud is estimated to cost the global food industry US $ 10-15 billion per year according to Grocery Manufacturers Association (GMA) (King et al., 2017). Incidents of food fraud are thus found to be detrimental to public health as well as the economy and therefore countermeasures for it need to be developed.

Food forensic is a branch of forensic chemistry that deals with food fraud investigations. Food forensic investigation involves, determining the active constituents, standards and quality assessments, detection of adulterants and contaminants, determination of geographical origin, identifying any safety or quality problems that may be associated with a food product, etc (Mermelstein, 2018). Basically, two main tasks can be done by the application of food forensics; food authentication and food traceability. Food authentication deals with the detection of adulterants and contaminants, quality assessments, and verifications of the labels associated with the food product. Food traceability helps in verifying the origin of the products or deals with finding out the raw materials associated with that food product (Aceto, 2015). To ensure customers' rights and to enforce food law; detection of adulterants and contaminants in foodstuffs is a very important task and forensic analysis can help authorities to gather, examine and interpret pieces of evidence against food fraud.

There are various methods of adulteration and contamination detection, which are physical, chemical, biological, or instrumental methods (Osman et al., 2019; Hong et al., 2017). The instrumental analysis based on the chemical composition of the sample can be divided into two categories– Spectroscopic and Chromatographic analysis.

The chromatographic analysis involves TLC (Jaiswal et al., 2016; Bhooma et al, 2019; Danciu et al., 2018), HPLC (Sebaei and Youssif, 2019), HPTLC (Vadivel et al., 2018), HPTLC-MS (Rani et al., 2015), LC- MS (Bessaire et al., 2019) and GC- MS (Vadivel et al., 2018; Bansal et al., 2015). Chromatographic techniques are the most common techniques applied in food analysis. This is because; chromatography can

be applied both to detect adulterations and to determine authenticity (Pastor et al., 2019). GC- MS has been used extensively in food analysis; as food presents a complex matrix and the chromatographic technique can readily and rapidly separate the molecules with extremely similar chemical characteristics even from complex matrices (Lees, 2000). In literature, there are many examples of GC- MS applications being used in food fraud analysis of spices, some recent examples being; establishing organic markers for certain common spices for spice authentication (Atkins, 2015), unknown spices identification and authentication, detection of plant- based adulterants in black pepper (Vadivel et al., 2018; Gul et al., 2017), saffron, fennel and cumin (Xiao-Dong et al., 2015; Farag et al., 2020), confirmatory analysis for electronic screening devices, detection of pesticide residues (Hakme et al, 2018) and also in origin differentiation of cumin & turmeric (Yichen et al., 2014). The motivation for spice fraud is largely attributed to economical interest to gain greater profit margins and such adulteration of spices can have serious implications for public health. Delhi, Uttar Pradesh and Hyderabad police busted a food contamination case of manufacturing fake cumin seeds. The accused reported that they first experimented with making cumin seeds with different types of grass and seeds from the forest. (Gurneel Kaur, 2019). Officers of the enforcement branch of the Kolkata police had on the basis of suspicion seized a large quantity of cumin seeds. The cumin seeds were mixed with sulfa seeds which are very cheap and resemble cumin seeds and is used as cow feed. The Spices Board of India got a huge export consignment of cumin from a Gujarat- based firm seized, after the testing at Kandla Port revealed adulteration and it was reported that there was presence of 23.33 per cent extraneous matter against maximum limit of 3 per cent in the sample which was a clear indication of adulteration (Press Trust of India. business-standard.com, 2018).

Table 1- Samples collected from different markets of Indian cities.

Spice	Sample type	Area of collection	Code
Black pepper (whole)	Local, opened	Local market, South Delhi	PD
		Local market, Meerut	PM
	Branded, closed	Grocery store, South Delhi	PB
Cumin (whole)	Local, opened	Local market, South Delhi	JD
		Local market, Meerut	JM
	Branded, closed	Grocery store, South Delhi	JB
Fennel (whole)	Local, opened	Local market, South Delhi	SD
		Local market, Meerut	SM
	Branded, closed	Grocery store, South Delhi	SB
Coriander (powder)	Local, opened	Local market, South Delhi	CD
		Local market, Meerut	CM
	Branded, closed	Grocery store, South Delhi	CB
Turmeric (powder)	Local, opened	Local market, South Delhi	TD
		Local market, Meerut	TM
	Branded, closed	Grocery store, South Delhi	TB

Spices and herbs, being high-priced commodities, have been often subjected to adulteration in many ways which reduces their quality and potentially has harmful health

implications. Adulteration is attributed primarily to increased demand or supply shortage of the spices.

The objective of the present study was to authenticate some household spices- black pepper, cumin, fennel, coriander & turmeric by identifying various active constituents and detecting any adulterant or contaminants in these local samples using the GC- MS technique. The chemical constituents of all the spices were compared with the chemical profile cited in the literature. This study may be helpful for the spice industry to detect the adulteration where physical and other chemical analysis have their own limitations.

Table 2- Volatile compounds detected in black pepper, hexane and ethanol extracts (PD-H, PD-E, PM-H, PM-E, PB-H & PB-E) by GC-MS analysis.

Compounds	PD-H	PD-E	PM-H	PM-E	PB-H	PB-E
3- Carene	✓	✓	✓	✓	✓	✓
4- Carene			✓			
7- epi- cis- Sesquisabinene hydrate		✓				✓
12- Methyl- E, E- 2, 13- octadecadien-1- ol		✓				✓
alpha- Pinene		✓		✓		✓
alpha- Sabinene	✓	✓			✓	✓
alpha- Terpinene			✓		✓	
Aromandendrene			✓		✓	
Azulene	✓					
beta- acorenol				✓		✓
beta- Pinene			✓		✓	
beta- Thujene			✓		✓	
beta- Selinene					✓	✓
Benzoic acid	✓				✓	
Caryophyllene	✓	✓	✓	✓	✓	✓
Caryophyllene oxide	✓		✓			✓
Cedrene						✓
cis- Verbenol, trimethylacetate			✓		✓	
cis- Z- alpha- Bisabolene epoxide			✓			✓
Copaene	✓	✓	✓	✓	✓	✓
D- Limonene	✓	✓	✓	✓	✓	✓
delta- Cadinol	✓	✓	✓	✓	✓	✓
delta- Elemene	✓	✓	✓	✓	✓	✓
Epicubebol		✓		✓		✓
gamma-Muurolene				✓		✓
Ingenol			✓			
Oleic acid	✓	✓	✓	✓	✓	✓
p- Menth- 8- en- 1- ol		✓				✓
p- Mentha- 1(7) ,8- diene						✓
Pellitorine			✓		✓	
Piperanine				✓		
Pipercitine				✓		
Piperidine		✓				✓
Piperine		✓		✓		✓
sia- Limonene				✓		✓
Tau- cadinol acetate		✓		✓		✓
trans- Z- alpha- Bisabolene epoxide				✓		✓

Materials and methods

Spices used

Spices used in the present study were black pepper, cumin, fennel, coriander and turmeric. Samples were collected from local markets of Indian cities of Delhi and Uttar Pradesh. Total of 15 spice samples were purchased from local markets; five of which were branded samples

sold in closed packets and the rest 10 were sold in loose open condition. For simplicity, the samples were coded according to Table 1.

Chemicals

Ethyl alcohol and Hexane of AR grade were procured from Hayman Exports Private Limited and SD Fine Chemical Limited, India respectively and were required for solvent extraction. Ethanol was used to extract hydrophilic and polar components while hexane was used to extract hydrophobic and non-polar components of the spices.

Glassware

The glass wares used were Glass vacutainer (15 ml), beaker (50 ml) and GC- MS glass vials (1.5 ml); which were procured from Borosil, India.

Sample preparation

One gram of each spice in ground form (the whole forms were roughly grinded using mortar & pestle) was taken into a glass vacutainer (15 ml). To this 15 ml solvent (ethanol/ hexane) was added and was covered tightly with a cork. The labeled vacutainers were than kept in a cold storage for one week, at 4 degree Centigrade. The supernatant was than collected in the GC-MS glass vials (1.5 ml). The extracts prepared were PD- H, PD- E, JD- H, JD- E, SD- H, SD-E, CD- H, CD- E, TD- H, TD- E, PM- H, PM-E, JM- H, JM- E, SM- H, SM- E, CM- H, CM-E, TM- H, TM- E, PB- H, PB- E, JB- H, JB- E, SB- H, SB- E, CB- H, CB- E, TB- H, & TB-E (H= hexane & E= ethanol).

Table 3- Volatile compounds detected in cumin, hexane and ethanol extracts (JD-H, JD-E, JM-H & JM-E, JB-H & JB-E) by GC-MS analysis.

Compounds	JD-H	JD-E	JM-H	JM-E	JB-H	JB-E
1, 3- p- Methadien- 7- al	✓		✓		✓	
1, 3, 8- p- Methatriene		✓				
1, 4- p- Methadien- 7- al		✓		✓		✓
2- decalinyl acetate				✓		✓
2, 5- octadecadiytioic acid				✓		✓
3- Caren- 10- al			✓			
3- p- Menthen- 7- al	✓		✓		✓	✓
4- (3, 3- dimethylbut- 1- ynyl)- 4- hydroxy- 2, 6, 6- trimethylcyclohex- 2- enone	✓		✓		✓	
beta- Caryophyllene	✓	✓			✓	✓
beta- Cymene			✓	✓	✓	✓
beta- Ionene						✓
beta- Pinene	✓	✓	✓	✓	✓	✓
Carotol	✓	✓	✓		✓	✓
Caryophylla- 4(12), 8(13)- dien- 5- beta- ol		✓		✓		✓
cis- Carveol		✓		✓		✓
Cuminaldehyde	✓	✓	✓	✓	✓	✓
Epicubebol				✓		✓
gamma- Muurolene	✓				✓	
gamma- Terpinene	✓	✓	✓	✓	✓	✓
o- Cymene	✓				✓	
Propanoic acid		✓				✓
Sabinene hydrate			✓		✓	
sia- Cumene					✓	

Instrumental parameters

GC- MS analysis was performed on an Agilent 7890B GC system having 7693 auto-sampler unit and fitted with 30 m long capillary column having 0.25 mm inner diameter and film thickness of 0.25 µm; coupled with Agilent MS- 5977A Mass Selective Detector (MSD). Helium gas is used as carrier gas at flow rate of 3 ml/ min and nitrogen was used as makeup gas. The oven temperature was programmed from

60 degree centigrade to 170 degree centigrade at 40 degree centigrade/ min and held for 10 min, then raised to 300℃ at 10 degree centigrade/ min and held for 5 min and finally the maximum temperature was 310 degree centigrade. It was operated on splitless mode. The injector, MS source & MS quad temperature were 60℃, 230℃ and 150℃ respectively. The injection volume was 1 µL. MS were taken at 70 eV with a mass scan range of m/z 40- 550. The compounds were then identified using NIST library database.

Result and discussion

Identification of active constituents of spices

From the GC- MS analysis of the spice samples, various active constituents were detected qualitatively. The results were represented in Tables 2- 6. The findings clearly shows that Black pepper contains piperine, piperidine, pipercitine, beta- carophyllene, 3-carene, alpha- pinene, copaene, delta- elemene, delta- cadinol, muurolene, etc; Cumin contains cuminaldehyde, 1, 3- p- menthadien- 7- al, beta-caryophyllene, pinene, o- cymene, beta-cymene, alpha- terpinene, gamma- terpinene, carotol, cis- carveol, etc; Fennel contains anethole, estragole, fenchone, d- limonene, p-acetonylanisole, etc; Coriander contains linalool, geranyl vinyl ether, nerolidol, oleic acid, lactic acid, leucine, nerolidyl acetate etc and Turmeric contains alpha- curcumene, beta-curcumene, turmerone, turmerol, curlone, atlantone, zingiberene, tumerone, nuciferol, zizanol, etc. Major flavor compounds, according to the spice board of India detected in the spices by the GC- MS analysis were Piperine, Carophyllene & 3- Carene in black pepper; Cuminaldehyde and 1, 3- methadien- 7-al in cumin; Anethole and Fenchone in fennel; Linalool and Geranyl vinyl ether in coriander and Turmerone and Zingeberene in turmeric.

Table 4- Volatile compounds detected in fennel, hexane and ethanol extracts (SD-H, SD-E, SM-H, SM-E, SB-H & SB-E) by GC-MS analysis.

Compounds	SD-H	SD-E	SM-H	SM-E	SB-H	SB-E
1- chloro- decane			✓		✓	
1- chloro- dodecane			✓		✓	
1, 2- Benzenedicarboxylic acid				✓		✓
2- bromo- octadecanal			✓		✓	✓
2- Tetradecanol				✓		✓
2, 3- dimethyl- 55- trifluoromethyl- phen- 1, 4- diol		✓				✓
3- chloro- 2- butanol		✓		✓		✓
3- Eicosanone	✓				✓	
(3E, 5E)- 2, 6- dimethylocta- 3, 5, 7- trien-2- ol		✓		✓		✓
3- ethyl-5-(2- ethylbutyl)- octadecane			✓		✓	
Anethole	✓	✓	✓	✓	✓	✓
beta -Terpineol				✓		✓
cis- Vaccenic acid			✓	✓	✓	✓
cis- Verbenol			✓		✓	
D- Limonene	✓	✓	✓		✓	✓
Estragole	✓	✓	✓	✓	✓	✓
Fenchone	✓	✓	✓	✓	✓	✓
Hexadecanoic acid						✓
Leucine		✓				
Linalyl acetate				✓		✓
Mesitylene			✓			
Nonacosan- 10- one	✓		✓			✓
Oleic acid		✓				✓
p- Acetonylanisole	✓				✓	
p- Menthan- 2- ol	✓					
p- Menth- 8- en- 1- ol		✓				✓
Phthalic acid						✓

Table 5- Volatile compounds detected in coriander, hexane and ethanol extracts (CD-H, CD-E, CM-H, CM-E, CB-H & CB-E) by GC-MS analysis.

Compounds	CD-H	CD-E	CM-H	CM-E	CB-H	CB-E
1-chloro-dodecane			✓		✓	
2-(1-cyclohexenyl)-clyclohexanone		✓				✓
2,4-Dimethylhexane	✓				✓	
2,5-Dihydrobenzoic acid					✓	
2-bromo-octadecanal	✓				✓	
2-Myristynoyl pantheine		✓				✓
9,12-Octadecadienoic acid (Z,Z)-				✓		✓
Acetoin		✓				
cis-7-Hexadecenoic acid				✓		✓
cis-10-Octadecenoic acid		✓			✓	✓
cis-13-Octadecenoic acid	✓				✓	✓
cis-Vaccenic acid				✓		✓
cis-Verbenol, trimethylacetate			✓		✓	
Enol						✓
Ethyl oleate		✓				✓
Geranyl vinyl ether		✓				✓
L-Lactic acid		✓				✓
L-Lucine			✓			
Linalool	✓	✓		✓	✓	✓
Nerolidol	✓				✓	
Nerolidyl acetate			✓		✓	
n-Hexadecanoic acid	✓	✓			✓	✓
Octadecyl vinyl ether						✓
Oleic acid	✓	✓		✓	✓	✓
Phorbol		✓				✓
Pterin-6-carboxylic acid				✓		✓

The following depictions (Fig. 1) show the TIC (total ion chromatogram) scan and MS (mass spectrum) Spectrum for the flavor compounds detected. The chemical profile generated for each spice extract is valuable in their identification and authentication. The samples which were considered authentic on the basis of this analysis were SM and CM.

Detection of adulterants and contaminants

Table 7 describes the qualitative detection of adulterants and contaminants in the samples. All the foreign materials detected in the analysis can be classified into adulterants, contaminants, agricultural contaminants, manufacturing contaminants, and industrial waste. The spices were collected randomly for conducting food forensic investigation, to detect any fraud. A total of 15 samples were analyzed. The samples analyzed as adulterated or contaminated products were PD, JD, CD, TD, SD, PM, JM, and TM. According to the analysis, out of 10 local samples 4 of them were found to be adulterated. In sample JD, the admixture of black cumin was observed; in TD, the admixture of wild turmeric was observed, in PM, the admixture of papaya seeds was observed and in JM, the admixture of aniseed was observed. All the adulterants detected in the study were plant- based, which is difficult to be detected by traditional analysis which involves physical and chemical tests. PD, SD, CD & TM were found to be contaminated by manufacturing, industrial and agrochemical contaminants. PD was contaminated by algae, *Sargassium vulgare*, which might be incorporated from contaminated water; SD was found to be contaminated by agrochemicals, ethylene oxide and carbanilide and also contain plastic waste, dibutyl phthalate; the CD was contaminated with agrochemicals, ethylene oxide and carbanilide, it was also infected by a soil fungus, *Pennicilum expansum* and also contain plastic waste, dibutyl phthalate, and beta- curcumene, ar- turmerone and curlone

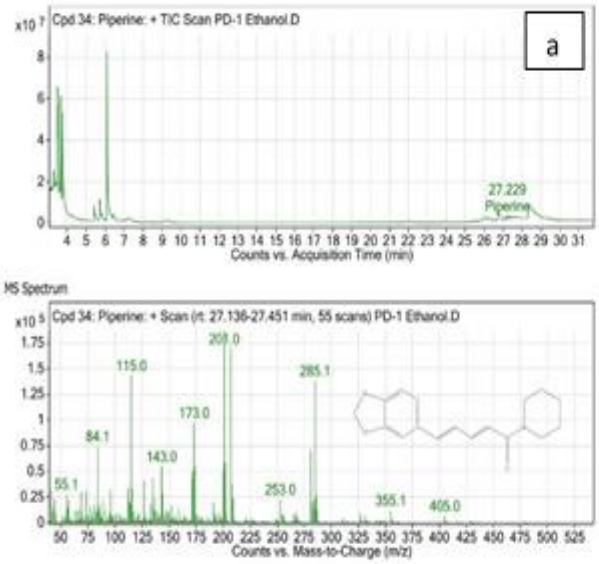
was present as manufacturing cross contaminant; TD was contaminated with a fumigating agent, ethylene oxide and TM was infected by a soil fungus, *Pennicilum expansum* and also found to contain plastic waste. They were also contaminated as a result of agricultural cross- contamination which is non-intentional and might be present due to the cultivation proximity or during distribution and storage of these products. Study on the types of adulterants added in the spices powder according to consumer's suspect has revealed that addition of low-grade raw spice with high grade was at the top position (Sattar et al., 2019).

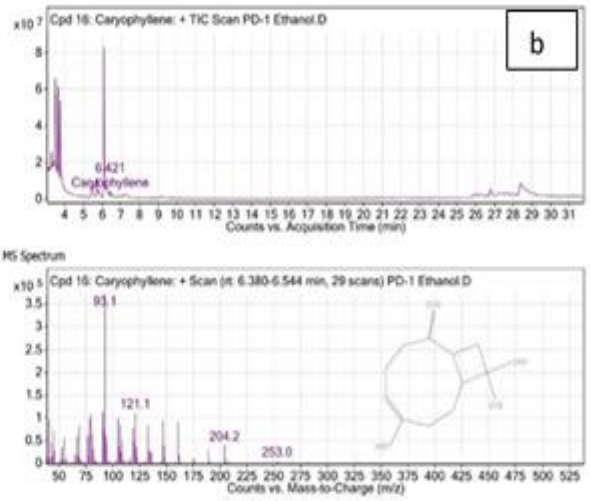
Table 6- Volatile compounds detected in turmeric, hexane and ethanol extracts (TD-H, TD-E, TM-H, TM-E, TB-H & TB-E) by GC-MS analysis.

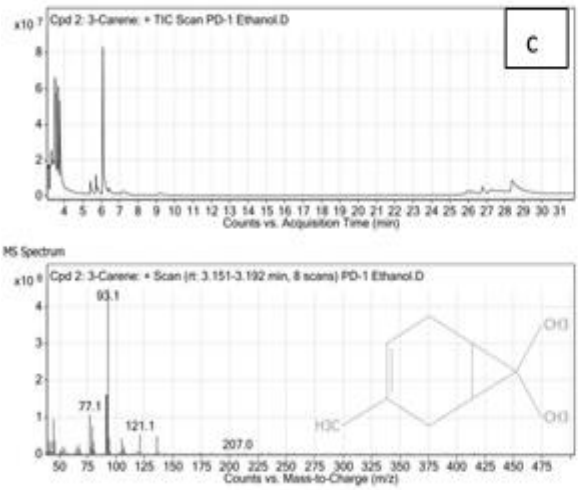
Compounds	TD-H	TD-E	TM-H	TM-E	TB-H	TB-E
1- (3- cyclopentylpropyl)- 2, 4- benzene	✓				✓	
1- Heptatriacotanol		✓				✓
3- Carene				✓		✓
4- Carene				✓		✓
4- epi- alpha- acoradiene	✓					
5`methyl- 2`hydroxy- acetophenone		✓				✓
6S, 7R- Bisabolone			✓		✓	
alpha- Curcumene	✓	✓	✓	✓	✓	✓
aR- Turmerol	✓	✓	✓	✓	✓	✓
aR- Turmerone	✓	✓	✓	✓	✓	✓
beta- Curcumen- 12- ol	✓	✓		✓	✓	✓
beta- Curcumene		✓	✓	✓	✓	✓
beta- Pinene				✓		✓
beta- Sesuiphellandrene	✓	✓	✓	✓		
beta- Turmerone	✓				✓	
Bisabolone				✓		✓
Caryophyllene			✓			
cis- Verbenol, trimethylacetate			✓		✓	
Curlone	✓	✓	✓	✓	✓	✓
Cyclohexene epoxide	✓	✓	✓		✓	✓
(E)- Atlantone	✓	✓	✓	✓	✓	✓
Eudesmol				✓		✓
Hexylene glycol					✓	
Humulenol- II			✓	✓	✓	✓
n- Hexadecanoic acid			✓	✓	✓	✓
Nuciferol		✓	✓	✓	✓	✓
Oleic acid			✓	✓	✓	✓
trans- Sesquisabinene hydrate		✓	✓			
trans- Z- alpha- Bisabolene epoxide			✓		✓	
Tumerone		✓	✓	✓	✓	✓
(Z)- gamma- Atlantone		✓	✓	✓	✓	✓
Zingiberene		✓	✓	✓	✓	✓
Zizanol		✓		✓		✓

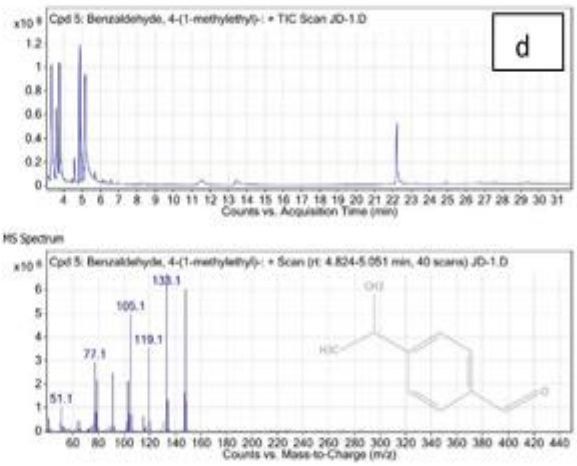
Detection of food fraud is an enormous challenge because consumers cannot detect them and those involved in the fraud business embrace innovative ways to avoid detection. New challenges with regard to addressing food fraud are related to astronomical growth of e-commerce of food. Two connected avenues for preventing and controlling food fraud are legal interventions and technological innovations. TLC method to detect the adulteration of black pepper powder with ground papaya seed has been reported (Paradkar et al., 2001). Detection of trans- anethole in the essential oil composition of cumin seeds has been reported as an indication towards the presence of contamination with low grade fennel seeds in

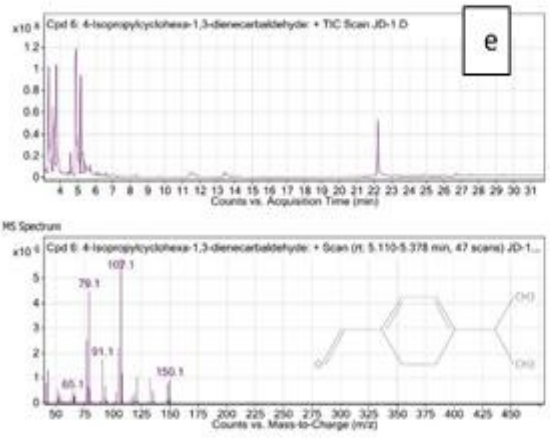
which trans- anethole is a major marker compound (Dinesh Singh Bisht et al., 2014).

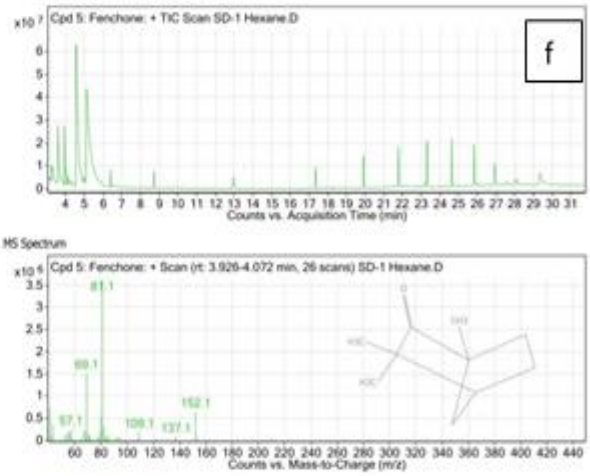












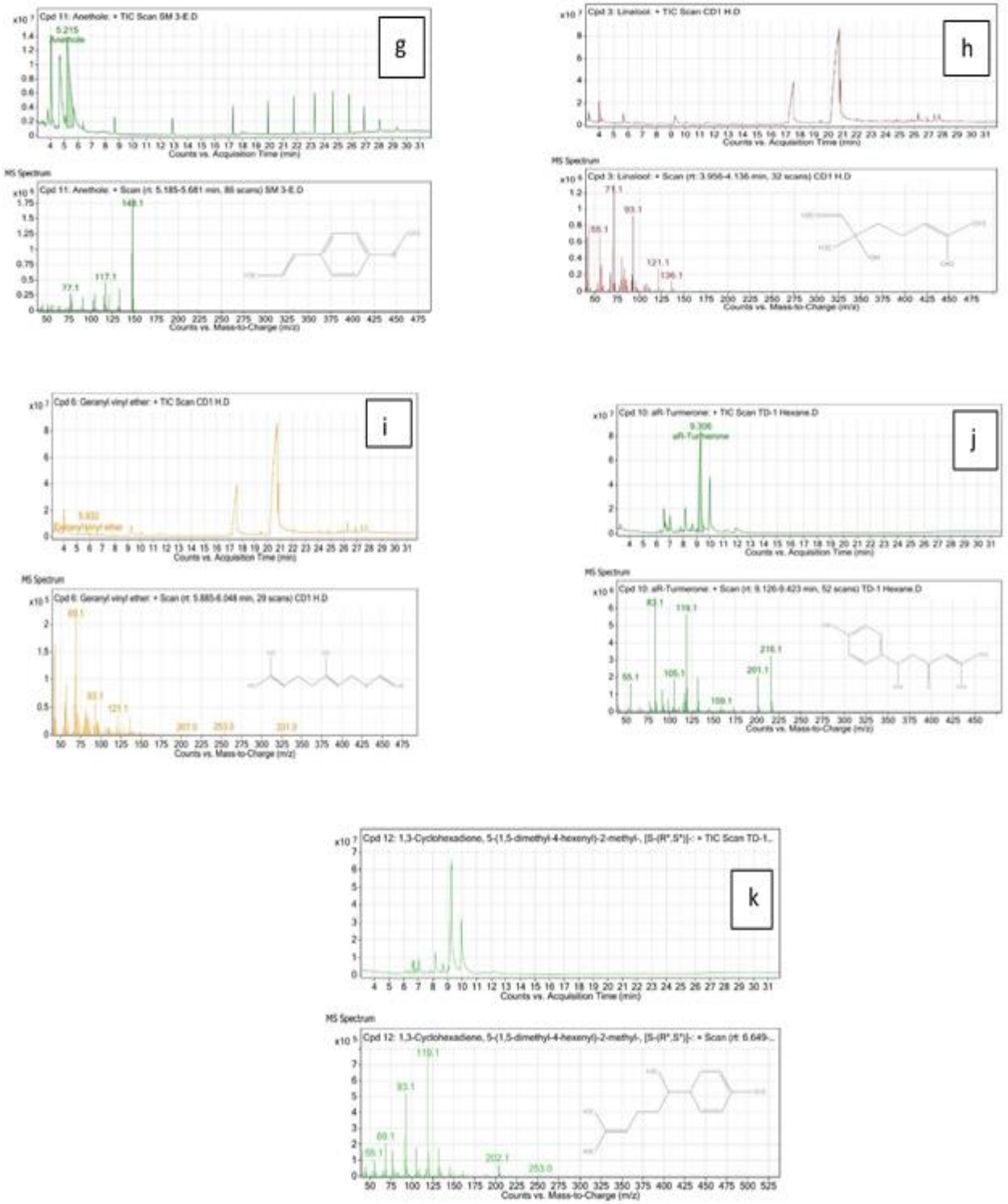


Fig. 1. Flavor compounds detected in the analysis.

TIC scan and MS spectrum of (a) Piperine, (b) Caryophyllene, (c) 3-Carene detected in *Piper nigrum* (black pepper); (d) Cuminaldehyde, (e) 1,3-p-menthadien-7-al detected in *Cuminum cyminum* (cumin); (f) Fenchone (g) Anethole detected in *Foeniculum vulgare* (fennel); (h) Linalool (i) Geranyl vinyl ether detected in *Coriandrum sativum* (coriander) and; (j) Turmerone, (k) Zingiberene detected in *Curcuma longa* (turmeric).

Table 7- Adulterants or contaminants detected in spice samples (*Abbreviations used are: "A" – Adulterant; "C" – Contaminant (including microbial infection and agrochemical residues); "G" – Agricultural cross contaminant; "M" – Manufacturing contaminant; "I" – Industrial waste.)

Compound	Type	Sample	Inference	Ref.
Valencene	G	PD-H PD-E	It is a metabolite of plant, *Piper betle (*Betle vine*)*. The sample might have been contaminated with this while agricultural practice.	Barata et al. (2021)
Dihydro- 3-isooctadecyl- 2, 5- Furandione	C	PD-H	It is a bioactive product of an alga, *Sargassium vulgare.* This was present as a contaminant, from contaminated water bodies.	Shreadah et al. (2018)
Limonen- 6- ol pivalate	A	JD-H	It is a bioactive product of *Nigella sativa* (Black cumin); it was present as an adulterant.	Hadi et al. (2016)
alpha-Longipinene	A	JD-H	It is a volatile of *Nigella sativa* (Black cumin); it was present as an adulterant.	Kabir et al. (2020)
Brassicasterol	G	JD-H	It is a product of rapeseed's EO; it was present as an agricultural cross contaminant.	Gul and Seker, (2006)
Ethylene oxide	C	SD-H CD-H TD-H	It is used as a fumigating agent, which is toxic in nature and thus banned in practice.	https://www.epa.gov/sites/production/files/2016-09/documents/ethylene-oxide.pdf> (assessed on 10.05.21) https://www.who.int/ipcs/publications/cicad/en/cicad54.pdf>(assessed on 10.05.21)
Carbanilide	C	SD-E CD-E	It was present as a pesticide residue.	https://patents.google.com/patent/EP1443045A9/en> (assessed on 10.05.21)
Dibutyl phthalate	I	SD-E CD-E	It is used as plasticizer, which might be present due to poor packaging material.	https://cameochemicals.noaa.gov/chemical/5717> (assessed on 10.05.21)
Lavandulol	G	CD-H	It is an active constituent of lavender's EO and was present as an agricultural cross contaminant.	https://pubchem.ncbi.nlm.nih.gov/compound/4-Hexen-1-ol_-5-methyl-2-_1-methylethenyl> (assessed on 10.05.21)
beta- Curcumen-12- ol	M	CD-H	It is an active compound of turmeric which might be present as a manufacturing cross contaminant.	[Table 6]
ar- Turmerone	M	CD-H CD-E	It is an active compound of turmeric which might be present as a manufacturing cross contaminant.	[Table 6]
Curlone	M	CD-H CD-E	It is an active compound of turmeric which might be present as a manufacturing cross contaminant. This might be because of using unclean industrial instrument during manufacturing.	[Table 6]
2- amino- 5- [(2-carboxyl)vinyl] – Imidazole	C	CD-E TM-E	It is a metabolite which belongs to fungus, *Pennicillum expansum*. It was present as a fungal contaminant.	Hamza et al. (2015)

Formic acid, 3, 7, 11- timethyl- 1, 6, 10- dodecatrien- 3- yl ester	G	CD-E	It is an active compound of *Citrus limonum* (lemon); it was present as an agricultural cross contaminant.	Ainane et al. (2018)
1H- 3a, 7- Methanoazulene	A	TD-H	It is a constituent of *Curcuma aromatica*, which is a wild turmeric species and it was a plant based adulterant which compromises the quality of product.	Umar et al. (2020)
7- (1, 3- dimethylbuta-1, 3- dienyl) -1, 6, 6- trimethyl- 3, 8 – dioxatricyclo [5.1.0.0(2,4)] octane	G	TD-E	It is a phytoconstituent of agarwood which is present in the sample as an agricultural contaminant.	Peng et al. (2020)
Linoleic acid	A	PM-H	It is a phytoconstituent present in papaya seeds. It is the most common adulterant used in black peppercorns.	https://phytochem.nal.usda.gov/phytochem/plants/show/346?qlookup=papaya&offset=0&max=20&et=> (assessed on 17.05.21)
2- bromo- octadecane	A	PM-H PM-E	It is a phytoconstituent present in papaya seeds. It is the most common adulterant used in black peppercorns.	https://phytochem.nal.usda.gov/phytochem/plants/show/346?qlookup=papaya&offset=0&max=20&et=> (assessed on 17.05.21)
Tricosenoic acid	A	PM-H PM-E	It is a phytoconstituent present in papaya seeds. It is the most common adulterant used in black peppercorns.	https://phytochem.nal.usda.gov/phytochem/plants/show/346?qlookup=papaya&offset=0&max=20&et=> (assessed on 17.05.21)
1, 4, 7- Cycloundecatriene, 1, 5, 9, 9- tetramethy- Z, Z, Z-	G	PM-H	It is a phytochemical of *Piper longum*; which is present in sample as an agricultural cross contaminant.	https://assets.researchsquare.com/files/rs-31834/v1/SupplementaryTableS1.docx> (assessed on 17.05.21)
Myristic acid	A	PM-E	It is a phytoconstituent present in papaya seeds. It is the most common adulterant used in black peppercorns.	https://phytochem.nal.usda.gov/phytochem/plants/show/346?qlookup=papaya&offset=0&max=20&et=> (assessed on 17.05.21)
Phthalic acid, butyl hex- 3- yl ester	A	PM-E	It is a phytoconstituent present in papaya seeds. It is the most common adulterant used in black peppercorns.	https://phytochem.nal.usda.gov/phytochem/plants/show/346?qlookup=papaya&offset=0&max=20&et=> (assessed on 17.05.21)
12- hydroxyoctadecanethioic acid, S- t- butyl ester	G	PM-E	*Radermachera xylocarpa*, present as an agricultural contaminant.	Ekade & Manik, (2014)
Artemisia alcohol	A	JM-H	It is an active constituent of aniseed (sweet cumin); which was used as an adulterant in this cumin sample.	Koul et al. (2017)

Thujyl acetate	A	JM-H	It is an active constituent of aniseed (sweet cumin); which was used as an adulterant in this cumin sample.	https://phytochem.nal.usda.gov/phytochem/plants/show/1502?qlookup=Pimpinella+anisum&offset=0&max=20&et=> (assessed on 17.05.21)
Linalool hydroxyl	A	JM-H	It is an active constituent of aniseed (sweet cumin); which was used as an adulterant in this cumin sample.	https://phytochem.nal.usda.gov/phytochem/plants/show/1502?qlookup=Pimpinella+anisum&offset=0&max=20&et=> (assessed on 17.05.21)
gamma-Cadinene	A	JM-H	It is an active constituent of aniseed (sweet cumin); which was used as an adulterant in this cumin sample.	https://phytochem.nal.usda.gov/phytochem/plants/show/1502?qlookup=Pimpinella+anisum&offset=0&max=20&et=> (assessed on 17.05.21)
cis-beta-Farnesene	A	JM-H	It is an active constituent of aniseed (sweet cumin); which was used as an adulterant in this cumin sample.	https://phytochem.nal.usda.gov/phytochem/plants/show/1502?qlookup=Pimpinella+anisum&offset=0&max=20&et=> (assessed on 17.05.21)
cis- Thujapsene	A	JM-H	It is an active constituent of aniseed (sweet cumin); which was used as an adulterant in this cumin sample.	https://phytochem.nal.usda.gov/phytochem/plants/show/1502?qlookup=Pimpinella+anisum&offset=0&max=20&et=> (assessed on 17.05.21)
Bisabolene	A	JM-H	It is an active constituent of aniseed (sweet cumin); which was used as an adulterant in this cumin sample.	https://phytochem.nal.usda.gov/phytochem/plants/show/1502?qlookup=Pimpinella+anisum&offset=0&max=20&et=> (assessed on 17.05.21)
alpha- Selinene	A	JM-H	It is an active constituent of aniseed (sweet cumin); which was used as an adulterant in this cumin sample.	https://phytochem.nal.usda.gov/phytochem/plants/show/1502?qlookup=Pimpinella+anisum&offset=0&max=20&et=> (assessed on 17.05.21)
Fumaric acid, 3-phenylpropyl-tridec- 2- yn- 1- yl ester	I	TM-H TM-E	It is a plastic waste which might have incorporated in the product due to poor packaging material.	Chiwara et al. (2018)
Cyclohexene, 4-isopropenyl- 1 –methoxymethoxy - methyl-	G	TM-E	It is present as an agricultural contaminant; it belongs to Artemisia annua.	Hameed et al. (2016)

Bicyclo [4.4.0]dec- 2- ene- 4- ol, 2- methyl- 9- (prop- 1- en- 3-ol- 2- yl)-	G	TM-E	It is a constituent of Ginger; which is present as a result of agricultural cross contamination.	Shareef et al. (2016)
Estra- 1, 3, 5 (10)- trien- 17- beta-ol	G	TM-E	It is a constituent of Ginger; which is present as a result of agricultural cross contamination.	Shareef et al. (2016)

Since quality control and safety monitoring has become a pressing issue for the spice industry. DNA barcoding in combination with morphological sorting and DNA metabarcoding, a combinatory approach of microscopy, chemical analysis and classical DNA barcoding of the isolated contaminants using the *matK* and *trnHpsbA* loci has been reported to provide qualitative and quantitative information on the amount of plant material responsible for the contaminations and the extent of the contamination (Willocx et al., 2014).

Literature survey reveals that the quality of spice is lost due to the presence of contaminants, filth matter, mycotoxins and heavy metals. Techniques like IRMS, NMR, FT- IR acts as a fingerprinting tool to effectively detect adulteration of spices and spice products even at trace levels (Bharathi et al., 2018). However, the major drawbacks in these technologies are their establishment and running cost. Thus the robustness of GC- MS as an analytical technique in detecting adulterants discussed in this paper is of significance in the forensic examination of seizures of spices. The adulterations identified in this study were economically motivated and the admixture of such cheaper and similar- looking plant materials does compromise the quality of the spices.

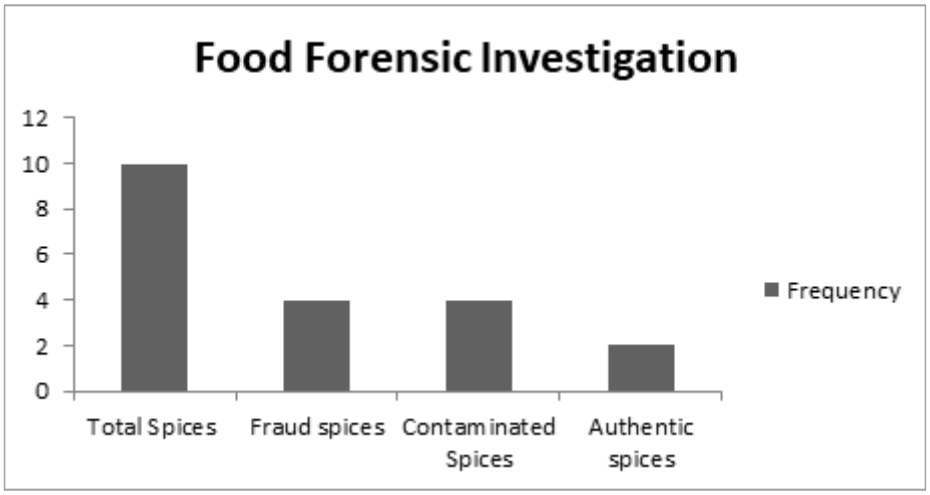


Fig. 2. Food forensic investigation; Bar graph showing the numbers of spices found to be adulterated, contaminated and those which were safe from any adulterants and contaminants.
Only the local products are taken into the consideration as branded samples were only used for reference.

The fraud detected in this investigation was adulteration; where adulterants that look similar to authentic spices were sold in the local markets. The algal or fungal contamination observed in PD, CD and TM is due to contamination of the environment surrounding cultivation, irrigation, distribution, or storage. Hygiene must be maintained in the food industry, it is important for the production of safe and high- quality products. The plastic waste or product of plasticizer detected in the GC- MS analysis represent the poor choice of

packaging which is done in local market and needs to be upgraded with much better biodegradable option. The agrochemicals detected in the sample were carbanilide and ethylene oxide; both are unsafe for human consumption. The food forensic investigation conducted can be summarized in the following chart (Fig. 2).

Differentiation of samples based on their source

Many phytoconstituents were detected through the GC- MS analysis of the spices. The spices were collected from two different regions Delhi and Uttar Pradesh in India. The differences between them can be established by the help of the chemical profiles of the spices. PD contains piperine and piperidine while PM contains piperine, pipercitine and piperanine. JD contains o- cymene and gamma- muurolene while JM contains beta- cymene and 3- caren-10- al. SD contains p- menthan- 2- ol, p-acetonylanisole and p- menth- 8- en- 1- ol while SM contains mesitylene and beta- terpineol. CD contains geranyl vinyl ether while CM contains nerolidyl acetate. TD contains beta- turmerone and TM contains beta- pinene and caryophyllene. Further comparisons of both the varieties, on the basis of number of active constituents detected and number of foreign compounds detected in the samples are represented in Fig. 3.

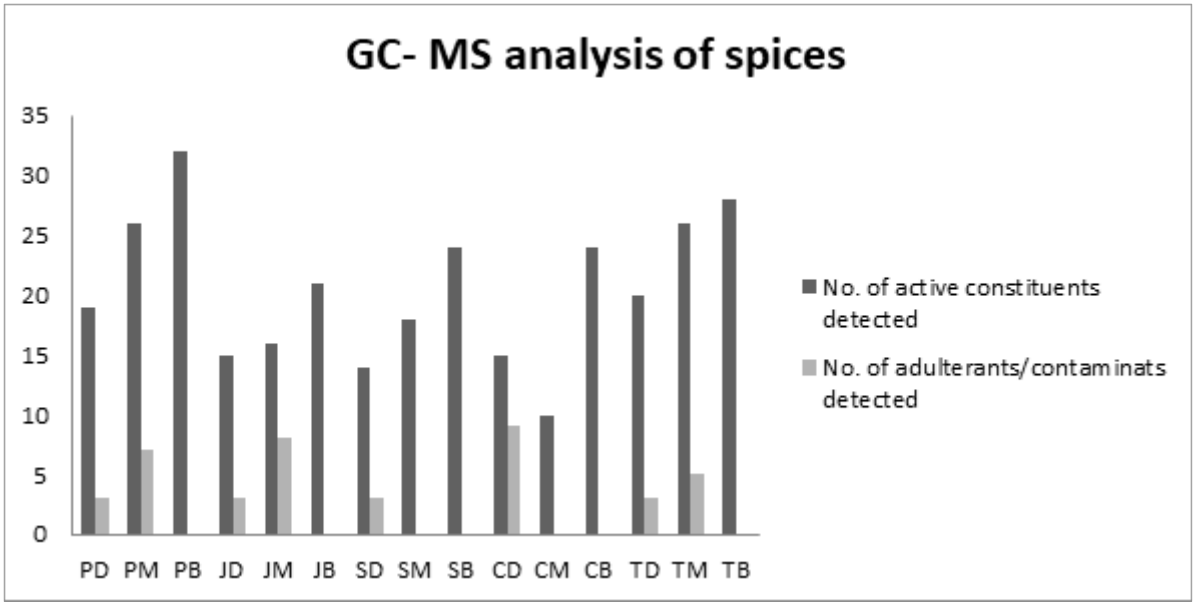


Fig. 3. Bar-graph showing the comparison done between the different samples.

The GC- MS qualitative analysis of spices results in formation of a chemical profile which can be used in identifying authentic products. Also it assists in detection of plant-based adulterants, agrochemicals, microbial infections and even plasticizers used in packaging. The instrument is very sensitive and can detect compounds in minute concentration and thus able to identify both primary and secondary metabolites of the spice samples. The chemical profile is useful for identification as well as differentiation of samples from different source. The volatiles of spice's extracts were readily analyzed using the GC-MS analysis and thus it proves to be an effective analytical tool in food analysis.

Conclusion

Spice adulteration is a highly dynamic fraudulent practice. Economically motivated adulteration is found in a wide variety of spices and adulteration is now a global challenge and at risk are spices and herbs as exemplified by the simple adulteration of cumin with anise

seeds. Despite having a regulatory body the food fraud instances are rising globally and especially during the pandemic covid- 19 there have been numerous instances of panic buying, buying from e- commerce websites which diluted the quality requirements and checks of spices used in the present study i.e. black pepper, cumin, fennel, coriander and turmeric. The food fraud investigations conducted by the food authorities are mostly targeted analyses. Therefore non- targeted analysis may prove to be more effective in food fraud detection. Analytical techniques like visual/ sensorial evaluations of plant material with microscopy, chromatographic and spectroscopic methods have been reported there to detect the adulteration of spices and herbs. Food forensic investigation conducted on the spices using GC- MS technique proved to be effective in detecting adulterants as well as contaminants and the qualitative results may be an important reference for law authorities to prevent and control food quality and health safety issues involved therein. The GC- MS qualitative analysis helps to authenticate the spices and the chemical profile generated can be a reference tool in further differentiation based on the sources and quality of the product. DNA- based techniques have now emerged as authentication tool for discrimination between closely related species and cultivars. Essential oil compositions of spices may be a future tool for predicting adulteration/ contamination and further studies on standardization and validation of active ingredients using various instrumentation techniques like GC- MS, HPTLC- MS, LC- MS can be undertaken for food fraud investigation.

Acknowledgement

The authors express their sincere gratitude to Mrs. Deepa Verma, Director, Forensic Science laboratory, NCT of Delhi, Shri K.C. Varshney, Head of office, Regional Forensic Science Laboratory, Chanakyapuri, New Delhi for their keen interest and encouragement. Research experience for postgraduate participant was supported by Department of Anthropology, University of Delhi.

Contribution of authors

Dr.Kanak Lata Verma: Conceptualizing, designing, supervising and interpreting the data pertaining to this research article.

Dr. Pallavi Choudhary: literature review, instrumental analysis, data analysis and participated in drafting the manuscript.

ArpitaSethi: literature review, data analysis and participated in drafting the manuscript.

References

1. Aceto, M. (2015). Food forensics. In *Comprehensive Analytical Chemistry* (Vol. 68, pp. 441-514). Elsevier. https://doi.org/10.1016/B978-0-444-63340-8.00009-1
2. Ainane, A., Khammour, F., Charaf, S., Elabboubi, M., Elkouali, M., Talbi, M., ... & Ainane, T. (2019). Chemical composition and insecticidal activity of five essential oils: *Cedrus atlantica*, *Citrus limonum*, *Rosmarinus officinalis*, *Syzygium aromaticum* and *Eucalyptus globules*. *Materials Today: Proceedings*, 13, 474- 485. https://doi.org/10.1016/j.matpr.2019.04.004
3. Arrests in Indian spice adulteration.https://www.intracen.org/blog/Arrests-in-Indian-spice-adulteration.Accessed onDecember 25,2021.
4. Atkins, P. (2015). Analysis of Organic Marker Compounds and Hazardous Organic Compounds by GC/MS to Identify Contamination, Counterfeiting and Adulteration of Spices, Inorganic and Organic Certified materials, SPEXCertiPrep, https://cdn.technologynetworks.com/ep/pdfs/analysis-of-organic-marker-compounds-and-hazardous-organic-compounds-by-gcms-to-identify.pdf
5. Attokaran M., Natural food flavors & colorants, Wiley-Blackwell, 2011

6. Bansal, S., Singh, A., Mangal, M., Mangal, A. K., & Kumar, S. (2017). Food adulteration: Sources, health risks, and detection methods. *Critical reviews in food science and nutrition*, 57(6), 1174-1189. DOI:10.1080/10408398.2014.967834
7. Barata, L. M., Andrade, E. H., Ramos, A. R., de Lemos, O. F., Setzer, W. N., Byler, K. G., ... & da Silva, J. K. R. (2021). Secondary metabolic profile as a tool for distinction and characterization of cultivars of black pepper (*Piper nigrum* L.) cultivated in Pará State, Brazil. *International journal of molecular sciences*, 22(2), 890.doi: 10.3390/ijms22020890
8. Bessaire, T., Savoy, M. C., Mujahid, C., Tarres, A., & Mottier, P. (2019). A new high-throughput screening method to determine multiple dyes in herbs and spices. *Food Additives & Contaminants*: Part A, 36(6), 836-850., doi: https://doi.org/10.1080/19440049.2019.1596320
9. Bhoomaa, V., Nagasathiya, K., Vairamani, M., & Parani, M. (2020). Identification of synthetic dyes magenta III (new fuchsin) and rhodamine B as common adulterants in commercial saffron. *Food chemistry*, 309, 125793.doi: https://doi.org/10.1016/j.foodchem.2019.125793
10. Braden D. T. (2014). Food fraud and adulterated ingredients – Background, Issues and Federal action, Nova science publishers
11. Carbanilide; https://patents.google.com/patent/EP1443045A9/en(assessed on 10.05.21)]
12. Chiwara, B., Makhura, E., Danha, G., Hlabangana, N., Gorimbo, J., & Muzenda, E. (2018). Optimization of the pyrolysis oil fraction: An attainable region approach. *Detritus*, (3), 68.doi: 10.31025/2611-4135/2018.13691
13. Danciu, V., Hosu, A., & Cimpoiu, C. (2018). Thin-layer chromatography in spices analysis. Journal of Liquid Chromatography & Related Technologies, 41(6), 282-300. https://doi.org/10.1080/10826076.2018.1447895
14. Dibutyl phthalate; https://cameochemicals.noaa.gov/chemical/5717(assessed on 10.05.21)
15. Dinesh Singh Bisht, A. K, Ramakrishna, A. G, Venugopal. (2022). Trans-Anethole Based Detection of Adulteration of Fennel (*FoeniculumVulgare Mill*.) Seeds in Cumin (*Cuminum Cyminum* L.) Seeds Using GC & GCMS. *International Journal of Innovative Research in Science & Engineering*, 2; 811-816.
16. Dr. Duke's Phytochemical and Ethnobotanical Databases; Chemicals found in Carica papaya; https://phytochem.nal.usda.gov/phytochem/plants/show/346?qlookup=papaya&offset=0&max=20&et= (assessed on 17.05.21)
17. Dr. Duke's Phytochemical and Ethnobotanical Databases; Chemicals found in Pimpinella anisum;https://phytochem.nal.usda.gov/phytochem/plants/show/1502?qlookup=Pimpinella+anisum&offset=0&max=20&et= (assessed on 17.05.21)
18. Ekade, P. P., & Manik, S. R. (2014). Investigations on secondary metabolites in different parts of Radermachera xylocarpa using GC-MS. Journal of Pharmacognosy and Phytochemistry, 2(6), 39-47. https://www.phytojournal.com/archives/2014.v2.i6.274/investigations-on-secondary-metabolites-in-different-parts-of-radermachera-xylocarpa-using-gc-ms
19. Ethyleneoxide; https://www.epa.gov/sites/production/files/2016-09/documents/ethylene-oxide.pdf> (assessed on 10.05.21)
20. Ethylene oxide; https://www.who.int/ipcs/publications/cicad/en/cicad54.pdf (assessed on 10.05.21)
21. Everstine, K., Spink, J., & Kennedy, S. (2013). Economically motivated adulteration (EMA) of food: common characteristics of EMA incidents. Journal of food protection, 76(4), 723-735., doi: 10.4315/0362-028X.JFP-12-399
22. Farag, M. A., Hegazi, N., Dokhalahy, E., & Khattab, A. R. (2020). Chemometrics based GC-MS aroma profiling for revealing freshness, origin and roasting indices in saffron spice and its adulteration. Food chemistry, 331, 127358.doi: https://doi.org/10.1016/j.foodchem.2020.127358

23. Flavor profile of spices, Spice Board India, https://www.indianspices.com/spices-development/properties/flavor-profile-spices.html (Accessed on December 25, 2021).
24. Food and Agriculture Organization Corporate Statistical Database (FAOSTAT), Crops, 2019, website <http://www.fao.org/faostat/en/#data/QC (assessed on 08.03.2021)
25. Food Contamination in India: Fake 'Cumin Seeds' Factory Busted,Gurneel Kaur,22.11.2019; https://www.grainmart.in/news/food-contamination-in-india-fake-cumin-seeds-factory-busted. Accessed onDecember 25,2021.
26. Food fraud– Intention, detection and management. Food safety technical toolkit for Asia and the Pacific No. 5. Bangkok,Food and Agriculture Organization of the United Nations Bangkok, 2021.
27. FSSAI enforcement report, website <https://www.fssai.gov.in/cms/enforcementreport.php (assessed on 10.03.2021)
28. Gul, I., Nasrullah, N., Nissar, U., Saifi, M., & Abdin, M. Z. (2018). Development of DNA and GC-MS Fingerprints for Authentication and Quality Control of *Piper nigrum* L. and Its *Adulterant Carica papaya* L. *Food analytical methods*, 11(4), 1209- 1222. https://link.springer.com/article/10.1007/s12161-017-1088-7
29. Gul, M. K., & Şeker, M. (2006). Comparative analysis of phytosterol components from rapeseed (*Brassica napus* L.) and olive (*Olea europaea* L.) varieties. *European Journal of Lipid Science and Technology*, 108(9), 759-765.doi:10.1002/ejlt.200600085
30. Hadi Mohammed, Y., Ghaidaa, J. M., & Imad, H. H. (2016). Analysis of bioactive chemical compounds of Nigella sativa using gas chromatography-mass spectrometry. *Journal of Pharmacognosy and Phytotherapy*, 8(2), 8-24.doi: https://doi.org/10.5897/JPP2015.0364
31. Hakme, E., Lozano, A., Uclés, S., Gómez-Ramos, M. M., & Fernández-Alba, A. R. (2018). High-throughput gas chromatography-mass spectrometry analysis of pesticide residues in spices by using the enhanced matrix removal-lipid and the sample dilution approach. Journal of Chromatography A, 1573, 28-41., doi: https://doi.org/10.1016/j.chroma.2018.08.046
32. Hamza, L. F., Kamal, S. A., & Hameed, I. H. (2015). Determination of metabolites products by Penicillium expansum and evaluating antimicobial activity. *Journal of Pharmacognosy and Phytotherapy*, 7(9), 194-220.doi:https://doi.org/10.5897/JPP2015.0360
33. Hameed I. H., Altameme H. J., and Idan S. A. (2016). Artemisia annua: Biochemical products analysis of methanolic aerial parts extract and anti-microbial capacity, *Research Journal of Pharmaceutical, Biological and Chemical Sciences*.
34. Hong, E., Lee, S. Y., Jeong, J. Y., Park, J. M., Kim, B. H., Kwon, K., & Chun, H. S. (2017). Modern analytical methods for the detection of food fraud and adulteration by food category. *Journal of the Science of Food and Agriculture*, 97(12), 3877-3896. https://doi.org/10.1002/jsfa.8364
35. https://www.indiatoday.in/crime/story/fake-cumin-made-from-broom-bits-sized-up-rae-bareli-1626995-2019-12-10.Accessed on December 25,2021.
36. Indian trade portal, spice industry in India, website <https://www.indiantradeportal.in/vs.jsp?lang=0&id=0,30,50,180#:~:text=India%20is%20the%20 world's%20largest,the%20global%20trading%20in%20spices (assessed on 08.03.2021)
37. Jaiswal, S., Yadav, D. S., Mishra, M. K., & Gupta, A. K. (2016). Detection of adulterants in spices through chemical method and thin layer chromatography for forensic consideration. Int J Dev Res, 6(08), 8824-8827.<https://www.journalijdr.com/sites/default/files/issue-pdf/5989.pdf>
38. Kabir, Y., Akasaka-Hashimoto, Y., Kubota, K., & Komai, M. (2020). Volatile compounds of black cumin (*Nigella sativa* L.) seeds cultivated in Bangladesh and India. *Heliyon*, 6(10), e05343. https://doi.org/10.1016/j.heliyon.2020.e05343
39. King P. G., Haughey S. A. & Elliott C. (2017). T., Herb and Spice Fraud; the Drivers, *Challenges and Detection, Food Control, 2017*, https://doi.org/10.1016/j.foodcont.2017.12.031

40. Koul, B., Taak, P., Kumar, A., Khatri, T., & Sanyal, I. (2018). The Artemisia genus: A review on traditional uses, phytochemical constituents, pharmacological properties and germplasm conservation. J. Glycom. *Lipidom*, 7, 1-7.
41. Lavandulol;https://pubchem.ncbi.nlm.nih.gov/compound/4-Hexen-1-ol_-5-methyl-2-_1-methylethenyl (assessed on 10.05.21)
42. Lees M., Food authenticity and traceability, CRC press, 2000
43. Marie Willocx, M., Van der Beeten, I., Asselman, P., Delgat, L., Baert, W., Janssens, S. B., ... & Vanhee, C. (2022). Sorting out the plants responsible for a contamination with pyrrolizidine alkaloids in spice seeds by means of LC-MS/MS and DNA barcoding: Proof of principle with cumin and anise spice seeds. Food Chemistry: *Molecular Sciences*, 4, 100070. https://doi.org/10.1016/j.fochms.
44. Matsushita, T., Zhao, J. J., Igura, N., & Shimoda, M. (2018). Authentication of commercial spices based on the similarities between gas chromatographic fingerprints. Journal of the Science of Food and Agriculture, 98(8), 2989-3000.doi:https://doi.org/10.1002/jsfa.8797
45. Mermelstein N.H., Food Forensics: Investigating Food Crime, Food Technology, IFT, 2018, website,https://www.ift.org/news-and-publications/food-technology magazine/ issues/2018/ january/columns/food-safety-and-quality-food-crimes-adulteration-food-fraud (assessed on 20.03.2021)
46. Msagati T. A. M., Food Forensics and Toxicology, Wiley Blackwell, 2018
47. Osman, A. G., Raman, V., Haider, S., Ali, Z., Chittiboyina, A. G., & Khan, I. A. (2019). Overview of analytical tools for the identification of adulterants in commonly traded herbs and spices. *Journal of AOAC International*, 102(2), 376-385.doi:https://doi.org/10.5740/jaoacint.18-0389
48. Pal, M., & Mahinder, M. (2020). Food adulteration: a global public health concern. Food Drink Indust, 38-40.
49. Paradkar, M. M., Singhal, R. S., & Kulkarni, P. R. (2001). A new TLC method to detect the presence of ground papaya seed in ground black pepper. *Journal of the Science of Food and Agriculture*, 81(14), 1322-1325.
50. Pastor, K., Ačanski, M., & Vujić, D. (2019). Gas chromatography in food authentication. In Gas Chromatography-Derivatization, Sample Preparation, Application (p. 109). IntechOpen.
51. Peng, D. Q., Yu, Z. X., Wang, C. H., Gong, B., Liu, Y. Y., & Wei, J. H. (2020). Chemical Constituents and Anti-Inflammatory Effect of Incense Smoke from Agarwood Determined by GC-MS. International Journal of Analytical Chemistry, 2020.doi: https://doi.org/10.1155/2020/4575030
52. Piper longum; https://assets.researchsquare.com/files/rs-31834/v1/SupplementaryTableS1.docx> (assessed on 17.05.21)
53. Raghavan S., Handbook of spices, seasonings and flavorings, CRC Press, 2006
54. Rani, R., Medhe, S., & Srivastava, M. (2015). HPTLC–MS based method development and validation for the detection of adulterants in spices. Journal of Food Measurement and Characterization, 9(2), 186-194. DOI: 10.1007/s11694-015-9223-x
55. Sattar, S., Das, P. C., Hossain, M. S., Sarower, K., & Uddin, M. B. (2019). Study on Consumer Perception towards Quality of Spices Powder Available in Bangladesh. Open Journal of Safety Science and Technology, 9(4), 137-144.https://doi.org/10.4236/ojsst.2019.94009
56. Seasoning and Spices Market Size, Share & Trends Analysis Report By Product (Herbs, Salt & Salts Substitutes, Spices), 2020; <Global Seasonings & Spices Market Size Report, 2020-2027 (grandviewresearch.com)> (assessed on 02.02.22)
57. Sebaei, A. S., Youssif, M. I., & Ghazi, A. A. M. (2019). Determination of seven illegal dyes in Egyptian spices by HPLC with gel permeation chromatography clean up. *Journal of Food Composition and Analysis*, 84, 103304. doi: https://doi.org/10.1016/j.jfca.2019.103304

58. Shareef, H. K., Muhammed, H. J., Hussein, H. M., & Hameed, I. H. (2016). Antibacterial effect of ginger (Zingiber officinale) roscoe and bioactive chemical analysis using gas chromatography mass spectrum. *Oriental Journal of Chemistry*, 32(2), 20-40.doi: http://dx.doi.org/10.13005/ojc/320207
59. Shreadah, M. A., El Moneam, N. M. A., Al-Assar, S. A., & Nabil-Adam, A. (2018). Phytochemical and pharmacological screening of *Sargassium vulgare* from Suez Canal, Egypt. *Food science and biotechnology*, 27(4), 963-979.DOI: 10.1007/s10068-018-0323-3
60. Spice Board halts export of adulterated cumin consignment. Press Trust of India. https://www.business-standard.com/article/pti-stories-board-halts-export-of-adulterated-cumin-consignment-118051001252_1.html. Accessed on December 25, 2021.
61. Tahri, K., Tiebe, C., El Bari, N., Hübert, T., & Bouchikhi, B. (2016). Geographical provenience differentiation and adulteration detection of cumin by means of electronic sensing systems and SPME-GC-MS in combination with different chemometric approaches. *Analytical Methods*, 8(42), 7638-7649. https://doi.org/10.1039/C6AY01906D
62. Traders arrested from Burrabazar on cumin dulterationcharges.https://www.telegraphindia.com/my-kolkata/news/trader-arrested-from-burrabazar-on-cumin-adulteration-charges/cid/1836396.
63. Umar, N. M., Parumasivam, T., Aminu, N., & Toh, S. M. (2020). Phytochemical and pharmacological properties of *Curcuma aromatica Salisb* (wild turmeric). *Journal of Applied Pharmaceutical Science*, 10(10), 180-194.doi:10.7324/JAPS.2020.1010018
64. Vadivel, V., Ravichandran, N., Rajalakshmi, P., Brindha, P., Gopal, A., & Kumaravelu, C. (2018). Microscopic, phytochemical, HPTLC, GC– MS and NIRS methods to differentiate herbal adulterants: Pepper and papaya seeds. Journal of Herbal Medicine, 11, 36-45. https://doi.org/10.1016/j.hermed.2018.01.004
65. Bharathi, S. K. V., Sukitha, A., Moses, J. A., & Anandharamakrishnan, C. (2018). Instrument-based detection methods for adulteration in spice and spice products–A review. *Journal of Spices and Aromatic Crops*, 27(2), 106-118.
66. Xiao Ma, D., Mao, W. W., Zhou, P., Li, P., & Li, H. J. (2015). Distinguishing *Foeniculum vulgare* fruit from two adulterants by combination of microscopy and GC–MS analysis. Microscopy Research and Technique, 78(7), 633-641. DOI: 10.1002/jemt.22523
67. Yichen, H, Y., Kong, W., Yang, X., Xie, L., Wen, J., & Yang, M. (2014). GC–MS combined with chemometric techniques for the quality control and original discrimination of *Curcumae longae* rhizome: Analysis of essential oils. *Journal of separation science*, 37(4), 404-411. DOI:10.1002/jssc.201301102

Iranian Food Science and Technology Research Journal
Vol. 18, No. 3, Aug. Sep. 2022, p. 31-51

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد ۱۸، شماره ۳، مرداد – شهریور ۱۴۰۱، ص. ۵۱-۳۱

شناسایی مواد تقلبی و آلاینده‌ها در فلفل سیاه، زیره، رازیانه، گشنیز و زردچوبه با استفاده از تکنیک GC-MS برای تحقیقات پزشکی قانونی

P. Choudhary[1], A. Seth[2], K. Lata Verma[3*]

تاریخ دریافت: ۱۴۰۰/۱۲/۱۲
تاریخ پذیرش: ۱۴۰۱/۰۱/۲۹

چکیده

تحقیقات پزشکی قانونی برای شناسایی مسائل کیفیت و ایمنی مرتبط با غذا و محصول آن بسیار حیاتی است. از GC-MS به‌طور گسترده در تجزیه و تحلیل مواد غذایی استفاده می‌شود. مطالعه حاضر، برای شناسایی ترکیبات فعال برخی از ادویه‌های خانگی و همچنین برای شناسایی مواد تقلبی و آلاینده‌هایی که ممکن است در نمونه‌ها وجود داشته باشد، استفاده شده است. ادویه‌های مورد استفاده در تحقیق حاضر فلفل سیاه، زیره، رازیانه، گشنیز و زردچوبه می‌باشد. عصاره ادویه‌ها با شناسایی ترکیبات فعال مختلف ادویه‌ها، مانند پیپرین، کاریوفیلن و ۳-کارن در فلفل سیاه، کومینالدئید و ۱، ۳- متادین- ۷- آل در زیره سبز، آنتول و فنچون در رازیانه، لینالول و ژرانیل وینیل اتر در گشنیز و تورمرون و زینجی برن در زردچوبه در احراز هویت این ادویه‌ها مفید بودند. مواد تقلبی شناسایی‌شده از نظر کیفی، تقلبی‌های گیاهی بودند. ثابت شده است که GC-MS یک ابزار موثر در تشخیص مواد تقلبی گیاهی، آلاینده‌های میکروبی، و پسماندهای شیمیایی کشاورزی و همچنین زباله‌های صنعتی و تولیدی است.

واژه‌های کلیدی: GC-MS، تقلب ادویه، احراز هویت ادویه، آلاینده‌های ادویه، کیفی.

۱و ۳- آزمایشگاه منطقه‌ای علوم پزشکی قانونی، دولت NCT دهلی، وزارت خانه، Chanakyapuri، دهلی نو، ۱۱۰۰۲۱، هند.
۲- گروه انسان شناسی، دانشگاه دهلی، دهلی، ۱۱۰۰۰۷، هند.

Iranian Food Science and Technology Research Journal
Vol. 18, No. 3, Aug. Sep. 2022, p. 53-68

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد ۱۸، شماره ۳، مرداد - شهریور ۱۴۰۱، ص. ۶۸-۵۳

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

Full Research Paper

Antioxidant and antibacterial properties of borage (*Echium amoenum* L.) and hollyhock (*Althaea rosea* var. *Nigra*) extracts obtained through soaking and ultrasonic-assisted extraction methods

M. Zamankhani[1], S. Moeini[1*], P. Mahasti Shotorbani[2], H. Mirsaeedghazi[3], A. Jafarpour[4]

Received: 2022.04.26
Accepted: 2022.06.08

How to cite this article:
Zamankhani, M., Moeini, S., Mahasti Shotorbani, P., Mirsaeedghazi, H., Jafarpour, A. (2022). Antioxidant and antibacterial properties of borage (*Echium amoenum* L.) and hollyhock (*Althaea rosea* var. *Nigra*) extracts obtained through soaking and ultrasonic-assisted extraction methods. *Iranian Food Science and Technology Research Journal*. 18 (3), 53-68.

Abstract

This study aimed to investigate antimicrobial and antioxidant activities of borage (*Echium amoenum* L.) and hollyhock (*Althaea rosea* var. *Nigra*) extracts. The extracts were obtained through soaking and ultrasound-assisted methods using water or methanol as a solvent. The total phenols and flavonoid, anthocyanin content, free radical scavenging activity, ferric reducing antioxidant power, and antibacterial capacity of the extracts were determined. Phenolic acids were identified using the HPLC chromatogram. It was found that the ultrasound-assisted extraction was more efficient compared to the soaking method. The results showed that in the TPC, anthocyanins, and the FRAP tests, the highest amount was related to the samples extracted using the ultrasound- assisted method with water as solvent. The highest amount of TFC was obtained through a soaking method using methanol as the solvent. Anti- radical activity of the samples indicated that using water as a solvent in the optimum method resulted in a higher antioxidant activity. Furthermore, bacterial alpha amylase inhibition test signified that the inhibitory effect was boosted by increasing the extract concentration. The HPLC analysis of the borage and hollyhock extracts revealed that gallic acid and Syringic acid were the most prominent phenolic compounds. Generally, the results showed a good antibacterial property against *Staphylococcus aureus* for borage and hollyhock extracts. The results give us valuable insight into the potential therapeutic and medicinal applications of borage and hollyhock as a natural preservative to improve immunity.

Keywords: Natural extracts, Soaking method, Ultrasound-assisted extraction, Polyphenols.

Introduction

A variety of palnt materials are known to be a rich sources of natural antioxidants. Dietary and medicinal plants, vegetables, and fruits can provide health benefits and inhibit oxidative damage through scavenging reactive free radicals (Shariffar et al., 2009). Furthermore, the application of antioxidant preservatives in the food processing leads to preserve freshness

1. Department of Food Sciences & Technology, Tehran North Branch, The Islamic Azad University,Tehran, Iran.
2. Department of Food Quality Control and Hygiene, Science and Research Branch, the Islamic Azad University, Tehran, Iran.
3. Department of Food Technology, College of Abouraihan, University of Tehran, Pakdasht, Tehran, Iran.
4. Department of Food Sciences & Technology, Garmsar Branch, the Islamic Azad University, Garmsar, Iran.
Corresponding Author: Sohrabmoeini1359@gmail.com)
DOI: 10.22067/IFSTRJ.2022.76413.1167

while avoiding browning and rancidity of foods. Such antioxidants can be used in particular to inhibit and delay deterioration of lipid products during processing, transportation, and storage (Al-Juhaimi et al., 2018). Historically, medicinal plants have been applied to control the microorganisms responsible for food spoilage in order to enhance the safety of products and also to prolong their shelf life (Nabavi et al, 2015). Borage (*Echium amoenum* L.) is a member of the *Boraginaceae* family and also one of the popular annual herbs used in traditional medicine in many countries. It is a member of t. Borage that is scattered in the northern Iran, Europe, and Mediterranean basin. The leaves and the stems of this herb are bristly and hairy with star- shaped bright blue flowers. Most parts of the plants are used for medical purposes (Abolhassani, 2010). The plant is commonly applied as a decoction whether used alone or combined with other herbs (Pilerood and Prakash, 2014). Traditionally, the plant is used also as an antidepressant, antifebrile, sedative, anti- inflammatory, and to treat influenza, infectious diseases, pulmonary and cardiovascular diseases. It is believed that the plant can be used for different types of cancers (Abolhassani, 2010). Researchers have conducted numerous in vitro, in vivo, and clinical studies to show the therapeutic effects assigned to the borage plant. The studies have shown that the flowers of this herb contain antioxidants (Abolhassani, 2010), antiviral (Ranjbar et al., 2006), and antibacterial activities against Gram-positive and Gram-negative bacteria. It also demonstrates anti-inflammatory effects (Karimi et al., 2018). Hollyhock is another important medicinal plant indigenous to Asia, Europe, and the United States which is traditionally used to treat throat irritation, dry cough, insect bites, mild gastritis, and skin burns. In addition, it can be used to treat inflammation, wound, abscess, constipation and diarrhea (Shah et al., 2011). Different parts of this plant contain various bioactive compounds such as mucilaginous compounds, starch, sucrose, betaine, flavonoids, coumarins, phenolic acid, essential fats, vitamin C, pectin, and carotene. A special variety of hollyhock, known as black hollyhock or *Althaea rosea* var. *Nigra* is widely used in traditional medicine because its dark purple flowers are rich in biologically active molecules especially anthocyanins such as delphinidin, petunidin, and malvidin (Dudek et al., 2006; Hosaka et al., 2012). The extraction yield and the bioactivity of the extracts from medicinal plants depend on the solvent and the method of extraction. Therefore, different methods and various solvents with different polarities such as water, methanol, ethanol, ethyl acetate, and petroleum ether have been used to obtain maximum yield and produce extract with good biological activity (Azwanida, 2015). The decoction of borage and hollyhock flower has been applied to treat a wide range of different illnesses and disorders in Iranian traditional medicine. Additionally, potential and industrial applications of these flowers need more extensive research. Therefore, the current study was conducted to assess anti- radical activity, bioactive compounds, and antibacterial properties of the extracts prepared from borage and hollyhock flower using soaking and ultrasonic-assisted methods in the presence of water or methanol as a solvent.

Materials and methods

Methanol, Folin- Ciocalteu, Sodium Carbonate, Gallic Acid, Hydrochloric Acid, Potassium Chloride, Sodium Acetate, TPTZ, Acarbose, α- amylase, Quercetin, Aluminum Trichloride, 2, 2- Diphenyl- 1- Picrylhydrazyl (DPPH), and Muller Hinton Broth were procured from Sigma-Aldrich (St. Louis, MO, USA). Borage was collected from the mountains of Mazandaran Province and Hollyhock was supplied by Zarband Pharmaceutical Company (Tehran, Iran).

Extraction methods

Echium Amoenum L. and *Althaea rosea* var. *Nigra* plants were oven- dried completely at 50°C, they were then ground before being stored in airtight containers. To extract using an

ultrasonic device, 10 g of the powder was homogenized and mixed in a ratio 1: 100 of solvent (water or methanol) in a cold oven and was then subjected to an ultrasonic device (AMMM, M.P.Interconsulting, Switzerland) for 10 minutes at 20°C. The solution tube was protected entirely by an aluminum foil against the light. Then, the extracts were placed on a shaker (Snijder, 34533) and after 24 hours, they were smoothed and kept at 4°C (Rabiei et al, 2012). In order to extract through the soaking method, 10 g of the powdered plant was mixed with 100 ml of the solvent (water or methanol) and stirred for 24h at ambient temperature. After extraction, the extracts were filtered and the solvents were removed through a rotary evaporator. Finally, the concentrated extracts were stored for further experiments at 4°C (Rafiee et al., 2012).

Extraction efficiency

The average extraction efficiency of the extracts from the soaking and ultrasound-assisted methods was determined using water and methanol solvents with four replicates per 100 g powder of the plant. For this purpose, the difference in the weight of the evaporative balloon (empty and after evaporation) was obtained by dividing it by the weight of the sample.

Total phenolic content (TPC)

Following Kiselova et al. with a few modifications, the total phenolic content of extracts was measured through the Folin-Ciocalteu colorimetric method. For this purpose, 1.0 g of each extract was mixed with methanol 80% and then it was centrifuged for 5 min at 3000 rpm. After that, 0.1 ml of the supernatant was mixed with 0.4 ml of methanol and 2.5 ml of Folin- Ciocalteu reagent (10% v/v) and it was incubated for 5 min at ambient temperature in a dark place. After that, 2 ml of sodium carbonate solution of 7.5% (w/v) was added to the mixtures. Following 2hrs of incubation at ambient temperature, the absorption level of the samples was determined at 765 nm using a UV/ Vis spectrophotometer. The results were expressed as milligrams of Gallic acid equivalent per gram of the extract (mg GAE/g) using the standard curve of Gallic acid (Kiselova et al., 2006).

Total flavonoid content (TFC)

To determine FC, 5.0 ml of 2% aluminum trichloride ($AlCl_3$) in methanol was mixed with the same volume of the given extracts. Then, the absorbance of the mixtures was measured at 415 nm using a spectrophotometer for 10 min against a blank sample composed of extract solution and 5.0 ml methanol without $AlCl_3$. Different concentrations of quercetin were used to prepare the standard curve. Therefore, flavonoids volume in the extract was determined as mg of quercetin equivalent per gram of extract (mg QUE/g) (Pilerood and Prakash, 2014).

Total anthocyanin content (TAC)

The anthocyanin content was identified qualitatively using ammonia HCl test according to Egbuna et al. 2 mL of the extract was mixed with ammonia and 2 mL of 2 N HCl. The change in color from pink -red to blue- violet was considered as an indicative of anthocyanin (Egbuna et al., 2018). Then, the total content of anthocyanin was specified using the pH differential method which is based on the structural changes in absorbance measurements at pH 1.0 and 4.5 and also chemical forms of anthocyanin. In total, 0.025 M hydrochloric acid potassium chloride buffer (pH= 1) and 0.4 M sodium acetate buffer (pH= 4.5) were used to dilute separately the crude extracts. To give an absorbance reading between 0.2 and 1.4 each sample was diluted with the buffers. The UV-Vis spectrophotometer showed the mixture absorbance at 700 nm. The total amount of anthocyanin content was expressed in the form of cyanidin- 3 glucoside equivalents according to the following equation (Anuar et al., 2013; Maran and Sivakumar, 2014; Shah et al., 2011; Zuo et al., 2002).

$$\text{Anthocyanin pigment (mg/L)} = \frac{A \times MW \times DF \times V \times 1000}{a \times l \times m} \quad (1)$$

In this equation, DF represents the dilution factor, MW represents the molecular weight of cyanidin- 3 -glucosode (449.2 g/mol), A represents the absorbance, V represents the solvent volume (mL), l is the cell path length (1 cm), and *a* represents the molar absorptivity (26,900 L. mol^{-1}. cm^{-1}).

Anti-radical activity of the extract based on DPPH method

Calculation of the anti-radical activity of the sample was conducted according to Oliveira et al. by using DPPH free radicals. In this method, 0.2 ml of the sample of different concentrations was mixed with 4 ml of DPPH methanolic solution with concentration of 6×10^{-5} mol/l and kept at dark place for 120 min. Then, the solution absorbance was read at 517 nm by a spectrophotometer. In this case, one sample containing 0.2 ml of methanol was used plus 4 ml of DPPH as a control. The radical inhibition activity rate was obtained as follows:

Inhibition (%)= $(A_{control}- A_{sample} /A_{control})\times 100$ (2)

In this formula, A_{blank} and A_{sample} represent the absorbance of the control and various concentrations of the extracts respectively. Then, the concentration of extract with a 50% radical inhibitory concentration was calculated by the graph and reported as IC_{50}. Clearly, the lower IC_{50} indicates a higher radical scavenging activity as a measure of antioxidant property of the extracts (Oliveira et al., 2008).

Ferric reducing antioxidant power (FRAP) assay

The decreasing power of the compound (antioxidant) as described formerly was the main factor in the assay. Some potential antioxidants reduce the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}); a blue complex (Fe^{2+}/TPTZ) is formed by the latter and increases the absorption at 593 nm. Breifly, the preparation of FRAP reagent was done by mixing acetate buffer (300 µM, pH 3.6), 10 µM TPTZ in 20 µM HCl, and 10 µM $FeCl_3$ at 10:1:1 (v/v/v). The sample solutions (10 µL) and reagent (300 µL) were mixed completely. The absorbance was measuredat 593 nm after 10 min. Different concentrations of Trolox were used to prepare the standard curve and then, the results of dilution were corrected and stated in micromolar Trolox per 100 g of dry weight (DW). The calculations were repeated three times.

α- Amylase inhibition assay

Based on the procedure outlined by Nowicka et al. the α-amylase inhibitory effect exerted by edible flowers extracts was assayed. By measuring the decreasing groups released from starch, the inhibition of α-amylase activity was determined. Acarbose was incorporated as the positive control. Reading of the results was done at 540 nm (α-amylase). The assays of enzyme inhibition were stated as IC50 value (mg/mL). The values expressed in mg/mL represents a quantitative measure indicating the concentration of edible flowers (mg/mL) required to inhibit, in vitro, a specific biological component solution by 50% (1 U/mL) (Nowicka et al., 2016).

Quantitative estimation of phenolic acids by HPLC

Dionex Ultimate 3000 liquid chromatography (Germany) including a manual sample injection valve equipped with a 20 µl loop, a diode array detector (DAD 3000) with 5 cm flow cell, and Chromeleon 6.8 system manager was used to conduct HPLC analyses. The fractionation process was conducted by a reversed-phase Acclaim TM 120 C18 column (5 µm particle size, 4.6× 250 mm). Additionally, following Hajlaoui et al. (2019), the HPLC was used to estimate phenolic acids.

Antimicrobial activity

In order to determine the minimum inhibitory concentration (MIC), 40 mg/ml of each extract was prepared. A series of ten tubes were used for each extract. Eight tubes were considered for different concentrations of each extract, one tube as positive control, and one

tube as negative control. To each of the tubes, 9 ml of the Muller Hinton Broth was added with a specific concentration of extract and 1ml of suspension of microorganisms (*Staphylococcus aureus*). The tubes were then placed in an oven at 35°C for 24hrs. After incubation, the turbidity caused by growth of microorganism in the tubes was determined. Among the tubes without bacterial growth, the one containing the lowest concentrations of herbal extracts was considered as MIC. In order to determine the minimum bactericidal concentration (MBC), one ml of tube without bacterial growth was mixed with 15 ml of Muller Hinton Broth and a molten agar with a temperature about 48°C was poured on the surface of plate. After agar being solidified, discs impregnated with different concentrations of extract were placed on the plate and incubated at 35°C for 24 hours. The least extract concentration without observing any bacterial growth was considered as the MBC of the extract (Rezaei et al, 2015).

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) carried out in the SPSS statistical package (SPSS 16.00). Duncan's multiple range test at $p< 0.05$ was also used to determine significant differences between the mean scores.

Results and discussion

Efficiency of extraction

According to the results (Fig. 1), the extraction efficiency for the hollyhock plant was significantly more than that of borage. Clearly, in both of the plants (i.e. borage and hollyhock), the ultrasound- assisted extraction was more efficient compared to the soaking method. Moreover, it was found that the higher extraction efficiency achieved by using methanol as a solvent compared to the water. Therefore, the highest extraction efficiency was found with the hollyhock extract which was prepared by ultrasonic- assisted method using methanol as a solvent.

The higher efficiency for ultrasound-assisted method compared to the soaking can be due to the fact that the ultrasound-assisted extraction method uses the shear force resulting from the cavitation bubbles implosion of ultrasonic waves in order to change material properties. Thus, it disrupts plant tissues and facilitates the extraction procedure to a greater extent (Dzah et al., 2020).

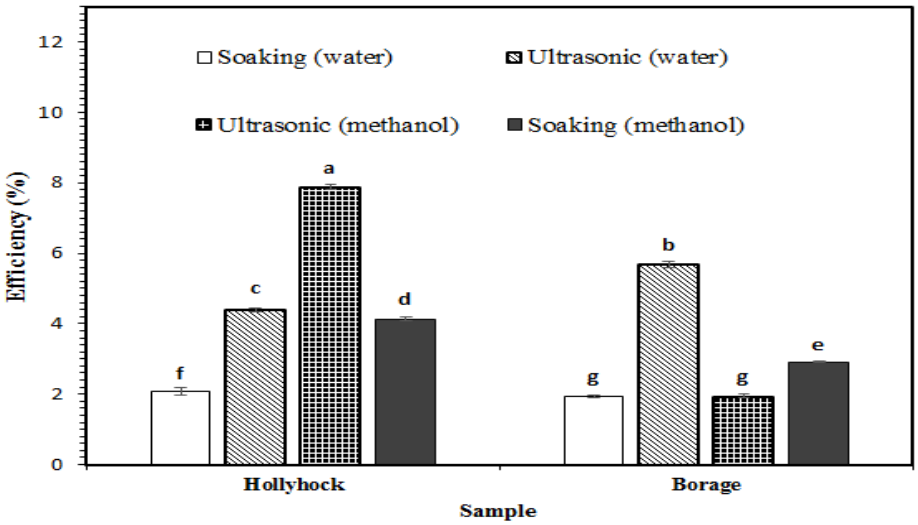


Fig. 1. The effects of the extraction method on the extraction efficiency
Means followed by different letters are significantly different ($p < 0.05$)

Therefore, the higher extraction efficiency of ultrasound-assisted method is due to solvent penetration, enhancement of cell disruption, and mass transfer (Chukwumah et al., 2009).

Consistent with our findings, Safdar et al. used different methods (ultrasound and maceration) of extracting polyphenols from mango peel (Safdar et al., 2017). According to their report, the highest extraction yield was associated with ultrasound-assisted extraction using methanol as a solvent. Generally, it can be concluded that the extraction efficiency of extracts from borage and hollyhock was a function of many factors including the type of method and the polarity of the solvent.

Phenolic content of extracts

Table 1 lists the TPC in different samples. In fact, results showed that as for the hollyhock, the greatest TPC was related to the extract which was prepared by ultrasound- assisted method with water as solvent suggesting high water solubility for the phenolic compounds of hollyhock extract. For the borage, the TPC content of extract prepared by ultrasound-assisted method was higher compared to those prepared by soaking method; however, the effect of solvent type (water and methanol) on the TPC of extracts produced by ultrasound-assisted extraction was insignificant ($p > 0.05$). Moreover, according to the results, the lowest TPC was related to the extracts which were prepared by soaking method with methanol as a solvent. Generally, it seems that the ultrasound-assisted extraction using water or methanol as a solvent is more efficient compared to a soaking method to produce extracts from borage and hollyhock with high content of phenolic compounds. The point that is consistent with our findings is that a higher TPC was reported for aqueous extract of *Echium amoenum* L. compared with the extract produced by acetone as a solvent (Pilerood and Prakash, 2014). This was attributed to the high degree of solubility of borage polyphenols in water. In addition, Goli et al. used different methods and solvents to prepare extracts from the pistachio green hull. Consistent with our findings, they also reported that the highest TPC was related to the extracts which were produced by ultrasound-assisted extraction using methanol or water as a solvent (Goli et al., 2005). In conclusion, with respect to the high TPC of extracts from borage and hollyhock as well as the many beneficial attributes of phenolic compounds such antioxidant and antimicrobial activity, the extracts produced in this study can be used as natural antioxidant and antimicrobial agents for developing food products with a wide range of health- promoting properties.

Flavonoid content of the extracts

The TFC of different extract samples prepared using soaking and ultrasound- assisted extraction methods using water and methanol as solvents is shown in Table 1. In both of the examined plants, the results showed that the extracts obtained by the soaking method using methanol as the solvent had the highest level of flavonoids. This observation suggested that the soaking method is a more efficient method in comparison with ultrasound-assisted extraction to prepare extracts with high content of flavonoids. The lower TFC of extracts through ultrasound-assisted method might be due to the formation of free radicals which may have an effect on the active unstable phytochemicals such as flavonoids (Azwanida, 2015). The results also indicated that methanol was a better solvent to extract flavonoids from borage and hollyhock compared to water. Therefore, it seems that the flavonoids found in these plants had a higher affinity to methanol compared to water. Consistent with our findings, Karimi et al. found a significant difference existed between methanol, ethanol, and water extracts of *Borago officinalis* L. flower for TFC (Karimi et al., 2018). The greatest content of flavonoids was found in the methanolic extract. This was ascribed to the greater polarity index of methanol relative to water and ethanol resulting in the extraction of more flavonoid compounds.

Anti-radical activity of the extracts

Antioxidants are chemicals that prevent and control the effects of free radicals thus helping to protect the human body against infections and diseases. Determining the antioxidant activity using DPPH assay as a common method of assessing the free radical scavenging

activity of plant extracts provides helpful information about the antioxidant potential that occur in plant materials (Fraczek et al., 2019; Safdar et al., 2017). The results showed the adequate ability of all extract samples to scavenge the free radicals of DPPH. The findings also showed that the extraction method and the solvents also had a significant effect on the antioxidant activity of the resulting extracts. As for hollyhock, the extracts prepared through the soaking method had a higher antioxidant activity compared to the extracts obtained through the ultrasound- assisted method. For borage, the anti- radical activity of extracts prepared using ultrasound- assisted extraction was higher than those extracted through soaking approaches. However, in both examined plants, using water as a solvent in the optimum method resulted in greater antioxidant activity than methanol. The antioxidant properties of these herbs may be connected to their biological active components such as phenolic and flavonoid compounds which are well-known as potent antioxidant and anti-radical agents (Hosaka et al., 2015; Karimi et al., 2018). Safdar et al. also indicated a higher DPPH radical scavenging activity in aqueous extracts of mango peel compared to those extracted by other solvents such as methanol and ethanol (Safdar et al., 2017). Pilerood and Prakash (2014) found that decreased power of extract and the free radical scavenging activity were measures of the antioxidant activity and associated with the type of the solvent which was employed to obtain the extracts. Generally, these findings suggested that the extracts from borage and hollyhock could be effectively applied as the natural antioxidant agents found in food products instead of the synthetic antioxidants which have many disadvantages.

Table 1- Total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity of different extract samples

Extract sample	TPC (mg GAE/g)	TFC (mg QUE /g)	IC_{50} (mg/ml)
Hollyhock + ultrasound (water)	535.07 ± 16.98^{a}	115.48 ± 1.89^{d}	0.50 ± 0.01^{bc}
Hollyhock + soaking (water)	421.80 ± 12.80^{b}	107.76 ± 5.84^{e}	0.32 ± 0.03^{cd}
Hollyhock + ultrasound (methanol)	332.50 ± 4.07^{e}	23.40 ± 0.70^{f}	0.67 ± 0.06^{b}
Hollyhock + soaking (methanol)	221.76 ± 2.97^{f}	337.03 ± 3.61^{c}	0.40 ± 0.00^{bcd}
Borage + ultrasound (water)	354.57 ± 5.66^{cd}	3.35 ± 0.21^{g}	0.13 ± 0.06^{d}
Borage + soaking (water)	344.93 ± 12.93^{de}	383.54 ± 3.27^{b}	1.70 ± 0.25^{a}
Borage + ultrasound (methanol)	363.03 ± 6.14^{c}	5.10 ± 0.11^{g}	0.18 ± 0.03^{d}
Borage + soaking (methanol)	117.52 ± 1.73^{g}	547.49 ± 2.05^{a}	1.83 ± 0.29^{a}

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

Anthocyanin content of the extracts

Total anthocyanidins are conventionally extracted from polar organic solvents. As shown in Figure 2 in the case of Hollyhock and Borage which were examined, the results showed that the extracts obtained by ultrasound- assisted method using water as a solvent had the highest level of Anthocyanin. In addition, these variations can be associated with the conditions of the extraction, raw material, and analysis conditions.

Ravanfar et al. (2015) reported that ultrasonic energy was effective in intensification and yields improvement of the anthocyanins' extraction. The type of plant pigments having relevant roles in plant defense mechanisms, plant propagation, and the color of fruits and vegetables is anthocyanins. As shown by studies, they have positive effects on human health. Pilerood and Prakash (2014) evaluated anthocyanins and the antioxidant activity of Borage (*Echium amoenum*). They indicated that borage is rich source of anthocyanins (104.4 mg/ 100 g). As for anthocyanin content, studies have reported that fruits contain anthocyanin in various amounts. For example, Liu et al. (2020) reported that the anthocyanin content of raspberries ranged from

0.17 to 57.0 mg/ 100 g, this range for grapes was reported by Bridle and Timberlake to be 30– 750 mg/ 100 g.

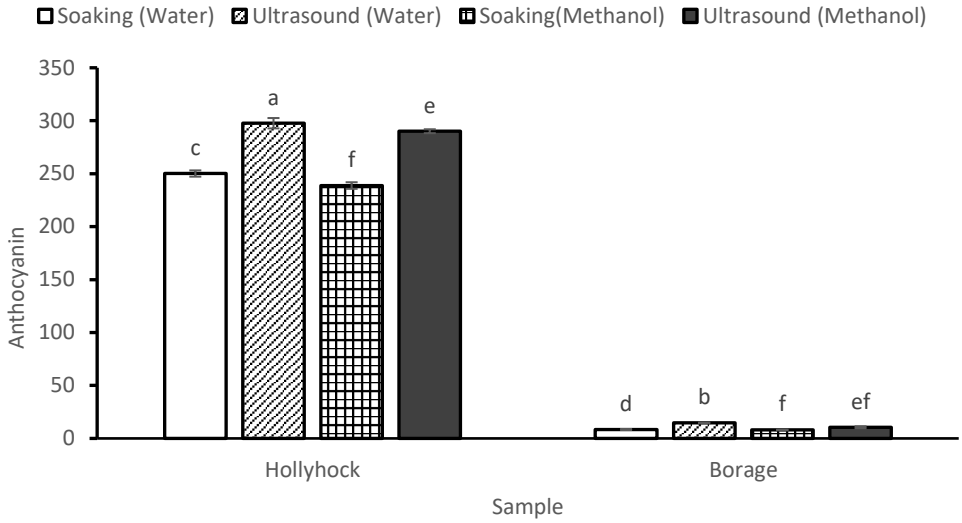


Fig. 2. The effects of the extraction method on the anthocyanin content of the extracts.
Means followed by different letters are significantly different ($p < 0.05$)

Ferric reducing antioxidant power (FRAP)

Total antioxidant activity results, as calculated using the FRAP method, are listed in Table 2 and compared to Vitamin C and TBHQ. Ultrasound- assisted method and water as a solvent had the main role and showed higher power in total antioxidant activity compared to natural and artificial antioxidants.

Table 2- Ferric reducing antioxidant power (FRAP) of different extract samples

Extract sample	FRAP
Hollyhock+ soaking (water)	1.88 ± 0.07^{b}
Hollyhock+ ultrasound (water)	2.19 ± 0.08^{a}
Borage+ soaking (water)	1.5 ± 0.07^{d}
Borage+ ultrasound (water)	1.61 ± 0.06^{c}
Hollyhock+ soaking (methanol)	1.68 ± 0.03^{c}
Hollyhock+ ultrasound (methanol)	1.92 ± 0.06^{b}
Borage+ soaking (methanol)	1.3 ± 0.08^{e}
Borage+ ultrasound (methanol)	1.49 ± 0.03^{d}
Vitamin C	1.31 ± 0.03^{e}
TBHQ	1.37 ± 0.03^{e}

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

Chaouche et al. (2011) indicated antioxidant activity in hydromethanolic root extracts of *E. pycnanthum* collected in southern Algeria. *E. vulgare* and *E. rubrum* have been tested in terms of antioxidant activity through metal-chelating (Fe^{2+}), FRAP, TAC, OH radical, DPPH and ABTS radical scavenging assays. In addition, the results indicated the high potency of *E. vulgare*, due to its high TPC and TFC values.

Bacterial alpha amylase inhibition

The α-amylase inhibition was used to investigate the edible flowers extracts in terms

of their inhibitory effect at various concentrations. Their biological activity was established and the IC50 values were calculated (Table 3). As for the human body, pancreatic α-amylase hydrolyzes dietary carbohydrates into monosaccharides which are considered suitable for absorption. One strategy applied to counteract metabolic abnormalities connected to type 2 diabetes and hyperglycemia is the inhibition of these enzymes, and thus using phytoextracts such as α-amylase inhibitors may provide an alternative to prevent diabetes mellitus. As shown in Fig 3., the inhibitory effect increases with increasing extract concentration.

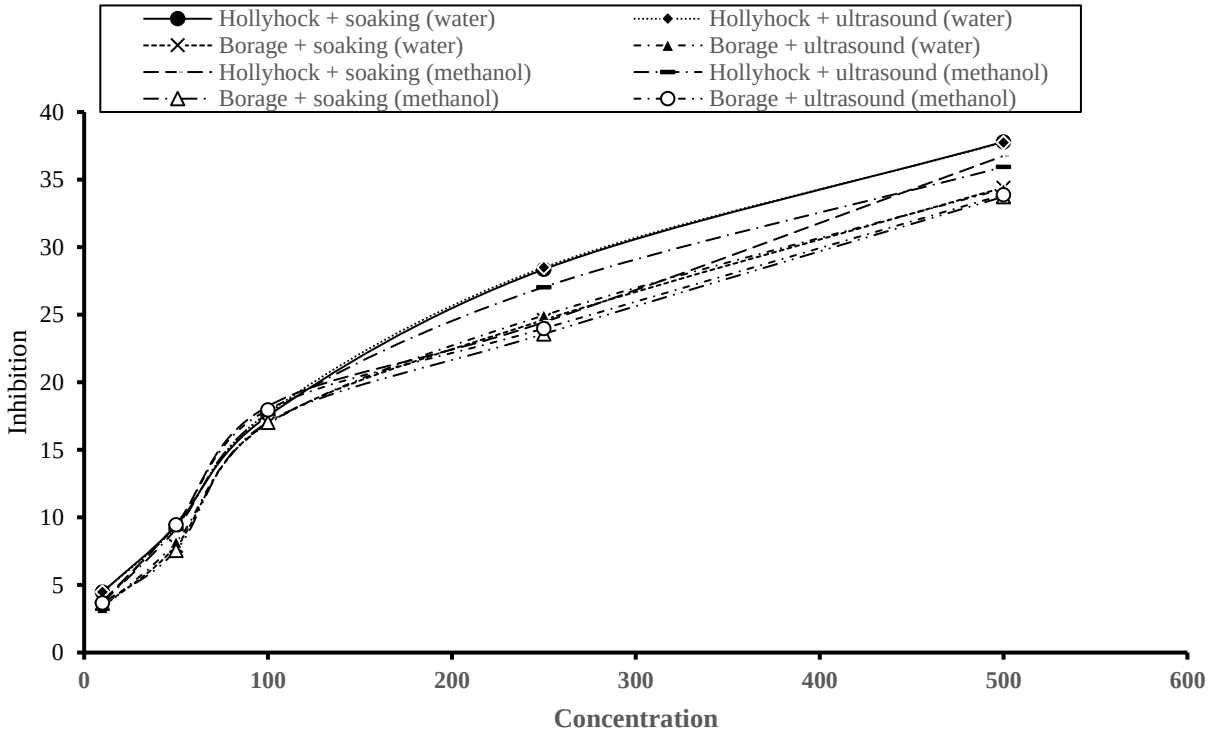


Fig. 3. The effects of Bacterial alpha amylase inhibition of the extracts.

Table 3- Bacterial alpha amylase inhibition of the extract samples

Extract sample	IC50
Hollyhock+ soaking (water)	3166.09 ±86.35^{f}
Hollyhock+ ultrasound (water)	3130.02 ±89.2^{f}
Borage+ soaking (water)	5520.72 ±101.66^{c}
Borage+ ultrasound (water)	5452.87 ±75.66^{c}
Hollyhock+ soaking (methanol)	4305.09 ±78.52^{d}
Hollyhock+ ultrasound (methanol)	3887.96 ±87.66^{e}
Borage+ soaking (methanol)	6715.49 ±70.12^{a}
Borage+ ultrasound (methanol)	6331.26 ±100.29^{b}

The α-amylase inhibition, presented by IC50 values, ranged from 3130.02 to 5520.72 mg/ml. The lowest value was for Hollyhock extract obtained by ultrasound- assisted and water as solvent and highest amount of IC50 was related to Borage extract obtained by ultrasound-assisted method and methanol as solvent.

An in-silico study investigating the inhibitory activities of certain flavonoids and phenolic acids on α-amylase and α-glucosidase, rosmarinic acid exhibited an IC50 value

equivalent to acarbose (Tolmie et al., 2021). McCue and Shetty (2004) also reported in support of these results. According to these researchers, rosmarinic acid has an in vitro inhibitory effect on porcine pancreatic amylase. Some reports in the literature have shown that some extracts contained chlorogenic acid as a major compound and exhibited significant inhibitory activity on digestive enzymes (Chokki et al., 2020; Liu et al., 2020a; Liu et al., 2020b). These findings support those from the present study.

Quantitative HPLC estimation of phenolic contents

Hollyhock+ ultrasound (water)

The HPLC analysis makes it possible to perform simultaneous fractionation and identify an extensive range of phenolics acids in a plant sample. In this study, it was found that an ultrasound- assisted method and water as solvent can extract higher phenolic compounds compared to soaking and methanol solvent. For this reason, the aqueous extract of plants extracted with ultrasound-assisted method was performed to identify phenolic compounds. Figure 4 illustrates the results of HPLC analysis of borage and hollyhock prepared through ultrasonic-assisted method and using water as solvent.

As can be seen in the Figure 4, syringic acid is considered as the main phenolic compound found in Hollyhock. The results showed that after syringic acids, two phenolic compounds, P-hydroxybenzoic acid, and P-coumaric acid had the highest concentrations in final Hollyhock extract. Similar to our research work, Dudek et al. examined the phenolic content of this species through HPLC method and determined the total volume of phenolic acids measured as caffeic acid in the ethereal fractions of the isopropanol extracts from the whole flower (Dudek et al., 2006).

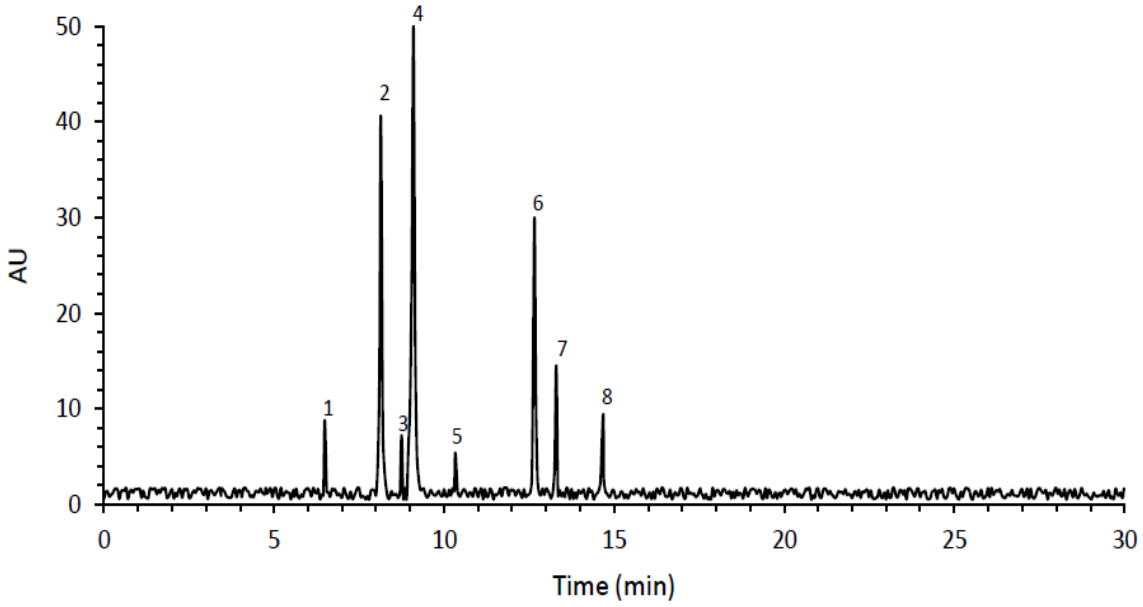


Fig. 4. The HPLC chromatogram of phenolic acids in the Hollyhock + ultrasound (water) Peaks (1) Chlorogenic acids, (2) P-hydroxybenzoic acid, (3) Caffeic acid, (4) Syringic acid, (5) m-hydroxybenzoic acid, (6) p-coumaric acid, (7) Ferulic acid, and (8) Isoferulic acid.

Borage+ ultrasound (water)

The results about identifying the phenolic compound in the aqueous extract of borage extracted by ultrasound-assisted method are presented in Figure 5.

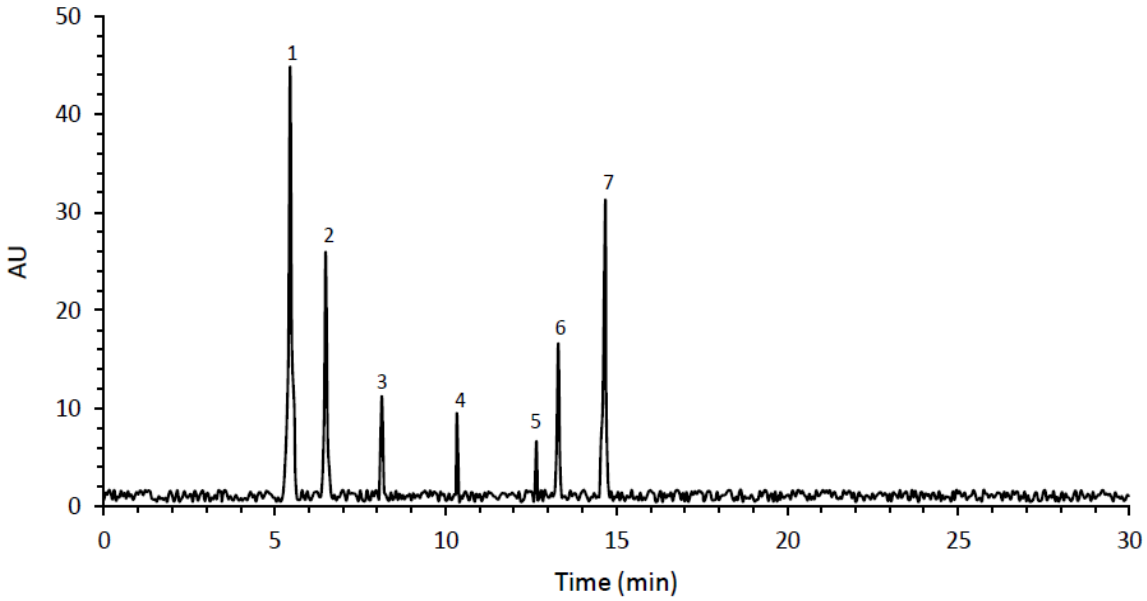


Fig. 5. The HPLC chromatogram of phenolic acids in the Borage + ultrasound (water) Peaks (1) Gallic acid, (2) Chlorogenic acid, (3) P-hydroxybenzoic acid, (4) m-hydroxybenzoic acid, (5) p-coumaric acid, (6) Ferulic acid, and (7) Isoferulic acid.

According to the results, gallic acid was the higher active ingredient in borage extract. The most abundant phenolic acid in medicinal plants is gallic acid. These findings are interesting given the samples pharmacological function. Chlorogenic acid and Isofric acid had the next highest concentrations in the extract. Bandoniene et al. (2005) used HPLC analysis of the aqueous extract of the borage and extracted rosmarinic acid as the major compound along with several minor components. El-Hallous (2019) used HPLC to examine Viper's Bugloss (*Echium Vulgare* L) extract as a natural antioxidant and found that the phenolic acids from *Echium vulgare* extract were gallic acid, benzoic acid, isoferulic acid, chlorogenic acid, vanillic acid, catechol, salicylic acid, ferulic acid, catechin, P-hydroxy-benzoic acid, protocatechuic acid, alpha coumaric acid, and p-coumaric acid.

Antimicrobial activity

The MIC and the MBC of the extracts of borage and hollyhock against *Staphylococcus aureus* are presented in Table 4. The extracts showed an inhibitory effect at concentrations of 1- 5 mg/ml and showed a bactericidal effect at the concentrations between 5- 10 mg/ml on *Staphylococcus aureus*. The results revealed lower MIC and MBC for the hollyhock extract compared to the borage extract. Moreover, it was found that the method and solvent did not have a significant effect on the MIC and MBC. Therefore, it can be concluded that the extract of hollyhock had an antimicrobial effect at lower concentrations and the antibacterial activity of this extract was significantly higher than borage extract. Antimicrobial activity of natural extracts appears to be due to their bioactive compounds especially phenolic and flavonoid compounds (Rezaei et al, 2015). Consistent with our results, Karimi et al. also reported a good antimicrobial activity for *Borago officinalis* L. flower against different foodborne pathogens, which was attributed to the existence of flavonoids and polyphenols in the extract (Karimi et al., 2018). These biologically active compounds possess antibacterial activity through the chemical barrier's induction against the invading microorganisms, interrupting energy supply of the metabolism, cytoplasmic membrane function, disrupting nucleic acid synthesis, non-specific reactions with carbohydrates in the cell

wall, and inactivation of adhesions and protein transport (Oliveira et al, 2008).

Table 4- Minimum inhibitory (MIC) and minimum bactericidal concentration (MBC) of borage and hollyhock extracts prepared using different methods and solvents.

Extract sample	MIC (mg/ml)	MBC (mg/ml)
Hollyhock+ ultrasound (water)	1.33± 0.58^{bc}	4.67± 0.58^{b}
Hollyhock+ soaking (water)	1.33± 0.58^{bc}	4.67± 0.58^{b}
Hollyhock+ ultrasound (methanol)	1.00± 0.00^{c}	4.67± 0.58^{b}
Hollyhock+ soaking (methanol)	2.00± 0.00^{b}	5.00± 0.00^{b}
Borage+ ultrasound (water)	4.67± 0.58^{a}	9.67± 0.58^{a}
Borage+ soaking (water)	4.67± 0.58^{a}	10.00± 0.00^{a}
Borage+ ultrasound (methanol)	5.00± 0.00^{a}	9.67± 0.58^{a}
Borage+ soaking (methanol)	4.67± 0.58^{a}	10.00± 0.00^{a}

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

Conclusions

The proper extraction of medicinal plants is essential to meet the increasing pharmaceutical industry demands for biologically active natural extracts. The extracts of medicinal plants with strong antioxidant activity increase pharmacological functions. Borage and hollyhock are rich sources of bioactive compounds with strong antioxidant activity. Different methods (ultrasound- assisted extraction and soaking) and solvents (methanol and water) were used to prepare extracts from these medicinal plants. The results showed that the ultrasound-assisted extraction and methanol were more efficient method and solvent compared to soaking method and water to prepare the extract. Moreover, the content of phenolic and flavonoid compounds as well as the antioxidant activity of the resulting extracts were different depending on the type of the method and the polarity of the solvents. The results of TPC, anthocyanins, and the FRAP tests showed that the highest extract yield was obtained through ultrasound- assisted method with water as solvent. The highest amount of TFC was reached by a soaking method using methanol as solvent. The anti- radical activity tests indicated that using water as a solvent in the optimum method resulted in a higher antioxidant activity. Furthermore, the bacterial alpha amylase inhibition test suggested that the inhibitory effect increased with increasing extract concentration. The HPLC analysis of the borage (*Echium amoenum* L.) and hollyhock (*Althaea rosea* var. *Nigra*) extracts revealed that gallic acid and syringic acid were the most prominent phenolic compounds. Indigenous herbs found in Iran and their effects on infectious agents such as *S. aureus* were addressed in this review. Moreover, the results showed that the borage and hollyhock aqueous extract had remarkable antimicrobial activity against *Staphylococcus aureus* which can be a subject of future studies to find their effective compounds contributing to the antibacterial activity. Therefore, borage and hollyhock can be considered as good sources of antioxidants and antimicrobial compounds aside from their medicinal properties. Thus, they can be applied as natural preservatives supplements in the food formulations in order to enhance the shelf-life through improving their stability against pathogens and by retarding the lipid peroxidation process.

References

1. Abolhassani, M. (2010). Antiviral activity of borage (Echium amoenum). Archives of Medical Science, 6(3), 366-369. https://doi.org/10.5114/aoms.2010.14256

2. Adel Pilerood, S., Prakash, J. (2014). Evaluation of nutritional composition and antioxidant activity of Borage (*Echium amoenum*) and Valerian (*Valerian officinalis) J. Food Sci. Technol*.; 51:845–854. https://doi.org/10.1007/s13197-011-0573-z
3. Al-Juhaimi. F, Ghafoor. K, Özcan. M. M, Jahurul. M. H. A, Babiker. E. E, Jinap. S. et al. (2018). Effect of various food processing and handling methods on preservation of natural antioxidants in fruits and vegetables. *J Food Sci Technol* 55(10):3872–3880. https://doi.org/10.1007/s13197-018-3370-0
4. Anuar, N., Mohd Adnan, A. F., Saat, N., Aziz, N., & Mat Taha, R. (2013). Optimization of extraction parameters by using response surface methodology, purification, and identification of anthocyanin pigments in Melastoma malabathricum fruit. *The Scientific World Journal*, 2013. https://doi.org/10.1155/2013/810547
5. Azwanida , N, N. A. (2015). Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. Med. *Aroma. Plants* 4, 1-6.
6. Bandoniene, D., Murkovic, M., & Venskutonis, P. R. (2005). Determination of rosmarinic acid in sage and borage leaves by high-performance liquid chromatography with different detection methods. *Journal of chromatographic science*, 43(7), 372-376. https://doi.org/10.1093/chromsci/43.7.372
7. Bridle, P, Timberlake, C, F. (1997). Anthocyanins as natural food colours–selected aspects. *Food Chem*. 58(1–2):103–109. https://doi.org/10.1016/S0308-8146(96)00222-1
8. Chaouche, T.; Haddouchi, F.; Bekkara, F. A. (2011). Phytochemical study of roots and leaves of the plant Echium pycnanthum Pomel. Der Pharm. Lett. 3, 1–4.
9. Chokki, M., Cudălbeanu, M., Zongo, C., Dah-Nouvlessounon, D., Ghinea, I. O., Furdui, B., ... & Baba-Moussa, F. (2020). Exploring antioxidant and enzymes (A-amylase and B-Glucosidase) inhibitory activity of Morinda lucida and Momordica charantia leaves from Benin. *Foods*, 9(4), 434. https://doi.org/10.3390/foods9040434
10. Chukwumah, Y. C., Walker, L. T., Verghese, M., Ogutu, S. (2009). "Effect of frequency and duration of ultrasonication on the extraction efficiency of selected isoflavones and trans-resveratrol from peanuts (*Arachis hypogaea*)," *Ultrason. Sonochem*., vol. 16, no. 2, pp. 293–299. https://doi.org/10.1016/j.ultsonch.2008.07.007
11. Dudek, M., Irena , M,. Maurycy, S,. (2006). "Phenolic acids in the flowers of *Althaea rosea* var. *nigra*." *Acta Pol Pharm*, 63(3): 207-211.
12. Dzah, C. S., Duan, Y., Zhang, H., Wen, C., Zhang, J., Chen, G., & Ma, H. (2020). The effects of ultrasound assisted extraction on yield, antioxidant, anticancer and antimicrobial activity of polyphenol extracts: A review. Food Bioscience, 35, 100547.https://doi.org/10.1016/j.fbio.2020.100547
13. Egbuna, C., Ifemeje, J. C., Maduako, M. C., Tijjani, H., Udedi, S. C., Nwaka, A. C., Ifemeje, M. O. (2018). Phytochemical test methods: Qualitative, quantitative and proximate analysis. *In Phytochemistry*: Volume 1: Fundamentals, Modern Techniques, and Applications, 1st ed.; Egbuna, C., Ifemeje, J.C., Udedi, S.C., Kumar, S., Eds.; Apple Academic Press: New York, NY, USA.
14. El-Hallous, E. (2018). Viper's Bugloss (*Echium Vulgare* L) Extract as A Natural Antioxidant and Its Effect on Hyperlipidemia. *International Journal of Pharmaceutical and Phytopharmacological Research (eIJPPR)*. February, Volume 8, Issue 1, Page 81-89.
15. Fraczek, B. Morawska, M. Gacek, M. Pogon, K. (2019). Antioxidant activity as well as vitamin C and polyphenol content in the diet for athletes. Ital. *J. Food Sci*. 31, 617-630. https://doi.org/10.14674/IJFS-1510

16. Goli, A. H., Barzegar, M., Sahari, M. A., (2005). "Antioxidant activity and total phenolic compounds of pistachio *(Pistachia vera*) hull extracts." *Food Chemistry* 92(3): 521-525. https://doi.org/10.1016/j.foodchem.2004.08.020
17. Hajlaoui, H. Arraouadi, S., Mighri, H., Chaaibia, M., Gharsallah, N., Ros, G., ... & Kadri, A. (2019). Phytochemical constituents and antioxidant activity of *Oudneya Africana* L. leaves extracts: evaluation effects on fatty acids and proteins oxidation of beef burger during refrigerated storage. *Antioxidants*, 8(10), 442. https://doi.org/10.3390/antiox8100442
18. Hosaka, H. Mizuno, T. Iwashina, T. (2012). Flavonoid Pigments and Color Expression in the Flowers of Black Hollyhock (*Alcea rosea 'Nigra*). *Bull. Natl. Mus. Nat. Sci., Ser*. B, 38(2), pp. 69–75.
19. Karimi, E., Oskoueian, E., Karimi A., Noura R., Ebrahimi, M. (2018). *Borago officinalis* L. flower: A comprehensive study on bioactive compounds and its health-promoting properties. J. Food Meas. Charact. 12:826–838. https://doi.org/10.1007/s11694-017-9697-9
20. Karimi, E., Oskoueian, E., Karimi, A., Noura, R., & Ebrahimi, M. (2018). *Borago officinalis* L. flower: a comprehensive study on bioactive compounds and its health-promoting properties. *Journal of Food Measurement and Characterization*, 12(2), 826-838.
21. Kiselova, Y., Ivanova, D., Chervenkov, T., Gerova, D., Galunska, B., & Yankova, T. (2006). Correlation between the in vitro antioxidant activity and polyphenol content of aqueous extracts from Bulgarian herbs. Phytotherapy Research: *An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 20(11), 961-965. https://doi.org/10.1002/ptr.1985
22. Liu, S. W., Yu, J. C., Guo, S., Fang, H. L., & Chang, X. D. (2020a). Inhibition of pancreatic alphaamylase by Lonicera caerulea berry polyphenols in vitro and their potential as hyperglycemic agents. LWT-*Food Science and Technology*, 126, 109288. https://doi.org/10.1016/j.lwt.2020.109288
23. Liu, X. C., Wang, Y. H., Zhang, J. C., Yang, L. L., Liu, S. Q., Taha, A. A., Wang, J., & Ma, C. (2020b). Subcritical water extraction of phenolic antioxidants with improved alpha-amylase and alpha-glucosidase inhibitory activities from exocarps of *Castanea mollissima* Blume. *Journal of Supercritical Fluids*, 158, 104747. https://doi.org/10.1016/j.supflu.2019.104747
24. Maran, J. P., Sivakumar, V., Thirugnanasambandham, K., & Sridhar, R. (2015). Extraction of natural anthocyanin and colors from pulp of jamun fruit. Journal of food science and technology, 52(6), 3617-3626. https://doi.org/10.1007/s13197-014-1429-0
25. McCue, P. P., & Shetty, K. (2004). Inhibitory effects of rosmarinic acid extracts on porcine pancreatic amylase in vitro. *Asia Pacific Journal of Clinical Nutrition*, 13(1), 101-106.
26. Nabavi. S. F, Di Lorenzo. A, Izadi. M, Sobarzo-Sánchez. E, Daglia. M, Nabavi. S. M. (2015). Antibacterial effects of cinnamon: from farm to food, cosmetic and pharmaceutical industries. *Nutrients* 7(9):7729–7748. https://doi.org/10.3390/nu7095359
27. Nowicka, P., Wojdyło, A., & Samoticha, J. (2016). Evaluation of phytochemicals, antioxidant capacity, and antidiabetic activity of novel smoothies from selected Prunus fruits. Journal of Functional Foods, 25, 397-407. https://doi.org/10.1016/j.jff.2016.06.024
28. Oliveira, I., Sousa, A., Ferreira, I. C., Bento, A., Estevinho, L., & Pereira, J. A. (2008). Total phenols, antioxidant potential and antimicrobial activity of walnut (*Juglans regia* L.) green husks. *Food and chemical toxicology*, 46(7), 2326-2331. https://doi.org/10.1016/j.fct.2008.03.017
29. Pilerood, S. A. and J. Prakash. (2014). "Evaluation of nutritional composition and antioxidant activity of Borage (*Echium amoenum*) and Valerian (*Valerian officinalis*)." *Journal of food science and technology*, 51(5): 845-854. https://doi.org/10.1007/s13197-011-0573-z
30. Rabiei, K., Bekhradnia, S., Nabavi, S. M., Nabavi, S. F., & Ebrahimzadeh, M. A. (2012). Antioxidant activity of polyphenol and ultrasonic extracts from fruits of Crataegus pentagyna

subsp. elburensis. *Natural Product Research*, 26(24), 2353-2357. https://doi.org/10.1080/14786419.2012.658799

31. Rafiee, Z., Jafari, S. M., Alami, M., & Khomeiri, M. (2012). Antioxidant effect of microwave-assisted extracts of olive leaves on sunflower oil. *Journal of Agricultural Science And Technology (JAST)*. Volume 14, Number SUPP; Page(s) 1497
32. Ranjbar, A., Khorami, S., Safarabadi, M., Shahmoradi, A., Malekirad, A. A., Vakilian, K., ... & Abdollahi, M. (2006). Antioxidant activity of Iranian Echium amoenum Fisch & CA Mey flower decoction in humans: a cross-sectional before/after clinical trial. Evidence-Based Complementary and Alternative Medicine, 3(4), 469-473.
33. Ranjbar, A., Khorami, S., Safarabadi, M., Shahmoradi, A., Malekirad, A. A., Vakilian, K., Mandegary, A., Abdollahi, M. (2006). Antioxidant activity of Iranian *Echium amoenum Fisch & C.A. Mey* flower decoction in humans: *A cross-sectional before/after clinical trial. Evid. -Based Complement. Altern. Med*. 3:469–473
34. Ravanfar, R.; Tamadon, A. M.; Niakousari, M. (2015). Optimization of ultrasound assisted extraction of anthocyanins from red cabbage using Taguchi design method. *J. Food Sci. Technol*. 52, 8140–8147.
35. Rezaei, M., Dadgar, Z., Noori-Zadeh, A., Mesbah-Namin, S. A., Pakzad, I., & Davodian, E. (2015). Evaluation of the antibacterial activity of the Althaea officinalis L. leaf extract and its wound healing potency in the rat model of excision wound creation. *Avicenna journal of phytomedicine*, 5(2), 105.
36. S. Shah, N. Akhtar, M. Akram, P. A. Shah, T. Saeed, K. Ahmed, H. M. Asif, (2011*). J. Med. Plant. Res*. 5, 5662-5666.
37. Safdar, M. N., Kausar, T., Nadeem, M. (2017). Comparison of Ultrasound and Maceration Techniques for the Extraction of Polyphenols from the Mango Peel: The Potential of Ultrasound Against Maceration. *Journal of Food Processing and Preservation*. 41(4). https://doi.org/10.1111/jfpp.13028
38. Shariffar, F, Dehghn-Nudeh, G, Mirtajaldini, M. (2009). Major flavonoids with antioxidant activity from *Teucrium polium* L. *Food Chem*, 112(4):885–888. https://doi.org/10.1016/j.foodchem.2008.06.064
39. Tolmie, M., Bester, M. J., & Apostolides, Z. (2021). Inhibition of alpha-glucosidase and alphaamylase by herbal compounds for the treatment of type 2 diabetes: A validation of in silico reverse docking with in vitro enzyme assays. *Journal of Diabetes*, 13,779–791. https://doi.org/10.1111/1753-0407.13163
40. Zuo, Y. Chen, H. and Deng, Y. (2002). "Simultaneous Determination of Catechins Caffeine and Gallic acids in Green, Oolong, Black and Puerr Teas using HPLC with a Photodiode Array Detector", *Talanta*, 57: 307-316. https://doi.org/10.1016/S0039-9140(02)00030-9

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد ۱۸، شماره ۳، مرداد - شهریور ۱۴۰۱، ص. ۶۸-۵۳

Iranian Food Science and Technology Research Journal
Vol. 18, No. 3, Aug. Sep. 2022, p. 53-68

بررسی فعالیت آنتی‌اکسیدانی و ضدمیکروبی عصاره گیاهان گل گاو زبان (*Echium amoenum L.*) و ختمی سیاه (*Althaea rosea var. nigra*) استخراج شده به روش خیساندن و فراصوت

مهشید زمانخانی[۱]- سهراب معینی[۱*]- پیمان مهستی شتربانی[۲]- حسین میرسعیدقاضی[۳]- افشین جعفرپور[٤]

تاریخ دریافت: ۱۴۰۱/۰۲/۰۶
تاریخ پذیرش: ۱۴۰۱/۰۳/۱۸

چکیده

هدف از این مطالعه بررسی فعالیت آنتی‌اکسیدانی و ضدمیکروبی عصاره گل گاوزبان (*Echium amoenum L*) و ختمی سیاه (*Althaea rosea* var. *Nigra*) بود. عصاره‌ها از طریق روش خیساندن و فراصوت با استفاده از آب یا متانول به‌دست آمد. سپس محتوای فنل و فلاونوئید، فعالیت مهار رادیکال‌های آزاد و ضدباکتریایی عصاره‌ها مورد بررسی قرار گرفت و آنالیز ترکیبات فنولی توسط دستگاه کروماتوگرافی مایع با کارایی بالا انجام گردید. نتایج به‌دست آمده نشان داد که بیشترین میزان ترکیبات فنلی و آنتوسانین و فعالیت آنتی‌اکسیدانی در روش FRAP در نمونه‌ای که به روش فراصوت و حلال آب استخراج شده است، مشاهده گردید و عصاره‌های استخراج شده با روش خیساندن و حلال متانول بیشترین میزان ترکیبات فلانوئیدی را دارا بودند. در بررسی فعالیت به دام‌اندازی رادیکال با دی فنیل پیکریل هیدرازیل، در هر دو عصاره به‌دست آمده به روش فراصوت غلظت کمتری IC50 تعیین گردید. نتایج حاصل از اثر مهارکنندگی عصاره‌ها بر فعالیت آنزیم آلفاآمیلاز نشان داد که با افزایش غلظت عصاره اثر مهارکنندگی آن نیز افزایش یافت. مواد موثره شناسایی شده با دستگاه HPLC نشان داد که اسید سیرینجیک و اسید گالیک ماده موثره اصلی در عصاره ختمی سیاه و گل گاوزبان به‌ترتیب بودند. نتایج به‌دست آمده از این تحقیق حاکی از آن بود که فعالیت ضدباکتریایی عصاره ختمی به‌طور معنی‌داری بیشتر از عصاره گل گاو زبان بوده است. این مطالعه پیشنهاد می‌کند که عصاره‌های گل گاو زبان و ختمی سیاه را می‌توان به‌عنوان یک نگهدارنده طبیعی در فرمول‌های غذایی گنجانید تا ویژگی‌های ارتقادهنده سلامت آنها را بهبود بخشد.

واژه‌های کلیدی: عصاره‌های طبیعی، روش خیساندن، استخراج به کمک فراصوت، پلی‌فنل.

۱- گروه علوم و صنایع غذایی، دانشگاه آزاد اسلامی، واحد تهران شمال، تهران، ایران.
۲- گروه کنترل کیفی، واحد علوم و تحقیقات، دانشکده علوم پزشکی، دانشگاه آزاد اسلامی، تهران، ایران.
۳- گروه علوم و صنایع غذایی، گروه فنی کشاورزی، پردیس ابوریحان، دانشگاه تهران، پاکدشت، ایران.
۴-گروه علوم و صنایع غذایی، دانشگاه آزاد اسلامی، واحد گرمسار، تهران، ایران.
(*- نویسنده مسئول: Email: Sohrabmoeini1359@gmail.com)

Iranian Food Science and Technology Research Journal
Vol. 18, No. 3, Aug. Sep. 2022, p. 69-84

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد ۱۸، شماره ۳، مرداد - شهریور ۱۴۰۱، ص. ۸۴-۶۹

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

Full Research Paper

Improving the survival of lactic acid bacteria in Tarhana soup as a non-dairy matrix: Improving the survival of probiotics

M. Azizkhani*[1], R. Karbakhsh Ravari[2]

Received: 2022.02.06
Accepted: 2022.03.06

How to cite this article:
Azizkhani, M., Karbakhsh Ravari, R. (2022). Improving the survival of lactic acid bacteria in Tarhana soup as a non-dairy matrix. *Iranian Food Science and Technology Research Journal*. 18 (3), 69-84.

Abstract

The objective of this study was to improve the survival of lactic acid bacteria (LAB) in Tarhana soup as a non-dairy matrix. *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophiles* were encapsulated in electrospun nanofiber mats fabricated from corn starch (CS) and sodium alginate (SA) and the protective effect of the nanofibers were investigated on the cells during the preparation of Tarhana and in the gastrointestinal tract. The moisture content of the control and nanofiber- loaded dried Tarhana samples was 8.75 and 8.71%, respectively; therefore, using nanofiber mats in the formulation had no significant effect on the moisture content of the samples. A negative zeta potential value of -15.1 mV was found for LAB- loaded nanofibers. The nanofibers mats prepared from SA and CS mix showed a bead- free and clean structure with uniformity in size. The diameter size of most of the fibers ranged from 175- 338 with an average of 265 nm. Loading nanofiber mats with *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* cells led to a uniform distributed beaded structure and the average diameter enhanced to approximately 763 nm. The viability of *L. delbrueckii* and *S. thermophilus* at the end of the electrospinning process was 92.82% and 95.83%, respectively, which indicating a slight loss in their population. Survival of nanoencapsulated *S. thermophilus* and *L. delbrueckii* was 93.50% and 89.16% respectively, while for free cells it was 85.3 and 76.4% that showed considerable protective effect of CS/SA fibers on the cells against dehydration of Tarhana medium. Nanofiber mats improved the stability of the cells against ordinary heat treatment used in preparing Tarhana soup. The survival rate of *S. thermophilus* was higher than *L. delbrueckii* subsp. *bulgaricus* and a significant difference was observed between the viability of free and nanoencapsulated bacteria. The survival of CS/SA nanoencapsulated *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* was 83.25% and 80.21%, respectively, which is indicative of the significant protective effect of fibers on the cells against the heating process. The nanofibers also provided good stability for the cells in the gastrointestinal tract as 10^6 to 10^7 $CFUg^{-1}$ of the cells were survived which is within the recommended level of potential probiotic dose to be effective. There was no significant difference in the color of all samples. Nanoencapsulation in CS/ SA nanofiber mats improved the protection of both LAB strains in simulated fluids of the stomach and intestine (Table 4). After continuous exposure to simulated gastrointestinal fluid, a significant loss of viable free LAB cells (higher than 4 log CFU/ml) was found while the population of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* encapsulated in CS/ SA nanofibers decreased only 0.45 and 0.37 log CFU after 120 min ($p> 0.01$), 0.93 and 0.80 log CFU after 180 min ($p< 0.01$), respectively. Tarhana soup prepared with probiotic– loaded nanofibers gained higher scores in terms of consistency, mouth feel, odor, taste, flavor, and overall acceptability attributes. Tarhana soup with nanofibers possessed much sour taste and flavor than samples prepared with free cells of probiotics. The results of the present study indicated that the protection obtained from CS/ SA capsules secured

1 and 2. Ph.D., Associate Professor and M.Sc. Student, Department of Food Hygiene, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Aftab 24 St., Haraz Av. Amol, Iran.
(Corresponding Author : azizkhani.maryam@gmail.com)
DOI: 10.22067/IFSTRJ.2022.75165.1145

around10^6 to 10^7 CFU/g of the probiotic cells which are within the recommended level of probiotic dose to be functional in consumers' body. Therefore, this product can be used by the consumers like vegetarians and lactose or milk peptide intolerants who do not consume dairy products but need potential fermented probiotic food.

Keywords: Biopolymer, Electrospinning, Encapsulation, Lactic acid bacteria, Tarhana.

Introduction

In recent years, consumers' awareness and interest in the important effect of diet composition and quality on health and well-being has significantly enhanced and resulted in increasing the demands for health- promoting and functional probiotic foods to provide nutrients and modulate one or more targeted physiological function of the body (Nyanzi, Jooste, & Buys, 2021). These types of foods are termed "functional foods" and are considered whole, enriched, or fortified foods that provide health benefits besides providing essential nutrients (like minerals and vitamins) when consumed at efficacious amounts as part of a regular diet (Sridharan & Das, 2019). Among the different types of functional foods, maximum attention has been paid to probiotic foods both as therapeutic supplements and health- promoting foods. Probiotic microorganisms ferment sugars and produce lactic and other organic acids. Furthermore, the enzymatic activity of probiotics changes the nature of food components in a way that exerts beneficial effects on the gastrointestinal tract.

Probiotics are "live microorganisms confer health benefits on the host when adequate level is digested" (Hotel & Cordoba, 2001); therefore, these microorganisms should reach the gastrointestinal tract alive and also in sufficiently high number to provide the health benefit. Prebiotic compounds are a category of complex carbohydrates that have a synergistic effect on probiotics and enhance their growth when administered together. Most of the prebiotics are originated from plant sources, like wheat, onion, garlic, etc. that metabolized by specific members of gut microbiota. So, prebiotics in plant foods can be used as suitable substrates for probiotic microorganisms in producing healthy functional foods (Sridharan & Das, 2019). Zendeboodi et al. (2020) proposed three main groups of probiotic as 'true probiotic' referring to active viable probiotic cells, 'pseudo- probiotic' referring to inactive viable cells, and 'ghost probiotic' referring to nonviable/ dead cells, in the forms of ruptured or intact. Each of these groups are divided into two subgroups according to their site of action/impact: external *(in vitro*) or internal (*in vivo*) (Zendeboodi, Khorshidian, Mortazavian, & da Cruz, 2020). The consumption of probiotic products is associated with multiple health benefits. Grom et al. (2020) evaluated the effect of some probiotic-enriched dairy matrices containing *L. casei* on *in vitro* and *in vivo* anti-hyperglycemic potential. They demonstrated that the type of food matrix has a considerable effect on health-promoting activity (Grom et al., 2020). Probiotic food products containing plant components as substrates for probiotics are good vehicles to deliver these organisms to the gut system. In addition, fermentation of plant ingredients by probiotic bacteria provides easy digestion of food, creates desired taste, degrades anti-nutritional compounds and flatulence- causing oligosaccharides, enhances protein digestibility in tannin- rich cereals, and improves the bio-availability of minerals (Karovičová & Kohajdova, 2007).

Most of the probiotic foods available in the market are fermented milk- originated products but some consumers prefer fermented non-dairy products due to trace cholesterol content or preferring plant- originated foods. Therefore, researchers and food industry are exploring probiotic plant foods to develop industrial-scale production of these types of products.

Tarhana, a traditional fermented cereal-based food popular in the West of Iran (province of Lorestan), is prepared with a

mixting of several types of cereal flours, vegetables, herbs, spices, and yogurt. Originally, it was produced by the Turkish in Middle Asia and then it extended to different parts of the world. The cereals flour and yogurt are mixed with the ratio of 2: 1, kneaded with dried vegetables and several spices, fermented with yogurt starter culture and baker's yeast (mainly *Saccharomyces cerevisiae*) (Demirci, Palabiyik, Ozalp, & Tirpanci Sivri, 2019). The Tarhana soup is prepared from dry or wet Tarhana and has a sour taste. Tarhana has high nutritional value due to being a good source of amino acids (of cereals and yogurt), minerals, B- group vitamins, and fibers. It is also considered functional food as it contains prebiotic and probiotic microorganisms. Tarhana dough which is prepared from yogurt, wheat flour, and barley whole meal found to be a rich source of lactic acid bacteria (LAB) as probiotics and β- glucan as prebiotic (Demirci et al., 2019; Ozdemir, Gocmen, & Yildirim Kumral, 2007). Some studies proposed that yogurt microorganisms (*S. thermophiles* and *L. delbrueckii)* could now be considered probiotics as they confer health benefits to the host (Akbar et al., 2018; Guarner et al., 2005; Mater et al., 2005). During preparation, storage, and digestion of Tarhana, probiotic microorganisms may lose their viability and functionality as the result of exposure to heat and high acidity. As some consumers (*e.g.* vegetarians and lactose or milk peptide intolerants) do not use dairy products but demand probiotic food, Tarhana containing a starter culture of yogurt could be a valuable alternative for them. There are several techniques to protect probiotics against harsh conditions of food processing and digestive tract like microencapsulation and nanoencapsulation (Akbar et al., 2018; López-Rubio, Sanchez, Wilkanowicz, Sanz, & Lagaron, 2012; Zupančič, Škrlec, Kocbek, Kristl, & Berlec, 2019). For example, a spraying method was used to microencapsulate and improve the stability and survival rate of a newly isolated probiotic *Lactococcus lactis* KUMS- T18 strain (originated from traditional Tarhana) during storage at 4°C and 25°C for four months. In their study, Tarhana as a microencapsulation matrix improved the quality and sensory properties of probiotic *L. lactis* enriched potato chips (Kiani et al., 2021). Also, in a recent work by Atraki and Azizkhani (2021), the electrospinning technique was used to nanoencapsulate some strains of lactic acid bacteria and bifidobacteria and improves their survival and viability in simulated condition of gastrointestinal tract (Atraki & Azizkhani, 2021). There is no data about applying electrospinning as a nanoencapsulation method to enhance the stability of yogurt culture bacteria (as potential probiotics) in a non- dairy food matrix (like Tarhana) and also in gastrointestinal tract. Therefore, in the present study, the effect of Nano encapsulation on the survival and viability of *S. thermophiles* and *L. delbrueckii* as Tarhana's potential probiotics during production, storage, and digestion was investigated.

Materials and methods

All culture media used in this work were obtained from Merck (Germany). The chemicals and reagents were purchased from Sigma- Aldrich and Merck (Germany). Wheat flour, onions, tomato, spices (turmeric, red pepper), vegetables (tarragon, mint, and oregano), salt, and baker's yeast used in Tarhana preparation were obtained from retail shops in Sari, Iran. Corn starch and sodium alginate were obtained from Anmol Chemicals Co. (India). Pepsin, trypsin, and bovine bile salt were purchased from Sigma- Aldrich (St. Louis, USA).

Bacterial strains

Commercial starter culture of lactic acid bacteria (LAB) for yogurt preparation, containing *L. delbrueckii* subsp. *bulgaricus* (DSM 24734) and *S. thermophiles* (DSM 24731), was purchased from Danisco/ DuPont (Denmark). The bacteria were cultured in de Man Rogosa and Sharpe (MRS) broth and incubated at 37± 1°C for 18 h under anaerobic conditions (10% carbon dioxide, 10%

hydrogen, and 80% nitrogen). The pellets were then precipitated by centrifugation (6000× g, 10 minutes), rinsed with sterile deionized water, diluted to one- hundredth (in their own broth), and stored at 30± 1°C till used for nanoencapsulation.

Nano encapsulation of the strains

Nano encapsulation of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* was performed by applying the electrospinning technique. At the first step, to prepare a coating solution, the optimum concentrations of corn starch (CS) and sodium alginate (SA) were obtained through evaluating the electrospinning capacity and mechanical properties to achieve desired nanofiber mats. The best spin ability rate was obtained at a concentration of 10% (w v^{-1}) CS (in pure deionized water at 100°C for 120 min) and 5% (w v^{-1}) SA (in pure deionized water). The spinning- dope solutions of CS/ SA to fabricate control (without LAB) nanofiber mats were produced via mixing CS and SA in the ratio of 5:1 (v v^{-1}), respectively. To prepare CS/SA/LAB spinning-dope solutions CSP and fabrication of nanofiber mats loaded with LAB, CS, SA, and CSP (10 log CFU ml^{-1}) were mixed in the ratio of 5:1:2 (v: v: v), respectively, and mixed further for 30 min to obtain homogeneous solutions. Then, each solution was transferred into the injectors (10 ml) attached to needles (Zupančič et al., 2019).

A laboratory- scale electrospinner (Vira System, Tehran, Iran) was used to fabricate nanofibers and the process was optimized to obtain desired nano- scale products: different voltage values (10 to 32 kV), flow rates (0.2 to 2.0 ml h^{-1}), distances between Taylor cone and the flat collector (5 to 15 cm), and also the combinations of these parameters were tested. The optimization results showed that the best condition would be as follows: the voltage of 24 kV, the flow rate of electrospinning dope solutions at 1.5 ml h^{-1}, the current on the needle to collect the fabricated nanofibers on the aluminum plate at 10 μA, and the distance between the needle and the collector was adjusted at 12 cm. The electrospinning process was conducted at 25± 1°C and the solutions were completely volatilized during the electrospinning. The electrospun nanofiber mats were freeze- dried after collecting from the collector to remove the remained water (A. Yilmaz et al., 2016).

Preparing Tarhana samples

Tarhana samples were prepared in two groups. Samples contained free LAB cells and samples which were produced with nanoencapsulated LABs. Tarhana samples were produced according to the traditional method in Lorestan province (Iran) with a slight modification as follows: wheat flour (1000 g), barely meal (300 g), tomato (150 g), onion (150 g), paprika powder (150 g), vegetables (thyme, mint, parsley, dill, native Tarhana herb, totally 200 g), salt (40 g), baker's yeast (10 g), and 0.25 g 10 L^{-1} of the free cells of the commercial starter (Elizaquível et al., 2011) or nanofiber mats loaded with the commercial starter. To prepare Tarhana dough, onion, tomato, vegetables, and paprika powder were completely smashed, mixed, and sieved (pore diameter: 1.5 mm). The mixture was pasteurized at 65± 1°C for 30 min and cooled to the ambient temperature. Then, free or nanencapsulated starter culture, yeast, wheat flour, and barley meal were added and this mixture was kneaded to obtain a homogenous dough. The dough was subsequently fermented at 30± 1°C for 7 days. After the fermentation step, the obtained Tarhana was dried at room temperature (Demirci et al., 2019).

Zeta potential of nanofiber mats loaded with LAB

The stability of a colloidal dispersion and electrophoretic mobility of the particles are determined by the zeta potential value. A Zetasizer Nano ZS (ZEN 3600, Malvern Instruments, Worcestershire, UK) was applied to determine the zeta potential of the nanofiber mats. The samples were prepared by dispersion of 1 mg of nanofiber mats in 5 ml of PBS and run 20 times at 25± 1°C.

Scanning electron microscopy (SEM)

One layer of two-sided tape was fixed to the sample stub of the scanning electron microscope and the freshly prepared nanofiber mat samples were sprayed onto one side of the tape following by gold spraying. The samples were observed on a high-resolution and low-vacuum scanning electron microscope (MIRA3 FEG-SEM, Tescan Co., Czech).

Survival of LAB during the electrospinning process

Since the viability and survival of LAB may be affected by the electrospinning process, the population of these microorganisms was determined at the end of nanoencapsulation in CS/SA nanofiber mats. The plate counting method was used for this purpose as follows: briefly, the LAB loaded-nanofiber mats were mixed with phosphate buffer saline (PBS) at the ratio of 1:1 and incubated for 1 h at 25± 1°C. Then, 10-fold serial dilutions were prepared and cultured on MRS agar. Free LAB strains were also cultured as described above. The plates were incubated at 37± 1°C in anaerobic condition (20% CO_2 in the atmosphere) for 48 h; the population of the free and nanoencapsulated strains was showed as log CFU/ml (López-Rubio et al., 2012).

Moisture content

The moisture content was determined for each sample as the percentage ratio of the weight loss to the initial weight of the sample as in Eq. 1. (AOAC, 2006). Samples were dried at 105ºC for 5 h.

$$MC = \frac{(W_i - W_f)}{(W_f)} \times 100 \quad (1)$$

W_i = initial weight; W_f = final weight, and MC = the moisture content.

Survival of LAB during the preparation of dried Tarhana

Tarhana samples were ground to powder and 25 g of each sample was diluted in 225 ml sterile PBS, homogenized in a stomacher (Stomacher® 400 Circulator, Seward Co., UK), decimal dilutions were prepared, and cultured on MRS agar. The plates were incubated at 37± 1°C for 48 h in anaerobic condition as mentioned above. The population of the LAB cells was reported as log CFU/ml (López-Rubio et al., 2012).

Survival of LAB during the preparation of Tarhana soup

Tarhana pieces were first soaked in cold water (1:5), allowed to dissolve and rehydrate for about 3 hours, and boiled for 20 minutes with occasional stirring. Then, the soup samples were cooled to room temperature, 10-fold serial dilutions were prepared, cultured on MRS agar. Plates were then incubated at 37± 1°C in anaerobic condition for 48 h; the population of the free and nanoencapsulated strains was showed as log CFU ml^{-1}.

Survival of LAB in the simulated gastrointestinal environment

The stability of LAB in Tarhana soup in the continuous model of gastrointestinal fluid was investigated. The simulated gastric fluid was prepared briefly as follows: the pH of the PBS solution was adjusted to 2.5 using 1 M HCl and then 3 $g.l^{-1}$ pepsin was added. To prepare simulated intestinal fluid, 13.6 g of dipotassium hydrogen phosphate, 77 ml of 0.2 M solution of NaOH, 10 g of trypsin, and 1 g of bovine bile salt were mixed with 250 ml deionized distilled water. The pH of the solution was adjusted to 6.80 using 0.2 M NaOH, and the final volume was adjusted to 500 ml using deionized distilled water. The simulated gastric and intestinal fluids were sterilized applying an MF-Millipore™ membrane filter (47 mm diameter, 0.22 µm pore size). Ten ml of Tarhana soup samples were, separately, added to 90 ml of the simulated gastric and incubated at 37ºC on a shaker (100 rpm) (Incu-Shaker, Benchmark Scientific, Canada) for 2 h. Then 25 ml of the gastric digested solution was transferred into 225 ml of simulated intestinal fluid. After 0, 30, 60, 120, and 180 min incubation at 37ºC, 100 µl from each sample was taken for viable cell counting. To count the viable cells, decimal

serial dilutions from each sample was prepared using PBS solution, cultured on MRS agar, and incubated under anaerobic conditions at 37°C for 48 h (Ji et al., 2019a; Yasmin, Saeed, Pasha, & Zia, 2019).

Sensory analysis

Sensory evaluation was performed on prepared Tarhana soups by twelve trained panelists (six male and six female, 20- 52 years old). The training of the panelist group was carried out for 6 h (6 sessions, each session 1h) in Amol University of Special Modern Technologies. The ingredients of the samples were explained to the panelists. Both groups of the soups were served to panelists in porcelain bowls at 50°C and the sensory evaluation was carried out in a room with daylight condition. Scoring of Tarhana soup samples was performed regarding color, odor, mouth feel, consistency, flavor, sourness, and overall acceptability applying the five-point hedonic scale (as 1= extremely disliked, 2= dislike a little, 3= neither like nor dislike, 4= like a little, and 5= extremely liked) (Demirci et al., 2019).

Statistical analyses

All the experiments were performed in triplicate. Data were analyzed by Independent Samples t-test at 95% confidence level ($p<0.05$) using SPSS (version 22.0) and presented as the mean± standard deviation. Mann-Whitney test was used as a non-parametric test to compare the sensory scores of two groups of Tarhana samples.

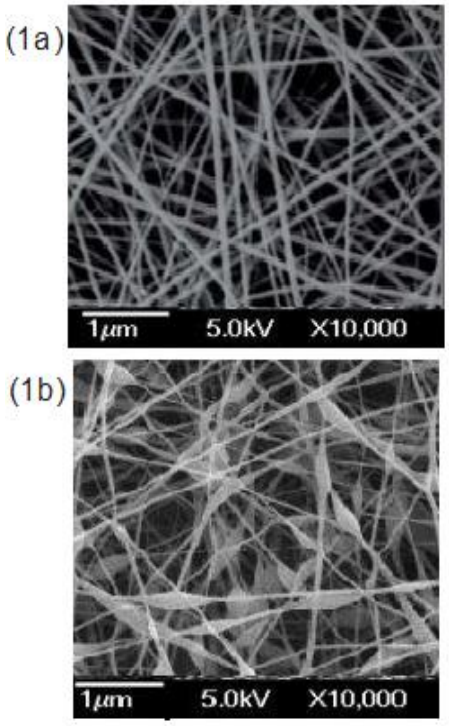


Fig. 1. SEM images of the LAB free (control) and probiotic-loaded nanofiber mats

Results and discussion

Zeta potential of nanofiber mats loaded with LAB

Zeta potential shows the particle surface charge and is used as a determining parameter in characterizing nano-scaled particles. Zeta potential presents the electrostatic potential value and it is claimed that zeta potential values of ±30 mV are representative of well-stabilized particles (Vogel et al., 2017). In this work, a negative zeta potential value of -13.84± 0.50 and -15.1± 0.77 mV was obtained for the control (nanofiber mats without LAB) and LAB-loaded nanofiber mats, respectively ($p<0.05$).

SEM images

Figure 1 shows the SEM images of the LAB-free (control) and LAB-loaded nanofiber mats. As seen, the nanofibers mats prepared from SA and CS mix showed a bead-free and clean structure with uniformity in size. The diameter size of most of the fibers was ranged from 175-338 with an average of 265 nm (Fig. 1a). Loading nanofiber mats with *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* cells led to a uniform distributed beaded structure as seen in Fig. 1b and the average diameter enhanced to approximately 763 nm.

Survival of LAB during the electrospinning process

A viability test was conducted to investigate the effect of encapsulation in electrospun fibers on LAB. According to the data presented in Table 1, the viability of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* cells at the end of the electrospinning were 92.82% and 95.83%, respectively, which indicated a slight loss in their population.

Table 1- Viability of the *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* during nanoencapsulation by electrospinning

	***L. delbrueckii* subsp. *bulgaricus* (log CFU ml^{-1})**	***S. thermophilus* (log CFU ml^{-1})**
Initial free cells	10.18± 0.75^{a*}	10.33± 1.15 ^{b}
Electrospun nanoencapsulated cells	9.45± 1.02 ^{a}	9.90± 0.83 ^{b}

*Different lowercase superscripts in a column express significant difference between means

Survival of LAB during the preparation of dried Tarhana

The moisture content of the control and nanofiber-loaded dried Tarhana samples was 8.75 and 8.71%, respectively; therefore, using nanofiber mats in the formulation had no significant effect on the moisture content of the samples ($p>0.05$). Survival of LAB during the drying step was measured to evaluate the effect of encapsulation within nanofibers on the stability of the bacteria in the dehydration process and low moisture content. As presented in Table 1, nanofiber mats had a protective effect on the cells, and *S. thermophilus* showed a higher viability rate in comparison to *L. delbrueckii* subsp. *bulgaricus* cells during the drying step ($p<0.05$). This might be due to lower water activity (~ 0.95) (Zhou et al., 2008) *S. thermophilus* needs to survive and proliferate in comparison to *L. delbrueckii* (~0.97) (Kamel, Gomma, Osman, & Hassan, 2018). At the end of the drying Tarhana samples, there was a significant difference between the survival rate of free and encapsulated bacteria ($p< 0.05$). The viability of nanoencapsulated *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* was 93.50% and 89.16%, respectively, while for free cells was 85.3 and 76.4%, respectively, that indicated the considerable protective effect of CS/SA fibers on the cells against dehydration of Tarhana medium.

Survival of LAB in Tarhana soup

To investigate the effect of nanoencapsulation within CS/SA fiber mats on the thermal stability of the bacteria in the heating process, the viability of LAB during preparing Tarhana soup was evaluated. As presented in Table 3, nanofiber mats improved the stability of the cells against ordinary heat treatment used in preparing Tarhana soup. The survival rate of *S. thermophilus* was higher than *L. delbrueckii* subsp. *bulgaricus* ($p< 0.05$) and a significant difference was observed between the viability of free and nanoencapsulated bacteria ($p< 0.05$). The survival of CS/SA nanoencapsulated *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* was 83.25% and 80.21%, respectively, which is indicative of the significant protective effect of fibers on the cells against the heating process.

Table 2- Viability of the *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* during drying process of Tarhana samples

	L. delbrueckii subsp. *bulgaricus* (log CFU ml^{-1})	*S. thermophilus* (log CFU ml^{-1})
Free cells in wet Tarhana	9.35± 0.75^{a*}	9.26± 1.15 ^{a}
Free cells in dried Tarhana	7.15± 1.02 ^{b}	7.90± 0.83 ^{b}
Nanoencapsulated cells in wet Tarhana	9.42± 0.75^{a}	9.89± 1.15 ^{a}
Nanoencapsulated cells in dried Tarhana	8.39± 1.02 ^{c}	9.24± 0.83 ^{a}

*Different lowercase superscripts in a column express significant difference between means

Table 3- Survival of the *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* during cooking process of Tarhana soup

	L. delbrueckii subsp. *bulgaricus* (log CFU ml^{-1})	*S. thermophilus* (CFU ml^{-1})
Free cells in dried Tarhana (before cooking)	7.15± 1.02^{a*}	7.90± 0.83 ^{a}
Free cells in Tarhana soup	3.85± 0.27 ^{b}	4.49± 0.61 ^{b}
Survival rate of free cells (%)	53.8	56.8
Nanoencapsulated cells in dried Tarhana (before cooking)	8.39± 1.02^{c}	9.24± 0.83 ^{c}
Nanoencapsulated cells in Tarhana soup	6.73± 0.80 ^{d}	7.69± 0.55 ^{a}
Survival rate of nanoencapsulated cells (%)	80.21	83.25

*Different lowercase superscripts in a column express significant difference between means

Survival of LAB in simulated gastrointestinal model

The free and CS/SA nanoencapsulated LAB were exposed to simulated gastrointestinal environment and the viability of the cells was studied. Nanoencapsulation in CS/SA nanofiber mats improved the protection of both LAB in simulated fluids of the stomach and intestine (Table 4). After continuous exposure to simulated gastrointestinal fluid, a significant loss of viable free LAB cells (higher than 4 log CFU/ml) was found while the population of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* encapsulated in CS/SA nanofibers decreased only 0.45 and 0.37 log CFU at 120 min ($p > 0.01$), 0.93 and 0.80 log CFU at 180 min ($p < 0.01$), respectively.

Table 4- Survival of free and nanoencapsulated *S. thermophilus* (*S*) and *L. delbrueckii* subsp. *bulgaricus* (*L*) upon exposure to continuous simulated gastrointestinal fluid.
Data are presented as mean± standard deviation log CFU.ml^{-1} from triplicate experiments (n =3)

	Exposure Time (min)				
	0	**30**	**60**	**120**	**180**
Free *S*	4.49± 0.61^{b*}	ND†	ND	ND	ND
Nanoencapsulated *S*	7.69± 0.55 ^{a}	7.50± 0.18^{b}	7.41± 0.35^{c}	7.32± 0.25^{c}	6.89± 0.47^{d}
Free *L*	3.85± 0.27 ^{b}	ND	ND	ND	ND
Nanoencapsulated *L*	6.73± 0.80 ^{d}	6.56± 0.71^{b}	6.39± 0.50^{c}	6.28± 0.10^{c}	5.80± 0.33^{c}

†ND: No cell detectable.
*Different lowercase superscripts in a row express significant difference between means during the incubation time ($p<0.01$).

Sensory properties

Table 5 presents the sensorial properties of the Tarhana samples. There was no significant difference in color of the samples ($p > 0.05$). Tarhana soup prepared with probiotic–loaded nanofibers gained higher scores in terms of

consistency, mouth feel, odor, taste, flavor, and overall acceptability attributes. Tarhana soup with nanofibers showed much sour taste and flavor than samples prepared with free cells of probiotics ($p< 0.05$).

Table 5- sensory analysis of Tarhana soup samples

Sensory properties	Tarhana soup samples	
	with free LAB	with nanoencapsulated LAB
Color	3.25± 0.28 [a*]	3.50± 0.31 [a]
Flavor	4.36± 050 [a]	3.95± 0.12 [b]
Taste	4.18± 0.35 [a]	3.77± 0.25 [b]
Odor	3.85± 0.10 [a]	3.80± 0.42 [a]
Sourness	4.55± 0.21 [a]	3.70± 0.18 [b]
Consistency	3.44± 0.16 [a]	2.91± 0.13 [b]
Mouth feel	3.57± 0.29 [a]	2.82± 0.34 [b]
Overall acceptability	3.64± 0.20 [a]	3.05± 0.45 [b]

*Different lowercase superscripts in a row express significant difference between Means during the incubation time ($p< 0.01$).

According to the results, the control and probiotic-loaded nanofiber mats had a negative zeta potential value of -13.84± 0.50 and -15.1± 0.77 mV, respectively. The negative charge and the resulted negative zeta potential of the nanofibers may be due to the detachment of protons from the acid groups of sodium alginate (Borumand, 2013) and also the presence of free carboxylic acid groups at the surface of alginate molecules (Borumand, 2013). Also, the corn starch molecule has a negative charge at the surface due to the de-protonation of some hydroxyl (OH) groups and dissociation of carboxylic (COOH) groups during the mixing-heating process (Robinson, Coustel, Abdelmoula, & Mallet, 2020). In a study by Wang et al., the nanoparticles prepared from starch and sodium alginate showed a zeta potential of -10.5 mV (Wang et al., 2017). Our findings demonstrated that loading nanofibers with probiotics caused lower zeta potential compared to unloaded nanofibers. It can be stated that loading nanofiber mats with probiotics increased the negativity of the zeta potential value. The higher negative zeta potential values of probiotic-loaded nanofiber mats can be explained by the net negative charge and negative zeta potential value of *Lactobacilli* cells (Dean, Leary, Sullivan, Oh, & Walper, 2019; Ji et al., 2019b; Murga, de Valdez, & Disalvo, 2000; Pérez, Minnaard, Disalvo, & De Antoni, 1998) that shows efficient nanoencapsulation of probiotics within nanofibers in our work.

The average diameter of the fibers was 265 nm and loading nanofiber mats with *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* cells led to a uniform distributed beaded structure with the average diameter of 763 nm, demonstrating the successful nanoencapsulation of the bacteria in the CS/ SA nanofibers. In research by Yilmaz et al. (2020), loading *L. paracasei* into the nanofiber mats (initial diameter size of 305 nm) resulted in a beaded structure of fibers with an increase of diameter to 842 nm (M. T. Yilmaz, Taylan, Karakas, & Dertli, 2020) which is similar to our findings Skrlec et al. (2019) prepared the poly (ethylene oxide) nanofibers loaded with *L. plantarum* cells with a diameter of 492 nm which had a lower size compared to the loaded nanofibers of our study (Škrlec et al., 2019) due to the difference between the coating material used to produce the nanofibers and the effect of the bacterial characteristics on the fiber diameter size.

The viability rates of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* cells during the electrospinning process was found 92.82% and 95.83%, respectively, which indicated a slight

loss in their population. Factors such as rapid evaporation of water content and high rate changes of osmotic pressure affect the survival of the cells during the electrospinning process (López-Rubio et al., 2012). In a previous study, a high viability rate was observed for *Bb. animalis* subsp. *Lactis Bb*12 and combination of *Streptococcus thermophilus*, *L. paracasei* and *Bb*-12 following encapsulation using the electrospun poly (vinyl alcohol) fibers (Akbar et al., 2018). Similar results were claimed by other researchers that prepared electrospun nanofibers from alginate (M. T. Yilmaz et al., 2020) and starch (Lancuški et al., 2017) to nanoencapsulate *L. paracasei* which provided good cell viability (higher than 85%). It is indicated that calcium alginate improved the viability of *L. rhamnosus, L. casei, L. acidophilus,* and *Bifidobacterium.* spp. in fermented dairy products and the survival rate was increased by combining calcium alginate and starch as the coating material (Chen, Wang, Liu, & Gong, 2017). The survival rate of the LAB cells in the present work was considerably higher than other studies that show good compatibility of the starch and sodium alginate to provide protection for probiotics.

In this work, there was a significant difference between the survival rate of free and encapsulated bacteria at the end of the drying process (93.50% and 89.16% for *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus,* respectively, verses 85.3 and 76.4%). The protecting activity of alginate capsules was also investigated by Albadran et al. (2015). They evaluated the stability of fluid bed and freeze-dried chitosan-coated alginate microcapsules loaded with LAB. The viability loss was about 0.8 and1.3 log for fluid bed and freeze-dried samples. In both samples, the moisture content and aw were lower than 10% w/w and 0.25, respectively, which is necessary for high stability during long storage (Albadran, Chatzifragkou, Khutoryanskiy, & Charalampopoulos, 2015). It is demonstrated by several studies that decreasing the moisture content and aw increases the survival of the dried encapsulated cells (Bora, Li, Zhu, & Du, 2019; Dianawati, Mishra, & Shah, 2016; Liu et al., 2019). Donthidi et al. evaluated the effect of starch on the viability of alginate encapsulated probiotics at different temperatures. The incorporation of starch improved the entrapment efficiency and the viability of encapsulated bacteria that is similar to our results (Donthidi, Tester, & Aidoo, 2010). The use of starch for the encapsulation of LAB can provide technological benefits like stability against high temperature and low aw. Furthermore, combining starch with alginate promotes a synergistic effect on the gelation of starch and provides further protection to LAB cells (de Araújo Etchepare et al., 2016; Donthidi et al., 2010; Mirzaei, Pourjafar, & Homayouni Rad, 2011).

The survival rate of CS/SA nanoencapsulated *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* (83.25% and 80.21%, respectively) showed the significant protective effect of fibers on the cells against the thermal processing. Mahmoud et al. (2020) studied the viability of alginate-microencapsulated *L. plantarum* during food processing. They reported that survival of the microencapsulated *L. plantarum* cells improved over the initial count, which was about 8 log CFU g^{-1} sample, upon exposure to 40 and 45˚C for 24 h and 30 min, respectively. In contrast, exposure to 65˚C for 30 min decreased the viability of the encapsulated bacteria (Mahmoud, Abdallah, El-Shafei, Tawfik, & El-Sayed, 2020). Also, Ouled-Haddar et al. (2016) found 100% and 90% survival of SA-encapsulated *L. plantarum* upon heat treatment at 40 and 50˚C for 20 min, respectively. SA-Skimmed milk encapsulating material maintained 10^6 CFU g^{-1} of *L. plantarum* after exposure to 65˚C for 30 min, which is the dietary recommended dose of probiotics in the food to exert functional effects (Bilenler, Karabulut, & Candogan, 2017; Teoh, Mirhosseini, Mustafa, Hussin, & Abdul Manap, 2011).

Nanoencapsulation in CS/SA nanofiber mats increased the viability of both LAB in simulated fluids of the stomach and intestine.

Our findings demonstrated that CS/SA nanofiber mats protected the LAB from adverse effects of gastric acid condition and bile salt. The technique used in our study to fabricate nanocapsules resulted in a higher level of protection in comparison to other methods (like microemulsification, nanoemulsification, extrusion, etc.) applied in previous studies (Coghetto, Brinques, & Ayub, 2016; Ji et al., 2019a; Liu et al., 2018; Yeung, Üçok, Tiani, McClements, & Sela, 2016). Mahmoud et al. (2020) reported the viability loss of 1.24, 1.71, and 2.47 log CFU for *L. plantarum* cells entrapped within SA/chitosan, SA/skimmed milk, and SA/dextran, respectively, upon 120 min exposure to gastrointestinal fluid. Also, combining our data with other studies 'data showed that the incorporation of sodium alginate and corn starch in nanofibers provides a higher survival rate in gastrointestinal fluids in comparison with alginate combined with other compounds (Ji et al., 2019a; Yeung et al., 2016; M. T. Yilmaz et al., 2020). In order to perform their functional activity in human body, probiotics must reach the small intestine and then colonize there in enough number, that is 10^6 -10^7 CFU g^{-1} and encapsulation seemed to be a promising method for increasing the viability of LAB cells in gastrointestinal tract conditions (Shori, 2017). Several studies have proved that combining SA with other polymers increases alginate's protective effect on probiotics. For example, it is reported that chitosan coating of alginate microcapsules resulted in a high level survival of *L. plantarum* (Fareez, Lim, Mishra, & Ramasamy, 2015). Ramirez et al. (2015) reported that alginate and starch granules exert a protective effect on each other (Ramírez et al., 2015) that can prevent or reduce the action of digestive enzymes on the encapsulated object. This finding is related to the electrostatic interactions between alginate and other molecules that forms a strong membrane and decreases the likelihood of leakage of the capsulated cells. The results also indicated that the protection provided by CS/SA maintained around10^6 to 10^7 CFU g^{-1} of the cells which is within the recommended level of probiotic dose to be functional and effective.

Tarhana soup prepared with probiotic–loaded nanofibers gained higher scores in terms of consistency, mouth feel, odor, taste, flavor, and overall acceptability attributes. This might be due to higher survival of nanoencapsulated bacteria which resulted in higher rate of fermentation and acid and flavoring metabolites production. For the overall acceptability, the panelists recorded lower scores for Tarhana samples prepared with free cells of *S. thermophilus* (*S*) and *L. delbrueckii* subsp. *bulgaricus*. The slight difference between consistency and mouth feel of Tarhana samples with nanofibers and the control is related to the presence of SA and CS (as bodying agents) that improved the consistency and texture of the samples. The overall sensorial data showed that encapsulating *S. thermophilus* (*S*) and *L. delbrueckii* subsp. *bulgaricus* in CS/SA nanofiber mats resulted in acceptable Tarhana soup properties according to the overall preference of the panelists.

Encapsulating *S. thermophilus* (*S*) and *L. delbrueckii* subsp. *bulgaricus* as the fermenting bacteria in preparation process of Tarhana improved their survival and viability during drying step and heat treatment during preparation of Tarhana soup and also provided a considerable protective effect on probiotics in gastrointestinal tract. The Tarhana soup prepared with encapsulated cells of *S. thermophilus* (*S*) and *L. delbrueckii* subsp. *bulgaricus* in corn starch and sodium alginate nanofiber mats was highly preferred in terms of the sensory properties in comparison to the samples containing free cells.

Conclusion

The results of the present study indicated that the protection obtained from CS/SA capsules maintained around10^6 to 10^7 CFU/g of the probiotic cells which are within the recommended level of probiotic dose to be functional in consumers' body. Therefore, this product can be used by the consumers like vegetarians and lactose or milk peptide

intolerants who do not consume dairy products but need potential fermented probiotic food.

Funding

This research was financially supported by a grant by Amol University of Special Modern Technologies.

Conflict of interest statement

No conflict of interest declared.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

References

1. Akbar, Z., Zahoor, T., Huma, N., Jamil, A., Ayesha, H., & JM, K. I. (2018). Electrospun probiotics: an alternative for encapsulation. *Journal of biological regulators and homeostatic agents, 32*(6), 1551-1556.
2. Albadran, H. A., Chatzifragkou, A., Khutoryanskiy, V. V., & Charalampopoulos, D. (2015). Stability of probiotic Lactobacillus plantarum in dry microcapsules under accelerated storage conditions. *Food Research International, 74*, 208-216.
3. AOAC. (2006). *Official methods of analysis of the Association of Official Analytical Chemists*. Retrieved from
4. Atraki, R., & Azizkhani, M. (2021). Survival of probiotic bacteria nanoencapsulated within biopolymers in a simulated gastrointestinal model. *Innovative Food Science & Emerging Technologies, 72*, 102750. https://doi.org/10.1016/j.ifset.2021.102750
5. Bilenler, T., Karabulut, I., & Candogan, K. (2017). Effects of encapsulated starter cultures on microbial and physicochemical properties of traditionally produced and heat treated sausages (sucuks). *LWT, 75*, 425-433. https://doi.org/10.1016/j.lwt.2016.09.003
6. Bora, A. F. M., Li, X., Zhu, Y., & Du, L. (2019). Improved viability of microencapsulated probiotics in a freeze-dried banana powder during storage and under simulated gastrointestinal tract. *Probiotics and antimicrobial proteins, 11*(4), 1330-1339. https://doi.org/10.1007/s12602-018-9464-1
7. Borumand, M. R. (2013). Preparation and characterization of sodium alginate nanoparticles containing ICD-85 (venom derived peptides). *International journal of innovation and applied studies, 4*(3), 534-542.
8. Chen, J., Wang, Q., Liu, C.-M., & Gong, J. (2017). Issues deserve attention in encapsulating probiotics: Critical review of existing literature. *Critical reviews in food science and nutrition, 57*(6), 1228-1238. https://doi.org/10.1080/10408398.2014.977991
9. Coghetto, C. C., Brinques, G. B., & Ayub, M. A. Z. (2016). Probiotics production and alternative encapsulation methodologies to improve their viabilities under adverse environmental conditions. *International journal of food sciences and nutrition, 67*(8), 929-943. https://doi.org/10.1080/09637486.2016.1211995
10. de Araújo Etchepare, M., Raddatz, G. C., de Moraes Flores, É. M., Zepka, L. Q., Jacob-Lopes, E., Barin, J. S., . . . de Menezes, C. R. (2016). Effect of resistant starch and chitosan on survival of Lactobacillus acidophilus microencapsulated with sodium alginate. *LWT-Food Science and Technology, 65*, 511-517.
11. Dean, S. N., Leary, D. H., Sullivan, C. J., Oh, E., & Walper, S. A. (2019). Isolation and characterization of Lactobacillus-derived membrane vesicles. *Scientific reports, 9*(1), 1-11. https://doi.org/10.1038/s41598-018-37120-6

12. Demirci, A. S., Palabiyik, I., Ozalp, S., & Tirpanci Sivri, G. (2019). Effect of using kefir in the formulation of traditional Tarhana. *Food Science and Technology, 39*(2), 358-364. https://doi.org/10.1590/fst.29817
13. Dianawati, D., Mishra, V., & Shah, N. P. (2016). Survival of microencapsulated probiotic bacteria after processing and during storage: a review. *Critical reviews in food science and nutrition, 56*(10), 1685-1716. https://doi.org/10.1080/10408398.2013.798779
14. Donthidi, A., Tester, R. F., & Aidoo, K. E. (2010). Effect of lecithin and starch on alginate-encapsulated probiotic bacteria. *Journal of microencapsulation, 27*(1), 67-77. https://doi.org/10.3109/02652040902982183
15. Elizaquível, P., Sánchez, G., Salvador, A., Fiszman, S., Dueñas, M. T., López, P., . . . Aznar, R. (2011). Evaluation of yogurt and various beverages as carriers of lactic acid bacteria producing 2-branched (1, 3)-β-D-glucan. *Journal of Dairy Science, 94*(7), 3271-3278. https://doi.org/10.3168/jds.2010-4026
16. Fareez, I. M., Lim, S. M., Mishra, R. K., & Ramasamy, K. (2015). Chitosan coated alginate–xanthan gum bead enhanced pH and thermotolerance of Lactobacillus plantarum LAB12. *International journal of biological macromolecules, 72*, 1419-1428. https://doi.org/10.1016/j.ijbiomac.2014.10.054
17. Grom, L., Rocha, R., Balthazar, C., Guimarães, J., Coutinho, N., Barros, C., . . . Silva, P. (2020). Postprandial glycemia in healthy subjects: Which probiotic dairy food is more adequate? *Journal of Dairy Science, 103*(2), 1110-1119. https://doi.org/10.3168/jds.2019-17401
18. Guarner, F., Perdigon, G., Corthier, G., Salminen, S., Koletzko, B., & Morelli, L. (2005). Should yoghurt cultures be considered probiotic? *British Journal of Nutrition, 93*(6), 783-786. https://doi.org/10.1079/BJN20051428
19. Hotel, A. C. P., & Cordoba, A. (2001). Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. *Prevention, 5*(1), 1-10.
20. Ji, R., Wu, J., Zhang, J., Wang, T., Zhang, X., Shao, L., . . . Wang, J. (2019a). Extending viability of Bifidobacterium longum in chitosan-coated alginate microcapsules using emulsification and internal gelation encapsulation technology. *Frontiers in microbiology, 10*, 1389.
21. Ji, R., Wu, J., Zhang, J., Wang, T., Zhang, X., Shao, L., . . . Wang, J. (2019b). Extending viability of Bifidobacterium longum in chitosan-coated alginate microcapsules using emulsification and internal gelation encapsulation technology. *Frontiers in Microbiology, 10*. https://doi.org/10.3389/fmicb.2019.01389
22. Kamel, D. G., Gomma, N. H., Osman, D. M., & Hassan, A. (2018). Effect of Water Activity on Growth of Certain Lactic Acid Bacteria. *Journal of Food and Dairy Sciences, 2018*, 97-102. DOI: 10.21608/jfds.2018.77762
23. Karovičová, Z. K.-J., & Kohajdova, J. (2007). Fermentation of cereals for specific purpose. *Journal of Food and Nutrition Research, 46*(2), 51-57.
24. Kiani, A., Nami, Y., Hedayati, S., Jaymand, M., Samadian, H., & Haghshenas, B. (2021). Tarkhineh as a new microencapsulation matrix improves the quality and sensory characteristics of probiotic Lactococcus lactis KUMS-T18 enriched potato chips. *Scientific Reports, 11*(1), 1-13. https://doi.org/10.1038/s41598-021-92095-1
25. Lancuški, A., Ammar, A. A., Avrahami, R., Vilensky, R., Vasilyev, G., & Zussman, E. (2017). Design of starch-formate compound fibers as encapsulation platform for biotherapeutics. *Carbohydrate polymers, 158*, 68-76. https://doi.org/10.1016/j.carbpol.2016.12.003
26. Liu, H., Cui, S. W., Chen, M., Li, Y., Liang, R., Xu, F., & Zhong, F. (2019). Protective approaches and mechanisms of microencapsulation to the survival of probiotic bacteria during processing,

storage and gastrointestinal digestion: a review. *Critical reviews in food science and nutrition*, *59*(17), 2863-2878. https://doi.org/10.1080/10408398.2017.1377684
27. Liu, H., Gong, J., Chabot, D., Miller, S. S., Cui, S. W., Zhong, F., & Wang, Q. (2018). Improved survival of Lactobacillus zeae LB1 in a spray dried alginate-protein matrix. *Food Hydrocolloids*, *78*, 100-108. https://doi.org/10.1016/j.foodhyd.2017.07.004
28. López-Rubio, A., Sanchez, E., Wilkanowicz, S., Sanz, Y., & Lagaron, J. M. (2012). Electrospinning as a useful technique for the encapsulation of living bifidobacteria in food hydrocolloids. *Food Hydrocolloids*, *28*(1), 159-167. https://doi.org/10.1016/j.foodhyd.2011.12.008
29. Mahmoud, M., Abdallah, N. A., El-Shafei, K., Tawfik, N. F., & El-Sayed, H. S. (2020). Survivability of alginate-microencapsulated Lactobacillus plantarum during storage, simulated food processing and gastrointestinal conditions. *Heliyon*, *6*(3), e03541. https://doi.org/10.1016/j.heliyon.2020.e03541
30. Mater, D. D., Bretigny, L., Firmesse, O., Flores, M.-J., Mogenet, A., Bresson, J.-L., & Corthier, G. (2005). Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus survive gastrointestinal transit of healthy volunteers consuming yogurt. *FEMS Microbiology Letters*, *250*(2), 185-187. https://doi.org/10.1016/j.femsle.2005.07.006
31. Mirzaei, H., Pourjafar, H., & Homayouni Rad, A. (2011). The effect of microencapsulation with calcium alginate and resistant starch on the Lactobacillus acidophilus (La5) survival rate in simulated gastrointestinal juice conditions. *Journal of Veterinary Research*, *66*(4), 337-342.
32. Murga, M. a. L. F., de Valdez, G. F., & Disalvo, A. b. E. (2000). Changes in the surface potential of Lactobacillus acidophilus under freeze–thawing stress. *Cryobiology*, *41*(1), 10-16. https://doi.org/10.1006/cryo.2000.2259
33. Nyanzi, R., Jooste, P. J., & Buys, E. M. (2021). Invited review: Probiotic yogurt quality criteria, regulatory framework, clinical evidence, and analytical aspects. *Journal of Dairy Science*, *104*(1), 1-19. https://doi.org/10.3168/jds.2020-19116
34. Ozdemir, S., Gocmen, D., & Yildirim Kumral, A. (2007). A traditional Turkish fermented cereal food: Tarhana. *Food Reviews International*, *23*(2), 107-121. https://doi.org/10.1080/87559120701224923
35. Pérez, P. F., Minnaard, Y., Disalvo, E. A., & De Antoni, G. L. (1998). Surface properties of bifidobacterial strains of human origin. *Applied and environmental microbiology*, *64*(1), 21-26. DOI: https://doi.org/10.1128/AEM.64.1.21-26.1998
36. Ramírez, C., Millon, C., Nunez, H., Pinto, M., Valencia, P., Acevedo, C., & Simpson, R. (2015). Study of effect of sodium alginate on potato starch digestibility during in vitro digestion. *Food Hydrocolloids*, *44*, 328-332. https://doi.org/10.1016/j.foodhyd.2014.08.023
37. Robinson, M. R., Coustel, R., Abdelmoula, M., & Mallet, M. (2020). As (V) and As (III) sequestration by starch functionalized magnetite nanoparticles: influence of the synthesis route onto the trapping efficiency. *Science and Technology of Advanced Materials*, *21*(1), 524-539. https://doi.org/10.1080/14686996.2020.1782714
38. Shori, A. B. (2017). Microencapsulation improved probiotics survival during gastric transit. *HAYATI Journal of Biosciences*, *24*(1), 1-5. https://doi.org/10.1016/j.hjb.2016.12.008
39. Škrlec, K., Zupančič, Š., Mihevc, S. P., Kocbek, P., Kristl, J., & Berlec, A. (2019). Development of electrospun nanofibers that enable high loading and long-term viability of probiotics. *European Journal of Pharmaceutics and Biopharmaceutics*, *136*, 108-119. https://doi.org/10.1016/j.ejpb.2019.01.013

40. Sridharan, S., & Das, K. M. S. (2019). A Study on Suitable Non Dairy Food Matrix for Probiotic Bacteria–A Systematic Review. *Current Research in Nutrition and Food Science Journal, 7*(1), 05-16.
41. Teoh, P. L., Mirhosseini, H., Mustafa, S., Hussin, A. S. M., & Abdul Manap, M. Y. (2011). Recent approaches in the development of encapsulated delivery systems for probiotics. *Food Biotechnology, 25*(1), 77-101. https://doi.org/10.1080/08905436.2011.547332
42. Vogel, R., Pal, A. K., Jambhrunkar, S., Patel, P., Thakur, S. S., Reátegui, E., . . . Broom, M. F. (2017). High-resolution single particle zeta potential characterisation of biological nanoparticles using tunable resistive pulse sensing. *Scientific reports, 7*(1), 1-13. https://doi.org/10.1038/s41598-017-14981-x
43. Wang, S., Yang, C., Tu, H., Zhou, J., Liu, X., Cheng, Y., . . . Xu, J. (2017). Characterization and metabolic diversity of flavonoids in citrus species. *Scientific Reports, 7*(1), 1-10. https://doi.org/10.1038/s41598-017-10970-2
44. Yasmin, I., Saeed, M., Pasha, I., & Zia, M. A. (2019). Development of whey protein concentrate-pectin-alginate based delivery system to improve survival of B. longum BL-05 in simulated gastrointestinal conditions. *Probiotics and antimicrobial proteins, 11*(2), 413-426. https://doi.org/10.1007/s12602-018-9407-x
45. Yeung, T. W., Üçok, E. F., Tiani, K. A., McClements, D. J., & Sela, D. A. (2016). Microencapsulation in alginate and chitosan microgels to enhance viability of Bifidobacterium longum for oral delivery. *Frontiers in microbiology, 7*, 494. https://doi.org/10.3389/fmicb.2016.00494
46. Yilmaz, A., Bozkurt, F., Cicek, P. K., Dertli, E., Durak, M. Z., & Yilmaz, M. T. (2016). A novel antifungal surface-coating application to limit postharvest decay on coated apples: Molecular, thermal and morphological properties of electrospun zein–nanofiber mats loaded with curcumin. *Innovative Food Science & Emerging Technologies, 37*, 74-83. https://doi.org/10.1016/j.ifset.2016.08.008
47. Yilmaz, M. T., Taylan, O., Karakas, C. Y., & Dertli, E. (2020). An alternative way to encapsulate probiotics within electrospun alginate nanofibers as monitored under simulated gastrointestinal conditions and in kefir. *Carbohydrate Polymers*, 116447. https://doi.org/10.1016/j.carbpol.2020.116447
48. Zendeboodi, F., Khorshidian, N., Mortazavian, A. M., & da Cruz, A. G. (2020). Probiotic: conceptualization from a new approach. *Current Opinion in Food Science, 32*, 103-123. https://doi.org/10.1016/j.cofs.2020.03.009
49. Zhou, K., Cui, T., Li, P., Liang, N., Liu, S., Ma, C., & Peng, Z. (2008). Modelling and predicting the effect of temperature, water activity and pH on growth of Streptococcus iniae in Tilapia. *Journal of applied microbiology, 105*(6), 1956-1965. https://doi.org/10.1111/j.1365-2672.2008.03969.x
50. Zupančič, Š., Škrlec, K., Kocbek, P., Kristl, J., & Berlec, A. (2019). Effects of electrospinning on the viability of ten species of lactic acid bacteria in poly (ethylene oxide) nanofibers. *Pharmaceutics, 11*(9), 483. https://doi.org/10.3390/pharmaceutics11090483

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد ۱۸، شماره ۳، مرداد - شهریور ۱۴۰۱، ص. ۸۴-۶۹

Iranian Food Science and Technology Research Journal
Vol. 18, No. 3, Aug. Sep. 2022, p. 69-84

افزایش بقای باکتری‌های اسید لاکتیک در سوپ ترخینه به‌عنوان ماتریکس غیرلبنی

مریم عزیزخانی*[۱]- رفعت کاربخش راوری

تاریخ دریافت: ۱۴۰۰/۱۱/۱۷
تاریخ پذیرش: ۱۴۰۰/۱۲/۱۵

چکیده

ترخینه، غذای تخمیری سنتی ایران بر پایه غلات، از مخلوطی از آرد غلات، سبزیجات، سبزی، ادویه‌جات و ماست تهیه می‌شود. هدف از این مطالعه بهبود بقای باکتری‌های اسید لاکتیک (LAB) در سوپ ترخینه به‌عنوان یک ماتریکس غیرلبنی بود. *لاکتوباسیلوس دلبروکئی* زیرگونه *بولگاریکوس* و *استرپتوکوکوس ترموفیلوس* در نانوالیاف الکتروریسی شده تهیه شده از نشاسته ذرت (CS) و سدیم آلژینات (SA) ریزپوشانی شدند و اثر محافظتی نانوالیاف بر سلول‌ها در طول تهیه ترخینه و نیز در دستگاه گوارش مورد بررسی قرار گرفت. نتایج نشان داد که مقدار پتانسیل زتا ۱۵/۱- میلی ولت برای نانوالیاف بارگذاری شده با LAB مشاهده شد. نانوالیاف حاوی LAB دارای ساختار مهره‌ای با توزیع یکنواخت و قطر متوسط ۷۶۳ نانومتر بودند. بقای *لاکتوباسیلوس دلبروکئی* و *استرپتوکوکوس ترموفیلوس* در پایان الکتروریسی ۹۲/۸۲ و ۹۵/۸۳ درصد بود که نشاندهنده کاهش جزئی در جمعیت آنها است. بقای *استرپتوکوکوس ترموفیلوس* و *لاکتوباسیلوس دلبروکئی* نانوکپسوله شده ۹۳/۵۰و ۸۹/۱۶ درصد بود، در حالی که برای سلول‌های آزاد ۸۵/۳ و ۷۶/۴ درصد بود که اثر محافظتی قابل توجه الیاف CS/SA را بر سلول‌ها در برابر دهیدراتاسیون محیط ترخینه نشان داد. نانوالیاف پایداری قابل توجهی برای سلول‌ها در برابر فرآیند حرارتی تهیه سوپ و همچنین در دستگاه گوارش فراهم آوردند، زیرا بقای $۱۰^{۶}$ تا $۱۰^{۷}$ سلول تامین شد که در محدوده دوز توصیه‌شده پروبیوتیک جهت تاثیرگذاری بر سلامت مصرف‌کننده قرار دارد. مصرف‌کنندگانی مانند گیاه‌خواران و افراد مبتلا به عدم تحمل لاکتوز/ پپتید شیر که لبنیات مصرف نمی‌کنند، لیکن به مصرف مواد غذایی پروبیوتیکی نیاز دارند، می‌توانند از این محصول استفاده نمایند.

واژه‌های کلیدی: الکتروریسی، باکتری های اسید لاکتیک، پلیمر زیستی، ترخینه، ریزپوشانی.

۱- دکترای تخصصی، دانشیار، گروه بهداشت مواد غذایی، دانشکده دامپزشکی، دانشگاه تخصصی فن‌آوری‌های نوین آمل، آمل، ایران.
۲- دانشجوی کارشناسی ارشد، گروه بهداشت مواد غذایی، دانشکده دامپزشکی، دانشگاه تخصصی فن‌آوری‌های نوین آمل، آمل، ایران.
(*- نویسنده مسئول: Email: m.azizkhani@ausmt.ac.ir)

Iranian Food Science and Technology Research Journal
Vol. 18, No. 3, Aug. Sep. 2022, p. 85-98

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد ۱۸، شماره ۳، مرداد - شهریور ۱۴۰۱، ص. ۹۸-۸۵

CC BY

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

Full Research Paper

Effect of ultrasound on the extraction of phenolic compounds and antioxidant activity of different parts of walnut fruit

S. H. Ziaolhagh[1*], S. Zare[2]

Received: 2021.11.15
Accepted: 2021.12.20

How to cite this article:
Ziaolhagh, S. H., Zare, S. (2022). Effect of ultrasound on the extraction of phenolic compounds and antioxidant activity of different parts of walnut fruit. *Iranian Food Science and Technology Research Journal*. 18 (3), 85-98.

Abstract

Walnuts have a high nutritional value because of their high levels of essential compounds for human health. Phenolic compounds have beneficial properties, including anti-cancer and antimicrobial properties. In this study, the amount of the extracted antioxidants from different parts of walnut, including the walnut kernel, hard shell and green husk by ethanol and water was compared with those of the ultrasound-assisted extraction. This study was performed by determining the amount of polyphenols present and the free radical scavenging power of DPPH. The results showed that the effect of all factors was statistically significant at 99% statistical level. The highest rate of extraction of phenolic compounds (1.09 mg Gallic acid/g) and the highest rate of free radical scavenging of DPPH (6.86%) was related to the use of ethanol solvent for extraction. It was also shown that the hard walnut shell has the highest amount of phenolic compounds (1.1 mg Gallic acid per gram of extract) and the walnut kernel has the highest antioxidant properties (7.99%). Ultrasonic pretreatment increased the extraction efficiency of phenolic compounds and antioxidant properties so that this process increased the extraction of phenolic compounds from the green husk of walnut by 1.22 mg Gallic acid/g and increased the antioxidant properties of the kernel walnut by 13.51% compared to other parts.

Keywords: Antioxidant activity, Ultrasonic extraction, Walnut husk, Walnut waste.

Introduction

Walnut (*Juglans regia* L.) is popular all over the world for its high nutritional value and health benefits. Different parts of walnut fruit, including green walnut husk, hard shell and its leaves are also used in various industries including food, cosmetics, health and pharmaceutical industries. Green husk and hard shell are considered agricultural wastes, while various uses may be created for them.Volatile compounds and phenols of walnut green skin have anti-inflammatory and antibiotic properties. On the other hand, tannins in walnut green skin have been shown to have antioxidant and antimicrobial properties. The hard, dark inner shell of the walnut, which divides the kernel into four parts, has been used in traditional Iranian medicine to treat high blood triglycerides (Dolatabadi et al., 2014; Vahdat Shariatpanahi et al., 2013). In 2019, Iran produced 321074 tonnes of walnuts and ranked third in the world (FAO, 2020). Walnut kernels are sensitive to oxidation due to their high levels of unsaturated fatty acids (Ziaolhagh et al., 2020). There is no official report of the amount of walnut waste and loss, but it is

1. Assistant professor, Agricultural Engineering Research Department, Agricultural and Natural Resources Research and Education Center of Semnan Province (Shahrood), AREEO, Shahrood, Iran.

2. MS Graduated student, Department of Food Science and Technology, Damghan Branch, Islamic Azad University, Damghan, Iran.
(Corresponding Author Email: h.ziaolhagh@areeo.ac.ir)
DOI: 10.22067/IFSTRJ.2021.72602.1114

estimated that about 20% of the produced walnuts are wasted because of their small size, molding and oxidation of walnut oils. On the other hand, the green husk and the hard shell contributes about 66% of the walnut fruit which are discarded after shelling the walnuts (Banaian, 2010). Researches have been done to reduce the waste and loss of nuts by proper packaging (Ziaolhagh, 2012; Ziaolhagh et al. 2020), but the waste are yet high.

Walnut kernels are rich in antioxidants such as tocopherols and phenolic compounds (namely proanthocyanidine, tannin and flavonoid) and contain more phenolic compounds than other nuts and thus can be considered a functional food (Kulacanin et al., 2020). Squalene is another compound in the walnut kernel that has antioxidant properties. Regular consumption of walnuts reduces cancer and cholesterol due to the presence of antioxidant compounds and omega- 3 and omega- 6 acids (Mohagheghi et al., 2010). Fernández-Agulló et al. (2020) recommended walnut leaves as a source of polyphenol compounds and antioxidant properties for industrial applications.

Oxidation is one of the most important and well-known causes of lipid degradation during storage or processing. The synthetic antioxidants BHA, BHT and TBHQ are used to delay or slow down the oxidation reaction process. Because their role in the development of diseases such as cancer and cardiovascular and liver diseases is well known, the replacement of these compounds with natural antioxidants of plant origin has received much attention (Ghaderi et al., 2012).

Factors such as solvent type and extraction time are very important in the extraction process of phenolic compounds. In addition, extraction by conventional methods such as soxhlet extraction and immersion requires a long time and a large amount of solvent. However, Noshirvani et al. (2015) showed that the Soxhlet extraction method leads to higher levels of polyphenols from the green husk of walnuts than the Maceration method. New extraction- assisted methods such as ultrasonic extraction and microwave extraction are fast and reliable methods for extracting effective compounds from plant tissue (Nasirifar et al., 2013).

Researchers have applied various methods to extract phenolic compounds from agricultural materials. Microwave- assisted extraction has been used for extracting phenolics from walnut leaves (Rezai Erami et al. 2015) and peanut shells (Ballard et al., 2010). Ultrasound-assisted extraction is another method that has been investigated by researchers on the extraction of phenolic, flavonoid compounds and the inhibitory power of DPPH free radicals from Daghdaghan fruit (*Celtis australis*) (Nasirifar et al., 2013), milled olive kernels (Jiménez et al., 2007), walnut leaf (Sanadgol et al., 2018), rosemary leaf (Rodriguez- Rojo et al., 2012) and pistachio green hull (Goli et al., 2005).

Polar or non-polar solvents may be used as extraction mediums. Dolat Abadi et al. (2014) investigated the effect of region, solvent and extraction time on the extraction efficiency of walnut green phenolic compounds by immersion at ambient temperature. They showed that these factors affected the amount of extracted phenolic compounds, and the activity and antioxidant properties depend on the concentration of phenolic compounds in the extract. Dolat Abadi et al. (2017b) found that a solution of 1000 ppm of walnut green husk extract showed more antioxidant activity than the synthetic antioxidant of BHA with a concentration of 200 ppm.

The results of many studies have shown that plants with high phenolic and flavonoid compounds have high antioxidant activity. The extracts of walnuts inner shells and green husk have a high antioxidant activity (Mirzaee et al., 2016). Oliveira et al. (2008) investigated the effect of solvents with different polarities on the properties of walnut green shell extract and reported that a mixture of polar and non- polar solvents increases the extraction efficiency of phenolic compounds and leads to increased antioxidant activity. As mentioned earlier, all

parts of walnut fruit may be used for different purposes. The green husk and the hard shell are the parts that are disposed as waste, while they may contain phenolic compounds with antioxidant properties. In the current study, to avoid the disposal of walnut husk and shell, we aimed to discover which parts of the walnut fruit have the most antioxidant activity and if the ultrasonic treatment could help the extraction of phenolics from different parts of the walnut fruit.

Materials and methods

In this study, walnuts were prepared from Dibaj area in Damghan, Semnan. Then the different parts of the walnut, including the walnut kernel, hard shell and green husk, were completely dried in an oven at 40ºC. Walnut kernels, hard shell and green husk were completely crushed after drying with a laboratory mill (Naniwa N 95, Iran).

For extraction, the crushed samples were mixed separately with water and ethanol (1:5) and stirred at room temperature for 48 h. Based on other studies, the extraction yields of ethanol and water in extracting total phenolic compounds are higher and these solvents are safer and less toxic compared to methanol and other organic solvents. Thus we selected them as the extraction solvents (Wang et al., 2020; Han et al., 2018; Chew et al. 2011). Two samples were taken from each extract. After 48 hours, a sample of each extract was passed through a strainer, and after filtration to remove the solvent, the aqueous and the ethanolic extracts were placed in a rotary evaporator (Heidolf, 4000, Germany) for 5 and 2 hours respectively. The concentrated extract was then placed in an oven (Froilabco, France) at 40℃ for 4 days to completely remove the solvent. The second sample of the extract was treated by ultrasound (Sonica, Italy) with a frequency of 100 kHz and a temperature of 25± 3℃ for 1 h.

To determine the per cent of total polyphenols in the extract, 0.01 g of the extract was mixed with 60% methanol and the resulting solution was made up to 10 ml with distilled water. One millilitre of this diluted extract was mixed with 1 millilitre of 10% Folin-Ciocalteu reagent and after 3 min, one mL of saturated sodium carbonate was added to the solution to make a volume of 10 ml with distilled water. The final solution was then placed in a dark environment for 90 minutes and its absorption was measured by a spectrophotometer (Genoa, UK) at a wavelength of 725 nm, which indicates the per cent of antioxidants extracted from the extract. Results were expressed based on mg of gallic acid per 100 g of dried sample (Rahimipanah et al., 2011).

DPPH method was used to measure the free radical scavenging power of antioxidants. To prepare a 0.4 M DPPH solution, 0.004 g of DPPH was mixed with 100 ml of methanol. Then 2 ml of the extract was mixed with 2 mL of DPPH solution prepared by the above method and kept in the dark for 30 minutes, after which the absorbance was measured at 517 nm (Yang et al., 2014). Then, using Equation 1, the percentage of free radical scavenging was calculated.

$$\text{percentage of free radical scavenging} = \frac{(AC-AS)}{AC} \times 100 \quad (1)$$

Where Ac and As are the adsorption of the control (without extract) and the sample, respectively.

The results were analyzed using factorial experiments in a completely randomized design with three replications. Factors included walnut parts at three levels (kernel, green husk and hard shell), solvent type at two levels (water and ethanol) and pretreatment at two levels (with or without ultrasound assistance). After analyzing the variance, the means were compared by Duncan's multiple range test. SPSS16 software was used to analyze the data and Excel software was used to draw the graphs.

Results and discussion

Total polyphenols

Table 1 shows that the effects of all treatments on the total phenols of the extracts

are significant. The extracts of different parts of the walnut showed different amounts of phenolic compounds. The highest and the lowest amounts of phenolics were found in the hard shell (1.11 mg Galic acid/g extract) and the green husk (0.76 mg Galic acid/g extract) of the walnuts, respectively (Fig.1). The results showed that the ethanol solvent was more effective in extracting the phenolic compounds from different parts of the walnuts than water solvent (Fig. 2). This is related to the different polarity of solvents that affects the extraction of phenolic compounds (Rezai Erami et al. 2015). The use of water as the extraction solvent creates a completely polar environment in which some phenolic compounds with lower degrees of polarity are extracted to a lesser extent, but adding water to organic solvents is accompanied by the formation of a relatively polar environment, and more types of phenolic compounds are ensured in these conditions. In addition, the aqueous extract contains large amounts of impurities such as organic acids, proteins and soluble sugars that can interfere with the detection and quantification of phenolic compounds (Chirinos et al., 2007). Fig 2. The effect of solvent type on the total phenolic compounds. Columns with similar letters are not significant. In the case of interaction between walnut parts and solvent type, the highest amount of phenolic compounds was observed for aqueous extract of walnut kernels, and the least was found for ethanolic extract of walnut kernels. The ethanol- soluble phenolics of the green husk were more than that of other samples (Fig. 3). Rezai Erami et al. (2012) showed that the microwave-assisted extraction of walnut green husk leads to increase the extracted phenols and in this method, the alcoholic solvent was more effective than the aqueous solvent in extracting the phenolics. In this regard, Noshirvani et al (2015) showed that the extraction method affected the extracted phenolics from the green husk of walnuts. They also showed that less concentration of green husk extract is more effective than higher concentrations in reducing oxidation.

Our results showed that the ultrasonic pretreatment was effective in extracting the phenolic compounds from all parts of the walnut, and the higher phenolics were obtained from the green husk. The extraction of phenolics from the hard shell was difficult in the absence of ultrasonic pretreatment (Fig. 4). It is attributed to the increased permeability of the cells. Ultrasound speeds up the mass transmission and diffusion of the phenolic compounds from the walnut parts into the solvent. Vapour pressure increase by ultrasonic waves and this will help the penetration and transportation of the phenolic compounds into the solvent (Wang et al. 2020).

Table 1- Analysis of variance of treatments on total phenols and DPPH

Source	df	Mean Square	
		Total Phenols	DPPH
Model	12	2.057**	88.905**
Walnut Parts	2	0.248**	68.206**
Solvent Type	1	0.656**	110.510**
PreTreatment	1	0.378**	15.974**
Walnut Parts× Solvent	2	0.040**	84.494**
Walnut Parts× PreTreatment	2	0.823**	41.103**
Solvent type× PreTreatment	1	0.258**	9.985*
Walnut Parts× Solvent× PreTreatment	2	0.271**	13.411**
Error	12	0.005	1.541
Total	24		
R^2		0.997	0.983

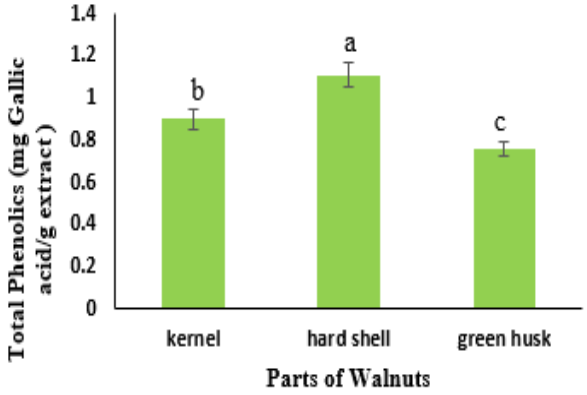


Fig. 1. Total phenolic contents of different parts of walnuts.
Columns with similar letters are not significant.

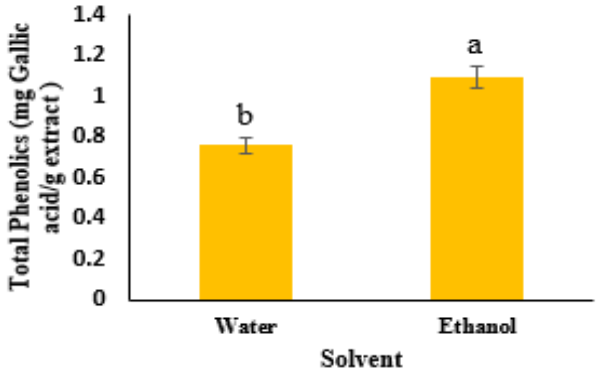


Fig. 2. The effect of solvent type on the total phenolic compounds.
Columns with similar letters are not significant.

In the ultrasonic- assisted extraction, the interaction between the ultrasound waves and the plant cells destruct the cell walls and more solvent penetrate into the cells. Ultrasound waves are sinusoidal, creating bubbles inside the environment that are full of solvent vapour. During the pressure cycle, these bubbles and the gas inside them compress and explode, increasing the pressure and temperature in the environment.

The result of the explosion of bubbles in the environment is a better mixing of solvent and plant material. In addition, ultrasound creates a mechanical force and increases the penetration of the solvent into the plant tissue. These two factors increase mass transfer and break down the cell wall (Albu et al. 2004). Ultrasound can also cause chemical changes due to the formation of free radicals during cavitation. (Paniwnyk et al., 2001). As a result, the penetration of solvents into the cells and mass transfer increases. Rapid changes in cavitation at temperature and pressure cause shear breakage and thinning of cell membranes, and these phenomena allow ultrasound to change the environment (Thompson and Doraiswamy, 1999). In accordance with the results of our research, Sanadgol et al. (2018), Rodriguez-Rojo et al. (2012) and Goli et al. (2005) respectively showed that ultrasound-assisted extraction is the best way to extract the phenolic compounds of walnut leaf, rosemary leaf and pistachio green hull. Dolat Abadi et al. (2017a) showed that the total phenolic content of the ultrasound- assisted extract of walnut green husk was more than that of extracted with the help of microwave or the maceration extraction. Ultrasound also reduces the particle size and increases the contact area, resulting in increased solvent diffusion into the tissue.

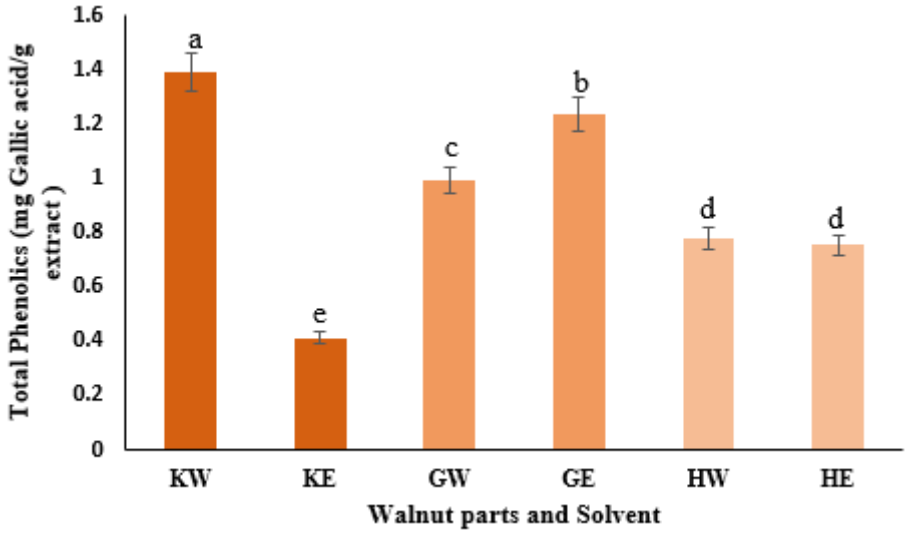


Fig. 3. The interaction effect of walnut parts and solvent on the amount of phenolic compounds. KW: Water extract from kernels, KE: Ethanol extract from kernels, GW: Water extract from green husk, GE: Ethanol extract from green husk, HW: water extract from hard shell and HE: Ethanol extract from hard shell.
Columns with similar letters are not significant.

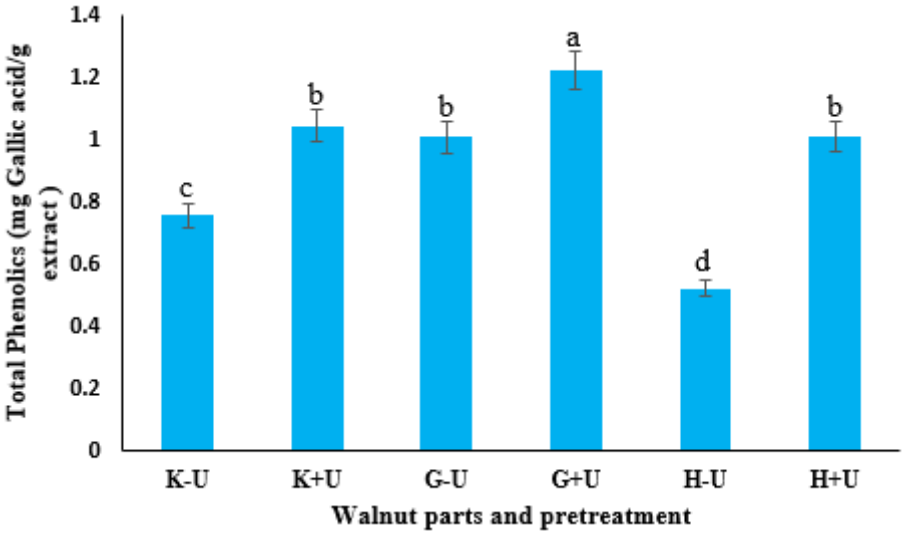


Fig. 4. The interaction effect of walnut parts and pretreatment on the amount of phenolic compounds. K-U:Kernels without ultrasonic pretreatment, K+U: Kernels with ultrasonic pretreatment, G-U: Green husk without ultrasonic pretreatment, G+U: Green husk with ultrasonic pretreatment, H-U: Hard shell without ultrasonic pretreatment and H+U: Hard shell with ultrasonic pretreatment.
Columns with similar letters are not significant.

DPPH radical-scavenging activity

As it is shown in Table 1, the effect of all factors on the DPPH radical- scavenging activity of the extracts was significant ($P<0.01$). The results showed that the kernel of walnuts had the highest DPPH radical-scavenging activity (7.9938%) followed by the hard shell (3.2287%) and the green shell (2.6875%) of the walnuts, but the difference between the DPPH radical- scavenging activity of the last two was not significant (Fig. 5).

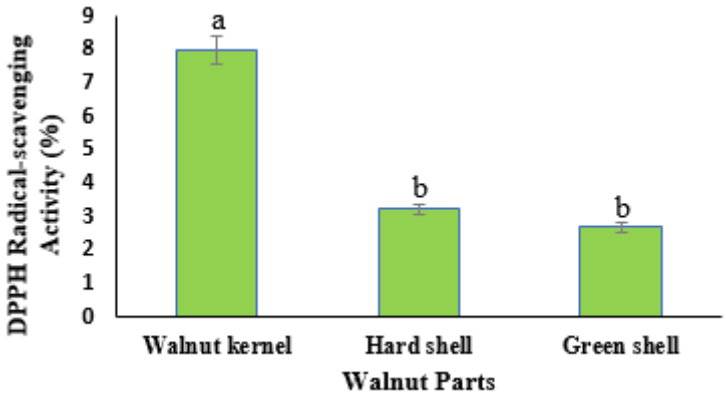


Fig. 5. The DPPH radical-scavenging activity of different parts of walnuts.
Columns with similar letters are not significant.

In addition, the ethanolic extract showed more DPPH radical- scavenging activity than the waterextract by 6.7625 and 2.4908% respectively (Fig. 6). In line with our results, Fernandez et al. (2013) showed that the highest inhibition of DPPH free radicals was related to the ethanolic extracts, which contained the most phenolic compounds. As the concentration of phenolic compounds increases, the inhibitory activity of DPPH free radicals increases, because at higher concentrations of phenolic compounds, due to the increase in the number of hydroxyl groups present in the reaction medium, the possibility of hydrogen donation to DPPH free radicals increases (Sun et al., 2007). Antioxidant activity is measured by different assays. Because the extracts contain different phenolic compounds, a number of them are identified in each method. Rezai Erami et al. (2012) showed that the antioxidant activity of the microwaved-assisted extracts were higher than that of macerated extracts, but in the conventional maceration method, the aqueous extract showed more antioxidant activity than the ethanolic extract.

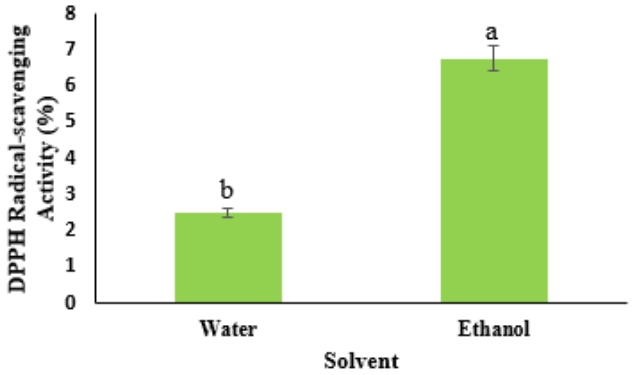


Fig. 6. The effect of solvent type on the DPPH radical-scavenging activity.
Columns with similar letters are not significant

The low level of non-polar compounds in plants is the reason for the low level of antioxidant activity of extracts prepared with non- polar solvents (Kamkar et al., 2010). In some cases, despite the high or low phenolic compounds, the antioxidant properties are not commensurate with these compounds, which indicates other influential factors that exist in these plants and affect the antioxidant properties during reactions (Mortazaie et al., 2013). Also, the phenolic content determined according to the Folin– Ciocalteu method is not a pure and definite measure of the amount of phenolic content. Different types of phenolic

compounds have a variety of antioxidant activities that depend on their structure. Different plant extracts may contain different types of phenolic compounds that have different antioxidant capacities (Kaur et al. 2014).

DPPH radicals interact with antioxidants or other radicals and decrease them. The extract of different parts of walnut is rich in different phenolic compounds which may inhibit free radicals due to their hydroxyl groups positioned along the aromatic ring (Zhang et al., 2009). The potential of a solvent in dissolving a group of antioxidants vary with its polarity. The different antiradical activity of different extracts may be related to several reasons. Position of phenolic hydroxyl groups, presence of other functional groups in the molecule such as double bonds and combination of hydroxyl groups and ketone groups play an important role in antioxidant activity (Rezai Erami et al., 2015). Shabanian et al. (2021) found that with increasing concentration of ethanol solvent, the per cent of phenolic compounds undergo a whole partial downward trend. The reason is the polarity of pure ethanol compared to a mixture of ethanol and water because polar solvents have a higher ability to extract phenolic compounds from the cell structure of plants (Gharekhani et al., 2010). Since there is a direct relationship between radical receptor activity and the per cent of phenolic compounds in fruits, so by increasing the per cent of phenolic compounds in all extracts, it is expected that the percentage of active radical inhibitory DPPH of the extracts will increase (Shabanian et al., 2021).

Differences in the antioxidant activity of different methods depend to a large extent on the hydrophilic and hydrophobic nature of the phenolic compounds present and their proportions. The DPPH radical scavenging test measures the antioxidant activity of water-soluble phenolic compounds (Rezai et al., 2012). With the increasing concentration of ethanol solvent, the DPPH activity of ethanolic extract decreased (Shabanian et al., 2021).

Ultrasonic pretreatment was effective in increasing the DPPH radical-scavenging activity of the extracts ($p<0.01$). Fig. 7 shows that the DPPH radical- scavenging activity of the extracts of the kernels and hard shell was increased by ultrasonic pretreatment, and the most DPPH activity was observed for the water extracted samples pretreated with ultrasound, as it can be seen from Fig. 8. In our study, we found that the extraction method had a significant effect on both the total phenolic content and the DPPH radical- scavenging activity. The phenolic content of all samples extracted by ultrasound was higher than that of the samples extracted by dipping in solvents, with the highest for walnut green husk. However, the antioxidant activity of green husk extract was not influenced by ultrasonic treatment. The ultrasonic- treated extract of walnut kernels showed the highest DPPH radical-scavenging activity. In the ultrasound treated extracts, the aqueous extracts showed more DPPH radical- scavenging activity than the ethanolic extracts.

To extract the compounds, the cell membranes must be broken. Bubbles created by ultrasound waves create shear forces that mechanically break down cell walls and improve material transfer (Dolat Abadi et al., 2017a). Dolat Abadi et al. (2017a**)** showed the highest DPPH radical scavenging activity for the ultrasound-assisted extraction. Sanadgol et al. (2018) also found that the highest rate of inhibition of DPPH free radicals in walnut leaf extract was related to the ultrasound method. The free radical DPPH in an alcoholic medium has a maximum absorption of 517 nm and produces a purple color. If this radical is neutralized, the intensity of the purple color decreases and changes to pale yellow. Therefore, the reduction of light absorption will be proportional to the ability of the extracts to neutralize DPPH radicals, in other words, the antioxidant power of the sample. In line with our results, Gohari et al. (2018) showed that the antioxidant activity of extract from walnut interstitial tissue decreased during the ultrasound process. They related this fact to the

increased extraction of impurities in ultrasonic treatment.

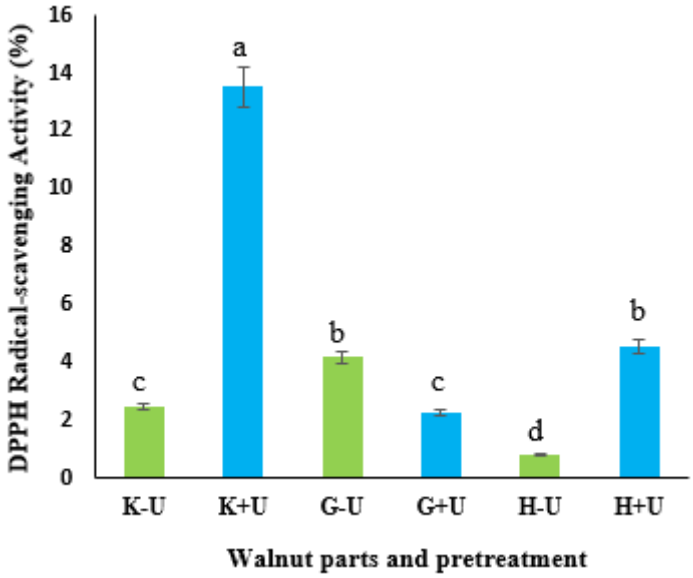


Fig. 7. The mutual effect of ultrasonic pretreatment and walnut parts on the DPPH Radical-scavenging activity. K-U:Kernels without ultrasonic pretreatment, K+U: Kernels with ultrasonic pretreatment, G-U: Green husk without ultrasonic pretreatment, G+U: Green husk with ultrasonic pretreatment, H-U: Hard shell without ultrasonic pretreatment and H+U: Hard shell with ultrasonic pretreatment.

Columns with similar letters are not significant

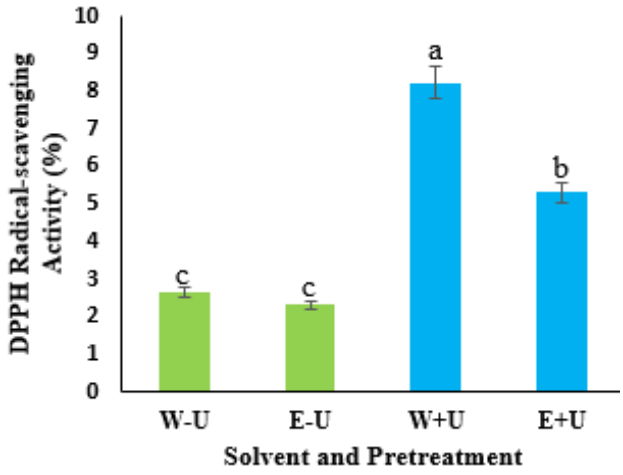


Fig. 8. The mutual effect of ultrasonic pretreatment and the solvent on the DPPH Radical-scavenging Activity. W-U: Water ectraction without ultrasonic pretreatment, E-U: Ethanol extraction without ultrasonic pretreatment, W+U: Water ectraction with ultrasonic pretreatment and E+U: Ethanol extraction with ultrasonic pretreatment.

Columns with similar letters are not significant

As it is shown in Fig. 9, the water extracts of kernels showed the most and the ethanol extract of the hard shell showed the least DPPH activity. Azadedel et al. (2018) found the lowest antioxidant activity of the pistachio green husk extract for the ultrasound-assisted extraction with ethanol solvent (which was in accordance with our results) but in the maceration method, the highest antioxidant activity was found for aqueous solvent. However, in our study, we

found no significant difference between aqueous and ethanolic solvents. Contrary to our results, Azadedel et al. (2018) obtained the highest antioxidant activity of pistachio hull extract by maceration extraction in water solvent. Therefore, the soaking method is more efficient than the method of using ultrasound waves and causes less degradation of phenolic compounds with antioxidant power.

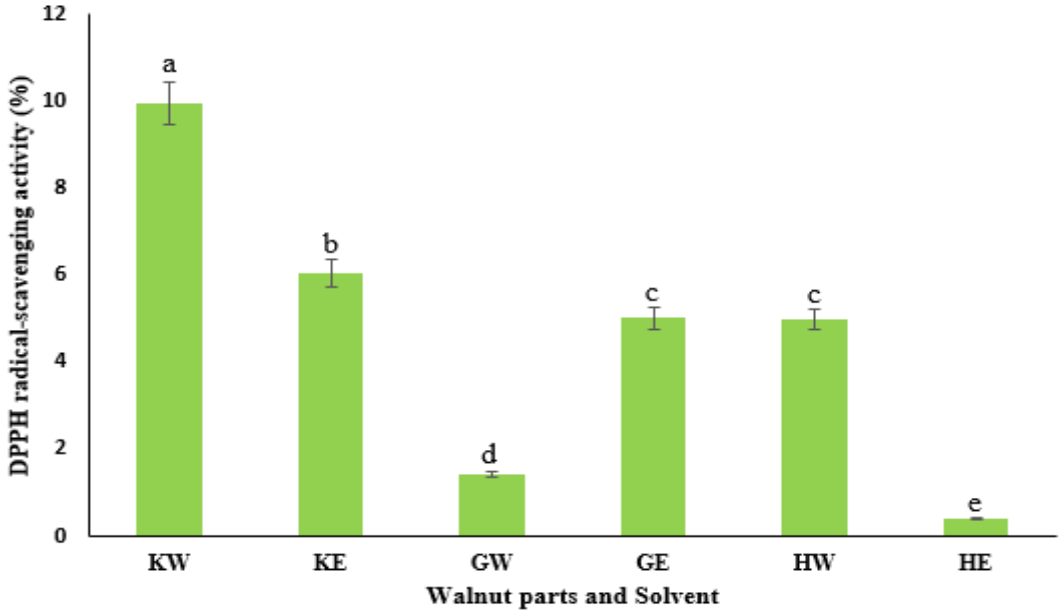


Fig. 9. The mutual effect of ultrasonic pretreatment and walnut parts on the DPPH Radical-scavenging Activity. KW: Water extract from kernels, KE: Ethanol extract from kernels, GW: Water extract from green husk, GE: Ethanol extract from green husk, HW: water extract from hard shell and HE: Ethanol extract from hard shell. Columns with similar letters are not significant.

Conclusion

Plants are a rich source of phenolic compounds (phenolic acids, flavonoids and tannins), which are the most important natural antioxidants. Antioxidants in the diet are important in protecting the body against oxidative stress and maintaining good health. In this study we found that ethanol was less effective than water in extracting phenolics from walnut kernels, but for green husk more phenolics were extracted by ethanol than water. For the hard shell of walnuts there were no significant difference between water and ethanol solvents. In the case of DPPH radical-scavenging activity the water extracts of kernels and hard shell were more effective than ethanolic extracts, but the ethanolic extract of green husk showed more antioxidant activity than aqueous extract.The results of the present study indicate that different walnut components (walnut kernel, green husk and hard shell) can be used as natural antioxidants. The green husk and hard shell of walnuts that are usually discarded as waste, are good sources of phenolics and could be used as an antioxidants source. Aqueous and ethanolic solvents are preffered for the extraction of phenolics from hard shell and green husk respectively. In addition, this study showed that ultrasound pretreatment increased the extraction yield of phenolics and antioxidant compounds.

References

1. Albu, S., Joyce, E., Paniwnyk, L., Lorimer, J. P. and Mason, T. J. (2004). Potential for the use of ultrasound in the extraction of antioxidants from *Rosmarinus officinalis* for the food and

pharmaceutical industry. *Ultrasonics Sonochemistry*, 11(3-4):261–265. https://doi.org/10.1016/j.ultsonch.2004.01.015
2. Azadedel, S., Hanachi, P. and Saboora, A. (2018). Investigation on antioxidant activity of pistachio (Pistacia vera L.) skin extraction. *Journal of Plant Research (Iranian Journal of Biology)*, 30(4):722-731.
3. Ballard, T. S., Mallikarjunan, P., Zhou, K.& O'Keefe, S. (2010). Microwave-assisted extraction of phenolic antioxidant compounds from peanut skins. *Food Chemistry*, 120(4): 1185-1192
4. Banaian, N. (2010). Estimating the energy of walnut waste and its recycling strategies. National Conference on Agricultural Waste and Waste Management.Tehran, Iran. https://civilica.com/doc/98938
5. Chew, K. K., Khoo, M. Z., Ng, S. Y., Thoo, Y. Y. and Aida, W. W. M. (2011). Effect of ethanol concentration, extraction time and extraction temperature on the recovery of phenolic compounds and antioxidant capacity of Orthosiphon stamineus extracts. *International Food Research Journal*, 18(4): 1427-1435.
6. Chirinos, R., Rogez, H., Campos, D,. Pedreschi, R. and Larondelle, Y. (2007). Optimization of extraction conditions of antioxidant phenolic compounds from mashua (Tropaeolum tuberosum Ruíz & Pavón) tubers. *Separation and Purification Technology*, 55(2):217-225. https://doi.org/10.1016/j.seppur.2006.12.005
7. Dolat Abadi, M., Raftani Amiri, Z. and Esmailzadeh Kenari, R. (2017a). Comparison of the effect of time and extraction methods on the phenolic compounds and antioxidant properties of walnut green husk of different regions of northern Iran. *Iranian Food Science and Technology Research Journal*. 13(2):273- 281.
8. Dolat Abadi, M., Raftani Amiri, Z. and Esmailzadeh, R. (2017b). Assessment of anti- microbial and anti- oxidant properties of Hezarjerib originated walnut green husk extract in refined soybean oil. *Journal of Food Research*, 27(4):23-35.
9. Dolat Abadi, M., Raftani Amiri, Z., Esmail Zadeh Kenari, R. (2014). Comparison of total phenols and antioxidant properties of walnut (*Juglans regia* L.) green husk of three regions of northern Iran (Shahrood, Bandar Gaz and Hzargarib). *Journal of Food Science and Technology (Iran)*, 11(45):183-192
10. FAO. (2020). Retrieved September 27 (2020), http://faostat.fao.org/site/339/default.aspx. Accessed 24 September 2021.
11. Fernández- Agulló, A., Castro-Iglesias, A., Freire, M. S. and González-Álvarez, J. (2020). Optimization of the extraction of bioactive compounds from walnut (Juglans major 209 x Juglans regia) leaves: Antioxidant capacity and phenolic profile. *Antioxidants*, 9(1):18.
12. Fernandez- Agullo, A., Pereira, E., Freirea, M. S., Valentao, Andrade, P. B., Gonzalez-Alvareza, and Pereira, J. A. (2013). Influence of solvent on the antioxidant and antimicrobial properties of walnut (*Juglans regia* L.) green husk extracts. *Industrial Crops and Products*, 42(2013):126–132. https://doi.org/10.1016/j.indcrop.2012.05.021
13. Ghaderi, G. M., Sadeghi, M. A., Alami, M., Azizi, M. H. and Ghorbani, M. (2012). Study on Antioxidant Activities of Phenolic Extracts from Fruit of a Variety of Iranian Acorn (*Q. castaneifolia* var castaneifolia). *Iranian journal of food science and technology*, 35(9):45-56.
14. Gharekhani, M., Ghorbani, M,. Ebrahimzadeh, M., Jaafari, S. and Sadeghi Mahoonak, A. (2010). 'Compare different methods of phenolic and flavonoid compounds extraction from Urtica dioica L.', *Iranian Journal of Medicinal and Aromatic Plants Research*, 26(3):389-405.
15. Gohari, A., Hakim Zadeh V. and Golmakani, E. (2018). Optimizing of Temperature, Time and Solvent Concentration in Extraction of Antioxidant Extract from Walnut Interstitial Tissue During Ultrasound Process. *Journal of food science and technology(Iran)*, 15(77):154-145

16. Goli, A. H., Barzegar, M. and Sahari, M. A. 2005. Antioxidant activity and total phenolic compounds of pistachio (Pistachia Vera) hull extracts. *Food Chemistry*, 92(3):521-525. https://doi.org/10.1016/j.foodchem.2004.08.020
17. Han, H., Wang, S., Rakita, M., Wang, Y., Han, Q. and Xu, Q. 2018. Effect of ultrasound-assisted extraction of phenolic compounds on the characteristics of walnut shells. *Food and Nutrition Sciences*, 9(8):1034-1045. DOI: 10.4236/fns.2018.98076
18. Jiménez, A., Beltrán, G., & Uceda, M. (2007). High-power ultrasound in olive paste pretreatment. Effect on process yield and virgin olive oil characteristics. Ultrasonics sonochemistry, 14(6):725-731.
19. Kamkar, A., Shariatifar, N., Jamshidi, A. H. Mohamadian, M. (2010). Evaluate the performance of the antioxidant water, methanol and ethanol extract cumin and Blghst in vitro. *Journal of Gonabad University of Medical Sciences and Health Services*, 16(2): 37-45.
20. Kaur, S. and Mondal, P. (2014). Study of total phenolic and flavonoid content, antioxidant activity and antimicrobial properties of medicinal plants. *Journal of Microbiol Experiment*, 1(1): 00005.
21. Kulačanin, D., Bijelić, S., Šućur, J., Bogdanović, B., Ercisli, S. and Malenčić, Đ. (2020). Antioxidant Activity and Polyphenolic Profile of Walnut (*Juglans regia* L.) Green Husks and Liqueurs. *Serbian Journal of Agricultural Sciences*, 69(1-2):5-11.
22. Mirzaee, Z. and Dehghan, G. (2016). The relationship between the antioxidant activity with different amounts of phenolic compounds. *Studies in Medical Sciences*, 27(4):321-329.
23. Mohagheghi, S. A., Poorazarang, H., Elhamirad, A. H., Dezashibi, Z. and Hematyar, N. (2010). Extraction of phenolic compounds from potato peel (Ramus variety) with solvent and ultrasound-assisted methods and evaluation of its antioxidant activity in soybean oil. *Iranian journal of food science and technology*, 8(1):81-91.
24. Mortazaie, S., Rafiyian, M., Ansari-samani, R. and Shahinfard, N. (2013). Compare the concentration of phenolic compounds and antioxidant activity of eight clinical plants. *Journal of Rafsanjan University of Medical Sciences*, 12(7):519-530.
25. Nasirifar, Z., Sadeghi, M. A. and Kamali, F. 2013. Effect of extraction condition with two ultrasonic methods on phenolic, flavonoids and DPPH free radical scavenging of Celtis australis extract. *Electronic journal of food processing and preservation*, 5(2):115-129.
26. Noshirvani, N., Fasihi, H. and Moradipayam, A. (2015). Study on the antioxidant effects of extract and powder of green walnut hulls on the oxidation of sunflower oil. *Iranian Journal of Nutrition Sciences & Food Technology*, 10(3): 79-90.
27. Oliveira, I., Sousa, A., Ferreira, I. C., Bento, A., Estevinho, L. and Pereira, J. A. (2008). Total phenols, antioxidant potential and antimicrobial activity of walnut (*Juglans regia* L.) green husks. *Food and chemical toxicology*, 46(7):2326-2331. https://doi.org/10.1016/j.fct.2008.03.017
28. Paniwnyk, L., Beaufoy, E., Lorimer, J. P. and Mason, T. J. (2001). The extraction of rutin from flower buds of *Sophora japonica*. *Ultrasonics Sonochemistry*, 8(3): 299–301. https://doi.org/10.1016/S1350-4177(00)00075-4
29. Rahimipanah, M., Hamedi, M. and Mirzapour, M. (2011). Analysis of some factors affecting the phenolic compounds extracted from green husk of walnut (*Juglans regia* L.). *Iranian Journal of Medicinal and Aromatic Plants Research*, 27(3): 419-430. DOI: 10.22092/IJMAPR.2011.6383
30. Rezai Erami, S., Jafari, S., Khamiri S. M. Bayat, H. (2015). Isolation of Shahmirzadi Husk Walnut Extract Using Microwave Assisted Extraction (MAE) and Evaluation of its Antioxidant Activity. *Journal of Food Technology and Nutrition*, 12(3):85-98.
31. Rezai, E. S., Jafari, S. M., Khomeiri, M. Bayat, H. (2012). Antioxidant activity of toyserkani variety of walnut husk and comparison of its antiradical activity with synthetic antioxidants. *journal of food research (university of tabriz)*, 22(1): 39-50.

32. Rodriguez-Rojo, S., Visentin, A., Maestri, D. Cocero, M. J. (2012). Assisted extraction of rosemary antioxidants with green solvents. *Journal of Food Engineering*, 109(1):98–103. https://doi.org/10.1016/j.jfoodeng.2011.09.029
33. Sanadgol, E., Raftani Amiri, Z. Mohammadzadeh Millani, J. (2018). Effect of different extraction methods on total phenolic content and antioxidant activity of the walnut (*Juglans regia* L.) leaves from Damavand, Shahrood and Hezarjarib. *Innovative Food Technologies*, 5(2), 349-359. DOI: 10.22104/JIFT.2017.2203.1510
34. Shabanian, M., Sari, A. A. and Daraei Garmakhany, A. (2021).Optimization of ethanolic extracts of walnut green peel extracted by Microwave method and investigation of their antioxidant properties. *Journal of Food Science & Technology (Iran)*, 18(115):283-298.
35. Sun, T., powers M. R. Tang, J. (2007). Evalurion of the antioxidant activity of asparagus, broccoli and their juices. *Food Chemistry*, 105(1): 101-106. https://doi.org/10.1016/j.foodchem.2007.03.048
36. Thompson, L. H. Doraiswamy, L. K. (1999). Sonochemistry: science and engineering. *Industrial & Engineering Chemistry Research*, 38(4): 1215-1249. https://doi.org/10.1021/ie9804172
37. Vahdat Shariatpanahi, Z. Shahbaazi, S. (2013). Effects of Inner Part of Walnut on Serum Triglyceride and HDL Cholesterol Level in Hypertriglyceridemic Subjects. *Journal of Islamic and Iranian Traditional Medicine*, 4(2):97-103.
38. Wang, S., Fu, W., Han, H., Rakita, M., Han, Q. Xu, Q. (2020). Optimization of Ultrasound-Assisted Extraction of Phenolic Compounds from Walnut Shells and Characterization of Their Antioxidant Activities. *Journal of Food and Nutrition Research*, 8(1): 50-57.
39. Yang, J., Chen, C., Zhao, S., Ge, F., Liu, D. (2014). Effect of solvents on the antioxidant activity of walnut (*Juglans regia* L.) shell extracts. *Journal of Food and Nutrition Research*, 2 (9): 621-626.
40. Zhang, Z., Liao, L., Moore, J., Wu, T. & Wang, Z. (2009). Antioxidant phenolic compounds from walnut kernels (*Juglans regia* L.). *Food Chemistry*, 113(1): 160-165.
41. Ziaolhagh, H. (2012). Study on the best packaging material and packaging conditions on the rancidity of almond kernels. *Acta. Horticulturae*, 963:113-118. DOI:10.17660/ActaHortic.2012.963.20.
42. Ziaolhagh, S., Fatemian, H. Goodarzi, F. (2020). The Effect of Thermal Pretreatment and Packaging Conditions on the Shelf-life of Walnut Kernels. *Journal of Nuts*, 11(2):159-168.

.

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد ۱۸، شماره ۳، مرداد - شهریور ۱۴۰۱، ص. ۹۸-۸۵

Iranian Food Science and Technology Research Journal
Vol. 18, No. 3, Aug. Sep. 2022, p. 85-98

اثر فرایند اولتراسوند بر استخراج ترکیبات فنلی و فعالیت آنتی‌اکسیدانی بخش‌های مختلف گردو

سیدحمیدرضا ضیاءالحق[1*]- سیما زارع[2]

تاریخ دریافت: ۱۴۰۰/۰۸/۲۴
تاریخ پذیرش: ۱۴۰۰/۰۹/۲۹

چکیده

گردو از نظر تغذیه، دارای ارزش غذایی بالایی است که برای سلامت انسان ضروری است. ترکیب‌های فنلی خواص سودمندی از جمله خاصیت ضدسرطانی دارند و از فعالیت میکروبی جلوگیری می‌کنند. استخراج به کمک امواج فراصوت (اولتراسوند) نسبت به سایر روش‌های استخراج مانند استخراج با مایکروویو هزینه کمتری دارد. در این پژوهش، میزان استخراج ترکیبات آنتی‌اکسیدان از قسمت‌های مختلف گردو شامل: مغز گردو، پوست چوبی و پوست سبز توسط حلال‌های اتانول و آب و با کمک دستگاه اولتراسوند مقایسه شد. این پژوهش از طریق ۲ آزمایش تعیین میزان پلی‌فنول‌های موجود و قدرت مهارکنندگی رادیکال آزاد DPPH اجرا شد. نتایج نشان داد که اثر تمام عوامل در سطح آماری ۹۹ درصد معنی‌دار بود. بیشترین میزان استخراج ترکیبات فنلی (۱٫۰۹ mg Galic acid/g) و بیشترین میزان مهار رادیکال آزاد DPPH (۶٫۸۶ درصد) مربوط به استفاده از حلال اتانول برای استخراج بود. همچنین نشان داده شد که پوسته سخت گردو بیشترین میزان ترکیبات فنلی (۱٫۱ میلی‌گرم اسیدگالیک بر گرم عصاره) و مغز گردو بیشترین خاصیت آنتی‌اکسیدانی (۷٫۹۹ درصد) را دارند. پیش‌تیمار اولتراسونیک باعث افزایش راندمان استخراج ترکیبات فنلی و خاصیت آنتی‌اکسیدانی شد، به‌طوری که این فرایند سبب افزایش استخراج ترکیبات فنلی از پوسته سبز گردو به میزان ۱٫۲۲ mg Galic acid/g و افزایش خاصیت آنتی‌اکسیدانی مغز گردو به میزان ۱۳٫۵۱ درصد نسبت به سایر قسمت‌ها گردید.

واژه‌های کلیدی: استخراج با اولتراسوند، پوست سبز گردو، گردو، فعالیت آنتی‌اکسیدانی.

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

بسم الله الرحمن الرحیم

مندرجات

نشریه پژوهش های 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 ۱۸ شماره ۳ مرداد- شهریور ۱۴۰۱

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.

صاحب امتیاز: دانشگاه فردوسی مشهد

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

سردبیر: دکتر فریده طباطبایی — استاد، میکروبیولوژی، دانشگاه فردوسی مشهد

اعضای هیئت تحریریه:

دکتر سید علی مرتضوی	استاد، میکروبیولوژی وبیوتکنولوژی، دانشگاه فردوسی مشهد
دکتر فخری شهیدی	استاد، میکروبیولوژی مواد غذایی، دانشگاه فردوسی مشهد
دکتر محمدباقر حبیبی نجفی	استاد، میکروبیولوژی، دانشگاه فردوسی مشهد
دکتر مرتضی خمیری	دانشیار، میکروبیولوژی، دانشگاه علوم کشاورزی و منابع طبیعی گرگان
دکتر سید محمد علی رضوی	استاد، مهندسی و خواص بیوفیزیک مواد غذایی، دانشگاه فردوسی مشهد
دکتر رضا فرهوش	استاد، شیمی مواد غذایی، دانشگاه فردوسی مشهد
دکتر بی بی صدیقه فضلی بزاز	استاد، میکروبیولوژی، دانشکده داروسازی دانشگاه علوم پزشکی مشهد
دکتر مهدی کاشانی نژاد	استاد، مهندسی مواد غذایی، دانشگاه علوم کشاورزی و منابع طبیعی گرگان
دکتر آرش کوچکی	استاد، تکنولوژی مواد غذایی، دانشگاه فردوسی مشهد
دکتر محبت محبی	استاد، مهندسی مواد غذایی، دانشگاه فردوسی مشهد
دکتر بابک قنبرزاده	استاد، مهندسی مواد غذایی، دانشگاه تبریز
دکتر ایران عالم‌زاده	استاد، بیوتکنولوژی مواد غذایی، دانشگاه صنعتی شریف
دکتر قدیر رجب‌زاده اوغاز	دانشیار، نانو فناوری مواد غذایی، مؤسسه پژوهشی علوم و صنایع غذایی
دکتر مهیار حیدرپور	دانشیار، زیست مولکولی، دانشکده پزشکی هاروارد
دکتر حمید بهادر قدوسی	دانشیار، میکروبیولوژی غذایی، دانشگاه متروپولیتن لندن
دکتر کیانوش خسروی	استاد، بیوتکنولوژی مواد غذایی، دانشگاه علوم پزشکی شهید بهشتی
دکتر مرتضی عباس‌زادگان	استاد، ویروس شناسی، دانشگاه آریزونا
دکتر محمدامین محمدی‌فر	استاد، مهندسی مواد غذایی، دانشگاه دانمارک
دکتر منوچهر وثوقی	استاد، بیوتکنولوژی مواد غذایی، دانشگاه صنعتی شریف
دکتر هادی الماسی	دانشیار، گروه علوم و صنایع غذایی، دانشکده کشاورزی، دانشگاه ارومیه
دکتر میلاد فتحی	دانشیار، گروه علوم و صنایع غذایی، دانشگاه صنعتی اصفهان
دکتر سلیمان عباسی	استاد، گروه علوم و صنایع غذایی، دانشگاه تربیت مدرس.
دکتر نونو بورخس	استاد، گروه علوم تغذیه و مواد غذایی، دانشگاه پورتو، پرتغال

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 پژوهش های علوم وصنایع 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

Iranian food science and technology Association

دانشگاه فردوسی مشهد

جلد ۱۸ شماره ۳

سال ۱۴۰۱

شماره پیاپی ۷۵

شاپا: ۴۱۶۱-۱۷۳۵

عنوان مقالات