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نشریه پژوهشهای علوم و صنایع غذایی ایران
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The effect of some processing parameters on mechanical and image texture properties of fried carrot

M. Fathi[1], Seyed M. A. Razavi[2*]

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Abstract

In this study, potential application of image texture analysis as a non-destructive method for automation and prediction of mechanical properties of carrot chips was investigated. Samples were fried at different processing conditions and moisture content, colour parameters (i.e. L^*, a^*, b^* and ΔE) and mechanical properties (i.e. hardness and apparent modulus) were determined. Hardness and apparent modulus increased by increasing frying temperature and time. Four image texture features namely contrast, correlation, energy and homogeneity were calculated using gray level co-occurrence matrix. The results showed contrast and energy of gray level images were well correlated with hardness of fried samples in compression and puncture tests. Correlation coefficients of 0.97 and 0.98 between four image texture features and hardness were obtained in compression and puncture tests, respectively. Results indicate that image texture analysis can be successfully applied as a non-destructive method for estimation of mechanical properties of carrot.

Keywords: Carrot; frying; image texture analysis; mechanical texture properties

Introduction

Carrot (*Daucus carota*) is one of the most important sources of natural antioxidants such as β-carotene and flavoniods and vitamins A, C and E (Alasalvar, Grigor, Zhang, Quantick, Shahidi, 2001). This agriculture product has been linked to inhibit certain types of cancer and chronic diseases (Rao, Agarwal, 1999). However, the carrot's shelf-life is confined due to its high moisture content which lead to the loss and oxidation of nutritional compounds such as vitamins and carotenoids and consequently, intensification of the bitterness flavor (Rao *et al.*, 1999). Deep-fat frying can be applied as a preservation method to prolong shelf-life and enhance taste, appearance and texture of carrot. Immersion of food materials into hot oil causes partial evaporation of the water and increase porosity and crispness, which lead to improve their palatability (Mohebbi, Fathi, Shahidi, 2011). Dueik *et al*. (2010) reported vacuum frying of carrot can maintain approximately 90 and 86% of its trans α-carotene and β-carotene, respectively.

Mechanical texture features and colour are recognized as the most important quality aspects affecting sensory perception of fried products. Temperature and time of frying have been reported to possess a critical effect on these properties. Pedreschi *et al*. (2007) studied colour components changes during potato frying and showed the colour changes (ΔE) enhanced with increasing frying time. Kita *et al*. (2007) investigated the effect of temperature on texture of fried potato and showed that the hardness decreases by increasing the processing temperature. Pedreschi and Moyano (2005) reported the lower frying temperature the crisper potato chips is produced. In spite of importance of mechanical texture properties of fried product, their applications are limited for on-line quality control evaluation due to destructive and time consuming natures of their evaluation instruments.

Image textures are important image features and have been recently applied in food engineering for quality evaluation as a non-destructive, objective and rapid method (Borah, Hines, Bhuyan, 2007; Dan, Azuma,

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Kohyama, 2007; Fathi, Mohebbi, Razavi, 2009; Gonzales-Barron, Butler, 2008; Zheng, Sun, Zheng, 2006). Image texture is defined as the spatial organization of intensity variations of pixels in gray level image, which corresponded to both brightness value and pixel locations (Pietikanen, 2000). Published researches revealed that image texture features can be correlated with mechanical properties of food materials (Qiao, Wang, Ngadi, Kazemi, 2007; Thybo *et al.*, 2004). Image texture features are usually classified into four categories namely, statistical, structural, model-based and transform-based textures (Bharati, Liu, MacGregor, 2004). In the food industry, statistical texture is the most commonly used method for quality evaluations. This method includes grey level co-occurrence matrix (GLCM), grey level pixel-run length matrix, and neighboring grey level dependence matrix (Zheng *et al*., 2006). The former that has been proposed by Haralick *et al*. (1973), is the widely applied statistical texture analysis method, in which texture features such as entropy, homogeneity, correlation and contrast are extracted by some statistical approaches from the co-occurrence matrix of gray scale image histogram. GLCM has been used for classification of cereal grain and dockage (Paliwal, Visen, Jayas, White, 2003), and apple (Kavdir, Guyer, 2002).

As mentioned above, determination of mechanical texture properties is both time consuming and destructive and development of a new replaced method is necessary. In spite of momentous application of image texture analysis, there is no published data in the literature on investigation of correlation between mechanical and image texture properties of food products. Therefore, the aims of this work were to determine mechanical and colour properties of fried carrot under different processing conditions and study the efficiency of image texture parameters (i.e. contrast, correlation, energy and homogeneity) to predict mechanical features of carrot chips.

Materials and methods

Sample Preparation

Fresh carrots (*Daucus carrota*) were purchased from local market and kept in refrigerator at 6-7 °C before frying. Carrots were cut into square dslices of 1mm in thickness and 30mm in length.

The frying process was accomplished in a thermostatically temperature controlled fryer (Black & Decker, USA). Sunflower oil was used for frying due to its high smoking point. The fryer was filled with 2.5L of sunflower oil. The fresh oil had been preheated at frying temperature for 30min before frying. Carrot samples were immersed in the frying oil at 140°C and 160°C. At the end of the frying times (3, 4, 5 and 6 min), the samples were removed from the fryer and were blotted with adsorbent paper to remove excess surface oil. Moisture content of fried carrots were measured by drying the samples in a convection oven at 105 °C until constant mass was achieved and reported as the wet basis.

Mechanical Measurements

Mechanical texture measurements of carrot chips were accomplished at room temperature (25 °C) by texture analyzer (QTS Texture analyzer, CNS Farnell, Essex, U.K.). In this study, puncture and compression tests were performed applying a crosshead speed of 70 $mm\,min^{-1}$ and using two probes with dimensions of 2mm and 35mm, respectively. The instrument's software was applied to determine the mechanical textural parameters, namely hardness (g) and apparent modulus ($g.s^{-1}$). The experiments were fulfilled in three replications.

Image Acquiring and Colour Changes

For each treatment, four fried samples were scanned with a Cannon (8800F) desktop flatbed scanner (at Optical Resolution of 4800 dpi × 9600 dpi) and the images were saved as BMP format. To study the effect of frying parameters (temperature and time) on colour changes (ΔE), the RGB colour space images were converted to *L*a*b* space and colour changes were calculated applying the

following equation:

$$\Delta E = \left[(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2 \right]^{\frac{1}{2}} \quad (1)$$

where L^* is lightness component, which ranges from 0 to 100 and parameter a^* (from green to red) and b^* (from blue to yellow) are two chromatic components, which range from -120 to 120. Subscripts *1* and *2* are referred to colour components before and after frying process, respectively (Fathi, Mohebbi, Razavi, 2011).

Grey Level Co-Occurrence Matrix and Image Texture Analysis

The first procedure for extracting image textural features was presented by Haralick *et al*. (1973). Each textural property is computed from a set of GLCM probability distribution matrices for a given image. The GLCM shows the probability that a pixel of a particular grey level occurs at a specified direction and distance from its neighboring pixels. Gray level co-occurrence matrix is represented by $P_{d,\theta}(i, j)$ where counts the neighboring pair pixels with gray values *i* and *j* at the distance of *d* and the direction of θ.

In this study, four image texture features namely, contrast, correlation, entropy and homogeneity were calculated based on equations 2-5 (Qiao *et al*., 2007). Contrast measures the local variation in an image (ranging from 0 to [size (GLCM, 1)-1]^2) and a high contrast value indicates a high degree of local variation. Correlation is an indicator of linear dependency of intensity values in an image (ranging from -1 to 1). For an image with large areas of similar intensities, a high value of correlation is measured. Energy (angular second moment) returns the sum of squared elements in the GLCM (ranging from 0 to 1) and homogeneity indicates the uniformity within an image (ranging from 0 to 1).

$$Contrast = \sum_{i=0}^{N-1} \sum_{j=0}^{N-1} (i-j)^2 P_{d,\theta}(i, j) \quad (2)$$

$$Correlation = \frac{\left[\sum_{i=0}^{N-1} \sum_{j=0}^{N-1} (ij) P_{d,\theta}(i, j) \right] - \mu_x \mu_y}{\sigma_x \sigma_y} \quad (3)$$

$$Energy = \sum_{i=0}^{N-1} \sum_{j=0}^{N-1} P_{d,\theta}(i, j)^2 \quad (4)$$

$$Homogeneity = \sum_{i=0}^{N-1} \sum_{j=0}^{N-1} \frac{P_{d,\theta}(i, j)}{1+|i-j|} \quad (5)$$

where μ_x, μ_y and σ_x, σ_y are the mean and standard deviation of the sums of rows and columns in the matrix, respectively, and *N* is the dimension of square matrix of GLCM. In this study, the four mentioned textural features were computed using the mean of the four values of different orientations (0°, 45°, 90° and 135°) at d = 1 applying a program developed in MATLAB 7.0.

Statistical Analysis

Analysis of variance (ANOVA) was performed using a computerized statistical program called "MSTAT" version C, and determination of significant differences of means was carried out by "Duncan" test at 95% confidence level using the above software program. Regression equations and coefficients of determination (R^2) between the mechanical and image texture features were obtained using SlideWrite software, version 2.

Result and discussion

The effect of frying conditions on moisture content of carrot was depicted in Fig. 1. The moisture content showed a classical drying profile and it was diminished by increasing frying time and temperature. The decrease of moisture content in result of increase of frying temperature is due to enhance of diffusion coefficient. Similar results were obtained by Moyano and Pedreschi (2006).

The results of analysis of variance for colour and mechanical properties of deep-fat fried carrot were summarized in Tables 1 and 2, respectively. Effect of frying temperature, time and their combination were significant (P<0.05) on colour components of L^* and b^* and colour changes.

The mean values of colour parameters as well as colour changes at different processing conditions were tabulated in Table 3. The lightness (L^*) decreased as the frying

temperature increased from 140 to 160° C. The darkening of carrot chips at higher processing temperature can be attributed to the acceleration of chemical reactions, e.g. Maillard reactions. Krokida *et al* (2001) reported that increasing frying temperature led to decrease of potato lightness. The results revealed that *b** component decreased with increasing frying temperature and time, indicating yellow colour deterioration. On the other hand, the samples were fried at higher frying temperature and longer time underwent more severe colour changes.

The effect of frying temperature was significant ($P<0.05$) on hardness and apparent modulus for both compression and puncture tests (Table 2). However, hardness was not statistically affected by frying time. Figures 2 & 3 show the mechanical properties of carrot chips determined by compression and puncture tests as a function of processing conditions. In all cases, hardness and apparent modulus of carrot chips raised as the frying temperature and time increased, which is due to fast moisture loss from the surface of the samples and consequently, forming of hard crust. Heredia *et al* (2014) studied textural properties of fried potato and reported a first stage of initial softening related to starch gelatinization followed by a second stage where the maximum force increased due to the gradual formation of a crust.

The image texture features (i.e. contrast, correlation, energy and homogeneity) of carrot chips were calculated from GLCM in four orientations and their average values in different frying temperatures and times were presented in Table 4.

Table 1. Successive mean squares from analysis of variance of color parameters of fried carrot

Source	Degree of freedom	Mean square			
		L*	**a***	**b***	**Δ*E***
Temperature	1	635.98^{Sig}	77.09^{NS}	600.13^{Sig}	215.04^{Sig}
Time	3	606.44^{Sig}	12.22^{NS}	214.20^{Sig}	182.68^{Sig}
Temperature*Time	3	566.87^{Sig}	11.81^{NS}	167.26^{Sig}	138.12^{Sig}
Error	24	676.41	17.29	10.85	10.85
Total	31				

^{Sig}, statistically significant at 5%; ^{NS}, not statistically significant.

Table 2. Successive mean squares from analysis of variance of hardness and apparent modulus for compression and puncture tests

Source	Degree of freedom	Mean square			
		Hardness (compression)	**Apparent modulus (compression)**	**Hardness (puncture)**	**Apparent modulus (puncture)**
Temperature	1	61509.37^{Sig}	1327784.41^{Sig}	11310.04^{Sig}	1821457^{Sig}
Time	3	32886.486^{Sig}	591673.47^{Sig}	40950.04^{Sig}	301986.6^{Sig}
Temperature*Time	3	452.49^{NS}	13671.0^{Sig}	2001.15^{NS}	43171.41^{Sig}
Error	16	10759.71	59972.49	3843.5	56816.02
Total	23				

^{Sig}, statistically significant at 5%; ^{NS}, not statistically significant.

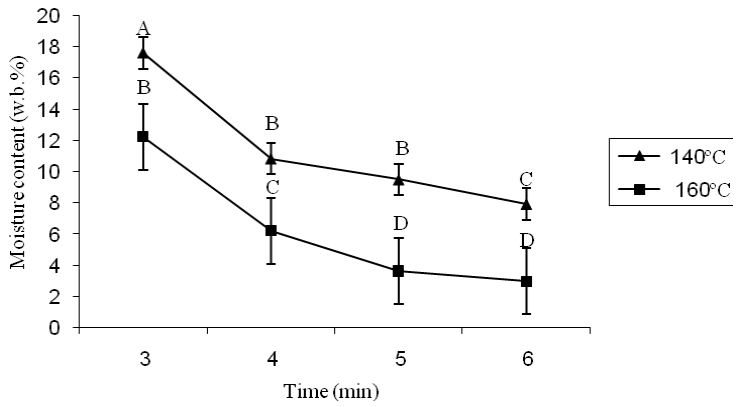

Fig. 1. Moisture content of carrot chips at different processing times and temperatures

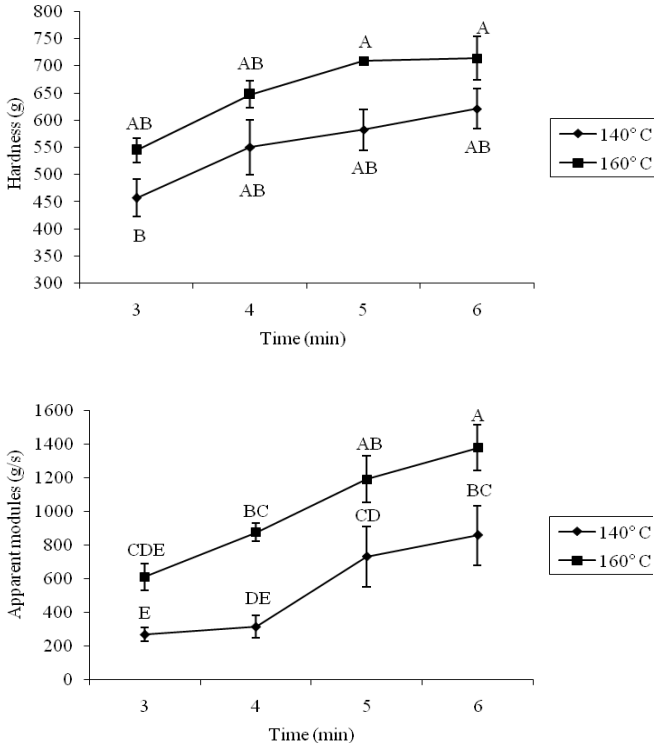

Fig. 2. Influence of frying temperature and time on the hardness and apparent modulus of carrot chips determined by compression test.

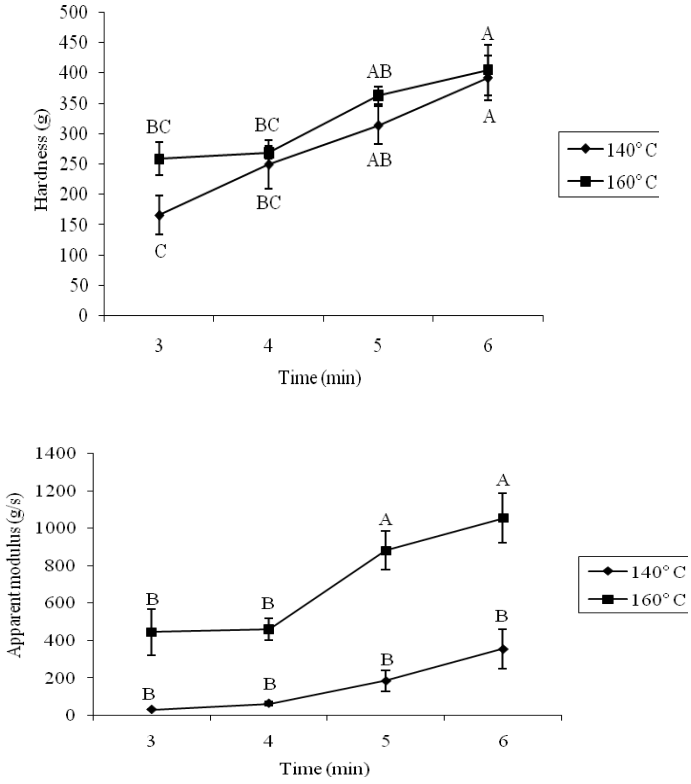

Fig. 3. Influence of frying temperature and time on the hardness and apparent modulus of carrot chips determined by puncture test.

The increase of the contrast as results of applying high frying temperature and long time indicates an enlargement of local variation of pixels in fried samples' images. However, the decrease trends of energy, correlation and homogeneity values reveal diminishing uniformity and smoothness of the images due to increase of black spot formation at higher frying temperature and time. As mentioned above, determination of mechanical properties of products is both destructive and time-consuming. Therefore, in this research an effort has been made to apply image texture analysis as a non-destructive and rapid method to predict mechanical properties of fried carrots. It was found that four image texture features were more correlated with hardness, rather than apparent modulus. Therefore, the obtained relationships between hardness values of compression and puncture tests and four image texture parameters including contrast, correlation, energy and homogeneity were presented in Figure 4.

It can be found that the hardness values of carrot chips in compression and puncture tests can be predicted using linear relationships of energy and contrast with superior correlation coefficients of -0.94 and +0.98, respectively. Qiao et al. (2007) studied the effect of frying condition on image texture properties of nugget. They stabilished multi-layer feed-forward networks to predict mechanical texture parameters based on image texture indices of samples. The results showed a strong potential for measuring mechanical and textural characteristics of fried nuggets using non-destructive image-based texture indices.

The results of multiple linear regression (MLR) between image texture features and hardness of fried carrots were also tabulated in Table 5. MLR relationships showed correlation coefficients of 0.97 and 0.98 for compression and puncture tests, respectively. These results reveal that image texture features can be potentially applied as a non-destructive method for quality control of mechanical properties of carrot chips.

Table 3. Average values of each color component and color changes of fried carrot at different frying conditions

Color parameter	140 °C				160 °C			
	3 min	4 min	5 min	6 min	3 min	4 min	5 min	6 min
L*	50.70^{AB}±0.70	59.11^{A}±4.22	54.70^{AB}±3.14	51.23^{AB}±1.31	55.66^{AB}±2.08	49.50^{B}±2.99	37.91^{C}±5.75	37.0^{C}±12.28
a*	21.84±0.71	19.50±2.28	20.04±1.46	19.77±1.43	23.79±2.86	23.06±0.41	20.68±1.36	20.56±10.97
b*	65.70^{A}±2.20	62.19^{A}±1.94	58.14^{AB}±2.16	55.12^{B}±1.74	59.18^{AB}±2.90	54.63^{B}±2.04	40.14^{C}±6.44	42.57^{C}±4.48
Δ*E*	33.23^{BC}±0.68	34.78^{BC}±1.57	35.93^{BC}±1.84	38.06^{B}±1.21	31.94^{C}±3.78	38.29^{BC}±1.57	49.98^{A}±6.58	49.15^{A}±4.50

The values in each row followed by different letters are statistically significant (P<0.05).

Table 4. Average values of image texture features of fried carrot at different frying conditions

Image texture feature	140 °C				160 °C			
	3 min	4 min	5 min	6 min	3 min	4 min	5 min	6 min
Contrast	0.173±0.024	0.199±0.023	0.225±0.031	0.254±0.010	0.191±0.030	0.208±0.056	0.255±0.038	0.277±0.036
Correlation	0.772±0.035	0.709±0.034	0.675±0.066	0.660±0.022	0.727±0.061	0.680±0.092	0.659±0.077	0.659±0.0120
Energy	0.350±0.012	0.348±0.009	0.291±0.024	0.250±0.018	0.30±0.051	0.250±0.012	0.227±0.012	0.210±0.012
Homogeneity	0.918±0.012	0.904±0.011	0.888±0.015	0.878±0.013	0.911±0.016	0.902±0.021	0.884±0.016	0.866±0.016

Table 5. Multiple linear regression (mlr) between image texture features and hardness of fried carrot in compression and puncture testes

Mechanical test	Relationship	Correlation coefficient	P value
Compression	Hardness= -3763.4 + (2809.2Contrast) - (944.4Correlation) - (655.7Energy) + (5119.6Homogeneity)	0.97	0.028
Puncture	Hardness = 335.8 + (1730.0Contrast) - (474.8Correlation) + (50.4Energy) - (116.5Homogeneity)	0.98	0.017

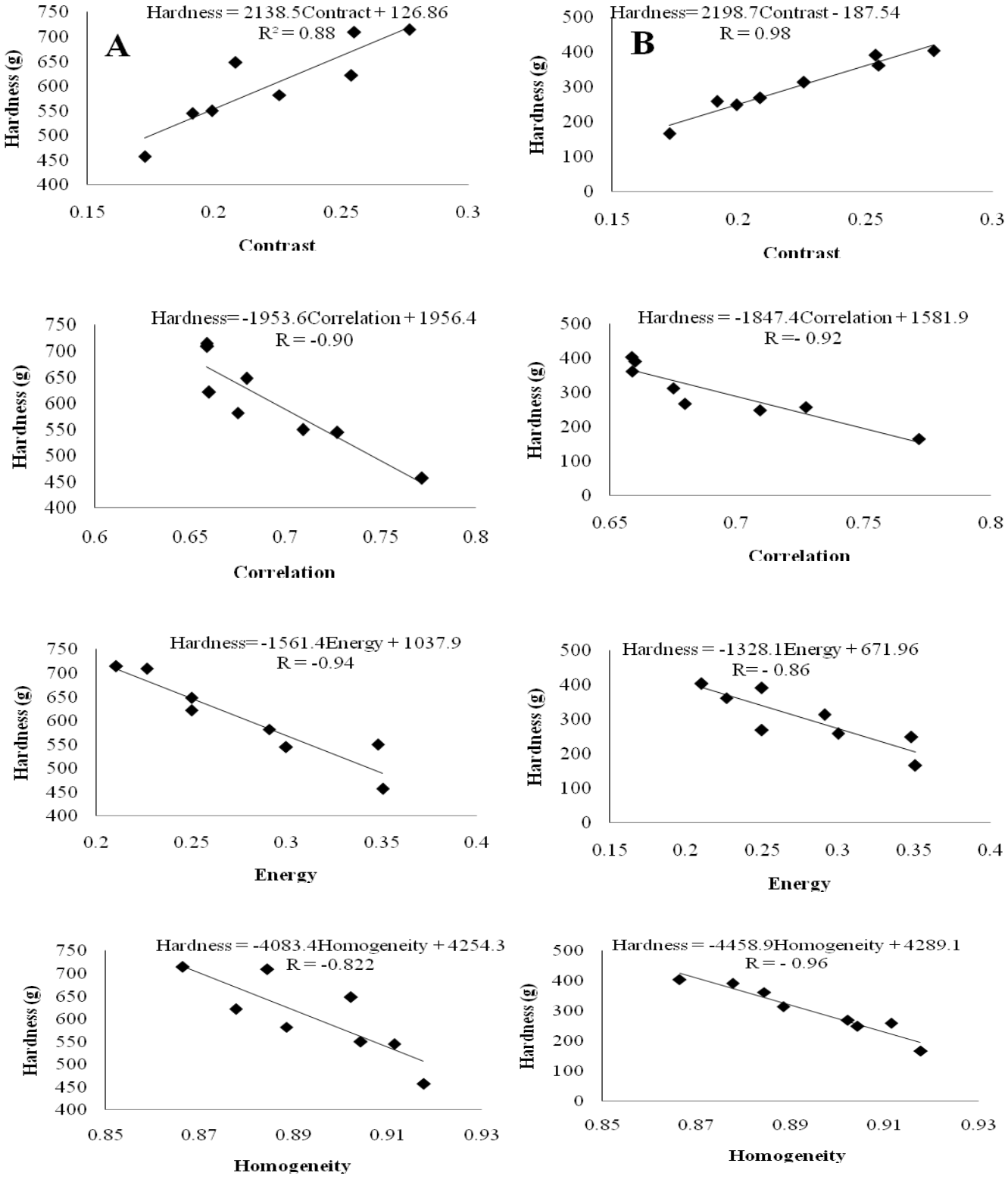

Fig. 4. Correlations of image texture features with hardness from compression (A) and puncture (B) tests

Conclusions

In this study, image texture analysis was applied to predict mechanical texture properties of carrot chips. Sliced samples were subjected to deep-fat frying at different processing conditions and moisture content, colour parameters (i.e. L^*, a^*, b^* and ΔE), mechanical properties (i.e. hardness and apparent modulus) and image texture features (i.e. contrast, correlation, energy and homogeneity) were determined. The results showed that moister content of fried samples (ranging from 2.97 to 17.60% w.b) decrease with increasing frying temperature and time. Nevertheless, the colour changes (ranging from 31.94 to 49.15) increased as the frying performed at the higher temperature for longer processing times. The results of mechanical texture analysis showed that hardness and apparent modulus significantly raised with increasing frying temperature and time. The image texture values, extracted from grey level co-occurrence matrix of fried carrots' images, showed that contrast and energy can be applied for estimation of hardness of samples for compression and puncture tests with high correlation coefficients of 0.94 and 0.98, respectively. The outcomes of this investigation suggest that image texture features can be successfully applied as a non-destructive and rapid method for mechanical texture prediction of carrot chips.

References

Alasalvar, C., Grigor, J.M., Zhang, D., Quantick, P.C., Shahidi, F. 2001. Comparison of volatiles, phenolics, sugars, antioxidant vitamins, and sensory quality of different colored carrot varieties. *Journal of Agricultural and Food Chemistry* 49, 1410-1416.

Bharati, M.H., Liu, J.J., MacGregor, J.F. 2004. Image texture analysis: methods and comparisons. Chemometrics and Intelligent Laboratory Systems 72, 57-71.

Borah, S., Hines, E.L., Bhuyan, M. 2007. Wavelet transform based image texture analysis for size estimation applied to the sorting of tea granules. *Journal of Food Engineering* 79, 629-639.

Dan, H., Azuma, T., Kohyama, K. 2007. Characterization of spatiotemporal stress distribution during food fracture by image texture analysis methods. *Journal of Food Engineering* 81, 429-436.

Dueik, V., Robert, P., Bouchon, P. 2010. Vacuum frying reduces oil uptake and improves the quality parameters of carrot crisps. *Food Chemistry* 119, 1143-1149.

Fathi, M., Mohebbi, M., Razavi, S.M.A. 2009. Application of image texture analysis for evaluation of osmotically dehydrated kiwifruit qualities, The International Symposium on Food Rheology and Structure, *Zurich*.

Fathi, M., Mohebbi, M., Razavi, S.M.A. 2011. Application of Image Analysis and Artificial Neural Network to Predict Mass Transfer Kinetics and Color Changes of Osmotically Dehydrated Kiwifruit. Food and Bioprocess *Technology* 4, 1357-1366.

Gonzales-Barron, U., Butler, F. 2008. Discrimination of crumb grain visual appearance of organic and non-organic bread loaves by image texture analysis. *Journal of Food Engineering* 84, 480-488.

Haralick, R.M., Shanmugam, K., Dinstein, I. 1973. Textural features for image classification. IEEE Transactions on Systems, Man, and Cybernetics 6, 610–621.

Heredia, A., Castelló, M.L., Argüelles, A., Andrés, A. 2014. Evolution of mechanical and optical properties of French fries obtained by hot air-frying. *LWT - Food Science and Technology* 57, 755-760.

Kavdir, I., Guyer, D.E. 2002. Apple sorting using artificial neural networks and spectral imaging. Transactions of ASAE 45 1995–2005.

Kita, A., Lisinska, G., Golubowska, G. 2007. The effects of oils and frying temperatures on the texture and fat content of potato crisps. *Food Chemistry* 102, 1-5.

Krokida, M.K., Oreopoulou, V., Maroulis, Z.B., Marinos-Kouris, D. 2001. Colour changes during deep fat frying. *Journal of Food Engineering* 48, 219-225.

Mohebbi, M., Fathi, M., Shahidi, F. 2011. Genetic algorithm–artificial neural network modeling of moisture and oil content of pretreated fried mushroom. *Food and Bioprocess Technology* 4, 603-609.

Moyano, P.C., Pedreschi, F. 2006. Kinetics of oil uptake during frying of potato slices:: Effect of pre-treatments. *LWT - Food Science and Technology* 39, 285-291.

Paliwal, J., Visen, N.S., Jayas, D.S., White, N.D.G. 2003. Cereal Grain and Dockage Identification using

Machine Vision. *Biosystems Engineering* 85, 51-57.
Pedreschi, F., Moyano, P. 2005. Effect of pre-drying on texture and oil uptake of potato chips. *LWT - Food Science and Technology* 38, 599-604.
Pedreschi, F., Moyano, P., Santis, N., Pedreschi, R. 2007. Physical properties of pre-treated potato chips. *Journal of Food Engineering* 79, 1474-1482.
Pietikanen, M.K. 2000. Texture analysis in machin vision World Scientific, London.
Qiao, J., Wang, N., Ngadi, M.O., Kazemi, S. 2007. Predicting mechanical properties of fried chicken nuggets using image processing and neural network techniques. *Journal of Food Engineering* 79, 1065-1070.
Rao, A.V., Agarwal, S. 1999. Role of lycopene as antioxidant carotenoid in the prevention of chronic diseases: A review. *Nutrition Research* 19, 305-323.
Thybo, A.K., Szczypinski, P.M., Karlsson, A.H., Dønstrup, S., Stodkilde-Jorgensen, H.S., Andersen, H.J. 2004. Prediction of sensory texture quality attributes of cooked potatoes by NMR-imaging (MRI) of raw potatoes in combination with different image analysis methods. *Journal of Food Engineering* 61, 91-100.
Zheng, C., Sun, D.-W., Zheng, L. 2006. Recent applications of image texture for evaluation of food qualities-a review. *Trends in Food Science & Technology* 17, 113-128.

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تاثیر برخی ویژگی‌های فرایند بر ویژگی‌های مکانیکی و بافت تصویر هویج سرخ شده

میلاد فتحی[۱] – سید محمد علی رضوی [۲*]

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چکیده

در این تحقیق کاربرد آنالیز بافت تصویر به‌عنوان یک روش غیرمخرب برای اتوماسیون و پیشگویی برخی ویژگی‌های مکانیکی چیپس هویج مورد بررسی قرار گرفت. نمونه‌ها در شرایط‌های مختلف سرخ شدندو رطوبت، پارامترهای رنگی (L*، a*، b* و ΔE) و خواص مکانیکی (سختی و مدول ظاهری) تعیین شد. سختی و مدول ظاهری با افزایش دما و زمان سرخ کردن افزایش یافتند. چهار ویژگی بافت تصویر (کنتراست، همبستگی، انرژی و یکنواختی) با استفاده از ماتریس هم‌وقوعی سطح خاکستری محاسبه شد. نتایج نشان داد کنتراست و انرژی سطح خاکستری تصاویر همبستگی بهتری با سختی نمونه‌های سرخ شده حاصل از آزمون فشاری سوراخ کردن داشتند. بترتیب ضرایب همبستگی ۰/۹۷ و ۰/۹۸ بین چهار ویژگی‌های بافت تصویر و سختی در آزمون فشاری و سوراخ کردن بدست آمد. نتایج نشان داد که آنالیز بافت تصویر می‌تواند به‌عنوان یک روش غیرمخرب برای تخمین ویژگی‌های بافتی هویج استفاده شود.

واژه‌های کلیدی: هویج، سرخ کردن، آنالیز بافت تصویر، ویژگی‌های بافتی مکانیکی

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Clarification of sour orange juice by ultrafiltration: Optimization of permeate flux and fouling resistances using response surface methodology

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Abstract

The turbidity of sour orange juice after juice extraction affects on quality, shelf-life and concentration of juice. Therefore, juice clarification is an important operation in the fruit processing industry. The goal of this study was evaluating the effect of membrane operation parameters including pressure (120-220 kPa) and temperature (25-35 ºC) on the permeate flux and hydraulic resistance of sour orange juice during membrane clarification. Response surface methodology (RSM) was used to optimizing the operating parameters. Results of the experiments showed that the permeate flux was raised with increasing of temperature, but total hydraulic resistance (R_T), concentration polarization resistance (R_{cp}) and gel layer resistance (R_g) was decreased in mentioned condition. The permeate flux, membrane resistance (R_m), R_T, R_{cp} and fouling index was raised with increasing in pressure. The R_m and fouling index are showed different behavior depending on temperatures level. Results of process optimization indicated that the best conditions to maximize of permeate flux, and to minimize of fouling index and R_T achieved at 35 ºC and 120 kPa for a maximum desirability of 0.761.

Keywords: Sour orange, Clarification, Response Surface Methodology, Ultrafiltration.

Introduction

The main applications of membrane operations in the food industry are in the beverage industry (wine, beer, fruit juices, etc.) and the dairy industry. In fruit juice processing, membrane technology is mainly used for several proposes: clarification of the juice by ultrafiltration (UF) and microfiltration (MF), deacidification by electrodialysis, recovery of aroma compounds by pervaporation and membrane distillation, and concentration or preconcentration of the juice by means of nanofiltration, reverse osmosis (RO), membrane distillation, or direct osmosis (Falguera & Ibarz, 2014). The sour oranges (*Citrus aurantium L.*) constitute a separate species of citrus fruit but are closely related to the sweet orange species. It is grown primarily for its peel, which is used in the manufacture of marmalades (Barrett, Somogyi, & Ramaswamy, 2005). The sour orange is popular for its characteristics, such as being a source of vitamin C, organic acids, mineral, soluble and insoluble fibers and also its antioxidant capacity. The sour orange juice is used as food additive, ingredient in salad dressing and also as a popular drink because of its rich flavor and aroma (Koshani, Ziaee, Niakousari, & Golmakani, 2014).

Thermal treatments are used in the preservation of fruit derivatives and in manufacturing operations. The negative effects of these treatments include non-enzymatic browning, nutrient loss and the formation of undesirable by-products such as 5-hydroxy methylfurfural (HMF) (Ibarz, Pagan, & Garza, 1999). Therefore, operation at low temperatures is of interest.

In pulpy juices, the raw juice obtained after pressing is very turbid, viscous, and of a dark color, and contains a lot of colloidal compounds that are stabilized in suspension by polysaccharides such as pectin, starch, cellulose, and gums. Therefore, many juices are clarified prior to their concentration.

Clarification is performed to remove the juice components that cause cloudiness, mainly pectin (Falguera & Ibarz, 2014). In the traditional clarification process, crude filtra-

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tion was performed directly after crushing the fruit. Pectinase was added to hydrolyze pectin, which reduced the viscosity of the juice before it was passed through a series of decantation and diatomaceous filtration steps to yield clear juice with a typical yield of about 90%. By replacing these final filtration steps with ultrafiltration, a very good-quality, almost-sterile product can be produced with a yield of almost 97% (Jansen, Feron, Hanemaaijer, & Huisjes, 2002; Prasad, Runkle, & Shuey, 1994).

UF is very promising alternatives to conventional clarification processes. The operational costs of using membrane processes are considerably lower than those of more traditional processes (Yazdanshenas, Tabatabaee -Nezhad, Soltanieh, Roostaazad, & Khoshfetrat, 2010). Moreover, UF membranes is able to retain microorganisms, avoiding the need for thermal pasteurization processes, and UF is able to remove polyphenol oxidases, which is effective in stabilizing the colors of fruit juices (Falguera & Ibarz, 2014).

Ultrafiltration had been investigated for the clarification of Apple (Onsekizoglu, Bahceci, & Acar, 2010), pear (Alis Cassano, Conidi, Timpone, D'avella, & Drioli, 2007), orange (Galaverna *et al.*, 2008), lemon (Chornomaz, Ochoa, Pagliero, & Marchese, 2011), kiwifruit (Tasselli, Cassano, & Drioli, 2007), chicory (Zhu *et al*., 2013), Black Currant (Pap et al., 2012) and pineapple juice (Laorko, Li, Tongchitpakdee, Chantachum, & Youravong, 2010).

One of the main drawbacks of membrane technology is the fouling of the membrane exhibited with total resistance, which is caused by the accumulation of solute molecules on the membrane surface or inside the pores. Membrane fouling causes a reduction of the permeate flux and also causes changes in selectivity and decreases the overall process productivity. The permeate flux can be restored by means of cleaning procedures, but the process must be stopped and large amounts of chemicals, energy, water, and time are consumed. Moreover, successive membrane cleaning operations can reduce the life of the membrane (Falguera & Ibarz, 2014).

RSM is a collection of statistical and mathematical techniques useful for developing, improving, and optimizing processes in which a response of interest is influenced by several variables and the objective is to optimize this response. RSM has important applications in the design, development and formulation of new products, as well as in the improvement of existing product design. It defines the effect of the independent variables, alone or in combination, on the process. In addition, in order to analyze the effects of the independent variables, this experimental methodology generates a mathematical model which describes the chemical or biochemical processes (Baş & Boyacı, 2007; Ruby Figueroa, Cassano, & Drioli, 2011).

Ruby Figueroa *et al*. (2011) evaluated the effect of process parameters including transmembrane pressure (TMP), temperature, and feed flow-rate on fouling resistances during ultrafiltration of orange press liquor and optimized Operation parameters. A strong interaction effect of temperature and feed flow-rate was observed on the permeate flux while interactions TMP-temperature and TMP-feed flow-rate were found to be less significant. In the case of fouling index, interactions TMP-temperature and TMP-feed flow-rate produced a significant effect (Ruby Figueroa *et al.*, 2011). Baklouti *et al*. (2013) evaluated the resistance-in-series model to analyze flux behavior, which involved the resistances of membrane itself, the fouling and solute concentration polarization. They concluded that the resistance due to solute concentration polarization (R_{cp}) dominated the flux decline (40–74%).

The fouling resistance (R_f) varied from 12 to 46%. The selected UF conditions of the compromise were as follows: three bars, 0.95 L min^{-1} and 30˚C. Optimal values of R_f, R_{cp} and permeate limit flux were equal to 18%, 72% and 19 L h^{-1} m^{-2}, respectively (Baklouti, Kamoun, Ellouze-Ghorbel, & Chaabouni, 2013). Nourbakhsh *et al*. (2014) evaluated the resistances (total, reversible, irreversible &

cake) during microfiltration of watermelon juice and red plum juice. Results showed that the total resistance decreased by about 45% when the feed velocity was increased during clarification of red plum juice due to change in cake resistance. MCE membrane had a lower cake resistance compared to PVDF membrane.

Examination of the microfiltration of watermelon juice showed that Rt decreased by about 54% when the feed temperature was increased from 20 to 50°C, partially due to the reduction of reversible fouling resistance by 78% (Nourbakhsh, Alemi, Emam-Djomeh, & Mirsaeedghazi, 2014).

The aim of this work was to evaluate the potentiality of Polyvinylidene fluoride UF membrane in the clarification of sour orange juice. As well as the effects of Operation parameters including pressure (120-220 kPa) and temperature (25-35 ºC) on the permeate flux and resistances investigated. Resistance in series model was applied to identify flux behavior in the UF process.

Materials and methods

Chemicals Material

Nitric acid (HNO_3) and sodium hydroxide (NaOH) were purchased from Merck Company. Sodium sulfite (Na_2So_3) was obtained from Sigma–Aldrich Company.

Preparation of sour orange Juices

Sour Orange fruit were purchased from a local market in Gorgan (Iran) and washed with tap water in order to remove foreign material from the skin and drained. Then, the juice was extracted by FMC juice extractors with a 2-mm-diameter perforated plate and placed in a tank. Extracted juices were 130 L from 600 kg sour orange fruit. 4 gr kg^{-1} Na_2So_3 was added to single strength juice to avoid browning reactions. The juice was stored at –18°C and was defrosted to room temperature before use. Preparation of fruits juices is shown in Figure. 1.

Ultrafiltration unit and procedures

UF experiments were performed by using a pilot plant unit equipped with a tubular Polyvinylidene fluoride membrane module[1] with a nominal MWCO[2] of 200 kDa. The characteristics of UF membrane are summarized in Table 1.

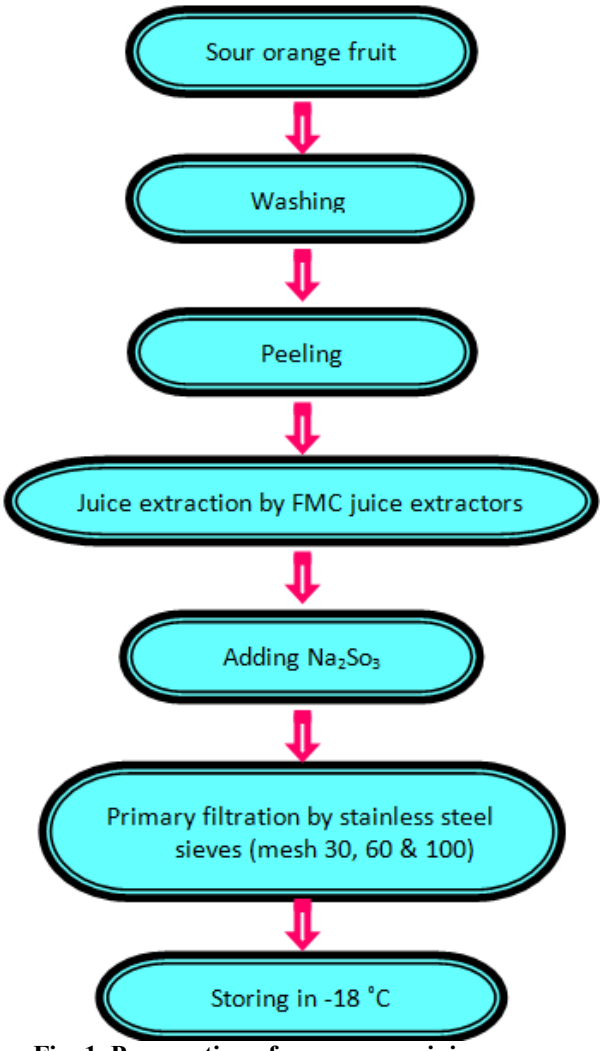

Fig. 1. Preparation of sour orange juice.

Table 1. Characteristics of UF membrane and module used in this study

Membrane type	F01740
Material	PVDF
Effective area	0.1 m^2
Length	120 cm
Range of pH tolerance	1.5- 10.5
Maximum temperature	60 ºC
Maximum pressure	1 MPa
Module	tubular

1. ITT PCI Membranes Technology Co., Ltd (Hampshire, United Kingdom)
2. Molecular Weight Cut-Off

The juice was clarified according to the batch concentration procedure in which the permeate is collected separately and the retentate is recycled to the feed tank. The operating pressure was in the range of 120–220 kPa and the temperature varied from 25°C to 35 °C. The permeate flux was measured every one min for 45 min. In Figure. 2 a schematic diagram of the UF experimental setup is illustrated.

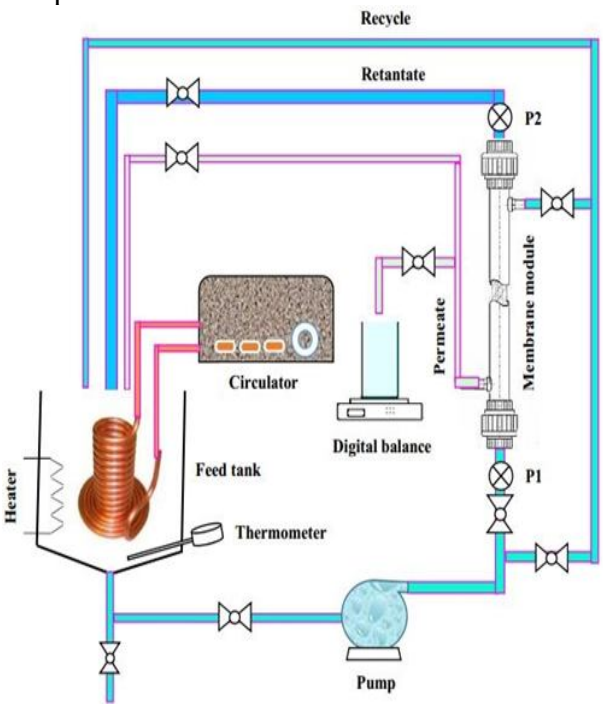

Fig. 2. Schematic of pilot plant ultrafiltration.

The membrane module was rinsed with distilled water for 20 min after the treatment of the juice; then it was submitted to a cleaning process with a NaOH solution (pH = 10, T= 50°C, pressure = 120 kPa, operating time = 0.5 h) followed by a cleaning with the HNO_3 (pH = 2, T= 50°C, pressure = 120 kPa, operating time = 0.5 h) and Backwashing with distilled water for 1 hour. A final rinse of the system with distilled water for at least 20 min was carried out. After each cleaning procedure the distilled water flux of the membrane module in fixed conditions (T= 35°C; pressure = 120 kPa) was measured.

Calculated parameters

The operating pressure calculated according to the following relationship:

$$DrivingForce = TMP = \frac{P_i + P_o}{2} - P_P \quad (1)$$

Where, TMP is the transmembrane pressure (Pa), P_i and P_o are inlet and outlet pressures, respectively, and P_p is permeate pressure. Since P_p is negligible, is not considered.

The permeate flux can be obtained from Darcy's law with assumption of resistance in series model as follows:

$$J_p = \frac{TMP}{\mu_p R_T} = \frac{TMP}{\mu_p (R_m + R_g + R_{cp})} \quad (2)$$

Where J_p is the permeation flux (kg/m^2.h), R_T is the total resistance (m^{-1}), μ_p is the dynamic viscosity of permeate (N.S/m^2), R_m is the Intrinsic or hydraulic membrane resistance, R_{cp} is the Concentration polarization resistance (m^{-1}) and R_g represents the Gel layer resistance due to (i) the internal fouling of the membrane by adsorption of macromolecules on the internal walls of membrane pores and (ii) a thin layer that blocks the membrane pores on the surface which is created by adhering the particles to the membrane surface (Kazemi, Soltanieh, & Yazdanshenas, 2013).

R_m was calculated by measuring the distilled water flux of clean membrane as follows:

$$R_m = \frac{TMP}{\mu_w J_w} \quad (3)$$

Where μ_w and J_w represents the viscosity and flux of distilled water, respectively. For this purpose, water flux in the temperature and pressure range used in this study was obtained and then R_m was calculated using equation (3). At the end of each stage filtration, water flux was measured to calculate R_g on the surface of membrane according to Equation (4).

$$R_g = \frac{TMP}{\mu_w J_{wf}} - R_m \quad (4)$$

Where, J_{wf} represents the distilled water flux at the end of filtration process (blocked membrane). R_{cp} Calculated as follows:

$$R_{cp} = R_T - (R_g + R_m) \quad (5)$$

The fouling index was calculated according to Equation (6):

$$Fouling Index\ (\%) = (\frac{J_w - J_{wf}}{J_w}) \times 100 \quad (6)$$

The hydraulic permeability of the membrane was determined as follows:

$$L_i^p = J_w / \Delta P \ (7)$$

Where, L_1^p, L_2^p and L_3^p are hydraulic permeability; after cleaning with distilled water, after cleaning with NaOH solution and after cleaning with HNO_3 solution, respectively.

The Viscosity was measured using a rotational type Brookfield LVDV-II digital viscometer (USA) at 25 ºC at 80 RPM. A sample of sour orange juice was loaded into cylindrical sample chamber (ULA-31Y) of 16 mL capacity for all experiments and was allowed to equilibrate at 25 ºC using a circulating water jacket (Model ULA-40Y Brookfield Engineering Laboratories). (Bodbodak, Kashaninejad, Hesari, & Razavi, 2013).

Experimental design and statistical analysis

The software Design-Expert (trial version 9.0.0.7, Stat-Ease Inc., Minneapolis, USA) was used for experimental design, analysis of data and plotting of graph. Response surface methodology was used to establish the relationships between operating parameters including pressure (120-220 kPa) and temperature (25-35 ºC) and ultrafiltration (UF) efficiency and thus to determine optimal conditions.Thirteen treatments Consisted of 5 replications at the central point were conducted base on the rotatable central composite design (table 2).

Response functions of measurement parameters were examined by fitting experimental data on linear (Y_1), 2FI (Y_2) and Quadratic models (Y_3) as follows:

$$Y_1 = b_0 + b_1 x_1 + b_2 x_2 \ (8)$$

$$Y_2 = b_0 + b_1 x_1 + b_2 x_2 + b_{12} x_1 x_2 \ (9)$$

$$Y_3 = b_0 + b_1 x_1 + b_2 x_2 + b_{11} x_1^2 + b_{22} x_2^2 + b_{12} x_1 x_2 \ (10)$$

Where, b_0; constant term, b_1 and b_2; linear effects, b_{11} & b_{22}; quadratic effects and b_{12}; interaction effects. The models were compared based on the coefficient of determination (R^2), adjusted coefficient of determination (R^2-adj) and predicted coefficient of determination (R^2-pred). The model with the highest values of R^2-adj & R^2-pred, was selected as the accurate model (Yolmeh, Habibi Najafi, & Farhoosh, 2014). Analysis of variance (ANOVA) was performed to assess the significant effects of process variables on each response.

Table 2. Experimental design results of central composite design (CCD)

Run	Temperature (˚C)	Pressure (kPa)	Average permeate flux (kg/m^2.h)
1	25	120	15.97
2	35	120	20.47
3	25	220	22.62
4	35	220	28.40
5	22.93	170	10.70
6	37.07	170	27.00
7	30	99.29	19.20
8	30	240.71	62.20
9	30	170	22.70
10	30	170	24.00
11	30	170	23.60
12	30	170	23.20
13	30	170	24.10

Result and discussion

Flux behavior

Permeate flux behavior during ultrafiltration of sour orange juice was shown in Figure. 3 The curve represented the evolution of permeate flux decline with time due to concentration polarization and gel formation. Permeate flux curve could be divided in three regions. An initial region in which a rapid decrease of permeate flux occurs; a second region, corresponding to a smaller decrease of permeate flux; a third region characterized by a small decrease of permeate flux up to a steady-state (A Cassano, Marchio, & Drioli, 2007).

hydraulic permeability

Hydraulic permeability is used for calculating of membrane fouling that is estimated with measuring the distillated water flux before and after the membrane filtration and after cleaning treatment. Figure. 4 represents the distillated water permeate flux of the membrane before and after cleaning treatments. According to figure. 4, after the

cleaning with water & NaOH, the hydraulic permeability of the membrane is 63 & 43% lower than the initial value (0.06 kg/m^2.h.kPa), respectively. Hydraulic permeability of the primary causes of acid wash with distilled water recovery percentage was 9/97. The cleaning with a HNO_3 solution permitted the recovery of about 97.9% of the initial distillated water permeability of the membrane.

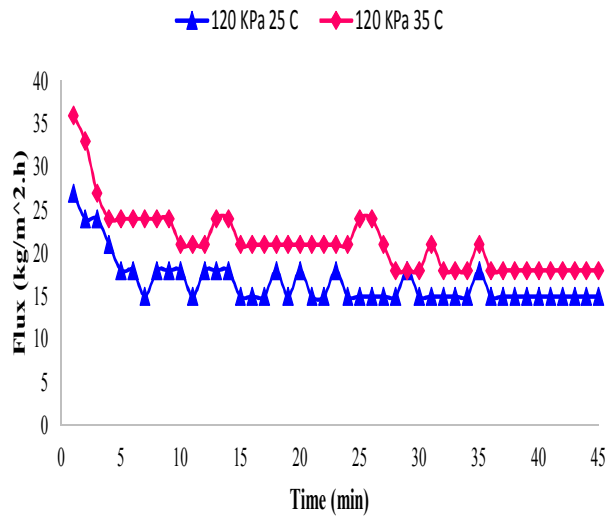

Fig .3. Time course of permeate flux during ultrafiltration of sour orange juice.

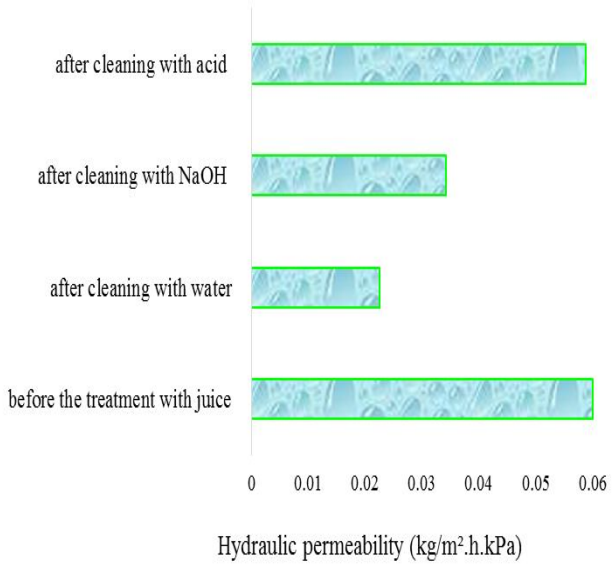

Fig .4. Hydraulic permeability of the UF membrane before and after cleaning procedures (T = 35°C; pressure =120 kPa).

Permeate flux

The results of ANOVA for permeate flux, showed that the effects of various operating parameters (temperature and pressure) on flux was significant. Highest and lowest fluxes were related to treatment of 30 ˚C; 240.71 kPa and 25 ˚C; 120 kPa, respectively. The maximum values of R^2, R^2-adj and R^2-pred obtained for linear model that revealed the adequate of this model for Prediction. The values of R^2, R^2-adj and R^2-pred for the permeate flux was 90.42, 88.51 and 82.24, respectively. The model as follows:

$$Flux=-1.86056+(0.47974\times temperature)+(6.11987\times pressure) \quad (11)$$

From equation (11), we found that the linear effect of pressure is the most effective factor in increasing of permeate flux. Ruby Figueroa et al (2011) during ultrafiltration of orange press liqueur reported that the linear coefficients of pressure were found to be the most significant effect to increase the permeate flux, followed by linear coefficient of temperature (Ruby Figueroa *et al*., 2011). Figure.5 represents effect of temperature and pressure on permeate flux. It seems that at such higher pressures, increasing the driving force led to increase in the flux, whereas, increasing the molecular diffusion and decreasing the viscosity led to flux improvement at such higher temperatures (Salehi & Razavi, 2012). The permeate flux was found to be pressure-depended in the pressure range studied.

An increase of temperature, enhanced permeate flux due to an increase of mass-transfer coefficient according to the film model (Vladisavljević, Vukosavljević, & Bukvić, 2003).

Fouling index (FI)

The results of ANOVA for FI, showed that the effects of various operating parameters on FI was significant. Highest and lowest FI were related to treatment of 30 ˚C; 240.71 kPa and 37 ˚C; 170 kPa, respectively. The maximum values of R^2, R^2-adj and R^2-pred obtained for quadratic model that revealed the adequate of this model for Prediction. The values of R^2,

R^2-adj and R^2-pred for the FI was 91.46, 85.35 and 67.74, respectively. From equation (12), it was found that the linear effect of temperature is the most effective factor in increasing of FI. The effect of temperature and pressure on the FI is shown in Fig. 6. The fouling index decreased with increasing temperature only at pressures higher than central point; at lower level the fouling index raised by increasing the temperature. The FI increased greatly with increasing pressure. In membrane processing which pressure is the driving force; also permeate flux can be raised with increasing in pressure; but, pressure is intensifies the membrane fouling index (Pabby, Rizvi, & Requena, 2009)

Resistance

Flux decline occurs during membrane filtration, because of several reasons including the gel layer formation and blocking the pores and the concentration polarization layer formation. This agents led to increase in the resistance of membrane against material passing.

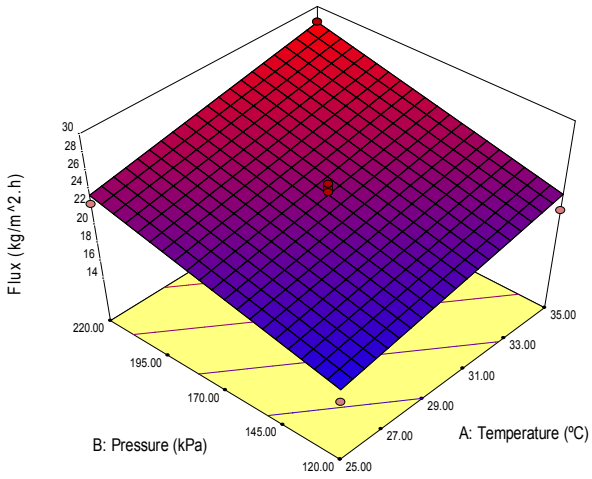

Fig .5. 3D response surface plot for the effect of pressure and temperature on permeate flux.

Total hydraulic resistance (R_T)

The results of ANOVA of total resistance showed that the effect of various treatment of operating parameters on R_T was significant. Highest and lowest R_T were related to treatment of 25 ˚C; 220 kPa and 30 ˚C; 99.29 kPa, respectively. The maximum values of R^2, R^2-adj and R^2-pred obtained for linear model that revealed the adequate of this model for Prediction. The values of R^2, R^2-adj and R^2-pred for the R_T was 92.94, 91.53 and 86.95, respectively. From equation (13), we found that the linear effect of pressure is the most effective factor in increasing of R_T. The R_T increased with increasing in pressure. In addition, the flux is raised with increasing in pressure. This suggests that pressure have more effect on flux compared to the R_T. Figure. 7 represent effect of temperature and pressure on R_T. At high pressure, the rate of deposition would be high and the high pressure would compress the rejected solutes into a thicker and denser fouling layer with an R_T (De Bruijn, Venegas, Martınez, & Bórquez, 2003). This result was also observed by Nourbakhsh *et al.* (2014) working with red plum and watermelon juices (Nourbakhsh *et al.*, 2014). Increasing in temperature, reduced viscosity and enhanced the diffusion coefficient of material from cake layer to feed, thus the RT decreased

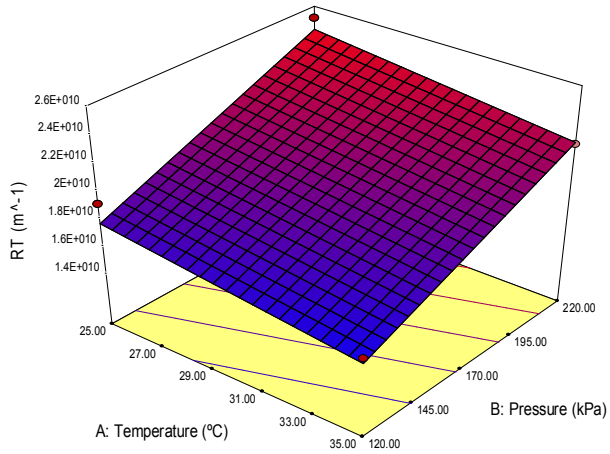

Fig .7. 3D response surface plot for the effect of pressure and temperature on R_T.

Membrane resistance (Rm)

The results of ANOVA of membrane resistance showed that the effect of various treatment of operating parameters on R_m was significant. The maximum values of R^2, R^2-adj and R^2-pred obtained for quadratic model that revealed the adequate of this model for Prediction. The values of R^2, R^2-adj and R^2-

pred for the R_m were found to be 89.92, 82.72 and 59.32, respectively. From equation (14), it was found that the linear effect of pressure on enhanced of R_m is more than the linear effect of temperature on reduced of this factor. The R_m changes during filtration by membrane fouling, which is due to either solute adsorption onto the membrane surface and membrane plugging (de Oliveira, Docê, & de Barros, 2012). The effect of temperature and pressure on the R_m is shown in Fig. 8. With increasing temperature and pressure, R_m was decreased and increased, respectively

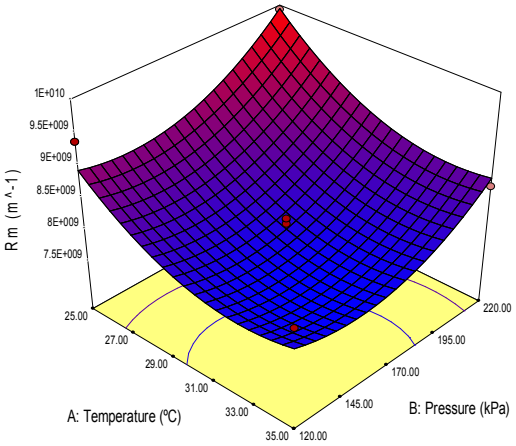

Fig .8. 3D response surface plot for the effect of pressure and temperature on R_m.

Gel layer resistance (R_g)

The results of ANOVA of R_g showed that the effect of various treatment of operating parameters on R_g was significant. In this study, the largest proportion of the total resistance (47.8%) were related to Rg. Baklouti *et al*. (2012) applied the Hermia model to describe the fouling during the filtration of enzymatic treated of pomegranate juice and reported that the gel layer formation was the major cause of fouling (Baklouti, Ellouze-Ghorbel, & Mokni, 2012). Domingues *et al*. (2014) reported that gel formation was the major fouling factor during the microfiltration of centrifuged and enzyme treated passion fruit juice with polieterimide hollow fibre Membrane (Domingues, Ramos, Cardoso, & Reis, 2014).

The maximum values of R^2, R^2-adj and R^2-pred obtained for linear model that revealed the adequate of this model for Prediction. The values of R^2, R^2-adj and R^2-pred for the R_g was 95.35, 94.41 and 90.4, respectively. From equation (15), we found that the linear effect of pressure on enhanced of R_g is more than the linear effect of temperature on reduced of this factor. Fig. 9 represents effect of temperature and pressure on R_g. increasing in pressure due to enhance of driving force and adsorption of particles on the membrane surface leads to increasing of R_g (Rai, Majumdar, Das Gupta, & De, 2007). According to the fig. 9, at higher temperatures, Rg slowly declined

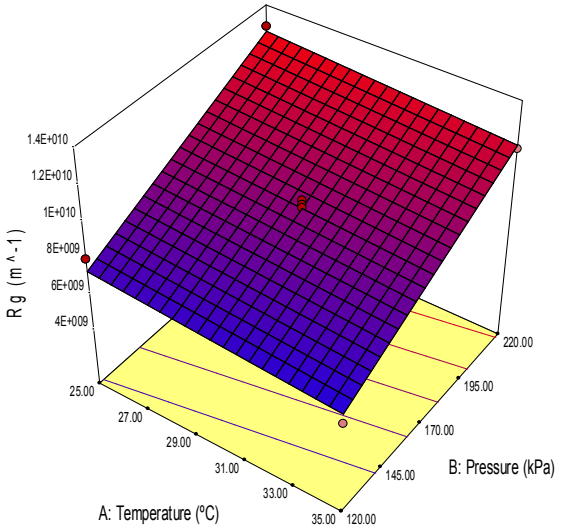

Fig .9. 3D response surface plot for the effect of pressure and temperature on R_g.

Concentration polarization resistance (R_{cp})

The results of ANOVA of R_{cp} showed that the effect of various treatment of operating parameters on R_{cp} was significant. In this study, the lowest proportion of the total resistance (9.39%) were related to R_{cp}. The maximum values of R^2, R^2-adj and R^2-pred obtained for linear model that revealed the adequate of this model for Prediction. The values of R^2, R^2-adj and R^2-pred for the R_{cp} was 79.25, 75.09 and 61.2, respectively. From equation (16), it was found that the linear effect of pressure on enhanced of R_{cp} is more than the linear effect of temperature on reduced of this factor. The effect of temperature and pressure on the R_{cp} is shown in Fig. 10. Concentration polarization occurs

in the all membrane processes which the driving force is the pressure. The accumulation of particles on the membrane surface may increase with increase of pressure. It enhances the membrane fouling via concentration polarization.
.

$$Fouling\ Index = -11.89560 + (0.99212 \times temperature) - (0.83645 \times pressure) - (0.00669349 \times temperature \times pressure) - (0.016689 \times temperature^2) + (0.69063 \times pressure^2) \quad (12)$$

$$R_T = 1.55245 \times 10^{10} - (2.24801 \times 10^8 \times temperature) + (6.56529 \times 10^9 \times pressure) \quad (13)$$

$$R_M = 3.07333 \times 10^{10} - (1.18969 \times 10^9 \times temperature) - (4.72727 \times 10^9 \times pressure) - (2.71050 \times 10^7 \times temperature \times pressure) + (1.18759 \times 10^7 \times temperature^2) + (1.88512 \times 10^9 \times pressure^2) \quad (14)$$

$$R_g = 3.39272 \times 10^9 - (9.12388 \times 10^7 \times temperature) + (5.21875 \times 10^9 \times pressure) \quad (15)$$

$$R_{cp} = 1.65555 \times 10^9 - (1.98456 \times 10^7 \times temperature) + (4.77544 \times 10^8 \times pressure) \quad (16)$$

An increase in temperature enhanced back diffusion of solutes into the bulk solution, reducing consequently the thickness of the polarized layer: therefore R_{cp} decreased with increasing in temperature (A Cassano, Mecchia, & Drioli, 2008). He *et al.* (2007) during clarification of apple juice over 20 hours, reported that membrane fouling to the filtration performance can be neglected; and the major factors influencing permeate flux were the reversible concentration polarization (in contrast to our study) related to feed concentration and viscosity, not the irreversible fouling such as internal plugging, silting, etc (He, Ji, & Li, 2007)

Optimizing

In order to maximize the permeate flux and minimize the fouling index and total resistance, the desirability function approach was applied to analyze the regression model equations. The optimized operating variables were found to be 120 kPa, 35˚C for an overall desirability of 0.761. In optimal condition, the permeate flux, fouling index and total resistance was 22.27 (kg/m^2.h), 2.09 % and 1.55×10^{10} (m^{-1}), respectively.

Optimization results for the UF of sour orange juice are summarized in table 3

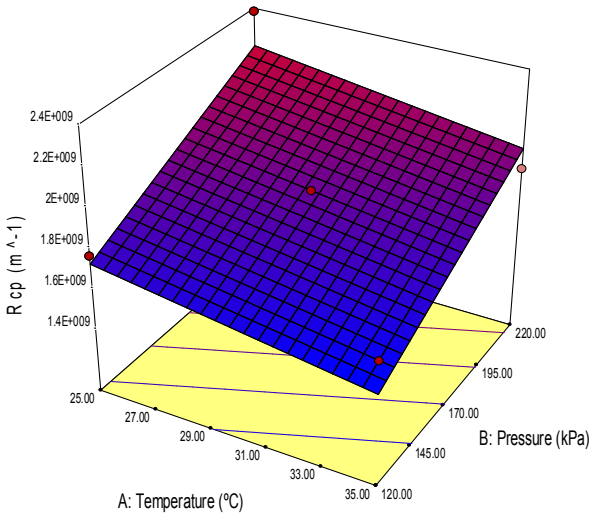

Fig .10. 3D response surface plot for the effect of pressure and temperature on R_{cp}.

Table 3. Predicted parameters and responses from RSM for optimized condition

			goal	The degree of importance
Predicted parameters/ responses	TMP	120 kPa	In range	-
	Temperature	35 ºC	In range	-
	permeate flux	22.27	maximizing	+++++
	fouling index	2.09 %	minimizing	+++++
	total resistance	1.55×10^{10}	minimizing	++++
	overall desirability		0.761	

Conclusions

Sour orange is a source of vitamin C that is cultivated in the north and central regions of Iran. The effect of operating parameters including of pressure and temperature on the performance of a UF membrane in the clarification of sour orange juice was studied with the response surface methodology. A central composite design was used for regression modeling and optimizing the UF operating parameters.

Results of the experiments showed that the permeate flux, was raised with increasing in temperature, but fouling index and all of resistances (R_T, R_M, R_{cp} & R_g), were decreased in this condition. Increasing in pressure, enhanced of permeate flux and all of the resistances. The permeate flux also decreased over the time. The best conditions to maximize of permeate flux, and to minimize of fouling index and R_T achieved at 35 ºC and 120 kPa.

References

Baklouti, S., Ellouze-Ghorbel, R., & Mokni, A. (2012). Clarification of pomegranate juice by ultrafiltration: study of juice quality and of the fouling mechanism. *Fruits*, 67(03), 215-225.

Baklouti, S., Kamoun, A., Ellouze□Ghorbel, R., & Chaabouni, S. (2013). Optimising operating conditions in ultrafiltration fouling of pomegranate juice by response surface methodology. *International Journal of Food Science & Technology*, 48(7), 1519-1525.

Barrett, D. M., Somogyi, L. P., & Ramaswamy, H. S. (2005). Processing fruits: science and technology: *CRC Press Boca Raton*, FL.

Baş, D., & Boyacı, İ. H. (2007). Modeling and optimization I: Usability of response surface methodology. *Journal of food engineering*, 78(3), 836-845.

Bodbodak, S., Kashaninejad, M., Hesari, J., & Razavi, S. (2013). Modeling of Rheological Characteristics of "MalasYazdi"(Punicia granatum L.) Pomegranate Juice. *Journal of Agricultural Science and Technology*, 15(5), 961-971.

Cassano, A., Conidi, C., Timpone, R., D'avella, M., & Drioli, E. (2007). A membrane-based process for the clarification and the concentration of the cactus pear juice. *Journal of food engineering*, 80(3), 914-921.

Cassano, A., Marchio, M., & Drioli, E. (2007). Clarification of blood orange juice by ultrafiltration: analyses of operating parameters, membrane fouling and juice quality. *Desalination*, 212(1), 15-27.

Cassano, A., Mecchia, A., & Drioli, E. (2008). Analyses of hydrodynamic resistances and operating parameters in the ultrafiltration of grape must. *Journal of food engineering*, 89(2), 171-177.

Chornomaz, P. M., Ochoa, N. A., Pagliero, C., & Marchese, J. (2011). Synthesis, characterization and performance of membranes for clarification of lemon juice. Desalination and Water Treatment, 27(1-3), 294-298.

De Bruijn, J., Venegas, A., Martınez, J., & Bórquez, R. (2003). Ultrafiltration performance of Carbosep membranes for the clarification of apple juice. *LWT-Food Science and Technology*, 36(4), 397-406.

de Oliveira, R. C., Docê, R. C., & de Barros, S. T. D. (2012). Clarification of passion fruit juice by microfiltration: Analyses of operating parameters, study of membrane fouling and juice quality. *Journal of food engineering*, 111(2), 432-439.

Domingues, R. C. C., Ramos, A. A., Cardoso, V. L., & Reis, M. H. M. (2014). Microfiltration of passion fruit juice using hollow fibre membranes and evaluation of fouling mechanisms. *Journal of food engineering*, 121, 73-79.

Falguera, V., & Ibarz, A. (2014). Juice Processing: Quality, Safety and Value-Added Opportunities: CRC

Press.
Galaverna, G., Di Silvestro, G., Cassano, A., Sforza, S., Dossena, A., Drioli, E., & Marchelli, R. (2008). A new integrated membrane process for the production of concentrated blood orange juice: Effect on bioactive compounds and antioxidant activity. *Food chemistry*, 106(3), 1021-1030.
He, Y., Ji, Z., & Li, S. (2007). Effective clarification of apple juice using membrane filtration without enzyme and pasteurization pretreatment. *Separation and Purification Technology*, 57(2), 366-373.
Ibarz, A., Pagan, J., & Garza, S. (1999). Kinetic models for colour changes in pear puree during heating at relatively high temperatures. *Journal of food engineering*, 39(4), 415-422.
Jansen, A. E., Feron, P. H. M., Hanemaaijer, J. H., & Huisjes, P. (2002). Apparatus and method for performing membrane gas/liquid absorption at elevated pressure*: Google Patents*.
Kazemi, M. A., Soltanieh, M., & Yazdanshenas, M. (2013). Mathematical modeling of crossflow microfiltration of diluted malt extract suspension by tubular ceramic membranes. *Journal of food engineering*, 116(4), 926-933.
Koshani, R., Ziaee, E., Niakousari, M., & Golmakani, M. T. (2014). Optimization of Thermal and Thermosonication Treatments on Pectin Methyl Esterase Inactivation of Sour Orange Juice (Citrus aurantium). *Journal of Food Processing and Preservation*.
Laorko, A., Li, Z., Tongchitpakdee, S., Chantachum, S., & Youravong, W. (2010). Effect of membrane property and operating conditions on phytochemical properties and permeate flux during clarification of pineapple juice. *Journal of food engineering*, 100(3), 514-521.
Nourbakhsh, H., Alemi, A., Emam-Djomeh, Z., & Mirsaeedghazi, H. (2014). Effect of processing parameters on fouling resistances during microfiltration of red plum and watermelon juices: a comparative study. *Journal of food science and technology*, 51(1), 168-172.
Onsekizoglu, P., Bahceci, K. S., & Acar, M. J. (2010). Clarification and the concentration of apple juice using membrane processes: A comparative quality assessment. *Journal of Membrane Science*, 352(1), 160-165.
Pabby, A. K., Rizvi, S. S., & Requena, A. M. S. (2009). Handbook of membrane separations: chemical, pharmaceutical, food, and biotechnological applications: *CRC press*.
Pap, N., Mahosenaho, M., Pongrácz, E., Mikkonen, H., Jaakkola, M., Virtanen, V., Vatai, G. (2012). Effect of ultrafiltration on anthocyanin and flavonol content of black currant juice (Ribes nigrum L.). *Food and Bioprocess Technology*, 5(3), 921-928.
Prasad, R., Runkle, C. J., & Shuey, H. F. (1994). Spiral-wound hollow fiber membrane fabric cartridges and modules having flow-directing baffles: *Google Patents*.
Rai, P., Majumdar, G., Das Gupta, S., & De, S. (2007). Effect of various pretreatment methods on permeate flux and quality during ultrafiltration of mosambi juice. *Journal of food engineering*, 78(2), 561-568.
Ruby Figueroa, R. A., Cassano, A., & Drioli, E. (2011). Ultrafiltration of orange press liquor: Optimization for permeate flux and fouling index by response surface methodology. *Separation and Purification Technology*, 80(1), 1-10.
Salehi, F., & Razavi, S. M. (2012). Dynamic modeling of flux and total hydraulic resistance in nanofiltration treatment of regeneration waste brine using artificial neural networks. Desalination and Water Treatment, 41(1-3), 95-104.
Tasselli, F., Cassano, A., & Drioli, E. (2007). Ultrafiltration of kiwifruit juice using modified poly (ether ether ketone) hollow fibre membranes. *Separation and Purification Technology*, 57(1), 94-102.
Vladisavljević, G., Vukosavljević, P., & Bukvić, B. (2003). Permeate flux and fouling resistance in ultrafiltration of depectinized apple juice using ceramic membranes. *Journal of food engineering*, 60(3), 241-247.
Yazdanshenas, M., Tabatabaee-Nezhad, S. A. R., Soltanieh, M., Roostaazad, R., & Khoshfetrat, A. B. (2010). Contribution of fouling and gel polarization during ultrafiltration of raw apple juice at industrial scale. *Desalination*, 258(1), 194-200.
Yolmeh, M., Habibi Najafi, M. B., & Farhoosh, R. (2014). Optimisation of ultrasound-assisted extraction of natural pigment from annatto seeds by response surface methodology (RSM). *Food chemistry*, 155, 319-324.
Zhu, Z., Luo, J., Ding, L., Bals, O., Jaffrin, M. Y., & Vorobiev, E. (2013). Chicory juice clarification by membrane filtration using rotating disk module. *Journal of food engineering*, 115(2), 264-271.

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جلد ۱۲، شماره ۳، مرداد - شهریور ۱۳۹۵، ص. ۳۶۱-۳۵۰

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شفاف‌سازی آب نارنج با استفاده از اولترافیلتراسیون: بهینه‌سازی شار تراوه و مقاومت گرفتگی با روش سطح پاسخ

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چکیده

کدورت آب نارنج بعد از آبگیری بر روی کیفیت، ماندگاری و تغلیظ آبمیوه موثر است. بنابراین شفاف‌سازی آبمیوه یک فرآیند مهم در صنعت فرآوری میوه است. هدف از این پژوهش بررسی پارامترهای عملیاتی غشاء شامل فشار (۲۲۰-۱۲۰ کیلوپاسکال) و دما (۳۵-۲۵ درجه سانتی‌گراد) بر شار تراوه و مقاومت هیدرولیک در حین شفاف‌سازی غشایی آب نارنج بود. روش سطح پاسخ برای بهینه‌سازی پارامترهای عملیاتی استفاده شد. نتایج آزمایشات نشان داد با افزایش دما شار تراوه افزایش یافت اما مقاومت هیدرولیک کل (R_T)، مقاومت پلاریزاسیون تغلیظ (R_{cp}) و مقاومت لایه ژل (R_g) در این شرایط کاهش یافت. شار تراوه، مقاومت غشاء (R_m)، R_T، R_{cp} و شاخص گرفتگی با افزایش فشار افزایش یافت. شاخص گرفتگی و R_m رفتار متفاوتی را با توجه به تغییرات دما نشان دادند. نتایج بهینه‌سازی فرآیند نشان داد بهترین شرایط برای به حداکثر رساندن شار تراوه و به حداقل رساندن شاخص گرفتگی و R_T در دمای ۳۵ درجه سانتی‌گراد و ۱۲۰ کیلوپاسکال با حداکثر مطلوبیت ۰/۷۶۱ بدست می‌آید.

واژه‌های کلیدی: نارنج، شفاف‌سازی، اولترافیلتراسیون، روش سطح پاسخ

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Dimensionless modeling of thin layer drying process of Aloe vera gel

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Abstract

This research, presents mathematical modeling of drying process of Aloe vera slices with dimensions of 7×4×0.5±0.1 cm. Peeled Aloe vera slices with the initial moisture content of 5750% (d.b) were osmosed for 5 hours in NACL solution of 10% and temperature of 40 °C at a constant solution to fruit ratio of 5:1. Osmosed and unosmosed Aloe vera samples were hot air dried at 55, 70 and 85°C with different air flow rates of 0.015, 0.036 and 0.054 m^3/s for 13200s. The moisture content of Aloe vera samples were measured over different intervals of drying time (1200, 2400, 6000, 9600, 13200s) for each experiment. The experimental results were used to obtain two different dimensionless models based on Buckingham's pi-theorem for both drying methods. To this end, three independent π terms were identified and then the relation between dependent π term and each independent π term was sought. Finally, the dimensionless models incorporating the effect of all the independent π terms on the dependent one derived and evaluated. The RMSE, (R^2), MRD and MBE for the modeling of osmotic-convective drying method were calculated as 0.0185, 0.99, 0.05 and 0.034, respectively. Also these statistical parameters for the convective drying method were as: 0.027, 0.98, 0.061 and 0.051, respectively. Therefore the dimensionless models could predict the moisture content of Aloe vera samples during drying, properly.

Keywords: Aloe vera, Osmotic-air drying, dimensionless model.

Introduction

Aloe vera is a traditional medicinal plant which used in food, pharmaceutical and cosmetic industries. Also it has been utilized to prepare health food drinks, beverage and confectionary. Aloe vera gel could be applied to make creams, lotions and soaps (Pisalkar *et al*, 2011). Some of plants such as Aloe vera leaf have high initial moisture content that it may lead to early spoilage. In other words, the main cause of the decay of fruits and vegetables is their high moisture content (Yadav and Singh, 2014). Drying is one of the most important methods to preserve the foods against decay and spoilage. Indeed, drying process reduces the water activity of the products and controls the microorganism growth (Zomorodian and Moradi, 2010). Also the drying mechanism causes to reduce the weight of the final products and therefore helps to transport the dried grains easier. There are different methods for dehydration of the Aloe vera gel. Osmotic drying is a suitable method because it helps to taste the final products, increasing the shelf life and maintain the quality of the dried gel (Pisalkar *et al*, 2011).

Mathematical modeling usually is used to predict the moisture content of the drying products instantaneously. The mathematical models classify into two categories: theoretical and empirical. The theoretical approach concerns either the diffusion equation or simultaneous heat and mass transfer equations. Having a theoretical model is hard because it needs to solve the governing equations using simplification hypothesizes and thus the derived model does not have admissible accuracy. In the empirical model, a direct relationship is established between the average moisture content and drying time (Akgun and Doymaz, 2005; Akpinar *et al*, 2003; Akpinar *et al*, 2003). Dimensional analysis is an empirical approach which introduces a dimensionless model to explain the relations between dependent and independent π terms.

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This method is a beneficial tool for providing guidance in mathematical modeling and specially simulating the complex phenomena such as those involved in fluid sciences (Giuseppe, 2006; Gibbings, 2011). Several dimensionless models have been proposed to simulate the drying process of bio-products (Melendez *et al*, 2002; Zare *et al*, 2012; Moradi and Karpaprvar fard, 2016). Zare *et al* (2012) developed a generalized dimensionless model of paddy drying from a validated partial differential equation (PDE) drying model using the dimensional analysis of Buckingham theorem.

They considered all drying parameters in an equation to predict the grain moisture content during the drying process. They mentioned that the obtained dimensionless model showed good agreement with the solution results of partial differential equation drying model.

In an another study, a dimensionless model was established and evaluated for drying of corn grains in a continuous dryer equipped with inert energy carrier particles. To do the simulation, five independent π terms which were responsible for the drying rate of the grains were identified and a dimensionless model, includes the effect of all independent π terms on the dependent π term, was derived and evaluated (Moradi and Karparvar fard, 2016). In this research, drying kinetics of Aloe vera gel was investigated at different operating conditions. Dimensional analysis technique was employed to make a predictive dimensionless model for the gel moisture content during drying based on experimental data.

Finding the dimensionless equations for the drying process of the food materials helps to perform a better design of the drying system thus this research is emphasizes to introduce the drying dimensionless models. As we know that no work has been reported on dimensionless modeling of Aloe vera gel during drying process, this study was mainly devoted to establish and evaluate new dimensionless models for thin layer drying of Aloe vera gel.

Therefore the main objective of this research is to define and evaluate the dimensionless model for the drying process of the Aloe vera gel based on Buckingham theorem.

Materials and methods

Aloe vera leaves harvested freshly at the early of morning. During the selection of the leaves for the harvesting, some of important factors such as their uniformity on thickness, color and maturity were considered. Each leaf harvested was cleaned at the start and then peeled by means of a knife to obtain a white gel. The gel samples were cut using the knife with the average dimensions of 0.5×4×7 (±0.1) cm. Aloe vera sample with initial moisture content of 5750% (d.b) was placed in a NACL solution of 10% at a constant solution to fruit ratio of 5:1 for 5 hours. During the osmotic dehydration, the sample with the brine was kept in an incubator at 40°C. Then the pretreated sample in the osmotic solution was dried using a cabinet dryer (Fig.1). In this dryer an electrical fan was used to introduce the ambient air through a heating channel to be heated and then blown to the drying chamber. The dry bulb temperature and the relative humidity of the ambient air were about 27 °C (±2 °C) and 30% (±1%), respectively. An electrical thermostat, with 0.1°C accuracy, in the output of heating channel was installed to fix the drying air temperature as desired value. Aloe vera samples were dried at three different levels of the drying air temperature (55, 70 and 85°C) and the drying air flow rate (0.015, 0.036 and 0.054 m^3/s). These values coincide with those applied in the literature for the osmosed and unosmosed Aloe vera samples. (Pisalkar *et al*, 2011). An adjustable plate was installed on the input of the electrical fan to set the air flow rate of the fan as favorite value.

Before the launching the experiments, intended air temperature and the air flow rate were calibrated by using a Testo 625 (Testo Company, Germany) thermometer with accuracy of ±0.5°C and a Testo 425 anemometer with accuracy of ±0.03m/s, respectively.

The experiments were performed at three

replications. All the above experiments replicated for the control samples (the samples without osmotic pretreatment). During the drying process, Aloe vera gel weighed by means of a digital weighing device (A&D) with 0.001g accuracy at different time intervals (1200, 2400, 6000, 9600, 13200s). The results were used to calculate the dry basis moisture content of the samples

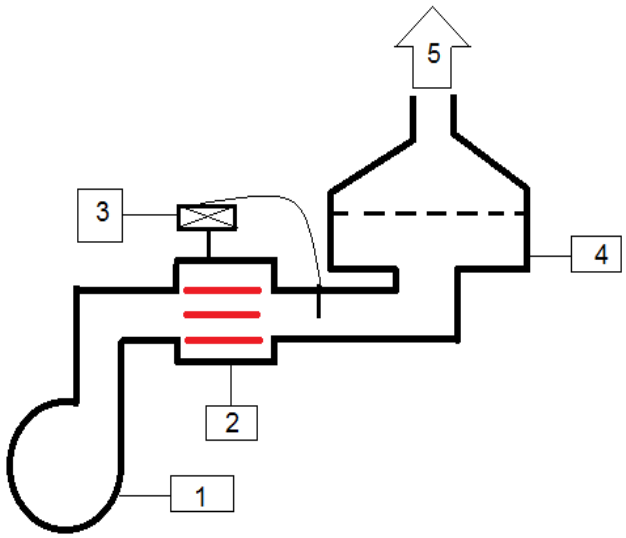

Fig.1.a. Schematic diagram of cabinet dryer

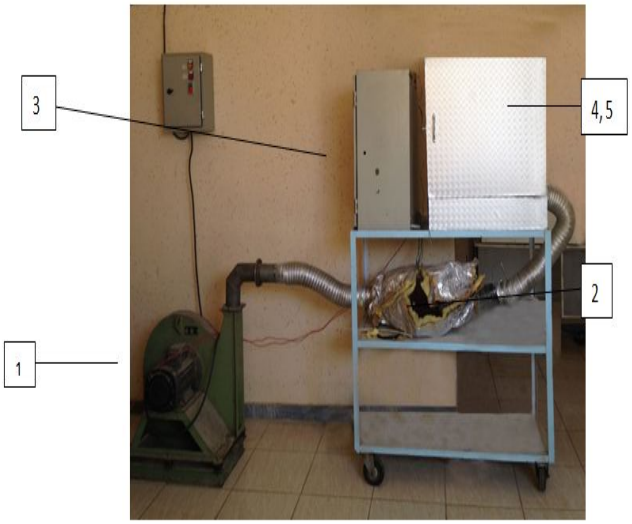

Fig. 1.a. The cabinet dryer used for convective hot-air drying

1-Blower fan 2- Heating canal 3- Air temperature control unit 4- Drying cabinet 5- Outlet of drying air

Osmosis characteristics including solid gain, water loss and weight reduction calculated by the following equations (Yousefi et al, 2013):

$$WR(\%) = \frac{W_0 - W}{W_0} \times 100 \quad (1)$$

$$SG(\%) = \frac{W(1-X_0) - W_0(1-X_0)}{W_0} \times 100 \quad (2)$$

$$WL(\%) = SG(\%) + WR(\%) \quad (3)$$

Where: WR: Weight reduction, SG: solid gain, WL: water loss, W_0: initial sample weight (g), W: final sample weight (g), X_0: initial moisture content of the sample (decimal), X: final moisture content of the sample (decimal).

Modeling

In order to obtain a dimensionless model, drying parameters must be introduced. The following factors (equation (4)), were recognized to be important in drying process of Aloe vera gel:

$$F(M, M_0, K_a, K_0, Q_a, D_{eff}, T_e, T_t) = 0 \quad (4)$$

Where:

M: moisture content of the sample (kg of water per kg of dry matter), M_0: initial moisture content of the sample (kg of water per kg of dry matter), K_a: temperature of drying air (°C), K_0: ambient air temperature (°C), Q_a: flow rate of drying air (m^3/s), D_{eff}: effective moisture diffusivity (m^2/s), T_e: elapsed time (s), T_t: total drying time (s).

Among these, two variables (M and M_0) were merged to produce one π term:

$$MR = M/M_0 \quad (5)$$

The remaining variables (K_a, K_0, Q_a, D_{eff}, T_e,T_t) included three principle dimensions, namely, L (length), T (time), and K (temperature), thus based on Buckingham's pi-theorem (6-3=3), three π terms were constructed as the following:

$$\pi_1 = K_a/K_0,$$

$$\pi_2 = T_e/T_t,$$

$$\pi_3 = (D_{eff} \times (T_t)^{1/3})/Q_a^{2/3} \quad (6)$$

Each of the above π terms were obtained based on their dimensionless process.

Consequently, equation (1) can be rewritten as:

$$MR = f[K_a/K_0, T_e/T_t, [D_{eff} \times (T_t)^{1/3}]/Q_a^{2/3}] \quad (7)$$

Three levels of K_a accompanied by the constant value of K_0=27°C were used to

calculate the different values of π_1. Five levels of elapsed time (1200, 2400, 6000, 9600, 13200s) during drying process were selected to measure the moisture content of the samples. To get the π_2 values, the ratios of these time levels to the total time duration of drying (T_t=13200s) were calculated. To obtain the quantities of π_3, at first the effective moisture diffusion coefficients of the drying gel were determined. To attain this goal, analytical solution of drying equation was considered (Khodabakhsh Aghdam et al, 2012):

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

Which; t: drying time (s), L: the half thickness of the slab in the sample (m)

If the variations of the (ln MR) versus (t) take in to account, slope of this variation could be calculated from the equation 6 (Doymaz, 2012):

$$k=\frac{-\pi^2 D_{eff}}{4L^2} \quad (9)$$

In the equation (9), the value of (k) was obtained from the experimental results thus the quantity of the D_{eff} can be easily calculated.

The amounts of diffusion coefficient for osmosed and unosmosed samples were ranged from 3.02×10^{-12} to 4.24×10^{-11} m^2/s and 8.97×10^{-12} to 1.19×10^{-10} m^2/s, respectively.

Three different values of the drying air flow rate (0.015, 0.036, 0.054 m^3/s) were applied at this research. This parameter was measured at the inlet vent of the air (7×7 cm^2) into the drying chamber. To compute the π_3 values, the average quantities of the D_{eff} in the each level of Q_a were used.

These π terms were computed for two different drying methods: the osmotic-convective and convective drying methods. All of the experiments were done at three replications and the average of these values was considered at each treatment.

The values of independent π terms for the osmotic-air dried samples have been presented in Table 1

The following relationship has been proposed to derive the final figuration of dimensionless model (Zare et al, 2012; Eric et al, 2015):

$$MR = A[(F_1(\pi_1))^B.(F_2(\pi_2))^C.(F_3(\pi_3))^D] \quad (10)$$

Where:

$F_1(\pi_1)$, $F_2(\pi_2)$ and $F_3(\pi_3)$ are the best fitted line equations between dependent π term (MR) and each independent π term. A, B, C and D are constants that must be determined.

Also Table 2 shows the values of independent π terms for the air dried samples.

Table1- Different levels of independent dimensionless groups for osmotic-convective samples

$\pi_1=K_a/K_0$	$\pi_2=T_e/T_t$	$\pi_3=(D_{eff}\times(T_t)^{1/3})/Q_a^{2/3}$
2.037	0.091	3.97×10^{-9}
2.593	0.182	4.71×10^{-9}
3.148	0.455	7.18×10^{-9}
	0.727	
	1	

Table2- Different values of independent π terms for air dried samples

$\pi_1=K_a/K_0$	$\pi_2=T_e/T_t$	$\pi_3=(D_{eff}\times(T_t)^{1/3})/Q_a^{2/3}$
2.037	0.091	6.75×10^{-9}
2.593	0.182	8.58×10^{-9}
3.148	0.455	9.87×10^{-9}
	0.727	
	1	

The increase

Results and discussion

Osmosis characteristics (WR, SG and WL), calculated by the equations 1 to 3, were as: 26.5%, 20.3% and 46.8%, respectively. Dimensionless modeling was selected as an empirical procedure to model the drying process of Aloe vera gel at different drying operations. Therefore Eq. 10 was considered as a basic equation to obtain the dimensionless models.

To get the Eq. 10 for each drying state, 80% of experimental data were used to construct the relations between dependent π term and each of the independent π term. The variations of the MR versus different independent π terms have been shown in Figs.2-4. Regression analysis technique was applied on the experimental data to find the best fit between dependent π term and each independent π term. The regression equations and coefficient of determination for both drying methods have been shown in Table3.

The figures 2 to 4 show the variations of MR versus each independent π terms. It can be seen from Fig.2 that MR has been decreased as the π_1 values increased. This may be because the higher air temperature causes to increase the heat transfer rate into the samples. The results are in good agreement with the results of other researchers which have reported increase in drying air temperature cause to decrease the moisture content of the sample (Yousefi et al, 2013; Zomorodian and Moradi, 2010). The best line which characterizes this behavior was also fitted to the experimental data. The relevant line equations have been shown in the Table 3 for two different drying methods (Eqs. 11 and 14).

In the Figure 3, the effects of the π_2 on the M.R of the Aloe vera gel for two drying methods have been illustrated. It was observed the greater drying time ratio (π_2), resulted in the lower MR. The best line, fitted to the experimental data, was also found based on the regression analysis. The line equations and the coefficients of determination have been brought in equations12 and 15. Similar results were obtained by Moradi and Karparvar fard (2016). They reported the variations in MR as the functions of dependent π terms. Also in another research, the variations of MR were shown as the functions of different dependent π terms. (Zare et al, 2012).

Fig.4 shows the changes in MR versus π_3 for two different drying methods. This figure also presents the best line which explains the behavior of the experimental data. The line equations and the coefficients of determination were mentioned in equations 13 and 16 (Moradi and Karparvar fard, 2016; Zare et al, 2012).

Table3- Regression equations and coefficient of determination

Drying state	Regression equations	R^2	Equation no.
Osmotic-convective drying	$F_1(\pi_1) = -0.2\pi_1 + 0.8853$	0.99	11
	$F_2(\pi_2) = 1.1011(\pi_2)^2 - 1.8917\,\pi_2 + 0.904$	0.99	12
	$F_3(\pi_3) = 3\times10^{7}\pi_3 + 0.1864$	0.99	13
Convective drying	$F_1(\pi_1) = -0.1613\,\pi_1 + 0.7728$	0.99	14
	$F_2(\pi_2) = 1.1016(\pi_2)^2 - 2.0388\,\pi_2 + 0.9624$	0.99	15
	$F_3(\pi_3) = 3\times10^{7}\,\pi_3 + 0.0651$	0.85	16

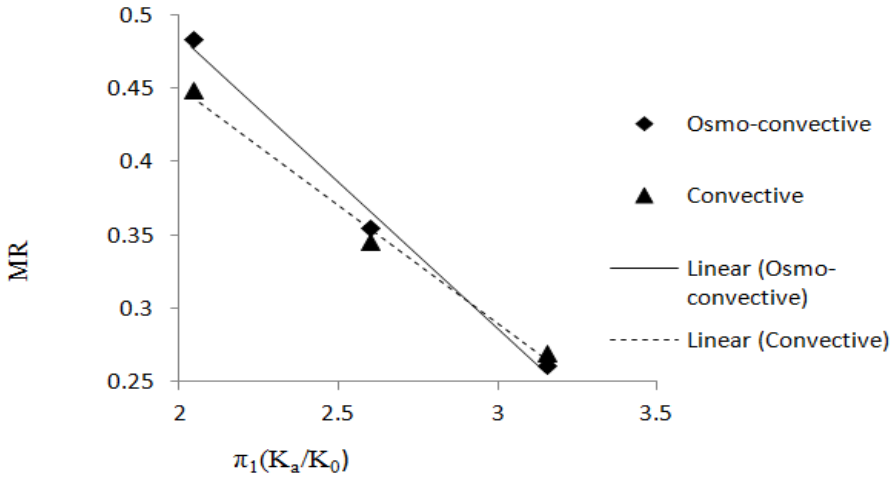

Fig.2. Variations of MR vs. π_1 for two different drying methods

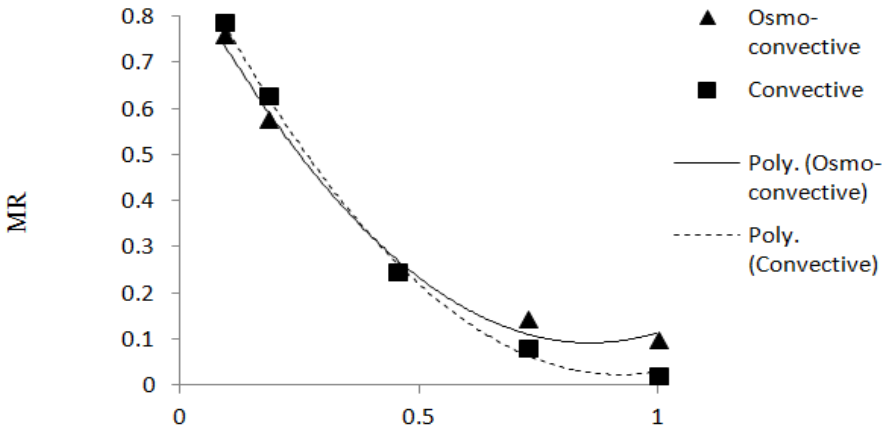

Fig3- Variations of MR versus π_2 for two different drying methods

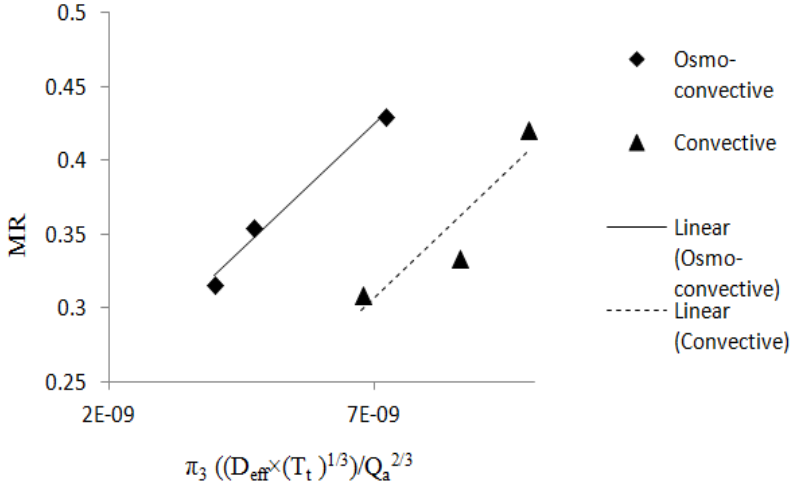

Fig4- Variations of MR versus π_3 for two different drying methods

In order to compute the constants of A, B, C and D in equation 10, an optimizing algorithm was used to reach a minimum value of mean bias error (MBE) (Eric et al, 2015):

$$\beta = \frac{1}{N}\sum_{i=1}^{N}\left|\frac{DR_i^{ex} - DR_i^{pre}}{DR_i^{ex}}\right| \qquad (17)$$

Where;

β: MBE, N: number of the experiments, MR_i^{ex}: experimental drying ratio, MR_i^{pre}: predicted moisture ratio.

Hence, the constants for two different drying methods were calculated as follow:

For the osmotic-convective drying method: A= 2.643, B=0.722, C=0.956, D=0.407.

For the convective drying method:

A= 3.514, B=0.594, C=0.956, D=0.568.

Prediction equation

By replacement of the equations 11, 12 and 13 to the equation 10, the dimensionless model for the osmotic-convective drying method was obtained as the equation 18.

$$MR= 2.643\times [-0.2\pi_1+0.8853]^{0.722}\times [1.1011(\pi_2)^2-1.8917\pi_2+0.904]^{0.956}\times [9\times10^7\pi_3+0.0404]^{0.407} \qquad (18)$$

Also the dimensionless model for the convective drying method was achieved by setting the equations 14, 15 and 16 in to the equation 10:

$$MR= 3.514\times [-0.1613\pi_1+0.7728]^{0.594}\times [1.1016(\pi_2)^2-2.0388\pi_2+0.962]^{0.956}\times [3\times10^7\pi_3+0.0651]^{0.568} \qquad (19)$$

The moisture content of Aloe vera gel can be computed by the equation (20):

$$M=M_0\times MR \quad (20)$$

Evaluating the predicted model

Evaluation of dimensionless models was performed based on comparison between the experimental and predicted moisture ratios. For this purpose, 20% of total experimental data, that did not incorporate in the modeling, were used to validate the derived models. Figs5 and 6 display the experimental MR versus the predicted values of MR for two different drying methods.

+20% and -20% lines show that all predicted data are in the appropriate range (Eric *et al*, 2015).

To validate the goodness of the modeling, three statistical criteria, MBE, RMSE[1], MRD[2] and R^2 were calculated using relations of 17, 21, 22 and 23, respectively:

$$RMSE = \left[\frac{1}{N}\sum_{i=1}^{N}(MR_i^{ex} - MR_i^{pre})^2\right]^{0.5} \quad (21)$$

$$MRD = \left[\frac{1}{N}\sum_{i=1}^{N}\left(\frac{MR_i^{ex} - MR_i^{pre}}{MR_i^{ex}}\right)^2\right]^{0.5} \quad (22)$$

$$R^2 = \frac{\left(\sum_{i=1}^{N}(MR_{exp,i} - \overline{MR}_{exp})(MR_{pre,i} - \overline{MR}_{pre})\right)^2}{\sum_{i=1}^{N}(MR_{exp,i} - \overline{MR}_{exp})^2 \sum_{i=1}^{N}(MR_{pre,i} - \overline{MR}_{pre})^2} \quad (23)$$

Where;

N: number of the observations, MR_i^{ex}: experimental moisture ratio, MR_i^{pre}: predicted moisture ratio, $\overline{MR}_{exp}$: the average of experimental moisture ratio, $\overline{MR}_{pre}$: the average of predicted moisture ratio.

The RMSE, R^2, MRD and MBE for the modeling of osmotic-convective drying method were calculated as 0.0185, 0.99, 0.05 and 0.034 respectively. Also these statistical parameters for the convective drying method were as 0.027, 0.98, 0.061 and 0.051. In a similar research, a generalized dimensionless model of paddy drying was developed from a validated partial differential equation and the statistical criteria were reported to be; R^2=0.866, MBE=0.0685 and RMSE=0.014 (Zare et al, 2012). In the another research, a dimensionless model was obtained to predict the moisture content of corn grains into a continuous dryer that R^2, MBE and RMSE were calculated as 0.85, 0.0648 and 0.018, respectively (Moradi and Karparvar fard, 2016). Therefore, the resulted dimensionless models can be used for predicting the moisture content of Aloe vera gel appropriately during thin layer drying process.

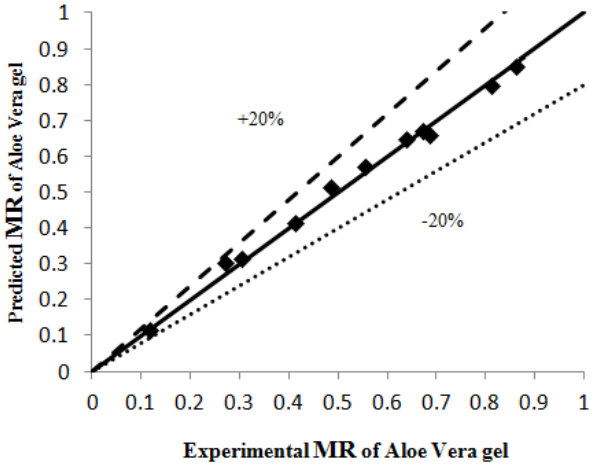

Fig5- Comparison of experimental and predicted MR values for osmotic-convective drying method

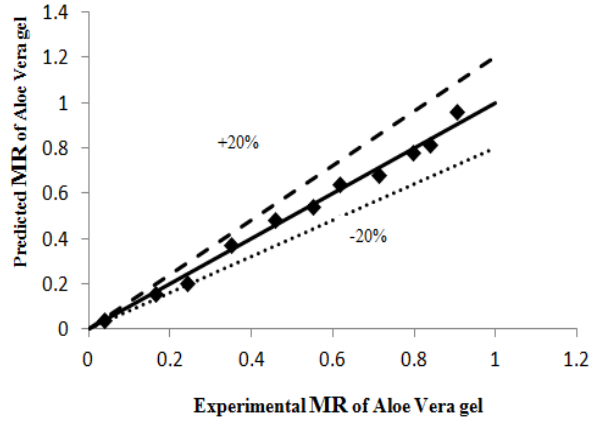

Fig6- Comparison of experimental and predicted MR values for convective drying method

Conclusions

In this research, osmosed and unosmosed Aloe vera samples were dried using a cabinet dryer. Different drying operations were applied on the dryer to obtain the drying kinetics of Aloe vera gels. Finally, two dimensionless equations for predicting the moisture content of Aloe vera gel as it was dried in two different drying methods were derived and evaluated. The statistical results showed good agreement between the

[1] Root Mean Square Error
[2] Mean Relative Deviation

experimental and predicted moisture content. Therefore the resulted equations can easily be used to design the dryer systems of Aloe vera gels.

References

Akgun, N., and Doymaz, I. 2005. Modeling of Olive Cake Thin-layer Drying Process. *Journal of Food Engineering*, 68: 455- 461.

Akpinar, E. K., Bicer, Y., and Midilli, A. 2003. Modeling and Experimental Study on Drying of Apple Slices in a Convective Cyclone Dryer. *Journal of Food Process Engineering*, 6(6): 515-541.

Akpinar, E. K., Bicer, Y., and Yildiz, C. 2003. Thin Layer Drying of Red Pepper. *Journal of Food Engineering*, 59: 99-104.

Doymaz, I. 2012. Evaluation of some thin layer drying models of Persimon slices (Diospyros Kaki L.). *Energy conversion and management*, 56:199-205.

Eric O., Karine, L. Celine, M., Guillaume, D., and Annie, M. 2015. Critical agitation for microcarrier suspension in orbital shaken bioreactors: experimental study and dimensional analysis. *Chemical Engineering Science*, 122: 545-554.

Gibbings, J.C. 2011. Dimensional Analysis, 1st Ed; Springer, London.

Giuseppe, D. P. 2006. An introduction to infinite-dimensional analysis, Springer, London.

Khodabakhsh Aghdam, Sh., Moradi, M., and Yousefi, A.R. 2012. Comparative study of mass transfer based on experimental data for Papaya fruit drying. *Iranian food science and technology research journal*, 8(3): 309-316.

Melendez, G. P., Hernandez, J. A., Salgado, M. A., and Garcia, M. A. 2002. Dimensionless analysis of the simultaneous heat and mass transfer in food drying. *Journal of food engineering*, 51: 347-353.

Moradi, M., and Karparvar fard S. H. 2016. Mathematical modeling of a corn grains drying process in a continuous dryer including inert particles. *Iranian journal of chemical engineering*, 14(83): 82-90.

Pisalkar, P.S., Jain, N. K., and Jain, S. K. Osmo-air drying of aloe vera gel cubes. 2011. *Journal of food science and technology*. 48(2): 183-189.

Yadav, A. K., and Singh, S. V. 2014. Osmotic dehydration of fruits and vegetables: a review. *Journal of Food Science and Technology*, 51(9):1654–1673.

Yousefi, A. R., Niakousari, M., and Moradi, M. 2013. Microwave assisted hot air drying of papaya (Carica papaya L.) pretreated in osmotic solution. 2013. *African journal of agricultural research*, 8(25): 3229-3235.

Zare D., Jayas, D. S., and Singh, C. B. 2012. A generalized dimensionless model for deep bed drying of Paddy. *Drying Technology*, 30: 44–51.

Zomorodian, A., and Moradi, M. 2010. Mathematical modeling of forced convection thin layer solar drying for Cuminum cyminum. *Jouranal of Agricultural Science and Technology*,12: 401-408.

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مدل سازی فرایند خشک شدن لایه نازک ژل آلوئه‌ورا

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چکیده

در تحقیق حاضر مدل سازی فرایند خشک شدن قطعات ژل آلوئه ورا با ابعاد ۰/۱±۰/۵×۴×۷ سانتیمتر ارائه می شود. قطعات پوست گیری شده ژل آلوئه ورا با محتوای رطوبت اولیه ٪۵۷۵۰ (برمبنای خشک) به مدت ۵ ساعت در معرض محلول آب نمک ٪۱۰ تحت دمای ثابت $40^{\circ}c$ و با نسبت وزنی ثابت محلول به ژل ۱:۵ قرار داده شدند. نمونه های اسمزی و غیر اسمزی آلوئه‌ورا با هوای خشک کننده تحت دمای ۵۵، ۷۰ و ۸۵ درجه سلسیوس و دبی ۰/۰۱۵، ۰/۰۳۶ و ۰/۰۵۴ متر مکعب بر ثانیه به مدت ۱۳۲۰۰ ثانیه خشک شدند. محتوای رطوبت آلوئه‌ورا در زمان‌های مختلف طی فرایند خشک‌شدن (۱۳۲۰۰، ۹۶۰۰، ۶۰۰۰، ۲۴۰۰، ۱۲۰۰ ثانیه) اندازه‌گیری شد. نتایج آزمایشگاهی جهت بدست آوردن دو مدل بی بعد بر اساس تئوری باکینگهام برای دو روش مختلف خشک‌شدن مورد استفاده قرار گرفت. به این منظور سه گروه بی بعد مستقل شناسایی و سپس رابطه گروه بی‌بعد وابسته با هر کدام از گروه‌های مستقل بدست آورده شد. سرانجام دو مدل بی بعد برای دو روش مختلف خشک شدن با مشارکت اثر همه‌ی گروه‌های بی بعد مستقل بر گروه بی بعد وابسته ایجاد و ارزیابی شدند. میانگین مربعات خطا (RMSE)، ضریب تعیین (R^2)، انحراف نسبی میانگین (MRD) و میانگین خطای انحراف (MBE) برای دو حالت خشک شدن اسمزی- همرفتی و همرفتی به ترتیب عبارتند از: ۰/۰۱۸۵، ۰/۹۹، ۰/۰۵، ۰/۰۳۴ و ۰/۰۲۷، ۰/۹۸، ۰/۰۶۱، ۰/۰۵۱. بنابراین مدل‌های بی بعد به دست آمده می توانند با دقت نسبتاً خوبی محتوای رطوبت ژل آلوئه ورا را حین خشک‌شدن پیش‌بینی کنند.

واژه‌های کلیدی: آلوئه ورا، خشک شدن اسمزی- همرفتی، مدل بی بعد.

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Effects of Different Manufacturing Methods on Yield, Physicochemical and Sensory Properties of Mozzarella Cheese

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Abstract

The effect of different mozzarella cheese manufacturing methods, i.e. direct acidification (DA), starter culture (SC) and their combination method (CM) on physicochemical, yield, texture, color and sensory properties of the product were compared. Chemical analyses of samples revealed that the SC cheese had higher fat, moisture, ash, titratable acidity, actual and adjusted yields and fat recovery than DA cheese. DA cheese showed higher springiness, cohesiveness, and hardness than CM and SC cheeses, due to denser and elastic protein network, whereas meltability and adhesiveness of DA cheese was lower than CM and SC samples. SC cheese had significantly higher b-value than DA sample. The sensory evaluation revealed that the SC cheese had higher sensory quality than other cheeses in fresh state and during 45 days of storage. In general, sensory scores of all mozzarella cheeses were acceptable up to 15th day of storage and thereafter decreased progressively till the end of storage period.

Keywords: Different manufacturing methods; Mozzarella cheese; Physicochemical; Textural properties; Sensory attribute.

Introduction

Mozzarella cheese belongs to the family of 'Pasta Filata' cheeses and has a specific manufacturing method which the curd is immersed and kneaded in hot water and then stretched several times to the desirable smooth texture (Abd El-Gawad *et al.,* 2012). Because the main application of mozzarella cheese is used as a topping on pizza, therefore the texture and particularly melting characteristics of cheese has significant effect on consumer acceptance. Mozzarella has a several desirable properties, such as medium firmness, appropriate melting and stretchability, and easy shredding (Jana and Mandal, 2011). These properties can be affected by different parameters such as moisture, pH and salt content of the cheese.

The variation of pH at different steps of manufacturing can affect the water holding capacity, composition and in addition promote the functional properties of the cheese (Guinee *et al.,* 2002). It also can affect the meltability and distribution of calcium in the cheese (Kindstedt *et al.,* 2001). Proteolysis which occurs in most varieties of rennet-curd cheeses has a significant effect on development of texture and flavor of the product. The proteolysis of casein is affected by calcium content and pH in diluted systems (Feeney *et al.,* 2002). The pH of 5.3 is a critical point in related to the yield and calcium contents of obtained cheese and by controlling/decreasing the calcium content of the cheese through lactic acid addition (e.g. with increasing the acidity of the curd from 0.5 to 0.8%), the meltability (Kiely *et al.,* 1992) and sensory scores (Jana and Mandal, 2011) of the final mozzarella cheese can be improved.

Milk pre-acidification with food grade acids before adding rennet causes the casein destabilization and removing the large amounts of calcium in casein micelles. This reduces the extent of calcium bonded between casein polymers and therefore the softer cheese with better and higher flow ability and stretching can be produced (Feeney *et al.,* 2002; Zisu and Shah, 2005).

Acid production rate is a critical issue that

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determines the cheese quality. Starter cultures used for promotion of acid production during curd manufacturing have also significant effects on flavor characteristics and textural properties of final cheese. A various variety of starter cultures such as, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* or *Lactobacillus helveticus* can be used alone or in combination for obtaining the appropriate properties of mozzarella cheese (Sameen *et al.,* 2010). Although the cheeses obtained by starter cultures may have more yield and flavors, but direct acidification method of cheese manufacturing can be a good choice for mechanization and continuous manufacturing of mozzarella cheese (Ernstrom, 1965).

Different manufacturing methods of mozzarella cheese can alter the quality and the quantity of the product. Though the effect of pre-acidification or addition of starter culture on the textural properties, sensory attributes, and quantity of the mozzarella cheese have been extensively investigated by many researchers (Dave *et al.,* 2003; Kiely *et al.,* 1992; McMahon *et al.,* 2005; Oommen *et al.,* 2002; Zisu and Shah, 2007), but comparison between these procedures along with their combination method (pre-acidifiacation and starter culture) have not yet been studied. Therefore, in the current research, effect of different methods of mozzarella cheese making, i.e. direct acidification, starter culture and their combination methods on physicochemical, yield, and sensory of the obtained cheeses were investigated.

Materials and Methods

Cheese Manufacture

The whole milk (3.2% fat) was pasteurized (72 ^{o}C for 15 s), cooled to 37 ^{o}C and divided into 10-litre quantities for each manufacturing method. Manufacturing methods of mozzarella cheese were done according to Dave *et al.* (2003) and Guinee *et al.* (2002) with some modifications. In direct acidification (DA) method, citric acid (10% solution) was used before and after curd formation. Before coagulation, pH of milk was reduced to 6.1 with using citric acid solution. Then commercial powdered microbial rennet (Rennilase, Meito Sangyo Co., Nagoya, Japan) at a level of 1.5 g per 100 kg of milk was added and after incubation at 37 °C for 30 min, curd was cut. At that time curd was maintained for about 10 min without stirring and then was gently stirred for further 10 min to facilitate whey expulsion and avoid fusion of freshly cut curd cubes. Then the half of whey was removed and temperature of remained whey was gradually raised up to 40 °C over 20 min. Afterward, another fourth of remained whey volume was removed and citric acid was added gradually and temperature was raised with time up to 43–44°C in at least 1h until the pH of whey and curd was reached approximately to 4.4 and 5.3, respectively. For starter culture (SC) method, about one percent starter culture (mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* at a ratio 1:1) was added to the milk at 42 °C and milk was incubated at 42 °C for 45 min. At the end of this incubation period, the pH of the milk was approximately reached to 6.3. Then, milk temperature was adjusted to 37 °C and rennet was added. After 30 min, the pH was reached to 6.1 and the curd was cut and further manufacturing method was sustained same as DA method. In combination method (CM), starter culture was added but milk pre-acidification was done using 10% citric acid solution and milk pH was adjusted to 6.3 before curdling. After curd formation, the reduction of pH was continued with starter culture and no citric acid solution was used.

In all three procedures, after attainment to final pH of curd, i.e. 5.3, whole amount of whey was drained and the curd was wrapped within a cheese-cloth for one hour. Afterward, the curd was stretched by hand in brine (2%) at 75 °C and after subsequent molding, the curd was immersed in cold brine (20% salt) for 1 h. Then brined curd was vacuum packed, stored at 4 °C and chemical composition, textural and sensory properties of cheeses were measured. All manufacturing methods were done at tree times.

Chemical Analysis

All cheese samples were analyzed for determination of pH at 20 °C (Metrohm, 827 pH lab, Swiss made), moisture content (102 °C for 4 h), acidity (NaOH 0.1 N), ash content (540 °C for 5h), protein content (Kjeldahl method, by multiplying the total nitrogen by 6.38) according to AOAC methods (2000). Fat content was measured through Gerber method according to the technique reported by Marshall (1993) with some modifications. For fat measurement, 10 ml of sulphuric acid was added to butyrometer, followed by adding the 3 ml of hot distilled water (60°C), 3 g of each cheese sample, 5 ml of distilled water (60°C) and 1ml amyl alcohol. In order to complete dissolving cheese particles, the butyrometer and its content were shacked extremely and then the butyrometer was centrifuged at 1300 rpm for 5 min. The fat content of each cheese sample was expressed as percentage by measuring the fat column.

Actual and Adjusted Yields of Cheese

Yield is an amount of cheese which is obtained from a known amount of milk and measured by weighing the obtained cheese before brining. The yield (%) can be expressed as (a) based on actual moisture (actual yield) which calculated by dividing the obtained cheese before brining on the weight of milk and (b) based on 55% moisture content according to following equations (Robinson and Tamime, 1996):

$$W_{55} = [(W \times TS)/0.45] \times 100 \quad (1)$$

Where W_{55} is a weight of cheese (kg) at 55% moisture; W is weight of cheese (kg); and TS is a weight of total solids in one kg of cheese.

$$Y_{55} = [W_{55}/Wm] \times 100 \quad (2)$$

Where Y_{55} is adjusted yield (%) at 55% moisture of cheese; W_{55} is a calculated amount in previous equation; W_m is weight of milk.

Fat and Protein Recoveries

Fat and protein recoveries (%) of cheese were calculated by dividing the total amount of fat or protein in obtained cheese on total amount of fat or protein in milk, respectively (Jooyandeh and Minhas, 2009):

Fat or protein recovery =((weight of cheese × fat or protein content of cheese)/(weight of milk × fat or protein content of milk)× 100) (3)

Meltability

Meltability of all cheese samples was measured according to the method of Madadlou *et al.* (2005).

Color Evaluation

Color evaluation of cheese samples were done by using Chroma meter (Chroma Meter CR-400, Konica Minolta, Japan made). The L, a and b-values are correspond to whiteness /darkness, redness/greenness and yellowness /blueness, respectively.

Texture Profile Analysis (TPA)

The texture evaluation of cheese was measured by using Texture Analyzer (TA-XT plus Texture Analyzer [Stable Micro System, Godalming, UK]). Testing conditions were set up according to method of Jooyandeh (2009) with some modifications. A aluminum cylindrical probe (5-mm diameter), 2 mm/s pre-test speed, 1 mm/s test speed, 1 mm/s post-test speed, rest time of 5 s, trigger force of 3 g, data acquisition 200 pps and 50% strain were used.

Sensory Evaluation

All cheese samples were encoded randomly and then evaluated by ten well trained panelists who were faculty members and students of food science and technology department of Ramin Agriculture and Natural Resources University. All cheese samples were evaluated for taste, odor, texture, color and appearance, and overall acceptability according to the nine-point hedonic scale test (1= dislike extremely to 9= like extremely (Lawless and Hymann, 1998)) after 0, 15, 30, and 45 days of storage. Samples were cut in pieces with a similar size (2 cm× 2 cm× 2 cm) and then obtained pieces were placed into airtight plastic container and

maintained at ambient temperature for 20 min before evaluation. The panelists used water for washing their mouth between testing each samples.

Statistical Analysis

All experiments were done at least in triplicate. The results obtained were subjected to statistical analysis to determine significant differences between three different cheese making procedures. The SAS software (version 9.1) were used for data analysis and differences between means were determined by Duncan's test at $P<0.05$.

Results and Discussion

Chemical Analysis

The chemical composition of mozzarella cheeses are shown in Table 1. Moisture is essential component of cheese which has a plasticizer effect in the protein matrix and therefore making it less elastic and more susceptible to fracture upon compression (Fox *et al.,* 2000). The moisture content of SC cheese was significantly ($P<0.05$) higher than DA cheese. These results are in agreement with those of Sheehan *et al.* (2005) and Zisu and Shah (2007), who reported the lower moisture in mozzarella cheeses produced with direct acidification. Katsiari *et al.* (2002) found that when adjunct cultures used for cheese manufacturing, the moisture content and hardness of cheese increased and decreased, respectively.

The SC cheese had lower protein content than DA and CM cheeses but showed higher fat content. The lower fat content in DA cheese is due likely to more and rapid interactions between proteins in this cheese sample (McMahon *et al.,* 2005) which causes the more compact protein network and therefore the lower moisture and fat content retained in final curd. These results are in agreement with those of Guinee *et al.* (2000), who reported that increased the volume fraction of the casein matrix is due to reduced fat content. The SC and CM cheeses had higher ash content due to their higher moisture content which may increase the amounts of soluble minerals including ionic K, Na, and Ca (McMahon *et al.,* 1999). The similar results were reported by Dave *et al.* (2003) and Sameen *et al.* (2008). The higher titratable acidity of SC and CM than DA cheese samples is likely due to the higher biochemical reactions and production of organic acids, particularly lactic acid by starter cultures which is in accordance with the results of Sameen *et al.* (2008).

Yield, Fat and Protein Recovery

Cheese yield is a one kg of obtained cheese from a one kg of milk, which is a very important parameter in cheese making. The higher recovery of solid material causes the higher cheese yield (El-Gawad and Ahmed, 2011). According to our results (Table 2), though SC cheese had higher actual yield than other mozzarella cheeses, their differences were not significant. However, for adjusted yield, magnitude variations were recognized and SC and CM samples had significantly higher adjusted yield than DA cheese. The higher yields of SC and CM cheeses were likely due to their higher moisture and fat contents. The proteolysis development which occurs during curd formation by starter culture may increase the water holding capacity of casein network and therefore causes the increase in weight/yield of cheese. Hence, CM and SC cheeses had the higher yield in compare with DA sample. These results are in agreement with those of Metzger *et al.* (2000), who reported that pre-acidification of milk before mozzarella making causes the decrease in cheese yield from 2.5 to 5.8% depend on pH of milk and type of used acid. Pre-acidification of milk and subsequent reduction of calcium, fat and protein recovery in cheese leads to lower actual and adjusted yields.

Fat and protein recoveries in cheese are very important parameters in cheese making industry, because their lessening result in cheese yields reduction. These parameters are significantly affected by the type of cheese making procedure/acidification. Different cheese making procedures had significant effect ($P<0.05$) on fat and protein recoveries.

SC cheese had higher fat and protein recoveries than DA cheese, but showed the lower protein recovery than CM cheese. The higher protein recovery and protein content of CM than SC cheese is likely due to the effect of partial acidification which causes more interactions between proteins (McMahon *et al.,* 2005). Increasing the acidity by direct acidification in DA cheese causes the decrease in cheese calcium and this has bilateral impact, lower the protein interactions between proteins and reduces the entrapped fat in casein matrix (Zisu and Shah, 2005). In agreement with our results, Metzger *et al.* (2000) observed that mozzarella cheeses produced by direct acidification of milk had lower fat and protein recoveries.

Table 1. The chemical composition of mozzarella cheeses.

Cheese sample	Protein content (%)	Fat content (%)	Moisture content (%)	Ash content (%)	Titratable Acidity (%)
SC	19.29±0.35^{b}	24.94±1.16^{a}	51.85±0.61^{a}	4.19±0.08^{a}	0.50±0.04^{a}
DA	23.05±0.27^{a}	21.95±0.84^{b}	47.83±1.14^{b}	3.18±0.06^{b}	0.35±0.01^{b}
CM	22.38±0.58^{a}	23.04±0.11^{ab}	49.94±0.30^{ab}	3.32±0.15^{b}	0.44±0.06^{a}

Means within the same column with different superscripts differ significantly (P<0.05); SC, Starter Culture; DA, Direct Acidification; and CM, their Combination Methods.

Table 2. Effect of different manufacturing methods on yield, fat and protein recoveries of cheese samples.

Mozzarella cheese	Actual Yield (%)	Adjusted Yield (%)	Protein recovery (%)	Fat recovery (%)
SC	10.85±0.63^{ns}	11.60±0.01^{a}	62.12±1.15^{ab}	72.12±2.51^{a}
DA	8.81±0.96	10.22±0.41^{b}	55.51±6.06^{b}	47.46±2.86^{c}
CM	10.07±0.86	11.21±0.14^{a}	65.12±4.38^{a}	59.80±2.00^{b}

Means within the same column with different superscripts differ significantly (P<0.05); SC, Starter Culture; DA, Direct Acidification; and CM, their Combination Method.

Color Evaluation

Food appearance is one of the most important properties of foods which are directly related to product quality and customer acceptance, and affected by its reflection, absorption or transmittance of light and these states are related to the physical structure and chemical nature of food (Rudan *et al.,* 1998). Results (Table 3) showed relatively higher but non-significant L value for SC cheese likely due to its higher fat globules content which increases the light scattering and therefore enhances whiteness of cheese (Rudan *et al.,* 1998). Rudan *et al.* (1999) also showed that by reducing the fat content, the amount of whiteness and opacity of cheese critically decreases. The b value of the SC cheese was significantly higher than other samples because of higher fat content. As well, SC and CM cheeses had higher moisture content and therefore the amount of serum in these cheese samples were higher than DA sample, resulted in greater greenish color (greater negative a value).

Table 3. The L, a, and b-values of cheese samples from different cheese samples from different manufacturing methods.

Cheese sample	L-value	a-value	b-value
SC	69.20±0.61^{ns}	-4.59±0.13^{b}	13.24±0.77^{a}
DA	67.28±1.91	-4.13±0.02^{a}	10.08±0.44^{b}
CM	67.01±1.54	-4.55±0.05^{b}	11.43±0.80^{b}

Cheese Texture Characterization

Meltability is a key functional property of mozzarella cheese (Sameen *et al.,* 2008) which defined as flowability and spreadability of cheese particles upon heating (Ma *et al.,* 2011). Fat amount and protein-protein interactions with water are two most important parameters which determine the meltability of

mozzarella cheese (McMahon *et al.,* 1999). Meltability of cheese samples are reported in Table. 4. The higher meltability of SC and CM samples is because of their higher fat content, which is in agreement with the results of Abd El-Gawad *et al.* (2012) and Ma *et al.* (2013). Khosrowshahi *et al.* (2006) reported that when fat content in cheese decreased, the obtained product has a compact texture, lower meltability, and undesirable sensory properties. The texture and functionality of mozzarella cheese also can be affected by its moisture contents. As, cheeses with higher moisture retention causes the softer and more pliable product with improved melting properties (Zisu and Shah, 2005).

Among the milk proteins, casein is the main factor which can affect the curd firmness, syneresis rate, moisture retention and finally cheese quality (Lawrence, 1993). Fat reduction in cheese causes the more elastic and dense protein network (Tunick *et al.,* 1995). According to our results (Table 4), hardness, cohesiveness and springiness of DA sample were higher than SC and CM samples, which are agreed with the results of Rudan *et al.* (1999), Sheehan and Guinee (2004) and Fife *et al*. (1996) who reported higher hardness, cohesiveness, elasticity, and springiness as a result of lower fat or moisture contents. Furthermore, the higher hardness and chewiness of DA cheese than other cheese samples (P<0.05) is likely due to its higher protein content and more compact texture.

The starter culture cheese had softer texture which could not completely restore its primary shape after 50% compression and showed lower springiness, cohesiveness, and hardness. Oommen *et al.* (2002) also reported that starter culture cheeses have a lower cohesiveness. The SC cheese had significantly higher adhesiveness than DA and CM samples, which is agreed with results of Zisu and Shah (2006), who observed that obtained cheese without pre-acidification has higher adhesiveness. Higher moisture content causes weakening of protein matrix and when this state is concomitant with proteolysis, the higher adhesiveness values can be obtained (McMahon *et al.,* 2005). Proteolysis rate in cheese is directly depends on concentration of starters and bacterial population in cheese. Hence, the lower hardness, chewiness, and cohesiveness of SC and CM cheeses are likely due to the higher proteolysis rate of added starter cultures (Jooyandeh, 2009). Firmness is inversely correlated with proteolysis, as decrease the firmness is concomitant with higher primary proteolysis (Sheehan and Guinee, 2004). The gumminess is result of hardness and cohesiveness of cheese (Tunick, 2000) and when cohesiveness or/and hardness of product decreases, its gumminess also reduces. The gumminess of SC cheese was significantly lower than DA cheese, because of probably the higher proteolysis.

Sensory Evaluation

Cheese body is gradually softened during proteolysis and produced water-soluble nitrogenous compounds which have a significant effect on cheese flavor. Cheese flavor, which numerous amounts of volatile and nonvolatile compounds contribute it, is very complex and result of lipolysis, proteolysis and glycolysis (Albenzio *et al.,* 2013).

Effect of storage times on color and appearance of the cheese samples are shown in Fig. 1A. Results revealed that SC sample had higher color and appearance scores than CM and DA samples at fresh state. Color and appearance of SC sample was intensely reduced over times of storage, because of probably its higher microbial activity due to starter cultures (Sameen *et al.,* 2008).

Proteolytic and peptidolytic activities in mozzarella cheese are due to residual coagulant, plasmin, or starter culture enzymes (Yazici *et al.,* 2010; Oommen *et al.,* 2002). As shown in Fig. 1B., SC and CM samples had higher odor scores than DA sample at 0 and 15 days of storage, probably due to their starter cultures activity which produces higher aromatic compounds.

But odor scores of all samples reduced over times, because of extreme and uncontrolled metabolic processes. Starter cultures have

direct and indirect contribution to sensory properties such as aroma, taste, texture, and flavor in cheese (Losito *et al.,* 2014). In terms of texture, results revealed significant differences (P<0.05) between texture of mozzarella cheeses while storage time had not momentous effect on this respect (Fig. 1C). Among cheeses, SC sample at 15th day of storage had higher score than the other cheeses during storage period. As it shown in Fig. 1D., there were no differences between taste of various cheeses at different storage period. However, SC cheeses had slightly better taste than DA and CM cheeses during all the storage period. With respect to overall accetability, results showed that the various mozzarella cheese making procedures had significant (P<0.05) effect on overal acceptability while storage time had no effect (Fig. 2). In general, SC cheese had higher overall acceptability than other cheeses at the beginning till the end of storage period.

Table 4. Texture profile analysis and meltability of various mozzarella cheeses.

Texture parameters	SC	DA	CM
Hardness (N)	3.83±0.44^{b}	5.25±0.24^{a}	4.25±0.04^{b}
Cohesiveness	0.58±0.04^{b}	0.64±0.02^{a}	0.55±0.00^{b}
Adhesiveness (J)	2.89±0.40^{a}	1.14±0.09^{c}	2.59±0.21^{b}
Springiness (mm)	6.48±0.61^{ns}	7.07±0.38	6.92±0.20
Gumminess (N)	2.22±0.19^{b}	2.88±0.15^{a}	2.74±0.11^{a}
Chewiness (J)	14.35±0.27^{b}	20.41±0.69^{a}	18.96±1.45^{a}
Meltability(mm)	75.50±2.08^{a}	64.75±2.71^{b}	67.25±3.02^{b}

Means within the same row with different superscripts differ significantly (P<0.05); SC, Starter Culture; DA, Direct Acidification; and CM, their Combination Method.

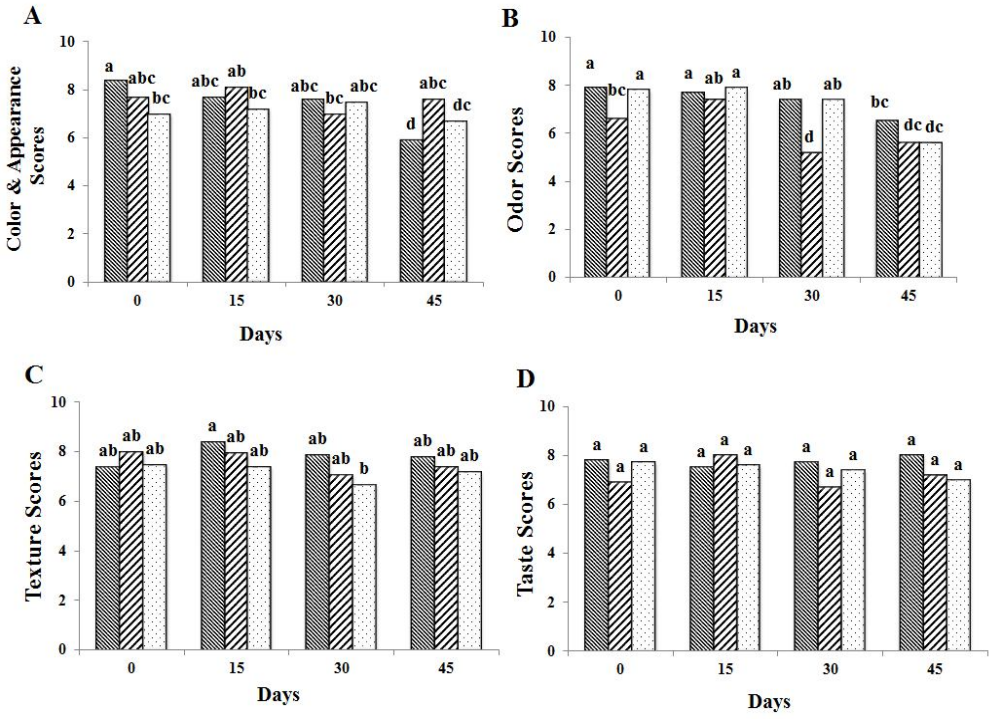

Fig. 1 Effect of different cheese making procedures and storage times on color and appearance (A), odor (B), texture (C) and taste (D) of mozzarella cheeses (SC ; DA ; CM).

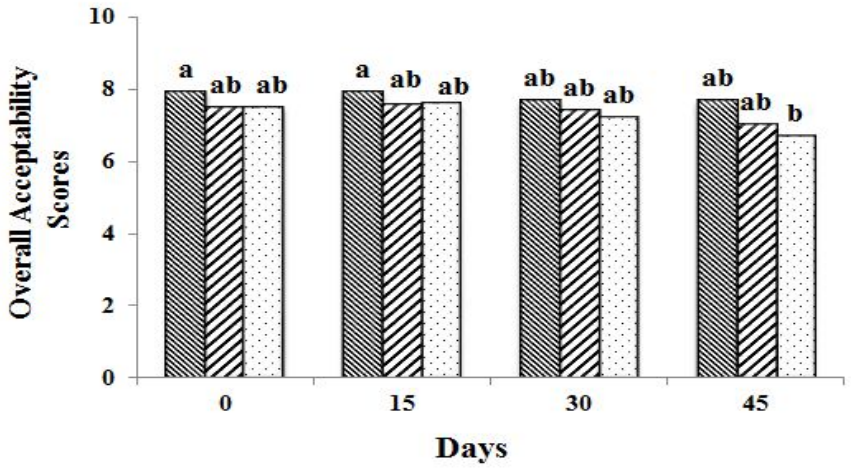

Fig. 2 Effect of different cheese making procedures and atorage times on overall acceptability of mozzarella cheeses (SC ▨ ; DA ▨ ; CM ⬚)

Mostly, sensory attributes (odor, taste, texture, color, and overall acceptability) of all mozzarella cheeses were higher at the beginning and 15th day of storage period. This is due to the fact that mozzarella cheese unlike many other cheeses is a un-ripened or non-aged cheese (Moneim and Sulieman, 2013; Yazici *et al.,* 2010) and therefore its sensory properties diminish over the storage period. However, fresh mozzarella cheese has an inappropriate meltability and is better to consume it after 1-3 weeks of storage (Rowney *et al.,* 1999). Whey protein films are excellent moisture, oxygen, aroma and oil barriers and may be used as a layer of protection to mozzarella cheese to protect its sensory characteristics during storage period (Jooyandeh, 2011).

Conclusions

The three different methods were used for mozzarella manufacturing and the starter culture method increased the fat, moisture, ash and titratable acidity of the cheese. The cheese making methods also affect the yield, fat and protein recoveries and SC method showed the higher actual yield and fat recovery. Color evaluation of cheese samples revealed that SC cheese had higher b- value than DA cheese probably due to its higher fat content. The texture of cheese samples was also influenced by cheese making methods. DA cheese had higher cohesiveness, hardness and springiness and lower meltability than other cheeses.

All mozzarella cheeses had almost higher sensory scores at 15th day of storage period. However, due to water movement from surface of all mozzarella cheeses during storage, the cheese color and appearance was diminished. Therefore, cheese coating with whey protein films could be suggested as a best solution, since these films are only moderate barriers to moisture.

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References

Abd El-Gawad, M. A. M., Ahmed, N. S., El-Abd, M. M. and Abd El-Rafee, S. 2012. Effect of homogenization on the properties and microstructure of Mozzarella cheese from buffalo milk. *Acta Sci. Pol. Technol. Aliment*., 11(2), 121–135.

Albenzio, M., Santillo, A., Caroprese, M., Braghieri, A., Sevi, A. and Napolitano, F. 2013. Composition and sensory profiling of probiotic Scamorza ewe milk cheese. *J. Dairy Sci.*, 96(5), 2792–2800.

AOAC, 2000. Official Methods of Analysis, 17th Ed., *Association of Official Analytical Chemists*, Gaithersburg, MD.

Dave, R. I., McMahon, D. J., Oberg, C. J. and Broadbent, J. R. 2003. Influence of coagulant level on proteolysis and functionality of mozzarella cheeses made using direct acidification. *J. Dairy Sci.*, 86(1), 114–126.

El-Gawad, M. A. A. and Ahmed, N. S. 2011. Cheese yield as affected by some parameters. *Acta Sci. Pol. Technol. Aliment.*, 10, 131–153.

Ernstrom, C. A. 1965. Mechanized pizza cheese making by means of a continuous, direct acidification method. *Mfd. Milk Prod. J.*, 57(7), 7.

Feeney, E. P., Guinee, T. P. and Fox, P. F. 2002. Effect of pH and calcium concentration on proteolysis in Mozzarella cheese. *J. Dairy Sci.*, 85(7), 1646–1654.

Fife, R. L., McMahon, D. J. and Oberg, C. J. 1996. Functionality of low fat Mozzarella cheese. *J. Dairy Sci.*, 79(11), 1903–1910.

Fox, P. F., Guinee, T. P., Cogan, T. M. and McSweeney, P. L. H. 2000. Fundamentals of cheese science. Aspen publishers Inc. Gaithersburg, Maryland, USA.

Guinee, T. P., Auty, M. A. and Fenelon, M. A. 2000. The effect of fat content on the rheology, microstructure and heat-induced functional characteristics of Cheddar cheese. *Int. Dairy J.*, 10(4), 277–288.

Guinee, T. P., Feeney, E. P., Auty, M. A. E. and Fox, P. F. 2002. Effect of pH and calcium concentration on some textural and functional properties of Mozzarella cheese. *J. Dairy Sci.*, 85(7), 1655–1669.

Jana, A. H. and Mandal, P. K. 2011. Manufacturing and quality of Mozzarella cheese: A review. *Int. J. Dairy Sci.*, 6(4), 199–226.

Jooyandeh, H. 2009. Effect of fermented whey protein concentrate on texture of Iranian white cheese. *J. Texture Stud.*, 40(5), 497–510.

Jooyandeh, H. 2011. Whey Protein Films and Coatings: A Review. *Pakistan J. Nut.*. 10 (3), 296-301.

Jooyandeh, H. and Minhas, K. S. 2009. Effect of addition of fermented whey protein concentrate on cheese yield and fat and protein recoveries of Feta cheese. *J. Food Sci. Technol.*, 46(3), 221–224.

Katsiari, M. C., Voutsinas, L. P., Kondyli, E. and Alichanidis, E. 2002. Flavour enhancement of low-fat Feta-type cheese using a commercial adjunct culture. *Food Chem.*, 79(2), 193–198.

Khosrowshahi, A., Madadlou, A., zadeh Mousavi, M. E. and Emam-Djomeh, Z. 2006. Monitoring the chemical and textural changes during ripening of Iranian White cheese made with different concentrations of starter. *J. Dairy Sci.*, 89(9), 3318–3325.

Kiely, L. J., Kindstedt, P. S., Hendricks, G. M., Levis, J. E., Yun, J. J., Barbano, D. M., ... and Rousseau, M. 1992. Effect of draw pH on the development of curd structure during the manufacture of Mozzarella cheese. Discussion. *Food Struct.*, 11(3), 217–224.

Kindstedt, P. S., Zielinski, A., Almena-Aliste, M. and Ge, C. 2001. A post-manufacture method to evaluate the effect of pH on Mozzarella cheese characteristics. *Aust. J. Dairy Technol.*, 56(3), 202.

Lawless, H.T. and Hymann, H. 1998. Sensory Evaluation of Food-Principles and Practices. Chapmann & Hall, New York.

Lawrence, R. C. 1993. Relationship between milk protein genotypes and cheese yield capacity. In: Factors affecting the yield of cheese. edn. D.B. Emmons. Int. Dairy Federation. Brussels. 121–127.

Losito, F., Arienzo, A., Bottini, G., Priolisi, F. R., Mari, A. and Antonini, G. 2014. Microbiological safety and quality of Mozzarella cheese assessed by the microbiological survey method. *J. Dairy Sci.*, 97(1), 46–55.

Ma, X., James, B., Zhang, L. and Emanuelsson-Patterson, E. 2011. Correlating Mozzarella cheese properties to production processes by rheological, mechanical and microstructure study: meltability study and activation energy. *Procedia Food Sci.*, 1, 536–544.

Ma, X., James, B., Zhang, L. and Emanuelsson-Patterson, E. A. 2013. Correlating mozzarella cheese properties to its production processes and microstructure quantification. *J. Food Eng.*, 115(2), 154–163.

Madadlou, A., Khosroshahi, A. and Mousavi, M. E. 2005. Rheology, microstructure, and functionality of low-fat Iranian white cheese made with different concentrations of rennet. *J. Dairy Sci.*, 88(9), 3052–3062.

Marshall, R. T. 1993. Standard methods for the examination of dairy products. 16th ed. American Public Health Association. Washington, DC.

McMahon, D. J., Fife, R. L. and Oberg, C. J. 1999. Water partitioning in Mozzarella cheese and its relationship to cheese meltability. *J. Dairy Sci.*, 82(7), 1361–1369.

McMahon, D. J., Paulson, B. and Oberg, C. J. 2005. Influence of calcium, pH, and moisture on protein matrix structure and functionality in direct-acidified nonfat Mozzarella cheese. *J. Dairy Sci.*, 88(11), 3754–3763.

Metzger, L. E., Barbano, D. M., Rudan, M. A. and Kindstedt, P. S. 2000. Effect of milk preacidification on low fat Mozzarella cheese. I. Composition and yield. *J. Dairy Sci.*, 83(4), 648–658.

Moneim, A. and Sulieman, E. 2013. Microbiological and sensory quality of Mozzarella cheese as affected by type of milk and storage. *J. Food Nutr. Disor.*, 2, 1.

Oommen, B. S., McMahon, D. J., Oberg, C. J., Broadbent, J. R. and Strickland, M. 2002. Proteolytic specificity of *Lactobacillus delbrueckii subsp. bulgaricus* influences functional properties of Mozzarella cheese. *J. Dairy Sci.*, 85(11), 2750–2758.

Robinson, R. K. and Tamime, A. Y. 1996. Feta and Related Cheeses. CRC Press.

Rowney, M. K., Roupas, P., Hickey, M. W. and Everett, D. W. 1999. Factors affecting the functionality of Mozzarella cheese. *Aust. J. Dairy Technol.*, 54(2), 94.

Rudan, M. A., Barbano, D. M., Guo, M. R. and Kindstedt, P. S. 1998. Effect of the modification of fat particle size by homogenization on composition, proteolysis, functionality, and appearance of reduced fat Mozzarella cheese. *J. Dairy Sci.*, 81(8), 2065–2076.

Rudan, M. A., Barbano, D. M., Yun, J. J. and Kindstedt, P. S. 1999. Effect of fat reduction on chemical composition, proteolysis, functionality, and yield of Mozzarella cheese. *J. Dairy Sci.*, 82(4), 661–672.

Sameen, A., Anjum, F. M., Huma, N. and Nawaz, H. 2008. Quality evaluation of Mozzarella cheese from different milk sources. *Pak. J. Nutr.*, 7(6), 753–756.

Sameen, A., Anjum, F. M., Huma, N. and Nawaz, H. 2010. Chemical composition and sensory evaluation of mozzarella cheese: influence by milk sources, fat levels, starter cultures and ripening period. *Pak. J. Agri. Sci.*, 47(1), 26–31.

Sheehan, J. J. and Guinee, T. P. 2004. Effect of pH and calcium level on the biochemical, textural and functional properties of reduced-fat Mozzarella cheese. *Int. Dairy J.*, 14(2), 161–172.

Sheehan, J. J., Huppertz, T., Hayes, M. G., Kelly, A. L., Beresford, T. P. and Guinee, T. P. 2005. High pressure treatment of reduced-fat Mozzarella cheese: Effects on functional and rheological properties. *Innov. Food Sci. Emerg. Technol.*, 6(1), 73–81.

Tunick, M. H. 2000. Rheology of dairy foods that gel, stretch, and fracture. *J. Dairy Sci.*, 83(8), 1892–1898.

Tunick, M. H., Malin, E. L., Smith, P. W. and Holsinger, V. H. 1995. Effects of skim milk homogenization on proteolysis and rheology of Mozzarella cheese. *Int. Dairy J.*, 5(5), 483–491.

Yazici, F., Dervisoglu, M., Akgun, A. and Aydemir, O. 2010. Effect of whey pH at drainage on physicochemical, biochemical, microbiological, and sensory properties of Mozzarella cheese made from buffalo milk during refrigerated storage. *J. Dairy Sci.*, 93(11), 5010–5019.

Zisu, B. and Shah, N. P. 2005. Low-fat mozzarella as influenced by microbial exopolysaccharides, preacidification, and whey protein concentrate. *J. Dairy Sci.*, 88(6), 1973–1985.

Zisu, B. and Shah, N. P. 2006. Role of microbial exopolysaccharides on moisture retention, texture and functionality of low-fat mozzarella cheeses. *Aust. J. Dairy Technol.*, 61(3), 253.

Zisu, B. and Shah, N. P. 2007. Texture characteristics and pizza bake properties of low-fat Mozzarella cheese as influenced by pre-acidification with citric acid and use of encapsulated and ropy exopolysaccharide producing cultures. *Int. Dairy J.*, 17(8), 985–997.

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تأثیر روش‌های مختلف تولید بر میزان راندمان و ویژگی‌های فیزیکوشیمیایی و حسی پنیر موزارلا

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چکیده

تأثیر روش‌های مختلف تولید پنیر موزارلا، یعنی روش اسیدی کردن مستقیم (DA)، استفاده از کشت آغازگر (SC) و تلفیقی از این دو روش (CM) بر میزان راندمان پنیرسازی و ویژگی‌های فیزیکوشیمیایی، بافت، رنگ و حسی محصول بررسی گردید. آنالیز شیمیایی نمونه‌های پنیر نشان داد که پنیر SC دارای مقادیر چربی، رطوبت، خاکستر، اسیدیته قابل تیتر، راندمان واقعی و تعدیل‌شده و همچنین بازیافت چربی بالاتری نسبت به پنیر DA می‌باشد. پنیر DA مقدار کشش‌پذیری، پیوستگی و سفتی بالاتری نسبت به نمونه‌های CM و SC به دلیل شبکه پروتئینی متراکم و الاستیک تر نشان داد، درحالی‌که قابلیت ذوب شدن و چسبندگی نمونه DA کمتر از نمونه‌های CM و SC بود. پنیر SC به طور معنی‌داری مقدار b-value بالاتری نسبت به نمونه DA داشت. ارزیابی حسی نمونه‌ها نشان داد که پنیر SC دارای کیفیت حسی بالاتری نسبت به سایر نمونه‌های پنیر در حالت تازه و طی ۴۵ روز نگهداری بود. به‌طورکلی، امتیازات حسی تمام پنیرهای موزارلای تولیدی تا ۱۵ روز اول نگهداری قابل قبول بود و سپس تا پایان دوره نگهداری به تدریج کاهش یافت.

واژه‌های کلیدی: روش‌های مختلف تولید، پنیر موزارلا، فیزیکوشیمیایی، خواص بافت، ویژگی‌های حسی

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Brief report

Antimicrobial Property of Lycopene Oleoresin on some Food Pathogens
Running Head: Lycopene oleoresin antibacterial potent

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Abstract

The aim of this work was to study the antimicrobial activity of tomato skin lycopene oleoresin against Pseudomonas aeruginosa ،*Escherichia coli* ،*Staphylococcus ureuse* ،*Salmonella typh* ، *L. monocytogenes* ، *Bacillus cereus* ،*Bacillus licheniformis*. Oleoresin was extracted from tomato peel. Lycopene content was measured by spectrophotometer. Lycopene oleoresin contained 2321mg lycopene/ 100 g oleoresin diluted in serial micro-dilution technique from 40,000 to 78.125 ppm. Microbial culturing was done in ELISA 96-well micro-titer plates in triplet and then MIC and MBC were determined. The results were shown that tomato peel oleoresin contained 2% lycopene, can inhibit and restrain the gram positive and negative bacteria.

Keywords: lycopene oleoresin, tomato skin, antimicrobial activity, ELISA, dilution method

Introduction

The medical properties of herbal extracts and essential oils against microbial [1, 2] and non-microbial [3, 4] diseases are known since ancient times .Many studies on different species of plant extracts or essential oils and their effects on microorganisms has been done. Antimicrobial properties of different plant extracts are reported [5, 6]. Antimicrobial effects of essential oils are reported on bacteria and yeasts more [5, 6] but fungi are also influenced by antimicrobial properties of essential oils [7, 8]. According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants [9].

Lycopene is dominant colorant in tomato, watermelon, papaya, and red pepper. The most important sources of lycopene is tomatoes. Depending on varieties they represent 65- 98% of total weight of carotenoids in tomato [10 and 16]. This pigment is a carotenoid with 11 conjugated double bands which besides the coloring ability, have antioxidant property and can inhibit the incidence of lung, prostate and stomach cancer. Today there are more trends in use of this natural and healthy component in human diet. Lycopene due to its antioxidant activity can play antimicrobial role. Lycopene is one of the popular pigments highly accepted by food industry as a food additive and also for its health benefits [17, 18]. As a red colorant and antioxidant agent, the demand for lycopene is still increasing. According to [17], total world consumption of lycopene was tripled to 15,000 tonnes in 2004 compared to 5000 tons in 1995.

Chandra *et al.* (2008) tested the effect of lycopene treatment in a randomized, double-blind study involving ten patients with clinical signs of gum inflammation. Half of the mount of each patient was also treated with oral prophylaxis. They found that lycopene treatment significantly decreased gum inflammation. The bleeding index of the gums treated with lycopene was further reduced by

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the oral prophylaxis. They also found that gum inflammation was strong negative correlated with uric acid level of the saliva [19]. Sung *et al.* (2007) also found that lycopene exerted potent antifungal activity on *Candida albicans* by causing significant damage to the cell membranes of the yeast. It showed little toxicity against human erythrocytes. More studies are required to demonstrate such effect in clinical experiment [20]. Al-Oqaili *et al.* (2014) studied the antibacterial activity of aqueous tomato extract on medical important pathogens. They found that the extract has inhibiting the growth of some isolated bacteria [21]. The objective of this study was analyzing the antimicrobial activity of tomato lycopene on Gram-positive and Gram-negative food-borne bacteria.

Materials and methods

Lycopene oleoresin preparation

At first ripened tomatoes immersed to boiling water for 1-2 minute and immediately cooled. Then peeled by hand. Skins were dried at 40^0C by convective oven. Extraction were carried out by hexane:ethanol:aceton (2:1:1) with 0.05% BHT as antioxidant by ratio of 1:10 (sample: solvent) by Sadler(1990) and Shi(1999) method at 30^0C with gently stirring [14, 15]. After 16 hr, the extracted solution was filtered by Watt man number 4 saturated with ethanol. 20% deionized water were added to filtrate and retained for 15 minute to divide into two phase. The upper phase is polar and contains lycopene and the under phase is aqueous and rejected. The upper phase is used for lycopene derivation [14, 15].

Lycopene quantity measurement

Spectrophotometer method was used to lycopene measurement [16, 22]. Lycopene absorption in hexane have three peak at 445 nm, 472 nm, and 503 nm which for reducing the intervention of other carotenoids and measuring total all-trans lycopene, quantification was carried out at 503 nm (λ_{Max}) in hexane (PG Instruments Ltd, UV/VIS T80) [16, 23]. The lycopene content in oleoresin, was calculated as below [23].

$$\text{Lycopene (mg)} = A \times dil \times ml \times 10 / E_{1cm}^{1\%} \quad (1)$$

wherein A is absorption in 1cm quvett; dil, dilution factor; ml, total volume of sample; and $E_{1cm}^{1\%}$, special extinction coefficient for lycopene in hexane (equals to 3450) [23].

Evaluation of antimicrobial activity

Antimicrobial activity of lycopene oleoresin was identified by determination of Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) tests. Seven important pathogenic bacteria includes *Pseudomonas aeruginosa (*ISIRI 275*) ،Escherichia coli(PTCC 1533) ، Staphylococcus ureuse (*PTCC 1112*) ، Salmonella typhi (PTCC 1609) ، L. monocytogenes (PTCC 1163) ، Bacillus cereus* (ATCC 11778*) ،Bacillus licheniformis (*PTCC 1525*)* were analyzed triplicate by micro-dilution technique in ELISA 96-well micro-titer plate. With this method we determined MIC and then MBC of lycopene oleoresin. Microbial species were cultured on Müeller-Hinton Agar and incubated at 37^0C for 24 hr (Figure 1).

Fig.1. Bacteria cultured on Müeller-Hinton Agar

Microbial population equal to 10^6 CFU/ml from each bacterium was prepared at log-phase growth by adjusting the turbidity of population according to 0.5 McFarland standard. Bacterial inoculum was prepared by suspension of freshly grown bacteria in sterile saline (0.85% NaCl w/v). Double serial dilutions of lycopene oleoresin was prepared by DMSO with concentrations between 40,000 to 78.125 ppm. Medium without lycopene,

medium contained DMSO and different lycopene concentrations without addition of bacteria were used as control for each of mentioned components, respectively. After preparation of micro-titer plates, they were incubated at 37^0C for 18-24 hr (Figure 2)

Fig. 2. ELISA 96-well micro-plate contained different concentrations of lycopene oleoresin

The turbidity were measured by ELISA READER (Bio-Tek Instruments) at 580 nm as a result of microbial growth and thus MIC were determined. MIC of lycopene oleoresin was determined by concentration with no visible bacterial growth. To detection of MBC, from wells without bacterial growth, point culturing was done at Nutrient Agar. After 24 hr incubation at 37^0C, the points without bacterial growth were reported as MBC.

Results and discussion

Oleoresin derived from tomato skin were extracted by solvents and its antimicrobial effect on *Pseudomonas aeruginosa* ، *Escherichia coli* ،*Staphylococcus ureuse* ، *Salmonella typhi* ، *L. monocytogenes* ، *Bacillus cereus* ،*Bacillus licheniformis* is shown in table 1. The results showed that lycopene oleoresin can inhibit and prevent the growth of studied bacteria. To prevent and inhibit the growth of Gram-positive bacteria, higher concentrations of lycopene oleoresin is needed with this evidence we can claim that rather than coloring and antioxidant ability of tomato lycopene oleoresin, it has very important property of antimicrobial activity on important food-borne bacteria. This can be a very important property for this component which increases its importance to use and application in food industry or pharmacy.

Table1- Antimicrobial effect of lycopene extracted from tomato peel on food-borne microorganisms

Bacteria	MIC (ppm)	MBC (ppm)
Escherichia coli (PTCC 1533)	2500	5000
L. monocytogenes (PTCC 1163)	20000	40000
Bacillus cereus (ATCC 11778)	1250	2500
Bacillus licheniformis (PTCC 1525)	2500	5000
Pseudomonas aeruginosa (ISIRI 275)	156.25	156.25
Salmonella typhi (PTCC 1609)	1250	2500

Al-Oqaili *et al.* (2014) showed that the antimicrobial activity of tomato extract is due to presence of active components of tomato in extract on some bacteria [21]. Dahan *et al.*2008 and Omodamiro *at al.* 2013 showed the effectiveness factor in tomato extract on bacteria, is lycopene that presence antimicrobial and antifungal activity [24, 25]. Lycopene oleoresin from tomato peel can exhibit the growth of *Pseudomonas aeruginosa* more than other bacteria. Both MIC and C are very low for this bactria. *But L. monocytogenes* was more sensible than others. The MIC of *L. monocytogenes* was 20000 ppm and the MBC was 40000 ppm. These amounts of MIC and MBC are high compared to other extracts. Mokami *et al.* (2014) showed the MIC and MBC of oils extracted from aerial parts of two medicinal plants, *Mentha longifolia* L. and *Rosmarinus officinalis* are lower than lycopene oleoresin. They found MIC and MBC of *Mentha longifolia* L. are 5000 and 5000 for *L. monocytogenes* and 10000 and 10000 for *Rosmarinus officials*, respectively [26]. They suggested this antibacterial power is due to oxygenated monoterpenes found in extracts.

Conclusions

This study revealed other than coloring and antioxidant activity, lycopene oleoresin can inhibit and prevent the growth of these very important studied bacteria in food. Today the demand for nature coloring agents is growing in developed countries, the lycopene extracted from tomato peel of food industries by-product, can play brilliant role in coloring,

antioxidant, and antibacterial activity. Thus we can look to lycopene rather than a coloring and antioxidant agent. Then we can recommit its use in diet as fresh using of its sources or its processed products and see its positive effects

References

Ciani, M., Menghini, L., Mariani, F., Pagiotti, R., Menghini, A. and Fatichenti, F., 2000, Antimicrobial properties of essential oil of Satureja montana L. on pathogenic and spoilage yeasts. *Biotechnology Lettters*, 22(12): 1007-1010.

Arrieta, J., Reyes, B., Calzada, F., Cedillo-Rivera, R.and Navarrete, A., 2001, Amoebicidal and giardicidal compounds from the leaves of Zanthoxylum liebmannianum. *Fitoterapia*, 72(3): 295-297.

Vikrant, V., Grover, J.K., Tandon, N., Rathi, S.S. and Gupta, N., 2001, Treatment with extracts of Momordica charantia and Eugenia jambolana prevents hyperglycemia and hyperinsulinemia in fructose fed rats. *Journal of Ethnology*, 76(2): 139-143.

Ngo, B.E., Schmutz, M., Meyer, C., Rakotonirina, A., Bopelet, M., Portet, C., Jeker, A., Rakotonirina, S.V., Olpe, H.R., and Herrling, P., 2001, Anticonvulsant properties of the methanolic extract of Cyperus articulatus (Cyperaceae). *Journal of Ethnology*, 76(2): 145-150.

Khan, M.R., Kihara, M., and Omoloso, A.D., 2001a, Antimicrobial activity of Harpulliua ramiflora. *Fitoterapia*, 72(3): 298-300.

Khan, M.R., Kihara, M., and Omoloso, A.D., 2001b, Antimicrobial activity of Picrasma javanica. *Fitoterapia*, 72(4): 406-408.

Bishop, C.D. and Thornton, I.B., 1997. Evaluation of the antifungal activity of the essential oils of Monarda citriodora var. citriodora and Melaleuca alternifolia on post harvest pathogens. *Journal of Essential Oil Research*, 9: 77-82.

Pandey, M.C., Sharma, J.R. and Dikshit, A., 1996, Antifungal evaluation of the essential oil of Cymbopogon pendulus (Nees ex St. eud) wats. CV. Prama. Flav. *Frag.* J, 11: 257-260.

Selvamohan, T. , Ramadas ,V.S. and Kishore ,S.S., 2012, Antimicrobial activity of selected medicinal plants against some selected human pathogenic bacteria .*Journal of Advances in Applied Science Research*. 3 (5):3374-3381.

Jay, James Monroe. *Modern Food Microbiology*.1998. Vol2.

Soliman, K. M., & Badeaa, R. I., 2002, Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. *Food and Chemical Toxicology*, 40, 1669–1675.

Vattem, D. A., Lin, Y. T., Labbe, R. G., & Shetty, K., 2004, Phenolic antioxidant mobilization in cranberry pomace by solid-state bioprocessing using food grade fungus Lentinus edodes and effect on antimicrobial activity against select food-borne pathogens. *Innovative Food Science and Emerging Technologies*, 5, 81–91.

Shi, J., 2001, Separation of carotenoids from fruits and vegetables. WO/2001/079355

Sadler G., and Davis, J., and Dezman, D., 1990, Rapid Extraction of Lycopene and β-Carotene from Reconstituted Tomato Paste and Pink Grapefruit Homogenates. *Journal of Food Science*. 55(5): 1460-146

Shi , J. , Maguer , M.L. , Kakuda , Y. , Liptay , A. , Niekamp , F., 1999, Lycopene degradation and isomerization in tomato dehydration . *Food Research International*, 32 , 15-21.

Lavecchia, R., and Zuorro, A., 2008, Improved lycopene extraction from tomato peels using cell-wall degrading enzymes. *European Food Research and Technology*. 228(1): 153-158.

Focus on Pigments., 2007, World spends more than $50 M on lycopene red. Focus Pigmentation, 4, 3–4.

Rao, A.V.; Argawal, S., 1999, Role of lycopene as antioxidant carotenoid in the prevention of hronic diseases: A review. *Nutrient Research*. 19, 305–323.

Chandra RV, Prabhuji ML, Roopa DA, Ravirajan S, Kishore HC., 2008, Efficacy of lycopene in the treatment of gingivitis: a randomised, placebo-controlled clinical trial. *Journal of Nutrient*. 138(1):49-53.

Sung WS, Lee IS, Lee DG., 2007, Damage to the cytoplasmic membrane and cell death caused by lycopene in Candida albicans. *Journal of Microbiol Biotechnol*. 17(11):1797-804.

Al-Oqaili. R. M. s. Mohammedi stabregh .B. B. Salman M. A. Al-satar asad D. A., 2014, In Vitro Antimicrobial activity of Solanum Lycopersicum Extract against some Pathogenic Bacteria. *Food Science and Quality Management*. 27: 12-17.

R. Choudhary, T.J. Bowser, P.Weckler, N.O. Manessb,W. McGlynn., 2008, Rapid estimation of lycopene

concentration in watermelon and tomato puree by fiber optic visible reflectance spectroscopy. *Postharvest Biology and Technology*. 7p.

Lu,C,H., J. Engelmann,N., Ann Lila,M., W. Erdman Jr,J., 2009, Optimization of Lycopene Extraction from Tomato Cell Suspension Culture by Response Surface Methodology. *Journal of Agriculture and Food Chemistry*. 56(17): 7710–7714.

Dahan K, Fennal M, Kumar NB., 2008, Lycopene in the prevention of prostate cancer. *Journal of Soc Integra Oncol*. 6:29-36.

Omodamiro O. D. and Amechi U., 2013, The phytochemical content, antioxidant, antimicrobial and anti-inflammatory activities of Lycopersicon esculentum (Tomato*). Asian Journal of Plant Science and Research*. 3(5):70-81.

Mohkami1, Z., Ranjbar, A., Bidarnamani, F., 2014, Essential Oil Compositions and Antibacterial Properties of Mint (Mentha longifolia L.) and Rosemary (Rosmarinus officinalis). *Annual Research & Review in Biology*. 4(17): 2675-2683.

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مقاله کوتاه پژوهشی

ویژگی ضدمیکروبی اولئورزین لیکوپن روی برخی پاتوژن‌های غذایی

عنوان کوتاه: توانایی ضدمیکروبی اولئورزین لیکوپن

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چکیده

هدف از انجام این تحقیق، مطالعه فعالیت ضد میکروبی اولئورزین لیکوپن پوست گوجه فرنگی در برابر *Pseudomonas aeruginosa*، *Escherichia coli*، *Staphylococcus ureuse*، *Salmonella typhi*، *L. monocytogenes*، *Bacillus cereus*، *Bacillus licheniformis* بود. الئورزین از پوست گوجه فرنگی استخراج شد. مقدار لیکوپن با اسپکتروفتومتر اندازه گیری شد. اولئورزین حاوی ۲۳۲۱ mg/۱۰۰g لیکوپن، به روش رقیق سازی میکرو، از غلظت ۴۰۰۰۰ ppm تا ۷۸/۱۲۵ ppm رقیق شد. کشت میکروبی در پلیتهای میکروتیتر ۹۶ خانه‌ای ELISA در سه تکرار انجام شد و سپس MIC و MBC تعیین شدند. نتایج نشان داد که اولئورزین پوست گوجه فرنگی حاوی ۲٪ لیکوپن، می تواند از رشد باکتریهای گرم مثبت و گرم منفی مورد مطالعه جلوگیری کند.

واژه‌های کلیدی: اولئورزین لیکوپن، پوست گوجه فرنگی، فعالیت ضد میکروبی، ELISA، روش رقیق سازی

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Brief report

Study of frequency of *eaeA*, *stx1* and *stx2* genes in *Escherichia coli* isolated from local cheeses in Maragheh city by multiplex PCR

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Abstract

Pathogens can be transmitted to the humans through the consumption of contaminated local dairy products such as cheese and, thus, cause pathogenic diseases. Shiga toxin produced by *Escherichia coli* can cause mild watery diarrhea as well as serious complications such as hemorrhagic colitis, and hemolytic uremic syndrome and may even lead to death. The present study was conducted to investigate the frequency of *eaeA*, *stx1* and *stx2* genes in *Escherichia coli* isolated from local cheese in Maragheh city through multiplex PCR. Thirty two *Escherichia coli* isolates from local cheese in Maragheh city were studied with regard to the frequency of *stx1*, *stx2* and *eaeA* genes through multiplex PCR. The frequency of *eaeA* gene in *Escherichia coli* isolates was 15.62% (5:32). *Stx1* and *stx2* genes were not found in any isolate. It was concluded that shiga toxin produced by *E.coli* exists in local cheeses and can pose risks to the human health in this region.

Keywords: *Escherichia coli*, Cheese, Multiplex PCR , Virulence genes

Introduction

Escherichia coli as an indicatoris used for determining the fecal contamination of water and food and presence of intestinal pathogens. This bacterium has different strains that its pathogen types are involved indifferent diseases in human and animals and play important role in public health. Shiga toxin producing *Escherichia coli* (STEC)serotypes arean important group of zoonotic and food borne pathogens that causes different diseases such as hemorrhagic diarrhea, hemorrhagic colitis, hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura(TTP) (Robinson &Batt Carl, 2000). STEC family is serologically diverse and more than 200 serotypes have been reported; out of which more than 100 are linked to human infections (Rammurthy, 2008). STEC contains different toxins the production of which is controlled by specific genes. *Stx1*,*stx2* and *eaeA* genes are the most important virulence factors in STEC. Shiga toxin 1 is 98% homologous to the stx produced by *Shigella dysenteriae* type 1, while *stx2* is about 60% homologous with *stx1* and is different from antigenic point of view (Tahamtan *et al*., 2010). The types of producing *stx1* and *stx2* toxins of *Escherichia coli* cause the mentioned syndromes. Shiga toxin genes in *Escherichia coli* generally are in specific phages. Various types of these toxins have been known such as: stx, *stx1*, *stx1*c, *stx2*, *stx2*c, *stx2*d, *stx2*e,*stx2*f (Friedrich *et al*., 2003). Intimin, a protein which is responsible for attachment of bacterium to intestine,is causing specific lesions calling attaching-effacing lesions in intestine epithelial cells. For this reason, the coding gene of this protein called eae (*E.coli* attaching and effacing) (Wales *et al*., 2005).Researches indicated that Shiga toxin-producing *Escherichia coli* strains are in ruminants that 40% of them are pathogen for human. This fact considers the ruminants as the important reservoir for these strains (Montenegro *et al*., 1990). Animal products such as unpasteurized dairy products such as domestic cheese are one of the most important transmission ways of STEC to human (Feng*et al*., 2011). According to

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different studies, 1-5% of food borne intoxications are associated with consumption milk and dairy products that 53% of food borne infections are due to consumption of contaminated cheese (Schrade&Yager, 2001). Since the Multiplex PCR has suitable sensitivity and specificity and it has high capability of identification of shiga toxin producing *E.coli* strains and possibility of direct application on clinical and food samples, it considers this method can be used as a common method in laboratories for the detection of suspicious samples.The aim of this study is to determine offrequency of *eaeA*, *stx1* and *stx2* genes in *Escherichia coli* isolated from domestic cheeses in Maragheh city by multiplex PCR

Materials and methods

Samples

Thirty two strains of isolated *E.coli* from local cheese in villages of Maragheh city were examined for occurrence of *stx1*, *stx2* and *eaeA* genes.

DNA extraction

DNA extraction was performed on 32 cultured isolates of*E.coli* in Brain Heart Infusion Agar (BHI) (Merck,Germany) medium. One ml of bacterial culture centrifuged in 5000g for 5 minutes and supernatant was poured off. Subsequent to addition of 1ml lysis buffer (Tris 1 M[pH=7.5], NaCl 5 M, EDTA 0.5 M and C-TAB 2%) on mixed pellet,it was put into 85ºc for 30 minutes(in water bath). In the next step, the supernatant was separated and 0.5 µl Rnase was added to it and ,later, it was kept at 37ºc for 30 minutes. Then an equal volume of isopropanol was added to the same and it was kept at -20ºc for 15 minutes and, later,it was centrifuged in 12000 g resulting in some DNA samples to be sedimented. Later, DNA samples were dried in lab temperature. Finally, dried DNA samples were dissolved in 50µl of double distilled water (Atashpaz *et al*., 2010).

Multiplex PCR

This reaction was performed with the help of selected primers of the bacteria under experimentation for 25µl reaction volume (Table 1).The mixed reaction contained master kit of PCR 12.5µl, specific primers (0.5M) and extracted DNA(1µl). Cycling condition for all were the following: 1 cycle at 95ºc for 4 min, 32 cycles at 95ºc for 1 min, 50ºc for 1 min and 72ºc for 1 min,with a final extension at 72ºc for 1 min. Electrophoresis of PCR product was conducted in 1.5% agarose gel. Indicator bacterial strain *E.coli* PTCC 1270 was used as positive control in PCR. Double distilled water was used as negative control.

Table1: Primers used in PCR for the detection of *stx1*,*stx2* and *eaeA* genes (Vidal *et al*., 2004).

Product size (bp)	Primer & Sequence	Gene
348bp	*Stx1* F; 5'CAG TTA ATG TGG TGG CGA AGG 3' *Stx1* R; 5' CAC CAG ACA ATG TAA CCG CTG 3'	*Stx1*
584bp	*Stx2* F; 5' ATC CTA TTC CCG GGA GTT TAC G 3' *Stx2* R; 5' GCG TCA TCG TAT ACA CAG GAG C 3'	*Stx2*
482bp	*eaeA* F; 5' TCA ATG CAG TTC CGT TAT CAG TT 3' *eaeA* R; 5' GTA AAG TCC GTT ACC CCA ACC TG 3'	*eaeA*

Results and Discussion

Among 32 isolated and biochemically characterized *E.coli*, none of the samples had *stx1* and *stx2* genes. Only standard bacterium (PTCC 1270) showed *stx1* gene. Five samples(15.62%) had only *eaeA* gene. None of the samples had 2 or 3 studied genes simultaneously. Standard bacterium (PTCC 1270) did not show both *stx2* and *eaeA* genes (Figure 1).

The present study, based on the literature, was the first study describing the detection and frequency of major virulence genes of STEC isolated from domestic cheese in Maragheh, Iran. *E.coli* is the normal gut flora of the human beings, but certain subsets of this

species have acquired virulence genes that enabled them to cause diarrhea and other extra-intestinal infections. Such is shiga toxigenic *E.coli* containing stx genes, which has direct enterotoxic properties resulting from selective targeting of Gb3 containing absorptive villus epithelial cells in the ileum (Feng*et al*., 2011). Among the *stx1* and *stx2*genes, *stx2* is considered to be the most important virulence factor associated with the human disease. It is about 400 fold more toxic to mice than *stx1* and also been shown to induce feto-placental re-absorption, intra-uterine haematoma, fibrin deposition and neutrophil infiltration (Islam *et al*., 2008). The spread of STEC infection among the humans could have been from contamination of food with water and sewage signifying poor level of hygiene maintained. One of the chief ways of transferring shiga toxin producing *E.coli* to human is food habits such as consuming half cooked or raw meats and unpasteurized dairy products that have close relationship with rural life (Mehrabiyan*et al*., 2013). Except of *E.coli O157*, other serogroups of STEC are the causative agent of 60% of shiga toxigenic *E.coli* that are widespread in many countries such as Argentina, Australia, Spain, Denmark, Chile and Germany (Johnson *et al*., 2006). The results of the presentstudy showed that the frequency of *eaeA* gene in *Escherichia coli* isolates were 15.62% (5/32). *Stx1* and *stx2* genes were not found in any isolate.Bonyadian*et al*., (2011) showed that among 14 *E.coli* strains isolated from unpasteurized cheese samples, none of them harbored*stx1*, *stx2* and *eaeA* genes. Also they showed that among 38 *E.coli* strains isolated from raw milk samples, none of them had *stx1* gene, but 3 isolates(7.89%) and 2 isolates(5.26%) had *stx2* and *eaeA* genes, respectively. In a study on shiga toxin producing *E.coli* strains isolated from milk tanks and new cheese samples in Spain, it was identified that only *E.coli* O157:H7 contained *eaeA*gene(Rey *et al*., 2006). In a study on 42 raw milk samples for occurrence of verotoxigenic *E.coli* in northern Ireland in 2003, only 4 cases carried both *stx2* and *eaeA* genes(McKee*et al*., 2003).

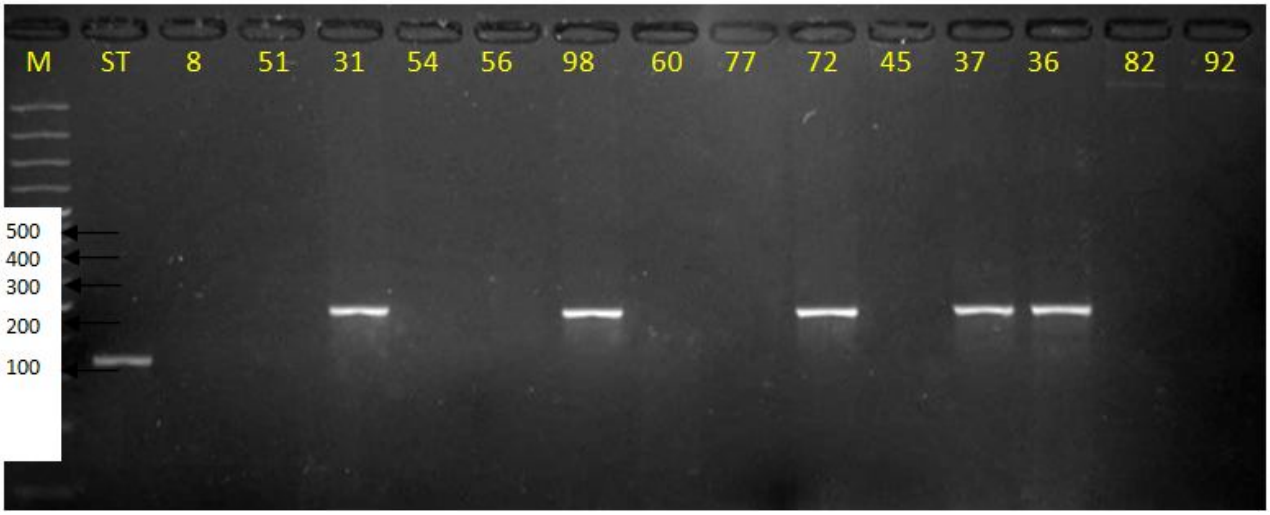

Fig 1: Lanes 31,98,72,37 and 36 are associated with *eaeA* gene(482bp). Lane ST indicates *E.coli* PTCC 1270 that bands within 348bp and is associated to *stx1* gene. The standard bacterium(ST) shows negative result for *eaeA* and *stx2* genes. Lane 8 is negative control (Double distilled water). Lane M is marker (100bp).

Mehrabiyan *et al*., (2013) reported that prevalence of *stx1*,*stx2* and *eaeA* genes in *Escherichia coli* isolated from sheep meat in Chaharmahal va Bakhtiari province were 11.1%,8.8% and 0, respectively. The reported investigations on contamination to virulence genes of *E.coli* in food in numerous areas of the world indicated the different rates (from low to up) of contamination to these genes. Shah Illi*et al*., (2010) reported that prevalence of *stx1* and *stx2* genes of shiga toxin-producing *Escherichia coli* from juice

purchase and vegetables in Shiraz city were 3.37% and 0, respectively. Moreover, 1.12% of isolated *E.coli* carried both *stx1* and *eaeA* genes. Simultaneous molecular investigations on environmental samples (such as water and soil), dairy products and ruminants fecal samples may indicate genetic association of strains and etiology of causative agents of disease. The results of present study, comparing to the previous studies, indicated the difference in dispersion of effective genes in *E.coli* virulence. This may be due to the geographical diversities and also difference in ecologic origin of isolated strains (Milk, human and different animals).

References

Atashpaz, S., Khani,S., Barzegari,A., Barar, J., Vahed,S.Z., Azarbaijani, R., and Omidi,Y, 2010, A robust universal method for extraction of genomic DNA from bacterial species. *Mikrobiologiia*, 79(4),562-566.

Bonyadian, M., Zahraei Salehi, T., Mahzounieh, M.R., and AkhavanTaheri, F, 2011, Virulence genes of verotoxigenic E.coli isolated from raw milk and unpasteurized cheese. *Journal of Veterinary Research*, 66(3),223-228(Persian).

Feng, P.C., Councell, T., Keys, C., and Monday, S.R, 2011, Virulence characterization of shiga-toxigenic Escherichia coli isolates from wholesale produce. *Applied and Environmental Microbiology*, 77(1),343-5.

Friedrich, A.W., Borell, J., Bielaszewska.M., Fruth. A., Tschape, H., and Karch, H, 2003, Shiga toxin 1c-producing Escherichia coli strains: phenotypic and genetic characterization and association with human disease. *Journal of Clinical Microbiology*, 41, 2448-2453.

Islam, M.A., Mondol, A.S., De Boer, E., Beumer, R.R., Zwietering, M.H., Talukdar, K.A., and Heuvelink, A.E, 2008, Prevalence and genetic characterization of shiga toxin producing Escherichia coli isolates from slaughtered animals in Bangladesh.*Applied and Environmental Microbiology*, 74,5414-5421.

Johnson, K.E., Thorpe, C.M., and Sears, C.L, 2006,The emerging clinical importance of non-O157 shiga toxin-producing Escherichia coli. *Clinical Infectious Diseases*, 43(12),1587-95.

McKee, R., Madden, R.H., and Gil Mou, A, 2003, Occurrence of verocytotoxin-producing Escherichia coli in dairy and meat processing environment. *Journal of Food Protection*, 66,1576-80.

Mehrabiyan, S., Tahmasby, H., Momtaz, H., Farahmandi, S., Monji, H., Farahmandi, K., andDavoudiJouneghani, Z, 2013, Multiplex PCR detection of Escherichia coli carrying shiga toxin genes in E.coli isolated from patients with diarrhea in Hajar hospital, Shahrekord, Iran. *Jentashapir Journal of Health Research* , 4(3),193-202(Persian).

Mehrabiyan, S., Tahmasby, H., Momtaz, H., Khosravi, N., Kaboli Boroujeni, H., Najafzadeh, V., TadiBeni, M., and Ansari, F, 2013, Multiplex PCR detection of *stx1stx2* and eaeA genes in Escherichia coli isolated from lambs in Chaharmahal va Bakhtiari, Iran. *Biological Journal of Microorganism*, 2(6),11-18(Persian).

Montenegro, M.A., Bulte, M., Trumpf, T., Aleksic, S., Reuter, G., Bulling, E., and Helmuth, R., 1990, Detection and characterization of fecal verotoxin producing Escherichia coli from healthy cattle. *Journal of Clinical Microbiology*, 28(6),1417-21.

Rammurthy, T., 2008, Shiga toxin producing Escherichia coli(STEC): the bug in our backyard. Indian *Journal of Medical Research*,128,233-236.

Rey, J., Sanchez, S., Blanco, J.E., Hermoso de Mendosa, J., Hermoso de Mendosa, M., Garsia, A., Gil, C., Tejero, N., Rubio, R., and Alonso, J.M, 2006,Prevalence,serotypes and virulence genes of shiga toxin-producing E.coli isolated from ovine and caprine milk and other dairy products in Spain. *International Journal of Food Microbiology*, 107,207-12.

Robinson, R.K., and Batt Carl, A, 2000,Encyclopedia of Food Microbiology. San Diego,USA: *Academic press*.

Schrade, J.P., andYager, J., 2001, Implication of milk and milk products in food disease in France and different industrialized countries.*International Journal of Food Microbiology*, 67,1-17.

Shah Illi, M., Kargar, M., Rezaeian, A.A., Homayoon, M., Kargar, M., and GhorbaniDalini, S, 2010, Evaluation of virulence genes of shiga toxin producing Escherichia coli from juice purchase and vegetables in Shiraz.*Journal of Microbial World*, 3(1),40-47(Persian).

Tahamtan, Y., Hayati, M., and Namavari, M.M, 2010,Prevalence and distribution of *stx1*, *stx2* genes in shiga

toxin producing E.coli(STEC) isolated from cattle. Iranian Journal of Microbiology, 2(1),8-13.
Vidal, R., Vidal, M., Lagos, R., Levine, M., and Prado, V, 2004,Multiplex PCR for diagnosis of enteric infectious associated with diarrheagenic Escherichia coli. *Journal of Clinical Microbiology*, 42(4),1787-9.
Wales, A.D., Wood Ward, M.J., and Pearson, G.R, 2005,Attaching-effacing bacteria in animals.*Journal of Comparative Pathology*, 132(1),1-26.

نشریه پژوهشهای علوم و صنایع غذایی ایران
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مقاله کوتاه پژوهشی

بررسی فراوانی ژن‌های *eaeA*، *stx1* و *stx2* در *اشریشیاکلی‌های* جداسازی شده از پنیرهای محلی شهر مراغه به روش Multiplex PCR

سامان مهدوی*

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چکیده

عوامل بیماری‌زا قادرند که از راه مصرف محصولات لبنی سنتی آلوده مثل پنیر به انسان منتقل شده و در نتیجه بیماری ایجاد کنند. *اشریشیاکلی* تولید کننده شیگاتوکسین، اسهال آبکی ملایم تا مشکلات جدی‌تر مثل التهاب کولون خونریزی‌دهنده و سندرم اورمی همولیتیک تا حتی مرگ را باعث می‌شود. مطالعه حاضر برای بررسی فراوانی ژنهای *eaeA*، *stx1* و *stx2* در *اشریشیاکلی*های جداسازی شده از پنیرهای محلی شهر مراغه به روش Multiplex PCR انجام شد. ۳۲ جدایه *اشریشیاکلی* از پنیرهای محلی شهر مراغه برای جستجوی ژنهای *eaeA* ، *stx1* و *stx2* به روش Multiplex PCR مورد بررسی قرار گرفتند. فراوانی ژن *eaeA* در جدایه‌های *اشریشیاکلی* ۱۵/۶۲٪ (۵/۳۲) بود. ژنهای *stx1* و *stx2* در هیچ جدایه‌ای مشاهده نشد. *اشریشیاکلی* تولیدکننده شیگاتوکسین در پنیرهای محلی این منطقه وجود داشته و برای سلامت انسان می‌تواند خطرناک باشد.

واژه‌های کلیدی: *اشریشیاکلی*، پنیر، مولتی‌پلکسPCR ، ژنهای حدت

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بسم الله الرحمن الرحیم

مندرجات

تاثیر برخی ویژگی‌های فرایند بر ویژگی‌های مکانیکی و بافت تصویر هویج سرخ شده ۳۴۹
میلاد فتحی- سید محمد علی رضوی

شفاف‌سازی آب نارنج با استفاده از اولترافیلتراسیون: بهینه‌سازی شار تراوه و مقاومت گرفتگی با روش سطح پاسخ ۳۶۱
محمد مهدی سیدآبادی - مهدی کاشانی‌نژاد - علیرضا صادقی ماهونک- یحیی مقصودلو- فخرالدین صالحی

مدل سازی فرایند خشک شدن لایه نازک ژل آلوئه‌ورا ۳۷۰
مهدی مرادی- مهرداد نیاکوثری- ارمغان اعتمادی

تأثیر روش‌های مختلف تولید بر میزان راندمان و ویژگی‌های فیزیکوشیمیایی و حسی پنیر موزارلا ۳۸۱
حسین جوینده- مجید نوشکام- امیر بهادر داوری

مقاله کوتاه پژوهشی

ویژگی ضدمیکروبی اولئورزین لیکوپن روی برخی پاتوژن‌های غذایی عنوان کوتاه: توانایی ضدمیکروبی اولئورزین لیکوپن ۳۸۷
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با شماره پروانه ۱۲۴/۸۴۷ و درجه علمی – پژوهشی شماره ۳/۱۱/۸۱۰ / ۸۸/۵/۱۰ از وزارت علوم، تحقیقات و فناوری

جلد ۱۲ شماره ۳ مرداد- شهریور ۱۳۹۵

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.

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جلد۱۲ شماره ۳

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عنوان مقالات

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