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Studying the effect of ozone gas and edible films on microbial features, qualitative properties of Mazafati date during storage period

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Abstract

Mazafati date is the most important export product in Kerman but it is difficult to be kept due to its high humidity and corruptibility. Fermentation and rancidity due to activity of molds and yeasts are considered as the most common problems of producers of Mazafati date. An edible film incorporated with vegetable oil extract and ozone gas is able to increase storage period of the fruit and to settle aforementioned problems. The treatments applied in this research include ozone gas, chitosan and zein films with thyme and carnation extracts. The effect of treatments was studied within 6-month storage in -3°C and 60% relative humidity. Results of data analysis showed that the increasing storage period increased the number of flavonoids. No change was seen in antioxidant activity and total number of phenols has increased for four months. Regarding the color, the effect of treatment was significant on L* component, a* component, number of microorganisms, molds and yeasts of the date but it had no significant effect on other variables. The increasing storage period increased the total number of samples except for the number of molds and yeasts. Use of edible films with vegetable oil extracts could prevent the growth of molds and yeasts especially *Aspergillus Niger* which is resistant against environmental conditions. Generally, results showed that ozone gas as well as edible films with 4000 ppm thyme and 1000 ppm carnation extracts was effective on protection of qualitative, quantitative and microbial properties.

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Introduction

Mazafati date is the third economic cultivar in Iran after Estemran and Shahani cultivars. It is cultivated in different regions. The main origin of this type of date is Bam, Kerman. Such cultivar is soft, semi-dried, blackish dark red and it is the best type of date in domestic market and it has been highly exported in recent years (Afshari Joibari & Farahnaki, 2011 Ahmad *et al*., 2016), But, it is difficult to be preserved due to high humidity and corruptibility of such cultivar. Removal of nectar, loss of humidity, stickiness, fermentation and rancidity due to activities of molds and yeasts are accounted as the most important problems during storage of such cultivar (Salajegheh *et al*., 2016). An edible film can increase fruit storage without increasing anaerobic interactions and it decreases fruit corruption and has no effect on its quality (El Ghaouth *et al.,* 1992). Edible films are divided into proteinic, polysaccharidic, lepidic films or a combination of them. Polysaccharidic films are used to delay loss of humidity in some foods within a short time. They are able to delay fruit maturity and to increase its storage without creating intense anaerobic condition for fruit maturity (Baldwin *et al*., 1995). Proteinic films such as Zein contain hard and shiny appearance, low permeability to water vapor compared to other films produced by vegetable proteins. In similar temperature and relative humidity, transfer of oxygen and dioxide carbon gases from Zein film is one or two times less than polyethylene (with low density), methyl cellulose and hydroxy propyl cellulose films. Such film is relatively similar to polyester films (Bautista *et al*., 2005 Ghanbarzadeh *et al*., 2010). Plasticizers are the main additives to formulation of edible films. Plasticizers have low molecular weights and thy play role in formation of polymeric films. Addition of plasticizers is not only effective on modulus of elasticity and other mechanical properties but also, they change edible films resistance against penetration of vapors and gases. Glycerol is one of plasticizers mostly used in production of edible films (Baldwin *et al*., 1995). In the study conducted by (Salajegheh *et al*., 1995 & Ghasemzadeh *et al*., 2008), edible films of chitosan and zein, pectin, resin were used to coat Mazafati data and two varieties of Thomson raisins were used to coat Shahani date. Microbial evaluation showed significant reduction of the growth of aspergillus, penicillium and Rhizopus. Chitosan film is a biopolymer and it can inhibit some bacterial and fungal strains. Chitosan tends to attack membranes with negative charges. On the other hand, they are less permeable to oxygen and humidity. Therefore, they are good inhibitors against gases and water vapor. This substance is also used in food package due to its antioxidant effect. It attracts metals which catalyze fat oxidation reaction thus it prevents oxidation. Maize zein is a class of prolamin protein and it is suitable for encapsulation of other films. Therefore, among proteinic films, such compounds produce a film with high tensile strength (ts) and low permeability to water vapor with proper heat seal property (Cho *et al.,* 2001). Moreover, corn zein acts to bind fatty acids because both are fat soluble. The coats manufactured from zein will reduce fat migration (Chinnan *et al.,* 1995). These films are brittle in structure, thus it is inevitable to add morpho-structures such as glycerin (Gennadios *et al*., 1994). Ozone is a strong oxidant and it has been used to reduce microbial population in dates in three concentrations (1, 3, 5ppm) and four different times (15, 30, 40 and 60min). Results indicated that 5 ppm ozone gas could reduce microbial population in fruits within 60min (Habibi Najafi *et al*., 2009). The efficiency of ozone gas, as a pesticide, has been evaluated in warehoused products (Farajzadeh *et al*., 2013). In a research conducted by Dostkhah (2005), ozone was used as a disinfectant for dates that were attacked by pests and different microorganisms. Results showed that the number of molds and yeasts, total number of microbes as well as coliform and Staphylococcus aureus were reduced significantly. Some medicinal plants and spices have been studied against many pathogens due to their antimicrobial activities. Medicinal plants are potential sources and their therapeutic and medicinal properties have been paid attention for a long time. Such compounds contain different components that reduce bacterial resistance against plant essences which have been used completely.

Use of carnation and thyme extracts is among safe methods for control of post-harvest diseases (Salajegheh *et al.*, 2013). The optimal concentration of the thyme is between 4000 and 6000 ppm because it contains antimicrobial, antioxidant and bioregulation properties and kills fungi (*Alternaria solani, Fusarium solani, Botrytis cinereal*). The minimum growth inhibitory concentration of such fungi was significant (P≤ 0.01). The minimum lethal concentration of carnation essence on aforementioned fungi was 800 microliter/liter. Oxidation reactions are risky for human health due to production of free radicals (Salajegheh *et al.*, 2013). Such radicals start oxidation reactions leading to either damage or death of cell **(**Kulshreshtha *et al.*, 2011). Different reactive oxygen types are produced during cell metabolism. If they are available as residual, they will be destructive and degrade biologic molecules such as proteins, fats and nucleic acid in oxidative process. Such damages can lead to different age-based diseases including cancer, Alzheimer and Parkinson (Hartmann & Oertel 1995). Recently, some efforts have been done to replace natural antioxidants obtained by biologic sources with artificial antioxidants (Buxiang & Fukuhara 1997). Flavonoids and phenolic acid are among natural antioxidants that have been identified in fruits such as date and vegetables and they showed high antioxidant activities (Einbond *et al.*, 2004).

The present research aims to use chitosan-zein films with 4000-6000ppm thyme (with the least effectiveness on fruit texture), 1000ppm carnation extracts as well as ozone on microbial features and color, phenolic compounds, flavonoids and antioxidant properties of Mazafati date within six-month storage.

Methods and materials

Materials

Mazafati date fruits cultivar was harvested from Azizabad station in Bam and dates with equal size were chosen for each treatment. Chitosan with low molecular weight, 75% or higher deacetylation degree, 20-300 centipoise viscosity was purchased. Zein was bought in form of yellow powder.

Thyme and carnation essences were purchased from Sirjan factory (under French license). Small cellulose box and polyethylene bag were bought for packing date. Glycerin was prepared from Merk Company in Germany.

Methods

After visiting groves surrounding Bam region, ten Mazafati date bunches (in Rotab stage) were harvested randomly from ten palm trees in Azizabad station. To avoid infection, the fruits were placed in special baskets. Then, bunches were transferred rapidly to laboratory of research center for agriculture and natural resource in Kerman and they were placed in fridges within 5^{o}C temperatures. Dates were removed from bunches & 24kg dates were divided into 5 parts. Then, treatments mentioned in table 1 were applied on the samples.

Table 1. Use of different treatments on Mazafati date.

row	Treatments	Use of ozone	Chitosan with 4000 ppm thyme extract	Zein with 1000 ppm carnation extract	Chitosan with 6000 ppm thyme extract	Control
1	A	+	-	-	-	-
2	B	-	+	-	-	-
3	C	-	-	+	-	-
4	D	-	-	-	+	-
5	E	-	-	-	-	+

Treatment with ozone gas

Ozone generator (manufactured by Ozone Ab Co, Model, AS-1200M, was used in this treatment. Portable indicator Model OZO21zx was used to determine the amount of ozone gas (0-10 parts per one million).

Pure oxygen was applied in the generator to produce ozone gas. The capacity of the ozone generator was 8g/h of ozone gas. The production of ozone gas was controlled by display screen. The date was placed in a special bag and it was transferred to the chamber of generator for use of ozone gas.

The date was exposed to ozone gas in terms of 5 parts per one million for one hour (Habibi *et al*., 2009).

Preparation of solutions of chitosan and zein

To prepare chitosan solutions, method of Kim *et al.,* 2006) was used. After preparation of chitosan solution via 1% acetic acid, it was placed in Ben Murray within 55°C for 2.5 hours and it was stirred uniformly. Then, 4000-6000 ppm thyme and 1000 ppm carnation extracts were used and 2% edible glycerol was added as a plasticizer. Regarding the nature of date and its soft tissue, dip coating is the best method for using edible film on the date. Date samples were dipped in chitosan solution for two minutes. Then, they were removed from the solution and were exposed to laboratory air flow for 30 minutes thus the film was dried completely. The method of Janes *et al*., 2002, was used to prepare 10% zein solution. The zein solution was prepared by 1000ppm carnation extract and 2% glycerol as plasticizer. Samples were dried as mentioned in previous method. The samples were kept in fridge with 3°C temperatures and 60% relative humidity for 6 months (Vargas *et al*., 2006).

Measuring phenolic compounds

To measure phenolic compounds of dates, they were kept in darkness and dried place (20°C) to reach a constant weight. The modified method of Chen *et al*., 2007, was used for extraction. The dried date was grinded then, they were sieved. The size of date particles was between 0.5 and 2mm. To extract the essence, 30ml of 80% methanol was added to 10g ground date, they were mixed with a magnate stirrer for 2-3 minutes and they were kept in darkness for 24 hours. The filtered sample was used to measure phenolic compounds, content of flavonoids and antioxidant activity. The amounts of phenolic, flavonoid and antioxidant compounds of the extract were determined by spectrophotometric method in wavelengths of 760, 430 and 517 respectively.

Measuring the color of date fruit

Hunterlab colorimeter (Konica Minolta, CR-400 Japan) was used to measure color of dates before and after coating and before and after being placed in fridge with three replications.

The colors were expressed in terms of white-black (L*), red-green (a*), yellow-blue (b*) using parameters of Hunterlab. Total color transformation (ΔE) was calculated using following formula (valle 2005).

$$\Delta E = \sqrt{(L^* - L^*_0) + (a^* - a^*_0) + (b^* - b^*)}$$

b*, Lo*, o* are color parameters related to date samples without films (freshly post-harvest date) and L*, a* and b* are color parameters related to date samples.

+ΔL*: samples lighter than fresh samples, -ΔL*: samples darker than fresh samples

+Δa*: samples redder than fresh samples, - Δa*: samples greener than fresh samples

+Δb*: samples yellower than fresh samples, - Δb*: samples bluer than fresh dark brown samples

Statistical analysis

The research was done in form of factorial test and complete random design with three replications. Agent A includes five films: 1- ozone gas, 2- chitosan and 4000ppm thyme extract, 3- zein and 1000ppm carnation extract, 4- chitosan and 6000ppm thyme extract, 5- control

Weight reduction

Three replications from each treatment were measured by digital scale with 0.01g precision at the beginning of experiment, after keeping and transferring to laboratory in given time intervals when keeping in incubator (AOAC1984). Weight reduction percent was calculated by following equation:

Weight Loss percent = $(W_1-W_2)/W_1 \times 100$

W_1 is the weight measured before keeping in the warehouse and W_2 is the weight measured after leaving the warehouse.

Microbial test of samples

Microbial tests include total count of microorganisms and fungi. Total number of microorganism was counted based on Pourplate method and Plate Count Agar. Yeast extract glucose oxytetracycline was used to count total number of fungi.

Results

Storage had significant effect on total phenolic compounds, flavonoids and antioxidant activity of date (P≤0.01) (table 2). As mentioned in introduction, the growth of microorganisms' causes' production of acid and rancidity in dates thus it is increased due to the increasing storage period. Fig. 1 shows the graph related to the increasing storage period between treatments applied on *Mazafati* date fruits.

The increasing storage period has increased the number of microorganisms.

Table 2. results of variance analysis of measured indices of post-harvest *Mazafati* date in Bam.

Source of change	Freedom degree	Mean squares		
		Phenolic compounds (mg per 100 g of sample)	Flavonoids (mg per 100 g of sample)	Antioxidant activity (mg per 100 g of sample)
Treatment	4	0.01*	1.38**	0.87**
Time	2	0.20*	14.65**	19.17**
Treatment * time	8	0.001	1.999	2.48**
Total	30	0.13	0.126	0.00047

Ns, * ,** means without and with significant difference in confidence levels of 5 and 1% respectively.

Table 3. comparing means of total phenol, flavonoids and antioxidant activity of post-harvest Mazafati date.

Row	Treatments	Total phenol (mg per 100 g of sample)	Flavonoids (mg per 100 g of sample)	Antioxidant activity (mg per 100 g of sample)
1	Zero time	0.02b	1.72c	3.71 a
2	Two months after storage	0.02b	1.76 c	3.68 a
3	four months after storage	0.03b	1.77 c	3.68 a
4	Six months after storage	0.02a	3.6 a	3.67 a

The numbers with different letters have statistically significant difference in probability level of 5%.

Table 4. Comparing mean number of microorganisms, molds, yeasts and color of Mazafati date within six-month storage.

Row	Treatments	Number of microorganisms	Mold & yeast	Color	Red-green	Yellow-blue	White-black
1	Ozone gas	2.14 bc	0.95 b	19.29 b	2.67 a	2.31 ab	1.22 ab
2	Zein & carnation film	2.02 c	0.69 c	29.46 a	1.29 c	0.61 c	0.49 c
3	Chitosan & 4000 ppm thyme film	2.3 b	0.83 c	19.84 b	1.7 bc	0.086 a	0.74 c
4	Chitosan & 6000 ppm thyme film	1.7 d	0.69 c	19.5 b	2.43 ab	0.082 a	1.13 b
5	Control	3.2 a	1.76 a	20.79 a	2.77 a	0.079 ab	1.4 a

As seen in table 3, the increasing storage period increases the number of flavonoids. No change was seen in antioxidant activity and total number of phenols increased for four months but they reduced within six months. The highest number of flavonoids is seen until four months. No changes were seen in antioxidant activity during storage. The highest amount of L* component (black color) was seen in zein + carnation film and control. Mean comparison of L* component showed that zein+ carnation film had class a. In other words, such film protected black color of date fruit. According to results of variance analysis, the effect of treatment color was significant on L* component, red color, number of microorganisms, molds and yeasts of date (P≤ 0.01). Storage period had no effect on L* component but it was not significantly effective on other variables (P≤0.01). The effect of treatment * time on L* component was small (P≤0.05). It was ineffective on red and yellow colors and it was significantly effective on the number of microorganisms, molds and yeasts of date (P≤ 0.01).

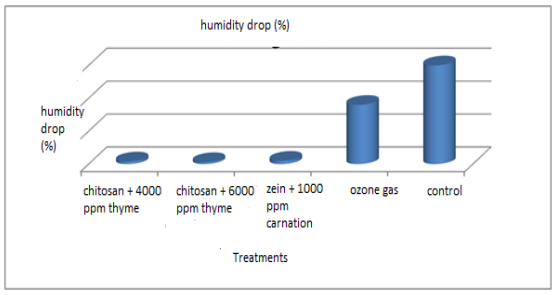

Fig. 1. mean comparison of humidity drop percent between different treatments applied on date during six-month storage.

The effect of treatments on humidity drop

As seen in Fig. 1, the highest humidity was seen in control. Edible films could reduce humidity drop to zero. Ozone treatment reduced humidity by 0.8% within six-month storage.

Table 5. mean comparison of the effect of treatment and storage period on microbial features and color of Mazafati date.

Treatment * time	Total number of microorganisms	Number of molds and yeast	Color	Color a	Color b
Zero time	1.43 gh	1.04 bcde	19.51 ed	2.3 abcd	2.07 def
Ozone gas two months after storage	1.70 efg	1.04 cde	17.69 e	3.05 ab	2.32 def
Ozone gas four months after storage	2.18 d	0.79 def	19.61 cde	2.93 abc	2.51 def
Ozone gas six months after storage	3.23 c	0.94 cde	20.38 de	3.1 a	2.52 def
Chitosan+ 4000 ppm thyme two months after storage	1.96 gh	0.79 def	19.28 e	1.36 de	2.12 f
Chitosan+ 4000 ppm thyme four months after storage	2.1 def	0.55 ef	19.35 e	2.26 abcd	1.21cdef
Chitosan+ 4000 ppm thyme six months after storage	3.85 ab	0.54 ef	20.1 cde	1.42 de	1.39 def
Zein+ 1000 ppm carnation two months after storage	1.04 hi	1.13 f	29.16 b	1.33 de	2.92 a
Zein+ 1000 ppm carnation four months after storage	1.73 efg	0.79 ef	32.44 a	0.63 e	1.85 abs
Zein+ 1000 ppm carnation six months after storage	3.75 b	0.55 ef	32.2 ab	0.62 e	1.82 a
Chitosan+ 6000 ppm thyme two months after storage	1.9 j	0.3 f	18.69 e	3.2 ab	2.29 def
Chitosan+ 6000 ppm thyme four months after storage	2.1 de	1.13 ef	19.24 de	1.6 cde	3.1 abcd
Chitosan+ 6000 ppm thyme six months after storage	3.48 bc	1.04 bcde	19.6 e	2.21abcd	1.5 f
Control two months after storage	0.54 fg	1.23 bcd	18.38 e	2.5 ab	2.08 def
Control four months after storage	2.1 c	1.51 b	20.5 de	2.66 abcd	1.89 def
Control six months after storage	4.23 a	2.95 a	20.9 de	2.69 abcd	2.4 cdef

Discussion

*The effect of treatment *storage time period on color of L* (darkness), a* (red), b* components (yellow) of Mazafati date*

According to variance analysis (table 2), the effect of treatments (edible films, ozone gas) applied on date color has been significant (P≤ 0.01). Table 5 shows changes in mean comparison of total color for coated samples within six-month storage.

Based on results, total color intensity increased until end of storage time period. The reason is due to formation of some chemical and biochemical reactions. The highest L* component is seen in zein + 1000ppm thyme treatment four months after storage (class a). Studies of Vargas *et al.,* (2006) indicated that L* component was increased by coating strawberry with chitosan and oleic acid.

Storage time period increased L* component. Color is an important feature which is effective on consumer's acceptance Taghadomi *et al* (2015) Ozone gas as well as chitosan + thyme film caused color stability during storage time period. The darkest color with the highest black and red values is the best color of date during storage time period (Habibi Najafi *et al.*, 2009 &Kashaninejad *et al.*, 2006). Research results of Taghadomi *et al.*, 2015) indicated that lightness coefficient of coated date reduced less than control group. Results of variance analysis showed that edible films, storage time period and all their reactions had significant effect on redness (color parameter a* was significant) (P≤ 0.01). Since the least amount of the component a* (redness) is seen in samples coated by carnation and thyme (table 6), it seems that the extracts with mentioned concentrations have antioxidant and inhibitory effects on browning reactions Javanmard (2008) reported that the addition of thyme essence to malvaceae mucilage in pears decreased peroxidase enzyme thus prevention from enzymic browning. Since the increasing storage time showed a little reduction in component a*, it seems that the browning reaction has reduced. Storage time period was significant on yellow color of component b* and the color increased by the increasing storage time period for four months then it reduced. The result was consistent with results of salajegheh *et al.,* 2013.

The effect of treatment and storage time period on phenolic compounds, flavonoids and antioxidant activity of Mazafati date fruits

Based on (table 2), of variance analysis, edible films and ozone gas had positive effect on protection of phenolic compounds during storage time period. Based on mean comparison of results in table 3, chitosan + 4000ppm thyme treatment and chitosan + 6000ppm thyme treatment had high phenolic compounds six months after storage. The lowest amount of total phenol was seen in zein+ carnation treatment after four-month storage and the lowest number of flavonoids was seen in zein + carnation treatment after two-month storage. The highest amount of antioxidant activity was seen in zero time. Chitosan + thyme treatment (in both concentrations) maintained this property.

Therefore, the type of package substance is one of important indices that increase antioxidant property of fruits such as date. Different researchers have conducted several studies including Singh *et al.*, 2012, and Chang *et al.*, 2002, No changes were seen in antioxidant properties of dates coated with chitosan, zein and ozone gas during storage time. A research was done in this regard in which protection of phenolic compounds was effective on attraction and neutralization of free radicals (Javanmardi *et al.*, 2003 & Bamdad & kramt2004). The phenolic compounds are among defense mechanisms of cells against unpleasant factors and they will decrease gradually via aging (Ayala- Zavala: 2004 & Taghvaei 2015). By creating a transformed atmosphere around the fruit and protection of CO2 higher than a natural level, the edible films reduce the respiration and the number of oxidation reactions of phenols by decreasing activity of polyphenol oxidase enzyme. Such effects are applied around the product via reduction of O2 Liu *et al.*, 2008. In a research on strawberry coated with chitosan, the effect of film on protection of phenolic compounds had a reducing power and it plays a considerable role in attraction and neutralization of free radicals in the body (Javanmardi *et al.*, 2003 & Mansouri *et al.*, 2005). Therefore, the richness of nutrients with food & drug compounds can be one of proper strategies for increasing the number of antioxidant compounds in the body. Concerning the increasing knowledge of consumers on unpleasant effects of synthesized antioxidants, the use of natural antioxidants is being increased (Al Farsi *et al.*, 2008 & Lee *et al.*, 2008).

The effect of treatments and storage time period on total number of microorganisms

In (table 4) of variance analysis, the effects of treatment, time, treatment * time on total number of microorganisms are shown in 1% significant difference. Experimental results showed that the increasing total number of microorganisms had direct relationship with increase of storage time. The result is consistent with results of Sarhadi *et al.*, 2015.

Among different treatments, the lowest and the highest numbers of microorganisms are seen in chitosan +6000ppm thyme films and control treatment respectively. Date samples were exposed to 5ppm ozone gas for 60 minutes. Then, the number of microorganisms changed from 3.2 log cfu/g to 2.14 log cfu/g. Such results are consistent with those of Habibi Najafi & Khodaparast (2009) with few differences. In addition, Öztekin *et al* (2006) reached similar results about dried figs. Regarding the number of microorganisms, the highest and lowest numbers of microorganisms were seen in control group and zein+ carnation and chitosan + thyme treatments.

In other words, the effect of independent variables of ozone gas, zein + carnation film, 4000ppm thyme + chitosan film, and 6000 ppm thyme + chitosan film on total number of microbes was 2.14, 2.02, 2.3 and 1.7 respectively. It suggests that above treatments played positive role in control of microbial load compared to the control group. Results of Moreira *et al,.* (2011) indicated that the number of microorganisms in samples treated with edible films is lower than 1.5 log cfu/g. similar results were reported by Durango *et al* .,2006.

As seen in Fig., the increasing storage time increases the number of microorganisms and molds and yeasts.

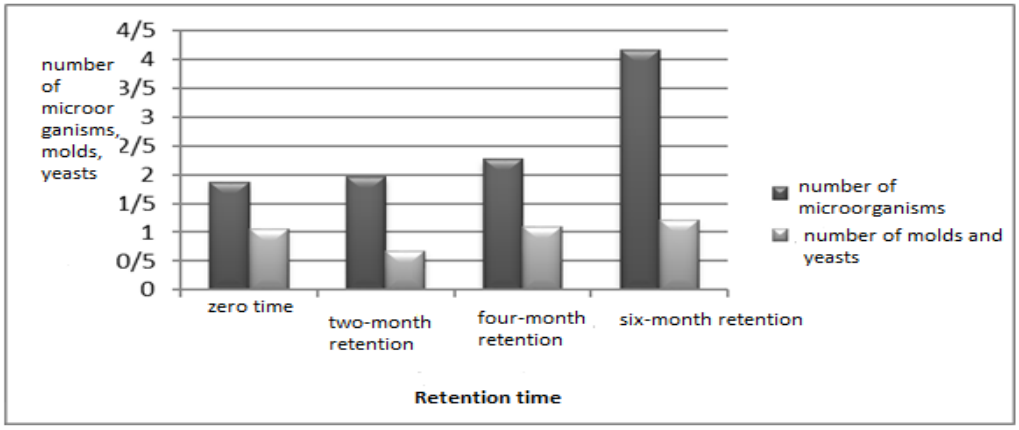

Fig. 2. The effect of storage time on number of microorganisms, molds and yeasts in Mazafati date.

The effect of treatments and storage time on number of molds and yeasts in Mazafati dates

Results of variance analysis (table 4) indicated that the total number of molds and yeasts was affected significantly by edible films and ozone gas, storage time and their interactions ($p \leq 0.01$). Among different treatments, the lowest and the highest numbers of molds and yeasts are seen in chitosan +6000ppm thyme, zein- carnation films and control treatment respectively (table 5). The presence of thyme, carnation extracts, glycerol and edible films reduced total number of molds and yeasts in dates. Use of ozone gas for four months after storage decreased total number of molds and yeasts then they increased. 4000ppm thyme + chitosan film and zein+ carnation film had controlling role in growth of molds and yeasts during storage period.

Salajegheh (2013), Salajegheh (2016), Hartmann *et al* (1999) studied the effect of thyme and carnation extracts on growth control of fungi (*Aspergillus Parasiticus*, *Botrytis cinereal*, *solani Fusarium and Aspergillus Niger)* in Round pistachio, Mazafati date and citrus. Results showed that the use of such extracts decreased significantly the total number of molds and yeasts during storage time ($P \leq 0.01$). According to Salajegheh *et al.,* (2013), use of thyme and carnation extracts was effective on control of fungi grown on Valencia and natural oranges.

The highest amount of L* component was seen in zein + carnation film and control group. Mean comparison of L* component showed that zein-carnation film had class A among different films. In other words, such film can protect black color of the date fruit.

Table 6. mean comparison of the number of microorganisms, molds and yeasts and color of Mazafati date during six-month storage.

Row	Treatments	Number of microorganisms, log cfc/g	Number of molds and yeasts, log cfc/g	Black-white	Red-green	Yellow-blue	White-black
1	Ozone gas	2.14 bc	0.95 b	19.29 b	2.67 a	2.31 ab	1.22 ab
2	Zein+ carnation film	2.02 c	0.69 c	29.46 a	1.29 c	0.61 b	0.49 c
3	Chitosan + 4000 ppm thyme film	2.3 b	0.83 c	19.84 b	1.7 bc	0.086 a	0.74 c
4	Chitosan + 6000 ppm thyme film	1.7 d	0.69 c	19.5 b	2.43ab	0.082 a	1.13 b
5	Control	3.2 a	1.76 a	20.79a	2.77 a	0.079ab	1.4 a

Edible films

Edible films have created a selective permeable obstacle against O2 and Co2 gases and a transformed atmosphere was created around the product via protection of Co2 higher than a natural level thus reduction of respiration, production and effect of ethylene and protection of compounds are resulted due to reduction of aging process. Therefore, consumption of organic acids and sugars is reduced and PH increase is prevented in products. The loss of fruit weight reduction associates with the crop respiration and water evaporation around the fruit. Furthermore, fruit weight reduction is due to water loss from fruit surface. Zein and chitosan films reduced post-harvest fruit weight (approximately zero) in dates regarding low storage temperature which is due to hygroscopic property of coatings that prevent water circulation between the fruit and its surrounding. Moreover, such coatings protect fruit peels against mechanical damages by sealing small lesions leading to delay on dryness.

References

Afshari Juibari H, Farahnaki A. 2011. Maturing expedition of date palm (mazafati cv.) using water and hot solutions of acetic acid and sodium chloride. J. Food Sci. Technol **30(8),** 45-52.

Ahmad K, qly born H, badzadh H, Hossein Pour R, hatmy F, bdshah K, rzayy, M, kazmy, M, milkweed Fazli M. 2016. Agricultural statistics, the third volume of Horticultural products. Ministry of Jihad and Agriculture Planning and Economic Department, Center for Information and Communication Technology Tehran.

Aider M. 2010. Chitosan application for active bio-based film production and potential in the food industry: review. LWT-Food Science and Technology **43,** 837-842.

Al-Farsi MA, Lee CY. 2008. Optimization of phenolics and dietary fibre extraction from date seeds. Food Chemistry **108,** 977-985.

AOAC. 1984. Official Methods of Analysis, 14th ed., Association of Officials Analytical Chemists, Washington DC.

Ayala-Zavala JF, Wang SY, Wang CY, Gonzales-Aguilar AG. 2004.Effect of storage temperature on Antioxidant capacity and aroma compounds in Strawberry fruit.LWT- Food Science and Technology **37,** 687-695.

Baldwin EA, Nisperos-Carriedo MO, Baker RA. 1995, Use of edible coatings to preserve quality Of lightly (and slightly) processed products, Critical Reviews in Food Science and Nutrition **35(6),** 509-52.

Bamdad F, kramt J. 2004. Determine the amount of phenolic compounds in two types of spices (cloves and caraway) and their antioxidant effects in model chemical systems. Conference medicinal plants. Shahed University, Tehran third number.

Bautista-Banos S, Hernandez-Lauzardo AN, Velazquez-del Valle MG, Hernandez-Lo pez M, Ait Barka E, Bosquez-Molina E, Wilson CL. 2006. Chitosan as a potential natural ompound to control pre and postharvest diseases of horticultural commodities. Crop Prot **25,** 108-118.

Buxiang S, Fukuhara M. 1997. Effects of co-administration of butylated hydroxytoluene, Butylated hydroxyanisole and flavonoide on the activation of mutagens and drug-metabolizing enzymes in mice.Toxicology **122,** 61-72.

Chang C, Yang M, Wen H, Chern J. 2002. Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods.Food and Drug Analysis 2002; **10,** 178-82.

Cheng G, Yang E, Lu W, Jia Y, Jiang Y, Duan A. 2009. Effect of Nitric Oxide on Ethylene Synthesis and Softening of Banana Fruit Slice during Ripening. Journal Agriculture Food Chemistry **57:** 5799-5804.

Chien P, Sheu F, Lin H. 2007. Coating citrus (*Murcott tangor*) fruit with low molecular weight chitosan increases postharvest quality and shelf life.Food Chemistry **100,** 1160-1164.

Chinnan MS, Balasubramaniam VM, Mallikarjunan P, Phillip RD. 1995. Edible film coatings for deep-fat frying of foods Institute of Food Technologists Annual Meeting 43-49.

Cho SY, Park, JW, Rhee C. 2001. Properties of laminated films form whey powder and sodium caseinate mixtures and zein layers. Lebensmittel-Wissenschaft and Technologie **35,** 135-139.

Del - valle V, Hernandez – Munoz P, Guarda A, Galotto MJ. 2005. Development of a cactus mucilage edible coating (*Opuntia ficusindica*) and its application to extend strawberry (Fragaria ananassa) shelf – life. Food Chemistry **91,** 751-756.

Dostkhah V. 2007. The effects of ozone on Microflora of Date fruit palm, received a master's thesis for the food industry, Islamic Azad University Sabzevar.

Durango A, Soares N, Andrade NT. 2006. Microbiological evaluation of an edible antimicrobial coating on minimally processed carrots. Food control **17,** 336-341.

Einbond LS, Reynertson KA, Luo XD, Basile MJ, Kennelly EJ. 2004. Anthocyanin antioxidants from edible fruits. Food Chemistry **84,** 23-28.

El Ghaouth A, Arul J, Ponnampalam R, Boulet M. 1992. Chitosan coating to extend the storage life of tomatoes, Horticulture Science **27(9),** 1016-1018.

Farajzadeh D, Qorbanpoor A, Rafati H, Isfeedvajani MS. 2013. Reduction of date microbial load with ozone. Jornal of Research in Medic Science. Apr **18(4),** 330-334.

Gennadios A, Mellugh TII, Weller CL, Krochta JM. 1994. Edible coatings and films based on proteins: Edible coating and films to Improve food Quality. Technomic Publishing ompany, PA, USA, 201-277. Industrial Crops and Products **14,** 11-22.

Ghanbarzadeh B, Almasi H, Zahedi Y. 2010. Biodegradable and edible biopolymers in pharmaceutical & food packaging. First Edition, Publisher: Amirkabir University of Technology: 521 (in Persian).

Ghasemzadeh R, Karbassi A, Ghoddousi HB. 2008. Application of Edible Coating for Improvement of Quality and Shelf-life of Raisins, World Applied Sciences Journal **3(1),** 82-87.

Habibi Najafi MB, Haddad Khodaparast M. 2009. Efficacy of ozone to reduce microbial populations in date fruits. Food control **20,** 27-30.

Hartmann A, Oertel WH. 1999. Analysis of the motor disorder in Parkinson's disease. In: Lewitt PA, Oertel WH, editrs. Parkinson's disease: the treatment options. London: Martin Dunitz Ltd; 1999. p. 39-50.

Janes ME, Kooshesh S, Johnon MG. 2002. Control of Listeria monocytogenes on the surface of refrigerated, ready- to- eat chicken coated with edible zein film coatings containing nisin and/or calcium propionate. Journal of Food Science **67,** 2754-2757.

Javanmard M. 2008. Shelf life of whey protein-coated pistachio kernel (*Pistacia vera* L.). Journal of Food Process Engineering **31,** 247-59.

Kashaninejad M, Mortazavi A, Safekordi A, Tabil LG. 2006. Some physical properties of pistachio (*Pistacia vera* L.) Nut and its kernel. J. Of Food engineering 2006 **72,** 30-8.

Kim SH, No HK, Kim SD, Prinyawiwatkul W. 2006. Effect of plasticizer concentration and solvent types on shelf- life of eggs coated with chitosan. Journal of Food Science **71,** S249-353.

Kulshreshtha M, Goswami MV, Rao C, Ashwlayan V, Yadav S. 2011. Estimation of antioxidant potential of aqueous extract of *Ficus bengalensis* leaf on gastric ulcer. Int J Pharmaceutical Sci Rev Res 2011; **9(1),** 122-6.

Liu J, Zhang J, Xia W. 2008. Hypocholesterolaemic effects of different chitosan samples in vitro and in vivo. Food Chem 2008; **107,** 419-25.

Mansouri A, Embarek G, Kokkalou E, Kefalas P. 2005. Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (*Phoenix dactylifera*). Food Chemistry **89,** 411-420.

Moreira Md R, Roura SI, Ponce A. 2011. Effectiveness of chitosan edible coatings to improve microbiological and sensory quality of fresh cut broccoli. LWT-Food Science and Technology **44,** 2335-2341.

Öztekin S, Zorlugenç B, Zorlugenç FKl. 2006. Effects of ozone treatment on microflora of dried figs. Journal of food engineering **75,** 396-399.

Salagegheh F, Golshan-Tafti A, Gafarzadah F, Beradaran G, Alimohamadei M. 2012. Evaluating Microbial quality of *Mozafati Rutab* upon storage Project, Agricultural Engineering Research Institute, 4-84-14-87006.

Salagegheh f, Panahi B, Tajaden B. 1395. The effect of edible coating of zein, chitosan, and *Trachyspermum copticumt* (L) essential oil on the quality properties of dates. Agricultural Research, Education and Extension organization Agricultural Engineering Research Institute, N 47926.

Salagegheh f, Shafei L, Aminaei M, Arjomand M, Hosanei G, Alimohamadi M. 1392. Effects of plants oil, curing and shrink wrapping on storage life of valencia and local oranges, Final Research Report. Agricultural Research, Education and Extension organization Agricultural Engineering Research Institute N44488.

Sarhadi H, Hadad khodaparast M, Sedaghat N, Mohebi M, Milani E. 2016. Studying The Efficiency of Temoerature of Ozone Gas, chitosan edible coating and temperature c during Palm date storage and optimizing storage conditions by The Method of response surface. Jornal of food Research (university of Tabriz) **261,** 49-60.

Singh V, Guizani N, Essa MM, Hakkim FL, Rahman MS. 2012. Comparative analysis of total phenolics, flavonoid content and antioxidant profile of different date varieties (*Phoenix dactylifera* L.) from Sultanate of Oman, lnt. Food Res., J **19,** 1062-1070.

Taghadomi-Saberi S, Omid M, Emam-Djomeh Z, Faraji-Mahyari Kh. 2015. Determination of Cherry Color Parameters during Rip ening by Artificial Neural Network Assisted Image Processing Technique, Agr. Sci. Tech. (2015) Vol. **17,** 589-600.

Taghvaei M, Jafari SM. 2015. Application and stability of natural antioxidants in edible oils in order to substitute synthetic additives, J Food Sci Technol. 2015 Mar; 52(3):1272-82.
DOI: 10.1007/s13197-013-1080-1. Epub 2013 Jul 6.

Vargas M, Albors A, Chiralt A, Gonzalez-Martinez C. 2006. Quality of cold-stored strawberries as affected by chitosan-oleic acid edible coatings. Postharvest Biol Technol 2006 **41,** 164-71.