

The Effect of Polylactic Acid Packaging Modified with Clay Nanoparticles on Quality and Shelf life of Mushroom

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ABSTRACT: One of the newest technologies for storing agricultural products is the biodegradable packaging method, in which certain materials are utilized on a nano scale. Edible mushroom is a nutritious agricultural product which can be considered a food source as well as having medicinal applications while having a short shelf life after harvesting. In this study, the mushroom was packaged in polylactic acid packages containing nanoparticles of clay and the effect of the packaging on the quality characteristics and shelf life of mushroom were determined. Aiming at determining the most optimal conditions for the packaging of the mushroom, the effects of the weight percent of nano clay (0, 3 and 6 wt%), time of storage (4, 8 and 12 day), and three different temperatures (4, 14, and 24 °C) on the quality characteristics and the shelf life of mushroom have been investigated. Different regression models were adopted to correlate the empirical results of dependent variables, and through statistical analyses the most reliable regression model was selected.

Keywords: *Mushroom, Nanoparticles, Packaging, Polylactic Acid, Shelf Life.*

Introduction

Mushroom is considered to be a medical and pragmatic food due to high levels of proteins, bioactive compounds, multiple carbohydrate and low cholesterol levels. Mushrooms are rich in nutrients such as essential amino acids (lysine and tryptophan), vitamin C and vitamin B complex, especially thiamin, riboflavin, niacin, biotin and pantothenic acid (Mahta *et al.*, 2011; Manzi *et al.*, 2001). Based on the absence of starch in the mushrooms, it is an ideal food for diabetic diseases and people who are on a diet (Chang, 2004).

Mushrooms are low in fat content (0.3%) and rich in linoleic acid (Mahta *et al.*, 2011). They are also rich in various minerals, including bioactive polysaccharides (beta-glucan), antioxidants, fiber and folate (Jagadish *et al.*, 2009). Researchers have reported that extracted compounds from mushrooms have antifungal and antibacterial properties. Mushrooms are suitable foods for patients, the elderly, pregnant women and children because of high concentration of proteins, sterols and micronutrients while containing low calories (Barros *et al.*, 2007). Physiologically, mushrooms are one of the most sensitive agricultural products after being harvested. Mushroom, due to the lack

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of cuticle on its surface, is very sensitive to moisture loss, physical damage, and microbial invasion (Zahedi and Sedaghat, 2011). Mushrooms have a short life time, less than three days at room temperature, and about 8-10 days under refrigeration conditions (Lee, 1999; Burton 1989). Dehydration, enzymatic browning and fungal growth lead to mushroom corruption (Aras *et al.*, 2007). Fresh mushrooms are metabolically active after being harvested. Therefore, the respiration rate increases with increasing speed of crop destruction and storage time (Aras *et al.*, 2007). Usually the quality of fresh mushrooms remain at an acceptable level for 12 hours after harvest at the temperature range of 21-25 °C. The effort to maintain quality and extend the storage time of fresh mushrooms is very important. Several methods have been investigated in order to maintain the quality of the mushrooms after harvesting. These methods include the use of cold storage, proper packaging, modified atmosphere packaging, irradiation, washing, etc. (Srivastava *et al.*, 2010). One of the new packaging techniques is based on nanotechnology and has created a great revolution in polymer packaging industry (Moraveji and Mostafavi, 2010). Depending on the food type, the use of different nanostructures could led to polymer packaging production with different levels of permeability to water vapor and gases. The packaging increases the shelf life, preserves the color, aroma and flavor of food, prevents the growth of microorganisms and facilitates transport operations and maintenance (Fathi and Mohebi, 2010). Nano packaging due to the quality of antimicrobial and barrier properties prevents discoloration, promotes better health and more control and less damage to the structure of the food. Packages containing clay nanoparticles have advantages in terms of mechanical, thermal and barrier properties. The nanoparticles have prevented

the passage of oxygen, carbon dioxide and humidity (Fathi and Mohebi, 2010). These nanoparticles have been studied widely in food industry. The application of clay nanoparticles in food packaging coatings has expanded due to its unique properties such as low toxicity, high thermal stability, low volatility and antimicrobial effects in food and medicine. Researchers have also reported that using low clay nanoparticles in food packaging is completely safe and the amount of nanoclay migration to the food is very low (Han *et al.*, 2011). Nanoclay can be used at the nano scale layer. The special feature of nanoclay can play an important role in nanocomposites production. These features include: opening layers from one another and their dispersion in the polymer matrix, modified surface for better interaction between the polymer matrix and nanoparticles (Azardo and Henrit, 2009). The use of a suitable polymer such as polylactic acid (PLA) as a biodegradable polymer has attracted the attention of many researchers. These polymers have a unique position in food applications because of special features such biological sources. Several studies have been performed on the use of PLA with nanoparticles of clay (Moraveji and Mostafavi, 2010). For example, Svagan *et al.* (2012) managed to improve the oxygen permeability of PLA films by adding nano clay, which has been reported as non-toxic. The most attractive aspect of polylactic acid is being biodegradable and its lack of toxicity. The combination of PLA and nanoparticles of clay may result in a nanocomposite with good barrier properties that is suitable for film packaging material. Polylactic acid is an environmentally friendly bio-polymer with excellent biocompatibility and need less energy to produce (Grober *et al.*, 2003).

In this study, mushrooms were packaged in PLA packages containing nanoparticles of clay, and the effect of the packaging on the quality characteristics and the shelf life of

mushrooms was determined. The aim of this research is to determine the most optimal condition for packaging of mushrooms that might affect the quality characteristics and shelf life of mushroom.

Materials and Methods

-Materials

The white-button mushrooms (*Agaricus bisporus*) were purchased from Shahriar agro-industry Company, IRAN. Polymer matrix was polylactic acid (PL0800) with density of 1.24 (gr/cm³) that was bought from Nature Work company, USA. Nanoclay was Cloisite 15A with density of 1.66 (gr/cm³) and was purchased from SOUTHERN CLAY Company, USA. The nanoclay was from minerals smectite families and groups of Montmorillonite that have been modified with alkyl ammonium ions. The modifier material has a hydrophilic and hydrophobic portion, and has the ability to easily exchange ions between layers. Nanoclay and polylactic acid were placed for two hours at 80 °C in a vacuum oven (Busolo *et al.*, 2010).

-Methods

-Nano composites polylactic acid with clay soil

This nanocomposite was prepared using a chloroform solvent (99% pure, Merck, Germany). The specific weight values of nanoclay were poured in 100 ml of chloroform, and were stirred for 24 hours at room temperature by a magnetic stirrer (Model RCT BASIC, IKA, Germany). This nanoclay particle suspension was subjected to ultrasound for half an hour to be uniformly distributed in chloroform. At the same time, the amount of 10 gr of polylactic acid was stirred in chloroform at room temperature for 24 hours by a magnetic stirrer. PLA is insoluble in water, ethanol, methanol and aliphatic hydrocarbons but it is soluble in chloroform (Muller *et al.*, 2017). The two solutions were mixed together and

were stirred for 12 hours by a magnetic stirrer. Nano-composite solution was placed for two hours in an ultrasonic bath (model P30H, Elmasonic Germany) with a frequency of 37 KHz. Finally, the nanocomposite PLA/nanoclay was poured into glass jars, and for 12 hours were allowed to the bulk of solvents leave from samples and for the complete withdrawal, the samples were placed in a vacuum oven (Model VO400, Memmert Germany) at 70 °C temperatures for 24 hours. Films were prepared by compression molding (manual press, Toyoseiki Japan) with 25MPa pressure and at 200 °C temperature. All the films were prepared at Iran's Polymer and Petrochemical Institute.

-Mushrooms packaging

The mushrooms were selected (*Agaricus bisporus*) and were free from contamination and decay. Mushrooms were crushed at the size of 30 to 40 mm in diameter by using a clean and disinfected knife. They were then placed in a covering with 30 cm in length, 25 cm in width and with 85 microns in thickness and sealed using a plastic sewing machine. All the packages (0, 3 and 6 wt% of nano clay) were stored at three temperatures (4, 14 and 24 °C) for 4, 8 and 12 days. Three holes were established below packages in order to prevent moisture condensation and growth of anaerobic bacteria.

-Quality analysis

Weight loss was monitored by the weight of the content of packages before and after the storage period using a digital balance (with an accuracy of ±10-1 g). Percent weight loss was calculated in the following equation (Zahedi and Sedaghat, 2011):

$$W = \left[\frac{(M_1 - M_2)}{M_1} \right] \times 100$$

In the above equations, W, M₁, and M₂ refer to the weight loss, the initial weight

immediately after harvest, and the weight at trial day, respectively.

The determination of moisture content was measured by hygrometer according to AOAC (1984). Moisture removal rate was obtained from the following equation:

$$W_L = \frac{w_0x_0 - w_1x_1}{w_0} \times 100$$

Where: WL is the percentage of water loss and W_0 , W_1 , X_1 are respectively the initial weight of sample, weight of dehydrated samples and moisture of dehydrated samples respectively.

Color samples were measured before and after packaging by Hunter lips devices (colorflex model, USA). Measurements are expressed as L^* (lightness), a^* (red/green), and b^* (yellow/blue). Color difference (ΔE) was calculated with respect to the standard plate parameters by using the following equation:

$$\Delta E = \sqrt{(L_0 - L^*)^2 + (a_0 - a^*)^2 + (b_0 - b^*)^2}$$

Where: L_0 , a_0 and b_0 indexes were measured before the test for fresh mushrooms color changes. In fact, fresh mushrooms were used as a control Unboxed and ΔE represents the color changes in comparison to fresh mushrooms.

The browning index (BI) was calculated according to the following equation (Asgari et al., 2006):

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

$$BI = \frac{[100(x - 0.31)]}{0.17}$$

Measuring of this index was conducted by the spectrophotometric calorimeter (Hunter lab). Browning index represents the purity of brown color and is considered to be an important parameter associated with

browning (Lopez-Malo, 1998).

The texture profile analysis for all the samples was performed using a Texture Analyser (Hounsfield-H5KS, UK). In this test, tissues of samples were evaluated by measuring the amount of stress at maximum force (the drilling method). Stress (MPa) was obtained from the following equation:

$$\delta = \frac{F}{A} \times 10^6$$

In this formula, δ , F and A show the stress (MPa), force (N) and area (m^2), respectively (Shamaee and Emam Jome, 2010).

Shrinkage is a major problem for the mushroom. To determine the amount of shrinkage, a cylinder was filled by 20 ml of toluene (Merck, Germany) and initial samples of mushrooms were put into a measuring cylinder and toluene volume changes were recorded. Samples sizes (after packaging) were measured in the same way, and the shrinkage rate is calculated according to the following equation (Shulka BD and Singh S P. 2007)

$$SH = \frac{V_0 - V_1}{V_0} \times 100$$

Where: SH, V_0 , and V_F refer to the shrinkage percentage, the change of initial sample volume (ml), and the change of packaged samples volume (ml), respectively.

Soluble solids content (SSC) was evaluated for each of the samples with an ATAGO refractometer (NAR-2T model, Japan) and the results were expressed as degree Brix.

-Statistical analysis

Response Surface Methodology (RSM) based on the Box-Behnken design (BBD) was employed to investigate the effects of the different operating conditions on quality characteristics of mushroom, but it is aimed

to reduce the number of samples required without confounding the importance of the primary factor excessively. The temperature, weight percent of nano clay and time of storage were considered as independent variables and the quality characteristics of mushroom was regarded as the dependent variable. In order to determine the relationship between the dependent variable and the independent variable, polynomial equation was expressed as a counter plot. The regression analysis is utilized here for understanding the scientific relationships between a dependent variable and several independent variables.

Results and Discussion

The experimental data have shown the effects of polylactic acid packaging based on nano clay particles on the quality characteristics of mushroom and its shelf life. The effects of the weight percent (wt%) of nano clay (0, 3 and 6 wt%) , time of storage (4 , 8 and 12 day), and three different temperatures (4, 14, and 24 °C) on the quality characteristics and the shelf life of mushroom have been reported.

The statistical results showed that temperature, storage time and concentration of nano clay in PLA packaging had significant effect on browning index of mushrooms. Browning index of mushrooms at different time storage (4, 8 and 12 days) in response to a percentage of nanoparticles and temperature are shown in Figures 1a, 1b, and 1c respectively. The results showed that the browning index of mushroom increases with increasing temperature and storage time. However, browning index showed reduction by increasing the weight percentage of clay nanoparticles. Experimental data showed that the PLA packaging based on nano clay had significant impact on reducing browning of mushrooms during storage. In the the packages without nanoparticles. Browning occurred very quickly due to oxygen entry,

and mushrooms changed color considerably. It appears that the nano clay particles used in the PLA packaging is capable of reducing metabolism of the mushrooms and extend the shelf life. Polyphenol oxidase (tyrosinase) is an enzyme responsible for browning of mushrooms. Oxidation of phenolic substances in mushrooms by enzymes caused brown color (melanin). In order to stop the activity of enzymes, it is necessary to reduce the rate of post-harvest metabolic activities of the mushroom tissues.

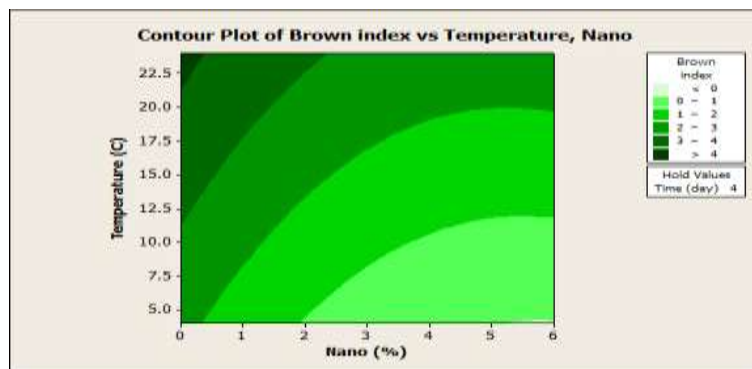
PLA packaging based on nano clay led reduce of the enzymatic activity and mushrooms' breathing. Researchers have shown that the presence of nanoparticles in packaging led to improve the inhibitory properties against oxygen and carbon dioxide (Aras *et al.*, 2007).

The shrinkage results of mushrooms have been considered at three different time storage (4, 8, and 12 day), and the results have been presented in Figure 2a, 2b, and 2c respectively. The results showed that temperature, storage time and weight percent of nanoparticles had significant effect on shrinkage of mushrooms. Figure 2 shows that shrinkage is increased with increasing temperature and storage time. However, shrinkage of mushroom decreases with increasing of clay nanoparticles. The experimental data showed that PLA packaging based on clay nanoparticles has a significant effect on reducing shrinkage of the mushrooms at three different storage times. Mushrooms are shrunk due to water loss during storage, therefore using one convenient package can prevent the moisture loss of mushrooms. Packaging based on clay nanoparticles prevents oxygen from penetrating the package and lower the outflow of moisture, therefore during storage, shrinkage is reduced in comparison to the PLA packaging that does not contain clay nanoparticles (Navgaran *et al.*, 2014).

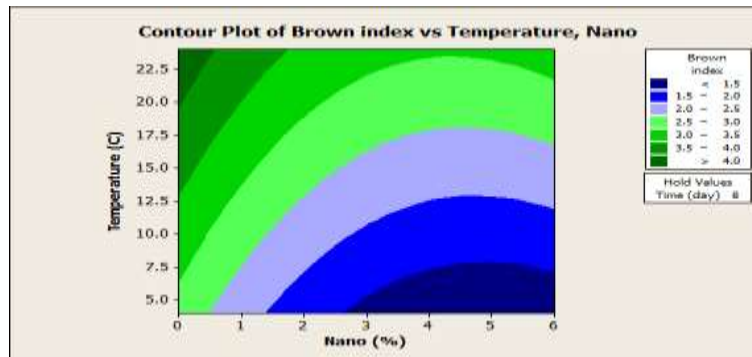
Some of the researchers have reported that the use of nanoparticles in food

packaging is growing over the past decade (Carbone *et al.*, 2015). Nanomaterials are mixed into the polymer matrix to improve the gas barrier properties such as polymer/clay nanocomposites. Metal nanoparticles with their potent antimicrobial properties are used as “active packaging” and the emerging metal nanoparticles with biocidal properties are Cu, Zn, Au, Ti, and Ag (Toker *et al.*, 2013). Among them clay nanoparticles have demonstrated to have the

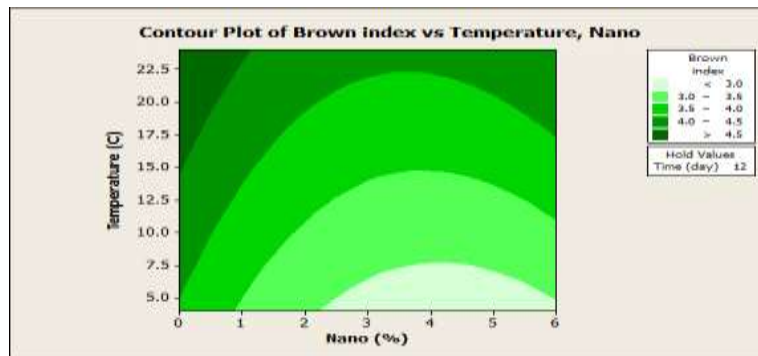
suitable properties for mushroom packaging. Clay nanoparticles is inexpensive in price as compared to the metallic silver. Furthermore, they exhibit low volatility and stability at high temperatures. Clay nanoparticles can be hosted in different matrices such as polymers and stabilizing agents through different strategies: they can be coated, absorbed, or directly incorporated in the synthesis processes (Martinez-Abad *et al.*, 2012).



(1a)

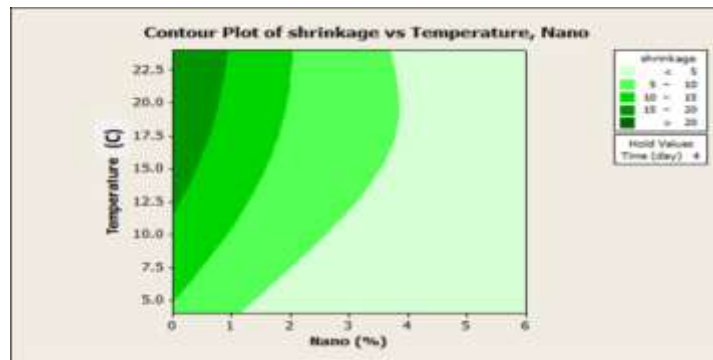


(1b)

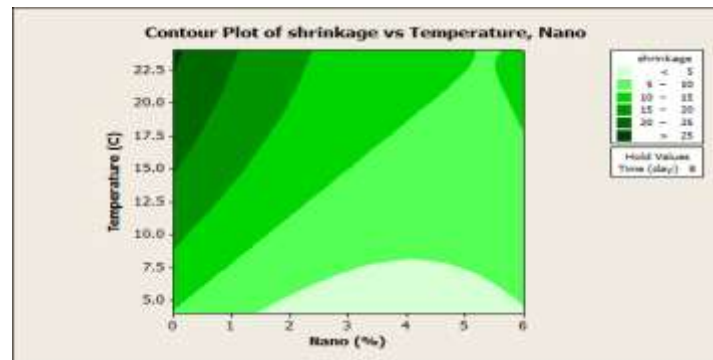


(1c)

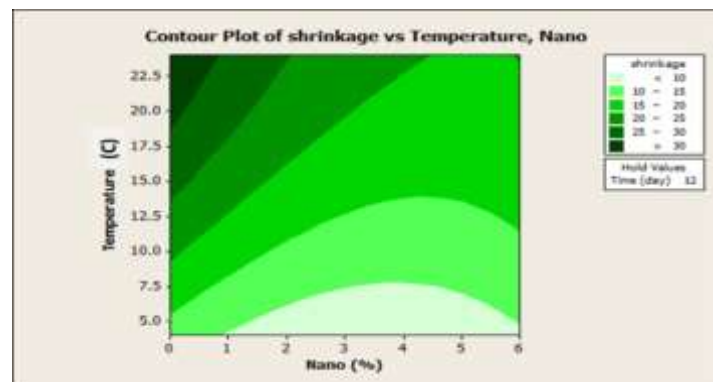
Fig. 1. Counter plot on the browning index of mushroom with the combined effects of nano clay weight percent and temperature (a:4, b:8 and c: 12 day of storage time)



(2a)



(2b)



(2c)

Fig. 2. Counter plot on the shrinkage of mushroom with the combined effects of nano clay weight percent and temperature (a:4, b:8 and c: 12 days of storage time)

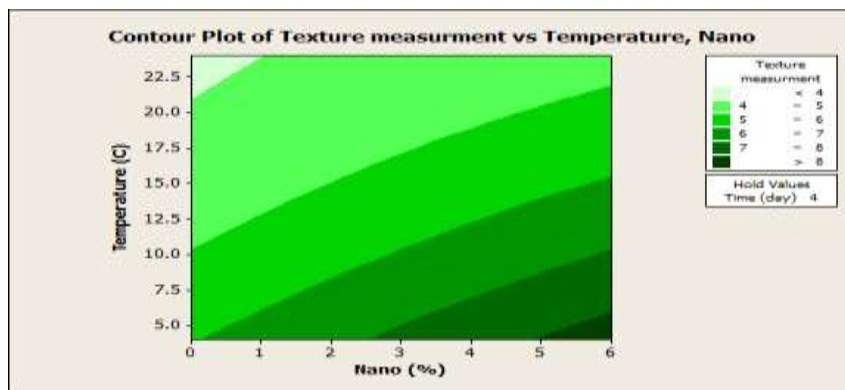
Figure 3 shows the values obtained for the textural property (firmness). The results of the mushroom firmness test showed that the highest firmness was obtained by PLA packaging based on nano clay, and the lowest firmness was observed by the use of PLA packaging. Figure 3 showed that the mushroom firmness increased in three different storage times with increases in weight percent of nano clay. During

maintenance, mushroom tissue will be damaged. The reduction of the stiffness and strength of the tissue might be due to enzymatic activity and the destruction of the cell wall. The presence of clay nanoparticles in packaging led to the improvement in the inhibitory properties against oxygen and carbon dioxide and shortness of breath and it also delayed softness of tissue (Chaudhry, 2010). The texture and tissue stiffness is

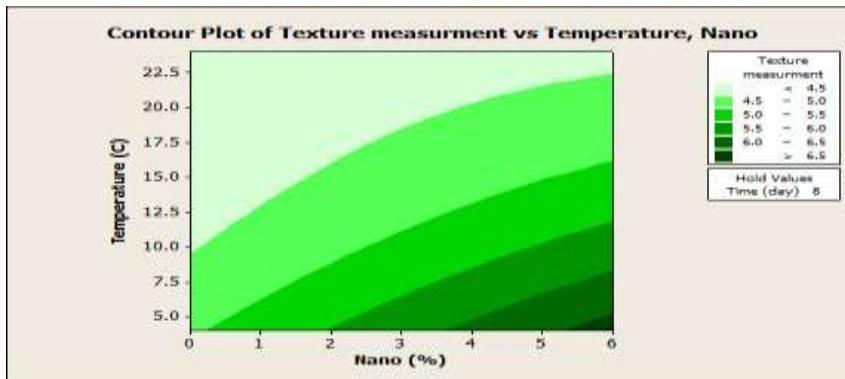
directly proportional to the amount of moisture (Zahedi and sedaghat, 2011). The moisture is preserved in mushrooms by PLA packaging that is based on clay nanoparticles, therefore firmness is increased as compared to the PLA packaging without nano particles.

The experimental data for the color difference (ΔE) on the mushrooms at different storage time (4, 8, and 12 day) are shown in Figures 4a, 4b and 4c respectively.

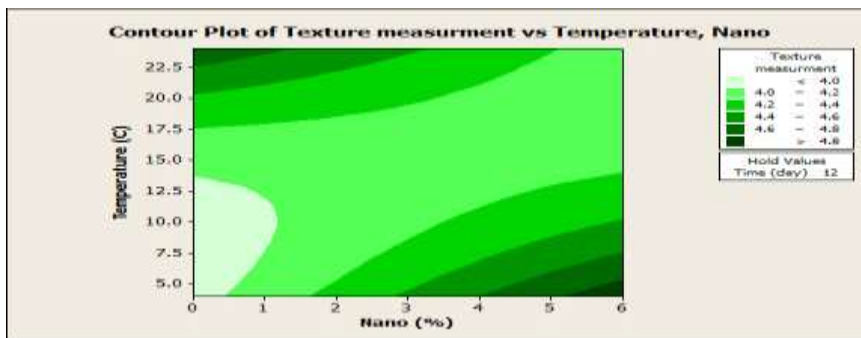
The results indicate that with an increase in the weight percent of nano particles at different storage times, the color difference of mushroom is reduced. In all the samples, the color difference of mushroom is increased with increase in temperature. It seems that by increasing the temperature of the samples, the enzymatic reaction and phenolic oxidation in the mushrooms has increased.



(3a)

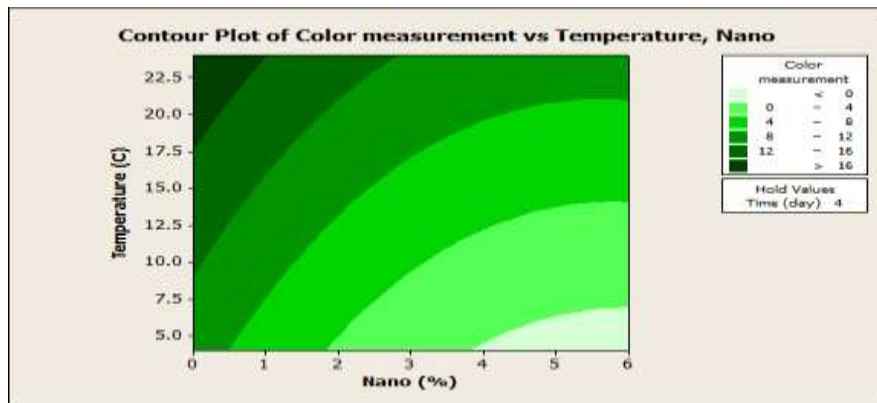


(3b)

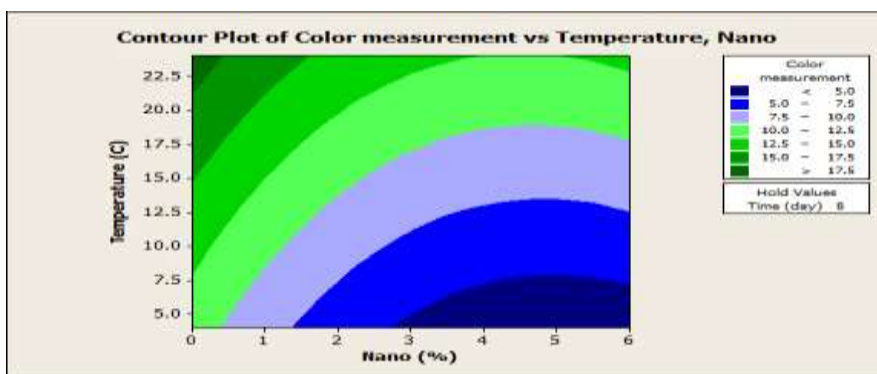


(3c)

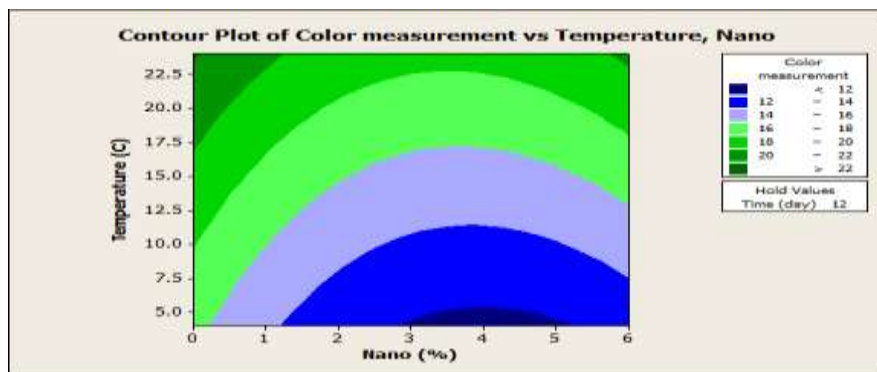
Fig. 3. Counter plot on the firmness of mushroom with the combined effects of nano clay weight percent and temperature (a:4, b:8 and c: 12 days of storage time)



(4a)



(4b)



(4c)

Fig. 4. Counter plot on the color difference of mushroom with the combined effects of nano clay weight percent and temperature (a: 4, b:8 and c: 12 days of storage time)

The effects of independent variables on the weight loss and moisture loss of mushrooms are presented in Table 1. The results have shown significant effect on the rate of weight and eventual moisture loss and moisture loss of mushrooms. The results indicate that with an increase in the weight percentage of nano particles, the weight and moisture loss of mushrooms decrease, and the reason for these two losses might be due

to the effects of two different factors:

A) Increasing the impermeability of to gases and moisture by PLA packaging based on clay nanoparticles

B) Improving the mechanical and heat resistance properties of packaging by nano particles.

The experimental data for the brix of mushrooms in PLA packaging containing nanoparticle (0, 3 and 6 wt%) at different

temperatures T = (4, 14, and 24 °C) are shown in Table 1. The results indicate that with a decrease in the weight percent of nanoparticle at different temperatures, the brix of mushrooms is increased. A change in the concentration of nano particles in PLA packaging can cause a change in the moisture loss of mushrooms at different storage times. Consequently, the results indicate that by increasing the moisture loss of mushrooms, the brix of mushroom is increased.

-Statistical analysis

Regression analysis is used for understanding scientific relations between a dependent variable and several independent variables. The experimental results were analyzed by statistical software SPSS (16), and MATLAB (R 2008a) software. To determine the relationship between the dependent variables and the weight percent of the clay-nanoparticles, as well as storage time and temperature, a regression analysis was conducted.

The different regression models,

correlated with experimental data, are detailed in the Table 2.

The optimal regression model was determined through coefficient of determination (R^2) defined by the following Equation:

$$R^2 = \frac{\sum_{i=1}^N (K_i^{EXPT.} - \bar{K})^2 - \sum_{i=1}^N (K_i^{EXPT.} - K_i^{MODEL})^2}{\sum_{i=1}^N (K_i^{EXPT.} - \bar{K})^2}$$

Where $K_i^{EXPT.}$ is the i th experimental value of the dependent variable; $K_i^{MODEL.}$ is the depended variable estimated by the regression model, and \bar{K} is the mean of the experimental data. The coefficient of determination (R^2) is a statistical measure evaluating the capability of a model to describe and predict the outcomes. The more the value of R^2 approximates to 1, the stronger the correlation between the experimental data and the regression model results is expected to be.

Table 1. Effect of nano clay (wt%), temperature (T) and storage time (t) on the wet loss, weight loss and brix of mushroom.

Nano clay (wt %)	T	t (day)	Wet loss (%)	Weight loss (%)	Brix
3	4	12	15.97	16.44	1.94
6	24	8	24.02	24.85	2.41
6	14	4	8.28	8.75	2.4
6	4	8	6.5	5.91	2.02
3	14	8	17.75	17.92	1.92
0	14	12	54.43	57.39	3.23
6	14	12	38.46	39.64	2.75
3	14	8	17.1	17.81	1.93
3	14	8	17.15	17.63	1.95
3	4	4	4.14	4.67	1.71
0	14	4	30.76	31.36	2.67
3	24	4	11.83	12.01	2.25
3	24	12	40.23	42.01	3.24
0	24	8	56.21	59.76	3.3
0	4	8	16.56	17.27	2.32

Table 2. The regression models for the dependent variables.

dependent variable	Regression Model	R ²
Brown Index	$1.75(Cn) + 0.13(T)0.05(t) + 0.06(Cn \times Cn)0.0002(T \times T) + 0.018(t \times t) + 0.0046(Cn \times T)$ $0.026(Cn \times t) - 0.0061(T \times t)$	0.984
Shrinkage	$8.65 - 4.22(Cn) + 0.79(T)1.19(t) + 0.54(Cn \times Cn)0.01(T \times T) + 0.08(t \times t)0.08(Cn \times T) + 0.06(Cn \times t)$ $+ 0.06(T \times t)$	0.985
Texture	$7.97 + 0.600(Cn) - 0.26(T) - 0.32(t) + 0.003(T \times T) - 0.01(Cn \times T) - 0.031(Cn \times t) + 0.018(T \times t)$	0.914
color	$10.25 - 4.40(Cn) + 0.52(T)1.06(t) + 0.303(Cn \times Cn) + 0.0014(T \times T) + 0.125(t \times t) + 0.01(Cn \times T)$ $+ 0.15(Cn \times t) - 0.02(T \times t)$	0.989
Weight loss	$17.16 - 9.89(Cn) + 1.74(T)2.98(t) + 1.37(Cn \times Cn) - 0.031(T \times T) + 0.26(t \times t) - 0.19(Cn \times T)$ $+ 0.10(Cn \times t) + 0.113(T \times t)$	0.968
Wet loss	$16.54 - 9.67(Cn) + 1.74(T) - 2.85(t) + 1.30(Cn \times Cn) - 0.03(T \times T) + 0.24(t \times t) - 0.18(Cn \times T)$ $+ 0.13(Cn \times t) + 0.1(T \times t)$	0.970
Brix	$3.13 - 0.32(Cn) + 0.002(T) - 0.28(t) + 0.05(Cn \times Cn) + 0.0005(T \times T) + 0.01(t \times t) - 0.004(Cn \times T)$ $- 0.0043(Cn \times t) + 0.0047(T \times t)$	0.980

T= Temperature t= time Cn= concentration of nanoparticles

Conclusion

In this study, the quality characteristics and shelf life of edible mushrooms have been reported using the PLA packaging containing clay-nano particles (0, 3 and 6 wt%). The PLA packaging containing nano clay showed its ability for increasing the storage time of mushrooms. The effect of temperature, storage time and concentration of nano clay were evaluated on the quality characteristics of mushrooms. All the studied parameters have shown that they have influence on the quality characteristics of mushrooms. The results of the experimental data showed that the storage time of mushrooms is increased by increasing the nanoparticles concentration, and also storage time is increased by decreasing the temperature. In general, with respect to the obtained results, using PLA packaging containing clay nanoparticles can be successful for increasing the shelf life of mushrooms. The application of PLA packaging based on 6 wt% clay-nanoparticles at the temperature of 4°C for 8 days is recommended as the best condition for the storage time of mushrooms. The results showed that the appearance and qualitative

characteristic of mushrooms in this selected package during the storage time have been significantly different from the control sample, therefore weight, moisture, firmness of tissue, freshness, and color of the mushrooms are maintained.

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