Effect of Modified Atmosphere Packaging and Storage Temperatures on Quality of Shelled Raw Walnuts

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Abstract—This study was aimed at analyzing the effects of packaging (MAP) and preservation conditions on the packaged fresh walnut kernel quality. The central composite plan was used for evaluating the effect of oxygen (0–10%), carbon dioxide (0-10%), and temperature (4-26 °C) on qualitative characteristics of walnut kernels. Also, the response level technique was used to find the optimal conditions for interactive effects of factors, as well as estimating the best conditions of process using least amount of testing. Measured qualitative parameters were: peroxide index, color, decreased weight, mould and yeast counting test, and sensory evaluation. The results showed that the defined model for peroxide index, color, weight loss, and sensory evaluation is significant (p < 0.001), so that increase of temperature causes the peroxide value, color variation, and weight loss to increase and it reduces the overall acceptability of walnut kernels. An increase in oxygen percentage caused the color variation level and peroxide value to increase and resulted in lower overall acceptability of the walnuts. An increase in CO_2 percentage caused the peroxide value to decrease, but did not significantly affect other indices (p ≥ 0.05). Mould and yeast were not found in any samples. Optimal packaging conditions to achieve maximum quality of walnuts include: 1.46% oxygen, 10% carbon dioxide, and temperature of 4 °C.

Keywords—Shelled walnut, MAP, quality, storage temperature.

I. INTRODUCTION

WALNUT (*Juglan sregia* L.) is a round shape fruit belonging to Juglandaceae family which has two shells; one of them is soft green shell which dries and wears out by time, and the other one is a hard and wooden shell [1], [2]. Walnut is among the best, most delicious, and most energizing kernels and can be consumed alone or along with other edible nuts [3]. Iran has some parts of vast habitats for walnut plant and it is being cultivated and harvested in large parts of the country [4]. The total walnut production of 2007 in the world was 1,694,889 tons, while Iran produced 170,000 tons of walnut and is considered as the fourth rank producer of this nut after China, US, and Turkey [5]. The main fatty acids in walnut kernel oil are: oleic acid, linolenic acid, and linoleic acid. The high levels of these unsaturated fatty acids in walnut kernel help to reduce high blood pressure and cholesterol level and also helps in body protection against heart and vain diseases and cancer. But, this oil is highly prone to oxidation due to existence of unsaturated bonds. The main reason for bad taste and reduced quality of walnut kernel is the oxidation of lipids [3], [5]. The oxygen concentration is among most important factors which affect oxidation of edible nuts. Fresh and perishable foodstuff can be packaged, while fresh using regulated atmosphere packaging and preserve in the same condition. Live and permanent processes can be slowed down using modified atmosphere packaging (MAP) method which is done through regulating the surrounding environment of foodstuff [6]. With this technology, the atmosphere inside the package is replaced with a mixture of gases specially intended for the certain product. Regulated atmosphere packaging maintains the chemical, microbial, and enzyme reactions inside the package and prevents or minimizes foodstuff decomposition during the preservation time [6]. The influence of various MAPs on fresh strawberry qualities was investigated and compared during storage [7].

One method for preventing decay due to oxidation and increasing product preservation time is to use suitable covering materials in the package and to maintain the required environment inside the package in order to preserve edible nuts [6]. This can be done through reducing oxygen concentration and replacing nitrogen gas and CO_2 inside the package in order to decrease the oxidation speed and level. Nitrogen gas and carbon dioxide cause lower hydrolysis due to their high solubility in oil. In addition to gases concentration in the product package, the preservation conditions like temperature and light will significantly affect the stability and preservation time of the edible nuts. The quality indices of the product suffer from undesirable effects due to increase of temperature and exposure to the light [8].

The effects of three types of coverings on the quality and preservation of fresh walnut have been studied: LDPE under air, PET/PE under nitrogen gas, and PET/SIOx/PE under nitrogen gas [3]. The time of dried walnut kernels was also evaluated using three types of coverings namely: PVC, LDPE/PA/LDPE, and PA/PP. Two levels of vacuum of 0.85 and 0.72 kg/cm^2 were applied inside the packages and were preserved for one year at room temperature [4].

Another investigation was conducted for analyzing the effects of packaging material on undesired changes of walnut flour due to oxidation under different temperature during 26 weeks, and we measured peroxide value during this period [9].

The goal of the present study was to evaluate the effects of atmospheric conditions inside the packaging with different percentages of nitrogen, oxygen, and carbon dioxide gases and also the preservation temperatures in order to obtain a product with suitable quality and long preserving shelf life of fresh and

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raw shelled walnut kernel.

II. MATERIALS AND METHODS

A. Walnut Kernel

Walnuts of Kermani cv. species (*J. regia* variety) had been provided by Namavarane Sanate Mehr Company (Kerman-Iran) and were stored in plastic bags until the day of test at 4 °C. Age of walnuts harvested prior to three-month storage period was 5 months.

B. Packaging Materials

Transparent polymer covering of PE/PET (polyethylene and polyethylene terephthalate) with 98 μm thickness that had permeability about 13.5 $cm^3/m^2/atm/24h$ was obtained from Pak plastics (Tehran, Iran) and was used for packaging the walnut kernels. Bag dimensions were "20cm × 30 cm".

C. Design of Experiment

This study uses the advantages of response level method for designing experiments, estimating the effects of process variables, and also optimization of analytic conditions.

To pack the walnut kernels, first the green shell was separated, and the walnuts were shelled; about 80 g of obtained kernels were packed in polymeric bags, and the pouches were transferred to packaging laboratory for gas injection using the packaging MAP machine (Henkelman200A model, Germany). Then, three gas mixtures of oxygen, nitrogen, and carbon dioxide with percentages pre-determined using response level method were injected into the pouches. Then, the prepared samples were kept in incubator machine (Heraeus, Germany) at temperatures of 4 °C, 15 °C, and 26 °C for three months. The intended tests for evaluating the characteristics of walnut kernels were done after this time on the samples.

D. Oil Extraction and Peroxide Analysis

Oil extraction and peroxide analysis was done according to National Iranian Standard No. 37. First, about 10 g of walnut kernel was crushed. Then, a paper filter was turned into a funnel, and the crushed walnut kernels were put inside and was slowly entered into soxhlet. 200 ml of hexan was poured into a round bottom flask, and it was fastened to the holder. A few boiling stones were also put into the flask. Next, soxhlet and cooler were installed on the flask, and water tap was opened, so water could flow through the cooler. Then, a metal lattice was put under the flask and Bunsen burner was ignited in below. The extraction was continued for 5h. The heat was cut after 5h, and the system was left to cool a little. Flask ingredients were poured into a beaker and left until the solvent got evaporated; the obtained substance is oil.

About 4 to 5 g of the fat extracted from walnut kernels was poured in a 250-ml grounded Erlenmeyer flask, and 30 ml mixture of acetic acid plus chloroform was added. Then, 0.5 ml saturated solution of potassium iodide was added and left in dark environment for 1 min. 30 ml of distilled water and a few drops of starch solution were added, and it was titrated with sodium hyposulfite or sodium thiosulfate 0.01 normal

until the blue color was faded out [10].

The peroxide value in ml equivalent oxygen per kg extracted oil was calculated according to:

$$P = (1000 \times N \times V) / W \tag{1}$$

E. Color Changes

The color was evaluated using the Hunter Lab system and colorimeter (model D65/10). Hunter Lab system is used on the basis of three indices: l*, a*, and b* for evaluating the color. About 20 g of sample is put on the transparent glass container and is put on top of the machine which states the color of it on the basis of black/white (l*), red/green (a*), and yellow/blue (b*) [3].

F. Decreased Weight

The sample was weighted before and after preservation time using digital scale (Sartorius PT210). Weight loss was evaluated according the following formula and was reported as a percentage [11].

$$percentage\ of\ weight\ reduction = \frac{M_1 - M_2}{M_1} \times 100 \tag{2}$$

M_1 = weight of first sample, M_2 = weight of second sample.

G. Sensory Evaluation

Sensory evaluation was done through help of 10 non-smoking people who did not suffer from any food allergy.

An acceptance test with 5-point Hedonic scale classification was used as the evaluation method. 20 samples were coded as W_1 to W_{20}, put in similar containers, and given to 10 evaluators. Each evaluator had to evaluate and rate the samples in sense of taste, color, odor, texture fragility, and finally general acceptability. The point of 5 meant very acceptable, and 1 meant unacceptable [12].

H. Mould Counting Test

This test is conducted according to National Standard of Iran No. 997. First, the walnut kernels are crushed completely using electrical mill (Mulinex, France). Then, 5 g of sample was weighed using a digital scale (Ouauz GT2100, Germany) and poured into Erlenmeyer flask which has to be sterilized before. Then, about 45 ml of thinner solution (ringer, physiological serum, distilled water) is added to the 5 g of sample inside the Erlenmeyer flask and left for 5 min. Next, about 1 ml of obtained thinned solution is poured into a sterilized plate. About 20 to 25 ml of sterilized cultivation environment (Temperature of 45 to 50 °C) is added to the plate containing the sample; the plate is shaken and left alone so that the cultivation environment gets solid. The plate is then kept in incubator with the temperature of 25 °C for three to five days. The results are to be analyzed after this time passed [13].

I. Statistical Analysis

The Minitab 15 software, Response Surface Package (Central Composite Design) was used to program the treatments. The number of treatments was 20 according to this

software.

The model used for RSM, is generally of complete second order or reduced second order. Y was used in this study as the dependent variable or response, and the following second order equation was used for analyzing the results:

$$Y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_{11}X_1^2+\beta_{22}X_2^2+\beta_{33}X_3^2+\beta_{12}X_1X_2+$$
$$\beta_{13}X_1X_3+\beta_{23}X_2X_3 \quad\quad (3)$$

in which, Y is the predicted response, β_0 is a fixed coefficient, β_1, β_2, β_3 are the linear effects, β_{11}, β_{22}, and β_{33} are the quadratic effects, and β_{12}, β_{13}, and β_{23} are the interactive effects. Minitab software was used for analyzing the information and creating the graphs using response level method [14], [15].

III. Results and Discussion

A. Peroxide Value

The level of oxidation of fatty acids in walnut was measured using Peroxide value. Results of variance analysis showed that the model defined for peroxide value is statistically significant ($p<0.001$). The significant terms of model pertaining to this response were O_2 ($p<0.001$), CO_2 ($p<0.001$), temperature ($p<0.001$), O_2-CO_2 interaction ($p<0.05$), O_2-temperature interaction ($p<0.05$), and also CO_2-temperature interaction ($p<0.05$). Variations of peroxide value are shown in Figs. 1-3. Simultaneous effects of two variables O_2 and CO_2 on the level of peroxide value at fixed temperature of 15 °C are shown in Fig. 1. On this basis, peroxide value level increases as O_2 percentage goes higher, while any increase in CO_2 percentage will cause the peroxide value to decrease; and considering the significance of O_2-CO_2 interaction effect, the peroxide level declines in this diagram. Fig. 2 shows simultaneous effects of two variables, O_2 and temperature, on the level of peroxide value at fixed level of 5% CO_2. According to this diagram, peroxide value level increases as temperature and O_2 percentage increase. And the peroxide level is increased here considering the significance of O_2-temperature interactive effect. The simultaneous effects of CO_2 and temperature at fixed rate of 5% O_2 is shown in Fig. 3. As one can see, the CO_2-temperature interactive effect has caused peroxide level to decline. According to previous studies, unsaturated fatty acids are prone to oxidation in contact with O_2 and produce peroxides which intensify the oxidation and increase the environment temperature in turn [16]. Presence of CO_2 impedes the hydrolysis and oxidation process and consequent rancidity of oil due to its high solubility [8]. In reference [3], Mexis and Kontominas studied packaging the walnut under N_2 and air inside three different types of packaging at the temperatures of 4 and 20 °C. They observed that peroxide level found in packaged walnuts in contact with air at 20 °C was higher. In [4], Tajeddin used two different levels of vacuum inside the packages of previously dried walnuts, and compared them with unpacked walnuts. He concluded that walnuts which were packaged under vacuum conditions maintained lower peroxide levels.

B. Weight Loss

The results obtained from statistical analysis showed that the proposed model for decreased weight is significant ($p<0.001$). Significant term of the model pertaining this response was temperature ($p<0.001$). Fig. 5 shows the simultaneous effects of oxygen and temperature variables on decreased weight level at fixed rate of 5% CO_2. The reduced weight level decreases as the temperature rises. Since losing water is a main reason for the weight loss, it is evident that walnut samples are subject to weight loss because of higher temperatures. In [17], Raii et al. studied the effects of atmosphere inside the package on physical and chemical characteristics of pistachio and obtained similar results; they found that higher temperature causes loss of moisture in pistachio. In [4], Tajeddin has evaluated physical and chemical characteristics of raisin inside different types of packages and concluded that preservation condition is a main cause of moisture loss. He observed that the raisins maintained at lower temperatures can keep relatively more moisture content.
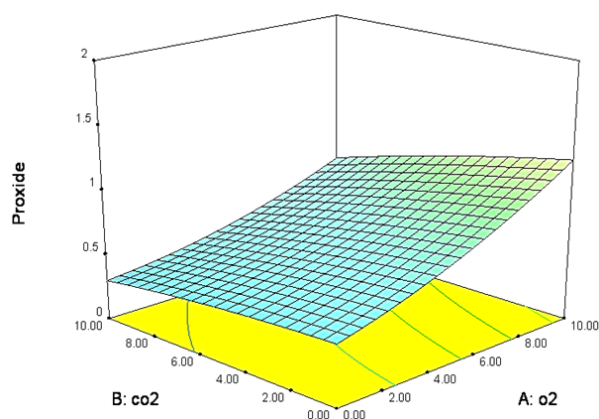

Fig. 1 3D diagram of simultaneous effects of two variables, O_2 and CO_2, on peroxide value
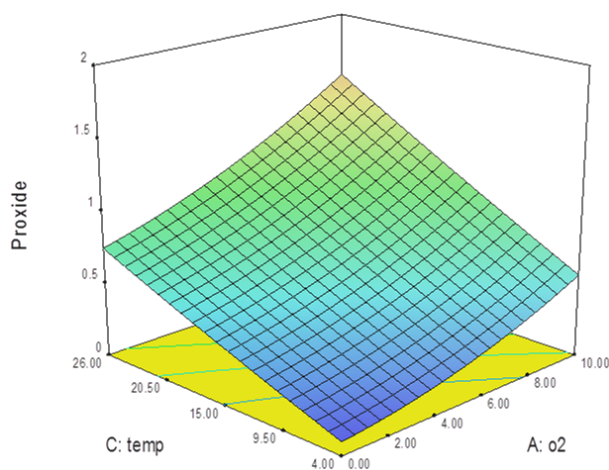

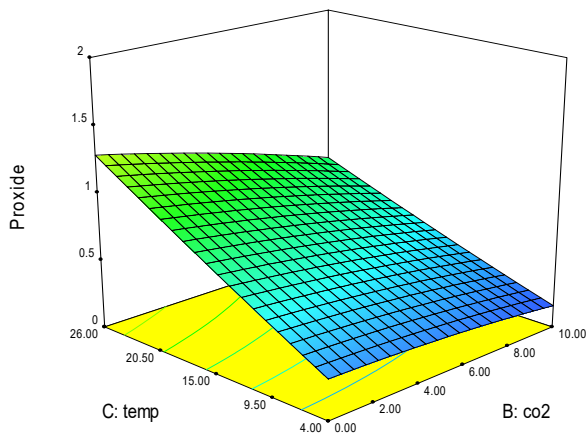Fig. 2 3D diagram of simultaneous effects of two variables, O_2 and temperature, on peroxide value

Fig. 3 3D diagram of simultaneous effects of two variables, CO_2 and temperature, on peroxide value
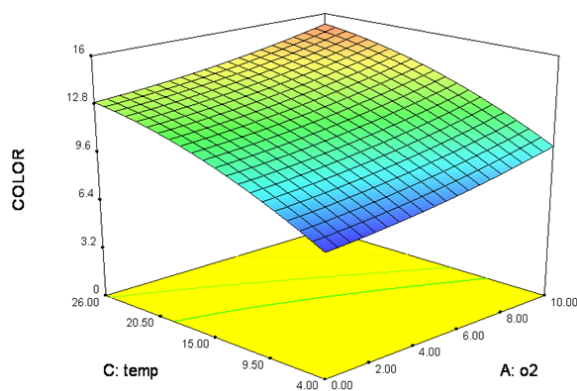

Fig. 4 3D diagram of simultaneous effects of O_2 and temperature variables on color variation
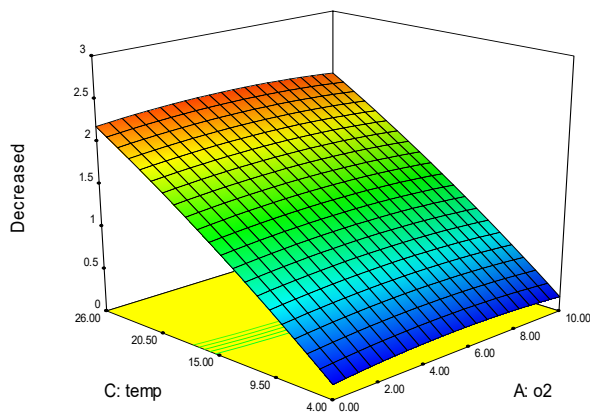

Fig. 5 3D diagram of simultaneous effects of two variables O_2 and temperature on decreased weight level

C. Sensory Evaluation

General acceptability of packaged walnuts was assessed using the Ranking method. The proposed model was significant (p<0.001) according to statistical analysis which was conducted using response level model. Significant terms regarding this response were: oxygen (p<0.01), and

temperature (p<0.001). Fig. 6 shows the simultaneous effects of oxygen level and temperature on the general acceptability of walnuts at a fixed rate of 5% carbon dioxide. As the diagram shows, general acceptability of walnut is decreased as temperature and oxygen percentage increase, also as shown in the figure, the effect of temperature on walnut acceptability decrease is bigger than the effect of oxygen percentage. According to the results of peroxide and color variation of walnut and the models of those responses being significant, we can justify these results such that an increase in oxygen level and temperature causes higher oxidation level of unsaturated fatty acids in walnuts, which in turn causes rancidity and changes smell and taste of the walnuts [16]. This situation decreases the level of its acceptability, also causes walnut color to turn darker in higher temperatures, and reduces its appeal [3].

D. Mould and Yeast Counting Test

Mould and yeast counting test was conducted in order to analyze the walnut packages for microbes. The results showed that no mould was found in any of walnut packages. In reference [17], Raii et al. studied pistachio packaging materials along with different gases, and concluded that high quality membranes and gases used in packaging and low humidity stopped moulds and pests from growing inside it, and that is the reason of pistachio durability
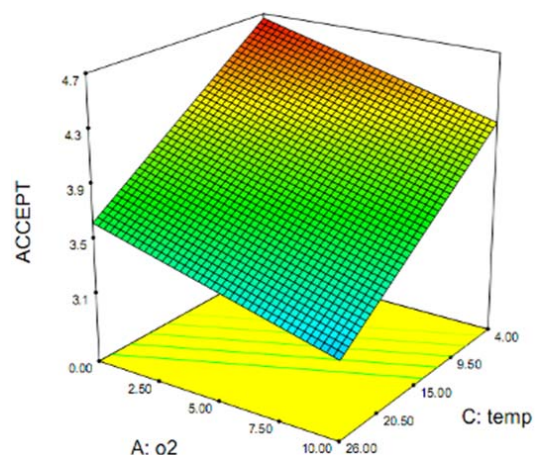

Fig. 6 3D diagram of simultaneous effects of O_2 and temperature level variables on general acceptability

E. Determining the Optimal Conditions

The optimal operating conditions were sought using the numeral optimization technique. To do this task, the optimization target was determined first, and then response levels and independent variable values were set. Consequently, the best responses were obtained. The settings applied on optimization process included minimum peroxide value, minimum color change, minimum decreased weight, and maximum general acceptability. The results of optimization process showed that the best optimal walnut packaging conditions are: 0.088 meqO$_2$/kg peroxide, color change of 8.43, decreased weight of 0.21%, and general

acceptability of 4.67, which are equivalent to 1.46% O_2, 10% CO_2, and temperature of 4 °C. The acceptability level would be 0.97 with that set of conditions.

IV. CONCLUSION

Storage quality of shelled walnut (*Juglans regia L.*) of packed in 50 μm PE/PET bag with different O_2/CO_2 compositions during three months at ambient temperature was studied. The test results were as follows: relatively low O_2 and high CO_2 storage conditions reduce the PV. Low O_2 and temperature reduce variation color and increase general acceptability of walnut, and low temperature reduces decreased weight.

ACKNOWLEDGMENT

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