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ScienceDirect
Journal of Food Engineering 86 (2008) 501–507
JOURNAL OF FOOD ENGINEERING
www.elsevier.com/locate/jfoodeng

Mathematical modeling, non-destructive analysis and a gas chromatographic method for headspace oxygen measurement of modified atmosphere packaged soy bread

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Received 12 June 2007; received in revised form 23 October 2007; accepted 3 November 2007
Available online 20 December 2007

Abstract

This study compared a mathematic model, non-destructive headspace analysis and gas chromatographic methods to monitor the oxygen concentration in modified atmosphere packaged soy bread with and without calcium propionate as a chemical preservative. The bread samples were packaged in low density polyethylene pouches with air as a control or flushed with 20% carbon dioxide:80% nitrogen mixture. Mold and yeast and aerobic plate counts in the bread were monitored when stored at 23 ± 2 °C and 38 ± 2% relative humidity. At 0, 2, 4, 6, 8, 10 and 12 days, samples were removed and the mold and yeast, aerobic plate count, non-destructive and destructive headspace oxygen contents determined. A mathematical model demonstrated that evolution of the package headspace gas composition resulted from the package permeation and respiration of the microorganisms. The mathematical model, non-destructive and gas chromatographic analytical methods can be used to predict the oxygen content in the headspace of the soy bread.

Keywords: Soy bread; Mold and yeast; Modified atmosphere packaging; Aerobic plate count; Non-destructive oxygen headspace monitoring

1. Introduction

Headspace oxygen within a packaged product is well known as a factor implicated in the deterioration of food quality (Rodriguez et al., 2000). The main factors affecting the amount of oxygen in the headspace of a packaged product (and therefore its shelf life) are the permeability of the material, any leaks in the container, chemical and microbial reactions within the product and the initial oxygen volume within the package (Johnson, 1997). Oxygen participates in many reactions which affect the shelf life of packaged foods. These include microbial growth, oxidation of lipids and consequent rancidity, and senescence of fruits and vegetables (Larsen et al., 2002). Thus, the oxygen headspace content of a package can be compared with the quality of the product if this headspace oxygen measurement is taken at the same time that sensory evaluation on the product is performed (Alli and Weddig, 1998).

Various methods have been used to monitor headspace oxygen in packaged foods. These include destructive and non-destructive methods. Non-destructive methods produce results in less time when compared with destructive ones. Also, in non-destructive methods, the package is not destroyed. This provides the advantage of testing the same sample repeatedly during shelf life experimentations. Another method to predict the headspace oxygen content of a package under specified conditions would be by the use of mathematical modeling. This technique reduces the need for both destructive and non-destructive testing methods. Mathematical modeling is cheap and does not require the need for expensive equipment or highly trained personnel. Irrespective of the method used to estimate the headspace oxygen in a test package, the accuracy and reliability of the method is essential.

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doi:10.1016/j.jfoodeng.2007.11.001

This study evaluated three different methods for predicting the headspace oxygen concentration in modified atmosphere packaged samples of soy bread. The results from this were compared with those from control samples packaged in air. The results from both the non-destructive method and the mathematical model were compared with those from a conventional gas chromatographic (GC) method for the headspace oxygen determination. The use of the GC method is well documented and has served the food industry and researchers well for this type of analysis (Alli and Weddig, 1998; Alves et al., 2001; Larsen et al., 2002; Miltz and Perry, 2005; Soares and Hotchkiss, 1999).

In predicting the headspace oxygen level in a hermetically sealed package during storage, several factors must be considered. These include the permeability of the material used to fabricate the package, the initial oxygen content within the headspace and its concentration in the surrounding atmosphere, the ambient temperature, the material's dimensions and any reactions that may occur between the packaged product and the oxygen within the headspace. During this study, these factors were considered and were factored into the mathematical model and into the calculations used for the oxygen headspace quantification.

The objectives of this study were: (1) to compare the use of a non-destructive technique to monitor headspace oxygen in modified atmosphere packaged soy bread with that of a traditional destructive gas chromatographic method and; (2) to develop a mathematical model to predict the headspace oxygen concentration of the soy bread packages and compare it with the experimental values obtained during the study.

2. Materials and methods

2.1. Soy bread

The soy bread samples used in this study, the ingredients used to prepare them and the baking protocol are explained in a research paper authored by Fernandez et al. (2006). After the baking step, the soy bread was cooled, sliced and packaged in a plastic bag. Each loaf had average weight, volume and density of 900 g, 1744 cm^3 and 0.52 g/cm^3, respectively. The average loaf volume (cm^3) was determined using the rapeseed displacement method (AACC 2000, Method 10-05). One hundred and twenty loaves were prepared.

2.2. Packaging materials

The film used to package the soy bread was a 50.8 μm thick low density polyethylene (LDPE) film obtained from Central Ohio bag and Burlap Inc., (Columbus, OH). To minimize and unify the headspace volume within each package, the film was cut into equal sizes and pouches measuring approximately 25.5 × 39 cm were fabricated using a Model 12SC/1 Sencorp impulse sealer (Sencorp Systems, Hyannis, MA). After the sliced soy bread samples were placed into these pouches, they were divided into three equal groups. All samples were hermetically sealed and packaged in at least one of two headspace gases. For atmospheric packaged samples (control), the pouches were sealed using the impulse sealer set at 207 kPa (30 psi), 14 V and 0.85 s dwell time. An Ultravac 2100D vacuum packaging machine (Koch LLC, Kansas City, MO) was used to package the samples with 20% CO_2:80% N_2 headspace gases obtained from Praxair (Columbus, OH). This sealer was set at 99% vacuum for 10 seconds, 30% headspace gas and a dwell time of 0.85 s. All samples were stored at 21 ± 3 °C and 38 ± 2% R.H. The mixed gases had a purity of 99.9%.

2.3. Microbial analytical method

The aerobic plate count (APC) was performed using a half of a slice (30 g of sample) of the bread from each sample loaf. It was homogenized in a Model STO-400 Tekmar stomacher (Lorton, VA) for 2 min with 270 ml sterile 0.1% peptone solution (homogenate). A 0.1 ml aliquot of the homogenate was removed from each sample and a tenfold dilution made using the peptone solution. A 0.1 ml aliquot from the final diluted solution was then removed and plated in duplicate on standard plate count agar (Difco Laboratories, Sparks, MD). After incubation for 1–2 days at 37 ± 1 °C in an incubator, the APC was determined and expressed as colony forming units (CFU) per gram of soy bread.

For the mold and yeast count (M + Y), approximately 100 g of the bread crust were similarly homogenized, but in this case, 400 ml of sterile 0.1% peptone solution were used. From this solution, 1 ml was removed and a tenfold dilution prepared. From the final dilution, a 0.25 ml aliquot was removed then plated in duplicate on standard plate count agar containing tetracycline (5 mg/ml) and chloramphenicol (5 mg/ml). The plates were all incubated for 4 days at 23 ± 1 °C, then the CFU counted and expressed as log CFU/g. All microbial analyses were performed on the soy bread samples after 0, 2, 4, 6, 8, 10 and 12 days of storage at 23 ± 2 °C. These samples were tested in triplicate.

2.4. Gas transmission rate determination

The oxygen gas transmission rate (OTR) for each film was determined using a two station Mocon Ox-Tran model 2/21 permeability tester produced by Mocon Inc. (Minneapolis, MN) according to the ASTM F 1927 method. The tester was equipped with a coulometric detector sensitive to an oxygen transmission rate of 0.01 cc m^{-2} day^{-1}. This detector was sensitive to the presence of oxygen molecules and produced an electrical signal proportional to its quantity. Measurements of the OTR for the film were carried out at 23 °C and 0% R.H. until steady-state oxygen transmission was achieved. The area of film exposed to

the oxygen was 5 cm^2. These films were tested by flushing the permeant side of the test cell on the equipment with 21% oxygen at 10 ml/min and at 1 atm. The Ox-Tran was connected to a desktop computer and output values were expressed as oxygen transmission rate in cc m^{-2} day^{-1}. Periodically, a standard film supplied by Mocon Inc. was used to check the accuracy of the equipment. The OTR measurements of this film were within ±1% of the expected value. This was considered acceptable since the manufacturer recommended a ±2% measurement for acceptable accuracy. The analyses of the test samples were performed in triplicate and the mean and standard deviation were recorded. The OTR was determined using the following equation:

$$\mathrm{OTR} = \frac{(E_e - E_0)}{(A \times R_L)} \times Q \tag{1}$$

where E_e is the steady-state voltage level with oxygen gradient applied to test film, E_0 is the zero voltage level, A is the specimen area, Q is the calibration constant and R_L is the value of load resistance.

The gas transmission rates for CO_2 and nitrogen were estimated after considering the permselectivity for generic LDPE as reported by Brandrup and Immergut (1989), and assuming that this material was tested at the same conditions as our LDPE samples. Accordingly, the CO_2 transmission rate is four times the OTR and for N_2 it is $\frac{1}{3}$ the rate of oxygen.

2.5. Non-destructive headspace oxygen analysis

All soy bread samples were analyzed for changes in headspace oxygen during storage, using an Oxysense™ 101 (Las Vegas, NV), non-invasive oxygen analyzer. The oxygen measurements were achieved by attaching an oxygen sensor (O_2 XYDOT)™ on the inside of each package using a 5 mm^2 piece of double sided tape. Once this was done, the package was sealed as described previously. During each headspace test, the voltage was set at 500 ± 30 by adjusting the gain control. The calibration constants for the sensor and sample temperature were entered into the program of the equipment. These constants are given by the manufacturer when each batch of oxygen sensors is purchased. These constants are used as a means of correcting for changes in temperature during a normal test. During each test, the transducer was placed directly over the sensor and the measurement taken under reduced ambient light. The partial pressure of oxygen in the headspace of the package is based on the effect of the oxygen on the fluorescence lifetime of an optically excited ruthenium complex. This complex was present in each sensor. When oxygen was present, the fluorescence was quenched due the collision of oxygen molecules with the excited ruthenium molecules. During the collision, energy was transferred from the ruthenium to the oxygen and this changes the emission intensity. This quenching resulted in a decrease in the fluorescence lifetime and was proportional to the oxygen partial pressure in the package (Draaijer and Konig, 2001). The headspace oxygen content expressed as a percentage was monitored for each sample during its storage until the day prior to removal from storage for the microbial analysis.

Typical fluorescent lifetime of the polymer described above varies between 1 μs in ambient air ($_pO_2 = 212$ mbar at sea level) and 5 μs in zero oxygen. Saini and Desautel (2002) described the monoexponential fluorescence decay by

$$\frac{I}{I_0} = \exp\left[\frac{(-t)}{(\tau)}\right] \tag{2}$$

where I is the fluorescence intensity at any time (t), I_0 is the fluorescence intensity at the start of the decay (in this case $t = 1$ μs), t is the time in μs and τ is the fluorescence lifetime or time constant.

This time constant was calculated from a monoexponential least squares fit of the fluorescence signals generated by the fluorescent polymer and the oxygen concentration was calculated from the time constant. There was therefore a relationship between the oxygen partial pressure and the fluorescence lifetime and it was determined from the Stern–Volmer equation:

$$\frac{\tau_0}{\tau} = 1 + K_{sv} \times {}_pO_2 \tag{3}$$

where τ is the time constant at current oxygen concentration, τ_0 is the time constant in the absence of oxygen, K_{sv} is the Stern–Volmer constant and $_pO_2$ is the oxygen partial pressure.

Since both the τ_0 and the Stern–Volmer constant depend on temperature, a correction compensates for changes in the ambient temperature and the software in the Oxysense™ non-destructive analyzer accounts for this in the headspace oxygen determination.

2.6. Mathematical model

A material balance was designed in order to develop the model to monitor the oxygen concentration of the modified atmosphere packaged soy bread. The gas composition inside the LDPE pouches was governed by the initial headspace gas composition, the transmission rate of the diverse gases through the packaging materials, the oxygen consumption and carbon dioxide emission rates of yeast and molds and the aerobic bacteria within the packaged soy bread. Therefore, the oxygen quantity change inside the package (in cc (STP)/day) can be expressed by

$$\begin{aligned}\frac{d(V(O_2))}{dt} &= \frac{d(V(O_2)_{permeation})}{dt} - \frac{d(V(O_2)_{micro})}{dt} \\ &= \frac{S \cdot G(O_2) \cdot ({}_p(O_2)^o - {}_p(O_2)^i)}{L} \\ &\quad - [N_M \cdot G_M \cdot {}_p(O_2)^i + N_B \cdot G_B \cdot {}_p(O_2)^i]\end{aligned} \tag{4}$$

where S is the package surface area (m^2), $G(O_2)$ is the oxygen permeability constant $\left(\frac{\text{cc mil}}{m^2 \text{ day atm}}\right)$, $({}_p(O_2)^o - {}_p(O_2)^i)$

expresses the difference in oxygen partial pressure (atm) between the external and the internal atmospheres of the package, L is the material thickness of the pouch (mil), G_M and G_B are the rate constants for oxygen consumption $\left(\frac{cc}{cfu\ day\ atm}\right)$ by the molds and bacteria, respectively, N_M and N_B are the moulds and bacteria counts per gram of bread. We make the assumption that the gases within the pouches are at standard conditions of 21.1 °C and 101.3 kPa, and remain at these conditions during the study. At these conditions, 1 mol of each constituent gas generally occupies 24.173 l. Similarly, a gas transmission rate of 1 cc/m^2 day converts to 4.137×10^{-5} mol/m^2 day.

The first term on the right-hand side of Eq. (4) is Fick's Law for mass transfer across a plastic film. The second term is the rate of oxygen consumption by yeast/mold and bacteria in the soy bread. Considering that microorganisms exhale CO_2 and consume O_2 in a 1:1 ratio, the equation for the evolution of CO_2 quantity in the headspace (expressed in cc (STP)/day) can be written as

$$\begin{aligned}\frac{d(V(CO_2))}{dt} &= \frac{d(V(CO_2)_{permeation})}{dt} + \frac{d(V(CO_2)_{micro})}{dt} \\ &= \frac{S \cdot G(CO_2) \cdot ({}_p(CO_2)^o - {}_p(CO_2)^i)}{L} \\ &\quad + [N_M \cdot G_M \cdot {}_p(O_2)^i + N_B \cdot G_B \cdot {}_p(O_2)^i] \end{aligned} \quad (5)$$

Finally, since the bag is flexible, and the volumes of O_2 and CO_2 exchanged do not necessarily balance, the potential exchange of nitrogen by permeation (expressed in cc (STP)/day) was determined by

$$\begin{aligned}\frac{d(V(N_2))}{dt} &= \frac{d(V(N_2)_{permeation})}{dt} \\ &= \frac{S \cdot G(N_2) \cdot ({}_p(N_2)^o - {}_p(N_2)^i)}{L} \end{aligned} \quad (6)$$

The partial pressure of oxygen and the number of microorganisms, variables in Eqs. (4)–(6) are time dependent and this dependency has to be considered in order to predict the variations of all gases. Also, the number of microorganisms within the package is time dependent and the growth kinetics of both yeast/molds and bacteria can be expressed by a Gompertz equation, as follows:

$$\log(cfu) = \log(cfu_0) + a \cdot \exp\left(-\exp\left(\frac{t_0 - t - t_1({}_p(O_2) - 0.21)/0.21 + t_2}{b - b_1({}_p(O_2) - 0.21)/0.21}\right)\right) \quad (7)$$

where cfu_0 is the initial count and the other three parameters of the equation are related to the initial growing time (t_0), the difference between the number of logs from the start to the stationary state (a) and the growth slope (b). The data for this Gompertz equation were obtained from the initial studies on this project as reported by Fernandez et al. (2006). Bread loaves, with and without preservative, were stored in diverse atmospheric conditions, and the microbial growth was monitored as described in the experimental section. Table 1 shows the experimental values used for the Gompertz parameters for aerobic bacteria, molds and yeasts. The parameters t_1 and b_1 in the exponential account for the delay and the rate decrease caused by the reduction of oxygen partial pressure in the modified atmosphere packaged samples, while the t_2 parameter reflects the delay caused by the presence of the preservative. Therefore, the number of bacteria (N_B) and yeast/molds (N_M) are

$$N_B = 10^{\log(cfu)_B} \quad \text{and} \quad N_M = 10^{\log(cfu)_M} \quad (8)$$

The increment with time of the number of microorganisms can be calculated by derivation of Eq. (8):

$$d(N_{B,M}) = \frac{d(N_{B,M})}{dt} \cdot dt \quad (9)$$

By integrating Eq. (4), a mathematical expression can be obtained to predict the headspace oxygen at different storage time for the diverse soy bread samples in the storage conditions. In this work, Eq. (4) has been numerically solved by using the finite increment method. The method consists of changing dt by Δt and considering that

$$\begin{aligned} {}_p(O_2)^i_{t+\Delta t} &= {}_p(O_2)^{i_{t+\Delta t}} + \Delta t \cdot \frac{d({}_p(O_2)^i)}{dt} \\ N_{B,M_{t+\Delta t}} &= N_{B,M_t} + \Delta t \cdot \frac{d(N_{B,M})}{dt} \end{aligned} \quad (10)$$

Thus, the final mathematical model to predict the quantity of oxygen in the package headspace is

$$\begin{aligned} V(O_2)_{t+\Delta t} &= V(O_2)_t + \Delta V(O_2) \\ &= \frac{S \cdot G(O_2) \cdot [{}_p(O_2)^o - {}_p(O_2)^i]_t}{L} \cdot \Delta t \\ &\quad - [(N_M)_t \cdot G_M W_b({}_p(O_2)^i)_t \\ &\quad + (N_B)_t \cdot G_B W_b({}_p(O_2)^i)_t] \cdot \Delta t \end{aligned} \quad (11)$$

Similar equations were obtained to describe the evolution of CO_2 and N_2. The headspace volume (V_{HS}) evolution is calculated as

Table 1
Parameters of the Gompertz equation used in Eq. (7)

Microorganisms	Gompertz parameters						
	a	b	t_0	cfu_0	t_1	t_2	b_1
Bacteria	5.7 ± 2.3	2.0 ± 0.2	0.9 ± 0.2	2.3 ± 0.3	0.5 ± 0.3	2.0 ± 0.7	1.0 ± 0.5
Molds and yeasts	6.7 ± 1.7	1.3 ± 0.3	3.0 ± 0.8	1.3 ± 0.3	0.5 ± 0.3	2.0 ± 0.7	1.0 ± 0.5

$$V_{HS,t+\Delta t} = V_{HS,t} + \Delta V(O_2) + \Delta V(CO_2) + \Delta V(N_2) \quad (12)$$

and therefore, the partial pressure of each gas at anytime can be determined by the ratio of the volume of the gas over the total headspace volume.

2.7. Destructive oxygen headspace determination

The headspace gas in each test package was analyzed for oxygen by removing a 100 μl aliquot through a self sealing silicon septum using a gas tight syringe. The self sealing septum was previously attached to the outside of each package. Headspace oxygen in the pouches was measured by injecting the 100 μl headspace gas into a Hewlett–Packard 5890 gas chromatograph (GC) equipped with a thermal conductivity detector. The GC was fitted with a stainless steel column (1.8 m × 0.32 cm) packed with a 80/100 Molecular Sieve 13× (Alltech Asso Inc., IL). The carrier gas used was high purity (99.995%) helium at a flow rate of 20 ml/min. The temperatures of the injection port, oven, and detector were 120, 40, and 150 °C, respectively. The gas chromatographic peak was measured by electronic count using a HP 3396A integrator.

For this destructive test, a total of 54 samples were analyzed. These were randomly drawn from the pool of 120 samples prior to the microbial analyses. Each sample was tested three times and the values averaged. This pool of 120 samples was made up of equal numbers of samples exposed to different levels of the preservatives and packaged in the control and mixed gas atmospheres.

2.8. Statistical analyses

The data collected from the microbial analyses were statistically analyzed using ANOVA with significance determined at $P < 0.05$. Samples that were designated as non-detectable counts were those with numbers of $\leqslant 2\log(CFU/g)$ (limits of detection for APC). The statistical analyses were conducted separately for the samples with and without preservative to determine the effect of preservative on the APC of the samples packaged with each of the two headspace gases. The statistical analyses were performed using the SAS computer software (SAS Institute, Inc., Cary, NC 1999), version 9.1. Means and standard deviations were calculated for the loaf volumes measurements using Microsoft Excel.

3. Results and discussion

The results for the oxygen permeation rate for the homopolymer LDPE packaging film was 6180 ± 180 cc mil/m^2/day. Each package had a headspace volume of approximately 900 ml. This also included the air trapped within the matrix of the bread. The surface area of the material used to fabricate each pouch was 0.139 ± 0.002 m^2.

Fig. 1 shows the mean headspace oxygen concentration in the samples with and without preservative and packaged in the control atmosphere. This figure shows that the headspace oxygen content in the samples decreased during the storage time. For samples without preservative, the reduction in the oxygen concentration was faster than for samples with preservative. The statistical analyses showed that the preservative had a significant effect ($P < 0.05$) on the headspace oxygen content in the soy bread packages. The results show that it took 7–8 days for the oxygen to be depleted in the packages without the preservative. For the packages with preservative, it took approximately 10 days for the oxygen depletion. Both these results were obtained by the use of the non-destructive oxygen headspace analytical method and the mathematical model. Fig. 1 shows that either method produced similar results.

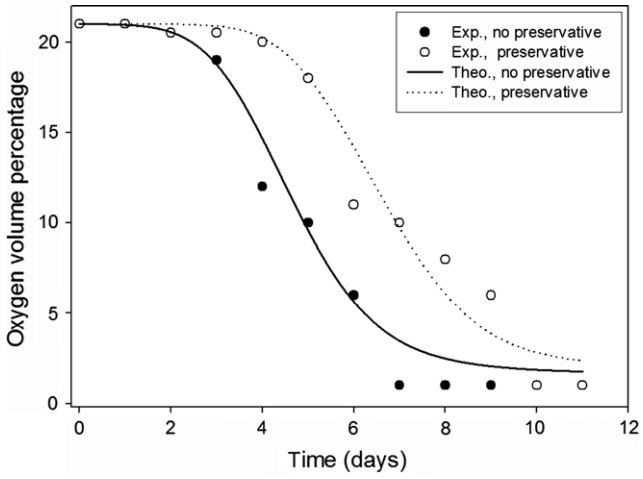

Fig. 1. Headspace oxygen analysis in soy bread with and without preservative initially packaged with air, tested by the experimental and mathematical model methods.

Fig. 2 shows the mean headspace oxygen concentration for the soy bread packaged in the modified atmosphere of 20% CO_2:80% N_2. Like Fig. 1, this figure shows that the

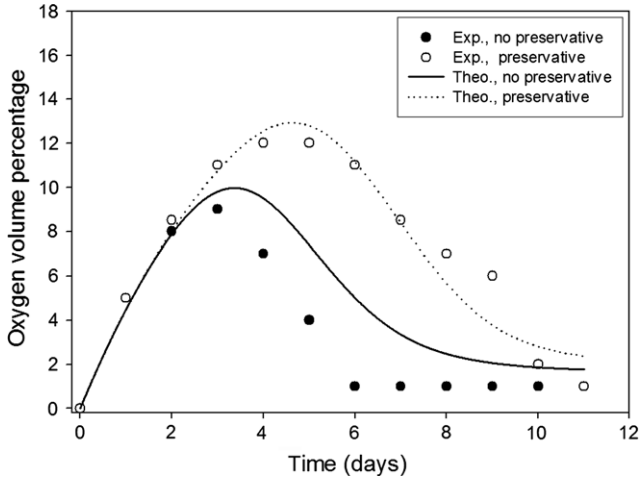

Fig. 2. Headspace oxygen analysis in soy bread with and without preservative initially packaged with the 20% CO_2:80% N_2 atmosphere, tested by the experimental and mathematical model methods.

packages with soy bread having the preservative had a lower headspace oxygen content when compared with the samples without the preservative. The statistical analysis also showed that there were significant differences ($P < 0.05$) between the headspace oxygen levels. Fig. 3 also shows that the oxygen level was almost depleted after 6 days of storage for the bread without preservative but it took 10–11 days for depletion in the samples with the preservative.

Both Figs. 1 and 2 show that the mathematical model produced similar results when compared with the experimental data. These figures thus show that the model could be relied upon to predict the headspace oxygen levels in the test packages. If the results in these figures (1 and 2) are compared with the Gompertz model for the microorganisms in Fig. 3, it can be seen that the APC was higher in the samples without the preservative. There thus appears to be a correlation between the APC, the preservative and the headspace oxygen concentration. Indeed, the statistical analysis does show that the preservative alone and in combination with the headspace oxygen concentration, significantly ($P < 0.05$) influenced the APC. This result also shows that an estimate of the headspace oxygen concentration can be achieved for soy bread packaged under modified atmosphere conditions when compared with bread packaged under normal atmosphere. This same approach could also be used to estimate the shelf life of soy bread prepared with calcium propionate (as a preservative) when compared with bread prepared without this additive. Since the mathematical model produced results similar to those of the experimental data collected for the headspace oxygen analysis, we could reasonable conclude that the mathematical model could be used as a predictive tool for soy bread quality control.

Fig. 4 shows the mold and yeast counts using the Gompertz and the experimental results. In general, this figure shows that the preservative had little effect on the molds and yeast counts whether in the control or the mixed gas atmosphere. The only exception to this was seen in the lower counts for the Gompertz model estimates in the mixed gas atmosphere. This count was lower that the values obtained for the other samples.

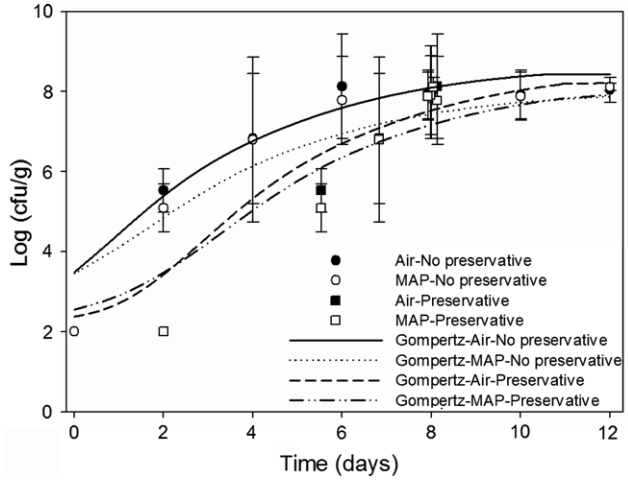

Fig. 3. The APC (expressed by the Gompertz model and experimental values) in soy bread with and without preservative in the control and mixed gas atmospheres.

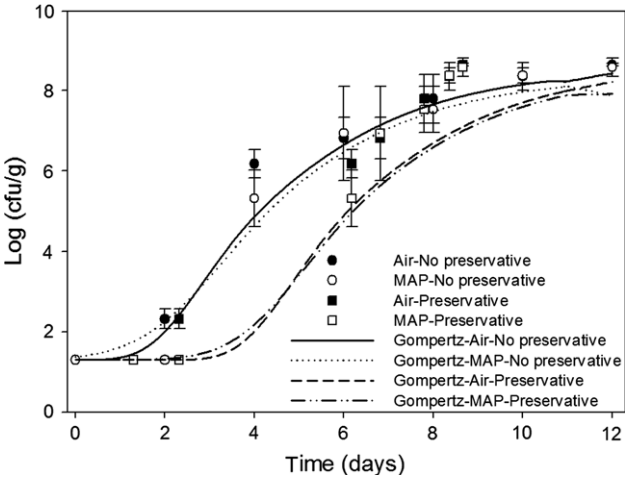

Fig. 4. The yeast and mold counts (expressed by the Gompertz model and experimental values) in soy bread with and without preservative in the control and mixed gas atmospheres.

Fig. 5 shows a comparison of the results obtained for the oxygen headspace analysis on random samples using the Oxysense™ non-destructive and the traditional GC destructive method. This figure shows that there was little difference between the values obtained by both methods. This shows that the non-destructive method for headspace oxygen analysis is as reliable as the traditional GC method. Since the mathematical model for predicting the oxygen levels in the headspace was comparable with that of the non-destructive method, we can also conclude that the mathematical model is also reliable.

An observation of the headspace oxygen results shows that the microorganisms in the samples with preservative depleted the available oxygen much slower than in the samples without preservative. This is so because the presence of the preservative limited the growth of these organisms. Thus, the greater the microbial counts, the quicker the oxygen consumption within the headspace of the package. Our results are similar to those obtained by Lambert et al. (1991) who reported that oxygen decreased more slowly within packaged pork samples subjected to a preservation technique when compared to those not subjected to the preservation technique.

When the headspace oxygen results for the samples packaged with the mixed gases are considered, it can be seen that the oxygen level in the headspace increased significantly before falling to near zero in at least 6–7 days. In the case of low barrier LDPE films, like the one used in this study, it can be deduced that the oxygen permeation into the package was high enough to compensate for the microbial consumption of oxygen during the early stage of storage. This deduction is supported by De Oliveira et al.

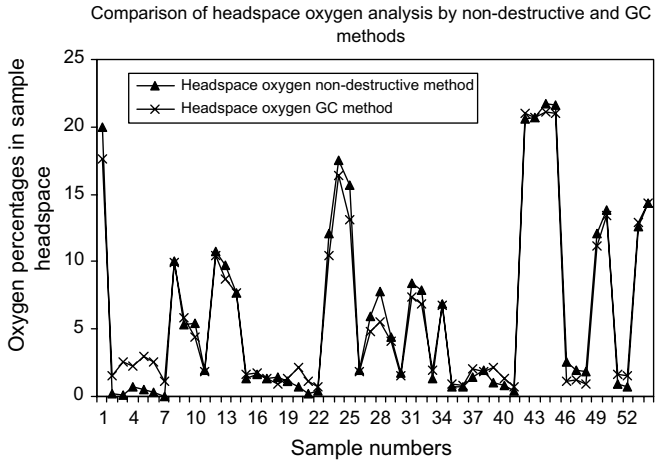

Fig. 5. Comparison of headspace oxygen analysis by the non-destructive and the GC methods.

(2001) who explained that the tendency for oxygen levels to increase in the headspace of MAP samples will occur if the barrier properties of the material are too low. This will allow the oxygen transmission into the package to exceed the quantities consumed by the microorganisms and any oxidation reactions that takes place within the product. Jansson et al. (2001) supports this explanation by stating that the rise in oxygen levels in MAP samples could result from the high permeability of the film. Our study also showed that after the initial increase, a continuous decrease in the headspace oxygen was observed and this happened because the oxygen depletion was greater than the oxygen ingression from the outside of the package. As explained earlier, the increase in the oxygen concentration from day 0 to day 1, might also be due to the diffusion and equilibrium of oxygen from the matrix of the bread to the headspace environment as explained by Pergiovanni and Fava (1997). Since bread is a spongy product, it was extremely difficult to evacuate all the oxygen from its headspace during the vacuum packaging process. Similar findings were reported by Fitzgerald et al. (2001) who found a great variability in the initial headspace oxygen contents in MAP bread samples.

4. Conclusions

From the data reported, it can be concluded that: (1) The microbial growth in soy bread with preservative was lower than the growth in samples without the preservative; (2) During storage, the oxygen headspace concentration decreased more in the packages with bread without the preservative when compared with samples treated with the preservative; (3) The mathematical model and the non-destructive method of headspace analysis using the Oxysense analyzer are as reliable as the traditional gas chromatographic method.

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