Oxygen and Carbon Dioxide Permeability of Wheat Gluten Film: Effect of Relative Humidity and Temperature

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The combined effect of relative humidity (RH) and temperature on O_2 and CO_2 permeability and selectivity (CO_2 to O_2 permeability ratio) of a wheat gluten film was evaluated using response surface methodology. Studied ranges of RH and temperature were 0−100% and 3−45 °C, respectively. CO_2 and O_2 permeabilities ranged from 88 to 55 580 and from 77 to 1970 amol m^{-1} s^{-1} Pa^{-1}, respectively. RH had an exponential effect on the CO_2 and O_2 permeabilities and selectivities of wheat gluten film. The effect of temperature appeared to be less pronounced in comparison with that of RH. High selectivity values (28 at 24 °C and 100% RH) of wheat gluten films would be very advantageous for fresh fruit and vegetable preservation under modified atmospheres.

Keywords: *Wheat gluten films; edible films; gas permeability; selectivity*

INTRODUCTION

Fruits and vegetables undergo progressive deterioration immediately after harvest. Postharvest deterioration occurs mainly through processes of physiological change and moisture loss (Pech et al., 1994; Kader, 1988). Modification of the atmosphere surrounding horticultural commodities is a method used to extend shelf life and to improve quality (Exama et al., 1993). In modified atmosphere packaging (MAP), the natural process of product respiration is used to reduce O_2 and increase CO_2 in conjunction with adequate gas exchange through a package. The low O_2 and increased CO_2 concentrations slow down respiration and thus ripening and senescence (Cameron et al., 1989; Chau and Talasila, 1994; Kader et al., 1989).

Polymeric films semipermeable to gases are the most popular to create MAP. However, for actively respiring fruits and vegetables commercially available polymeric films have not been very successful, because these films are not sufficiently permeable to O_2 (Yam and Lee, 1995; Exama et al., 1993). Thus, the quantity of O_2 in the package drops rapidly, inducing detrimental anaerobic conditions (Kader et al., 1989). To overcome this problem, the potential of edible films to generate a favorable MAP has been considered (Gennadios and Weller, 1990; Gontard and Guilbert, 1994; Koelsch, 1994; Guilbert et al., 1996). Edible films based on wheat gluten proteins have proved to have good film-forming and gas permeability properties (Gontard, 1991; Gennadios and Weller, 1990).

Barrier properties of films are affected by temperature and, in the case of synthetic or natural hydrophilic films, by humidity (Pascat, 1986; Mannheim and Passy, 1985). Gas permeability measurements of wheat gluten films, generally conducted at fixed temperature and dry conditions (Gennadios and Weller, 1990; Aydt et al., 1991; Gennadios et al., 1993a), have shown high oxygen barrier properties. In another study O_2 permeability of wheat gluten films was determined at different temperatures and 0% relative humidity (RH) (Gennadios et al., 1993b). Results showed very good agreement with the Arrhenius model. Recent studies have shown that O_2 and CO_2 permeabilities and gas selectivity (CO_2 to O_2 permeability ratio) of wheat gluten film are very sensitive to RH (Gontard et al., 1996). These authors also found that wheat gluten film tends to have a high gas selectivity value, which is of particular importance for MAP applications to fresh fruits and vegetables.

At present, no work has dealt with the effects of both temperature and RH on gas permeability of wheat gluten film, whereas this knowledge is required, particulary in a range of climates and handling conditions, to evaluate their real applicability in MAP systems.

The effect of temperature and RH on physical and mechanical properties of wheat gluten film has been related to glass transition. Under these conditions, the films exhibit so-called anomalous or non-Fickian sorption and diffusion behavior with the penetrants (Gontard, 1991).

Due to the lack of gas permeability data and the complexity of the combined effect of temperature and RH on gas permeability of protein-based films, further research is necessary. Therefore, to obtain an overall view and acquire practical information, the present study was undertaken to investigate the combined effect of temperature and RH on oxygen and carbon dioxide permeabilities of a wheat gluten film, using response surface methodology.

MATERIALS AND METHODS

Film Preparation. The Gontard et al. (1992) procedure was used. The film-forming solution (100 mL) was prepared using 7.5 g of gluten (Amylum Aquitaine, Bordeaux, France), 0.025 g of sodium sulfite (Prolabo, Montpellier, France), 45 mL of ethanol (Carlo Erba, Milano, Italy), 1.5 g of glycerol (Labover, Montpellier, France), and distilled water. pH of the solution was brought to 4 using acetic acid (Baker, Deventer, Holland). All of the components were mixed vigorously and warmed at 45 °C. The film-forming solution was then spread onto a plexiglass leveled surface using a thin-layer chromatography applicator and dried at 30 °C in a ventilated oven for 12 h.

Film Thickness. The thickness of the film was measured with a micrometer (Braive, Checy, France). Ten readings were taken at random positions around the film, and the results were averaged. Films used in permeation tests had a thickness of 80 ± 3 μm.

Film Conditioning. To shorten the time needed to reach steady state during permeability measurements, each gluten

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Table 1. Coded and Real Values for the Variables of the Central Composite Design

coded value	−1.41	−1	0	1	1.41
T (°C)	3	9	24	39	45
RH (%)	0	14.6	50	85.3	100

film was placed in a desiccator with one of several saturated salt solutions (Multon et al., 1991) and kept for 5 days in a constant-temperature chamber before being used for the permeation tests.

Permeability Measurement. Oxygen and carbon dioxide permeability measurements were carried out according to the isostatic method as described by Gontard et al. (1996).

The permeation cell consisted of two stainless steel chambers separated by a film sample. The testing gas was directed into one chamber, and pure nitrogen gas was directed into the other. Pressures in the chambers were equalized and maintained at atmospheric pressure. The nitrogen and oxygen or carbon dioxide streams were humidified by incorporating a humidifying device in each stream. It consists of two glass flasks containing a saturated salt solution through which a stream of gas was bubbled, before entering into the cell chamber. When necessary, instead of a saturated salt solution, the second bubbling flask contained a glycerol-distilled water solution (ASTM, 1954) to fit the exact RH.

The concentration of permeant gas in the carrier stream was measured using a gas analyzer PAK 12X (Abiss, Villemoisson, France). The gas analyzer is equipped with an electrochemical oxygen detector and an infrared sensor to determine the carbon dioxide concentration. Flow rates were measured with a Chromoptic flow meter (Auxerre, France).

At steady state (constant oxygen or carbon dioxide concentration in the nitrogen stream), flow rate and gas composition of the nitrogen stream were measured and used to calculate oxygen or carbon dioxide permeability. Permeability (*P*) was calculated as described by Chao and Rizvi (1988)

$$P = J(\Delta x)/A(\Delta p) \quad (1)$$

where P is the CO_2 or O_2 permeability (amol s^{-1} m^{-1} Pa^{-1}), J is the transmission rate of CO_2 or O_2 (amol/s), Δx is the film thickness (m), A is the surface area of the film (m^2), and Δp is the differential partial pressure of the permeant gas across the film (Pa) (amol = 10^{-18} mol).

Selectivity is the CO_2 permeability to O_2 permeability ratio.

Experimental Design. A central composite design was chosen for this study (Gacula and Sing, 1984). The variables are temperature and RH. Each variable was coded at five levels: −1.414, −1, 0, 1, and 1.414 (Table 1).

Statistical Analysis. Statgraphics (1988) software was used to conduct the response surface methodology. A second-order polynomial expression of two variables was fitted

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \quad (2)$$

where Y represents the response (O_2 permeability, CO_2 permeability, or selectivity); X_1 and X_2 are the codified values of temperature and relaive humidity, respectively; b_0 is a constant; b_1 and b_2 are linear coefficients; b_{12} is the cross-product coefficient; and b_{11} and b_{22} are quadratic coefficients.

RESULTS AND DISCUSSION

The results are summarized in Table 2. CO_2 permeability ranged from 88 to 55 580 amol m^{-1} s^{-1} Pa^{-1} and O_2 permeability from 77 to 1970 amol m^{-1} s^{-1} Pa^{-1} when RH changed from 0 to 100%, at 24 °C. CO_2 permeability values are higher than those of O_2. The gas selectivity ranged from 1 to 28 in the tested conditions.

Model Fitting. Regression analysis with experimental data and with their logarithms was carried out. Logarithmic transformation was done due to the high variation of permeability values with temperature and RH. The regression coefficients, statistical analysis, and level of significance of the selected models are presented in Table 3. The coefficients of determination of the models obtained with the logarithm of permeability values are highly significant (R^2 = 0.99 and 0.98), indicating that the models correctly fit the data. With regard to the selectivity (CO_2 permeability to O_2 permability ratio), the best fitted model was obtained with actual permeability values (R^2 = 0.97).

Table 2. Central Composite Design Arrangement and Responses

variable levels		responses		
T (°C)	RH (%)	CO_2 permeability (amol s^{-1} m^{-1} Pa^{-1})	O_2 permeability (amol s^{-1} m^{-1} Pa^{-1})	selectivity
9	14.6	258	111	2.3
39	14.6	314	131	2.4
9	85.3	11475	1011	11.3
39	85.3	22353	863	25.9
3	50	317	181	1.7
45	50	1026	233	4.4
24	0	88	77	1.1
24	100	55580	1970	28.2
24	50	536	159	3.3
24	50	545	152	3.5

Table 3. Regression Coefficients and Level of Significance for the Fitted Model[a]

coeff	log O_2 permeability (amol s^{-1} m^{-1} Pa^{-1})	log CO_2 permeability (amol s^{-1} m^{-1} Pa^{-1})	CO_2 to O_2 permeabilty ratio
b_0	2.19	2.73	3.47
b_1	0.02	0.16*	2.29**
b_2	0.47***	0.93***	8.85***
b_{12}	−0.04	0.05	3.62**
b_{11}	0.08	0.10	0.20
b_{22}	0.21***	0.37**	6.00***
R^2	0.99	0.98	0.97

[a] *, significant at 10% level; **, significant at 5% level; ***, significant at 1% level.

Regression coefficient values and significance showed that RH was the predominant factor influencing the CO_2 and O_2 permeabilities and selectivity of wheat gluten film (Table 3). Positive b_2 and b_{22} coefficients indicate that increases in RH increased gas permeability and selectivity. Note that temperature was a significant factor in CO_2 permeability and selectivity models. Significant interaction between temperature and RH (b_{12}) was observed for selectivity.

The second-order polynomial equations were then used to represent the data in three-dimensional plots as seen in Figures 1, 2, and 4.

Permeability to Oxygen. Figure 1 shows the predicted O_2 permeability of wheat gluten film as a function of temperature and RH. Within the studied temperature range, O_2 permeability remained low and fairly constant (100 amol m^{-1} s^{-1} Pa^{-1}) for RH <50%. At RH >50%, O_2 permeability increased exponentially and approached very high values.

RH dependence of O_2 permeability of protein-based films was observed by McHugh and Krochta (1994) and Gontard et al. (1996). This high sensitivity to RH is shared with other films made of nylon 6 (Hernández, 1994), cellulose (Paine and Paine, 1983), cellophane (Krochta, 1992), methylcellulose (Rico Peña and Torres, 1990), and fruit puree (McHugh et al., 1996).

The rise in O_2 permeability as a function of RH may be related to the hydrophilicity of wheat gluten proteins due to their high content of amide groups (Gontard et al., 1992). Interactions between water molecules and

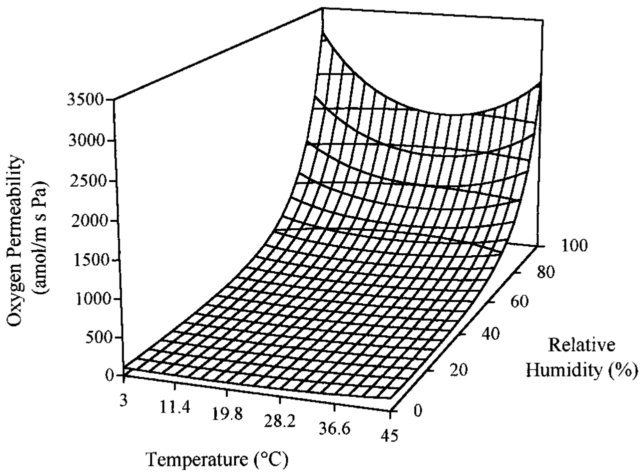

Figure 1. Variation of O_2 permeability of a wheat gluten based film as a function of RH and temperature.

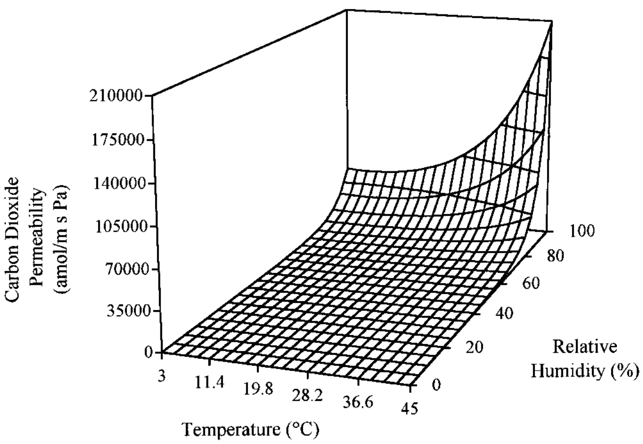

Figure 2. Variation of CO_2 permeability of a wheat gluten based film as a function of RH and temperature.

amide groups contribute to a high water content in the film at high RH (increasing gas solubility) and to a modification in the protein network structure. The disruption of hydrogen bonds may create additional sites for the dissolution of oxygen and increase mobility of the O_2 molecules within the polymer bulk phase (Gennadios and Weller, 1990; Gontard et al., 1993, 1996). This modification would correspond to a change from a glassy to a viscoelastic state (Gontard et al., 1996). Under dry conditions, interchain hydrogen bonds of the gluten proteins form a strong network structure. The glass transition temperature (T_g) is one of the more important properties of polymers directly related to structure modifications that affect the diffusivity and, subsequently, the permeability of gases. A lower mobility of the protein chains makes the O_2 diffusion difficult (Krochta, 1992; Gontard et al., 1993; Gennadios et al., 1993b; Slade and Levine, 1995).

The statistical analysis (Table 3) showed that temperature did not significantly influence O_2 permeability. Therefore, conclusive discussions on the O_2 permeability variations observed in the response surface plotted with the developed empirical model (Figure 1) cannot be made. The dependence of the O_2 permeability on temperature could not be described with the second-order polynomial. As expected from theoretical considerations, the gas permeability of films should vary with temperature following an Arrhenius-type relationship (Paine and Paine, 1983; Mannheim and Passy 1985)

$$\ln P = \ln P_o + (E_P/R)(1/T) \quad (3)$$

where E_P is the activation energy of permeability (J/mol), R is the universal gas constant (8.314 J/mol K), T is the absolute temperature (K), and P_o is the Arrhenius constant (amol/m s Pa).

This unexpected behavior of O_2 permeability may be partly due to the relative changes in diffusivity (D) and solubility (S) of oxygen, as they vary with temperature (Samaniego-Esguerra and Robertson, 1991):

$$D = D_o \exp(-E_D/RT) \quad (4)$$

$$S = S_o \exp(-\Delta H_S/RT) \quad (5)$$

where E_D and $-\Delta H_S$ are the energy of diffusion and heat of solution, respectively.

O_2 diffusion increases and O_2 solubility decreases as temperature rises; hence, their contribution to permeability values, which is defined as the product of D and S (Pascat, 1986; Chao and Rizvi, 1988), will be the result of a coupled overall complex effect of temperature on permeability of a hydrophilic film, such as wheat gluten film. The low-temperature effect could also be masked by the predominant effect of RH.

Permeability to Carbon Dioxide. As for O_2 permeability, CO_2 permeability revealed an upward trend with increasing RH (Figure 2). CO_2 permeability remained low at low RH (<50%), whatever the temperature, and rose suddenly beyond 50% RH. Note that carbon dioxide permeability seems to be very sensitive to changes in RH. CO_2 permeability at 100% RH and 24 °C (55580 amol m^{-1} s^{-1} Pa^{-1}) was 29 times higher than O_2 permeability in the same conditions.

The difference in CO_2 and O_2 permeabilities may be partially explained in terms of solubility of these gases in water. In dry conditions (0% RH and 24 °C, Table 2), CO_2 and O_2 permeability values are very close (88 and 77 amol m^{-1} s^{-1} Pa^{-1}, respectively). Henry's law constants for CO_2 and O_2 in water at 25 °C are 7.4×10^7 and 2.6×10^9 Pa mol $fraction^{-1}$ respectively, indicating that CO_2 is 35 times more soluble than O_2 in water (Perry et al., 1984). This value was close to that of the CO_2 to O_2 permeability ratio, observed at 100% RH and 24 °C (Table 2). Hence, the higher solubility in the water may enhance the transport of CO_2 through the wheat gluten film with an increased water content.

The significant and positive effect of the temperature on CO_2 permeability is more evident in high RH conditions (Figure 2). It is presumed that the increase of CO_2 diffusion with temperature is more important than the decrease of CO_2 solubility.

Predicted CO_2 permeabilities were used to generate Arrhenius plots at different RH values (over the temperature range of 9−39 °C), by using eq 3. Figure 3 shows that a change occurred in the slope of the Arrhenius plots, which were lower at low temperatures. Hence, energy of permeation (E_P) values were different for high and low temperatures. E_P was calculated, above and below the discontinuity range temperature. E_P values were close to zero at temperatures below the inflection point whatever the RH and ranged from 26 kJ/mol at 0% RH to 46 kJ/mol at 100% RH above the inflection point. These E_P values were comparable with those of synthetic films such as polyamides (15−48 kJ/mol), *trans*-polyisoprene (12−45 kJ/mol) (Arvanitoyannis et al., 1992; Arvanitoyannis and Blanshard, 1993),

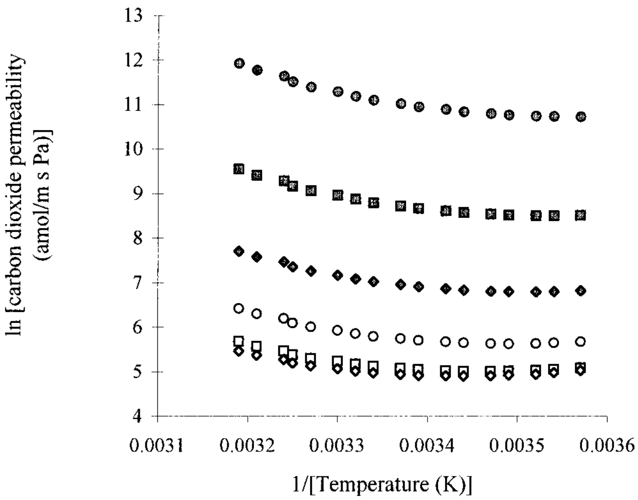

Figure 3. Arrhenius plot for carbon dioxide permeability of wheat gluten film at different RH values: (●) 100% RH; (■) 80% RH; (◆) 60% RH; (○) 40% RH; (□) 20% RH; (◇) 0% RH.

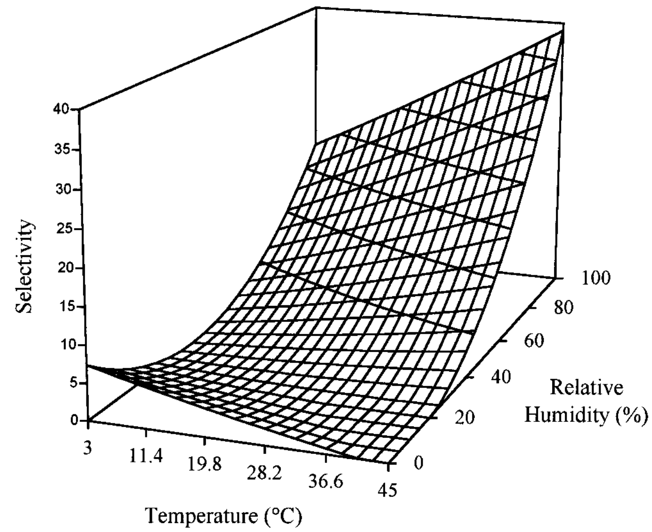

Figure 4. Variation of the gas selectivity of a wheat gluten based film as a function of RH and temperature.

and poly(vinyl chloride) (41.5 kJ/mol) (Doyon et al., 1991) but higher than those of edible starch films (0.1−2.6 kJ/mol) (Arvanitoyannis et al., 1994).

Such discontinuity, also noticed by Arvanitoyannis et al. (1994) for the permeation of O_2 and CO_2 in potato and rice starch films, could be correlated to the glass transitions of polymer network (Kumins, 1965; Gennadios et al., 1993b; Gontard and Ring, 1996). The temperature at which the inflection point occurred decreased when RH increased. This observation is in agreement with the evolution of T_g with RH (Gontard and Ring, 1996). Nevertheless, to obtain a valuable correlation between these phenomena, further and precise investigations are required in the region of T and RH, where glass transition takes place. It would also be convenient to evaluate separately the effects of temperature, RH, and water content on gas solubility and diffusivity (the cofactors defining permeability), to gain a better understanding of the phenomena occurring during gas permeation through the wheat gluten film. Research on these subjects is actually in progress in our laboratory.

Selectivity and Potential Application of Wheat Gluten Films. The selectivity is one of the most descriptive parameters of a packaging film. It determines the possible combination of oxygen and carbon dioxide concentrations inside the film that should be in correspondance with fruit and vegetable needs (Kader and Zagory, 1989).

Wheat gluten film selectivity was also found to be highly dependent on RH (Figure 4). An exponential increase of the selectivity is also observed when RH increases beyond 50%. The shape of the response surface was characteristic of interaction between temperature and RH. A wide range of selectivity values was exhibited by the wheat gluten film (3−28), unlike with most polymeric films, the selectivity of which usually lies between 4 and 6 (Zagory and Kader, 1988; Yam and Lee, 1995).

Fruits and vegetables increase their respiration rate and CO_2 production with temperature. Inside the package, high CO_2 concentrations can be harmful to CO_2-sensitive products. A packaging film leading to low injury levels would be necessary (Kader et al., 1989; Church, 1994). This film should change its diffusion properties in correspondance with the change in the product respiration rate. In that respect, it seems that wheat gluten film could act as an "active packaging" (Labuza and Breene, 1989; Gontard et al., 1996), by increasing CO_2 and O_2 permeabilities and selectivity concomitantly with the rise in respiration. Research is being carried out to test the wheat gluten films performance in preservation of fresh mushroom and tropical fruits by MAP.

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