Permeability of d-Limonene in Whey Protein Films

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ABSTRACT

The effects of temperature, relative humidity (%RH) and permeant concentration on aroma permeability were investigated in edible whey protein isolate (WPI) films. An orthogonal regression was performed to ascertain significance of these factors. Temperature and %RH had exponential effects on d-limonene permeability, interacting synergistically to influence aroma transport in WPI polymer films. Permeability of d-limonene in WPI polymer films was not influenced by permeant concentration in the range 62 to 226 ppm (mol/mol). The predictive equation generated by regression analysis could be potentially useful for edible packaging design within the given temperature, %RH, and concentration ranges. The Arrhenius-type format also provided insight into the temperature-sensitivity of WPI films and confirmation of the influence of %RH on permeability activation energy.

Key Words: aroma, limonene, permeability, edible film, whey protein

INTRODUCTION

DEPLETION OF INDIVIDUAL AROMA COMPOUNDS can result in alteration of characteristic food flavors and aroma and lower food quality. Commercial food processing includes packaging used to extend the shelf-life by controlling aroma transport. A range of synthetic polymers and laminates are available which are excellent aroma barriers. Some edible packaging supplements have many of the same properties and effectiveness of some synthetic polymers which they augment.

Edible polymer films may be used as food coatings or as stand-alone film wraps and pouches to supplement synthetic packaging. The oxygen and aroma barrier properties of edible polymer films indicate good potential as food packaging supplements (Miller and Krochta, 1997a). Such edible film properties would reduce the functions of the synthetic polymer to providing a barrier to moisture loss and protecting the food from external contamination. The amount of synthetic packaging would be reduced and recyclability would be increased because the need for synthetic laminates to improve oxygen and aroma barrier properties would be diminished.

Aroma permeability of polymers is often correlated with their oxygen permeability (DeLassus, 1994). Oxygen and aroma permeability were comparable within the rubbery or glassy polymer categories, but not between such polymer categories.

Knowledge of the transport properties of edible films is critical to the development of generalized theories to describe aroma transport behavior which may then be applied to food packaging problems. Kester and Fenninga (1996) concluded that much of the edible film and coating published information is of limited value due to “lack of quantitative data on barrier characteristics of the coatings.”

Published reports are scarce concerning aroma barrier properties of edible films. The study of aroma transport properties of edible films relies on reviews of synthetic polymer research for guidance (Felder and Huvard, 1980; Hernandez et al., 1986; DeLassus, 1992; Nielsen and Giacin, 1994; Miller, 1997). Debeaufort et al. (1994) were the first to publish aroma permeability coefficients for edible polymer films. They concluded that gluten (wheat protein) film was a better barrier to 1-octen-3-ol (mushroom aroma) than low density polyethylene (LDPE) or methylcellulose but not as good a barrier as cellulose. Debeaufort et al. (1995) attempted to explain differences in 1-octen-3-ol transport among such films. However, aroma flux did not correlate with the amount of aroma adsorbed, the hydrophobicity of the polymer, or trends in diffusion coefficients. Variations in aroma permeability were presumed to be due to a moisture plasticization and the “sweeping” action of water vapor (Debeaufort et al., 1995). Miller and Krochta (1997b) found whey protein isolate (WPI) film containing 25% glycerol (dry basis) plasticizer was comparable to ethylene vinyl alcohol copolymer (EVOH) as a barrier to d-limonene at similar temperature and humidity conditions. No other work has been published regarding aroma permeability in edible films.

The diffusion coefficient, D, is defined by Fick’s First Law in one dimension...

\[ J = -\frac{dM}{dt} = -D \frac{dc}{dx} \]  

where J is the permeant flux represented by the permeant mass transfer rate (dM/dt) per cross-sectional area (A), M is the amount of permeant, t is time, c is the permeant concentration, and x represents distance (film thickness). The solubility coefficient, S, is defined from an adaptation of the Nernst distribution function relating c at the film surface to the permeant partial pressure, p, as...

\[ c = Sp \]

When D and S are independent of concentration and steady-state conditions are attained, the permeability coefficient, P, was derived by Crank (1975) from Eq. (1) and (2) as...

\[ P = D S = \frac{(dM/dt)_{steady\ state}}{A \Delta x} \]  

which incorporates both kinetic (D) and thermodynamic (S) properties of the polymer-permeant system providing a gross mass transport property. The negative sign indicates that permeability occurs in the direction of decreasing permeant partial pressure.

Our objective was to examine the effects of temperature, relative humidity and permeant concentration on d-limonene permeability in edible WPI films.

MATERIALS & METHODS

AROMA PERMEABILITY COEFFICIENTS WERE DETERMINED using a recirculating constant-volume method. We utilized a specially designed permeability cell and a quasi-isostatic measurement technique, as described by Miller and Krochta (1997b) (Fig. 1). Headspace samples (1 mL) were removed periodically from both the isolated downstream cell chambers (197 mL) and the recirculating upstream cell chambers (4,055 mL) and injected into a Hewlett Packard 5890 Series II gas chromatograph (Hewlett Packard, Palo Alto, CA) equipped with a
flame ionization detector. The sample was cryo-focused with liquid nitrogen for 1 min prior to elution on a DB-5ms capillary column (J&W Scientific, Folsom, CA). For each headspace sample removed, an equal volume of humidified nitrogen was injected back into the chamber to alleviate any instantaneous pressure gradient due to sampling.

The permeant was allowed to accumulate in the isolated downstream chambers to a concentration 3% of the upstream concentration to assure that downstream permeant concentration remained negligible (Baner et al., 1986). The GC peaks obtained from the headspace injections were quantified by comparison with a standard curve. A plot of the amount of permeant transmitted vs time was generated for each film at each test condition. The steady state transmission rate was determined by performing a linear regression (StatView 4.5, Abacus Concepts, Berkeley, CA) on the linear portion of the data with the slope of the regression line giving the transmission rate.

The permeability coefficient was calculated from the steady state transmission rate as shown (Eq. 3). Permeability coefficients for aroma compounds are reported in Modified Zobel Units (MZU) which are related to SI units as follows:

$$1 \text{ MZU} = 10^{-20} \text{kg-m/(m}^2\text{-s-Pa)}$$

The test permeant, $d$-limonene (Sigma Chemical Co., St. Louis, MO), is a primary aroma component of citrus and one of the most studied aromas. The GC technique allowed reproducible measurement of $d$-limonene concentrations in the range 1 ppm (mol/mol).

WPI films were cast from an aqueous solution containing 10% (by weight) WPI (Davisco International, Inc., Le Seuer, MN) and 25% (dry basis, by weight) glycerol (Fisher Scientific, Fair Lawn, NJ) which was used as a plasticizer. The solution was prepared by solubilizing dry WPI powder in deionized water, degassing the solution with a vacuum pump, heating the solution at 90°C for 30 min to denature the protein, cooling followed by addition of glycerol, and a final degassing.

The solution was then placed on high density polyethylene casting plates and dried on a leveled surface overnight at room temperature. Film thickness was controlled by the amount of total solids deposited on the casting plate. The thickness of each WPI film was calculated by averaging 10 measurements taken with a Mitutoyo model 2804-10 micrometer (Mitutoyo, Japan). The WPI film thickness ranged from $1.016 \times 10^{-4}$ m to $1.52 \times 10^{-4}$ m.

Three levels each for temperature, relative humidity and permeant concentration were studied to detect possible nonlinear trends in aroma permeability. All combinations of the specified levels of temperature, $T$ (40°, 50°, and 60°C), relative humidity, %RH (40, 60, and 80) and permeant vapor concentrations, $C$, (62, 144, and 226 ppm, mol $d$-limonene/mol air) were examined. Since all three factors were quantitative, it was desirable to develop a relationship between permeability and the three independent factors, as well as establishing their relative influence. Therefore, a polynomial regression (StatView 4.5, Abacus Concepts, Berkeley, CA) approach was applied. To control experimental error, four subsamples were used. These subsamples were films tested at the same time under identical temperature, relative humidity, and permeant concentration combinations.

**RESULTS & DISCUSSION**

**Regression analysis**

The independent variables ($T$, %RH, and $C$) were all evenly spaced and could, therefore, be transformed into orthogonal (uncorrelated) variables to minimize multicollinearity (Draper and Smith, 1981). Such multicollinearity (linear dependency) between independent variables in a regression model would occur when the independent variables have high correlation coefficients. A high correlation coefficient would be indicative of some relationship between the presumed independent variables. If the correlated variables are not independent, discerning their relative importance becomes difficult since changing one results in variations in the others. Since a polynomial regression includes higher order terms composed of the main independent variables, (e.g., $T^2$ or $T \times %RH$), multicollinearity can diminish its effectiveness and reliability.

The permeability coefficients for $d$-limonene in WPI films were compared (Fig. 2 and Fig. 3).
The orthogonal regression model composed of \(\%RH\), \(T^\star\%RH\), \(\%RH^2\), and \(T^\star\%RH^2\) was highly significant (\(P<0.0001\)), indicating nonzero coefficients for these variables. However, the adjusted coefficient of multiple determination (adjusted \(R^2\), an unbiased measure of goodness of fit, was only 0.757. Additionally, examination of the residuals (difference between observed and predicted values) for this model showed that they increased as the predicted values increased. This implies nonconstancy of variance, suggesting a need for transformation of the data. A logarithmic transformation of the permeability (\(P\)) data was performed based on a possible Arrhenius-type relationship of the form...

\[
P = P_0 e^{-(E_p / RT)}
\]

where \(P_0\) is a pre-exponential factor, \(E_p\) is the energy of activation for permeability, \(R\) is the gas constant, and \(T\) is the absolute temperature (°K).

An Arrhenius relationship between aroma permeability and temperature has been suggested (DeLassus et al., 1988; Strandburg et al., 1990; Torres, 1994). Additionally, the transformation is supported by a previously suggested exponential relationship between aroma transport and moisture content of hydrophilic films (Schwartzberg, 1986). The transformation of \(T\) (°K), to its inverse, \((1/T)\), prevents the use of orthogonal analyses (data no longer evenly spaced). Thus, attention must be placed on the correlation coefficients to identify multicollinearity in the transformed variables.

Examinations on the correlation coefficients of the transformed data showed some multicollinearity in the higher order terms. Using the initial orthogonal regression as a guide, only the main effects of \((1/T)\), \(\%RH\), and \(C\) and their first order interactions (i.e., \((1/T)\times\%RH\)) were considered. The presence of multicollinearity among independent variables does not necessarily preclude a good fit for the regression equation or predictions of new observations, as long as these inferences are made within range of the initial observations. However, the corresponding regression coefficients tend to have a larger variability (Neter et al., 1990).

The regression analysis on the transformed data shows that \((1/T)\), \(\%RH\), and the \((1/T)\times\%RH\) interaction all significantly influenced \(d\)-limonene permeability and that the regression equation...

\[
P = \exp[0.60 (\%RH) – 1100(1/T) – 160 (\%RH)(1/T)]
\]

is highly significant (\(p<0.0001\)) (Tables 1 and 2). Additionally, the adjusted \(R^2\) of 0.958 indicates a very good fit between the regression equation and observed permeability values.

### Table 1—ANOVA table for the polynomial regression of the transformed \(d\)-limonene permeability in WPI edible films

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>3</td>
<td>207.448</td>
<td>69.149</td>
<td>206.778</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Residual</td>
<td>24</td>
<td>8.026</td>
<td>.334</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>215.474</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\*Significant at = 0.0001.

### Table 2—Polynomial regression coefficients for independent variables significantly affecting transformed \(d\)-limonene permeability in WPI films

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Coefficient</th>
<th>Standard error</th>
<th>Standard coefficient</th>
<th>t-Value</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(T^{-1})</td>
<td>-1111.891</td>
<td>136.751</td>
<td>-0.047</td>
<td>-8.131</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>(%RH)</td>
<td>0.597</td>
<td>0.071</td>
<td>5.305</td>
<td>8.439</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>(%RH\times T^{-1})</td>
<td>-162.765</td>
<td>22.942</td>
<td>-4.498</td>
<td>-7.095</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>


### Effect of permeant concentration, temperature, and relative humidity

Permeant concentration did not influence \(d\)-limonene permeability in any of the regression analyses. In synthetic polymers, aroma permeants often plasticize the films. In such cases, increasing permeant concentration results in sharp increases in film permeability. The lack of influence of permeant concentration on \(d\)-limonene permeability was clear.
Table 3—Limonene permeability in WPI films compared to synthetic aroma barrier polymers

<table>
<thead>
<tr>
<th>Film</th>
<th>Limonene conc (ppm-mol/mol)</th>
<th>Temp (°C)</th>
<th>%RH</th>
<th>P (MZU)</th>
<th>Std Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>co-VDC</td>
<td>83</td>
<td>55</td>
<td>—</td>
<td>18000</td>
<td>±5000</td>
</tr>
<tr>
<td>EVOH</td>
<td>109</td>
<td>55</td>
<td>50</td>
<td>0.9</td>
<td>±0.3</td>
</tr>
<tr>
<td>WPI</td>
<td>144</td>
<td>50</td>
<td>40</td>
<td>1.2</td>
<td>±0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>60</td>
<td>10.1</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>80</td>
<td>74.9</td>
<td>20.4</td>
</tr>
</tbody>
</table>


ly shown by the scatter of data and lack of definitive trends associated with concentration variations (Fig. 2 to 4).

Combining the last two terms in Eq. (5) to correspond to the usual Arrhenius-type relationship gives...

\[
P = \exp\left[0.60 \times \left(\frac{\%RH}{E_p/RT}\right)\right] \tag{6}
\]

where R is the gas constant (1.987E-3 kcal/mol, °K), T is absolute temperature (°K), and \(E_p\) is the Arrhenius activation energy for permeability (kcal/mol), which is a function of relative humidity...

\[
E_p = 2.2 + 0.32 \times \left(\%RH\right) \tag{7}
\]

Other researchers have suggested a relationship between relative humidity and \(E_p\) (Labuza and Contreras-Medellin, 1981). Equation (5) indicates that the temperature influence on \(d\)-limonene permeability in WPI films increased as the relative humidity increased. The activation energy \(E_p\) is indicative of the temperature-sensitivity of \(d\)-limonene permeability in WPI films. The higher the % RH, the larger \(E_p\) and the greater the influence of temperature on permeability. This synergistic influence of temperature and relative humidity is evident upon examination of our data (Fig. 5 to 7).

Activation energies for gas and moisture permeability in polymers usually range from 0-15 kcal/mol (Torres, 1994). Activation energies of permeability for hydrophobic ethylene vinyl alcohol copolymer (EVOH) at 0% RH ranged from 17.1 kcal/mol for trans-2-hexenal (DeLassus et al., 1988) to 32.5 kcal/mol for ethylheptanoate (Strandburg et al., 1990). Activation energies for \(d\)-limonene permeability in WPI films at 50% RH, 60% RH, and 80% RH were 15.1, 21.6, and 28.0 kcal/mol, respectively. These results seem to indicate that aroma permeability is less temperature sensitive in hydrophobic polymers at lower humidities. Thus, WPI films probably would be less temperature-sensitive than EVOH, given that the reported \(E_p\) for EVOH at 0% RH is similar to the calculated \(E_p\) for WPI at 40% RH. Confirmation of this would require calculation of \(E_p\) for WPI and EVOH at identical temperature and relative humidity for the same permeant.

Permeability coefficient data for \(d\)-limonene in WPI films were compared to ethylene vinyl alcohol copolymer (EVOH) and vinylidene chloride copolymer (co-VDC) films (Table 3). Even at very high relative humidity (80%), WPI polymer films have \(d\)-limonene permeability coefficients three orders of magnitude lower than the industry standard for hydrophobic aroma barriers, co-VDC. The WPI polymer films also yield \(d\)-limonene permeability coefficients that are comparable to EVOH, the industry standard for hydrophobic aroma barriers.

CONCLUSIONS

WPI POLYMER FILMS SHOW GREAT PROMISE AS supplemental aroma barriers for packaging of low moisture foods. Temperature and relative humidity have exponential effects on \(d\)-limonene permeability, interacting synergistically to influence aroma transport in WPI films. Permeability of \(d\)-limonene in WPI polymer films was not influenced by permeant concentration in the range 62-226 ppm (mol/mol). The predictive equation for \(d\)-limonene in WPI films would be potentially useful for packaging design within the given temperature, relative humidity, and concentration ranges. The Arrhenius-type format of the regression equation also provided useful insight into the temperature-sensitivity of WPI films and confirmation of the influence of relative humidity on \(E_p\).

REFERENCES


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