# Tempering Influence on Oxygen and Water Vapor Transmission through a Stearyl Alcohol Film

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**Stearyl alcohol was layered on a filter paper support and tested for resistance to $O_2$ and water vapor transmission following tempering. Tempering at 48C for 14 or 35 days caused the resistance to $O_2$ and water vapor transmission to increase. The resistance to $O_2$ and water vapor transport was increased 80% and 50%, respectively, after 35 days. Likely mechanistic explanations include the healing of crystal imperfections and the development of a more extensive and better-linked arrangement of lipid crystalline platelets.**

Previous studies have confirmed that the flux of gases and vapor through lipid films is influenced by lipid type, polymorphic state, temperature and pressure differential across the film (1-4). An additional factor of potential importance is tempering or annealing. Intuitively, improved barrier characteristics of lipid films would be anticipated following tempering since this would afford an opportunity for film defects to be healed. Previous studies, however, are not in agreement concerning the influence of tempering on barrier properties of lipid films. Fox (5) studied the resistance of paraffin wax films to water vapor transmission and found that permeability decreased significantly with storage time at an elevated temperature. The author attributed this to a healing of microscopic defects in the crystal structure and the growth of crystalline wax platelets parallel to the film surface. Contrary to Fox's results, Landmann et al. (6) studied cocoa butter in its most stable polymorphic form and found that permeability to water vapor increased more than 10-fold during storage at room temperature for 3 weeks. The authors postulated that the increased permeability resulted from the growth of lipid crystals during aging and a resultant increase in intercrystalline porosity.

Clearly, the effect of tempering on the resistance of lipid films to gas and vapor flux has not been satisfactorily resolved. The objective of this study was to clarify the effect of tempering, without polymorphic transformation, on the barrier properties of lipid films to oxygen and water vapor.

## MATERIALS AND METHODS

*Film fabrication.* Stearyl alcohol (99%; Sigma Chemical Co., St. Louis, MO) was used to prepare lipid films according to the procedure described by Kester and Fennema (1). The fatty alcohol was applied to Whatman 50 (W50) filter paper by immersing the filter disc in molten lipid, allowing the disc to drain and then cooling it to room temperature. This procedure added approximately 3 mg stearyl alcohol/cm$^2$ of filter area. An additional 1 mg/cm$^2$ was applied by spreading molten lipid uniformly over one surface of the disc with a preheated, thin-layer chromatography spreader. Finished stearyl alcohol-W50 films contained 4.0 ± 0.2 mg lipid/cm$^2$ of film area ($\bar{X}$ ± SD), as determined by weighing filter discs before and after lipid application. Thickness of stearyl alcohol-W50 films was 0.11-0.12 mm.

*Tempering.* The tempering of stearyl alcohol-W50 films was performed at 48 ± 2C for 0 days, 14 days and 35 days, during which time the films were maintained in a flat, horizontal position. Before and after tempering, powder x-ray diffraction patterns of stearyl alcohol scraped from the surface of W50 films were determined to identify the polymorphic form and to confirm the absence of a change in polymorphic form during tempering (1).

*Measurement of resistance to oxygen and water vapor transmission.* Resistances of tempered and untempered films of stearyl alcohol-W50 to oxygen [$r(O_2)$] and water vapor [$r(H_2O)$] transmission were determined as described previously (1, 2). Units of resistance are sec·m$^{-1}$.

The diffusion of simple gases, such as $O_2$, through films generally obeys Fick's first law, however, diffusion of water vapor through polar film matrices generally does not (2). Therefore, it is appropriate to express moisture barrier characteristics of stearyl alcohol-W50 films in terms of an "effective" resistance to water vapor transmission [eff $r(H_2O)$], which is accurate under the given environmental conditions of the determination (i.e., % relative humidity, temperature). Similarly, temperature dependence of eff $r(H_2O)$ is expressed in terms of an "apparent" activation energy ($E_{app}$).

At least five replicates of film samples receiving each tempering treatment (0 days, 14 days and 35 days) were evaluated for $r(O_2)$ and eff $r(H_2O)$ at 25, 30, 35 and 40C.

The Student's t-distribution was used to compute 95% confidence intervals for $r(O_2)$ and eff $r(H_2O)$ and to evaluate the statistical significance ($P \leq 0.05$) between calculated activation energies of $O_2$ and water vapor transport (7).

*Scanning electron microscopy (SEM).* Surface morphologies of stearyl alcohol-W50 films were observed with SEM as described previously (1), with the exception that a thicker coating (20 nm) of gold-palladium alloy was applied to the film surface.

## RESULTS AND DISCUSSION

Stearyl alcohol can be directly and easily solidified from the melt (m.p. 57.9C) in its $\beta'$-polymorphic form, characterized by an orthohombic arrangement of hydrocarbon chains and maximum thermodynamic stability (1,8). It will remain in the $\beta'$ form unless the temperature is raised to the range of 54-57.9C which will cause conversion to the $\alpha$-polymorphic form, characterized by a hexagonal arrangement of hydrocarbon chains (9). Stearyl alcohol was chosen for study because 1) its melting point permits tempering to be conducted at a temperature sufficiently high (48 ± 2C) so that effects of annealing and growth of crystals on film permeability, if such effects exist, can be

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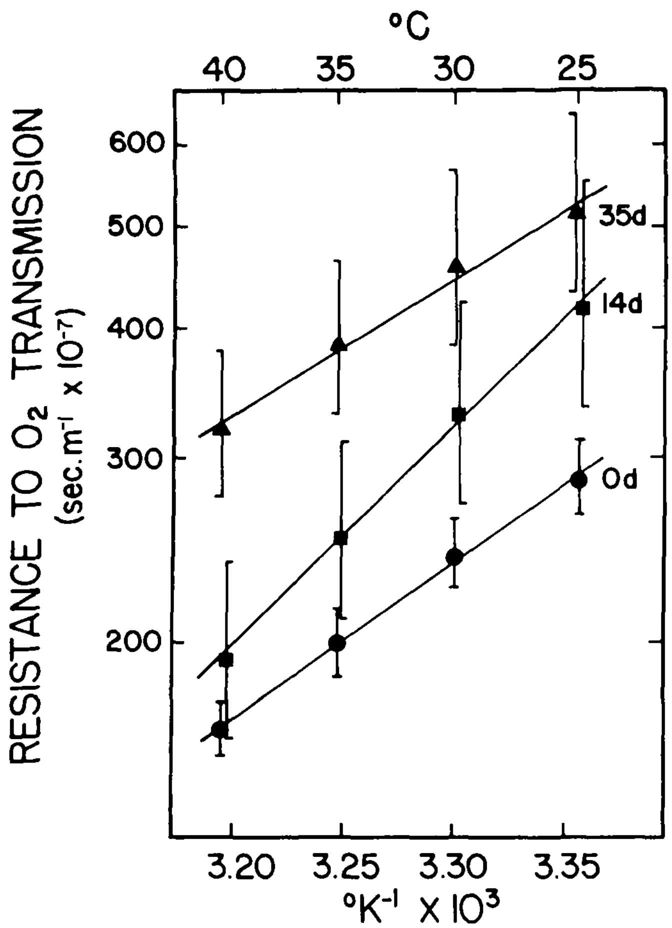


FIG. 1. Temperature dependence of the resistance to oxygen transmission [$r(O_2)$] of stearyl alcohol-W50 films as influenced by prior tempering. Films were tempered 0 (●), 14 (■) and 35 (▲) days at 48 ± 2C. Numerals on ordinate have been reduced by a factor of $1 \times 10^7$. Amount of lipid per unit area of film was 4.0 ± 0.2 mg/cm² ($\bar{X} \pm SD$). Each data m point is a mean of at least five replicates. Vertical bars represent 95% confidence intervals for resistance. Least squares regression lines are drawn through each set of data. See Table 1 for regression equations and correlation coefficients.

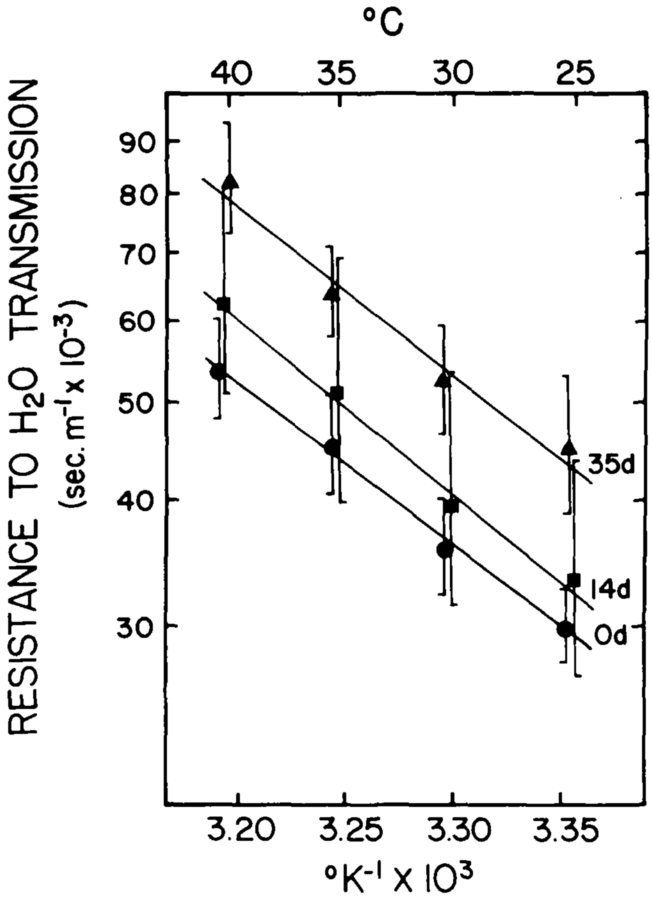


FIG. 2. Temperature dependence of the effective resistance to water vapor transmission [eff $r(H_2O)$] of stearyl alcohol-W50 films as influenced by prior tempering. Films were tempered (●), 14 (■) and 35 (▲) days at 48 ± 2C. Numerals on ordinate have been reduced by a factor of $1 \times 10^7$. Amount of lipid per unit area of film was 4.0 ± 0.2 mg/cm² ($\bar{X} \pm SD$). Each data m point is a mean of at least five replicates. Vertical bars represent 95% confidence intervals for resistance. Least squares regression lines are drawn through each set of data. See Table 1 for regression equations and correlation coefficients.

easily detected and 2) it can be easily solidified and maintained in the $\beta'$-polymorphic form, thus enabling the effect of tempering to be studied unambiguously. Arrhenius' plots illustrating temperature dependence of $r(O_2)$ and eff $r(H_2O)$ of stearyl alcohol-W50 films as influenced by tempering are presented in Figures 1 and 2, respectively. Linear regression parameters and calculated activation energies for the transport processes are listed in Table 1.

Mean resistance to transmission of both $O_2$ and water vapor, at all temperatures from 25 to 40C, increased with the time of tempering at 48C. The $r(O_2)$ at 25C increased from $286 \times 10^7$ sec·$m^{-1}$ for untempered films to $417 \times 10^7$ and $517 \times 10^7$ sec·$m^{-1}$, respectively, after 14 and 35 days of annealing. The eff $r(H_2O)$ of stearyl alcohol films at 25C increased from $29.7 \times 10^3$ sec·$m^{-1}$ (untempered) to 33.0 $\times 10^3$ after 14 days and $44.6 \times 10^3$ sec·$m^{-1}$ after 35 days. Thus, barrier performance of stearyl alcohol to $O_2$ and water vapor diffusion improved approximately 80 and 50 percent, respectively, after tempering at 48C for 35 days. These results are in general agreement with those of Fox (5), who reported a significant reduction in water vapor permeability of paraffin wax films after tempering at an elevated temperature.

The slopes of the Arrhenius' plots in Figures 1 and 2 are proportional to activation energies (E) of the transport processes (1,2). Positive slopes and, hence, activation energies were obtained for $O_2$ transport (Figure 1, Table 1). For each tempering time, the calculated E value for $O_2$ transport was within the range of 5-16 kcal/mole which Lebovits (10) states is typical of most permeation processes, such as $O_2$ transport, that occur by molecular diffusion. Activation energies for $O_2$ transmission through films conditioned 0 days and 35 days did not differ significantly ($P \leq 0.05$). Stearyl alcohol films conditioned 14 days, however, displayed an E that was significantly

**TABLE 1**

**Activation energies and parameters of regression equations for resistance of stearyl alcohol-W50 films to the transmission of oxygen and water vapor as affected by prior tempering at 48C[a]**

| Resistance[b] ($sec \cdot m^{-1}$) | Tempering time (days) | Regression[c] constant, a | Regression[c] coefficient,b | Correlation coefficient | Activation energy[d] |
|---|---|---|---|---|---|
| | | | | | (E) |
| | 0 | 4.38 | 1513.17 | 0.9987 | $7.0 \pm 0.3^A$ |
| $r(O_2)$ | 14 | 2.56 | 2105.21 | 0.9987 | $9.5 \pm 1.5^B$ |
| | 35 | 5.29 | 1319.87 | 0.9941 | $6.1 \pm 0.8^A$ |
| | | | | | ($E_{app}$) |
| | 0 | 9.92 | -1624.35 | -0.9982 | $-7.4 \pm 0.8^A$ |
| eff $r(H_2O)$ | 14 | 10.43 | -1762.58 | -0.9970 | $-8.0 \pm 1.1^A$ |
| | 35 | 10.23 | -1665.71 | -0.9917 | $-7.6 \pm 0.9^A$ |

[a]Stearyl alcohol was in the $\beta'$ polymorphic form throughout the study. W-50 is Whatman 50 filter paper. Lipid coverage was $4.0 \pm 0.2$ mg ($\overline{X} \pm$ SD)/cm$^2$ of W-50 filter paper.
[b]Resistance to water vapor transmission is expressed in terms of effective resistance [eff $r(H_2O)$] and resistance to oxygen transmission is simply $r(O_2)$.
[c]Regression equation is $\log r = b\,(1/T) + a$. Regression lines are plotted in Fig. 1 and Fig. 2.
[d]Units of E for resistance to oxygen transmission and $E_{app}$ (apparent activation energy) for resistance to water vapor transmission are kcal/mole; data are means $\pm$ SD; within each group (i.e., $O_2$ or $H_2O$), values with different superscript letters are significantly different ($P \leq 0.05$).

larger than those obtained at 0 days and 35 days. This observation is puzzling and no explanation is obvious.

Arrhenius' plots for water vapor transmission displayed negative slopes (Figure 2); therefore, apparent activation energies ($E_{app}$; Table 1) were also negative. This has previously been described as a consequence of the moisture sorption behavior of the hydrophilic supporting matrix (2). There is no significant difference between $E_{app}$ values for the three tempering treatments (Table 1).

SEM micrographs of stearyl alcohol-W50 films conditioned at 48C for 0, 14 and 35 days are presented in Figure 3. The untempered film (Figure 3A) displayed a layered lipid morphology with large platelets partially projecting out of the bulk lipid. This was also reported in a previous study (1). In fact, the effective resistance of stearyl alcohol to the transmission of $O_2$ and water vapor, relative to other lipids, was attributed to the extensive layered structure, much of which is oriented horizontally to the film surface. According to Fox (5), lipids with sizeable plate crystals oriented perpendicular to the direction of gas or vapor flow exhibit the greatest resistance to gas and vapor transport.

SEM micrographs of tempered films show a progressive increase in the number and size of fatty alcohol platelets with increased tempering time (Figures 3B and 3C). Growth of large crystals at the expense of smaller ones is thermodynamically favorable and can be interpreted as movement toward a state of lower free energy.

Although the mechanisms by which tempering improves the barrier properties of stearyl alcohol to oxygen and water vapor are not known, at least two factors are probably involved. The first relates to healing of both film imperfections and crystal lattice dislocations which are believed to exist in all crystalline materials. Partial healing of these flaws during tempering would logically increase resistance to gas and vapor transmission by reducing the effective diffusion constants for the respective permeants.

A second factor likely contributing to enhanced resistance of stearyl alcohol to gas and vapor transport during tempering is growth of lipid crystals. This could have occurred by recrystallization (conversion of small crystals to larger ones or by a growing together of crystals) or by crystallization of previously uncrystallized material. Both events probably occurred since they are common in many kinds of samples. The possibility that the crystalline fraction of the sample increased during tempering is likely because the high viscosity of the sample would tend to preclude achievement of solid-liquid equilibrium during the initial solidification process.

Fox (5) attributed the improved water vapor barrier properties of paraffin wax films following tempering, in part, to growth of crystalline platelets positioned parallel to the film surface, i.e., perpendicular to the direciton of vapor flow. Although SEM did, indeed, show an increase in the size and number of stearyl alcohol platelet structures with increased tempering time, it appears from the micrographs that the visible platelets (those on the surface) are not uniformly arranged perpendicular to the direction of gas and vapor flow (Figures 3B and 3C). However, nothing is known about the arrangement of crystals below the surface. Furthermore, it is possible that those platelets crystals not aligned perpendicular to the direction of gas or vapor flow also provide effective resistance to transport. In this regard, leaves and fruits possess surface deposits of lipids that are crucial for controlling water loss. In many instances, these lipids assume the form of rods, tubules or other crystalline structures that project out from the surface and yet function as effective barriers to water vapor movement (11,12). For example, Possingham (13)

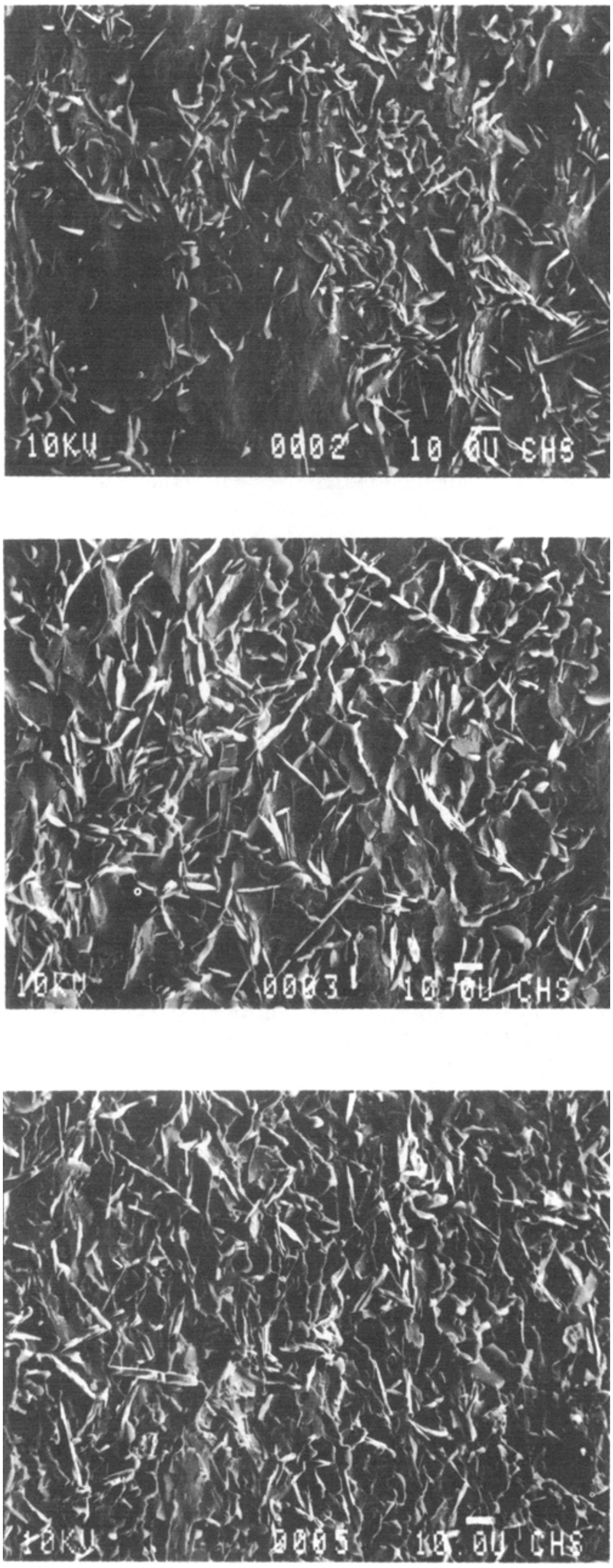


**FIG. 3. SEM micrographs of stearyl alcohol-W50 films tempered at 48 ± 2C for 0 (A), 14 (B) and 35 (C) days. Amount of lipid per unit area of film was 4.0 mg/cm$^2$. Micrographs were taken at a 45° angle to the film surface. The white bars are 10 $\mu$m in length.**

reported that lipids on the surface of sultana grapes are arranged in an elaborate series of overlapping platelets oriented in all directions, including many with their major planes perpendicular to the surface. Consequently, it is quite reasonable to suggest that the several "protruding" types of crystalline lipid structures just mentioned, including those on the surface of the stearyl alcohol film, can contribute effectively to the barrier properties of a lipid layer by increasing the length and tortuosity of the gas or vapor diffusion pathway. The net effect would be a reduction in the effective diffusion constant of the permeant (14). Proliferation of these structures on the surface of the stearyl alcohol film during tempering may be an important mechanism by which resistance to $O_2$ and water vapor transport is increased. It is likely, however, that this mechanism, and healing of crystalline imperfections and growth of lipid crystals all are involved in improving the gas/vapor barrier properties of stearyl alcohol films during tempering.

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