Modified Atmosphere Packaging for Prevention of Mold Spoilage of Bakery Products with Different pH and Water Activity Levels

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ABSTRACT

A sponge cake analog was used to study the influence of pH, water activity (a_w), and carbon dioxide (CO_2) levels on the growth of seven fungal species commonly causing bakery product spoilage (Eurotium amstelodami, Eurotium herbariorum, Eurotium repens, Eurotium rubra, Aspergillus niger, Aspergillus flavus, and Penicillium corylophilum). A full factorial design was used. Water activity, CO_2, and their interaction were the main factors significantly affecting fungal growth. Water activity at levels of 0.80 to 0.90 had a significant influence on fungal growth and determined the concentration of CO_2 needed to prevent cake analog spoilage. At an a_w level of 0.85, lag phases increased twofold when the level of CO_2 in the headspace increased from 0 to 70%. In general, no fungal growth was observed for up to 28 days of incubation at 25°C when samples were packaged with 100% CO_2, regardless of the a_w level. Partial least squares projection to latent structures regression was used to build a polynomial model to predict sponge cake shelf life on the basis of the lag phases of all seven species tested. The model developed explained quite well (R^2 = 79%) the growth of almost all species, which responded similarly to changes in tested factors. The results of this study emphasize the importance of combining several hurdles, such as modified atmosphere packaging, a_w, and pH, that have synergistic or additive effects on the inhibition of mold growth.

Bakery products are important staple foods in most countries and cultures. However, mold growth and staling are two problems that limit the shelf lives of both high- and intermediate-moisture bakery products (35). Fungal contaminants have been isolated from bakery products and identified as species of Eurotium, Aspergillus, and Penicillium. Other species, such as those of Cladosporium, Mucor, and Rhizopus, have been found less frequently (3).

Through the use of good sanitary conditions, it is possible to reduce the number of unwanted fungal spores on processed bakery products. However, the presence of such spores is expected, and special attention must be paid to them for food preservation. Many traditional preservation methods are based on the application of different “hurdles,” or “barriers,” that act synergistically to inhibit or retard microbial growth (21). pH and water activity (a_w) are among the most common variables used to ensure the microbical stability of a particular foodstuff. Also, the addition of weak organic acids, such as sorbic, propionic, and benzoic acids, is widely used in the preservation of food products (5). However, it is well known that the antimicrobial activity of these acids depends on their undissociated molecule. Several researchers have reported the inefficacy of this type of preservative in preventing the spoilage of products with a near-neutral pH (18, 23, 24). A more recent and increasingly popular way of preserving foods is controlled atmosphere storage or modified atmosphere packaging (MAP). These methods take advantage of a combination of the inhibitory effects of low oxygen levels and elevated carbon dioxide levels on many deterioration processes in foods and are effective in preventing microbial spoilage (15, 19).

The three major gases used in commercial MAP are oxygen (O_2), nitrogen (N_2), and carbon dioxide (CO_2) (8). Nitrogen is an inert, tasteless gas that displays little or no antimicrobial activity on its own. Because of its low solubility in water, the presence of N_2 in MAP food can prevent packages from collapsing for products that can absorb CO_2. CO_2 is the most important gas in the gas mixture; it is both bacteriostatic and fungistatic (7). Its preserving effect varies with concentration, incubation temperature, organism, and the a_w of the medium (15, 33). El Halouat and Debevere (10) reported that a reduction in the a_w level increased the inhibitory effects of high levels of CO_2 on the germination and mycelium growth of Aspergillus niger, Eurotium amstelodami, Penicillium chrysogenum, and Fusarium oxysporum.

Since molds are strictly aerobic, for the attainment of long shelf lives the levels of residual O_2 must be kept below 1% (27). Abellana et al. (2) reported that the CO_2 concentration in the headspace, a_w, and the interaction between CO_2 and O_2 levels were the most significant factors affecting Eurotium spp. growth on a sponge cake analog. To attain a residual O_2 level below 1%, the lowest vacuum level has to be reached before the shielding gas is reinjected into the package. The film permeability for CO_2 and the
TABLE 1. Mean oxygen concentrations for cake analogs as affected by \( a_w \) and modified atmosphere packaging, measured after 28 days of incubation at 25°C

<table>
<thead>
<tr>
<th>Atmosphere</th>
<th>( 0.80 )</th>
<th>( 0.85 )</th>
<th>( 0.90 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>17.65 A</td>
<td>1.14 AB</td>
<td>1.07 A</td>
</tr>
<tr>
<td>100% N(_2)</td>
<td>0.60 b</td>
<td>0.18 b</td>
<td>0.68 A</td>
</tr>
<tr>
<td>70% N(_2), 30% CO(_2)</td>
<td>0.52 b</td>
<td>0.19 AB</td>
<td>0.46 A</td>
</tr>
<tr>
<td>50% N(_2), 50% CO(_2)</td>
<td>0.77 b</td>
<td>0.67 AB</td>
<td>0.03 A</td>
</tr>
<tr>
<td>30% N(_2), 70% CO(_2)</td>
<td>0.75 b</td>
<td>0.70 AB</td>
<td>0.53 A</td>
</tr>
<tr>
<td>100% CO(_2)</td>
<td>1.05 b</td>
<td>1.35 AB</td>
<td>1.20 A</td>
</tr>
</tbody>
</table>

*Means with different letters in the same column are significantly different by the Duncan test.

characteristics of the product, such as its ability to trap O\(_2\) molecules inside its structure, have to be considered (27, 36).

The MAP technique involving various mixtures of CO\(_2\), O\(_2\), and N\(_2\) has been used to extend the chemical and microbiological shelf lives of several food products, such as meat and fish (9), peanuts and pecans, fish, rice, and bakery products (12, 15, 34). Much literature has described the effects of gaseous environments on fungal germination and growth on food and synthetic media. However, reports on the use of MAP to control fungal spoilage of bakery products are scarce.

Despite the inherent complexities associated with the quantification of fungal growth (17), predictive modeling of filamentous fungal growth has been carried out by several researchers (4, 29, 31). Many of these researchers have focused on the development of models for the growth rate or lag phase as a function of temperature, pH, and \( a_w \), and only a few studies have included carbon dioxide or oxygen concentration as a factor (16).

The objectives of the present work were (i) to determine the antifungal effects of different CO\(_2\) concentrations in the MAP of a sponge cake analog, representative of a Spanish bakery product with a near-neutral pH, at different \( a_w \) and pH levels and (ii) to develop models for the prediction of spoilage in bakery products.

MATERIALS AND METHODS

Fungal isolates. Seven isolates from different bakery products were used. Five of them, Eurotium amstelodami (3.205), Eurotium herbariorum (3.209), Eurotium rubrum (3.228), Aspergillus flavus (3.226), and Aspergillus niger (3.227), were isolated from Spanish bakery products by Abella et al. (3). These isolates belong to the Food Technology Department microorganism collection of Lleida University. The other two isolates, Eurotium repens (IBT18000) and Penicillium corylophilum (IBT6978), were kindly provided by the Department of Biotechnology of the Technical University of Denmark and had been isolated from Danish bakery products.

Experimental design. The factors used in this study included \( a_w \) (at levels of 0.80, 0.85, and 0.90), pH (at 6 and 7.5), and different gas compositions (air [21% O\(_2\), 70% N\(_2\)], 100% N\(_2\), 70% N\(_2\) and 30% CO\(_2\), 50% N\(_2\) and 50% CO\(_2\), 30% N\(_2\) and 70% CO\(_2\), and 100% CO\(_2\)). The \( a_w \) and pH levels were selected on the basis...
of the range of levels that might occur in bakery products. Colony diameter was the response recorded. A full factorial design was used, and all treatments were carried out three times separately.

Preparation of the analog. Sponge cake analogs were prepared as previously reported by Abellana et al. (1). Each analog was composed of 273 g of wheat flour, 211 g of vegetable oil, 258 g of eggs, and 4 g of baking powder. After baking, the cake had a pH of about 7.5, and its initial a_w value was ca. 0.75. To attain the required pH (6.0), citric acid was added to the mix of solid ingredients before baking, whereas a_w was adjusted by placing the cake analogs in petri plates containing water-glycerol agar (105.8, 69.0, and 39.1 g of glycerol in 100 ml of distilled water plus 1.5% of agar to adjust the a_w values of cake analogs to 0.80, 0.85, and 0.90, respectively). Previously, two calibration curves were constructed, one to determine the amount of citric acid necessary to reach the desired pH (24), and another to determine the concentration of glycerol in the agar needed to increase and maintain the a_w of the dough during the incubation period (data not shown). After baking, the cakes were aseptically cut into square pieces (5 by 5 cm) and placed in the sterile 9-cm petri dishes containing water-glycerol agar at the desired a_w and pH levels and the cakes were sealed with Parafilm for 48 h for the equilibration of moisture between the agar and the analogs.

Inoculation conditions. Fungi were grown on DG18 (10 g of glucose, 5 g of peptone, 1 g of KH_2PO_4, 0.5 g of MgSO_4·7H_2O, 220 g of glycerol, 15 g of agar, 0.2 mg of dichloran, and 100 mg of chloramphenicol in 1,000 ml of distilled water) for 14 days at 25°C to obtain sporulating cultures. Spores were harvested with a loop (3 mm) and transferred to sterile distilled water (0.005% Tween 80). The concentration of spores was determined with a Thoma chamber and adjusted to 1×10^6 to 5×10^6 spores per ml. Cake analogs were needle-inoculated in the center. Analogs with the same a_w and pH levels were inoculated with the seven different isolates and packed in the same bag (eight plates [seven isolates and one blank] per bag). The uninoculated plate (blank) was included in each bag to check a_w and pH levels at the end of the incubation period. The plastic bags used were composed of a polyethylene and polyamide coextrusion mix film (FontPack, Comercial del Paper Font S.A., Spain), with a total thickness of 90 μm. The transmission rates for the bags were as follows: oxygen, 19.913 cm^3 m^2 24 h^{-1} atm^{-1} [1 atm = 101.29 kPa]; carbon dioxide, 164.903 cm^3 m^2 24 h^{-1} atm^{-1}; water vapor, 2.60 cm^3 m^2 24 h^{-1} atm^{-1}. The bags were filled with air or the desired mixture of N_2 and CO_2 (Carburos Metálicos, S.E. de Carburos Metálicos S.A., Barcelona, Spain), at a product/gas ratio of 1:3 (vol/vol). The packaging was carried out with an EGAR VAC Basic-9 Digital compensated vacuum machine (Egarvac S.C.P., Terrassa, Barcelona, Spain) by creating a vacuum in the bag and then flushing the gas mixture at around 150 kPa prior to heat sealing. The bags were then stored at 25°C for 28 days.
**Growth measurements.** Colony diameters were measured daily or as required with the aid of a binocular magnifier oriented in two directions at right angles to each other. At the end of the experiment, the gas composition in the headspace of each package was sampled and analyzed by gas chromatography. A micro-gas chromatograph CP2002 (Chrompack International, Middelburg, The Netherlands) fitted with a thermal conductivity detector was used. A packed column COP-Molsieve (5 A, 4 m by 0.32 cm, thickness of stationary phase [df] = 10 μm) was kept at 90 kPa for the quantification of O₂ and N₂ (55°C). The CO₂ concentration was analyzed with a Pora-PLOT Q column (10 m by 0.32 mm, df = 10 μm) at 75°C and 140 kPa.

**Data analysis.** An analysis of covariance of colony radiiuses measured during the storage period with time as a covariable was carried out for each fungal species separately in order to determine significant differences between the levels of the factors assayed and their interactions. For this purpose, the Statistical Analysis System package (SAS, Version 8.02, SAS Institute Inc., Cary, N.C.) was used. The Gompertz model was used as the fitting equation (25) to estimate time before visible growth (lag phase), and this parameter was analyzed by partial least squares projection to latent structures (PLS) in order to establish suitable secondary predictive models. PLS is a regression extension of principal components analysis, which is used when it is of interest to connect the information in two blocks of variables to each other (14). The analysis gives the percentage of variance of the response explained by the model ($R^2$) and the predictive power of the model according to cross validation ($Q^2$). The latter analysis was performed with MODDE software (Version 4.0, Umetrics, Umea, Sweden).

**RESULTS**

**Oxygen levels in packages.** Table 1 presents the mean concentrations of O₂ measured in inoculated bags at the end of the incubation period. At $a_w$ levels of 0.85 and 0.90, all gas atmospheres contained <1.35% O₂, and almost no significant differences were observed among them. However, at an $a_w$ level of 0.80, the level of O₂ detected was nearly 17% for air-packaged samples, suggesting that the limiting factor for fungal growth was water availability. The exhaustion of O₂ in air-packaged samples at high $a_w$ levels
(0.85 to 0.90) was probably the reason fungal growth stopped after some days of incubation.

**Impact of CO\textsubscript{2} headspace concentration on colony radius.** The analysis of covariance revealed that MAP (air and 0 to 100% of CO\textsubscript{2} balanced with N\textsubscript{2}), a\textsubscript{w} (at levels of 0.80, 0.85, and 0.90), and their interaction had a statistically significant effect (P < 0.01) on fungal growth (Table 2). Despite the significant effect of pH or the interaction of pH and atmosphere on the growth of some species (*E. amstelodami, E. herbariorum, E. repens, E. rubrum, A. niger, A. flavus*, and *P. corylophilum*), no clear link was found between CO\textsubscript{2} antifungal activity and pH level. In general, all species were affected in the same way: they grew faster as the a\textsubscript{w} level increased and the level of CO\textsubscript{2} in the headspace decreased. Some slight differences among fungal species’ levels of sensitivity to gas composition were found, with *P. corylophilum* being the species that was most affected by the limiting factors applied, followed by *Aspergillus* spp. and *Eurotium* spp. Under the conditions that were most favorable for fungal growth (presented by cakes with high a\textsubscript{w} levels packaged in air), the maximum colony radii were ca. 25 mm for *Eurotium* spp. and 5 mm for *Aspergillus* spp. and *P. corylophilum*.

Water activity had a significant influence on fungal growth and determined the level of CO\textsubscript{2} needed to prevent cake analog spoilage (Figs. 1 through 3). It should be noted that the maximal colony radii for cake analogs (Table 3) were ca. 5 mm for *Eurotium* spp., no clear link was found between CO\textsubscript{2} antifungal activity and pH level. In general, all species were affected in the same way: they grew faster as the a\textsubscript{w} level increased and the level of CO\textsubscript{2} in the headspace decreased. Some slight differences among fungal species' levels of sensitivity to gas composition were found, with *P. corylophilum* being the species that was most affected by the limiting factors applied, followed by *Aspergillus* spp. and *Eurotium* spp. Under the conditions that were most favorable for fungal growth (presented by cakes with high a\textsubscript{w} levels packaged in air), the maximum colony radii were ca. 25 mm for *Eurotium* spp. and 5 mm for *Aspergillus* spp. and *P. corylophilum*.

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Impact of CO\textsubscript{2} headspace concentration on lag phases. In general, longer lag phases were estimated for the conditions that were the most critical for fungal growth (Table 3). A linear correlation between the inverse of the lag phase and the growth rate was found for all isolates, with R\textsuperscript{2} values ranging from 0.84 to 0.96 depending on the isolate. Longer lag phases corresponded to lower growth rates and smaller maximal colony radii. It is important to make use of these kinds of experimental data by constructing models with which, given a combination of factors and a storage period, one can predict the approximate degree of spoilage. Although approximate, these models can help food technologists to better predict shelf life and safety.

Lag phases for the seven isolates were modeled simultaneously with the use of all factors (pH, a\textsubscript{w}, and CO\textsubscript{2} concentration) and their quadratic terms and cross-term interactions. However, since pH was nonsignificant (data not shown), a new regression model including only significant terms (a\textsubscript{w} and CO\textsubscript{2} concentration, their interaction, and quadratic terms) was calculated. This new PLS regression led to two principal components models (PC1 and PC2) (Fig. 4). PC1 explained most of the variance in the lag phase date (R\textsuperscript{2} = 75.9%). PC2 is represented by a line orthogonal to PC1 and improves the approximation of the data set as much as possible, carrying the maximum residual information (not taken into account in PC1) (1.8% variance explained by PC2). The predictive power of the model (Q\textsuperscript{2}) was near 72.0%.

The model explained the variation for almost all species quite well, with fungal responses located close to one another in the loadings plot indicating that all species responded to changes in tested factors in similar ways (Fig. 4). The greater the distance of a factor or response from the origin, the stronger its effect on the model. Thus, responses were well explained, and a\textsubscript{w}, CO\textsubscript{2} concentration, and their interaction had a strong influence of on lag phases, while quadratic terms had little effect on PC1, which meant that they had little effect on lag phases. If a factor is located opposite a fungus, a negative correlation between the levels of the factor and lag phase of that fungus exists.
Lag phases were described by a polynomial model equation with six coefficients \( b_0, b_1, \ldots, b_5 \) (Table 4): lag phase \( = b_0 + b_1a_w + b_2CO_2 + b_3a_wCO_2 + b_4a_w^2 + b_5CO_2^2 \). It should be noted that these coefficients are unscaled and uncentered in order to allow the direct calculation of lag phases, so they cannot be used to assess the significance of the effects of the factors on lag phases.

Most of the models are highly significant, with \( R^2 \) values of >70%. The response of \( P. corylophilum \) was slightly different, and the model explained only 58.8% of the lag phase variance for this organism (Table 4). This result can also be observed in the loadings plot (Fig. 4), in which \( P. corylophilum \) appears quite separate from the other fungal species.

The models were subsequently used to generate two-dimensional contour plots. Plots of the combined effects of \( a_w \) and \( CO_2 \) are shown in Figure 5. Since similar plots were obtained for all \( Eurotium \) species and for all \( Aspergillus \) species, one species of each genus is represented. Lag phases for \( Eurotium \) spp. at an \( a_w \) level of 0.87 increased two-fold when the level of \( CO_2 \) in the headspace increased from 0 to 70% (e.g., \( E. amstelodami \) growth started after 20 days). Storage in 100% \( CO_2 \), irrespective of the \( a_w \) level, resulted in lag times of >20 days. When Figure 5 is considered, it should be taken into account that the absence of growth is equivalent to a lag phase of 28 days, the duration of the experiment. Thus, when a shelf life of ca. 28 days is predicted from the models, it is probable that no spoilage will occur.

**DISCUSSION**

In this study, the use of MAP combined with different pH and \( a_w \) values to prevent the spoilage of bakery products by \( Eurotium, Aspergillus \), and \( Penicillium \) species was evaluated. Bakery products with near-neutral pHs were used for the experiment. The results obtained corroborate those from other studies, which show that \( CO_2 \) has an important fungistatic effect (2, 15, 26). Spoilage was prevented with 70% \( CO_2 \) in the headspace (with a residual \( O_2 \) level of <1.35%) for products with an \( a_w \) level of 0.80, while spoilage was significantly delayed for products with \( a_w \) levels of 0.85 to 0.90. These results confirm those of previous studies in which reductions in the \( a_w \) level were shown to increase the effect of high levels of \( CO_2 \) (10, 19, 20, 22). It was found that with 30 to 50% \( CO_2 \) in the headspace, \( Eurotium \) spp. growth was totally prevented at an \( a_w \) level of 0.80;

<table>
<thead>
<tr>
<th>Isolate</th>
<th>( Q^2 )</th>
<th>( R^2 )</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>( E. amstelodami )</td>
<td>81.5</td>
<td>86.5</td>
<td>lag phase = -391.58 + 1,200.93( a_w ) - 1.56( CO_2 ) - 851.29( a_w^2 ) + (4.96 \times 10^{-4})(CO_2)^2 + 1.96a_wCO_2</td>
</tr>
<tr>
<td>( E. herbariorum )</td>
<td>64.9</td>
<td>67.8</td>
<td>lag phase = -630.02 + 1,763.63( a_w ) - 1.75( CO_2 ) - 1,181.92( a_w^2 ) + (8.28 \times 10^{-4})(CO_2)^2 + 2.11a_wCO_2</td>
</tr>
<tr>
<td>( E. repens )</td>
<td>81.5</td>
<td>85.7</td>
<td>lag phase = -111.52 + 511.90( a_w ) - 1.21( CO_2 ) - 431.05( a_w^2 ) + (1.11 \times 10^{-4})(CO_2)^2 + 1.60a_wCO_2</td>
</tr>
<tr>
<td>( E. rubrum )</td>
<td>80.9</td>
<td>84.3</td>
<td>lag phase = -64.09 + 388.73( a_w ) - 1.11( CO_2 ) - 350.36( a_w^2 ) + (5.03 \times 10^{-5})(CO_2)^2 + 1.48a_wCO_2</td>
</tr>
<tr>
<td>( A. niger )</td>
<td>71.2</td>
<td>76.3</td>
<td>lag phase = -571.49 + 1,610.86( a_w ) - 1.57( CO_2 ) - 1,076.31( a_w^2 ) + (7.61 \times 10^{-4})(CO_2)^2 + 1.89a_wCO_2</td>
</tr>
<tr>
<td>( A. flavus )</td>
<td>82.3</td>
<td>84.8</td>
<td>lag phase = -745.34 + 2,069.57( a_w ) - 1.98( CO_2 ) - 1,377.98( a_w^2 ) + (9.85 \times 10^{-4})(CO_2)^2 + 2.37a_wCO_2</td>
</tr>
<tr>
<td>( P. corylophilum )</td>
<td>44.7</td>
<td>58.8</td>
<td>lag phase = -497.24 + 1,341.13( a_w ) - 0.96( CO_2 ) - 852.60( a_w^2 ) + (6.98 \times 10^{-4})(CO_2)^2 + 1.08a_wCO_2</td>
</tr>
</tbody>
</table>

\(^a\) \( Q^2 \), percentage of variation predicted by the model; \( R^2 \), percentage of variation explained by the model.
as water availability increased, growth was only delayed. Only a 100% CO₂ atmosphere prevented spoilage by almost all isolates regardless of the a_w level. The inhibitory effect of CO₂ on microorganisms in a culture medium or in a food system depends on many factors. These factors include partial CO₂ pressure, O₂ concentration, headspace gas volume, temperature, acidity, and a_w (15). The effect of temperature was not assayed in this study, and the temperature level chosen was the one that was most likely to be encountered during distribution and storage in the retail market. The preserving effect of CO₂ strongly depended on a_w, while interaction with pH was not important, probably because of the similar pH values involved. Similarly, Ellis et al. (11, 12) demonstrated that the growth of A. flavus under different atmospheric CO₂ and O₂ concentrations was highly dependent on a_w and temperature. However, these authors did not find important differences between the effects of gas composition on fungal growth at pH 6 and those at pH 8. Haasum and Nielsen (20), in a study of the growth of fungi in a cheese environment, also found no significant differences in the inhibitory effects of CO₂ at different pH levels (4–8). The effectiveness of CO₂ also varies with the type of organism under consideration, with molds being more sensitive than yeasts (19, 22, 30).

In this study, all O₂ concentrations measured at the end of the incubation period except those for air-packaged bags were between 0.2 and 1.35%. Since molds by definition are strict aerobes, sufficient residual oxygen must be present in the package headspace to allow mold growth (10, 34). It has been demonstrated that molds can tolerate and even grow in air with headspace oxygen concentrations as low as 1 to 2% (13, 32). Moreover, several studies have shown that molds can grow in the presence of elevated CO₂ levels if O₂ is present (11, 12). Smith et al. (34) reported that a minimum of 0.4% O₂ was needed for the growth of A. niger and Penicillium spp. in a CO₂/N₂ (60:40) atmosphere. Abellana et al. (2) also found that low levels (0.02 to 0.5%) of O₂ did not modify fungal growth when levels of CO₂ in the bags were high. However, these authors found that as the CO₂ concentration decreased, O₂ levels had more influence on growth kinetics (small differences in O₂ levels could make fungi grow). Similarly, Nielsen and Rios (26) found that the growth of Aspergillus and Penicillium species was delayed only at reduced O₂ levels (0.02 to 0.03%) in either pure nitrogen or 50% N₂ and 50% CO₂. However, in pure CO₂, the molds were strongly inhibited, despite the residual O₂ in the package. The total elimination of air during MAP represents a serious hurdle owing to both the film’s permeability and the product’s capacity to trap O₂ molecules (28). When a vacuum evacuation of the package is carried out prior to gas injection, the level of residual O₂ in the headspace can be <1% (30). However, the smooth and fragile texture of sponge cakes must be taken into account; the cakes could be deformed (27). An innovative method for controlling oxygen without physically deforming products involves the incorporation of O₂ absorbers into the package (32).

CO₂ is both water and lipid soluble, and when gaseous CO₂ is applied to a product, part of the CO₂ goes to the liquid phase (water or oil) as carbonic acid (about 2%) (7). This development can produce a small decrease in pH, resulting in changes in the organoleptic characteristics of the product (15, 30). The solubility of this gas in a culture medium or in a food system increases as pH increases, as a_w increases, as the volume of headspace gas increases, or as temperature decreases. The pHs of cake analogs packaged aerobically and with the different gas compositions tested were measured, and no appreciable differences were found (data not shown). This result may suggest that the solubility of CO₂ in the cake analog used was not signifi-

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**FIGURE 5.** Contour plots based on models obtained by PLS regression showing lag phases (days) as affected by CO₂ level and a_w for (a) E. amstelodami, (b) A. niger, and (c) P. corylophilum.
cant or was not sufficient to produce a decrease in the analog pH.

In the last few years, predictive microbiology has become an important area of research. The advantage of the response surface modeling approach is that it allows the simultaneous examination of several variables. Also, mathematical models can be generated to predict the packaging and environmental storage conditions that would control fungal growth (11). Abellana et al. (2) predicted lag phases, radial growth rates, and maximum growth in relation to aw, O2, and CO2 concentrations for three Eurotium species on a sponge cake analog. A model such as that described here may be an important tool to aid food technologists in predicting shelf lives of products or may give an idea of the effects of certain changes in a product’s formulation. Also, this study emphasizes the importance of combining several barriers, such as MAP, aw, and pH, whose effects on mold growth are synergistic or additive.

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REFERENCES


