Modeling *Botrytis Cinerea* Spores Growth in Carbon Dioxide Enriched Atmospheres

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ABSTRACT: This study simulates the conditions in which *Botrytis* may appear in a modified atmosphere packed horticulture product, such as strawberry, so as to elaborate a predictive model that could allow us to estimate the shelf-life of a contaminated food product in such atmosphere conditions (0 to 40% CO_2). The estimated shelf-lives obtained at 18 °C were 92, 164, and 236 h in storage atmospheres of 0, 10, and 20% CO_2, respectively, very close to observed values; no growth was observed above 30% CO_2. The elaborated predictive model allows us to: (a) control development of this fungi if the food product is maintained in an atmosphere containing more than 20% CO_2 and (b) predict the time taken for potential colonies to become visible (3 mm dia) and, thus, cause immediate rejection by consumers.

Keywords: *Botrytis cinerea,* modeling, controlled atmosphere

Introduction

BOTRYTIS CINEREA IS A FUNGUS THAT FREQUENTLY SPOILS PLANT products such as tomatoes, grapes, and strawberries, particularly during humid periods. This infection is known by producers as gray mold rot. Spoilage is one of the main visible parameters consumers associate with loss of quality. Fungal growth occurs in plant products as soon as the tissue structure becomes suitable for such growth, either because of tissue softening due to ripening or because of fruit surface bruising.

Modeling enables us to predict what will happen during the storage of a product according to factors affecting microorganism growth. If growth data are obtained through a combination of growth-affecting factors, these can be used to build an empirical model to predict limit conditions for growth, growth rate, or the time required to achieve a particular microbial density or colony size in relation to shelf-life. Although many studies have focused on the development of models for bacterial growth as a function of temperature, pH, and a_w (Ratkowsky and others 1982; Geeraerd and others 2000; McKellar 2000; Ross and others 2000; Soboleva and others 2001; Uyttendaele and others 2001), to our knowledge, only a few studies have examined models that describe the growth physiology of common food spoilage molds growing on solid surfaces (Gibson and others 1994; Baranyi and others 1996; Cuppers and others 1997; Valik and others 1999; Santour and others 2001; Valik and Piecková 2001), while fewer still have included carbon dioxide or oxygen as a factor (El Halout and Debevere 1997; Hertog and others 1999).

As mentioned previously, strawberries are one of the main horticultural products affected by *Botrytis cinerea* (Browne and others 1984; Ghaouth and others 1991; Chambroy and others 1993; Vaughn and others 1993; Saks and others 1996). This fruit is one of Spain's main exports. It is grown mainly in the province of Huelva (south-western Spain), and its primary fresh-market destinations extend across central Europe. Strawberries are a highly perishable commodity; their shelf-life is usually only about 7 d, partly due to high respiration and transpiration rates and a morphology that renders them susceptible to crushing and gray mold fruit rot. When infection occurs, the infected tissue acquires a dull pinkish-brown color, and this may extend to the whole fruit without disintegration and with little sweating. After a while, white mycelia become visible on the fruit surface, which later turn gray as sporulation takes place. The infection of a healthy fruit occurs as the result of colonization from mycelia-infected neighboring fruits or by spore germination. In the latter case, the spores require free water on the fruit surface to germinate; this water comes from splashes or condensation on the fruit and occurs over a wide range of temperatures (Olías and others 1998). Techniques that enable even a short extension of shelf-life can have a profound effect on fresh-market strawberries. Atmospheres with CO_2 contents of 15 to 20% and O_2 contents of 5 to 10% have been reported as optimal for strawberry storage in terms of the different factors that may affect strawberry quality, such as *Botrytis cinerea* infection (Reyes and Smith 1986; Li and Kader 1989; Agar and others 1990; García and others 1996).

The aim of this study was to elaborate a model of the spores growth of *Botrytis cinerea* in function of the CO_2 concentration, and to evaluate its possibilities of prediction of shelf-life of a contaminated food product. In the case of packed strawberries, the appearance of just 1 fungus colony would give rise to consumer rejection.

Material and Methods

Samples

The strain used here, *Botrytis cinerea* (CECT 210), was restored in brain heart infusion (BHI, Oxoid) and inoculated repeatedly in PDA (Potato Dextrose Agar, Difco). Inoculum was prepared using the methodology described by García and others (1996), at a concentration of 10^3 cfu/ml. Conidia were counted using a Bürker camera and the final concentration was adjusted by dilution.

Petri dishes containing PDA were inoculated with 0.1 ml of fungus suspension containing 100 spores, and introduced in groups of 6 into 5 L flasks conditioned at CO_2 contents of 0% (air), 10%, 20%, 30%, and 40%, and stored at 18° C for 10 d. Colony dia was measured daily. The experiment was repeated twice.

Atmosphere composition analysis

Gas composition inside each flask was analyzed every d. CO_2 and O_2 contents were measured using a Hewlett-Packard 5890 gas chromatograph equipped with a thermal conductivity detector on a stainless steel Carbosieve S-II (3 m x 3 mm i.d.) column and with helium as carrier gas. CO_2 was analyzed isothermally at 225° C and O_2 at 33° C. Gas samples were 0.5 ml.

Shelf-life criteria

According to Gibson and others (1994), a 3 mm colony dia is deemed to be the size at which a consumer would reject a plant product. The end of shelf-life was therefore considered to be the point at which the colony reached this size, since it would become visible to the naked eye and the product would consequently be rejected.

Predictive model

Colony growth data were fitted applying Gompertz equations (Gibson and others 1988) and Baranyi and Roberts (1995) using DMFit program . Colony growth rate and lag-time were calculated by the same program. The next step is to study the relationship between the kinetic parameters estimated (colony growth rate and lag-time) with the factor affecting *Botrytis cinerea* growth, the CO_2 concentration. This was developed by applying the equations of Ratkowsky and others (1982) and Arrhenius (McMeekin and others 1993), as well as other possible mathematical transformations, searching that with less estimation error.

Statistical analyses of means, standard deviation and variance were performed using the STATISTICA/w 5.1 program (StatSoft Inc., Tulsa, Okla., U.S.A.).

Results and Discussion

The colony fungal growth during storage at 18° C is shown in Figure 1. An inhibitory effect of CO_2 on microbial growth was observed, mainly at CO_2 concentrations higher than 20%, since spores did not germinate within the storage period of 240 h studied here; complete growth inhibition was observed in atmospheres with CO_2 contents of 30 and 40% (Table 1). It is difficult to compare the results obtained here with those reported by other authors, since no similar studies have been found; most authors report experiments focusing on the development of the microorganism in horticultural products. Other authors, studying behavior in foods such as strawberries, report that when stored in atmospheres with 16% CO_2 and 8% O_2 for 7 d, *B. cinerea* was visible in 65% of cases. However, in foods stored at concentrations slightly higher than 20%, for example at around 23% CO_2 and 5% O_2 , this fungi grew in only 36% of the samples studied (Sanz and others 1999). This agrees with our findings, which suggest that germination of the microorganism was strongly inhibited in atmosphere with a CO_2 content of over 20%. Wszelaki and Mitcham (2000) also conclude that the most effective treatment for inhibiting the growth of *B. cinerea* mycelia is storage in an atmosphere with a CO_2 content of 15% and, as observed by other authors, this gas, rather than acting as a fungistat, delays germination (García and others 1996; El Halout and Debevere 1997; Hertog and others 1999; Sanz and others 1999).

García and others (1996), in a study of strawberries packed in plastic film, observed that in samples stored in atmospheres with a higher accumulated CO_2 concentration (52%), the development of *B. cinerea* was inhibited almost completely; however, by the 4th d of storage at 18° C, 34% of samples were affected by the microorganism. These authors also report that in strawberries stored in the open air (0% CO_2), *Botrytis cinerea* was visible in 25% of inoculated samples within 24 h, and after 4 d all were infected by the microorganism. This clearly demonstrates the inhibitory effect of CO_2 on this microorganism.

Table 1—Colony growth rate (μ), lag time (λ), time needed for the *Botrytis cinerea* to reach 3 mm (ts) and predictive values (ts pred) by the model.

CO_2	μ (h^{-1})	λ (h)	ts (h)	ts pred (h)
0%	0.8997	179	96	92
10%	0.6892	192	156	164
20%	0.7963	287	240	236
30%	NG[a]	NG	NG	NG
40%	NG	NG	NG	NG

[a]NG: not growth data

Agar and others (1990) studied the growth of *B. cinerea* in culture medium at different atmospheric CO_2 and O_2 concentrations, using already-formed mycelia measuring around 5 mm in dia; hence, the growth of this microorganism in atmosphere rich in CO_2 (30 to 40%) could be observed. This difference, with respect to the results obtained here, may be due to the fact that CO_2 affected the germination rather than the growth of this microorganism.

The equations of Baranyi and Roberts and Gompertz were each applied to colony growth data and the equation yielding the best fit was selected. Using these equations, colony growth rate (m, h^{-1}) and lag-time (l, h) were calculated by DMFit program.

Kinetic parameters (colony growth rate and lag-time) and CO_2 contents were compared using different equations, such as Ratkowsky and Arrhenius. Several other mathematical transformations were also used. The growth rate parameter at the 3 CO_2 concentrations was very similar and was fitted to a polynomial equation:

$$\mu = 0.0016 * (\%CO_2)^2 - 0.0369 * (\%CO_2) + 0.8997 \qquad r^2 = 1$$

The best fit for lag-time was as follows:

$$\ln(\lambda) = 0.235 * (\%CO_2) + 5.1338 \qquad r^2 = 0.85$$

CO_2 content was found to have a greater and more linear effect on lag time than growth rate; these findings agree with those reported by other authors (Agar and others 1990). The development of fungal spores in the plant product can be avoided, or at least delayed, if high concentrations of CO_2 are applied as soon as possible after harvest (Agar and others 1990 , García and others 1996, 1998).

The results described by Agar and others (1990) were fitted to the Baranyi and Roberts formula and kinetic parameters were estimated in the same way as the data obtained here, in order to compare both sets of data (Table 2). Agar and others (1990) report faster lag-times than those obtained here, perhaps because they used mycelia as starting material and the cultures were prepared at 20 °C; in this study, spores were used as starting material, since these were considered more likely to appear on the surface of strawberries than mycelia that have already formed. Growth rate parameters were more similar, although Agar's study recorded higher values than those obtained here in storage conditions with CO_2 contents of 0 and 10% (Table 1 and 2).

The product shelf-life (ts) was calculated for different CO_2 concentrations, by fitting to colony growth curves. These param-

Table 2—Colony growth rate (μ), lag time (λ), time needed for the *Botrytis cinerea* colony to reach 3 mm (ts), estimated from data obtained by Agar and others (1990).

% CO_2	μ (h^{-1})	λ (h)	ts (h)
0	1.073	0.000	3.78
10	0.746	0.000	5.13
15	0.578	0.000	6.49
20	0.529	10.092	15.67
25	0.486	21.240	27.31
30	0.413	33.856	40.99
40	0.106	63.300	91.00

eters, determined when colonies reached a dia of 3 mm, were 96 h, 156 h, and 240 h for 0%, 10%, and 20% CO_2, respectively. Linear regression of the data enabled the theoretical product shelf-life to be determined, according to the possible growth of this fungus, with CO_2 contents of between 0 and 20% and using the following equation:

$$ts\ (h) = 7.2^* (\%CO_2) + 92 \qquad r^2 = 0.99$$

The model therefore enabled us to accurately predict the time taken for the mycelium to reach a visible size in comparison with observed values (Table 1). Comparison of estimated lag-time and ts values revealed the following linear relationship:

$$\lambda\ (h) = 0.75^* ts\ (h) + 96.333 \qquad r^2 = 0.8388$$

This linear relationship was also evident when comparing the estimated data reported by Agar and others (1990), which gave the following equation:

$$\lambda\ (h) = 0.7497^* ts\ (h) - 2.034 \qquad r^2 = 0.9836$$

As described above, ts was estimated using the data reported by Agar and others (1990) (Table 2) and using the shelf-life model employed here (Table 1), with significant differences apparent between the 2. This was to be expected, as explained earlier, since different starting-materials were used to chart *B. cinerea* behavior. The advantages of building up the model with spore starter inoculum instead of mycelium is that it is more realistic and simulates better what would occur in the food product, which will be contaminated by spores rather that a mycelium already formed. This brings the consequence that the predictions of shelf-life will be greater, though the spores will have to germinate and the colony has to acquire the enough size to be seen (3 mm), and the model will not be so over-conservative that it will conduce to reduction of benefits to the producer with no justified reason.

In contrast, Hertog and others (1999) report shelf-life estimates, based on evidence of *B. cinerea,* of between 19 h and 36 h for strawberries packed and stored at 18 °C. Depending on the number of strawberries initially affected, these authors report a value substantially lower than that calculated using our model for 0% CO_2 that is the shorter shelf-life, or 96 h (Table 1).

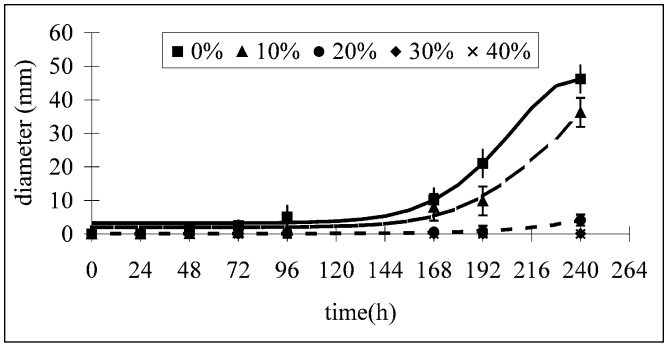

Figure 1—Mathematical fit of *Botrytis cinerea* colony diameter growth at different CO_2 concentrations.

Conclusion

THIS STUDY SIMULATES THE CONDITIONS IN WHICH *BOTRYTIS* MAY appear in a product such as the strawberry, by means of spores, which is a more realistic view than studying mycelium growth. Hence, this model enables us (a) to know that, in order to inhibit fungal development, the product should be kept in an enriched atmosphere with more than 20% CO_2 content; and (b) to estimate shelf-life in function of CO_2 content of the packed, as the time taken for any colonies to become visible, thus giving rise to immediate rejection by consumers.

References

Agar T, García JM, Miedtke U, Streif J. 1990. Effect of high CO_2 and low O_2 concentrations on the growth of *Botrytis cinerea* at different temperatures. Gartenbauwissenschaft 55:219-222.
Baranyi J, Gibson A, Pitt J, Eyles M, Roberts T. 1996. Predictive models as means of measuring the relatedness of some *Aspergillus* species. Food Microbiol 14:347-351.
Baranyi J, Roberts T. 1995. Mathematics of predictive food microbiology. Int J Food Microbiol 26:199-218
Browne K, Geeson J, Dennis C. 1984. The effects of harvest date and CO_2 –enriched storage atmospheres on the storage and shelf-life of strawberries. J Hortic Sci 59:197-204.
Chambroy Y, Guinebretiere M, Jacquemin G, Reich M, Breuils L, Souty M. 1993. Effects of carbon dioxide on shelf-life and post harvest decay of strawberries fruit. Sci Aliments 13:409-423.
Cuppers HGAM, Oomes S, Brul S. 1997. A model for the combined effects of temperature and salt concentration on growth rate of food spoilage molds. Appl Environm Microbiol 63(10):3764-3769.
El Halout A, Debevere J. 1997. Effect of water activity, modified atmosphere packaging and storage temperature on spore germination of moulds isolated from prunes. Int J Food Microbiol 35:41-48.
García J, Aguilera C, Jimenez A. 1996. Gray moldi and Quality of Strawberry Fruit following Postharvest Heat Treatment. Hort Sci 31:255-257.
García JM, Medina R, Olías JM. 1998. Quality of strawberries automatically packed in different plastic films. J Food Sci 63:1037-1041
Geeraerd A, Herremans C, Van Impe J. 2000. Structural model requirements to describe microbial inactivation during a mild heat treatment. Int J Food Microbiol 59:185-209.
Ghaouth A, Arul J, Grenier J, Asselin A. 1991. Glucanohydrolases and inhibitory activity to *Botrytis cinerea* in extracts from strawberry fruits. Can J Plant Pathol 13:315-320.
Gibson A, Baranyi J, Pitt J, Eyles M, Roberts T. 1994. Predicting fungal growth: the effect of water activity on *Aspergillus flavus* and related species. Int J Food Microbiol 23:419-431.
Gibson AM, Bratchell N, Roberts TA. 1988. Predicting microbial growth: growth responses of salmonellae in a laboratory medium affected by pH, sodium chloride and storage temperature. Int J Food Microbiol 6:155-178.
Hertog M, Boerrigter H, van der Boogaard G, Tijskens L, van Schaik A. 1999. Predicting keeping quality of strawberries (cv. Elsanta) packed under modified atmospheres: an integrated model approach. Postharvest Biol Technol 15:1-12.
Li C, Kader A. 1989. Residual effects of controlled atmospheres on postharvest physiology and quality of strawberries. J Am Hort Sci 114:629-634.
McKellar RC. 2000. A heterogeneous population model for the analysis of bacterial growth kinetics. Int J Food Microbiol 36:179-186.
McMeekin T, Olley J, Ross T, Ratkowsky D. 1993. Predictive microbiology. Theory and application. Taumton, Somerset, England: Research Studies Press LTD. p 96-110.
Olías JM, Sanz C, Pérez AG. 1998. Postcosecha de la Fresa de Huelva. Principios Básicos y Tecnología. Huelva, Spain: Imprenta Oromana, S.A.L. p 21.
Ratkowsky DA, Olley J, McMeekin A, Ball A. 1982. Relationship Between Temperature and Growth Rate of Bacterial Culture. J Bacteriol 149:1-5.
Reyes A, Smith R. 1986. Controlled atmosphere effect on the pathogenicity of fungi on celery and on the growth of *Botrytis cinerea.* Hort Sci 21:1167-1169.
Ross T, Dalgaard P, Tienungoon S. 2000. Predictive modeling of the growth and survival of *Listeria* in fishery products. Int J Food Microbiol 62:231-245.
Saks Y, Copel A, Barkai-Gola R. 1996. Improvement of harvested strawberry quality by illumination: color and *Botrytis* infection. Postharv Biol Technol 8:19-27.

Sanz C, Pérez A, Olias R, Olías JM. 1999 Quality of strawberries packed with perforated polypropylene. J Food Sci 64 (4):748-752.
Sautour M, Danatigny P, Divies C, Bensoussan M. 2001. A temperature-type model for describing the relationship between fungal growth and water activity. Int J Food Microbiol 67:63-69.
Soboleva TK, Filippov AE, Pleasants AB, Jones RJ, Dykes GA. 2001. Stochastic modeling of the growth of a microbial population under changing temperature regimes. Int J Food Microbiol 64:317-324.
Uyttendaele M, Taverniers I, Debevere J. 2001. Effects of stress induce by suboptimal growth factors on survival of *Escherichia coli* O157:H7 Int J Food Microbiol 66:31-37.
Valík L, Baranyi J, Görner F. 1999. Predicting fungal growth: the effect of water activity on *Penicillium roqueforti* Int J Food Microbiol 47:141-146.
Valík L, Piecková E. 2001. Growth modeling of heat-resistant fungi: the effect of water activity. Int J Food Microbiol 63:11-17.
Vaughn S, Spencer G, Shasha B. 1993. Volatile compounds from raspberry and strawberry fruit inhibit postharvest decay fungi. J Food Sci 58:793-796.
Wszelaki AL, Mitcham EJ. 2000. Effects of superatmospheric oxygen on strawberry fruit quality and decay. Postharvest Biol Technol 20:125-133.
MS 20010539Submitted 9/28/01, Accepted 2/5/02, Received 2/6/02

This work was partly financed by the CICYT ALI98-0676-C02-01, and the Research Group AGR-170 HIBRO. The authors would also like to thank the Spanish Ministry of Science and Technology for a Research Staff Training grant. To Józef Baranyi, who kindly provided the DMFit program.

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