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A Review of Effects of Carbon Dioxide on Microbial Growth and Food Quality

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ABSTRACT

Carbon dioxide is effective for extending the shelf-life of perishable foods by retarding bacterial growth. The overall effect of carbon dioxide is to increase both the lag phase and the generation time of spoilage microorganisms; however, the specific mechanism for the bacteriostatic effect is not known. Displacement of oxygen and intracellular acidification were possible mechanisms that were proposed, then discounted, by early researchers. Rapid cellular penetration and alteration of cell permeability characteristics have also been reported, but their relation to the overall mechanism is not clear. Several researchers have proposed that carbon dioxide may first be solubilized into the liquid phase of the treated tissue to form carbonic acid (H_2CO_3), and investigations by the authors tend to confirm this step, as well as to indicate the possible direct use of carbonic acid for retarding bacterial spoilage. Most recently, a metabolic mechanism has been studied by a number of researchers whereby carbon dioxide in the cell has negative effects on various enzymatic and biochemical pathways. The combined effect of these metabolic interferences are thought to constitute a stress on the system, and result in a slowing of the growth rate. The degree to which carbon dioxide is effective generally increases with concentration, but high levels raise the possibility of establishing conditions where pathogenic organisms such as *Clostridium botulinum* may survive. It is thought that such risks can be minimized with proper sanitation and temperature control, and that the commercial development of food packaging systems employing carbon dioxide will increase in the coming years.

The first observations regarding the effect of carbon dioxide on retarding bacterial growth were made nearly 100 years ago *(23,34)*. Since then, the potential for retarding spoilage through application of carbon dioxide has been explored in relation to a number of commodities. A partial list includes: fruits *(30,38,48)*, vegetables *(47)*, eggs *(12)*, carbonated beverages (28), pork *(1)*, poultry *(41,50)*, beef *(17)*, and seafoods *(7,18,26)*. Through these, and other studies, carbon dioxide has been shown to be effective for foods whose spoilage flora is dominated by gram-negative, aerobic, psychrotrophic bacteria. For this reason, as well as economic considerations, recent research has centered on use of carbon dioxide with fresh meats, poultry, and seafoods. The observation that high concentrations of carbon dioxide can cause darkening in tissues by combining with myoglobin to form metmyoglobin *(15)* has discouraged use of the technique with meats containing high levels of myoglobin, and has further focused attention on its use with poultry and seafoods.

In general, application of carbon dioxide increases both the lag phase and the generation time in the growth cycle of microorganisms *(51)*. These effects vary with the concentration of carbon dioxide, incubation temperature, organism, and water activity of the medium *(55)*. Despite over 100 years of study, and numerous publications exploring the effect of carbon dioxide on foods and bacterial cultures researchers have been unable to determine conclusively the method(s) by which carbon dioxide exerts an inhibitory effect on bacterial growth. This paper will review the major theories that have been put forth to explain the bacteriostatic action of carbon dioxide and its effect on food spoilage, and discuss the possible role of carbonic acid in that mechanism.

MECHANISM OF CARBON DIOXIDE ACTION

Displacement of oxygen

One of the first explanations for the action of carbon dioxide was that it displaced some or all of the oxygen available for bacterial metabolism, thus slowing growth by a proportional amount. This possibility was discounted early in the study of this system by experiments which showed that anaerobic bacteria were also inhibited by carbon dioxide atmospheres *(23)*. Callow *(16)* confirmed these findings by replacing the bacterial growth atmosphere with 100% nitrogen. He did not observe the degree of inhibition equal to that of when carbon dioxide was present. Although reducing available oxygen may have some effect on bacterial growth, it does not appear to be the most limiting factor.

Influence on pH

Most studies on carbon dioxide atmospheres and bacterial growth make the observation that the pH of the

growth medium is decreased *(9,32,52)*. A brief review of the behavior of carbon dioxide in solution illustrates the chemical species present that can account for this acidification. It should first be noted that the solution of gaseous carbon dioxide into an aqueous mixture obeys Henry's law very closely at moderate temperatures and low pressure. The values for the Henry's law constant for carbon dioxide were determined to be 0.797 atm/mole fraction at 10°C, 1.039 atm/mole fraction at 18°C, and 1.255 atm/mole fraction at 25°C. Table 1 illustrates the relationship between the partial pressure of carbon dioxide above a solution, and its solubility in the aqueous phase, as well as the fact that solubility increases as temperature decreases *(43)*.

Table 2 shows the primary reactions that occur when gaseous carbon dioxide is dissolved in water. The value of the equilibrium constant (K_{eq}) for hydration of carbon dioxide indicates that the percentage of carbonic acid present in solution is very small compared to the percentage of dissolved carbon dioxide gas. Several researchers *(13,43)* have estimated the proportion of carbonic acid to be about 2.0%.

While the hydration of carbon dioxide to form carbonic acid proceeds very slowly, the K'_a value for step (2) shows that the dissociation of the acid to form bicarbonate ion (HCO_3^-) and hydrogen ion (H^+) occurs very quickly, indicating that carbonic acid is a moderately strong acid. The second dissociation, which produced additional hydrogen ions, and carbonate ions (CO_3^{--}) does not generally occur to any great extent as evidenced by the K''_a value of 5.61×10^{-11}. The overall dissociation of carbonic acid to the various ionic species is dependent on the hydrogen ion concentration of the solution. Figure 1 illustrates that below pH 5, carbon dioxide in solution exists primarily as dissolved carbon dioxide gas and carbonic acid. Between pH 8 and 9.5 the carbonic acid dissociates (step 2) to form the bicarbonate and hydrogen ion species. While above pH 11.5 the second dissociation (step 3) is a factor resulting in the presence of hydrogen and carbonate ions.

Several researchers have suggested that when gaseous carbon dioxide is applied to a biological tissue, it is first dissolved into the liquid phase of the tissue, then absorbed as carbonic acid in the undissociated form *(7,25,39,45)*. This mechanism for movement of carbon dioxide into a cell would help to explain the observation by many researchers that application of carbon dioxide atmospheres also causes a rapid pH drop in the tissue *(2,32,42)*. After exclusion of oxygen was rejected as a major mechanism for the action of carbon dioxide, many early researchers suggested internal acidification as the cause of its bacteriostatic effect. Once again, however, a few relatively straightforward experiments demonstrated

TABLE 2. *Reactions of carbon dioxide in aqueous solution.*

(1) CO_2 (dissolved) + $H_2O \longrightarrow H_2CO_3$

$$K_{hyd}\ (298°K,\ 1\ atm) = \frac{[H_2CO_3]}{[CO_2]} = 2.6 \times 10^{-3}$$

(2) $H_2CO_3 \longrightarrow HCO_3^- + H^+$

$$K'_a\ (298°K,\ 1\ atm) = \frac{[H^+][HCO_3^-]}{[H_2CO_3]} = 4.3 \times 10^{-7}$$

(3) $HCO_3^- \longrightarrow CO_3^{--} + H^+$

$$K''_a\ (298°K,\ 1\ atm) = \frac{[H^+][CO_3^{--}]}{[H_2CO_3^-]} = 5.61 \times 10^{-11}$$

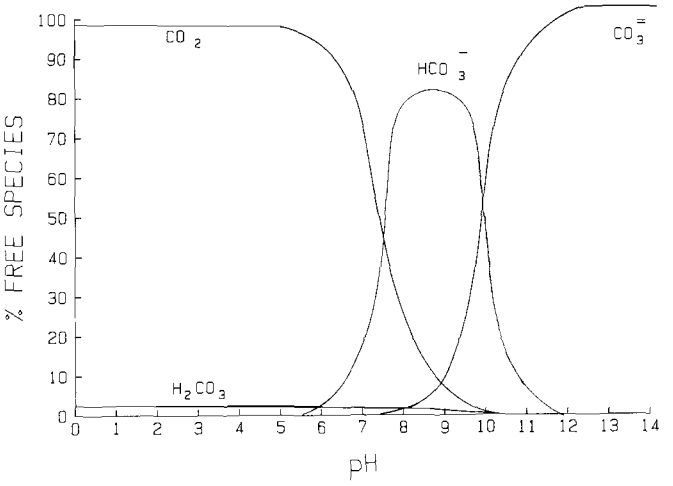

Figure 1. *Fraction of carbonate, bicarbonate, and carbon dioxide ions in solution as a function of pH* (3).

TABLE 1. *Solubility of carbon dioxide in water at various pressures and temperatures (volumes of CO_2 and 1 ATM/volume of water).*[a]

Pressure (psig)	Temperature (°C)				
	0	12	20	26	32
15	3.46	2.20	1.71	1.84	1.27
25	4.58	2.04	2.29	1.93	1.70
35	5.80	3.69	2.86	2.42	2.13
45	6.95	4.43	3.44	2.91	2.56
55	8.11	5.17	4.02	3.40	2.99
60	8.71	5.53	4.31	3.64	3.20
70	9.86	6.27	4.89	4.14	3.63
80	11.02	7.00	5.46	4.62	4.06
90	12.18	7.74	6.04	5.12	4.49
100	13.34	8.40	6.62	5.60	4.91

[a]From Quinn and Jones *(43)*.

that the observed effects were not due to acidification alone. Coyne *(19)* adjusted the pH of bacterial growth media to standard levels (approximately pH 5.8) then grew pure cultures of *Achromobacter, Pseudomonas,* and *Bacillus* under atmospheres of air, and others under carbon dioxide. In all trials, the carbon dioxide treatments produced a far greater degree of inhibition, as measured by culture growth. In another investigation, Becker *(9)* studied other acids that produced equal acidification in the cells, but found that they were not able to inhibit growth to the levels achieved through application of carbon dioxide.

The observation by several investigators that the effect of carbon dioxide is increased at low temperatures tends to support the theory that carbon dioxide acts first by dissolving in the liquid phase. Barnett et al. *(7)* reported this to occur to a minimum temperature of 1°C, below which no additional bactericidal effect was gained. Golding *(25)* stated that increased inhibition at lower temperatures has been correlated to increased solubility of the gas in the water phase. This was more recently confirmed by Wolfe *(56)* who found an increased effect at refrigerated temperatures and attributed it to increased solubility of carbon dioxide. Recent investigations by the authors indicate that direct application of carbonic acid to fresh fish fillets was effective in reducing surface microbial growth during refrigerated storage. Fresh cod fillets were dipped in cold carbonic acid that was prepared by dissolving solid carbon dioxide in distilled water. Controls included samples not treated, and those dipped for an equal time in distilled water alone. The dipped fish were also packaged in a retail 'tray-pack', using a semi-permeable polymeric film for overwrap. Figure 2 shows that the carbonic acid/packaged fish had approximately a one-log cycle reduction in surface microbial count over water dipped samples after 3 weeks in storage, and approximately a two-log cycle reduction over untreated samples. A subsequent trial compared the effectiveness of the carbonic acid treatment to one where the packages were flushed with a 98% carbon dioxide atmosphere before sealing. Figure 3 shows that although the gaseous carbon dioxide treatment resulted in a slower increase in microbial count over the first 2 weeks of the test, by the third week the levels of the carbonic acid and carbon dioxide treatments were nearly identical. Both treatments resulted in a significant reduction as compared to the microbial levels measured on the untreated controls. These results tend to support the hypothesis that the mechanism through which carbon dioxide acts on bacterial growth involves solubilization of the gas into the liquid phase of the system, and raises the possibility that carbonic acid may be used directly instead of the more difficult and costly practice of extending the shelf-life of perishable commodities through application of gaseous modified atmospheres.

Cellular penetration

Many researchers have documented the rapidity with which carbon dioxide in solution penetrates into the cell. Krogh *(36)* discovered that the rate is 30 times faster than

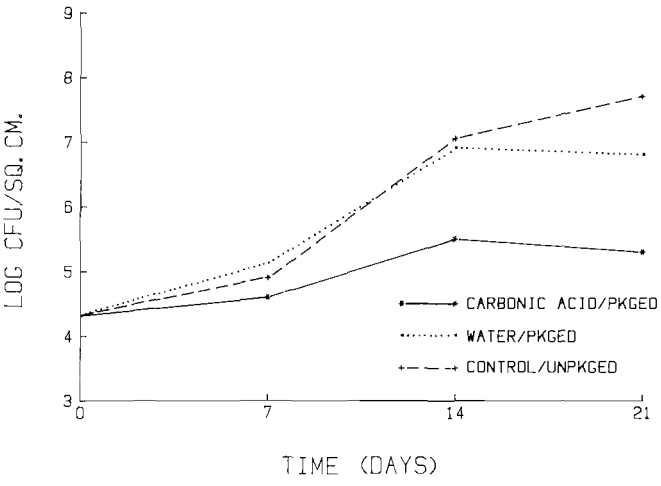

Figure 2. *Surface microbial growth for treated and control cod fillets stored at 1°C.*

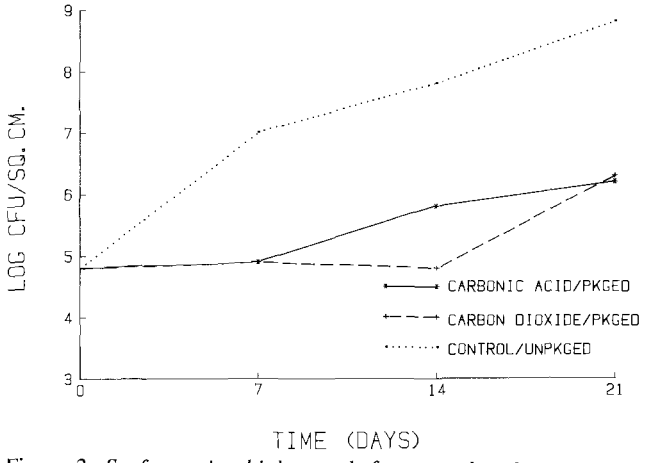

Figure 3. *Surface microbial growth for treated and control cod fillets stored at 1°C.*

for oxygen under most circumstances. This particular characteristic appears unique to carbon dioxide, and while undoubtedly related to the magnitude of the response, does not reveal any additional information as to the mechanism of inhibition once the effective compound is in the intracellular fluid.

An alternative theory suggests that carbon dioxide and bicarbonate ions may alter contact between the cell and its external environment by affecting the structure of the cell membrane. Sears and Eisenberg *(45),* using a model system, observed that the concentration of bicarbonate ions influenced the molecular arrangement at the interface between lipid droplets and water. They further reported that higher concentrations caused a decrease in the interfacial tensions and increased hydration of the 'membrane.' In the presence of carbon dioxide, the researchers found a tendency toward dehydration of the 'membrane.' They concluded that the bicarbonate would cause an increase in the membrane's permeability to ionic species, and could alter the balance in the internal and external metabolic processes. If carbon dioxide is first dissolved into the form of carbonic acid, then bicarbonate ion would be present as a dissociation product, and thus be available to produce these changes in cell permeability.

Metabolic interference by carbon dioxide

A broad category to which many recent investigators studying the mechanism of carbon dioxide/cellular interactions have alluded is that once in the cell, it interferes, either directly or indirectly, with the various chemical processes of metabolism. Elsden *(20)* first reported that *Bacillus* showed an increased rate of succinate formation in the presence of carbon dioxide, and that removal of carbon dioxide resulted in a decrease of succinate concentration. Foster and Davis *(22)* reported the specific inhibition of fumaric acid formation from glucose in *Rhizopus nigricans* when grown anerobically under high concentrations of carbon dioxide. In addition, these same authors reported that oxaloacetate decarboxylase was inhibited by the presence of carbon dioxide *(22)*. Fenestil et al. *(21)* reported that carbon dioxide stimulated mitochondrial ATPase activity, and that such an action would have an uncoupling effect on oxidative phosphorylation, resulting in a decreased level of energy available to the organism in the form of ATP for metabolism and growth. King and Nagel *(32)* found different growth rates for *Pseudomonas* grown on various substrates, and postulated that carbon dioxide may interfere with formation of exoenzymes that break down the substrate before absorption by the bacteria. King and Nagel *(33)* later attempted to determine whether carbon dioxide has a general effect on all enzymes, or a specific effect on selected enzymes. They detected no inhibition by carbon dioxide on the enzymatic rates of oxaloacetate decarboxylase, fumarase, succinate dehydrogenase, or cytochrome c oxidase, but concluded that carbon dioxide at concentrations above 50% inhibits the activity of isocitrate dehydrogenase and malate dehydrogenase. From these results, the authors concluded that carbon dioxide has a specific effect on particular enzymes, and inhibits certain decarboxylation enzymes through a mass action effect.

Barnett et al. *(8)*, studying the storage of shrimp in saturated carbon dioxide brines, observed an approximate doubling of storage time, as well as a slowing of the development of tissue blackening. Their report attributes the effect on the darkening reaction to inhibition of an enzymatic reaction by the carbon dioxide in solution.

More recently, Mitsuda et al. *(39)* stated that on the basis of work with a model system, carbon dioxide interacts with enzymes to cause a transient inactivation, particularly to the many hydrolases that cause autolysis after death. Wolfe *(56)* related disruption of normal intracellular activity to pH changes related to absorption of carbon dioxide.

Although there appears to be ample evidence that carbon dioxide inhibition is related to enzymatic interference, Gill and Tan *(24)* expressed the consensus by stating that the basis of such inhibition is not known, although the specific inhibition of certain enzymes may be involved.

The research to date regarding inhibition of bacterial growth in the presence of carbon dioxide, may be summed up as follows: *(a)* The exclusion of oxygen by replacement with carbon dioxide may contribute slightly to the overall effect, by slowing the growth rate of aerobic bacteria. *(b)* The ease with which carbon dioxide penetrates the cell may facilitate its chemical effects on the internal metabolic processes. *(c)* Carbon dioxide is able to produce a rapid acidification of the internal pH of the cell with possible ramifications relating to metabolic activities. *(d)* Carbon dioxide appears to exert an effect on certain enzyme systems. Such effects do not appear to be similar among species, and may well be affected by different growth conditions among members of the same species.

APPLICATION OF CARBON DIOXIDE FOR FOOD PRESERVATION

Two aspects concerning use of carbon dioxide need to be addressed in regard to food preservation. One is the concentration of carbon dioxide needed to produce optimal inhibition of bacterial growth; the other is the potential for growth of pathogenic bacteria under high carbon dioxide concentrations.

With regard to optimal concentration, there is considerable ambiguity among various researchers as to reported values, as well as methodologies for approaching the question. Valley *(53)* reported that concentrations only slightly above atmospheric can actually stimulate bacterial growth, but that in still higher concentrations bacterial growth is inhibited. Haines *(27)* reported that concentrations as low as 10 to 20% were sufficient to inhibit growth of *Pseudomonas* and *Achromobacter*. Shewan *(46)* recommended concentrations between 30 and 40% for improving the quality of whitefish. Brown et al. *(14)* studied storage of rockfish fillets and salmon steaks in both 20 and 40% carbon dioxide atmospheres and reported superior quality in the higher concentration. Tarr *(49)* recommended a minimum of 40 to 50% to derive maximum benefit in the storage of fresh fish. Coyne *(19)*, in one of the original studies on using carbon dioxide atmospheres to prolong fish quality, recommended concentrations between 40 and 60%, and suggested that above the upper concentration no additional benefits could be derived. Callow *(16)* studied the effect of carbon dioxide on pork and bacon using 100% carbon dioxide atmospheres. Although this author makes no recommendations as to optimal level or commercial applications, it represents the extreme of the numerous concentrations that can be found in the literature. There is some evidence to support the observation that the bacterial inhibition increases with the concentration of carbon dioxide present in the system. King and Nagel *(32)* controlled the various growth factors for pure cultures of *Pseudomonas aeruginosa*, and found a linear relationship between generation time and carbon dioxide level. This relationship was more recently confirmed by Blickstad et al. *(10)*, who concluded that the bacteriostatic/preservative effect of carbon dioxide increases with increasing concentration.

While it may be impossible to prove that in CO_2-rich atmospheres the potential for contamination with toxin from *Clostridium botulinum* does not exist, there is evi-

dence that it may not present a significant risk provided proper sanitation and temperature controls are employed. Zak *(57)* found that haddock *(Melanogrammus aeglefinus)* contain an antimicrobial polypeptide which inhibits growth of *Clostridium*. This factor may be present in other species as well. Johannsen *(29)* suggested that those bacterial species which predominate in carbon dioxide atmospheres, such as the lactobacilli, form peroxides and acids that may inhibit growth of *Clostridium*. Schmidt et al. *(44)* reported 3.3°C as a minimum temperature for growth and toxin production by *C. botulinum* type E. The potential for contamination could be minimized by maintaining temperatures below this level. *Clostridium* growth and toxin production has been detected in vacuum-, and carbon dioxide-packaged fish, but in all instances, the food was spoiled beyond all hope of human consumption *(6)*. Wilhelm *(54)* cautioned that until the safety from botulism can be demonstrated, use of vacuum- and carbon dioxide-packaging that involve low oxygen concentrations cannot be recommended for retail use. Licciardello et al. *(37)* reminded that even if the toxin is formed, normal cooking operations would inactivate it.

Carbon dioxide has been used by the food industry for many years, particularly for preservation of highly perishable, and certain high-value commodities. For instance, controlled atmosphere warehousing of fresh fruits has been practiced since the 1920s *(11)*. This application of carbon dioxide does not use the bacteriostatic effects described in this review, but benefits from retardation of ripening, that is caused by a mass action effect on respiration of the stored fruit. This technique is still widely used, and has been extended to the long distance transport of different fruits and vegetables in air-tight rail cars, trucks, and sea-board containers *(30)*. Though not yet as widespread, carbon dioxide has also found application in others foods where bacterial spoilage must be controlled. This effort has been spurred by recent advances in the packaging industry that have led to development of highly specialized polymeric packaging films, and so have brought about a renewed interest in use of carbon dioxide and other gases for modified atmosphere packaging (MAP). The European food industry is currently using MAP for many commodities such as beef, pork, chicken, fish, breads, pasta, and others *(4)*. Development of similar commercial applications is also beginning to gain momentum in the United States. A trend toward using modified atmospheres in smaller unit volumes has been observed over the last decade, to the point where it is now being employed for individual retail packages *(5)*. A recent market study recognized this packaging approach as one with growing potential and identified a number of promising products including meats, seafoods, cheese, potato chips and other snack foods, and cereals *(35)*. In most of the applications reported to be under development, carbon dioxide is mixed with other gases, such as oxygen and nitrogen, that are intended to serve different purposes. For instance, in the packaging of red meats under MAP, oxygen is used to retain the desirable red color in the product, and nitrogen is often included as an inert filler *(4)*.

It is likely that with continuing development of packaging materials and equipment, and further research into optimizing the technique's effectiveness, carbon dioxide and other gases will see increased use for preservation of food quality in the food manufacturing and marketing industries.

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REFERENCES

1. Adams, J. R., and D. L. Huffman. 1972. Effect of controlled gas atmospheres and temperatures on quality of packaged pork. J. Food Sci. 37:869-872.
2. Aickin, C. C., and R. C. Thomas. 1975. Micro-electrode measurement of the internal pH of crab muscle fiber. J. Physiol. 52:803-807.
3. Anonymous. 1981. Carbon dioxide electrode. Form 95-021M/1870. p 18. Orion Research Incorporated Cambridge, MA.
4. Anonymous. 1984. Controlled atmosphere packaging. Food Eng. 56:52-53.
5. Anzuetto, C. R., and S. S. H. Rizvi. 1984. Microatmosphere packaging of apples. Paper presented at Institute of Food Technologists annual meeting. June 10-13. Anaheim, CA.
6. Banner, R. 1978. Vacuum-packaging for fresh fish; scaling the first hurdle. Food Eng. 50:92-93.
7. Barnett, H. J., R. W. Nelson, P. J. Hunter, and H. Groninger. 1971. Studies on the use of carbon dioxide in refrigerated brine for preservation of whole fish. Fish. Bull. 69:433-441.
8. Barnett, H. J., R. W. Nelson, P. J. Hunter, and H. Groninger. 1978. Use of carbon dioxide dissolved in refrigerated brine for the preservation of pink shrimp (*Pandalus* spp.). Mar. Fish. Rev. 40:24-28.
9. Becker, Z. E. 1933. A comparison between the action of carbonic acid and other acids upon the living cell. Protoplasma 25:161-175.
10. Blickstad, E., S-O Enfors, and G. Molin. 1981. Effect of hyperbaric carbon dioxide pressure on the microbial flora of pork stored at 4°C or 14°Celcius. J. Appl. Bacteriol. 50:493-504.
11. Brecht, P. A. 1980. Use of controlled atmosphere to retard spoilage of produce. Food Technol: 34 45-50.
12. Brooks, J., and D. J. Taylor. 1955. Eggs and egg products. Dept. of Scientific and Industrial Research Food Investigation Special Report no. 60. HMSO, London.
13. Brinkman, R., R. Margaria, and F. J. W. Roughton. 1933. The kinetics of the carbon dioxide-carbonic acid reaction. Proc. Roy. Soc. Lond. A. 232:65-73.
14. Brown, W. D., M. Albright, D. A. Watts, B. Heyer, B. Spruce, and R. J. Price. 1980. Modified atmosphere storage of rockfish

(Sebastes miniatus) and silver salmon *(Oncorhynus kisutch)*. J. Food Sci. 45:93-96.
15. Brown, W. D., and L. B. Mebine. 1969. Autoxidation of oxymyoglobins. J. Biol. Chem. 244:6696-6701.
16. Callow, E. H. 1932. Gas storage of pork and bacon. Part I - Preliminary experiments. J. Soc. Chem. Ind. (London) 51:116T-119T.
17. Clark, D. S., and C. P. Lentz. 1973. Use of mixtures of carbon dioxide for extending shelf-life of prepackaged fresh beef. J. Inst. Can. Scien. Tech. Aliment. 6:194-199.
18. Coyne, F. P. 1932. The effect of carbon dioxide on bacterial growth with special reference to the preservation of fish. Part I. J. Soc. Chem. Ind. (London) 51:119T-121T.
19. Coyne, F. P. 1933. The effect of carbon dioxide on bacterial growth with special reference to the preservation of fish. Part II. J. Soc. Chem. Ind. (London) 52:19-24.
20. Elsden, S. R. 1938. The effect of carbon dioxide on the production of succinic acid by *Bact. coli commune*. Biochem J. 32:187-193.
21. Fanestil, D. D., A. B. Hastings, and T. A. Mahowald. 1963. Environmental carbon dioxide stimulation of mitochondrial adenosine triphosphatase activity. J. Biol. Chem. 238:836-842.
22. Foster, J. W., and J. B. Davis. 1949. Carbon dioxide inhibition of anaerobic fumarate formation in molds *Rhizopus nigricans*. Arch. Biochem. 21:135-142.
23. Frankel, H. R. 1889. As cited in: Killeffer, D. H. 1930. Carbon dioxide preservation of meat and fish. Ind. Eng. Chem. 22:140-143.
24. Gill, C. O., and K. H. Tan. 1979. Effect of carbon dioxide on the growth of *Pseudomonas fluorescens*. Appl. Environ. Microbiol. 38:237-240.
25. Golding, N. S. 1945. The gas requirements of molds. IV. A preliminary interpretation of the growth rates of four common mold cultures on the basis of absorbed gases. J. Dairy Sci. 28:737-750.
26. Gray, R. J., D. G. Hoover, and A. M. Mvir. 1983. Attenuation of microbial growth on modified atmosphere-packaged fish. J. Food Prot. 46:610-613.
27. Haines, R. B. 1933. The influence of carbon dioxide on the rate of multiplication of certain bacteria as judged by viable counts. J. Soc Chem Ind. (London) 52:13T-15T.
28. Insalata, N. F. 1952. Carbon dioxide versus beverage bacteria. Food Eng. 24:84-85,190.
29. Johannsen, A. 1965. Public health aspects of prepackaged fish for retail. p. 271-278. *In* Fish Handling and Preservation. OECD. Paris.
30. Kadar, A. A. 1980. Prevention of ripening in fruits by controlled atmospheres. Food Technol. 34:51-54.
31. Killeffer, D. H. 1930. Carbon dioxide preservation of meat and fish. Ind. Eng. Chem. 22:140-143.
32. King, A. D., and C. W. Nagel. 1967. Growth Inhibition of a Pseudomonas by carbon dioxide. J. Food Sci. 32:575-579.
33. King, A. D., and C. W. Nagel. 1975. Influence of carbon dioxide upon the metabolism of *Pseudomonas aeruginosa*. J. Food Sci. 40:362-366.
34. Kolbe, H. 1882. As cited in: Killeffer, D. H. (1930) Carbon dioxide preservation of meat and fish. Ind. Eng. Chem. 22:140-143.
35. Koski, D. 1984. Costs and benefits of controlled atmosphere packaging. Presented at Controlled Atmosphere Packaging conference. Univ. of Del., March 25-26.
36. Krogh, A. 1919. The rate of diffusion of gases through animal tissues with some remarks on the coefficient of invasion. J. Physiol. 52:391-408.
37. Licciardello, J. J., J. T. R. Nickerson, C. A. Riblich, and S. A. Goldblith. 1967. Thermal inactivation of type E botulinum toxin. Appl. Microbiol. 15:249-256.
38. Littlefield, N. A., B. N. Wankier, D. K. Salunkhe, and J. N. McGill. 1966. Fungistatic effects of controlled atmospheres. Appl. Microbiol. 14:579-581.
39. Mitsuda, H., K. Nakajima, H. Mizuno, and F. Kawai. 1980. Use of sodium chloride for extending shelf-life of fish fillets. J. Food Sci. 45:661-666.
40. Morgan, O. M., and O. Maass. 1931. An investigation of the equilibria existing in gas-water systems forming electrolytes. Can. J. Res. 5:162-199.
41. Ogilvy, W. S., and J. C. Ayres. 1951. Post-mortem changes in meats II. The effects of atmospheres containing carbon dioxide in prolonging the storage life of cut-up chicken. Food Technol. 5:97-102.
42. Parkin, K. L. 1979. The use of modified atmospheres for the preservation of fresh seafood products. M. S. Thesis, Univ. Cal., Davis.
43. Quinn, E. L., and C. L. Jones. 1936. Carbon dioxide. Reinhold Pub. Corp. New York, NY.
44. Schmidt, C. F., R. V. Lechowich, and J. F. Folinazzo. 1961. Growth and toxin production by type E *Clostridium botulinum* below 40°F. J. Food Sci. 26:626-630.
45. Sears, D. F., and R. M. Eisenberg. 1961. A model representing a physiological role of carbon dioxide at the cell membrane. J. Gen. Physiol. 44:869-887.
46. Shewan, J. M. 1949. Some bacteriological aspects of handling, processing, and distribution of fish. J. Roy. Sanit. Inst. 69:394-421.
47. Singh, B., C. C. Yang, and D. K. Salunkhe. 1972. Controlled atmosphere storage of lettuce. I. Effects of quality and respiration rate on lettuce heads. J. Food Sci. 37:48-51.
48. Smith, W. H. 1963. The use of carbon dioxide in the transport and storage of fruits and vegetables. p. 96-99. *In* Advances in food research, vol. 12. Academic Press. New York, NY.
49. Tarr, H. L. A. 1954. Microbiological deterioration of fish postmortem; its detection and control. Bacteriol. Rev. 18:1-15.
50. Thomson, J. E.,, and L. A. Risse. 1971. Dry ice in various shipping boxes for chilled poultry: Effect on microbiology and organoleptic quality. J. Food Sci. 36:74-77.
51. Tomkins, R. G. 1932. The inhibition of the growth of meat-attacking fungi by carbon dioxide. J. Soc. Chem. Ind. 51:261T-267T.
52. Valley, G., and L. F. Rettger. 1927. The influence of carbon dioxide on bacteria. J. Bacteriol. 14:101-137.
53. Valley, G. 1928. The effect of carbon dioxide on bacteria. Quart. Rev. Biol. 3:209-213.
54. Wilhelm, K. A. 1982. Extended fresh storage of fishery products with modified atmospheres: A survey. Mar. Fish. Rev. 44:17-20.
55. Wodzinski, R. J., and W. C. Frazier. 1961. Moisture requirements of bacteria. IV. Influence of temperature and increased partial pressure of carbon dioxide on requirements of three species of bacteria. J. Bacteriol. 81:401-413.
56. Wolfe, S. K. 1980. Use of CO and CO_2 enriched atmospheres for meats, fish, and produce. Food Technol. 34:55-58.
57. Zak, J. M. 1970. Factors affecting the outgrowth of *Clostridium botulinum*, type E. PhD Thesis, Mass. Inst. of Technol. Cambridge.