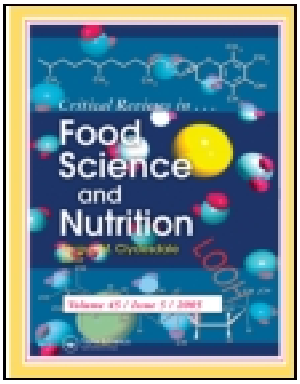

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Shelf Life and Safety Concerns of Bakery Products—A Review

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Bakery products are an important part of a balanced diet and, today, a wide variety of such products can be found on supermarket shelves. This includes unsweetened goods (bread, rolls, buns, crumpets, muffins and bagels), sweet goods (pancakes, doughnuts, waffles and cookies) and filled goods (fruit and meat pies, sausage rolls, pastries, sandwiches, cream cakes, pizza and quiche). However, bakery products, like many processed foods, are subject to physical, chemical and microbiological spoilage. While physical and chemical spoilage limits the shelf life of low and intermediate moisture bakery products, microbiological spoilage by bacteria, yeast and molds is the concern in high moisture products i.e., products with a water activity (a_w) >0.85. Furthermore, several bakery products also have been implicated in foodborne illnesses involving Salmonella *spp.,* Listeria monoctyogenes *and* Bacillus cereus*, while* Clostridium botulinum *is a concern in high moisture bakery products packaged under modified atmospheres. This extensive review is divided into two parts. Part I focuses on the spoilage concerns of low, intermediate and high moisture bakery products while Part II focuses on the safety concerns of high moisture bakery products only. In both parts, traditional and novel methods of food preservation that can be used by the bakery industry to extend the shelf life and enhance the safety of products are discussed in detail.*

Keywords bakery products, spoilage, pathogens, food borne illness, shelf life, safety, microbial control

INTRODUCTION

Bakery products have been an important part of a balanced diet for thousands of years. Indeed, cereal grains, mixed with water and cooked by fire, may have been our ancestors' first "bread type" product. Today, the production of bread and other bakery products has evolved from a primitive, cottage industry into a large-scale, modern manufacturing industry, generating billions of dollars in revenue and employing thousands of personnel. In 1998, sales of bakery products in the United States exceeded 10 million metric tons and had a market value of ∼$27 billion dollars—a 14.5% increase over the previous four years.[145] In Canada, the bread and bakery industry shipped ∼$2.3 billion dollars (Cdn) of products in 2000, an increase of 36.3% from 1988 levels, an amount which accounted for 4.2% of the total food and beverage processing sector shipments.[4] This sustained growth has been driven by consumer demand for convenient, premium baked goods which are fresh, nutritious, conveniently packaged, and shelf stable. Moreover, this increased demand is being met by various new processing and packaging technologies, including modified atmosphere packaging, a technology that has increased the availability and extended both the shelf life and market area of a wide variety of bakery products. At the same time, there has been an increase in in-store bakeries and a renewed interest in "organic," ethnic, and artisan type bakery products.

A wide variety of bakery products can be found on supermarket shelves, such as breads, unsweetened rolls and buns, doughnuts, meat pies, dessert pies, pizza, quiche, crackers, cookies, and other products. Several methods can be used to classify these products. Classification can be based on product type, i.e., unsweetened, sweetened, or filled goods, as shown in Table 1, or on their method of leavening, e.g., biological, chemical or unleavened. However, from a technological viewpoint, bakery products can be classified on the basis of their pH, moisture content, and water activity (a_w).[77,213]

Bakery products can be conveniently classified by pH into three groups: (i) high acid bakery products with pH < 4.6, (ii) low acid bakery products with pH > 4.6 but <7, and (iii) nonacid or alkaline bakery products with pH > 7. Examples of various products within these pH categories are shown in Table 2.

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Table 1 Categories of bakery products found on supermarket shelves

Categories of bakery products	Types within each category
Unsweetened goods	Bread: sliced, crusty, par-baked, ethnic
	Rolls: soft, crusty
	Crumpets
	English muffins
	Croissants
	Pizza base
	Raw pastry
Sweet goods	Large cakes: plain, fruited
	Pancakes
	Doughnuts
	Waffles
	Cookies
	Biscuits
	American muffins
	Buns
	Wafers
Filled goods	Tarts: fruit, jam
	Pies: meat, fruit
	Sausage rolls
	Pasties
	Cakes: cream, custard
	Pizza
	Quiche

Adapted from Blakistone.[27]

Smith and Simpson[223] also classified bakery products on the basis of their a_w as (i) low moisture bakery products with $a_w < 0.6$, (ii) intermediate moisture bakery products with a_w between 0.6 and 0.85, and (iii) high moisture bakery products with $a_w > 0.85$ and generally between 0.95 and 0.99. Examples of products within each a_w category are shown in Table 3.

Most bakery products are marketed fresh and are stored at ambient temperature. However, other products, such as cream, fruit, and meat filled pies and cakes, are stored under refrigerated or frozen storage conditions to achieve a longer shelf life. Bakery products, like most processed foods, are subject to physical, chemical, and microbiological spoilage. Furthermore, classification of products on the basis of their pH and a_w is helpful in recognizing the spoilage and safety potential of bakery products. While physical and chemical spoilage problems limit the shelf life of low and intermediate moisture bakery products, microbiological spoilage is the main concern of intermediate and high moisture products. High moisture unfilled, and filled, bakery products have also been implicated in outbreaks of foodborne illness and, therefore, pose safety concerns. This review will be divided into two parts. Part I will focus on the shelf life concerns of low, intermediate and high moisture bakery products, while Part II will review the safety concerns of high moisture products only. In both parts, the strategies used by the industry to extend shelf life and enhance the safety of bakery products will be discussed in detail.

Table 2 pH range of selected bakery products

Product	pH range	Reference
High acid		
Sourdough bread	4.2–4.6	Martinez-Anaya et al.[168]
Apple pie	4.2	Smith and Simpson[223]
Low acid		
White bread	5.7	Rosenkvist and Hansen[199]
Whole wheat bread	5.6	Rosenkvist and Hansen[199]
Chocolate nut bread	6.2–6.6	Denny et al.[71]
Date nut bread	6.1–6.7	Denny et al.[71]
Nonacid		
Crumpets	6–8	Jenson et al.[132]
Banana nut bread	7.2–7.9	Aramouni et al.[12]
Carrot muffin	8.7	Smith and Simpson[223]

Table 3 Water activity (a_w) range of selected bakery products

Product	a_w
Low moisture content	
Cookies	0.2–0.3
Crackers	0.2–0.3
Intermediate moisture content	
Chocolate coated doughnuts	0.82–0.83
Danish pastries	0.82–0.83
Cream filled cake	0.78–0.81
Soft cookies	0.5–0.78
High moisture content	
Bread	0.96–0.98
Pita bread	0.9
Fruit pies	0.95–0.98
Carrot cake	0.94–0.96
Cheese cake	0.91–0.95
Pizza crust	0.94–0.95
Pizza	0.99

Adapted from Doerry,[76] Smith and Simpson.[223]

PART I

A. SPOILAGE CONCERNS OF BAKERY PRODUCTS

Spoilage refers to any change in the condition of a food that makes it less palatable at the time of consumption. The spoilage problems of bakery products can be sub-divided into 1) physical spoilage (moisture loss, staling), 2) chemical spoilage (rancidity), and 3) microbiological spoilage (yeast, mold, bacterial growth). The predominant spoilage problem is influenced by inter-related factors, specifically storage temperature, relative humidity, level of preservatives, pH, packaging material and gaseous environment surrounding the product and, most importantly, by the moisture content and a_w. The common spoilage concerns of bakery products will be briefly reviewed.

1. Physical Spoilage

Moisture loss and gain is a serious problem in many bakery products that can result in textural changes and may even promote chemical and microbiological spoilage in low and intermediate moisture products. However, both moisture loss or gain can be overcome by packaging products in materials with selective moisture and gas barrier properties, such as low density polyethylene (LDPE). However, the use of such films may result in conditions conducive to mold growth, particularly in high moisture bakery products.

A more serious physical spoilage problem in bakery product is staling. Staling has been defined as "almost any change, short of microbiological spoilage, that occurs in bread or other products, during the post baking period, making it less acceptable to the consumer."[115] The major changes that occur after baking are moisture redistribution, starch retrogradation, increased firmness, and loss of aroma and flavor.[192] The staling mechanism has been the subject of many investigations. Several studies have suggested that staling is due to moisture migration from the crumb to the crust and, more specifically, from swollen starch to gluten. Products with a higher moisture content, e.g., bread and cakes, stale faster than intermediate or low moisture products, such as cookies or crackers. Staling, however, is not simply due to moisture loss or migration.[147] It has been shown that the degree and rate of crystallization (association) of starch components, specifically of the non-linear amylopectin fraction, is mainly responsible for staling. Complex formation between starch polymers, lipids, and flour proteins is thought to inhibit the aggregation of amylose and amylopectin.[147] Thus, the content of these components can influence the rate of staling. Cookies and biscuits, for example, have a higher lipid content than bread and tend to stale more slowly. However, these products are more susceptible to lipid oxidation and the development of rancid flavor.

Staling is economically important to the bakery industry. In a market where ~20 billion pounds of bread are produced annually, an estimated return of 3% (600 million pounds) of bread, due to staling, poses an economic concern to both the bakery industry and consumers.[115]

Several commercial methods are used to delay the staling of bakery products, including reformulation with lipids and shortenings, surfactants, emulsifiers, gums, and mono- and diglycerides. More recently, an antistaling enzyme, Multifresh™, developed by Enzyme Bio-Systems Ltd., Beloit, Wisconsin, has been commercially used to delay staling in many products. This enzyme is active at temperatures above starch gelatinization and works by hydrolyzing the amylopectin fraction, thereby, preventing re-crystallization and, hence, staling.[32] While extensive research has been done on methods to delay staling, the common experimental approach in most of these studies has been to investigate the effect of variables one at a time. However, the examination of several variables individually is laborious and time consuming. It results in large quantities of data that are difficult to interpret and, in addition, it fails to measure interaction effects. To overcome the limitations of this experimental approach, a process optimization technique that involves factorial designs and multiple regression techniques, called Response Surface Methodology (RSM), can be used. The advantages of an RSM approach are that it examines variables simultaneously, it is less time consuming and more cost effective, and it explains any synergies between variables and their effects on staling. Furthermore, the results may be illustrated graphically in easy to undestand 2-D contour and 3-D response surface plots.

While staling is usually delayed through the addition of chemical additives, the use of CO_2 enriched atmospheres, which are employed commercially to extend the mold free shelf life of many bakery products, has also been investigated as a means of retarding staling. Knorr and Tomlins[144] showed that the compressibility of bread packaged in 100% CO_2 was lower than bread packaged in air, suggesting that CO_2 decreased the rate of firming. Avital et al.[17] also showed that CO_2 delayed bread firming and proposed that the sorption properties of gas packaged bread were responsible for this effect. These authors reported that after one day at ambient temperature, the amylose fraction of bread was in a crystalline state, while the amylopectin fraction had water binding sites. They proposed that CO_2 dissolved in these sites, thereby, reducing hydrogen bonding between amylopectin branches, resulting in less firm and stale bread.[17] However, the effectiveness of CO_2 atmospheres as an antistaling agent has been refuted by other studies. Doerry[76] showed that bread crumb became firm regardless of the storage atmosphere, i.e., 100% CO_2 or 100% N_2. Black et al.[26] also reported no uniform pattern of firming over time in pita bread packaged under various gas atmospheres, while Brody[33] reported that the staling rate of white and whole wheat bread was not significantly reduced when packaged in CO_2, N_2 or air. Thus, while the antimycotic effect of CO_2 enriched atmospheres has been well researched and documented, further research is required to determine its role, if any, in delaying staling.

2. Chemical Spoilage

Bakery products, especially those with a high fat content, are also subject to chemical spoilage or rancidity. Rancidity is characterized by lipid degradation resulting in off-odors and off-flavors, which render products unpalatable and decrease shelf life. Two types of rancidity problems can occur—oxidative and hydrolytic.

Oxidative rancidity results in the breakdown of unsaturated fatty acids by oxygen through an autolytic free-radical mechanism. Consequently, malodorous aldehydes, ketones, and short chain fatty acids are formed. These free radicals and peroxides, formed during lipid oxidation, may lead to even more detrimental effects on food quality by bleaching pigments (e.g., lycophene in tomato paste in pizza), destroying certain vitamins, such as vitamin A and E, and protein degradation.

Hydrolytic rancidity, unlike oxidative, occurs in the absence of O_2 and results in the hydrolysis of triglycerides and the

subsequent release of glycerol and malodorous fatty acids. This type of rancidity is enhanced by the presence of moisture and endogenous enzymes, such as lipases and lipoxygenases. These enzymes, commonly found in vegetables, wheat flour, spices, and cheese, catalyze the oxidation of unsaturated fats, producing peroxides and heat stable compounds that survive the baking process.

Chemical spoilage is usually prevented by the addition of antioxidants, such as butylated hydroxyanisole (BHA), and butylated hydroxy toluene (BHT), α-tocopherol, ascorbic acid, and its salts and certain gums. The displacement of atmospheric O_2 by gas packaging in 100% N_2 may also be an alternative approach in delaying rancidity in low moisture bakery products, where microbiological problems are not a concern.

3. *Microbiological Spoilage*

While physical and chemical spoilage problems occur in many bakery products, microbiological spoilage is often the major factor limiting the shelf life of high and intermediate moisture bakery products and is also a major cause of economic loss to the bakery industry. It has been estimated that in the U.S. alone, losses due to microbiological spoilage are ~1 to 3% or over 90 million kg of products each year.[177] This loss is based on in-plant and in-store spoilage only, and if losses at the consumer level were also taken into account, the total loss would reach a staggering proportion.[177]

The most important factor influencing microbiological spoilage of bakery products is water activity (a_w). The minimum a_w for growth of spoilage microorganisms in selected baked products is shown in Figure 1. For low moisture baked products ($a_w < 0.6$), microbiological spoilage is not a problem. In intermediate moisture products (a_w 0.6–0.85), osmophilic yeasts and molds are the predominant spoilage microorganisms. In high moisture products (a_w 0.94–0.99), almost all bacteria, yeasts, and molds are capable of growth.[222]

a. *Bacterial Spoilage*

Since most bacteria require a high a_w for growth, bacterial problems are limited to bakery products with a high moisture content. The major bacterial problem in bread is "rope," caused by *Bacillus subtilis*, a spore forming bacteria. This microorganism, which is usually present in raw ingredients, (e.g., flour, sugar, and yeast), survives the baking process, germinates upon cooling, and grows under both aerobic and anaerobic packaging conditions. "Ropey" bread has a characteristic flavor similar to ripe cantaloupe. The bread crumb becomes discolored and sticky, due to protein and starch degradation during growth of the bacteria.[222] Rope problems can usually be overcome by the use of chemical preservatives (propionates) or by natural preservatives (acetic acid).

Unfilled pastries can undergo similar deterioration to that of bread. However, when pastries are filled, they are subject to other types of microbial spoilage. Many fillings can support the growth of food pathogens, especially if they contain egg or dairy products.[212] Custard-filled products are a potential health hazard, due to the growth of *Bacillus cereus* and *Staphylococcus aureus*.[212] This latter pathogen has also been implicated in food-borne poisoning outbreaks from cream-filled bakery products. The safety of high moisture bakery products will be discussed in more detail in Part II of this review.

b. *Yeast Spoilage*

Yeast problems occur mainly in intermediate and high moisture bakery products. According to Legan and Voysey,[154] the yeast problems of bakery products can be subdivided into two broad types: (i) visible growth of yeasts on the surface of the products (white or pink patches); and, (ii) fermentative spoilage of a wide range of products or ingredients manifested by alcoholic, esteric, or other odors and/or visible evidence of gas production, such as gas bubbles in jams and fondants or expansion of flexible packaging.

Visible yeast growth is generally associated with products of high a_w and short shelf life, while fermentative spoilage is usually associated with low a_w and long shelf life products, e.g., fruit cakes and Christmas puddings.[154] Yeasts, which cause surface spoilage of bread, are mainly *Pichia burtonii* ("chalk mold") and, to a lesser extent, *Candida guilliermondii*, *Hansenula anomala* and *Debaromyces hansenii*.[154] The most common osmotolerant yeast that causes spoilage of high sugar coatings and fillings such as jam, marzipan, and mincemeat, is *Zygosaccharomyces rouxii*. Contamination of products by osmophilic yeasts normally results from unclean utensils and equipment. Therefore, maintaining good manufacturing practices will minimize contamination by osmophilic yeasts. Preservatives, such as sorbates, benzoates, and parabens are also effective in inhibiting yeast spoilage.

c. *Mold Spoilage*

A perennial problem limiting the shelf life of intermediate and high moisture bakery products is mold growth. Many molds are capable of growing at a_w values of >0.8, while a few xerophilic molds are capable of growth at a_w values as low as 0.65. Losses due to mold spoilage vary between 1 and 5% of products depending on season, type of product, and method of processing.[165] Mold spoilage of bakery products is, therefore, of serious economic concern to the bakery industry. Although freshly baked products are free of viable vegetative molds and mold spores, products soon become contaminated as a result of post baking contamination by mold spores from the air, bakery surfaces and equipment, food handlers, and raw ingredients such as glazes, nuts, spices, and sugars.[130,212,213] Mold problems are more troublesome during the summer months, due to airborne contamination and the warmer, more humid storage conditions. Furthermore, products may be wrapped prior to being completely cooled. This results in moisture condensing

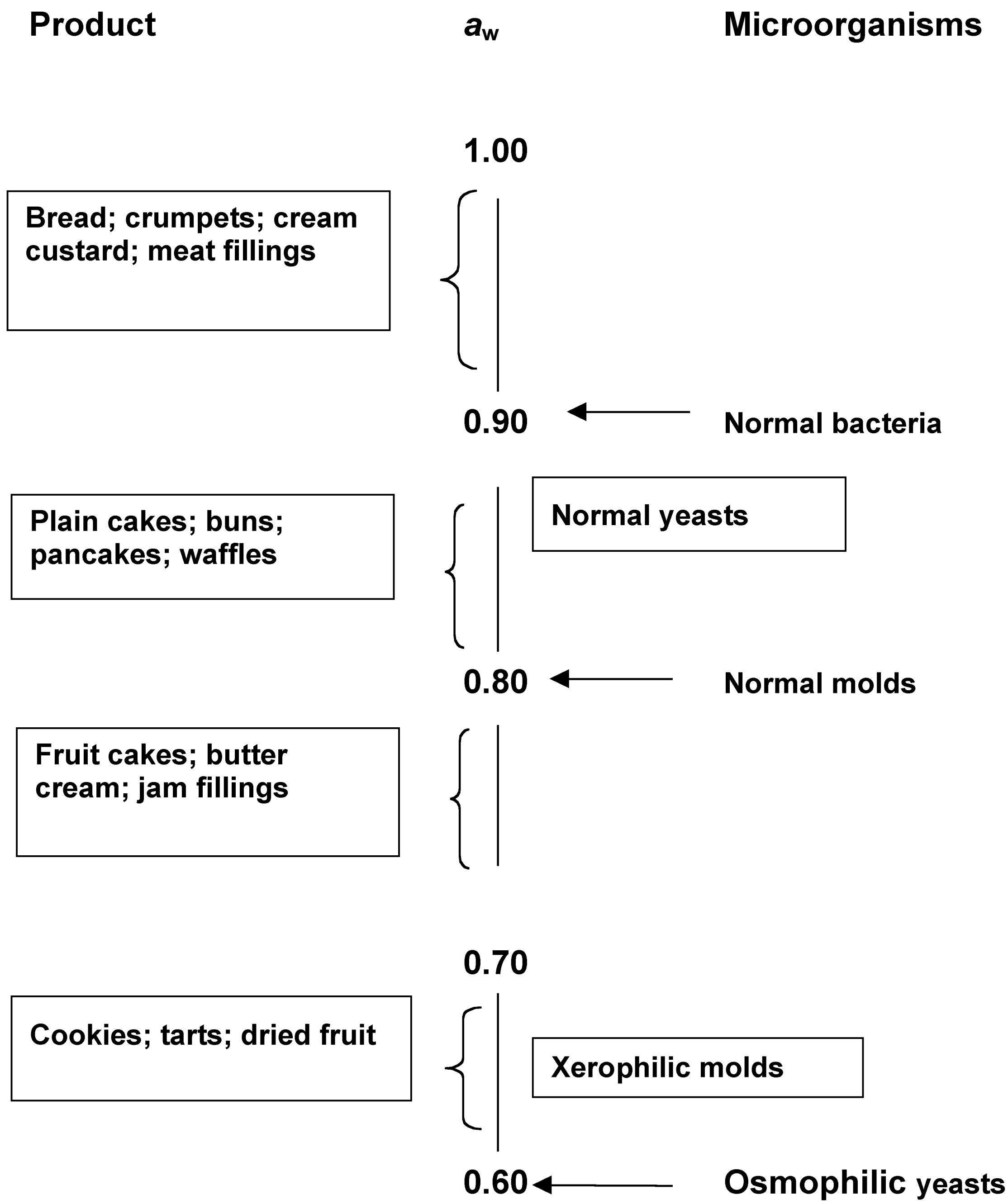

Figure 1 Minimum a_w values for the growth of spoilage microorganisms in selected bakery products (Blakistone).[27]

inside the package and on a product's surface—conditions that are conducive to mold growth.

Several factors influence the types and numbers of mold spores found on bakery products, including product type and equilibrium relative humidity (erh) and season. The most common mold contaminants found on cake in the United Kingdom were *Wallemia sebi, Penicillium* spp., *Cladosporium* spp., Eurotium *(Aspergillus) glaucus* group, and other Aspergilli.[211] *Penicillium* spp. particularly, *P. notatum, P. expansum*, and *P. viridicatum*, were the predominant spoilage molds in products with a high erh, i.e., >86%. However, for products with erh values below this level, *Eurotium glaucus* spp. and, particularly, *Eurotium amstelodami*, predominate.[211] During a one year study from bread obtained from 46 bakeries and stored in plastic bags at 22°C for 5–6 days, *Penicillium* spp. were present in nearly all of the loaves, while *Aspergillus* spp. and *Cladosporium* spp. occurred on approximately half of the loaves.[154] Chalk molds (*P. burtonii*) were isolated from 5 to 30% of all loaves, but peaked at 50% in the month of September. In another study with bread stored at 25°C and 70% erh, visible yeasts caused less than 7%

spoilage, while *Penicillium roquefortii* caused 85% of the observed spoilage and other molds accounted for the remainder.[233] *P. roquefortii* is a rare contaminant in North American and British bread, but it is a common contaminant in German bread, due to the sourdough bread making process.[233] Al-Mohizea et al.[7] studied the shelf life of Arabic bread purchased from local bakeries in Saudi Arabia. For pita bread stored at ambient temperature and relative humidity (rh) of 43%, the mold free shelf life ranged from 9 to 12 days. *Penicillium* and *Aspergillus* spp., were the predominant mold contaminants (39–42%) of pita bread, while *Rhizopus* and *Neurospora* spp. were isolated from 13 to 15% of samples. Thus, the efficacy of any method to control mold spoilage is influenced by the types and levels of mold spores present on a product, as well as product characteristics.

B. STRATEGIES TO CONTROL MICROBIOLOGICAL SPOILAGE OF BAKERY PRODUCTS

Since microbiological spoilage is often the main factor limiting the shelf life of intermediate and high moisture bakery products, methods to control microbiological spoilage, particularly mold growth, are of significant economic importance to the bakery industry. According to Seiler,[214] three basic strategies can be used to extend the microbiological shelf life of bakery products:

1. Prevention of post baking contamination by packaging prior to baking or immediately after baking under aseptic conditions.
2. Destruction of post baking contaminants on the surface of products after packaging.
3. Controlling the growth of post baking contaminants in the packaged products.

Each of these approaches will be briefly reviewed.

1. Prevention of Post Baking Contamination

The most important spoilage problem of high moisture bakery products, especially bread, is mold, spoilage, followed by staling. Since mold spores are destroyed during baking, mold spoilage occurs as a result of post baking contamination from the bakery atmosphere, equipment, such as slicing machines, and bakery personnel. Therefore, the mold free shelf life of many bakery products could be increased if post baking contamination were prevented. This could be achieved by either packaging products prior to baking or as soon as possible after baking. However, the disadvantage of the former approach is that special heat resistant packaging material would be required, while condensation problems would occur on the surface of products using the latter method.[210]

Another approach to prevent post-baking contamination would be to cool, slice, and package products in a sterile atmosphere. Air filtration/circulation systems have now been developed that are capable of removing all microorganisms from air while blowing sterile air into the bakery environment. While such systems may prevent post processing contamination of products, the major disadvantage of such aseptic packaging conditions is cost.[210]

2. Destruction of Post Baking Contaminants

Despite improvements in bakery design, cooling, and packaging systems, attempts to prevent postbaking contamination of bakery products with mold spores has been met with limited success. Therefore, attention has focused on methods to destroy and control any post-processing contamination of products with mold spores.

Several traditional and novel methods have been investigated to destroy post baking contaminants of high and intermediate moisture bakery products including ultraviolet light, infrared radiation, microwave heating, low dose irradiation, pulsed light technology and ultra high pressure.

a. U.V. Light

The ultraviolet (UV) region of the spectrum extends below wavelengths of 450 nm, with the most effective region for the destruction of microorganisms being around 260 nm.[131] UV light results in DNA mutations in microorganisms; furthermore, it causes adjacent thymine molecules to covalently link into dimers, thus, blocking further replication of DNA.[131] UV light has been used in bakeries since before World War II as a means of reducing the levels of mold spores in the bakery environment and, hence, reduces post baking contamination of products.[70] However, the major disadvantage of UV light in controlling mold spoilage of contaminated products is that it lacks penetration, and it is limited to unfilled and uncoated products with a smooth surface. As a result, its not very effective in extending the mold free shelf life of products with porous, uneven crusts (e.g., bread) or in controlling mold growth on the side and bottom surfaces of products.[210] Other disadvantages of UV light that limit its use as a method of increasing the mold free shelf life of products include cost, secondary mold contamination due to inadequate sealing of the packaging film, and safety concerns.[149,210]

b. Infrared Radiation

Infrared radiation (IR) has been investigated to extend the mold free shelf life of high moisture, pre-packaged cakes. Seiler[210] reported that when cake surfaces were heated by IR to 160°F (71.1°C), the mold free shelf life increased by 200–300%, without adversely affecting product appearance. The time required to reach the desired temperature depended on the film

thickness and the distance between the infrared source and the surface of the product.[210] However, a major limitation of these early IR studies was the lack of availability of plastic films with optimum thermoplastic properties. Seiler[210] successfully used high gas barrier cellophane films to withstand the heat treatment; however, low gas barrier cellophane films tended to crinkle upon cooling.[210] Currently, cellophane films are seldom used in the bakery industry and have been replaced by packaging films with improved thermoplastic properties. Despite the availability of such films, IR has not been used commercially to destroy mold contaminants on bakery products, due to the additional cost of these thermally resistant packaging materials and the cost of the heating system, which may be substantial if designed to heat all sides of a product.[210]

c. Microwave Heating

Microwave energy causes food molecules with a dipole or charge to oscillate when placed in an electromagnetic field, creating an intermolecular friction that is manifested as heat. Most microwave food research has been carried out at two frequencies −915 and 2450 megacycles/second.[131] Experimental treatment of packaged bread with high frequency microwave energy for 45 to 60 seconds has been shown to extend the mold free shelf life of bread.[184] Seiler[210] investigated both static and conveyorized microwave ovens at 2450 megacyles/second to treat the surfaces of cakes packaged in high and low barrier cellophane films. While the heat treatment extended the mold free shelf life of products, it had an adverse effect on the functional properties of the low gas barrier cellophane film. Furthermore, severe condensation problems occurred in products packaged in the high gas barrier cellophane film.[210] Goldblith[101] also reported uneven heating in fruit cakes, due to variations in the dielectric properties of these products. However, despite the widespread availability of packaging films with selective heat resistance, as well as gas and moisture and barrier properties, microwave heating of pre-packaged products is not widely used in the bakery industry to extend the mold free shelf life of pre-packaged products.

d. Low Dose Irradiation

The use of gamma irradiation is currently being employed commercially in the U.S. to extend the shelf life and to keep the quality of a range of products, including strawberries, poultry, crustaceans, and ground beef. It has also been investigated as a means of extending the mold free shelf life of Arabic bread. When pita bread was treated post-baking with a low dose of gamma irradiation (0.2 Mrads), all post-baking contaminants (molds and yeasts) were inactivated, and the level of heat resistant spores was reduced from 3.5×10^3 CFU/g to 1×10^1 CFU/g.[108] Furthermore, the irradiated bread remained mold free for more than 7 days at ambient temperature, while the non-irradiated bread developed visible mold growth within 1 to 2 days. Sensory evaluation found no difference between non-irradiated and irradiated bread.[108] However, while low dose irradiation is being used increasingly as a means of extending the shelf life of many perishable foods, it has not yet been considered as a commercially viable method to extend the mold free shelf life of pre-packaged bakery products.

e. Pulsed Light Technology

Pulsed light technology is a relatively new technology that was developed by PurePulse Technologies in San Diego, California in the 1990s. The technology uses flashes from a broad-spectrum light of wavelengths, ranging from the UV spectrum (200 nm) to the infrared spectrum (1000 nm). Each pulse or flash of light lasts only a few microseconds and has a light intensity 20,000 times more intense than sunlight at the earth's surface.[81] Pulsed light has been investigated as a means of extending the shelf life of baked goods, seafood, meat products, and fruits and vegetables.[81] Bread treated with pulsed light had a mold free shelf life of more than two weeks at ambient temperature, while control loaves were visibly spoiled with mold growth after one week.[81] However, the authors did not report the effect of pulsed light on the sensory properties of bread or on the functional properties (permeability, seal strength) of the packaging film surrounding the product. Nevertheless, pulsed light technology shows promise as a novel method to extend the mold free shelf life of pre-packaged bakery products.

f. High Pressure Technology

Another novel technology that shows promise as a method of extending the shelf life and keeping quality of pre-packaged food products is high pressure processing (HPP), also referred to as ultra high pressure (UHP) processing. High pressure processing can inactivate microorganisms by damaging the cell membrane and inactivating key enzymes involved in DNA replication and transcription.[122] However, the extent of inactivation depends on a number of interrelated factors, including level and duration of pressure, microbiological types and numbers, processing temperature, and substrate composition.

Studies were conducted in our laboratory to determine the effect of high pressures on the mold free shelf life of pita bread. Freshly baked pita was inoculated with spores of *A. niger* and *P. notataum* (10^2 spores/g), packaged in a high gas barrier film, and subjected to pressures ranging from 0 to 400 MPa for 15 minutes. Mold growth appeared in all control pita bread stored at ambient temperature after only 4 and 7 days in bread treated with low pressures (5–10 MPa). At higher pressures (30–70 MPa), mold growth did not appear until day 14, while mold growth was completely inhibited in all products subjected to pressures >200 MPa.[85] However, such high pressures had an adverse effect on the sensory quality of pita bread and also resulted in delamination of the packaging material at the sealant layer.[85] While lower pressures had no effect on film characteristics, mold growth was evident in all products within one week at ambient temperature. While high pressure technology is used commercially in Japan to produce jam, jellies, and surimi type

products, it is unlikely, due to cost, processing, and packaging constraints, to become a commercially viable technology in extending the mold free shelf life of pre-packaged, high moisture bakery products.

3. Controlling the Growth of Post Baking Contaminants

While the bakery industry has a wide choice of traditional and novel methods to destroy post-processing mold contaminants, they are not widely used to extend the mold free shelf life of bakery products. The most practical, common, and cost efficient approach used by the bakery industry to achieve this objective is to control the growth of any post-baking contamination in the packaged products. This can be achieved through reformulation to reduce product a_w, the use of chemical preservatives (e.g., sorbates or propionates) incorporated directly into the product or applied to the surface as a spray or modified atmosphere packaging (MAP), using gas packaging or interactive packaging sachet technology.

a. Product Reformulation

The microbiological shelf life of high moisture bakery products is related to their pH and water activity (a_w). Therefore, product reformulation to reduce both pH and a_w could be used to increase shelf life. Reduction of pH can be achieved through the use of acidulants, such as organic acids (e.g., citric, lactic, acetic acids) or cultures of lactic acid bacteria (e.g., sour dough cultures). Reduction of a_w can be achieved through the addition of solutes, such as sugars, salts, polyalcohols, or fractionated milk products. However, a limitation of this approach, particularly to control mold spoilage, is that the inhibitory level of solutes required for inhibition may result in adverse changes to the sensory and textural properties of products. While the use of humectants (e.g., glycerol) may overcome this problem, it is a more costly approach. However, this approach was used by Smith et al.[227] to prevent secondary spoilage problems by *Leuconsotoc mesenteroides* and to improve the sensory properties of gas packaged English style crumpets.

b. Preservatives

While reformulation is one option to extend product shelf life, chemical preservatives are most commonly used as additional barriers in controlling both mold and bacterial spoilage problems in bakery products. However, due to consumer concerns regarding the use of chemicals in food products, the use of natural preservatives, such as cultured products, vinegar, or raisin juice, is increasing. They are typically used in baked products marketed as "preservative-free." A good chemical or natural preservative should possess the following features, as described by King[141]: possess a broad antimicrobial spectrum; be non toxic to humans; be effective at low concentrations; have a minimal effect on pH of products; should not affect the odor, color, and flavor of products at the intended level of use; be available in a dry form; have good water solubility; be non corrosive; be stable during storage; have no adverse effects on fermentation or loaf characteristics; and be cost effective.

i. Chemical Preservatives. Chemical preservatives are most commonly used by the bakery industry to prevent or retard microbiological spoilage. The Code of Federal Regulations (CFR) defines a chemical preservative as, "any chemical that tends to prevent or retard deterioration when added to food."[54] The chemical preservatives used in bakery products include calcium and sodium propionate, sorbic acid, potassium sorbate, sodium diacetate, methylparaben, propylparaben, sodium benzoate, and acetic acid. The minimum levels of each preservative required to inhibit the growth of common spoilage microorganisms of concern in bakery products are shown in Table 4. Each of these preservatives will be briefly reviewed.

a) Propionic Acid and Propionates. Propionic acid and its salts (propionates) are used to control mold spoilage and "ropiness" caused by *B. subtilis*.[175] Although propionic acid has a higher antimicrobial activity, propionates (sodium and calcium) are more commonly used in bakery products, due to their greater solubility and odor-free characteristics. They are effective against molds, but have little activity against bacteria (with the exception of *B. subtilis*) and have no activity against yeasts. This selective antimicrobial activity makes them ideal as mold inhibitors in yeast leavened products. The maximum levels of propionic acid and propionates permitted in bakery products are

Table 4 Antimicrobial spectra of preservatives used in foods

Organic acids	Yeasts	Molds	*Enterobacteriacea*	*Micrococcaceae*	*Bacillaceae*
			Minimum levels required for inhibition (% w/w)		
Acetic acid	0.5	0.1	0.05	0.05	0.1
Benzoic acid	0.05	0.1	0.01	0.01	0.02
Citric acid	>0.005	>0.005	>0.005	0.001	>0.005
Lactic acid	>0.01	>0.02	>0.01	>0.01	>0.03
Propionic acid	0.2	0.05	0.05	0.1	0.1
Sorbic acid	0.02	0.04	0.01	0.02	0.02
			Parabens		
Methyl	0.1	0.1	0.2	0.4	0.2
Ethyl	0.1	0.05	0.1	0.1	0.1
Propyl	0.01	0.02	0.1	0.05	0.05

Adapted from Chichester and Tanner.[50]

Table 5 Suggested levels of propionates in baked goods

Product type	Propionate level oz/100 lb. flour
Breads	Ca/Na propionate
White breads, buns, rolls, specialties	2.5–5
Dark bread, whole or cracked wheat	3–6
English muffins	6.5–12
Pita bread	4–5
Tortillas/soft tacos	4–5

Adapted from Hebeda and Zobel.[115]

shown in Table 5. A compound called "Soprodac®" is also commercially available. It is comprised of a 70% aqueous solution of equimolar amounts of sodium propionate and propionic acid and is commonly used as an antimycotic agent in pizza crust. However, with increasing consumer concerns about preservatives, acetic acid and its salts are now replacing propionic acid in many applications.

b) Sorbic Acid and Sorbates. Sorbic acid and its sodium, calcium, and potassium salts are permitted preservatives in bakery products. The sorbates have been shown to be effective against yeasts, molds, and various bacteria in baked goods. However, due to solubility and cost considerations, potassium sorbate is most commonly used in bakery products.

Sorbic acid and potassium sorbate have a broad-spectrum of activity against yeasts and molds, but little activity against most bacteria, with the exception of *B. subtilis*. Sorbic acid and potassium sorbate are effective antimycotic agents at pH values up to 6. Hence, the antimicrobial activity of sorbates, as well as propionates, increases with a decrease in pH. Sorbates, on a weight per weight basis, have been shown to be twice as effective as propionates to control mold growth in bakery products.[210] However, they have an adverse effect on yeast activity and reduce loaf volume, as well as make the dough sticky and difficult to process.[154] The suggested levels of sorbates used in baked goods are shown in Table 6.

To overcome their inhibitory effect on yeast fermentation during baking, sorbates can be applied to bakery products in various ways. These include encapsulation, spraying onto the product as an aerosol, or incorporating it into the packaging material.[231] Sorbic acid can be encapsulated with fatty acids, such as palmitic acid, to produce sorboyl palmitate. This ester has been shown to be effective in controlling mold growth without interfering with the fermentation process. During baking, sorboyl palmitate is hydrolyzed, and sorbic acid is released into the product to inhibit mold growth during storage.[231] A recent innovation involves coating with mono- or di-glycerides or emulsifiers. While one advantage of this method of encapsulation is that less yeast is used during baking, disadvantages include dispersion problems, resulting in the formation of black spots on the crust.

Surface application of potassium sorbate as a spray has been shown to be effective in controlling mold spoilage in bread, English muffins, and brown'n'serve rolls.[69,165] A disadvantage of this mode of application of potassium sorbate is that it is not as effective as a mold inhibitor in products with porous, uneven surfaces.

Other studies have shown that sorbates can be impregnated onto packaging materials, commonly referred to as interactive packaging. Seiler[208] showed that sorbic acid and its potassium salts, impregnated to vegetable parchment, was effective in controlling mold growth in cakes. However, this method was only effective when there was a good contact between the impregnated packaging material and the surface of the cake, allowing adequate transfer of preservatives from the packaging material to the product. To overcome this migration problem in

Table 6 Suggested levels of sorbates in baked goods

Product	Level oz/100 lb. flour	Method of application
Breads		
Breads, buns, rolls, etc.	1–6	Surface spraying
Tortillas	1.25–3	Dry blend
	% of batter weight	Dry blend with
Cakes		
Cheese cake	0.1–0.3	Sugar/milk powder
Chocolate cake	0.1–0.3	Flour
Fruit cake	0.1–0.4	Flour
Pies		
Pie crusts	0.05–0.1	Flour/dough
Pie fillings	0.05–0.1	Flour/add and agitate after heating

Adapted from Hebeda and Zobel.[115]

products with an uneven surface, heat can be applied to vaporize the inhibitor and coat the surface of the baked product. This approach was used with brown'n'serve rolls, which were packaged in cellulose acetate impregnated with 5 g sorbic acid/1000 in^2 and flash heated at 450°F (230°C) for 30 seconds. Control samples became moldy after 5 days when stored at room temperature, whereas the sorbic acid treated rolls remained mold-free for 15 days.[104] In another study, bread wrapped with sorbic acid treated crêpe paper (2 g/m^2) and overwrapped in polyethylene bags remained mold free for greater than 6 months, when heat processed at 95–100°C (203–212°F) for 30 minutes. Bread stored without heat processing and containing a similar concentration of sorbic acid in the crêpe paper became moldy within 3 weeks. However, when used at these levels, sorbic acid had an adverse effect on the organoleptic quality of bread.[97]

Similar studies have been carried out using packaging material impregnated with propionic acid. Mälkki and Rauha[165] showed that when wheat buns were packaged in laminated or coated cellophane, containing 3–6% propionic acid and heat treated at 230–302°F (110–151°C), they remained mold-free for nearly 3 months at ambient temperature. While such interactive packaging systems appear effective in controlling mold spoilage, the limitations of this approach for bakery products include the cost and the heat required to volatize preservatives from the packaging material to the uneven surface of many bakery products.

c) Acetic Acid and Acetates. While propionic acid and its salts commonly have been used to control both rope and mold problems in bakery products, they are being replaced by acetic acid and its salts (sodium acetate/di-acetate) because of their lower cost and lower toxicity. An additional advantage is that they are technically chemical preservatives and are considered by consumers to be a natural preservative. Acetic acid and its salts are polyfunctional, having not only good antimicrobial activity, but also they can be used as acidulants, flavoring agents, and sequesterants. Like most preservatives, acetic acid and its salts are most effective in baked goods with a $pH < 6.0$ and have a greater activity against yeasts and bacteria than molds. However, they are less effective, on a weight per weight basis, at controlling mold spoilage than either propionic or sorbic acid.

d) Sodium Benzoate. Sorbates and propionates are commonly used in bakery products with $pH > 5.0$; however, sodium benzoate is more effective in products or ingredients with low pH (2.5–4.0). While it is not commonly used as an inhibitor in unfilled bakery products, sodium benzoate is used to delay yeast and mold spoilage in high acid fillings such as fruits, jams, and jellies.

A disadvantage of most organic acid preservatives is that they are only effective at $pH < 6.5$. This limitation exists for most organic acid preservatives due to their low dissociation constants (pKa). However, since several bakery products have alkaline pH values (e.g., carrot cake, ginger bread cake, and some English style muffins), the choice of chemical preservatives to control microbial spoilage in these products is restricted. Solutions to this problem include the use of acidulants to reduce product pH or the use of preservatives, such as parabens and sorbic hydroxamic acid, which have higher dissociation constants.

e) Parabens. Parabens are alkyl esters of *p*-hydroxybenzoic acid that do not dissociate until ~pH 8.0. The most common forms of parabens are methyl and propyl-paraben, which are both GRAS (Generally Regarded as Safe) inhibitors. They are more effective antimicrobial agents against molds and yeasts than against bacteria. Parabens are used as antimicrobial agents in cakes (particularly fruitcakes) pie crusts, non-yeast pastries, icings, fruit jellies, and toppings at levels ranging from 0.01 to 0.1%.

f) Sorbic Hydroxamic Acid. While parabens could be employed to extend the mold free shelf life of alkaline pH bakery products, they are expensive and can only be used in non-yeast leavened products.[78] An alternative to parabens is sorbic hydroxamic acid, an ester of sorbic acid, which remains undissociated at even higher pH values than parabens.[78] Sorbic hydroxamic acid has been shown to inhibit the growth of various mold species (*Aspergillus niger, Penicillium notatum*, and *Rhizopus* spp.) in grape juice at pH values ranging from 3.6–9.2, while sorbic acid was not effective at pH 5.7 and above.[78] Studies in our laboratory, with the common mold contaminants of bakery products, have confirmed the antimycotic spectra of sorbic hydroxamic acid over a wide pH range (5.0–9.0). Furthermore, it has been shown to be effective against *Bacillus* spp. of spoilage and safety concern in high pH products. Therefore, sorbic hydroxamic acid has the potential to be used as an antimicrobial agent in bakery products with pH values >7. However, further studies are required to determine the long-term toxicity of sorbic hydroxamic acid before it is approved for use.

ii. Natural Preservatives. While the bakery industry has conventionally relied on chemical preservatives for the shelf life extension of products, its attention is now focusing on natural preservatives. One such preservative is nisin, a bacteriocin produced by *Lactococcus lactis*, which has been widely used as a food preservative in the dairy industry for many years. It is accepted as safe for food in many countries and is GRAS in the United States. Nisin has proved to be effective against gram-positive bacteria, especially spore formers, such as *Bacillus and Clostridium* spp. In vegetative cells, nisin acts as a surface-active cationic detergent, destabilizing the cytoplasmic membrane. In spores, it facilitates germination, but inhibits spore outgrowth. However, since nisin is more soluble and stable at pH levels <4, its antimicrobial activity is diminished at pH levels >8. Nisin's efficacy is also dependent on its dispersion in food systems, which is affected by the food composition, specifically fat content.

While nisin is most commonly used in dairy and meat products, it has been investigated in high moisture baked products, such as crumpets and pikelets, to control the outgrowth of bacterial spores. When added to crumpet batter at levels of 3.75 $\mu g/g$, nisin restricted the growth of *B. cereus* at ambient temperature.[132] Other studies have shown that 12 psychrotrophic strains of

B. cereus were inhibited in skim milk stored at 7°C containing 6.25 μg/ml of nisin, while growth of 2 strains occurred in milk containing 1.25 μg/ml.[79]

Other commercially available biopreservatives are Alta® 2341 and Perlac® 1911, produced by Quest International in Montreal, Canada. Alta® 2341 is a pediocin produced as a by-product of fermentation when *Pediococcus acidlactici* is grown on yeast extract, corn syrup, and vegetable protein. Perlac® 1911 is produced by *Lactococcus lactis* and is derived from the fermentation of glucose solids. They are multifunctional ingredients that improve flavor, water retention, and shelf life of various foods. While both these biopreservatives are used in high acid products, such as mayonnaise and salad dressings, they have proved ineffective in controlling the growth of *B. cereus* in broth studies at all concentrations (0–3,000 ppm) and pH levels (5–9) examined.[86] However, their antimycotic effect is unknown and warrants investigation.

One natural preservative that has been investigated as an alternative to organic acids as mold inhibitors is Upgrade.® This product comprises of 79% soy flour, 17.5% whey, and 3.5% calcium sulfate. In studies at the American Institute of Baking [AIB],[9] Upgrade® was as effective a mold inhibitor in both white and whole wheat bread when used at levels of 1–2%, compared to calcium propionate (0.125–0.25%). Raisin juice (2%) was also investigated as a natural preservative in this study, but was less effective than either Upgrade® (1%) or calcium propionate (0.125–0.25%) as a mold inhibitor in white and whole wheat bread.[9] While natural preservatives have proven effective as antimycotic agents, the levels required to control mold spoilage are generally higher than those required using traditional preservatives and, therefore, may impart undesirable color and flavor changes to many products.

c. Modified Atmosphere Packaging (MAP)

An alternative approach to chemical preservatives controlling mold spoilage problems in bakery products, regardless of their a_w and pH, is Modified Atmosphere Packaging. MAP has been defined as “the enclosure of a food product in a high gas barrier film in which the gaseous environment has been changed or modified to slow respiration rate, reduce microbiological growth, and retard enzymatic spoilage with the intent of extending shelf life.”[267] MAP technology has been more successful in European countries than in North America. Currently, the United Kingdom is the leader in MAP technology, accounting for about half of the European market, followed by France, which has approximately 25% of the MAP packaged food market. It is estimated that between 300–500 companies use MAP technology for shelf life extension and distribution of food.[177] With respect to the North American market, MAP is still in its infancy. This is mainly due to consumer/regulatory concerns about the safety of this technology. However, it is estimated that the demand for MAP foods in North America could reach 11 billion packages by the year 2005.[177] Several factors have played a role in the growth of MAP technology, such as:

i. Developments in New Polymeric Barrier Packaging Materials. The success of any packaging technology as a means of extending shelf life is dependent on the permeability characteristics of the packaging material surrounding a product. Initially, the choice of polymers with selective barrier properties to gases and moisture vapor and heat sealing characteristics was limited. However, developments in polymer chemistry have resulted in packaging films, such as polyvinylidene chloride (PVDC) and ethylene vinyl alcohol (EVOH). Both these films have excellent barrier properties to water vapor and gases. Furthermore, they can be laminated to other polymers to give films with the desired strength, heat sealability, and permeability characteristics to enable food to be packaged/maintained under MAP conditions.[224] In addition, developments in high speed, continuous, and thermoforming packaging equipment, compatible with the machineability characteristics of these films, have also promoted the growth of MAP.

ii. Extended Market Areas. Primarily, the aim of the bakery industry is to satisfy the needs and demands of consumers on a regional basis. However, bakery products are now marketed on a national and international basis. To meet the demands of this global market, bakery products can be packaged under MAP conditions to extend their shelf life and to keep quality. A good example is Forecrest Foods of Calgary, Alberta, which produced crumpets. Prior to gas packaging, this product had a mold-free shelf life of ~4 days. However, the mold-free shelf life was extended for ~1 month at ambient temperature through product reformulation and gas packaging in 60% CO_2. This allowed the distribution of crumpets throughout Canada and into U.S. markets. Consequently, sales of crumpets quadrupled.[224]

iii. Consumer Concerns About Preservatives. Consumers are becoming more aware and concerned about the use of food preservatives and are demanding preservative-free food. In a recent survey conducted by Agriculture and Agri-Food Canada, 75% of consumers were concerned about the levels of preservatives in food products, while only 19% were concerned about irradiation. The response by the food industry to these concerns has been to use MAP to extend the shelf life of bakery products. Furthermore, the gases used in MAP (CO_2, N_2) are natural and do not need to be declared on a product’s label.[224]

iv. Increasing Energy Costs. Increasing energy costs associated with traditional methods of food preservation/storage, such as freezing, have resulted in the growth of less energy intensive and more economical methods of short and long term preservation (e.g., MAP). It has been estimated that MAP can reduce energy consumption by 18–20%, compared to freezing for the shelf life extension of bakery products.[2]

v. Consumer Perception of MAP. A recent consumer survey conducted by Agriculture and Agri-Food Canada on consumers’ perception of MAP indicated that Canadian consumers favored MAP technology over other methods of preservation and were willing to pay a premium for MAP food products.[224] Most consumers agreed with the idea of foods being preserved by natural gases, such as CO_2. They also believed that MAP would save money by reducing waste, and that the MAP process

represented a significant improvement over current packaging technologies.

As a result of these inter-related factors, MAP is fast emerging as the preservation technology of the future for many foods, including bakery products. The methods used to modify the gas atmosphere within packaged products and the application of MAP to extend the shelf life of bakery products will now be reviewed in greater detail.

C. METHODS OF ATMOSPHERE MODIFICATION

Several traditional and novel methods can be used to modify the atmosphere within a package. Traditional methods include vacuum packaging and gas packaging (injection of gas mixtures), while novel methods include interactive sachets, such as O_2 absorbents and ethanol vapor generators. Each of these methods of atmosphere modification will be briefly reviewed.

1. Vacuum Packaging

In vacuum packaging of bakery products, the product is packaged in a film of low O_2 permeability, and then air is removed under vacuum. Under good vacuum conditions, oxygen in the package headspace is reduced to $<1\%$, i.e., levels which delay mold growth. Vacuum packaging is not a suitable technology to extend the mold free shelf life of most soft bakery products, due to its crushability effect. It has been used, however, to prevent mold problems in flat breads (naan, pita) and pizza crusts and to prevent rancidity problems in shortbread, a high fat product with a hard texture.

2. Gas Packaging

An alternative to vacuum packaging is gas packaging, a technology widely used in both the United Kingdom and Europe, to extend the shelf life of bakery products. Gas packaging involves packaging of products in high gas barrier films with the appropriate mixture of gases, followed by heat sealing of the package. Both these operations are achieved using continuous or thermal forming gas packaging equipment. The former method is used for soft products, such as bread and rolls, while the latter method is used for products such as cakes, doughnuts, pies, and pizzas.[223] Gases used in packaging mainly include nitrogen and carbon dioxide.

Nitrogen is an inert gas that has no antimicrobial effect. It is used mainly as a filler gas to prevent package collapse and oxidative rancidity problems in low water activity bakery and snack food products, where microbiological spoilage is not a concern.

Many gases (e.g., carbon dioxide, carbon monoxide, ozone, ethylene oxide, propylene oxide, nitrous oxide, and sulfur dioxide), have an antimicrobial effect, of these, only carbon dioxide is permitted for use in bakery products for reasons of stability, low toxicity, lack of effect on the organoleptic quality of products, and low cost.

Carbon dioxide is both bacteriostatic and fungistatic, i.e., it inhibits bacterial and mold growth. It can also prevent insect growth in packaged and stored food products. It is highly soluble in water and fats, where it forms carbonic acid, which may result in a reduction of a product's pH.[224] Furthermore, CO_2 can be absorbed by the food, resulting in package collapse, if a high concentration of gas is used in conjunction with a flexible high barrier film. This problem can be overcome by either using nitrogen in the gas mix or a more gas permeable film. Several factors influence the antimicrobial activity of CO_2, including product type, numbers and types of spoilage microorganisms, gas concentration, surface to product ratio, storage temperature, and packaging film permeability.

3. Factors Influencing the Antimicrobial Effect of CO_2

a. Types of Microorganisms

The numbers and types of microorganisms present in a food product influence the antimicrobial effect of CO_2. Microorganisms differ considerably in their sensitivity to CO_2. It has been shown that CO_2 is most effective against aerobic bacteria and molds. Concentrations of CO_2 as low as 5–10% are being used to suppress growth of these microorganisms.[177,222]

Generally, gram-negative bacteria are more sensitive to CO_2-enriched atmospheres than gram-positive types. However, certain gram-positive bacteria (e.g., lactic acid bacteria and certain *Bacillus* species) are very resistant to CO_2 and can tolerate and even grow in atmospheres containing 75–100% CO_2. Anaerobic bacteria, such as *Clostridium botulinum*, are not inhibited by elevated levels of CO_2 in the package atmosphere, and indeed, their growth may actually be stimulated by CO_2. Therefore, if pathogenic species of *Bacillus* and *Clostridium* are present, they could possibly grow to hazardous levels in high moisture, MAP bakery products, particularly at temperature, abuse storage conditions.[222]

Mold species also vary in their sensitivity to the inhibitory effects of CO_2. The *Eurotium* (*Aspergillus*) *glaucus* group of molds was observed to be more susceptible to the effect of CO_2 than the *Penicillium* species, the predominant spoilage molds in bakery products with an equilibrium relative humidity (erh) of 86% and above.[214] Furthermore, certain *Penicillium* species, particularly, *P. roquefortii*, a common contaminant in German rye bread, was more CO_2 resistant than others. Studies with products of differing erh, inoculated with the same *Penicillium* species, failed to show that the activity of CO_2 increases as the erh decreases.[214] This study showed that the type of mold present was a more important factor affecting the antimycotic effect of CO_2 than the erh of the product.[214]

The numbers and age of the microbial population also influence the inhibitory effect of CO_2. Gas packaged baked products contaminated with a high spore load will have a reduced shelf

life, compared to similarly packaged products contaminated with a low spore load. Therefore, gas packaging should not be regarded as a panacea for poor manufacturing practices. The shelf life of gas packaged bakery products will only be as effective as the manufacturing conditions under which they are produced, cooled, and packaged. Furthermore, as microorganisms move from the lag phase to the log phase, the inhibitory effects of CO_2 are reduced.[177] Thus, the antimicrobial effect of CO_2 will be enhanced the earlier a product is gas packaged.

While gas packaging can be used to prevent mold growth, secondary spoilage problems by yeasts or lactic acid bacteria can occur, due to the microaerophilic, fermentative nature of these spoilage microorganisms and their resistance to high levels of CO_2. Surface growth of the filamentous yeast, *P. burtonii,* limited the shelf life of gas packaged rye bread, while the shelf life of gas packaged apple turnovers was limited, due to gas production and swelling of packages by CO_2 resistant yeasts.[221] Lactic acid bacteria, particularly *Leuconostoc mesenteroides*, were responsible for the spoilage and termination of shelf life of gas packaged crumpets.[226] These studies clearly indicate that a knowledge of a product's background microflora is critical to the success of MAP in achieving a desired product's shelf life.

b. *Concentration of CO_2*

Early experiments clearly demonstrated that the success in controlling the growth of aerobic spoilage microorganisms in food was not simply due to the elimination of O_2, but, rather, due to a definite need for CO_2 in the gas atmosphere.[60,253] Furthermore, the growth of aerobic bacteria and molds can be inhibited by low concentrations of CO_2. For example, the growth of *Aspergillus niger, Rhizopus nigricans*, and *P. expansum* could be controlled for ∼2 to 3 days in bread stored in 10 and 17% CO_2 at 28°C.[218] However, a concentration of 50% CO_2 was needed for complete inhibition of mold growth on bread. Upon transfer from the CO_2 enriched atmosphere to air, mold growth occurred as if no treatment had been applied, confirming the fact that CO_2 exerts a fungistatic effect rather than a fungicidal effect.[218] Similar observations have been reported in gas packaging studies of crumpets.[176] Mold growth was evident after 7 days in products packaged under 25% CO_2 and stored at 25°C. However, higher concentrations of CO_2 in the package headspace (50–100%) resulted in complete inhibition of mold growth throughout 14 days storage at 25°C.[176]

It is evident from these studies that the concentration of CO_2 in the gas mix is critical, if the desired extension in shelf life of a product is to be achieved. For most products, a minimum of 20–30% CO_2 (v/v) is required to inhibit the majority of aerobic spoilage microorganisms, while for longer shelf life extensions, a concentration of ∼60% should be used. This is based on studies that have shown that the inhibitory effect of CO_2 increases linearly with increasing concentration, with little or no increased effect being shown at concentrations above 50–60%.[176] However, while there is little or no increased antimicrobial effect or extension in product shelf life at concentrations of CO_2 above 50–60%, slightly higher concentrations (70–80%) are sometimes used to compensate for losses of CO_2 through packaging films, or its absorption by products. However, higher concentrations of CO_2 (100%) can result in the formation of a partial vacuum within the product and package collapse.[176] While this problem can be overcome by using mixtures of CO_2 and N_2, Seiler[214] believes there is little justification in using gas mixtures for the preservation of bakery products, apart from lower cost benefits. Seiler recommends the use of 100% CO_2, in conjunction with packaging films that are less permeable to CO_2 to prevent/reduce the vacuum package effect.[214]

c. *Temperature*

Storage temperature also affects the antimicrobial activity of CO_2. It has been shown that CO_2 is a very effective antimicrobial agent at low storage temperatures, but less effective at higher temperatures. This increased inhibitory effect at lower storage temperatures has been attributed to the greater dissolution of CO_2 in the aqueous phase of products and resultant changes in the intracellular pH and enzymatic activities of microorganisms. Therefore, the decrease in inhibition at higher storage temperatures results from the lower solubility of CO_2 in the aqueous phase of a product. MAP should not be regarded as a substitute for proper storage temperature. While MAP slows down the deterioration of a food product, it never totally arrests these deteriorative reactions.

The effects of temperature abuse on gas packaged products are two-fold. First, microorganisms will overcome the inhibitory effect of the CO_2-enriched atmosphere, and spoilage may occur before the "best buy" date of the product. Secondly, higher temperatures may change the permeability characteristics of a high gas barrier film, resulting in an increase in headspace O_2. This will enhance both microbiological spoilage and chemical spoilage (oxidative rancidity) problems in gas packaged products.

Temperature control is also essential if gas packaging is used with any meat- or cream-filled baked products. Several studies have shown that *Salmonella* and *Staphylococcus* species can grow under anaerobic conditions at 10 to 12.5°C, i.e., conditions of moderate temperature abuse.[180]

d. *Film Type*

One of the most important factors influencing the antimicrobial effect of CO_2 is packaging film permeability. The success or failure of MAP foods depends on both the O_2 and CO_2 impermeability of packaging materials necessary to maintain the correct gas mixture in the package headspace. In addition, films, used in gas packaging should also have low water vapor transmission rates to prevent moisture loss or moisture gain. Polymers commonly used for the gas packaging of food include polyamide (nylon), polypropylene (PP), PVDC, EVOH, and low and high density polyethlylene LDPE/HDPE.[225] If only a short

shelf life (e.g., 2–3 days) is desired for a gas packaged bakery product, such as bread, LDPE/HDPE bags are suitable. However, if a longer shelf life is desired, individual polymers are laminated to one another, since all the desired characteristics of a packaging film for MAP applications, i.e., strength, impermeability, and heat sealability, are seldom found in one polymer. Examples of laminated structures for gas packaging include nylon/PE, nylon/PVDC/PE, or nylon/EVOH/PE. These composite structures have all the desired characteristics of a packaging film for gas packaging applications, specifically, strength, provided by the outermost layer of nylon, gas and moisture vapor impermeability, provided by EVOH or PVDC, and heat sealability, provided by LDPE or ethylene vinyl acetate (EVA) or an ionomer (Surlyn). The important attributes of laminated films for MAP foods are high lamination bond strength; consistent and uniform thickness; consistent seal strength; and consistent barrier to O_2 and moisture vapor.[225] This latter attribute is critical, since the O_2 level in the package headspace may soon reach concentrations of 1% or more, even using high barrier films. Several studies have shown that molds can tolerate and grow in such low concentrations of headspace O_2, even in the presence of elevated levels of CO_2.[88,220,221]

Laminated films commonly used for the gas packaging of bakery products are nylon/LDPE (medium gas barrier film) and PVDC-coated polypropylene laminated with polyethylene or ionomer (high gas barrier film). Surlyn is preferable to polyethylene as the heat sealant layer, since the ionomer has been shown to give consistent seals when crumbs and other debris are lodged in the heat sealing layers.[214]

e. Use of Gas Packaging for Shelf Life Extension of Bakery Products

The use of CO_2-enriched atmospheres for the shelf life extension of food is not a new concept in food preservation. As early as the 19th century, scientists discovered that increased levels of CO_2 and reduced levels of O_2 retarded catabolic reactions in respiring foods and inhibited the growth of aerobic spoilage microorganisms. The potential for CO_2-enriched atmospheres to extend the mold-free shelf life of bakery products was demonstrated in early experiments with bread.[218] They showed that mold growth could be partially inhibited in bread stored under 17% CO_2 and completely inhibited when stored under 50% CO_2. Extensive research on the use of CO_2-enriched atmospheres for the shelf life extension of bakery products was done by Seiler, at the Flour Milling and Bakery Research Association in England. In detailed studies with bread and cake stored under various concentrations of CO_2 (0–60%) at 21 and 27°C, the mold free shelf life of products increased as the concentration of CO_2 in the atmosphere increased. This effect was greater with lower storage temperature.[209] Furthermore, similar extensions in the mold free shelf life of both bread and cake were observed, regardless of the erh of the products.[209] Substantial increases in shelf life have also been observed for bread and cake packaged in mixtures of CO_2 and N_2 and in 100% N_2.[29] However, if the residual O_2 concentration was $>1\%$ in the N_2 packaged bread, mold growth was evident after only 5 days, compared to 100 days for bread packaged in an atmosphere of 99% CO_2 and 1% O_2.[29] This study confirmed the need for CO_2 in the package headspace and not solely the displacement of headspace oxygen to prevent mold in bakery products.

More recently, the effect of different concentrations of CO_2 (0–100%) on the shelf life of a variety of bakery products (Madeira cake, crumpets, par-baked bread, rye bread, and fruit pies) stored at 21 and 27°C was investigated[214] Once again, the mold free shelf life increased with increasing concentrations of CO_2 in the package headspace. However, contrary to previous observations, increases in shelf life were similar at both storage temperatures. Furthermore, the mold free shelf life of products with lower a_w was greater than those with higher a_w products, such as crumpets, fruit pies, and bread, particularly, at higher concentrations of CO_2 (80–100%).[214] Seiler concluded that the difference in shelf life extension was due to the types of molds present, rather than the enhanced antimicrobial effect of CO_2 at reduced relative humidities. In low moisture ($a_w < 0.85$) products, *Eurotium* species predominated, while in higher a_w products (0.92–0.96), (e.g., crumpets and fruit pies), *Penicillium* species were more common.[214]

Extensive studies have been done to investigate the suitability of gas packaging involving a CO_2:N_2 (60:40) gas mixture to extend the shelf life of a variety of bakery products and to evaluate secondary problems which might be encountered in these products, including doughnuts, crumpets, waffles, crusty rolls, muffins, butter tarts, and various fruit pies and cream cakes (Table 7).[177,220,226] These products were selected because they were representative of products with different formulations and, hence, physical and chemical characteristics. Preliminary studies indicated that gas packaging was an effective means of extending the physical, chemical, and microbiological shelf life of most of these products (Table 7). However, both minor and major secondary spoilage problems were encountered in certain gas packaged bakery products.

Minor problems encountered were related to the texture, color, and taste of the gas packaged products. A “stale” taste was encountered in crumpets and waffles after 3–4 weeks storage at ambient temperature (Table 7). They also became slightly discolored at the end of the storage period. However, both of these problems were overcome by toasting the products prior to consumption. The shelf life of butter tarts, mini-blueberry pies, and apple pies was decreased, due to textural problems during extended storage (Table 7).[177] A different type of shortening was recommended to overcome this textural problem in these gas packaged products. Cake doughnuts also became sticky during storage, probably due to moisture migration from the inside of the doughnut. This problem could be overcome by using a less hygroscopic sugar or sweetening agent or anti-caking agent.[177]

Major problems encountered in some products were due to mold, yeast, or bacterial growth resulting in off-odors and, in certain cases, swelling of packages. Mold growth limited the shelf life of gas packaged crusty rolls to 14 days at ambient storage

Table 7 Evaluation of suitability of gas packaging for some bakery products

Product	a_w	pH	Moisture%	Swelling	CO_2 level	Mold	Yeast	Bacteria	Problem
a. Dough or batter									
1. Cake donut	0.82	6.60	17.85	−	−	−	−	+	T,C
2. Crumpet	0.97	6.00	47−52.41	−	+++	−	−	+++	B,S
3. Crusty roll	0.95	5.60	29.28	−	−	+(14d)	−	++	M(leak?)
4. Yeast donut	0.91	6.40	27.98	+	++	−	+	+++	B,Y,S(14d)
5. Waffle	0.94	7.20	65.79	−	+	−	−	++	Not much
b. Cake/pastry:									
1. Chocolate danishes	0.83	6.28	22.54	−	−	−	−	−	None
2. Carrot muffin	0.91	8.70	35.14	−	−	−	−	+	
3. Butter tart	0.78	5.70	19.95	−	−	−	−	+	
c. Cake (layer):									
1. Strawberry layer cake	0.90	6.66	37.24	+	+	−	++	++	Y,B,S,(21d)
2. Cherry cream cheese cake	0.94	4.51	49.90	++	++	−	++	−	Y,S(14d)
d. Pie									
1. Mini blueberry pie	0.94	3.78	40.20	−	−	−	−	−	T,C
2. Apple turnover	0.94	4.60	35.12	+	++	−	+++	+++	Y,B,S(14d)
3. Apple pie (baked)	0.95	4.21	47.82	−	−	−	+		B,Y,T,C
4. Apple pie (unbacked)	0.96	4.25	54.75	+++	+++	−	++	+++	Y,B,S(7d)

Adapted from Ooraikul.[177]
T = Texture, C = Color, B = Bacteria, Y = Yeast, M = Mold, S = Swelling of package.

temperature. This problem was due to the inability of the packaging system to reduce the headspace O_2, levels to <0.6% and to maintain it at this level.[220] Studies have shown that *Aspergillus* species and *Penicillium* species, the common mold contaminants of crusty rolls, can tolerate and grow in the presence of a low concentration of O_2, even in the presence of high concentrations of CO_2.[220] The mold problem was resolved using an Ageless® O_2 absorbent (Mitsubishi Gas Chemical Co.) to ensure that the residual O_2 was completely scavenged from the package headspace.

By contrast, the shelf life of certain products, specifically crumpets, apple turnovers, strawberry layer cake, yeast doughnut, and cherry cream cheese cake had a blown appearance (Table 7). The shelf life of crumpets was terminated after 14 days, due to the growth and heterofermentative activity of lactic acid bacteria, specifically, *L. mesenteroides*, while the shelf life of apple turnovers and cheesecakes was terminated after 16 days, due to CO_2 production by *Saccharomyces cerevisiae*.[221,226] Subsequent studies were done using an RSM approach to determine the levels of a_w, and pH, and CO_2 to control both mold spoilage and gas production by *L. mesenteroides* in gas packaged crumpets. Based on these studies, products were reformulated to the appropriate levels of a_w and pH and packaged under a suitable CO_2 concentration to inhibit both mold and bacterial problems, thereby extending the ambient storage life of crumpets beyond 21 days.[227] Similar extensions in shelf life were possible for apple turnovers and cream filled products using ethanol vapor generators.[177] These methods of additional control for shelf life extension of specific bakery products using O_2 absorbent technology and ethanol vapor generators will be discussed in more detail in a subsequent section. These extensive studies clearly demonstrate that MAP may not be suitable for all types of bakery products, and that a knowledge of a product's physical, chemical, and microbiological characteristics are critical to the success of this technology. Furthermore, they emphasize the importance of combining technologies to increase the shelf life of bakery products and the usefulness of optimization techniques, such as RSM, which are used sparingly as research tools in multivariable food microbiology studies.

Examples of other possible gas mixtures to extend the shelf life of selected bakery products are shown in Table 8. While gas

Table 8 Typical gas mixtures used in gas packaged bakery products

	Gas (% v/v)		
Product	CO_2	N_2	O_2
Sliced bread	100	—	—
Rye bread	100	—	—
Buns	100	—	—
Brioche	100	—	—
Cakes	100	—	—
Madeira cake	65	35	—
Madeira cake	80	20	—
Tea cakes	50	50	—
Danish pastries	50	50	—
Crêpes	60	40	—
Croissants	100	—	—
Crumpets	100	—	—
Crumpets	60	40	—
Pita bread	99	1	—
Pita bread	73	27	—

Adapted from Goodburn and Halligan[103]; Ooraikul.[177]

Table 9 Advantages and disadvantages of gas packaging of food

Advantages	Disadvantages
• Increased shelf life	• Initial high cost of films, equipment
• Increased market area	• Discoloration of meat pigments
• Reduction in production/storage costs	• Leakage
• Improved presentation	• Fermentation and swelling
• Fresh appearance	• Potential growth of organisms of public health significance
• Clear view of the product	
• Easy separation of slices	

Adapted from Smith and Simpson.[223]

packaging appears to be a relatively simple technology, the optimum blend of gases for a specific product cannot be determined by trial and error, but only through a detailed, systematic study of the variables influencing product shelf life. When such empirical data is available, the mold-free shelf life of baked products can be extended for 3 weeks to 3 months at room temperature, using appropriate mixtures of CO_2:N_2 (Table 8).

f. Advantages and Disadvantages of Gas Packaging of Bakery Products

The gas packaging of bakery products has several advantages, including an extended product shelf life and associated increase in market area, improved product presentation, and a reduction in processing, transportation, and storage costs of MAP products, compared to freezing storage costs (Table 9). For example, the production, storage and transportation costs of frozen crumpets was 45% more energy intensive than MAP crumpets.[2] This was reduced to an overall cost advantage of 14% over freezing, due mainly to the higher cost of packaging materials used in gas packaging. Therefore, for a bakery with an annual production of 1,000,000 kg of crumpets, a saving of $34,730 could be achieved by using MAP instead of freezing.[2]

Presently, more than 150 European bakery firms are using gas packaging technology to extend the mold-free shelf life and to keep the quality of rolls, cakes, pizza, baguettes, and sliced bread. In addition, several bakery companies in the U.S. are now reaping the benefits of extended product shelf life through MAP technology.[9]

Some of the disadvantages of MAP technology (Table 9) include the initial cost of packaging equipment, slower throughput of product, secondary fermentation problems caused by CO_2 resistant microorganisms, and the potential growth of microorganisms of public health concern, particularly *C. botulinum*. While this latter topic has been the subject of several investigations in muscle foods packaged under MAP conditions, there is little conclusive evidence that MAP represents a significantly greater hazard than packaging in air, particularly under conditions of temperature abuse where CO_2 is less effective. It is commonly believed that the inclusion of O_2 in the package headspace may prevent the growth of *C. botulinum* in products that may be susceptible to contamination by this pathogen. However, recent studies have refuted this claim and have shown that the inclusion of O_2 in the package headspace offers no additional protection against *C. botulinum* type A and B spores and may even enhance toxin production by this pathogen.[150] Nevertheless, these safety concerns have limited the use of MAP technology in the North American marketplace. The public health concerns of gas packaged bakery products will be discussed in greater detail in Part II of this review.

4. Oxygen Absorbents

While gas packaging has been used to slow down or inhibit aerobic deteriorative changes in bakery products, mold spoilage can still occur in such products, depending on the level of residual O_2 in the package headspace. The level of residual O_2 in gas packaged products could be due to a number of factors, such as, oxygen permeability of the packaging material; ability of the food to trap air; leakage of air through poor sealing; and inadequate evacuation and/or gas flushing.[220]

A novel and innovative method of O_2 control and an alternative to vacuum or gas packaging, as a method of atmosphere modification, involves the use of O_2 absorbents. Oxygen absorbents can be defined as "a range of chemical compounds introduced into the MAP package (not the product) to alter the atmosphere within the package."[4] Developed in Japan, O_2 absorbents were first marketed by Mitsubishi Gas Chemical Co. under the trade name "Ageless®," while the Toppan Paper and Printing Company in Japan also produced a range of absorbents under the trade name "Freshilizer.®" In North America, Multisorb Technology in Buffalo, NY produced a range of O_2 absorbents sachets and labels under the trade names Freshpax® and Freshmax®, respectively.

a. Ageless® Absorbents

Ageless® absorbents consist of a range of gas scavenger products designed to reduce O_2 levels to less than 100 ppm in the package headspace.[225] While both organic types (based on ascorbic acid or catechol) and inorganic types (based on iron powder) are available, the inorganic types are most commonly used in the Japanese market. The basic system is made up of finely divided powdered iron, which, under appropriate humidity conditions, uses up residual O_2 to form non-toxic iron oxide (rust). Several

Table 10 Types and properties of ageless oxygen absorbents

Type	Function	Moisture status	Water activity	Absorption speed (day)
Z	Decreases O_2	Self reacting	<0.65	0.5–2
S	Decreases O_2	Self reacting	>0.65	0.5–2
SS	Decreases O_2	Self reacting	>0.85	2–3 (0–4°C) 10 (−25°C)
FX	Decreases O_2	Moisture dependent	>0.85	0.5–1
FM	Decreases O_2	Moisture dependent	>0.85	0.5–1
E	Decreases O_2 increases CO_2	Self reacting	<0.3	3–8
G	Decreases O_2 increases CO_2	Self reacting	0.3–0.5	1–4
SE	Decreases O_2/increases ethanol	Moisture dependent	>0.85	1–2

Adapted from Smith and Simpson.[223]

types and sizes of Ageless® sachets are commercially available and can be used with high, intermediate, or low moisture bakery products, as well as with high fat products (Table 10). A rapid and efficient method of monitoring package integrity throughout the storage period is through the incorporation of the Ageless® eye redox indicator. When placed inside the package alongside an Ageless® sachet, the color of the indicator changes from blue (oxidized state), to pink (reduced state), when the O_2 content of the container reaches less than 0.05%. If the indicator reverts to its blue color, it is indicative of poor packing integrity caused by poor sealing of film or minute pinholes in the film.[225]

b. Freshilizer® Absorbents

Freshilizer® absorbents consist of two series, the F and the C series.[225] Series F uses mainly ferrous metal and absorbs only O_2. Three types are commercially available: FD, FH, and FT. Type FD is designed for use in food products with a_w values less than 0.8 (nuts, chocolate, etc.), while type FH is suitable for use in products with an activity in the range of 0.6–0.99 (used mainly with beef jerky and salami to maintain color). Type FT works best in foods with a_w greater than 0.8, such as pizza crusts. Series F absorbents can absorb 20–300 ml O_2, corresponding to a package volume of 100–1500 ml of air. The C series consists of types, C, CW, and CV. These sachets consist of non-ferrous metal particles and can, therefore, be used in products that must pass through a metal detector. Types C and CW absorb O_2 and generate an equal volume of CO_2, thereby, preventing package collapse. Type C is used in foods with an a_w of 0.8 or less (nuts), while type CW is suitable for foods with higher a_w values (>0.8). Type CW is commonly used to prevent mold growth in sponge cakes. Type CV absorbs both O_2 and CO_2 (used with roasted or ground coffee).[225]

c. FreshPax® and FreshMax® Absorbents

FreshPax® oxygen absorbents also provide an alternative to gas/vacuum packaging as a means of improving shelf life and product quality, while simultaneously reducing costs. Produced in sachet forms, FreshPax® absorbs headspace O_2 to less than 0.01%, using safe nontoxic ingredients that rapidly absorb O_2 before the food deterioration process begins.[225] The types of FreshPax® absorbents, along with their functional properties and specific uses, are listed in Table 11.

Multisorb Technology in Buffalo, NY also produce oxygen absorbents in a label form, marketed under the trade name FreshMax®. These are mainly used in conjunction with MAP foods to scavenge low levels of residual oxygen in the packaging headspace. They are widely used in the UK to maintain the color quality of cured meat products.[225]

d. Oxygen Absorbents for Shelf Life Extension of Bakery Products

Several studies have shown the potential of oxygen absorbents to extend the mold free shelf life of bakery products. The mold free shelf life of white bread packaged in polypropylene film can be extended from 5 to 45 days at room temperature by incorporating an O_2 absorbent sachet into the package. Pizza crust, which molds in 2–3 days at 30°C, can also remain mold free for more than 14 days at this temperature, using an appropriate

Table 11 Types and properties of FreshPax oxygen absorbents

Type	Function	Moisture status	Water activity (a_w)	Absorption speed (day)	Comments and /or specific uses
B	Decreases O_2	Moisture dependent	>0.65	0.5–2	Moist, semi-moist foods
D	Decreases O_2	Self reacting	<0.7	0.5–4	Dehydrated/dried foods
R	Decreases O_2	Self reacting	0.3–0.95	0.5–1	Refrigerated/frozen foods
M	Decreases O_2	Moisture dependent	<0.65	0.5–2	Moist or semi-moist, gas flushed products

Adapted from Smith and Simpson.[223]

O_2 absorbent.[225] Studies in our laboratory have shown O_2 absorbents to be 3 times more effective than gas packaging for increasing the mold free shelf life of crusty rolls.[220] When Ageless® (S or FX) was packaged alongside crusty rolls, either alone or in conjunction with gas packaging, headspace O_2 never increased beyond 0.05%, and the product remained mold free for over 60 days at ambient storage temperature. While a longer extension in the mold free shelf life was possible using O_2 absorbents, mold problems occasionally arose in the Ageless® packaged product. This was due to absorption of headspace gas and package collapse, resulting in some products being tightly wrapped with the package film. This created pockets of localized environments between product surface and the film where the O_2 concentration may have increased to a level sufficient to permit mold growth. This clearly demonstrates the need for a free flow of gas around the product if Ageless® is to be totally effective as an O_2 scavenger. A mold free shelf life of one year has been achieved for specialty gluten-free bread. The bread was packaged in a co-polymer film of Mylar/EVOH/Surlyn film with 100% CO_2 and O_2 absorbent/CO_2 generator; type CW supplied by Toppan Company. Oxygen absorbent technology is now used by the U.S. Army for meal ready-to-eat pouch bread.[186] In this system, an O_2 scavenging system, FreshPax® (Multisorb Technology, Buffalo, NY) was enclosed in a pouch made of polyester/aluminum foil/high density polyethylene with baked ready-to-eat bread. Under these packaging conditions the bread kept mold free for 13 months at 25°C. This system was used commercially by two U.S. bakery companies (Sterling Foods and Franz Bakery Company) to produce the extended shelf life of bread and rolls for the troops in the Gulf War Crisis of 1990–1991 (AIB, personal communication). Extensive studies in our laboratory have also shown that O_2 absorbents can extend the mold free shelf life of bagels,[14] pizza crust,[111] and pita bread.[85]

The advantages and disadvantages of O_2 absorbents are summarized in Table 12. Oxygen absorbent technology is a huge commercial success in Asian markets and is widely used to extend the shelf life and keep the quality of foods, including bakery products. However, the commercial success of this innovative method of atmosphere modification has been limited in North American and the European marketplace due to the cost of the sachets, which range from 5 to 25 cents depending on sachet size and the consumer resistance to the inclusion of sachets in packaged product. This consumer resistance centers around (a) fear of ingestion of the contents of the sachet, even though the contents are safe and the label on each sachet says "Do not eat," and (b) spillage of sachet contents into food and adulteration of product. These concerns have been addressed by incorporating the oxygen scavenging capacity in a label format or directly into the packaging film surrounding the products. However, a disadvantage of the label format is its limited oxygen scavenging potential. Therefore, for optimum results they are best used in conjunction with gas flushing or vacuum packaging to absorb any residual oxygen in the package headspace.

5. *Ethanol Vapor Generators*

The use of ethanol as an antimicrobial agent is well documented. Alcohol was used by the Arabs over 1000 years ago to preserve fruits from mold spoilage. Seiler [210,214] also demonstrated the potential of ethanol in the vapor phase in delaying mold spoilage in bread and cakes, by spraying ethanol directly onto the products' surface prior to packaging in a plastic film. However, a more practical and safer method of generating ethanol vapor is through the use of ethanol vapor generating sachets. These interactive sachets are manufactured by the Freund Company Ltd. of Japan and sold under the trade name Ethicap® or Antimold® 102 (Table 13). Ethicap consists of food grade alcohol (55% by weight) absorbed on to silicon dioxide powder (35%) and contained in a sachet made of a copolymer of paper/ethyl vinyl acetate copolymer. Vanilla is also added to mask the smell of ethanol. Sachet sizes range from 0.6 to 6G, containing 0.3 to 3 g ethanol that can be evaporated. The choice of sachet depends on product weight, product a_w, and desired

Table 12 Advantages and disadvantages of oxygen absorbents

Advantages	Disadvantages
• Inexpensive and simple to use • Non-toxic and safe to use • Prevent aerobic microbiological spoilage • Extend shelf life of product	• Free air-flow around sachet is needed • Cause package collapse • May promote growth of *C. botulinum*
• Prevent rancidity/off-flavors in fats and oils	• May cause flavor changes in: • High moisture food • High fat foods
• Maintain flavor by preventing oxidation of flavor compounds • Maintain quality without additives • Increase product shelf life • Increase distribution radius • Reduce distribution losses • Replace chemical pesticides to prevent insect damage of foods	• Consumer resistance to sachet use in packages. Possible misuse of the sachet

Adapted from Smith and Simpson.[223]

Table 13 Types of ethanol vapor generators

Type	Function	Application	a_w of product
Ethicap®	Generates ethanol vapor	Cakes/bread	>0.85
Negamold®	Absorbs O_2/generates ethanol vapor	Cakes/bread	>0.85

Adapted from Smith and Simpson.[223]

product shelf life. When food is packed with an Ethicap® sachet, moisture is absorbed from the food, and ethanol vapor is released from encapsulation and permeates the package headspace. However, both the initial and final level of ethanol vapor in the package headspace is a function of sachet size and product a_w. Another type of ethanol vapor generator produced by Freund is Negamold®. This dual functional sachet absorbs oxygen and generates ethanol, albeit it is ∼50% of the ethanol vapor generated by Ethicap® sachets.

a. Use of Ethanol Vapor Generators to Extend the Shelf Life of Bakery Products

Ethanol vapor generators have been shown to be effective in controlling at least 10 species of molds, including *Aspergillus* and *Penicillium* species; 15 species of bacteria including *Staphylococcus, Salmonella* and *E. coli* spp.; and three species of spoilage yeast.[225] Ethanol vapor generators have been used extensively in Japan to extend the mold free shelf life of high moisture bakery products. A 5–20 times extension in mold free shelf life has been observed for high moisture cakes depending on the size of Ethicap® used.[225] Results also showed that ethanol vapor had an antistaling effect. Ethanol vapor generators are widely used in Japan to extend the mold free shelf life of bread, doughnuts, and sponge cake stored at ambient temperature. The mold free shelf life of Madeira cake was also extended for ∼6 weeks at room temperature using a 3G sachet of Ethicap®. [179] However, there was a significant change in cake quality after 3 weeks, characterized by loss of moistness, firmness, and the development of soapy and rancid flavors. Therefore, while Ethicap® could be used to extend the mold free shelf life of products, the sensory shelf life was not significantly extended.[179] Black et al.[26] examined the combined effect of gas packaging and Ethicap® (size unknown) to extend the shelf life of pita bread. They reported a 14 day mold free shelf life for pita bread packaged in 40% CO_2 (balance N_2) or 100% CO_2. However, the shelf life was doubled when an Ethicap® sachet was included with the gas packaged products. These authors reported that ethanol had little antistaling effect on pita bread.[26] Furthermore, they observed mold spots in some products after 28 days. They hypothesized that this may have been caused by ethanol vapor acting as a plasticizer, thereby, increasing the flim's permeability to both O_2 and CO_2.[26]

Ethicap® has also been shown to prevent secondary spoilage problems in gas packaged par-baked bakery products.[221] Apple turnovers with an a_w of 0.93 had a shelf life of 14 days when packaged in a CO_2:N_2 (60:40) gas mixture at ambient temperature, due to the growth of and CO_2 production by *S. cerevisiae*. However, when Ethicap® was included with the packaged product, yeast growth was completely suppressed, and all packages appeared normal at the end of the 21 day storage period. Ethicap has also proved effective in controlling secondary spoilage problems by *S. cerevisiae* in gas packaged strawberry and vanilla layer cakes and cherry cream cheese cake.[177]

The advantages and disadvantages of ethanol vapor generators are summarized in Table 14. One main disadvantage of using ethanol vapor for shelf life extension of bakery products is its absorption from the package headspace into the product, resulting in sensory rejection of the product.[221] However, the concentration of ethanol found in products, such as apple turnovers, crumpets, or pizza crusts can be reduced to less than 0.1% by heating products at 375°F prior to consumption.[221] Therefore, while a longer shelf life may be possible by packaging products with Ethicap®, its use as a food preservative may be limited to "brown'n'serve" products. An alternative approach to minimize these sensory problems may be to replace Ethicap® with dual functional sachets (Negamold®) that absorb O_2 and generate lower levels of ethanol into the package headspace.

In conclusion, interactive packaging technology involving gas absorbents, gas/vapor generating sachets, appears to be, at first glance, a relatively simple concept in atmosphere modification and the shelf life extension of food. However, the appropriate choice of sachet requires careful evaluation of the chemistry and microbiology of the food product in relation to the dynamics of the microenvironment within the packaged product. Nevertheless, these interactive sachets offer the bakery industry a viable

Table 14 Advantages and disadvantages of ethanol vapor generators

Advantages	Disadvantages
• Ethanol vapor can be generated without having to spray ethanol directly onto product surface prior to packaging • Sachets can be conveniently removed from the package and discarded at the end of the storage period • Eliminates the need for preservatives such as benzoates and sorbates to control yeast spoilage • Controls mold spoilage and delay staling in bakery products	• Absorption of ethanol from the package headspace into the product

Adapted from Smith and Simpson.[223]

alternative to gas packaging in extending the mold free shelf life of products. Furthermore, they could be used in developing countries to extend the shelf life of products without the need for expensive packaging systems.

PART II

A. SAFETY CONCERNS OF BAKERY PRODUCTS

To date, this review has focused on the physical, chemical, and microbiological spoilage problems limiting the shelf life of bakery products. However, high moisture bakery products have also been implicated in several outbreaks of foodborne illness and, therefore, pose safety concerns.

Each year, thousands of North American consumers suffer from some form of foodborne illness with symptoms ranging from mild to fatal. Mead et al.[170] estimated that in the United States there are approximately 76 million foodborne illnesses, 325,000 hospitalizations, and 5,000 deaths each year. While foods such as meat, fish, poultry, eggs, and dairy products are the most common vehicles of foodborne illnesses worldwide, bakery products have also been implicated in foodborne disease outbreaks.[248] In the United States between 1988 and 1992, baked foods accounted for 29 outbreaks, involving 820 cases out of a total of 2,423 reported outbreaks of foodborne disease.[21] In Canada, pizza, cheesecake, pies and tarts, bread, and muffins have all been implicated in outbreaks of foodborne illnesses.[248] Seiler[212] estimated that between 1969 and 1972, 30% of foodborne illnesses in the U.K. were attributed to bakery products, with *S. aureus* being involved in most of these outbreaks. Pizza and noodles were responsible for 2 of the 72 outbreaks reported in Australia between 1980 and 1995.[57] More recently, *B. cereus* has been implicated in outbreaks of foodborne illness involving high moisture English style crumpets.[132]

The rest of the world is not immune to foodborne disease outbreaks caused by bakery products. Todd[248] reported that 35–47% of all foodborne disease outbreaks in Poland, Portugal, Bulgaria, and Switzerland were caused through bakery products. Cuba reported 186 outbreaks, involving 8,813 patients in the first six months of 1990. The major food vehicles were beef, pork, chicken, and cake. Between 1988 and 1990 in Brazil, several outbreaks were traced to white cheese and cream filled cakes, with *S. aureus* being the main organism involved.[185]

There are several reasons why bakery products are implicated in outbreaks of foodborne disease:

1. Processing Conditions

In order to achieve desirable textural and quality attributes, most bakery products only receive a moderate heat processing treatment. For example, bread is baked at high temperature; however, during baking, the temperature in the center of the crumb rarely exceeds 100°C for a few minutes. Furthermore, some baked products include cream, cold custard, icing, spices, nuts, or fruit toppings or fillings, which may be prepared without any heating. According to Bryan et al.[35] vegetative pathogenic microorganisms should be readily destroyed during baking, due to their low thermal resistance (D values) as shown in Table 15. However, spores of spore forming bacteria, will readily survive baking due to their high D values (Table 15) and may grow to levels of public health concern, if packaging and storage conditions are conducive to their growth.[35] While vegetative microorganisms should be destroyed during baking, products may be subject to post baking contamination from the air, equipment, and handlers.[235] Furthermore, cross contamination may occur if bakery products are prepared or stored in the same area as raw foods, such as eggs, meat, or milk.

Table 15 Heat resistance of some specific bacteria

	Heat resistance in minutes		
Bacteria	$D_{70°C}$	$D_{90°C}$	$D_{121°C}$
Bacillus cereus		10	—
Campylobacter jejuni	0.0001	—	—
Clostridium botulinum (Group 1)	—	—	0.2
Clostridium botulinum (Group 2)	—	1.5	—
Clostridium perfringens	—	—	0.15
Escherichia coli	0.001	—	—
Listeria monocytogenes	0.3	—	—
Salmonella spp.	0.001	—	—
Staphylococcus aureus	0.1	—	10
Vibrio parahaemolyticus	0.001	—	—
Yersinia enterocolitica	0.01	—	—

Adapted from Report on Vacuum Packaging and Associated Processes.[195]

2. Hazardous Products/Ingredients

Many bakery products and their ingredients have a pH of >4.6 and an a_w of >0.85 i.e., conditions that are conducive to the growth of pathogenic bacteria.

Several bakery products/ingredients have pH and a_w levels that restrict microbiological growth, while others have levels conducive to microbiological growth. For example, the pH of custard used in many filled baked products is 5.8–6.6 and is ideal for the growth of *Salmonella* spp.[36] However, it is also important to note that both pH and a_w may change during storage. For example, icing, which has a low a_w, is not usually a microbiological problem; however, the interface between the cake and icing may have a much higher a_w, due to moisture migration that encourages microbiological growth at the interface. Silliker and McHugh[216] reported such an incident, in which *S. aureus* grew at the interface of cake and icing.

Table 16 Growth requirements of pathogenic bacteria

	Minimum required			
Bacteria	Temperature °C	pH	a_w	Gaseous conditions
B. cereus	4	4.3	0.91	Facultative
C. jejuni	32	4.9	0.99	Microaerophilic
C. botulinum (Group 1)	10	4.6	0.95	Anaerobic
C. botulinum (Group2)	3.3	5.0	0.97	Anaerobic
C. perfringens	15	5.0	0.95	Facultative
E. coli	7	4.4	0.95	Facultative
L. monocytogenes	0	4.3	0.92	Facultative
Salmonella spp.	6	4.0	0.94	Facultative
S. aureus	6 (10 for toxin)	4.5 (5.2 for toxin)	0.86 (0.90 for toxin)	Facultative
V. parahaemolyticus	5	4.8	0.94	Facultative
Y. enterocolitica	−1	4.2	0.96	Facultative

Adapted from Report on Vacuum Packaging and Associated Processes.[195]

3. Storage Conditions

Most bakery products, with the exception of cream, custard, and meat filled products, are held at ambient temperature for maximum storage quality; however, such storage conditions may be conducive to microbiological growth and may compromise safety. Furthermore, since most products are "cook and hold" and are not heated prior to consumption, there is no safety margin for destroying bacteria that may survive the baking process or may have been introduced during handling or storage. English style crumpets, a high moisture snack food product held at ambient temperature, have been implicated in several food poisoning outbreaks involving *B. cereus*. For in-store bakeries, products are often displayed in bins or are loosely wrapped in paper. While customers like this form of product presentation, there is a potential for contamination of these products from self-serve bins, if they are handled without the use of tongs or glassine paper.

Products, such as cream, meat, and cheese filled cakes have an established history as vehicles of foodborne illness. While holding at refrigeration temperatures will delay microbiological growth in these filled products, it may not be sufficient to prevent the growth of psychrotrophic pathogens, such *L. monocytogenes*. Furthermore, there is always the potential of temperature abuse at all stages of processing, distribution and storage chain, and in the home. If products are frozen, bacterial growth will be slowed, but once the product is thawed, growth may resume, as has been shown in outbreaks involving *Salmonella* spp.[205]

4. Modified Atmosphere Packaging

Modified atmosphere packaging (MAP) using CO_2 enriched gas atmospheres can extend the mold free shelf life and keep the quality of a wide variety of bakery products stored at ambient temperature. Examples of gas packaged products on the marketplace include bread, pita bread, crumpets, sandwiches, pizza, and muffins. However, there are concerns about the safety of this technology, since many pathogens can grow under a wide range of a_w, pH, temperature, and packaging conditions, as shown in Table 16. One pathogen of concern in MAP products is spores of *C. botulinum*, which will readily survive the baking process. This concern would appear justified since this pathogen has been shown to grow to hazardous levels in gas packaged food stored at ambient temperature, yet products were still organoleptically acceptable to the consumer.[91,117] While gas packaging is widely used in Europe and is gaining acceptance in North America to extend the shelf life of high moisture bakery products, there is a paucity of data on the safety of such goods stored at ambient temperature.

5. Recent Market Trends

Recent consumer trends have resulted in novel products, such as preservative free, low fat, and reduced calorie baked goods. However, modification of a product's formulation may also influence its a_w or pH to levels conducive to the growth of foodborne pathogens. Such novel products may be safe, but their safety must be assessed on an individual basis. This is even more critical, if such products are packaged under modified atmospheres and stored at ambient temperature.

The second part of this review will focus on the specific microorganisms associated with foodborne disease outbreaks in high moisture bakery products and the strategies used to enhance the safety of such products.

B. SPECIFIC MICROORGANISMS OF CONCERN

1. Salmonella Species

a. Sources of Contamination

Salmonellosis is a common gastrointestinal foodborne disease which, although generally self-limiting, can result in chronic complications in the very young, the old, or the immunocompromised.[63] *Salmonella* spp. causing foodborne disease are

commonly isolated from animals, their food products, and their processing environments.[82,240,261] *Salmonella* spp. may be also introduced into bakery products through a wide range of ingredients. Furthermore, *Salmonella* spp. can also be easily spread by cross contamination when ingredients or finished bakery products are in contact with other animal foods or contaminated surfaces during production, storage, and transportation.

Although eggs are invaluable for their foaming, emulsifying, and binding properties they are a major source of *Salmonella* spp. in bakery products. *Salmonella* spp. may be found on the eggshell[28]; however, *Salmonella enteritidis* has also been found inside eggs as a result of transovarial transmission during formation. The United States Department of Agriculture (USDA) estimates that one in 100,000 eggs is contaminated with *S. enteritidis*. Higher numbers are found more often in whites than in yolks, but contaminated eggs occasionally contain extremely large numbers of *S. enteritidis*. While the pasteurization of egg products effectively destroys *S. enteritidis* and maintains its functional properties, the use of unpasteurized shell eggs in small bakeries or in the home increases the risk of the contamination of bakery products by *Salmonella* spp.[94] Furthermore, handling contaminated eggs often results in the widespread contamination of work surfaces, equipment, and hands.[123,124]

While eggs are one of the most common vehicles of transmission of *Salmonella* spp. in baked products, other ingredients may pose safety concerns. Pasteurization of milk destroys *Salmonella* spp.; however, dairy products including fresh or dried milk, butter, cream, and cheese may contain *Salmonella* spp. through inadequate pasteurization or through post pasteurization contamination in the dairy environment.[5,8,84,133] *Salmonella* spp. have also been found in flour. Richter et al.[196] reported that 1.3% of 4000 samples of wheat flour contained *Salmonella* spp. Although flour is too dry for growth, cells can remain viable for several months.[64]

Other bakery ingredients from which *Salmonella* spp. have been isolated, many of which have been implicated in foodborne illness, include cocoa and chocolate, particularly milk chocolate,[62,250] coconut,[100] peanuts and peanut butter,[204] fruit,[102,190] spices, and yeast flavorings.[134,155] *Salmonella* spp. are resistant to desiccation and can survive for long periods of time on work surfaces and in foods with low a_w, particularly those with a high fat content. Once hydrated, *Salmonella* spp. may grow rapidly in such products held at ambient temperature.

Since many raw bakery ingredients may contain *Salmonella* spp., constant vigilance is required to prevent their growth in these ingredients and to minimize cross contamination of other ingredients and final baked products.

b. Associated Outbreaks

The symptoms of salmonellosis in people who are not at high risk may be mild, and it is estimated that the disease is significantly under-reported.[247] Outbreaks from infected chocolate and cheese suggest that the infective dose may be fewer than 10 *Salmonella* cells per 100 g of food.[8,119]

Most reported outbreaks of salmonellosis caused by eating contaminated bakery products have involved *S. enteritidis* PT4, *S. enteritidis* PT7, and *S. typhimurium*. In most outbreaks, eggs were confirmed as the suspected vehicle of transmission.

The use of raw shell eggs in unbaked products has been the causative agent in several salmonellosis outbreaks. Unbaked products prepared with raw shell eggs involved in such outbreaks include tiramisu, mousse, and charlotte ruse.[190,191] Marshmallow cones, which typically have an $a_w < 0.7$, have been associated with one foodborne illness outbreak.[158] The marshmallow was made by cold setting raw egg white combined with a commercial mix containing sugar and dried egg whites. The wrapped filled cones were kept at room temperature, and *Salmonella* spp. may have grown during storage at the interface between the cone and the marshmallow or may have remained viable in the uncooked product. Consumers eating the cones became ill and *S. enteritidis* PT4 was isolated from the marshmallow, but not from the mix or cones. This outbreak illustrates the complex nature of the food matrix and food safety. Even although this product had an initial a_w to prevent the growth of *Salmonella* spp., moisture absorbtion or migration may have resulted in pockets of a_w that were conducive to their growth. If a product is to be regarded as safe with respect to this pathogen, all components should have an $a_w < 0.9$ and be stored under suitable conditions to prevent moisture absorbtion/migration.

Many egg based bakery products receive a mild heat treatment to provide a light and tender product. Such products include sweet and savory custard fillings, pies, mousse, meringues, and cheesecake. Such mild heat treatments may be insufficient to completely destroy all *Salmonella* spp., depending on their level in the raw ingredients and the time/temperatures processing conditions.[109] Outbreaks are common in such foods, especially when prepared with unpasteurized shell eggs. The practice of cooking and holding at room temperature, prior to the consumption of products, facilitates the growth of surviving organisms. Custard pies, prepared commercially and held for 21 hours prior to consumption, resulted in a large outbreak of salmonellosis in which one person died.[44] Other similar products that have been implicated in outbreaks include bread pudding,[44,105] custard filled cakes and pastries,[19,190] quiche,[121] meringue pies,[263] mousse, banana puddings,[44,120] and cheesecake.[47,264]

Infected eggs or other ingredients may also cross contaminate equipment, resulting in multiple outbreaks. Evans et al.[89] reported consecutive salmonellosis outbreaks traced to the same bakery. The first outbreak was attributed to cross contamination during the preparation of a cold-custard mix in a bowl previously used for shell eggs, while inadequate cleaning and disinfecting of nozzles used for piping cream was the source of cross contamination in the second outbreak.[89] Similarly, four outbreaks were caused through cross contamination by raw eggs used in the production of gateaux and cheesecake.[264]

While low pH can be used to control the growth of pathogenic bacteria, there is no guarantee that acidic products, such as fruit pies, will not be implicated in foodborne disease outbreaks. Apple pie was implicated in an unusual outbreak of salmonellosis,

involving *S. enteritidis* PT4. The commercially prepared pie was prepared from canned filling, and the crust was brushed with a raw shell egg—milk glaze and baked. The 20 cm pies were cooled and held for five hours at room temperature prior to consumption. The baking conditions were adequate for the destruction of *Salmonella* spp., and the low pH (3.6) of the baked pie filling should have been an additional barrier to control the growth of this pathogen.[30] However, cross contamination of pies may have occurred during packaging, or the egg glaze may have seeped through the high fat pastry, resulting in the growth of *S. enteritidis* at the product's interface.

Salmonella spp. can also resist desiccation and can survive for long time periods in foods of very low a_w. Outbreaks involving *Salmonella* spp. have been reported in cake mixes made with dry eggs,[217] snack foods,[139,155] and toasted oat cereal.[45]

c. Control Measures

Since raw eggs are a common source of *Salmonella* spp., the use of pasteurized whole egg, egg white, and egg yolk is critical to control salmonellosis in bakery products. Pasteurized eggs should always be used in unbaked or lightly cooked products or in products that require the pooling or holding of eggs. If raw eggs are to be used, cracked eggs should be avoided to reduce the risk of foodborne illness.[248] However, in the interest of food safety, it may be prudent to assume that all eggs are potentially hazardous ingredients, and they should be stored under proper refrigerated storage conditions to prevent the growth of any *Salmonella* spp. present.

Processing conditions also play a role in the destruction of S*almonella* spp. Baking or cooking should destroy any *Salmonella* spp. present, but the degree of inactivation will depend on a number of inter-related factors, such as the composition of the bakery product (pH, a_w, preservative), level of contamination, and time-temperature of the heat process. During the baking of retail pumpkin pie with a pH 5.6 and an a_w of 0.97, an internal temperature of 108°C for one minute was sufficient to destroy 10 CFU/g of inoculated *S. typhimurium*.[265] A similar inoculum of *S. enteritidis* was destroyed during the baking of cheesecake (pH 4.9, a_w 0.98), but when higher numbers (10^6 CFU/g) were inoculated into cheesecake, some *Salmonella* spp. survived baking. In custard, a temperature of 60°C for 19 minutes was required to kill 10^7 CFU/g of *Salmonella* spp. However, if the temperature was increased to 65.7°C, similar levels of *Salmonella* could be inactivated in only 3.5 minutes.[10] Sumner et al.[237] studied the heat resistance of *S. typhimurium* in sugar syrups having a_w of 0.98 to 0.83 and found that at 65.6°C, the decimal reduction time ($D_{65.6^\circ C}$) varied from less than 30 seconds to more than 40 minutes. Simply reboiling custard fillings after thickening ingredients were added or the rebaking of pies after filling, were also effective methods of inactivating *Salmonella* spp.[42] Temperature/time combinations should also be monitored by thermometers to ensure that products are processed properly for the adequate destruction of *Salmonella* spp. While these studies again illustrate that food safety is dependent on a number of variables, it is important that all baked products or their ingredients are cooled rapidly to 2–5°C within 30 minutes of preparation, regardless of their heat treatment.

Strict temperature control is also critical in ensuring the safety of bakery ingredients containing fresh cream. The refrigeration (4°C) of cream filled pastries has been shown to prevent the growth of *Salmonella* spp.[36] However, studies have shown that *Salmonella* spp. remain viable during refrigerated or frozen storage.[205] Therefore, temperature abuse at any stage during processing, distribution, and storage may compromise the safety of cream filled bakery products or their ingredients. The importance of strict temperature control cannot be overemphasized, as *Salmonella* spp. can grow as low as 6°C. While additional barriers, such as potassium sorbate (2500 ppm), may be used to limit the growth of *Salmonella* spp. in cream filled products,[265] preservatives should not be regarded as a substitute for proper refrigerated storage conditions.

Strict personal hygiene, good manufacturing practices, ongoing education of personnel, and implementation of a Hazard Analysis Critical Control Point (HACCP) approach are also critical to reduce cross contamination of products by *Salmonella* spp. and to limit the spread of this foodborne pathogen. However, while medium and large sized bakery companies will have the financial resources and technical support to implement these control programs, small companies and family owned operations may not.

2. *Staphylococcus aureus*

a. Sources of Contamination

Staphylococcus aureus is a gram positive facultative bacterium of which 50–70% of strains are estimated to be enterotoxigenic.[251] Staphylococcal food poisoning results in susceptible individuals following consumption of food in which *S. aureus* has grown to sufficient numbers ($\sim 10^6$/g) to produce enterotoxin. Enterotoxin A is implicated in 75% of *S. aureus* outbreaks; however, outbreaks are occasionally attributed to enterotoxin B.[23] The illness is characterized by nausea, vomiting, abdominal pain, and diarrhea 2–6 hours after eating the food containing staphylococcal enterotoxin. Recovery takes 1–3 days and death is rare.[251] As symptoms of the illness are usually mild, medical attention is not always sought. Furthermore, in many countries, staphylococcal food poisoning is under-reported. It has been estimated that only 1–5% of cases are reported, but only when a large number of individuals is involved in an outbreak.[128]

The major reservoirs of *S. aureus* are humans and animals. *S. aureus* is carried by 30–50% of humans in nasal passages, throats, and on skin.[23] It is also ubiquitous in air, water, milk, sewage, and on food contact surfaces.[128] Humans harboring *S. aureus* are a major source of contamination of products during preparation or post preparation handling. Post preparation contamination is also possible from air, surfaces, and cross contamination. Ingredients may also be sources of high numbers

of *S. aureus*. Milk from mastitic cows can contain high levels of *S. aureus*. Although milk in North America is generally screened for mastitis, milk in some temperate countries is a source of *S. aureus*.[23] In Brazil, surveys have reported an average of 10^3 CFU/g of *S. aureus* in milk, while 50% of pasteurized milk and cream have been shown to be contaminated with this pathogen.[203] Other ingredients, such as reconstituted dried milk, whey, and cream also have been found to contain *S. aureus* enterotoxin.[23]

b. Associated Outbreaks

In the United States, cream filled bakery products made from fresh or synthetic creams and custard fillings were once the main cause of all food poisoning outbreaks involving *S. aureus*. While these products are seldomly implicated in outbreaks of *S. aureus* food poisoning in North America due to good manufacturing practices, they continue to be a major source of illness in many temperate countries where refrigeration is still a problem.[73] Outbreaks in Brazil, involving 122 cases, were caused by eating tainted cream puffs, while other outbreaks have been attributed to chocolate éclairs served on a flight from Rio de Janerio to New York City. An outbreak of staphylococcal food poisoning, involving 215 cases, also occurred on a Caribbean cruise ship sailing from the United States, with cream pastries being the implicated food.[43,258] One survey found that 55% of cream pies stored at room temperature from commercial establishments in Brazil were contaminated with *S. aureus* and 19.5% of the products contained levels $>10^5$ CFU/g.[11] Desai and Kamat[72] reported that 85.7% of 7 pastry and biscuit creams from commercial establishments in India were positive for *S. aureus*, with average counts of $\sim10^4$ CFU/g. Leela et al.[153] found that enterotoxigenic Staphylococci in bakery products in India were always associated with cream and coconut fillings. *S. aureus* ($3 \times 10^1 - 6.5 \times 10^4$ CFU/g) producing enterotoxin A, B, and E were found in cakes, sweet puffs, vegetable puffs, and cream buns from five Indian bakeries. Breads and buns from the same bakeries were negative for *S. aureus*.[202]

These outbreaks show that cream type fillings are an excellent medium for the growth of *S. aureus*. In cream pie fillings inoculated with 10^2 CFU/g of FR-100, enterotoxin was first detected after 35 hours at 20°C (3.9 μg/g). In fillings stored at 37°C, 50 μg/g of enterotoxin was present after 14 hours.[118] McKinley and Clarke[169] demonstrated that imitation cream fillings used in bakery products are also capable of supporting the growth of enterotoxigenic strains of *S. aureus*. Although imitation cream on its own does not contain sufficient nutrients to support the growth of this pathogen, growth can occur at the interface of the cream and the baked product.[169] Surkiewicz[238] also showed that imitation cream pies spoiled within 48 hours at ambient temperature, with counts of *S. aureus* up to 10^6 CFU/g.

Other products have been shown to be contaminated with this pathogen. Sumner et al.[236] isolated *S. aureus* from 9.8% of 214 bakery products, including oatmeal raisin cookies, apple muffins, cream puffs, and long johns. The a_w of the cookies was too low for the growth of *S. aureus*. However, 3.3% of apple muffins, 30% of cream puffs, and 11% of long johns were positive for *S. aureus* with enterotoxin, produced by 7 isolates. Concern was raised over enterotoxigenic strains of *S. aureus* isolated from low moisture products, i.e., muffins, which are generally considered to be low risk products. This concern would seem justified, since dry flat bread was responsible for an outbreak of staphylococcal food poisoning in Norway.[1]

Pizza is also frequently involved in foodborne outbreaks involving *S. aureus*.[248] Contamination usually occurs through poor manufacturing practices, and *S. aureus* can grow in high salt concentrations found in many pizza ingredients.

Although *S. aureus* is destroyed by heating, the enterotoxin is heat resistant and is not inactivated by pasteurization.[23] Hand made tortillas had counts of approximately10^8 CFU/g after preparation. The reheating of products resulted in only a 1 to 2 log reduction in counts, while *S. aureus* enterotoxin survived the heating process.[38] Therefore, food poisoning outbreaks by *S. aureus* may still occur, even in the absence of viable cells, if preformed enterotoxin is present in the product, as a result of temperature abuse of the ingredients/fillings prior to baking. Potential problems also exists if the contaminated fillings are uncooked, e.g., whipped cream fillings and toppings. *S. aureus* is a poor competitor; however, if contamination occurs after heating, in which most other vegetative organisms have been destroyed, conditions will favor rapid growth.

There is an increasing variety of goods available from in-store bakeries that are held at ambient temperature. These products are often handled manually in self serve bins by employees and customers. This increases the potential for contamination by *S. aureus* and subsequent growth and/or enterotoxin production at ambient storage temperature.

c. Control Measures

The number of staphylococcal food poisoning outbreaks, attributed to the consumption of cream and custard filled pastries in the United States, has dramatically decreased in recent years, due to improved sanitation, temperature control, modification of product formulations, and the use of preservatives.[87] Good manufacturing practices (GMPs) have effectively reduced the level of contamination of *S. aureus* in frozen cream pies in North America. In a survey of the frozen cream pie industry, the Food and Drug Administration (FDA) concluded that operating under good sanitary conditions resulted in products with no *S. aureus* in 0.1 g samples.[238] A survey of all plants manufacturing frozen pies in the United States reported that levels of *S. aureus* were consistently <10 CFU/g. Guidelines have been developed in Canada.[249] Warburton and Weiss[257] found that aerosol cream filling substitutes and powdered whipped toppings contained <5 CFU/g of *S. aureus*, due to GMPs.

Methyl paraben, sodium benzoate, potassium sorbate, and calcium propionate have also been shown to inhibit *S. aureus* at pH 5.2–5.6 and a_w 0.86–0.90.[31] Schmidt et al.[206] found that both potassium sorbate and sodium benzoate (1000 ppm) inhibited

the growth of *S. aureus* in synthetic cream pies at 22 and 37°C and at pH 4.5–5.0, but not at pH 5.2. The growth of *S. aureus* was found to be inhibited in fillings made from chocolate or cocoa. While inhibition was initially attributed to a reduction in pH, substances in the non-fat part of the chocolate were later found to be responsible for the inhibition of *S. aureus*.[41] D and L-serine have also been shown to inhibit the growth of this pathogen in cream pie fillings.[39]

Heating immediately after preparation can destroy *S. aureus* in fillings and filled products. Stritar et al.[234] reported that heating custard filled pastries previously inoculated with 10^5 CFU/g of *S. aureus* to 190.6°C for 25 minutes resulted in complete inactivation. Other temperature-time processes have been recommended.[10,99,125,137] Cathcart et al.[42] found that simply reboiling custards after the thickening mix had been added also destroyed the inoculated *S. aureus*. However, care must be taken to avoid subsequent recontamination.

A strict temperature control (4°C) of finished products and their ingredients is also critical to inhibit the growth and enterotoxin production of *S. aureus*.[36] Bergdall[23] reported that cakes held at room temperature resulted in food poisoning, while those held at 4°C did not. Cream filled cakes inoculated with 10^6 CFU/g of an enterotoxin producing strain of *S. aureus* did not support growth, nor was enterotoxin detected in cakes held under refrigeration. At room temperature (27–29°C), enterotoxin was detectable in cakes inoculated with 10^2 and 10^3 CFU/g after only 24 and 18 hours, respectively. Such time-temperature abuse is not uncommon for cream filled bakery products.[11]

Since *S. aureus* can grow facultatively, MAP will not inhibit its growth or enterotoxin production.[117] Park et al.[181] demonstrated the importance of refrigerating fresh pasta stored under modified atmospheres. In pasta stored at 5°C, counts of *S. aureus* declined, and no enterotoxin was detected. For fresh MAP pasta products with an $a_w < 0.95$, a reduction in the product's a_w and pH is recommended as an additional safety barrier against the growth of *S. aureus*.[40] Some products, such as meringue and butter creams, may need to be formulated to an a_w of <0.86, since *S. aureus* can tolerate and even grow in products with a high sugar content and a low a_w.[189] However, pockets of high moisture and, hence, a high a_w, may still occur within such products and encourage the growth of this pathogen. Therefore, storage under strict temperature and humidity control is essential in preventing moisture migration and the growth of this pathogen.

3. *Bacillus Species*

a. *Sources of Contamination*

Bacillus cereus has been implicated in several outbreaks of foodborne illnesses involving bakery products. There is also evidence that *B. subtilis* and *B. licheniformis,* well known as the rope forming spoilage bacteria of bread, can also cause foodborne illness.[146,242,246] *B. cereus* has two distinct forms of toxin-mediated gastroenteritis. The emetic type is generally associated with cereal based foods, while the diarrheal type is most frequently associated with proteinaceous foods.[162]

Bacillus species form spores, which are ubiquitously found in soils, dust, and water and are commonly isolated from plants and animal products.[106] *Bacillus* spp. attach to wheat, which is milled into flour.[142] Graves et al.[107] examined flour from 11 U.S. mills and found that 18.2% of samples contained *B. cereus*, 9.1% contained *B. licheniformis*, and 45.3% contained *B. subtilis*. The level of contamination of species *B. cereus* in wheat flour was generally less than 10^3 spores/g.[136,140,198] Therefore, *Bacillus* spores are commonly found in flour and flour based products, as well as in the bakery environment. Spores of *Bacillus* spp. are heat resistant and will survive baking and, under favorable conditions, may grow to levels associated with toxin production. The survival of spores during baking depends on the type of product, the internal temperature reached during baking, as well as the thermal resistance of the spore.

Spores of *Bacillus* spp. have also been found in milk and can survive pasteurization. Hence, they are a source of concern in dairy products, such as cream, dried milk,[151,219] and whey concentrates.[183] Although *B. licheniformis* was initially found in higher numbers than *B. cereus* when milk products were held at room temperature, *B. cereus* rapidly dominated and reached levels associated with enterotoxin production.[61] Harmon and Kautter[110] reported a six- to seven-fold increase in *B. cereus* in reconstituted non-fat dried milk held at room temperature for 10.5 hours. Cold adapted strains can even produce toxin at refrigerated temperatures,[93] especially in aerated products, such as whipped cream.[51]

Spices, such as ginger, mace, allspice, cinnamon, garlic, and pizza spice usually contain low levels of *Bacillus* spores; however, higher levels of spores, associated with toxin production, have occasionally been found in these bakery ingredients.[143,178,187]

Other bakery ingredients that may be sources of *Bacillus* spp. include dried eggs,[215] soy protein,[22] rice,[52] yeast and improvers,[18,55,242] dried fruits,[6,172] and cocoa.[96,242]

b. *Associated Outbreaks*

The levels of *B. cereus* required to produce toxin is approximately 10^5 spores/g of food, while higher spore levels (10^6–10^9 spores/g) are required for *B. licheniformis* and *B. subtilis*.[162] Foodborne illness caused by *Bacillus* spp. is under-reported, as symptoms are generally mild and self-limiting.[243]

Although low numbers of spores may be present initially in flour,[136] spores that survive baking can grow rapidly in products held under suitable conditions.[199] While *B. cereus* does not easily survive the baking of bread, the more heat resistant *B. subtilis,* that has a $D_{100°C}$ of 14 minutes does.[157] Kaur[136] reported that *B. cereus,* inoculated with $\sim 10^4$ spores/g, did not survive baking in 400 g loaves, but survived baking in 800 g loaves. Although the actual number of *B. cereus* that survives baking is dependent on the loaf size and oven temperature/time combinations, the risk of foodborne illness from bread is minimal.[55,136,199,244] Kaur[136] estimated that it would take three days at 27.5°C for *B. cereus*

that survived the baking process to reach levels of 10^5 CFU/g in bread.

Some strains of *B. subtilis* and *B. licheniformis* cause "ropiness," resulting in the sensory rejection of the product. However, this is not always the case, and bread may have high numbers of *B. subtilis*, yet still be organoleptically acceptable to the consumer. Illness attributed consumption of bread with high numbers of *B. subtilis* or *B. licheniformis* has been reported.[229,244,247] Therefore, control of *B. cereus*, *B. subtilis*, and *B. licheniformis* is critical to the safety of bread products.

Although the potential health hazard of bread is minimal, *B. cereus* is of greater concern in bakery products that receive a surface heat treatment. Examples of such griddle baked bakery products include ethnic flat breads, crumpets, and waffles. Outbreaks of *B. cereus* gastroenteritis have been attributed to naan bread,[59] crumpets,[152] and pikelets.[173] Growth of *B. cereus* in these products is difficult to control, since the heat treatment is insufficient to destroy spores and may actually heat shock spores, which enhance their growth at ambient storage temperature. There is also concern for their growth in dry reconstituted rice cereals, particularly when reconstituted with milk.[129]

B. cereus has also caused illness in bakery products containing dairy based custards or creams. Pinegar and Buxton[182] showed that custard used in the production of vanilla slices contained low numbers of spores ($<$500/g). However, immediately after cooking, levels of *B. cereus* increased to $>10^6$ CFU/g. *B. cereus* has also been isolated from prepared bakery products, including meat filled bakery products,[138] bread,[201] pies, and pastry.[182,266]

c. *Control Measures*

Conventional methods of control include proper sanitation and the testing of raw materials to reduce initial spore counts; however, these measures will not eliminate all spores or prevent germination and the growth of *Bacillus* spp. in finished products. The growth of *Bacillus* spp. in final baked products can, however, be controlled with preservatives. Propionic acid, calcium or potassium propionates, and calcium acetate can delay germination and the growth of some *Bacillus* spp., particularly rope producers.[136,142,200] Thompson et al.[245] reported that vinegar was more effective in preventing rope production in white bread than calcium propionate. Potassium sorbate, at a level of 2500 ppm, prevented the growth of *B. cereus* in pumpkin pie,[266] while 2000 ppm of sorbic acid or 4000 ppm of potassium sorbate inhibited *B. subtilis* and *B. cereus* in the rice filling of Karelian pastry.[194]

Lactic acid bacteria may also inhibit *Bacillus spp*. Corsetti et al.[58] reported that 33% of 232 lactobacilli isolated from Italian sour dough inhibited strains of *B. subtilis*. Control of *B. subtilis* and *B. licheniformis* in bread was achieved by the addition of 10 to 15% sourdough fermented with *Lactobacillus plantarum* or *Lactobacillus sanfrancisco* L99. Bread had an increased acidity, due to the production of lactic and acetic acids and resulted in an increased shelf life, without the addition of preservatives.[200]

Some strains of lactic acid bacteria also produce bacteriocins. Nisaplin® is a commercially available bacteriocin (nisin), produced by *Lactococcus lactis*, and is the only bacteriocin shown to have an antimicrobial effect against *Bacillus* spp. The growth of *B. cereus* in high moisture English style crumpets was controlled in crumpets for up to 5 days at ambient storage temperature by the addition of 1 to 5 μg/g of nisin.[132] However, nisin at levels up to 100 μg/g, had no effect on the growth of either *B. subtilis* or *B. licheniformis* in wheat bread.[200]

Spores of *Bacillus* are known for their heat resistance. Typical $D_{100^\circ C}$ values for *B. cereus, B. subtilis*, and *B. licheniformis* have been reported as 40, 14, and 56 minutes, respectively.[157,266] Therefore, spores can readily survive the mild heat processing conditions of baking. However, thermal resistance is influenced by a product's pH. The $D_{90^\circ C}$ value of *B. cereus* spores decreased from 3.7 to 3.1 minutes, when the pH of custard was decreased from 7.2 to 6.2.[20] A high pH ($>$9) may also provide some measure of control, however, very few bakery products are formulated to such pH levels.[86,157]

Cold storage may be a suitable control measure for some products, such as cream and custard type products. However, up to 14% of *B. cereus* strains may be psychrotrophic.[106] Therefore, temperature alone is not a practical control measure. Custards and fillings should be prepared in small batches, cooled rapidly, and stored at 4°C.

Modified atmosphere packaging with CO_2 cannot control the growth of *Bacillus* spp. alone. Smith et al.[226] reported that *B. subtilis* and *B. licheniformis* grew to 5×10^8 CFU/g after 3 days at 37°C or after 2 weeks at 20°C in crumpets packaged under modified atmospheres. El-Khoury[86] controlled the growth of *B. cereus* in crumpets stored at 30°C for $>$28 days, using a combination of 100% CO_2 and 600S Negamold®, an oxygen absorbent-ethanol vapor generator. Products, however, were organoleptically unacceptable, due to the high levels of ethanol absorbed from the package headspace.[86]

A multibarrier approach has also been investigated by other researchers as the optimum strategy in controlling the growth of *B. cereus*. Sutherland et al.[239] examined the combined effects of ambient temperature, pH, salt, and CO_2 on the growth of *B. cereus* in carbohydrate based foods. Quintavalla and Parolari[193] also modeled the effects of pH, a_w, and temperature on the growth of *Bacillus* spp., isolated from bakery products. They reported a 12–15 day shelf life for products stored at 20°C and reformulated to pH 5.2 and a_w 0.93. Reformulation to pH 4.3 and a_w 0.92 extended product shelf life to 30 days at a similar storage temperature. However, the limitations of such models are that the data cannot always be extrapolated to the food, due to the complex nature of the food matrix.

4. *Clostridium botulinum*

a. *Sources of Contamination*

Foodborne botulism is a rare, potentially fatal neuromuscular illness that can result from the consumption of food in

which *C. botulinum* has grown and produced neurotoxin.[112,171] Infant botulism results when germinating spores of neurotoxigenic clostridia colonize the infant intestine and produce neurotoxin in situ.[46,74,75] Botulism has also resulted from similar colonization in people who are immunocompromised.

The most likely contaminants of bakery products are *C. botulinum* type A and B spores, since these are ubiquitously found in soil and in agricultural and animal products. However, few surveys have been carried out to determine the levels of *C. botulinum* in bakery ingredients or finished bakery products. While flour has been assumed to contain the occasional spore of *C. botulinum*,[87] there are no sound empirical data to confirm this assumption. However, spores have been detected in other ingredients, when appropriate methodology was used to detect this pathogen in foods. Dairy products, which are used in many bakery formulations, have an extremely low incidence of *C. botulinum* spores and are seldom implicated in outbreaks of foodborne botulism.[56] A recent survey of dairy foods in Italy found no spores of *C. botulinum* in raw, pasteurized or clotted milk, butter, pasteurized cream, or ricotta cheese. However, higher levels (<10 spores/g) were found in mozzarella, soft and processed cheeses, which are commonly used as pizza ingredients. Furthermore, 32% of the 1,017 samples of mascarpone cream cheese were contaminated with *C. botulinum* spores.[95] Cheese, previously involved in an outbreak of foodborne botulism,[15] also contained neurotoxin type A.[95] Fruits, vegetables, and spices may also be contaminated with *C. botulinum* spores. Outbreaks of foodborne botulism have been caused by a variety of fruits and vegetables (peppers, tomatoes, potatoes, mushrooms, onions, garlic, olives, peanuts, and hazelnut puree) used in the production of sweet and savory bakery foods.[114,174] Surveys of honey have shown levels of contamination of <1–10^1 spores/kg,[74] although higher levels (10^3–10^4 spores/kg) have been reported in honey associated with infant botulism.[113] For this reason, it is best to assess the botulism risk of each bakery product independently, based on the ingredients used in its formulation.

b. Associated Outbreaks

Although there have been no reported outbreaks of botulism involving bakery products in North America, several challenge studies have shown the potential of high moisture bakery products to support the growth of this pathogen. Spores of *C. botulinum* are heat resistant, with proteolytic spores having a reported $D_{100^\circ C}$ value of 25 minutes.[75] Since the internal temperature of bread during baking rarely reaches $>100^\circ$C, spores of proteolytic *C. botulinum* will survive baking[167,232] and have the potential to germinate, grow, and produce toxin in products stored under favorable conditions. Daifas et al.[65] showed that the heat treatment required to bake crumpets of acceptable quality represented only 1% of the treatment necessary for complete spore destruction. Whether spores are present in ingredients prior to baking or are incorporated into products post processing, through fillings or toppings or from environmental contamination, they have the potential to germinate, outgrow, and produce neurotoxin, if conditions are suitable.

Bakery products with a pH > 4.6 or an $a_w > 0.95$ can support growth of *C. botulinum*. Other conditions that may allow for growth and toxin in bakery products include gas packaging, which excludes oxygen, or vacuum packaging in cans or jars, or packaging in airtight conditions, in which spoilage organisms may consume residual headspace oxygen.[12,16,65,66] However, the inclusion of oxygen in the package atmosphere does not ensure the safety of MAP products. There is evidence that toxin may be formed in foods containing high levels of oxygen in the package headspace (20–100% v/v) and even in food stored aerobically.[53,80,228] Once germination is initiated, growth may be rapid, as the redox potential is rapidly reduced. Furthermore, pockets of food may be sufficiently anaerobic or have redox potentials conducive to germination and growth. Daifas et al.[66] have shown that *C. botulinum* produced neurotoxin in English-style crumpets at approximately the same time (4–6 days), when stored at ambient temperature, regardless of the packaging atmosphere. Subsequent studies showed that changes in the redox potential of bakery products may be more important for growth and toxin production by proteolytic strains of *C. botulinum* than the gas atmosphere surrounding the product.[68] While concern has been expressed about the safety of MAP bakery products, our studies have shown that high-a_w and high-pH air packaged bakery products could pose similar public health risks, if contaminated with *C. botulinum* spores and stored at ambient temperature.

The potential of bakery products in supporting the growth of proteolytic strains of *C. botulinum* has been well established in inoculation experiments.[83] Bever and Halvorson[24] also demonstrated that neurotoxin could be produced by *C. botulinum* in a medium of sterile bread crumbs containing calcium or sodium propionate at levels of 0.2–1.4%, when the pH of the medium was between 4.5 and 9.6. Concern was also expressed about the safety of canned bread intended for military rations.[232] Subsequent studies confirmed that canned bread could support growth and neurotoxin production and determined that the conditions necessary for the safe production of this product would be a pH < 5.4 and a moisture content of 35% (corresponding to an a_w of $\sim$0.95).[135,232,252] However, increasing the pH of the product resulted in toxigenesis. Growth and toxin production was observed in inoculated canned bread with a pH of 5.8 and a moisture content >36% and in canned steamed chocolate nut bread with a pH of 6.8 and 36% moisture.[255,256] Bread with a pH of 4.8 was determined to be safe, as no growth occurred, and the number of inoculated spores decreased during ambient storage in an erh of 97%.[127] However, the safety of the bread depended on maintaining its pH throughout storage, as there were still viable spores after six months. Temperature differences in the canned bread during freezing, thawing, or heating resulted in moisture migration and uneven moisture distribution in the bread.[259] The pH of thawed canned bread was unchanged, but the pH of the moisture that developed within the can was high enough (pH 5.5–6.7) to support the growth of *C. botulinum*.

These early studies demonstrated the need for multiple barriers into a food product to ensure its safety, particularly in products stored at ambient temperature. Bread is no longer canned for military rations, however, canned breads are commercially available in Japan and in South Africa.[160]

Home style quick breads, containing fruits and vegetables and baked in Mason jars, are also of public health concern. Such breads are available by mail order and are promoted in newspapers and on the Internet and are claimed to have a shelf life at room temperature for 6 months or longer. Aramouni et al.[12] demonstrated the survival of inoculated spores of *C. sporogenes* in canned home style banana bread. They concluded that further work was needed to determine the safe processing procedures for this type of product. Recent studies in conjunction with Health Canada have shown that pumpkin bread (a_w 0.973 pH 7.3) and zucchini bread (a_w 0.974, pH 8.1), packaged under a modified atmosphere, may be a potential hazard. When breads were challenged with proteolytic spores of *C. botulinum,* packaged with an oxygen absorbent and stored at room temperature, products became toxic within 28 days. However, *C. botulinum* failed to grow in cranberry bread (a_w 0.963, pH 5.3), packaged and stored under similar conditions (unpublished data).

More recently, English style crumpets, a high moisture snack food that has been implicated in several outbreaks of *B. cereus* in Australia, were investigated for their potential to support the growth of *C. botulinum*. Crumpets were inoculated with proteolytic strains of *C. botulinum*, packaged in various gas atmospheres (60% CO_2/40% N_2, in air, or in air with an oxygen absorbent), and stored at ambient temperature. *C. botulinum* increased from 2.6 to 7 log CFU/g and produced toxin in less than one week in all crumpets, regardless of the packaging atmosphere.[66] Sensory analysis indicated that crumpets were sensorially acceptable at the time of toxin detection[66]. When crumpets were packaged in 100% CO_2, toxin production was slightly delayed, regardless of the crumpet's pH (6.5 and 8.3); however, toxin production again preceded spoilage.[67] All of these challenge studies clearly demonstrate that many high moisture bakery products, depending on their a_w and pH, are ideal substrates for the growth of *C. botulinum*. Furthermore, if contaminated with spores of proteolytic *C. botulinum*, pre- or post-baking, they could grow and produce toxin, regardless of the packaging atmosphere surrounding the product.[66–68]

Fortunately, there have been no reported outbreaks of botulism caused by bakery products in North America. However, in India, a suspected outbreak of foodborne botulism, affecting 34 children, including three fatalities, was attributed to the growth and production of neurotoxin type E by *C. butryicum*, in sevu, a crisp flat bread prepared from gram (pulse) flour. Growth and neurotoxin production was attributed to the improper storage of the implicated crisp bread.[48] In Italy, an outbreak of botulism resulted from the consumption of tiramisu made with contaminated mascarpone cheese.[15] However, this botulism outbreak demonstrated that overt spoilage cannot always be regarded as a reliable barrier of food safety, since the tiramisu was described as "malodorous" at the time of consumption.[15] Clearly, more research is required on the safety, shelf life, and sensory characteristics of high moisture bakery products, particularly those packaged under modified atmospheres. Such information is critical in determining if spoilage precedes toxigenesis or vice versa and the margin of safety in such MAP products.

c. *Control Measures*

Although cases of foodborne botulism are rare, the severity of the intoxication and the potential for growth and toxin production by *C. botulinum*, particularly in high moisture MAP bakery products, may warrant the use of additional barriers to ensure the safety of these products stored at ambient temperature. Additional barriers that can be used alone or in combination with each other, to restrict the growth of *C. botulinum* in such products, are a_w, pH and preservatives. These traditional barriers have proved effective against the growth of *C. botulinum* and should ensure the safety of most high moisture MAP bakery products stored at ambient temperature.

The a_w of bakery products is one of the most important factors influencing the growth and toxin production of *C. botulinum.* Denny et al.[71] demonstrated the importance of a_w on the growth of proteolytic C. *botulinum*. In seven varieties of inoculated canned and low acid fruit and vegetable bread, stored for up to six years, toxin was produced at $a_w > 0.95$ but not at $a_w < .95$. *C. botulinum* did not grow or produce toxin in low acid apple coffee cake or spice cake with an a_w of 0.93, when inoculated with 10^5 spores/g and incubated at 30°C.[188] More recently, high moisture and low acid crumpets, pizza, and bagels were inoculated with 10^4 spores/g of *C. botulinum* and stored at ambient temperature. Crumpets (a_w 0.990) and pizza (a_w 0.960) became toxic, while bagels (a_w 0.944) did not.[65]

Another barrier that can be used to enhance the safety of bakery products is pH. Growth and toxin production was inhibited in cooked shelf-stable noodles acidified to a pH < 4.6. However, toxin production occurred in one sample of noodles in which the pH had increased as a result of microbiological growth, demonstrating the importance of incorporating multiple barriers in preventing the growth of *C. botulinum*.[126] Recent challenge studies have shown that the growth and toxin production of *C. botulinum* could be inhibited in high moisture crumpets stored at ambient temperature, if products were reformulated to pH 4.6, regardless of the packaging atmosphere (unpublished results).

Sorbates and propionates are used in bakery products to delay or prevent mold spoilage. However, propionic acid or its salts at permitted maximum levels (2000 ppm), were ineffective in preventing the toxin production of *C. botulinum* in canned bread (pH 5.4) or in sterile bread crumbs.[24,135] While higher levels of propionates (6%) may delay the growth of *C. botulinum,* such high levels, if legally permitted, would result in off flavors and the sensory rejection of baked products. However, since most bakery products, with the exception of sourdough, have pH values >5.0, chemical presevatives lose their antimicrobial effect, due to their low dissociation constants.

While ethanol is well known for its antimycotic effect, it has been investigated as a potential alternative barrier to pH and a_w reduction and chemical preservatives in inhibiting the growth of *C. botulinum*. Recent studies have confirmed the antibotulinal action of ethanol vapor in crumpets challenged with proteolytic strains of *C. botulinum* and packaged with ethanol vapor generators (Ethicap®), sizes 2, 4 and 6G.[68] Toxin was detected after 5 days in all English style crumpets inoculated with 500 spore/g or *C. botulinum* and packaged in air. Packaging with Ethicap® (2G) delayed toxin for 10 days at ambient temperature, while complete inhibition for 21 days was observed in all crumpets packaged with Ethicap® 4 or 6G. However, all crumpets were organoleptically unacceptable, due to their absorption of ethanol (>2% w/w) from the package headspace. Nevertheless, these initial studies have shown that ethanol, in the vapor phase, has the potential to enhance the safety of high moisture English style crumpets stored at ambient temperature.[68]

Other novel barriers that may warrant an investigation as antibotulinal barriers are biopreservatives (e.g., nisin), antimicrobial essential oils, (such as oil of mastic), and sorbic hydroxamic acid. These barriers both have proved to be effective in our laboratory in controlling the growth of *B. cereus,* a pathogen of concern in high moisture crumpets and English style crumpets.

Based on previous empirical data, high moisture MAP bakery products would need to be reformulated to an a_w of <0.95 to prevent the growth of *C. botulinum* and ensure their safety. However, reformulation to such low a_w may result in textural changes in the final baked product. To overcome these problems, bakery products of concern could be reformulated to a higher a_w and a lower pH. The combined use of these barriers (a_w 0.97, pH < 5.0) have proved effective in our studies in preventing the growth of spores of proteolytic *C. botulinum* in gas packaged crumpets stored at 25°C (unpublished results).

Ideally, the two recommended barriers (a_w, pH) should be incorporated into a HACCP program, and these two Critical Control Points (CCPs) should be monitored on a regular basis to ensure product safety. The level of concern in failing to control these CCPs must also be identified. MAP bakery products may be regarded, based on their safety history, to be of low or no concern, if these two CCPs (a_w and pH) are not controlled for the specific hazard of concern (*C. botulinum*). However, a good safety history does not always guarantee that this new generation of MAP bakery products, with extended shelf life, will not be involved in botulism outbreaks in the future. Therefore, these two recommended CCPs, if incorporated into high moisture MAP bakery products, should be monitored on a regular basis to ensure that they are always within specification. Such product control will ensure regulatory and consumer confidence in the safety of MAP products, at all stages of the distribution chain. It may also be prudent to restrict the shelf life of any MAP bakery product with a_w of 0.95 or greater in any component or to carry out appropriate challenge tests on the finished product to establish its safe shelf life with regard to *C. botulinum*.

5. *Other Microorganisms of Concern*

a. *Listeria monocytogenes*

Listeria monocytogenes is widespread in nature, occurring in soil, vegetation, water, and many animal and plant products.[161] Furthermore, it can grow over a wide pH, a_w, and temperature ranges. Outbreaks of listeriosis have resulted from consumption of soft cheese, cream, and butter.[159,163] Therefore, *L. monocytogenes* is of concern in bakery products containing dairy ingredients. *L. monocytogenes* is also classified as a psychrotrophic pathogen and can readily grow at refrigerated storage temperatures that cream, cheese or butter based iced or filled pastries, and cakes are stored and consumed without heating. Furthermore, since *L. monocytogenes* is ubiquitous in the bakery environment, post processing contamination of finished products is possible.

Gifford et al.[98] analyzed 28 bakery products for *L. monocytogenes*; however, no products were contaminated with this pathogen. In another survey of 300 pastries from 100 bakeries in France, Ferron and Michard[92] found that 14% of pastries were contaminated with *L. monocytogenes*. One sample contained 7×10^5 CFU/g, levels that can cause listeriosis. The authors concluded that the risk of listeriosis from pastry was at least equivalent to that of meat and delicatessen products.

Richter et al.[197] demonstrated that heating flour inoculated with *L. monocytogenes* for 5 minutes at 80°C was sufficient to destroy this pathogen. However, when inoculated, non-thermally treated flour was stored at room temperature. *L. monocytogenes* survived for at least two weeks. *L. monocytogenes* is also unlikely to survive boiling, in the preparation of custards used in certain filled products.

Strict hygiene and temperature control are essential to prevent contamination and growth of this pathogen. However, since temperature abuse is commonplace in the food industry, temperature alone is not an adequate barrier to ensure the safety of products contaminated with *L. monocytogenes*. Furthermore, it can grow under both low and high O_2 conditions, as well as in the presence of elevated levels of CO_2. Since *L. monocytogenes* can grow at low pH/a_w levels (~5.2, 0.92, respectively) product reformulation is not commercially viable, since it may result in sensorial and textural changes in the finished product.

b. *Mycotoxigenic Molds*

While bacteria are most commonly implicated in outbreaks of foodborne illness, molds, which often limit the shelf life of high and intermediate bakery products, can also be of public health concern. Although moldy bakery products will be rejected by consumers, molds may secrete mycotoxins into bakery products without visible signs of spoilage. Some molds, including *Alternaria, Aspergillus, Fusarium*, and *Penicillium* spp. may secrete one or more toxins, which may be teratogenic and carcinogenic.[241,254]

Mycotoxins have been found in many foods, including cereals and grain products, seeds, nuts, fruits, vegetables, and

dairy products.[166] In a survey of flour, Weidenborner et al.[260] found that *Aspergillus* species were the predominant isolates, with 93.3% of isolates being toxigenic. Increased mycotoxin levels in grains are influenced by climatic conditions (drought, rain) and improper storage. Abouzied et al.[3] surveyed 92 samples of grains from retail stores for aflatoxin B1, zearalenone, and deoxynivalenol (DON [vomitoxin]). Only one sample of buckwheat flour was positive for aflatoxin B1, while Zearalenone was found in 26% of samples. DON was found in 50% of samples at levels in excess of the FDA recommended level of 1 ppm. Furthermore, 88% of corn cereals, wheat flour, muffin mixes, and rice cereals tested positive for DON.[3]

Control measures include the proper storage of wheat to avoid moisture gain/moisture migration, thereby, preventing mold growth. However, outbreaks of mycotoxicosis have occurred from flour made from rain damaged wheat.[25] Antimycotic agents, such as propionates and sorbates, greatly reduce the risk of mold growth and mycotoxin production in bread.[156] Genetic engineering is also playing a role in the production of grains with increased resistance to DON producing molds.[37] Oxygen absorbent technology has also been used to control the growth and aflatoxin production of *Aspergillus flavus* and *Aspergillus parasicitcus*.[88]

c. Viruses

Norwalk-like viruses (NLVs), also known as small round-structured viruses, (SRSV) are ubiquitous in the environment and are highly resistant. They cause viral gastroenteritis illnesses from foods prepared or handled under unsanitary conditions.[116] An active carrier of NLVs can contaminate large quantities of food in a short time period. Therefore, outbreaks can involve a large number of cases. One outbreak in Minnesota, which involved 3000 cases, was attributed to the consumption of frosted bakery products.[148,164] Norwalk-like viral gastroenteritis, involving U.S. Army trainees in Texas, was associated with the consumption of contaminated crumb cake, pie, and rolls.[13] Another outbreak involving 250 cases of school children was associated with hamburger buns and cookies contaminated by symptomless excreters of the virus.[21] Custard slices prepared from dried custard mix and inadvertently prepared with contaminated water were associated with a large scale community outbreak of Norwalk virus.[34]

Another virus that can be transmitted from infected handlers to bakery products is hepatitis A. Outbreaks of Hepatitis A have been attributed to unbaked sherry trifle,[49] breads, rolls, and sandwiches[230] and pastries covered with glaze or icing, applied after baking.[207] More recently, a community outbreak in 1994 in New York was traced to an infected bakery worker, who contaminated cooked doughnuts while applying a sugar glaze.[262]

Reduction of viral transmission from infected bakery workers to food depends on education, good personal hygiene, and proper sanitation. Furthermore, all ill workers should be excluded from production areas and, if possible, food handlers should be immunized.

CONCLUSION

Mold growth and staling remain two of the major spoilage problems, limiting the shelf life of many intermediate and high moisture bakery products. The traditional approach to extend the mold free shelf life of these products is through the use of chemical preservatives. However, this approach has several limitations. Chemical preservatives are only effective in bakery products with a pH < 6.5. Furthermore, they are less effective in the presence of high number of mold spores and cannot replace good sanitation and cleanliness in the bakery environment. Consumer resistance to the use of chemical preservatives is also of concern to the bakery industry. While this latter issue may be addressed through the use of "natural" preservatives, such as raisin juice and oil extracts of certain spices, the levels required to inhibit mold growth may affect the organoleptic quality of the final product. To overcome the limitations of chemical preservatives, the bakery industry can now consider several emerging technologies to achieve a longer product shelf life. Gas packaging, O_2 absorbent technology, and pulsed light technology have all proved effective in extending the mold free shelf life of many packaged bakery products. While these technologies may delay mold growth in bakery products for several weeks at ambient temperature, they do not delay staling. However, advances in enzyme technology can now enable bakery products to remain stale free for several weeks at ambient temperature. Such enzyme systems could be used in conjunction with MAP/pulsed light to extend both the stale free and mold free shelf life of bakery products for several months. To date, however, few studies have examined the combined effects of such systems for the shelf life extension of bakery products. Such combination technology is necessary to obtain the desired product shelf life for today's global marketplace.

Despite improvements in bakery technology and manufacturing practices and stricter food hygiene and safety regulations, there has been no significant decrease in the number of reported foodborne illnesses each year. Bakery products have contributed to this trend for a number of reasons, including:

1. The production of bakery products with lower levels of traditional hurdles to microbiological growth (e.g., preservatives, salt, sugar) in response to consumer demands for low calorie preservative free "fresh" food.
2. The increased time between the preparation and consumption of bakery products particularly cream and meat filled products, which increase the potential of temperature abuse.
3. The increased globalization of sources of raw materials, food production and distribution, and the centralization of food operations.
4. The emerging pathogens and changes in mechanisms of transmission, infective doses and microbiological resistance to temperature, acid, and antimicrobial agents.
5. The increased surveillance and awareness of foodborne illnesses.[57,90]

6. The novel methods of packaging, such as modified atmosphere packaging, which may promote the growth of certain pathogenic bacteria.

While MAP technology can be used to extend the shelf life and to keep the quality of high moisture bakery products, there are regulatory concerns about the safety of these products with extended shelf life. A major microbiological hazard of concern in MAP high moisture bakery products is the growth and toxin production by proteolytic strains of *C. botulinum*. The botulism concerns of high moisture MAP bakery products are due to the ability of these spores, if present in flour or other ingredients, to survive the baking process; the anaerobic packaging conditions in the MAP products, which would be conducive to the growth of *C. botulinum*; and the inhibition of mold growth, which is the common indicator of spoilage in high moisture bakery products. Recent product recalls of MAP gluten free breads, containing potato flour and naan breads would appear to exacerbate these safety concerns.

High moisture bakery products have been implicated in several outbreaks of foodborne disease; however, they have been mainly caused by *Salmonella* spp., *S. aureus*, and *B. cereus*. While there are concerns about the safety of high moisture MAP bakery products with respect to *C. botulinum*, to our knowledge, there have been no reports of botulism in such products that have been on the marketplace for over twenty years. These regulatory concerns and recommendations for additional safety barriers appear to be based on either previous challenge studies with high moisture bakery products packaged in hermetically sealed containers and stored at ambient temperature or on predictive modeling studies. These challenge studies confirm that such packaged high moisture bakery products have the potential to support the growth of *C. botulinum* and may pose a safety risk, if contaminated with this pathogen. Predictive modeling studies in broth or solid media have also shown that the probability of toxin production is influenced by spore inoculum, a_w, and pH. However, these modeling studies have certain limitations regarding the safety of bakery products. The limitations in these studies are that they are done using pure cultures of *C. botulinum* spores and do not consider the background microflora of bakery products, and they do not consider the sensory aspects of a product to determine if toxigenesis preceded spoilage or vice-versa. Furthermore, a major limitation of all challenge and modeling studies to date is that they used high inoculum levels of *C. botulinum* (10^2–10^4 spores/g), i.e., levels that are unlikely to occur in bakery ingredients or final baked products. Based on these limitations, it is difficult to extrapolate these "worst case scenario" challenge and modeling studies to all high moisture MAP bakery products. It cannot be assumed, based on a limited number of challenge and modeling studies, that all high moisture MAP bakery products, if contaminated with spores or vegetative cells of *C. botulinum,* would support their growth. Farber[91] reported that *C. botulinum* type E produced toxin in synthetic media at 4 and 8°C, but not in crabmeat under the same experimental conditions. Furthermore, nitrogen packed hamburger sandwiches were capable of supporting the growth and toxin production of *C. botulinum* type E at 12°C, whereas turkey or sausage sandwiches were not.[91]

In conclusion, both food spoilage and food safety are complex issues involving many inter-related variables, including, a_w, pH and preservatives, microbial ecology of the food matrix, redox potential, packaging film permeability characteristics, gaseous atmosphere surrounding the product, and storage temperature. While new and emerging processing technologies may extend the shelf life of bakery products, they should not compromise product safety. Therefore, continuing research is required at the industry, government, and university levels on the contribution of these novel processing/packaging technologies, to both shelf life and safety of high moisture bakery products. When such empirical data are available, it will be possible to accurately determine the shelf life of such products with extended shelf life and to make realistic guidelines concerning the levels of additional barriers required, if any, to ensure their continued safety at ambient storage temperature.

Continuing education in food hygiene and safety for bakery personnel, food handlers, and, indeed, the general public is also a critical component in the battle against foodborne disease. This can be achieved through training courses, programs, such as FightBAC®, education through videos, newspaper and magazine, articles, and the internet. Implementation of a HACCP program is also essential to minimize contamination and the spread of foodborne illnesses in high moisture bakery products. Newer technologies, such as the rapid screening methods of pathogens, time/temperature indicators to monitor temperature abuse of refrigerated bakery products, and smart films that change color as a function of food spoilage could also be used as aids to facilitate HACCP and ensure the safety of high risk bakery products with extended shelf life.

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