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AN OVERVIEW OF STERILIZATION METHODS FOR PACKAGING MATERIALS USED IN ASEPTIC PACKAGING SYSTEMS

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In aseptic packaging systems, packaging materials are sterilized by various methods in order to kill microorganisms contained in the packages during forming and transport through the machine prior to filling. Experimental data as well as theoretical results from several years of research in the area of sterilization methods for effective inactivation of microorganisms on surfaces of aseptic packaging materials are compiled and presented in order to choose the right method of sterilization by the food processing industry for a successful operation. Hydrogen peroxide, with concentrations up to 30%, temperatures of up to 80°C and contact times up to 15 s, with or without wetting agent, has been found to be successful for in-line aseptic packaging. The final product must not contain greater than 0.5 ppm H_2O_2. Economic considerations and non-uniform dose delivery to pre-formed containers inhibit commercial adoption of ionizing radiation sterilization in-line with aseptic packaging systems.

Keywords: aseptic packaging; sterilization; thermal processes; radiation; light-pulse; chemical methods.

INTRODUCTION

Packaging plays an important role in the food manufacturing process. It makes food more convenient and gives the food greater safety assurance from microorganisms and biological and chemical changes such that the packed foods can have longer shelf life. In order to meet the huge demand of processed food with longer shelf life, various new methods of packaging are being used in the food processing industry. Aseptic packaging is one of them. Aseptic packaging involves the filling and sealing of microbiologically stable (i.e. commercially sterile) product into sterilized containers under conditions that prevent microbial recontamination of the product, the containers, and their closures (i.e. under aseptic condition). It is a technology of continuous food sterilization and packaging that is of considerable current interest to the US and European food industries. A typical flow diagram is illustrated in Figure 1. In aseptic packaging, raw or unprocessed product is heated, sterilized by holding at high temperature for a predetermined amount of time, then cooled and delivered to a packaging unit for packaging, while packaging material and equipment surface may be sterilized by various methods such as heat, hydrogen peroxide, irradiation, infrared light etc. and combinations of methods. Some of these methods are given in Table 1. Aseptic packaging can be used to package a wide range of products such as milk, juice, drinks, concentrates, wine, tea, mineral water, nutritional beverages, sauces, dairy and tomato products. Aseptic packaging offers advantages to the consumer as well as to distribution channels (lower distribution and storage costs, extended shelf life, relief of pressure on chilled cabinet, cost effectiveness and freedom from additives).

Sterilization of packaging material is a critical step in the aseptic packaging system. Therefore, the sterilization process should meet the following requirements for sterilization of packaging materials:

- rapid microbicidal activity;
- compatibility with surfaces treated, especially packaging material and equipment;
- easily removed from surface, minimum residue;
- present no health hazard to the consumer;
- no adverse effect on product quality in the case of unavoidable residue or erroneous high concentration;
- present no health hazard to operation personnel around the packaging equipment;
- compatibility with environment;
- non-corrosive to surfaces treated;
- reliable and economical.

When using sterilants that do not leave any residue on the food contact surface, the Food and Drug Administration (FDA) considers sterilization as a process, which is regulated only when used on low acid foods (Code of Federal Regulations, 1986, Title 21, Part 113). However, when plastic packaging materials and chemical sterilants are used, the process is regulated as an indirect additive to food (Code of Federal Regulations, 1986, Title 21, Part 174). The process of obtaining FDA approval for the use of chemical sterilants in food packaging has eased considerably since the concept was introduced in the early 1970s. Scientific evidence for process adequacy must be filed by

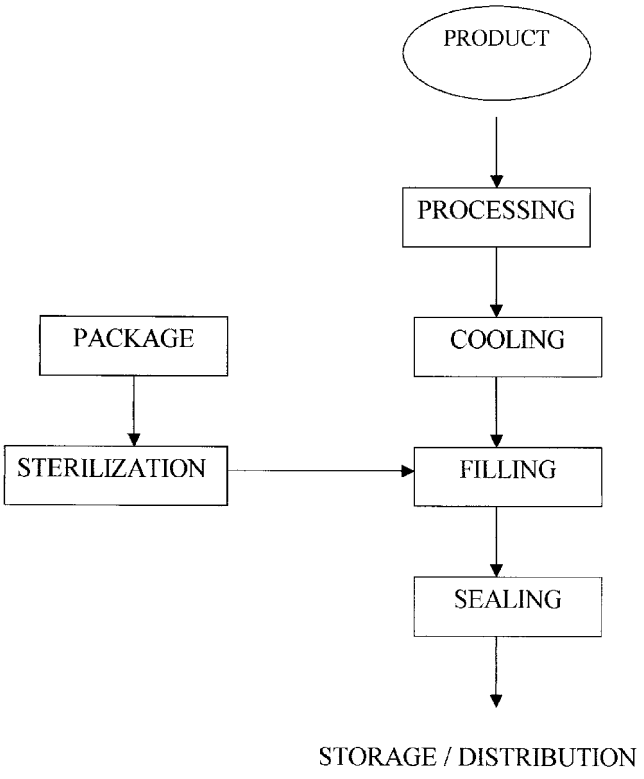

Figure 1. Schematic flow diagram of aseptic packaging system.

processors before a system is placed into commercial operation. For successful operation of aseptic packaging systems, parameters for process adequacy can be effectively designed. A major concern in aseptic packaging of low-acid foods (pH > 4.6) is the assurance of microbial safety in the final product, which supports the growth of *Clostridium botulinum.*

Hydrogen peroxide is now the only chemical sterilant for sterilization of packaging materials that has been proved to be acceptable in the USA. The FDA regulations specify that a maximum concentration of 35% H_2O_2 may be used for sterilizing food contact surfaces. In a properly designed aseptic packaging system a good microbicidal effect using hydrogen peroxide can be achieved and the level of residue can be effectively controlled to within permissible limits. The final product must not contain greater than 0.5 ppm H_2O_2. Initially, the use of hydrogen peroxide as a sterilant for packaging material that directly contacts food was approved only for polyethylene [*Federal Register*, 1981, 46(6), 2341]. The approval was extended to include all polyolefins in March 1984 (Code of Federal Regulations, 1984, Title 21, Part 178, 1005), and in 1985 approval was extended to include polystyrene, modified polystyrene, ionomeric resins, ethylene methyl acrylate copolymer resin, ethylene vinyl acetate copolymer resin and polyethylene tetrapthalate (Code of Federal Regulations, 1986, Title 21, Part 178, 1005). In January 1987 approval was extended to include ethylene acrylic acid copolymers [*Food Chemical News* 1987, 28(44), 28]. The first successful aseptic filling system for cartoning, the aseptic Tetra Pak of 1961, used a combination of hydrogen peroxide and heat for the sterilization of the surface of container material (Burton, 1988).

Development of acceptable sterilization methods suitable for different types of packaging materials and consumer demand for good quality microbiologically safe, shelf-stable food, encouraged commercial adoption of aseptic packaging systems. The present work is a critical review of literature on currently used and new methods for in-line sterilization of packaging materials mentioned in the scientific and patent literatures. The limited body of literature directly related to the effects of sterilization methods on product quality and storage stability also has been discussed.

STERILIZATION METHODS FOR PACKAGING MATERIALS AND EQUIPMENT

Various methods for the sterilization of packaging materials are currently used in aseptic packaging systems. The sterilization process employed should be established in terms of numbers of log cycle reductions of the most resistant organisms. Packaging material is usually sterilized either inside the packaging machine or externally and introduced aseptically into the aseptic zone of the packaging machine. Many aseptic packaging systems utilize plastics,

Table 1. Methods for sterilizing aseptic packages.

Methods	Application	Advantages/disadvantages	Reference
Superheated steam	Metal containers	High temperature at atmospheric pressure. Microorganisms are more resistant than in saturated steam	Collier and Townsend (1956)
Dry hot air	Metal or composite juice and beverage containers	High temperature at atmospheric pressure. Microorganisms are more resistant than in saturated steam	Denny and Mathys (1975)
Hot hydrogen peroxide	Plastic containers, laminated foil	Fast and efficient method	Denny *et al.* (1974)
Hydrogen peroxide/UV light combination	Plastic containers (pre-formed cartons)	UV increases effectiveness of hydrogen peroxide	Bayliss and Waites (1982)
Ethylene oxide	Glass and plastic containers	Cannot be used where chlorides are present or where residuals would remain	Blake and Stumbo (1970)
Heat from co-extrusion process	Plastic containers	No chemicals used	—
Radiation	Heat-sensitive plastic containers	Can be used to sterilize heat-sensitive packaging materials. Expensive. Problems with location of radiation source	—

paperboard or foil containers to pack the sterilized product and sterilize by physical methods and chemical reagent.

Thermal Processes

Microorganism inactivation has traditionally been accomplished by heating. Microorganisms, especially spores, show greater thermal resistance when exposed to dry heat than moist heat. Spores of *Bacillus stearothermophilus* are extremely resistant to heat (Russell, 1982). Spores of *Bacillus subtilis* ATCC 9372 are used as indicators in dry heat (Anonymous, 1995) and these spores are used commercially in sterility testing of aseptic fillers (e.g. the spore-strip kit of North American Science Associates, Northwood, OH, USA). *D*-values (the time required to reduce the number of microorganisms by one log cycle) and *Z*-values (the temperature rise necessary to reduce the *D*-value by a factor of 10) are significantly higher for dry heat sterilization than for steam sterilization. Dry heat produces microbial death as a result of dehydration followed by protein oxidation. Death by moist heat is caused by denaturation and coagulation of essential cell proteins (Parrott, 1970). Reaction velocity for their destruction depends on how rapidly the heat from the thermal carrier can be transferred to the cell. When heat is used, the nature of the surface must be considered. Plastics or carton packaging with their low conductivity are more difficult to thermally sterilize than are metal containers. In addition, plastic materials generally have a low thermal stability and can be permanently deformed by the time/temperature sequences necessary to achieve sterilization. Thermal processes do not deposit any hazardous or undesirable residues on the surface being treated and do not present environmental hazards.

Saturated steam

Saturated steam is a form of moist heat. Sterilization of metal cans and lids by saturated steam under pressure was used as early as 1920 in the USA (Reuter, 1988) and is used today for sterilizing thermostable plastic cups. To attain adequate destruction in the short time available in high-speed packaging units, the surface temperature of the material must reach 135°C. The technological problem is to obtain a sufficiently high surface temperature to achieve the required sterility in a time consistent with high production rates and to avoid softening and deformation of the material. Moulded polystyrene cups and foil lids are subjected to saturated steam at 165°C and 6 bars immediately after deep drawing. At the same time the external cup surface is cooled to limit the effect of the short time heat application on the material. It should be noted that *Bacillus subtilis* is not generally recommended for validation of steam sterilization (Larousse and Brown, 1997). Polypropylene cups that have higher thermal tolerance can be treated on surfaces at lower temperatures and longer times, 140–147°C for 4–6 s, in a pressure chamber. Lid foil can be treated continuously by passage through a pressure lock. Moist heat could cause blistering or delamination of paper-based packaging materials and impair the heat-sealing characteristics of plastics. Atmospheric steam can only be used on non-paper-based pre-formed containers. Saturated steam is a preferred treatment method for sterilizing metal food contact surfaces downstream from the hold tube, including sterile hold tank, homogenizers, fillers and the aseptic packaging zone. Equipment sterilization can be attained by exposure of the surfaces to an appropriate time/temperature sequence (e.g. 30 min at a surface temperature of at least 121°C) by superheated water, saturated steam, superheated steam or other appropriate treatments.

Superheated steam and hot air

Dry heat delivered by superheated steam or hot air is not as effective as moist heat at the same temperature for sterilization, therefore higher temperatures are required. The Martin–Dole process continuously sterilizes tinplate cans by passage through 220–256°C-superheated steams at normal pressure for 45 s (Larousse and Brown, 1997). When paper-based packaging materials are used, hot air is preferred as a sterilant over superheated steam. Combi-cans of cardboard laminates (i.e. aluminium and paper) are sterilized in hot air at 145°C for 3 min (Reuter, 1988). This method has only been found suitable for products that have a pH of <4.6.

When sterilizing by hot air or superheated steam, there is no effect on packaging material quality. Furthermore, the temperature within the entire aseptic filling chamber may be maintained at a level lethal to microorganisms, thus assuring asepticity. The high temperature of the headspace gas at the time of sealing also reduces the level of oxygen in the sealed package and can contribute towards prolonging product shelf life. Hot air sterilization units are commercially available for juice and beverages. Dry heat sterilization is slow and not suitable for heat-sensitive materials like many plastic items.

Heating by extrusion

Aseptic packaging system utilizing heat coextrusion process for sterilization is a form–fill–seal packaging system that relies on the temperatures reached by thermoplastic resin, during the co-extrusion process used to produce multi-layer packaging material, to produce a sterile product contact surface. Temperatures of 180–230°C can be reached during the extrusion of granulated plastics as a pretreatment for a subsequent blow moulding operation. The high temperature produced by extrusion produces sterile surfaces. During production, the multi-layer package material is fed into the machine where it is delaminated under sterile conditions. This removes a layer of material and exposes the sterile product contact surface. The container material is then thermoformed into cups. The lid material that is also delaminated is then sealed onto the cup after filling. The sterility of the forming, filling and sealing areas is maintained by sterile air under positive pressures. Reports as to the efficacy of this heating method (Reuter, 1988) vary considerably. Therefore, aseptic filling into extruded containers appears only suitable for acidic products with pH less than 4.6. For products with pH greater than 4.6, extruded containers should be post-sterilized with hydrogen peroxide or with mixtures of peracetic acid. It would be prudent to validate any application of this method on a case-by-case basis.

Radiation

When neither heat nor chemicals can be used to sterilize a given material, radiation is considered as an option.

Electromagnetic radiation characterized by a frequency, a wavelength, penetrating power and an energy range are infrared, ultraviolet, γ rays, etc. The dosage and type of radiation sterilization is a function of the type and amount of the microbial load that has to be removed from a given materials and equipment. As irradiation does not leave any residue on the treated surface nor affect the immediate environment, it appears at first sight to be a perfect solution for sterilization of packaging material. The products used for packaging in radiation sterilization recommended by the International Atomic Energy Agency (IAEA) are polyethylene, polyester, polypropylene, nylon, PVC, etc. Irradiation may indirectly affect chemical or biological properties, leading to interaction with the packaged product, changing its efficacy or usability.

UV ray

UV irradiation has been successfully applied to the sterilization of air and of water which is free from suspended matter. With the development of more powerful sources of UV radiation (Bachmann, 1975), attempts have been made to use it for surface sterilization. The microbicidal effects of UV rays lies in the range of 200–315 nm, with an optimal effect being between 250 and 280 nm, the so-called UV-C range. UV light at a wavelength of 253.7 nm is an effective germicide against yeast, moulds, bacteria, viruses and algae. The lethal irradiation dose is defined as the product of irradiation intensity and time and expressed in terms of milli Watts second per square centimeter ($mW\,s\,cm^{-2}$). High intensity sources of UV radiation are very effective in killing unprotected microorganisms suspended in air or on the surface of materials and products. Data published by Bachmann (1975) on dose levels required to inactivate spores of moulds and bacteria indicated excellent potential of the process for sterilizing aseptic packaging materials. Maunder (1977) suggested the use of high-intensity UV for aseptic packaging material sterilization. Both vegetative cells and spores are sensitive to UV radiation. Microbial resistance to UV radiation increases in the order: vegetative bacterial cells $\ll$ yeasts $<$ bacterial spores $<$ mould spores. Gram-positive bacteria require twice the dosage of Gram-negative bacteria, and 5–10 times for bacterial spores. Mould spores, particularly those that have dark colours (e.g. *Aspergillus niger*) are especially resistant and require dosage levels 20–100 times higher (Wallhauser, 1978). Covered, shaded or shielded organisms will not be affected. Dust particles present on the surfaces reduce the effectiveness of UV irradiation for sterilization of aseptic packaging materials. The penetration ability of UV rays is very limited, as shown in Table 2.

A typical UV radiation intensity is between 0.5 and $1.5\,W\,cm^{-2}$ at a distance of 10 cm. Laboratory tests using an energy density of $30\,mW\,cm^{-2}$ on artificially contaminated smooth, even, dust-free surfaces and an irradiation time of 4–6 s showed a 4–5 log cycle reduction (Cerny, 1977). Kodera (1983) obtained a patent for both the method (UV light plus organic acid or hot water) and apparatus for sterilizing packaging film. The reported advantage of this method is that organic acids and water are not toxic. UV irradiation and a citric acid solution resulted in approximately a six-log cycle inactivation of *Bacillus subtilis* spores within 5 s. Doyen (1973) described an aseptic packaging machine that used both an alcohol bath and high intensity UV radiation for sterilization of flexible plastic pouches. The film was washed in 95% ethanol for 20 s and then passed under a flow of $10\,mW\,cm^{-2}$ of UV for 30 s. The rate of microbial reduction in the sterilization of smooth surface plastic cups and lids by UV irradiation at a constant radiator output depends upon a number of factors (Cerny, 1977). UV irradiation under practical conditions only always count reduction of 2–3 log, and mould spores can be particularly resistant. This low count reduction is insufficient for aseptic packaging; the process is therefore merely suitable for a filling and packaging process with a relatively low contamination count.

Table 2. Penetration of UV light.

Substrate	Distance that UV will penetrate (cm)
Air	300–500
Water	30
Window glass	<0.1
Transparent plastic foil	<0.01
Fruit juice	0.1
Fluid milk	0.01

Combination of hydrogen peroxide with UV

The ability of UV and hydrogen peroxide to inactivate bacteria has been well known for a long time. The process of simultaneously applying hydrogen peroxide solution and UV will provide adequate sporicidal activity to relatively low concentrations of hydrogen peroxide solution, minimizing the problem of removal of residues after sterilization. The Liquid-Pak System uses this effect with a carton forming, filling and closing system in a sterile chamber. Many researchers have considered sterilization of packaging materials using peroxide and UV irradiation. It has been found that the lethal action of peroxide solutions with and without the application of heat is increased by simultaneous UV irradiation, and that the overall lethal effect is greater than the sum of the effects of the peroxide and irradiation alone (Bayliss and Waites, 1979a, 1982). They subsequently demonstrated the same type of synergy with a variety of *Bacillus* and *Clostridium* (Bayliss and Waites, 1979b), but found that mild heat was also needed for effective killing of more resistant spores tested. Higher count reduction can be obtained by prior treatment of the surface with cold hydrogen peroxide and by ensuring that the surface is dust free (Bernard, 1983; Cerny, 1977; Ito and Stevenson, 1984; Wainess, 1982). The effect is optimum at a relatively low peroxide concentration, between 0.5 and 5% and at higher concentration the peroxide appears to have a protective effect, and the greater the UV intensity the higher is the optimum peroxide concentration. Four decimal reductions of *Bacillus subtilis* spores of a strain very resistant to H_2O_2 were easily obtained by irradiation with an UV dose of $1.8\,W\,cm^{-2}$ in 2.5% peroxide followed by a short heat treatment (Bayliss and Waites, 1982). Subsequent heating to 80°C increased the kill further. Maunder (1977) used a conventional UV lamp to sterilize pouches; also with the advent of high intensity UV-C lamp (Brandli, 1975), very high lethal effects have been reported; up to 5 decimal reductions on flat board (Cerny, 1977) and 7 decimal reduction for *Bacillus* spores on inoculated strips placed into food packaging cartons (Sturm and Gilliand, 1974).

Decontamination of pre-formed cartons for aseptic packaging poses problems which are not encountered with flat surfaces. Therefore, UV irradiation of a pre-formed carton must be considerably more intense than for flat boards to ensure that all surfaces are effectively irradiated (Bachmann, 1975). Experiments with cartons artificially contaminated with *Bacillus subtilis* spores, sprayed with 1% H_2O_2 and then irradiated for 10 s with a high-intensity UV source above the carton, showed 5 decimal reductions with polyethylene lined material and 3.5 decimal reductions with a polyethylene/aluminium foil laminate (Stannard *et al.*, 1983). No heat was applied. This combination of hydrogen peroxide with UV has now been applied commercially to carton sterilization during aseptic filling.

Infrared ray

Infrared rays in the waveband $0.8–15 \times 10^{-6}$ are easily generated. On an absorbent surface they are converted into sensible heat, producing an increased temperature. The energy impact on the surface that is convertible into heat is dependent on the same geometric conditions as those outlined for UV rays. Thus, infrared irradiation is best applied to a smooth even surface with vertical radiation incline. This method has been used to treat the interior of aluminium lids with a plastic coating on the exterior surface. As the temperature rise can cause a softening of the plastic, the maximum temperature and exposure time are limited. Count reductions on the same order as those for UV irradiation has been found.

Ionizing ray

Ionizing radiation is an excellent sterilizing agent and penetrates deep into objects. Ionizing radiation of primary interest includes α-rays, X-rays, β-rays, electron beams and γ-rays. Bags used in bag-in-box aseptic packaging are currently sterilized with γ-rays from a ^{60}Co source. Radiation kills bacteria in the same logarithmic fashion as heat, and *D*-values for many organisms have been reported. A dose of 4.7 Mrad is considered a 12*D* level for *Clostridium botulinum*, which are the most radiation-resistant spores of public health and spoilage significance (Urbain, 1978). The effect of water activity on irradiation by ^{60}Co γ-rays was investigated by Harmulv and Snygg (1973). Dosage levels to inactivate 90% of *Bacillus subtilis* spores increased from 0.23 to 0.42 Mrad as the water activity (a_w) decreased from 1.00 to 0.00. For a 99.9% reduction (3 log) of *Bacillus stearothermophilus* spores, the dosage increased from 1.7 Mrad at an a_w of 1.00 to 3.00 Mrad at 0.00.

Gamma irradiation has been used to sterilize plastic bags for bulk packaging acid foods using an aseptic bag-in-box system (Nelson, 1984). The bag made of plastic laminates are treated in a specialized irradiation plant, and given a radiation dose of 25 kGy (2.5 Mrad) or more, which is sufficient to ensure sterility. However, the difficulty of maintaining sterility during the transfer operation to the filling spout of an aseptic filling machine, raises some doubts as to the practicality of gamma irradiation sterilized packaging materials for use on low-acid foods. The heavy shielding necessary to prevent radiation leakage from gamma ray source makes the system impractical to use for in-line sterilization. Gamma radiation has not yet been widely employed in the USA because of the cost and concerns about the effects of γ-radiation on food.

Electron beam sterilization of packaging material is a proven, state-of-the art technology, which outperforms any legislative and customer requirements. Studies have shown that depending on the level of microorganisms, a dose of 5–7 kGy was found to be effective against yeast, mould and spores and no viable microorganism was found after treatment. Unfavourable effects of electron beam treatment were not observed. High-energy electron beams are not as penetrating as γ-rays, and therefore do not require as much shielding as a γ-ray sources. The irradiation process takes just a few seconds and does not leave any type of residue. However, with pre-formed containers, the geometry of the delivery system makes it difficult to apply a uniform dose on the whole container. In-line processing, heavily demanded by industry, requires a careful process design. Technological and economic optimization of electron beam source and shielding are the most important tasks to be accomplished before in-line electron decontamination of food packaging may be applied on a large scale. A major impediment to adoption of this technology in the food industry is the high initial and operating cost of the units.

Care must be used when using ionizing radiation for sterilization of aseptic packaging materials. Although aluminium foil is not affected by irradiation, paper and plastic could be affected, with results ranging from discolouration to loss of pliability and mechanical strength. While radiation can be quite effective, the equipment is expensive, as is the protection for workers and the environment. Workers are not usually willing to work in such an environment. It will probably be more extensively employed in the future.

Light-pulse

The technology of utilizing short pulses of light is an attractive alternative for sterilizing packaging materials and processing equipment in aseptic packaging. The spectrum of light used for sterilization purposes includes wavelengths in the ultraviolet to wavelengths in the near-infrared region. The material to be sterilized is exposed to at least one pulse of light having an energy density in the range of about 0.01–50 J cm^{-2} at the surface. The duration (Anonymous, 1994) of pulses ranges from 1 μs to 0.1 s. The packaging materials are typically exposed to between 1 and 20 pulses of light-intensity, short-duration light. A few flashes, applied at a rate of 1–10 per second, provide very high microbial kill levels, making the system ideal for continuous in-line sterilization. It is desirable for certain aseptic processes that packaging material be treated with pulses having a relatively high UV content to minimize the total fluence (J cm^{-2}) necessary to achieve the desired reduction in microbial population (Dunn *et al.*, 1991). Comparison of the antimicrobial effects obtained using pulsed light with those obtained using non-pulsed or continuous wave conventional UV sources shows a significantly higher inactivation for pulsed light. More than 7 log cycles of *Aspergillus niger* spore inactivation result with few pulsed light flashes. A variety of microorganisms including *Bacillus subtilis* are inactivated by using between 1 and 35 pulses of light with intensity ranging between about 1 and 12 J cm^{-2}. Greater inactivation is obtained when full-spectrum light rather than glass-filtered spectrum light is used. Thus, the UV component of light is needed to inactivate microorganisms (Dunn *et al.*, 1991). Spores of *Bacillus*

subtilis, *Bacillus pumilus*, *Bacillus stearothermophilus* and *Aspergillus niger* were inactivated completely from 6–8 logs of colony forming units (CFU) with 1–3 pulses (Bushnell *et al.*, 1998). *Bacillus subtilis*, for example, is sterilized (99.999%) by about 42,600 $\mu W\,s\,cm^{-2}$ of UV while requiring a dose of only 4500 $\mu W\,s\,cm^{-2}$ under pulsed light. Microorganisms are inactivated by a combination of photochemical and photothermal mechanisms.

Light pulses apparently do not affect the nutrient retention in food, although a detailed study is not available. Pulsed light treatment costs are very favourable (Dunn *et al.*, 1995). Packaging materials compatible with this process will transmit light over the broad spectrum employed. LLDPE, LDPE, nylon, Aclar, HDPE and PP have all been used. Package geometry should not allow any shadowing on the product or the light exposure may not be sufficient.

Chemical Methods

Thermal destruction is by far the most common and efficient mode of sterilization. Dry or moist heat for sterilization is also not always feasible. Chemical methods use a wide variety of chemicals in the form of liquids and gases to disinfect and sterilize equipment and packaging materials.

Hydrogen peroxide

Hydrogen peroxide is one of the most widely used sterilants used for sterilizing packaging materials. The first successful aseptic filling system for cartoning the aseptic Tetra Pak of 1961 used a combination of hydrogen peroxide and heat for the sterilization of the surface of container material (Burton, 1988). Many aseptic packaging systems use hydrogen peroxide at concentrations from 30 to 35% as a sterilant for packaging materials and other food contact surfaces followed by hot air (60–125°C) to augment the sterilizing effect and to dissipate residual hydrogen peroxide. Sterilizing performance increases with both peroxide concentration and temperature. At ambient environmental temperatures, the activity of hydrogen peroxide is relatively slow; however, this can be increased considerably by raising the temperature to 85–90°C and/or increasing concentration (Smith and Brown, 1980; Swartling and Lindgren, 1968). Therefore for effective sporicidal action in food processing environment, treated with hydrogen peroxide (at 30%) is followed by application of hot air (Yokoyama, 1990). Swartling and Lindgren (1968) found that four decimal reductions were obtained after suspension in 20% at 80°C for 15 s. Ito and Stevenson (1984) state that the challenge organisms of choice are *Bacillus stearothermophilus* for aseptic packaging systems that use heat sterilization and strains of *Bacillus subtilis A* or *Bacillus subtilis* var. *globigii* for filler surfaces that sterilize with hydrogen peroxide and heated air.

The following relationship holds for destruction of spores of *Bacillus stearothermophilus* (Shapton and Shapton, 1998):

$$\log D = 17 - 1.3 * \log(\%\ H_2O_2)$$

where D is the decimal reduction time in minutes.

Spores resistant to hydrogen peroxide have been reviewed by Ito (1973), and Stevenson and Shafer (1983). Toledo *et al.* (1973) reported that dry spores of *Bacillus subtilis* var. *globigii* (NCIB 8058) were less resistant than the wet spores. Consistent with observations for heat and irradiation, dry spores are more resistant than wet spores. Since sterilization processes used on aseptic packaging systems for low-acid foods must have documentation for adequacy to inactivate pathogenic and spoilage microorganisms, a unit must be tested using an acceptably resistant organism before it can be placed in commercial production. In commercial aseptic packaging equipment which uses roll stock, the packaging material is immersed in hydrogen peroxide solution followed by heating to vaporize the peroxide before the packages are filled. Contact time with the solutions, which contain a wetting agent, is often less than 1 min. A large amount of the sterilizing liquid is removed mechanically, e.g. by rollers or air blasts, and the remainder is generally removed by drying with hot or sterile air or radiant heat. The nascent oxygen thus produced due to breakdown of peroxide reacts with the oxidizable cell components of the microorganisms and causes bactericidal effect. Commercial packaging systems employ hydrogen peroxide at concentrations of 10–35% at either room or elevated temperature (Von Bockelmann, 1974; Von Bockelmann and Von Bockelmann, 1972) in the form of a spray or immersion bath. The sporicidal action of hydrogen peroxide also has been reviewed by Von Bockelmann and Von Bockelman (1972). Hydrogen peroxide kills a wide variety of organisms (Wang and Toledo, 1986) and viruii (Mental and Schmidt, 1973). Both linear (Swartling and Lindgren, 1968; Toledo *et al.*, 1973) and nonlinear (Cerf and Hermier, 1972) relationships between log number of survivors and time of exposure to different concentrations of hydrogen peroxide have been reported and Toledo *et al.*'s (1973) data show that 1 min in 30% hydrogen peroxide at ambient temperature produced only 2.5 decimal reductions of mould spores and bacterial spores were not affected at all. Thus, in current commercial practice, the microbicidal action is primarily due to the heated film of hydrogen peroxide at the start of the drying phase of the sterilization process. For this reason, wetting of the packaging material and the presence of a uniform film of liquid on the material surface are critical factors.

When sterilizing pre-formed containers, hydrogen peroxide is sprayed or atomized into the container. A measured amount of hydrogen peroxide is metred into each nozzle which delivers the solution into each container to ensure that a uniform film coats the inside surface of the package. For acid food products, relatively low microbial lethality required makes it easy to achieve the desired level of microbial inactivation and meet the residue tolerance level. However, for low-acid foods, a system must be carefully designed such that both criteria of commercial sterility and adequate sterilant removal are achieved.

Hydrogen peroxide-sterilized aseptic packaging systems that have been approved for commercial packaging of low-acid foods have been proven to produce an acceptable level of residues. The Food and Drug administration of the USA has ruled that the level of hydrogen peroxide that may be present in product packaged in material sterilized by hydrogen peroxide must not be greater than 0.5 ppm (Reuter, 1988). An improperly designed system may result in more incidence of non-compliance with regulations pertaining to residues. Prolonged contact between liquid hydrogen peroxide and the packaging material also increased the difficulty of removing residues.

Hydrogen peroxide is frequently used to sterilize the packaging material as it decomposes to a product which will neither taint the food nor render it toxic. It is reported that the traces of residual of hydrogen peroxide left in food is less than 0.25 ppm and normally this amount does not have any harmful effect when consumed (Hedrick, 1973; Smith and Brown, 1980). Residual hydrogen peroxide and that trapped in the package headspace at the time of sealing has an adverse effect on product stability particularly on ascorbic acid degradation in fruit juices (Toledo, 1986). When the concentration of hydrogen peroxide at the time of packaging exceeded $0.1\,\mu g/ml^{-1}$ a marked increase in ascorbic acid degradation in bottled orange juice and orange juice concentrates was observed by Toledo (1975, 1986). Aseptic packaging of foods containing ascorbic acid in a system that uses hydrogen peroxide for sterilization of material is not desirable, unless some means is used to remove hydrogen peroxide vapours from the package head space and packaging material is completely free from hydrogen peroxide residues.

There are three commercial methods for applying hydrogen peroxide to packaging materials: dipping, spraying, and rinsing.

Dipping: the packaging material (i.e. plastic laminates with cardboard, films of thermoformable plastics and laminates) are taken from a reel and dipped into a bath of aqueous 30–33% hydrogen peroxide (Reuter, 1988). Count reductions on the order of 4–5 logs are claimed. Wetting agents are added to ensure uniform wetting of the surfaces. Excess solution is removed by squeeze rolls or air jets after removal of the material from the bath, which leaves a thin film of solution that is then dried by the application of hot air. To increase the efficacy, especially in the case of dusty or slightly soiled material, prior treatment of the material with rotating brushes, sterile compressed air jets or ultrasound applied to the bath may be added.

Spraying: this is the method (Reuter, 1988) for preformed containers in which the hydrogen peroxide is sprayed in the form of small dispersed droplets into the container. Spraying does not result in a cohesive film due to the hydrophobic characteristics of plastics; rather only 30–40% of the inner surfaces of the container are covered and thereby it does not ensure good sterility of film. A conventional spray gives drops of over 30 μm diameters on the surface, and only 30–40% of the surface area is covered. An ultrasonic system can be used to give particle sizes of only 3 μm diameter, which will give an average surface cover of about 60%. The droplet size distribution, between 2 and 80 μm, and the distribution of droplets on the inner surfaces of the various geometric shapes of the containers is problematical. The efficacy is dependent upon the volume of solution sprayed; however, the larger the volume, the longer the drying time. The drying must be carried out with hot sterile air. Improved aerosol sprayers can limit the droplet size to 2–4 μm and increase the coverage to 60% while permitting reduced volume of peroxide solution to be applied; hence the drying time. This method is being replaced by the use of a mixture of hot air and vapourized peroxide. Sterilization by hydrogen peroxide vapour would be a cost-effective alternative as the least amount of hydrogen peroxide is used. Furthermore, the amount of hydrogen peroxide adsorbed on the treated surface from the vapour phase will be several orders of magnitude smaller than a liquid film. Therefore flushing the vapour-treated surface with low-temperature sterile air free of hydrogen peroxide vapours can effectively eliminate residues.

Low levels of hydrogen peroxide, which remain in the vapour phase in air at near ambient temperature has been shown to have sporicidal activity suitable for in-line sterilization of aseptic packaging materials (Wang and Toledo, 1986). Air saturated with hydrogen peroxide vapour carry relatively low concentration of hydrogen peroxide ($7.6\,mg\,l^{-1}$ at 70°C) but can induce 6 decimal reductions of spores of *Bacillus subtilis* var. niger in 1.2 min. In comparison the same organism requires 1.2 min to achieve one decimal reduction in hot air alone at 150°C. Since the amount of hydrogen peroxide vapourized from solution is less than that from water, the solution is enriched during prolonged operation and a system must be devised to add make-up water to maintain the desired concentration.

Rinsing: prefabricated, intricately shaped containers for which the spraying process is unsuitable can be rinsed with an aqueous 30–33% H_2O_2 solution. For ambient temperature sterilization this can be combined with peracetic acid. The containers are drained, allowed to drip dry, and then completely dried by hot sterile air. Glass containers, metal cans, and blow-moulded plastic bottles are treated in this manner.

Ethylene oxide

Implementation of ethylene oxide sterilization has made possible the use of sterile, low-cost, disposable thermoplastic devices for industrial application. It has been used to presterilize paperboard cartons (Hedrick, 1973) and plastic packaging materials (Alguire, 1973). However, no commercial system uses ethylene oxide as a sterilizing agent in aseptic packaging of low acid foods for ambient temperature storage and distribution. The vapour form is flammable and explosive (Merck, 1989) and hence employed as a mixture with an inert gas such as dichlodifluoromethane. Alguire (1973) reported that 100% ethylene oxide has better microbicidal properties than an ethylene oxide inert gas mixture. An important consideration in bulk sterilization of preformed packaging materials is ethylene oxide's ability to permeate and contact all surfaces that need to be sterilized. The use of lower concentration of ethylene oxide is safer than use of 100% ethylene oxide from standpoint of potential for injury to personnel. The *D*-value for *Clostridium botulinum* 62 *A* require a minimum of 72 min for a 12*D* process, thus making it impractical to use ethylene oxide for in-line sterilization of aseptic packaging materials in high-speed packaging lines. Ethylene oxide is effective against bacteria, moulds, yeasts, as well as viruses. The most probable activity is alkylation of nucleic acids and other cellular components (Phillips, 1977). It is a particularly effective sterilizing agent because it rapidly penetrates packaging material. The ethylene oxide concentration, humidity and temperature influence the rate of sterilization. A clean object can be sterilized if treated for 5–8 h at 38°C or 3–4 h at 54°C when the relative humidity is maintained at 40–50% and the EtO concentration at $700\,mg\,l^{-1}$. Extensive aeration of the sterilized materials is necessary to remove residual EtO because it is so toxic. However, one disadvantage of it is its comparatively slow rate of action against microorganisms. Spores of *Bacillus subtilis* var. niger ATCC 9372 are used as indicators in dry heat and ethylene oxide sterilization (Anonymous, 1995, 1999).

Other sterilization methods

There are many other chemicals such as peracetic acid, beta propiolactone, alcohol, chlorine and its oxide and ozone etc. that have been suggested as having potential for use in sterilizing aseptic packaging materials. Peracetic acid is a peroxide of acetic acid and on decomposition it forms acetic acid and water. The low pH and oxidizing properties make it an excellent sporicidal agent. It has all the advantages of hydrogen peroxide and is unaffected by catalase and peroxidases. Peracetic acid is a liquid sterilant which is particularly effective against spores of aerobic and anaerobic bacteria and is effective at lower temperatures than hydrogen peroxide. In practice, it is used in a solution which also contains hydrogen peroxide. The solution containing peracetic acid and hydrogen peroxide is effective against resistant bacterial spores even at 20°C, for example a 1% solution will eliminate 10^{-7}–10^{-8} of most resistant spore stains in 5 min at 20°C , and the most resistant strains in 60 min. The maximum usable temperature is 40°C when the sterilization times are about 5 times shorter. In spite of its sporicidal properties, peracetic acid is not an approved sterilant for use on aseptic packaging materials. Its vapour is very pungent and irritating. Therefore a system that utilizes this compound must be airtight to prevent environmental release. Unlike hydrogen peroxide vapours for which a tolerance is allowed inside sealed packages, no such tolerance is allowed for peracetic acid and vapours in the headspace can cause disagreeable vinegar like off-flavour in some food products. In spite of these problems, this compound deserves attention as a possible sterilant for aseptic packaging materials because of its effectiveness as a sporicidal agent. The presence of small amounts of acetic acid may not be considered off-flavours. At ambient temperature and halogen concentration of 100 ppm, exposure times for effective sterilization would be on the order of hours. Under these conditions, it would be impractical to use these compounds as in-line sterilants in aseptic packaging. One major problem with the use of strong chlorine preparations for sterilization is that available chlorine reacts with organic material. Reaction between chlorine and the material being sterilized may produce off-flavours.

Beta propiolactone is bactericidal, sporicidal, fungicidal and virucidal. It lacks the penetrating power of the ethylene oxide, but it is considerably more active against microorganisms. Only 2–5 mg of beta propiolactone per litre is required for sterilization compared with 400–800 mg of ethylene oxide per litre. However, its poor penetration of materials and its cancer-inducing properties have restricted its use as a practical sterilizing agent.

Ethanol is the only industrially significant alcohol used as bactericide and fungicide but not for sporicide. Their mechanism of action appears to involve protein denaturation and membrane lipids dissolution. Ethanol liquid and vapour have been suggested (Doyen, 1973; Mita, 1985). However, ethanol is effective only against vegetative cells and not against fungal conidia or bacterial spores therefore its use in packaging of foods is limited to extension of shelf life of packaged foods, which are normally stored under refrigeration. The two most popular alcohol germicides are ethanol and isopropanol, usually used in about 70–80% concentration.

Chlorine and its oxide and ozone are used mainly for water disinfection. Ozone can be used as a sterilizing agent because of its high oxidizing capability. Death of almost all microorganisms usually occurs within 30 min. Ozone was recommended recently as an alternative to chlorine (Kim, 1998) and hydrogen peroxide (Khadre and Yousef, 2001). They found that hydrogen peroxide at 10,000-fold higher concentration was less effective than ozone against *Bacillus* spores. Antimicrobial power of ozone increases as temperature decreases below ambient (Herbold *et al.*, 1989). Comparatively low concentration is needed to eliminate large population of spores at ambient temperature in short time period and this makes ozone best suited for an industrial setting.

CONCLUSIONS

Sterilization of packaging materials in-line of processing poses certain difficulties. Based on the review presented it is evident that hydrogen peroxide sterilization followed by hot air appears to have the most potential for use as in-line sterilants for packaging materials. This combination is time-tested and no adverse report seems to have put any doubt over the method's acceptability. Sterilization of packaging materials using hydrogen peroxide and followed by ultraviolet irradiation also has been accepted for industrial application. Dry heat, saturated steam and superheated steam can be effective sterilants, but the degree of heat damages many packaging materials so they find limited application. Infrared rays cannot be applied as there is a temperature rise of the packaging material due to infrared application and this results in softening of the plastics. Ionizing rays are not accepted as they have harmful effect on personnel. Light pulses have not been adequately studied so far as their effect on food material is concerned. Ethylene oxide requires a very long time which precludes its use for in-line applications. Peracetic acid produces an off-flavour in food if residual deposit is enclosed in the container. Beta propiolactone lacks the penetrating power of ethylene oxide and ethanol is not effective against spores.

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