

ANTIMICROBIAL EFFECTS IN FOOD AND PREVENTION OF CONTAMINATION IN FOOD INDUSTRY

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INTRODUCTION

The most commonly recognized food-borne infections are caused by *Campylobacter jejuni* and *Salmonella* spp. In the past 25 years, *Listeria monocytogenes* has become increasingly important as a food-associated pathogen. Though, an annual incidence of human listeriosis is between two and ten reported cases per million in EU countries. Because of its high case fatality rate, listeriosis ranks among the most frequent causes of death due to food-borne illness. *L. monocytogenes* and *C. jejuni* has been shown to adapt to different environmental stress factors, including disinfectants. *Salmonella enteritidis*, *C. jejuni* and *L. monocytogenes* may also form a biofilm which helps to survive in the environment. For that reason, the food industrial hygiene has become important to avoid contamination of raw food and food products with these pathogens.

The aims of the present report are to describe and discuss different antimicrobial methods that prevent the contamination and transmission of food-borne pathogens. The antimicrobial methods could be divided such as physical activities (agents) and chemical agents (e.g. sanitizers, antibiotics).

PHYSICAL ACTIVITIES (AGENTS)

CLEANING OF EQUIPMENT

Since cleaning and sanitizing may be the most important aspect of a sanitation program, sufficient time should be given to outline proper procedures, and parameters. Detailed procedures must be developed for all food-product contact surfaces (equipment, utensils, etc.) as well as for non-product surfaces such as: non-product portions of equipment, overhead structures, shield, walls, ceilings, lighting devices, refrigeration units, heating, ventilation and air conditioning (HVAC) systems, and anything else which could impact food safety.

Equipment cleaning methods can be categorized with regard to cleaning method as follows: a) Mechanical cleaning often referred to as cleaning-in-place (CIP) requires no disassembly or partial disassembly; b) Cleaning-out-of-place (COP) can be partially disassembled and cleaned in specialized COP pressure tanks & c) Manual cleaning requires total disassembly for cleaning and inspection.

THERMAL SANITIZATION

Thermal sanitization involves the use of hot water or steam for a specified temperature and contact time. On the other hand, the effectiveness of an antimicrobial agent depends on its chemical and physical state, treatment conditions (such as water temperature).

Hot-water sanitizing, through immersion (small parts, knives, etc.), spray (dishwashers), or circulating systems, is commonly used. The time required is determined by the temperature of the water. Typical industrial requirements for use of hot water in dishwashing and utensil sanitizing applications specify: immersion for at least 30 sec. at 77 °C for manual operations; a final rinse temperature of 74 °C in single tank, single temperature machines and 82 °C for other machines.

The primary advantages of hot-water sanitization are: relatively inexpensive, easy to apply and readily available, generally effective over a broad range of microorganisms, relatively non-corrosive, and penetrates into cracks and crevices. Hot-water sanitization is a slow process which requires come-up and cool-down time; can have high energy costs; and has certain safety concerns for

employees. The process also has the disadvantages of forming or contributing to film formations, and shortening the life of certain equipment or parts thereof (gaskets, etc.).

FILTRATION

The guarantee of food quality, and particularly food safety, places an onerous responsibility on all food processors. EU Regulations (EN 29000–29004) define this responsibility not only in terms of the quality of the end produce, but also the precautions which must be taken in the preparation and processing of foods to minimize the risk of contamination.

For all food products, and especially for those which are heat sensitive (e.g. milk or egg-based) and subject to minimal heat treatment, all aspects of the process must be rigorously controlled to prevent microbial contamination. This includes not only the processing equipment, but also the processing environment including the air, which may reduce product shelf life, and in certain circumstances, pose a serious hazard to food safety.

While air filtration is now standard practice in food processing environments, significant increases in aerial microbial counts may occur intermittently due to failure to adhere to Good Manufacturing Practices and/or failure of the air filtration system due to design, inadequate maintenance, malfunction, etc.

Frequent and effective monitoring of the air sterility, in the process environment is therefore essential to alert processors of the potential risks, and the possible need for corrective action. However, the lack of reliable quantitative air sampling techniques, coupled to the uncertainty about the behaviour or control of micro-organisms within air filtration systems, present serious obstacles to effective control of processing environments.

FREEZING IN FOOD PRESERVATION

According to EU Directive 89/108, the temperature of quick-frozen foods must be stable and maintained at -18 °C or colder at all points. The temperature of frozen foods must be -12 °C or colder, and this temperature must be maintained. Directive 89/108 requires that after quick-freezing the product temperature must

be $-18\text{ }^{\circ}\text{C}$ or colder after thermal stabilization. This is useful wording, as it means that the freezing process can be stopped before the core temperature is $-18\text{ }^{\circ}\text{C}$. It is sometimes recommended that for practical purposes the freezing process can be stopped when the core temperature is $-10\text{ }^{\circ}\text{C}$ or colder. For most products, this would mean that the average temperature is the same or colder than the stipulated storage temperature.

The freezing process can be considered ended when all points in the food are $-18\text{ }^{\circ}\text{C}$ or colder. The length of this stabilization period depends on the type of food (thermal conductivity), how it is packaged, and how the temperature distribution in the food is at the beginning of stabilization. In some countries, there has been legislation requiring that a core temperature of $-18\text{ }^{\circ}\text{C}$ must be reached in less than 1 hour for small pieces (steaks), 2 to 6 hours for medium sized pieces, e.g. poultry and roasts, and 24 hours for large packs, e.g. cartons with boned meat. Frozen foods must be stored at $-12\text{ }^{\circ}\text{C}$ or colder, quick-frozen foods at $-18\text{ }^{\circ}\text{C}$ or colder. Some frozen foods, e.g. beef, broilers, butter, have a fairly long storage life even at $-12\text{ }^{\circ}\text{C}$, while foods such as lean fish require storage temperatures around $-28\text{ }^{\circ}\text{C}$ in order to reduce the quality loss and have a long storage life.

The EU Directive 92/1 requires that storage facilities must have installed a temperature recording device. The refrigerating capacity must be sufficient to obtain a maximum difference between evaporator temperature and room temperature of $7\text{ }^{\circ}\text{C}$. The QFF Directive requires that the temperature of quick-frozen foods must be maintained at $-18\text{ }^{\circ}\text{C}$ or colder at all points in the product, with possibly brief upward fluctuations of no more than $3\text{ }^{\circ}\text{C}$ during transport. It is often recommended, e.g. given in the cooking instructions on the label, that if frozen foods must be thawed before cooking, this should be done in a refrigerator.

PASTEURIZATION

Pasteurization is achieved by a treatment involving (i) a high temperature for a short time (at least $72\text{ }^{\circ}\text{C}$ for 15 s); (ii) a low temperature for a long time (at least $63\text{ }^{\circ}\text{C}$ for 30 min); or (iii) any other combination of time and temperature conditions to obtain an equivalent effect, such that the products show, where applicable, a negative reaction to an alkaline phosphatase test immediately after such treatment.

Ultrahigh temperature (UHT) treatment is achieved by a treatment (i) involving a continuous flow of heat at a high temperature for a short time (not less than 135 °C in combination with a suitable holding time) such that there are no viable micro-organisms or spores capable of growing in the treated product when kept in an aseptic closed container at ambient temperature; and (ii) sufficient to ensure that the products remain microbiologically stable after incubating for 15 days at 30 °C in closed containers, or for 7 days at 55 °C in closed containers, or after any other method demonstrating that the appropriate heat treatment has been applied.

PRESSURE

Food preservation using high pressure is a promising technique in food industry as it offers numerous opportunities for developing new foods with extended shelf-life, high nutritional value and excellent organoleptic characteristics. High pressure is an alternative to thermal processing. The resistance of microorganisms to pressure varies considerably depending on the pressure range applied, temperature and treatment duration, and type of microorganism. Generally, gram-positive bacteria are more resistant to pressure than Gram-negative bacteria, moulds and yeasts; the most resistant are bacterial spores. The nature of the food is also important, as it may contain substances which protect the microorganism from high pressure. Despite the introduction of food standards obligatory in EU countries, epidemiologists believe that 75% of food-borne diseases are caused by bacteria.

High pressure is an alternative to thermal processing. The resistance of microorganisms to pressure varies considerably depending on the pressure range applied, temperature and treatment duration, and type of microorganism. The first high pressure processed food products appeared in Japan in the early 1990s. In Europe, high pressure processing (HPP) of foods was rather at the stage of research or pilot production in the last decade. EU legislation included HPP foods in the “novel food” category. EC Novel Food regulation (EC 258/97) has introduced a statutory pre-market approval system for novel foods across the whole of the European Union. Recently, rapid progress of HPP toward commercial exploitation has been achieved, but still the process requires close collaboration between researchers, food and equipment manufacturers, as well as proper financial support.

SONICATION

Ultrasound is energy generated by sound waves of 20,000 or more vibrations per second. Presently, most developments of ultrasonic (sonication) for food applications are nonmicrobial in nature. High frequencies in the range of 0.1 to 20 MHz, pulsed operation and low power levels (100 mW) are used for non-destructive testing. Industrial applications include texture, viscosity and concentration measurements of many solid or fluid foods; composition determination of eggs, meats, fruits and vegetables, dairy and other products; thickness, flow level and temperature measurements for monitoring and control of several processes; and non-destructive inspection of egg shells and food packages. Researchers also listed direct process improvements such as cleaning surfaces, enhancement of dewatering, drying and filtration, inactivation of microorganisms and enzymes, disruption of cells, degassing of liquids, acceleration of heat transfer and extraction processes and enhancement of any process dependent upon diffusion. It is evident that ultrasound technology has a wide range of current and future applications in the food industry.

The bactericidal effect of ultrasound is generally attributed to intracellular cavitation. It is proposed that micro-mechanical shocks are created by making and breaking microscopic bubbles induced by fluctuating pressures under the ultrasonication process. These shocks disrupt cellular structural and functional components up to the point of cell lysis.

PULSED ELECTRIC FIELD

Pulsed electric field (PEF) processing is a non-thermal method of food preservation that uses short bursts of electricity for microbial inactivation and causes minimal or no detrimental effect on food quality. PEF can be used for processing liquid and semi-liquid food products. PEF processing offers high quality fresh-like liquid foods with excellent flavour, nutritional value, and shelf-life. Since it preserves foods without using heat, foods treated this way retain their fresh aroma, taste, and appearance. PEF processing involves treating foods placed between electrodes by high voltage pulses in the order of 20–80 kV (usually for a couple of microseconds). The applied high voltage results in an electric field that causes microbial inactivation.

ELECTROPORATION

This method is used to transform a wide variety of microorganisms and requires a brief exposure to a high-voltage electric field to introduce genetic material into a microorganism. Electroporation is the most popular technique for introducing genetic material in microorganisms because of its simplicity, efficacy and versatility. Electroporation is the phenomenon in which a cell exposed to high voltage electric field pulses temporarily destabilizes the lipid bilayer and proteins of cell membranes. The plasma membranes of cells become permeable to small molecules after being exposed to an electric field, and permeation then causes swelling and eventual rupture of the cell membrane. The main effect of an electric field on a microorganism cell is to increase membrane permeability due to membrane compression and poration.

OHMIC HEATING

Ohmic heating is an advanced thermal processing method wherein the food material, which serves as an electrical resistor, is heated by passing electricity through it. Electrical energy is dissipated into heat, which results in rapid and uniform heating. Ohmic heating is also called electrical resistance heating, Joule heating, or electro-heating, and may be used for a variety of applications in the food industry. Like thermal processing, ohmic heating inactivates microorganisms by heat. Additional non-thermal electroporation type effects have been reported at low-frequency (50–60 Hz), when electrical charges can build up and form pores across microbial cells however, it is not necessary to claim such effects since heating is the main mechanism. The shelf life of ohmically processed foods is comparable to that of canned and sterile, aseptically processed products. In various countries, including Italy, Greece, France, ohmic heating has been used to produce a low-acid particulate product in a can, as well as pasteurized liquid egg.

CHEMICAL AGENTS

SANITIZERS

The selection of a sanitizer depends on the type of equipment to be sanitized, the hardness of the water, the application equipment available, the effectiveness of the sanitizer under site conditions, and cost. Sanitizing compounds which

contain phenols impart strong undesirable odors and flavors to foods and should not be used. Thorough cleaning is essential before using a sanitizer. Sanitizers are less effective when food particles or dirt are present on equipment surfaces. Typical disinfecting agents used in the food industry include quaternary ammonium compounds (QACs), chlorine, alcohols, hydrogen peroxide, peracetic acid and iodofors.

Legislation concerning disinfectants include Directive 98/8/EC of the European Parliament and of the Council on the placing on the market of biocidal products. Member States had to transpose the rules before 14 May 2000 into national law. The Biocidal Product Directive aims to harmonise the European market for biocidal products and their active substances and to protect humans, animals and the environment (<http://ec.europa.eu/environment/biocides/index.htm>). The new REACH Regulation (EC) No 1907/2006 of the European Parliament and of the Council and Directive 2006/121/EC of the European Parliament and of the Council were published in the Official Journal on 30 December 2006. The aims of the proposed new Regulation are to improve the protection of human health and the environment while maintaining the competitiveness and enhancing the innovative capability of the EU chemicals industry. REACH will also give greater responsibility to industry to manage the risks from chemicals and to provide safety information on the substances. REACH will enter into force on 1 June 2007 (http://ec.europa.eu/enterprise/reach/index_en.htm).

Many commonly used disinfectants have been shown to be effective against *L. monocytogenes* in suspension. Other factors affecting on efficacy of disinfectants against *L. monocytogenes* include e.g. the concentration and effect time as well as temperature and pH of the use solution. *L. monocytogenes* strains can vary in resistance against disinfectants. *L. monocytogenes* has been shown to adapt to different environmental stress factors, including disinfectants. The adaptation may occur especially when the disinfectant is present in the environment in sub lethal amounts. Differences in the susceptibility and adaptive response of strains to disinfectants have been observed, and it has been suggested that such differences could influence the survival of the strains in the food processing plants. Generally, the recommendations of use-concentration of disinfectants should be followed. However, these may not always be sufficient against *L. monocytogenes*.

Future investigations include further studies on mechanisms of adaptation and differences between strains. Besides disinfectants, other technologies for reducing use of water and chemicals in disinfection in food plants should be investigated.

ANTIBIOTICS

Antibiotics are chemical substances, either produced naturally by microorganisms or manufactured synthetically, that are lethal to other bacteria. Antibiotics are used for the treatment of bacterial infections in both humans and animals. The emergence of antibiotic resistance as a serious problem in human medicine has prompted concerns about the public health implications of antibiotic use in agriculture. Antimicrobial resistance has emerged among *Campylobacter* mainly as a consequence of the use of antimicrobial agents, especially fluoroquinolones, macrolides, and tetracyclines in food animal production. In the recent study a high number of *Campylobacter* isolates from Estonian poultry had increased antimicrobial resistance to ciprofloxacin, tetracycline. The use of the antibiotics as growth promoting agents is forbidden in EU countries since January 2006 (Regulation (EC) No 1831/2003 of the European Parliament and of the Council).

FUTURE NEEDS

New technologies are needed for surveillance of food-borne disease and food monitoring. These include typing pathogens, different *in vitro*, animal and clinical testing. New research and development are required in food industry such as application of antimicrobial surface materials and green technologies (enzymes).

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