Int.J.Curr.Microbiol.App.Sci (2018) *7*(3): 2833-2838

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 7 Number 03 (2018)**
Journal homepage: http://www.ijcmas.com

Review Article https://doi.org/10.20546/ijcmas.2018.703.326

Microbial Behavior against Newer Methods of Food Processing and Preservation: A Review

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Keywords

Food processing, Thermal processing, Preservation

Article Info

Accepted:
24 February 2018
Available Online:
10 March 2018

A B S T R A C T

There has been a great advancement in food processing methods over the years from traditional thermal processing to various non-thermal processings like high-pressure, electric field and radiations based methods. These methods have been found more effective and less damaging to food quality. This review describes the mechanism of inactivation of microbes due to these newer methods of food processing. These methods kill vegetative microbes but fail to effectively kill spores, but a combination of methods can be used to achieve the objective. These methods, however, can meet the demands of consumers for safe, nutritious, improved taste, texture and ready-to-eat food products.

Introduction

Food Processing is the conversion of raw materials or ingredients to a final product. According to Connor (1988) food processing is that branch of manufacturing that starts with raw animal, plant or marine materials and transforms them into intermediate foodstuffs or edible products through the application of labor, machinery, energy and scientific knowledge. Thermal pasteurization and sterilization had been in use in the food industry for a long time for their efficacy and product safety record. Excessive heat used in these processes, may, however, cause undesirable quality changes in food like browning, protein and fat deterioration, loss of certain nutrients etc. The alternative technologies are non-thermal as these do not employ heating of food directly, thus, minimizing the damaging effects on food quality. The newer methods includes High hydrostatic pressure (technique that destroys the microorganisms with the intense pressure in the range 100-1000 MPa), Pulse electric field (delivery of pulses at high electric field intensity 5-55 kV/cm for a few milliseconds), gamma radiations also known as cold sterilization (employs doses of 2-10 kGy), ultraviolet radiations (germicidal properties at wavelengths in the range of 200- 280 nm), ultrasound (20 to 100 kHz; which is referred to as ‘‘power ultrasound’’, has the ability to cause cavitation, which has uses in food processing to inactivate microbes) (Zhang *et al.*, 1995; Kuo *et al.*, 1997; Piyasena *et al.*, 2003; Gervilla *et al.*, 2001). These methods employ different mechanisms of inactivation of microbes. Very few of these new preservation methods are until now implemented by the food industry. The aim of this article is to reflect the mechanisms of inactivation of these newer methods and lighting up the research efforts made in direction of use of such less food damaging techniques.

High Hydrostatic pressure

Certes, in 1883, was the one who succeeded in relating the effects of high pressure on microorganisms (Knorr, 1995). The principle demonstrates that food product is compressed under uniform pressure in every direction and regains it’s original shape as the pressure is released (Yordanov and Angelova, 2014). High pressure processing is comprised of the following units: a) pressure vessel b) pressure generating device c) material handing system d) temperature controls. The food package is loaded onto vessel and the top of vessel is closed. The pressure medium (generally water) is allowed to pump into the vessel from the bottom. As the desired pressure is reached, pumping is stopped. The valves are closed and pressure is maintained. The pressure was applied in an isostatic manner so that all the food in the container experiences a uniform pressure throughout (Mertens, B. 1995; Doona and Feeherry, 2008).

High pressure has a lethal effect on vegetative microorganisms and that is the result of numerous changes that take place in the membrane of a microbial cell. The membrane is the most probable site of disruption in a microbial cell. The active and passive transport functions of membrane are altered by the high pressure treatment which ultimately disturbs the internal physiochemical balance of the cell. The lethal pressure is approximately above 180 MPa after which there is observed loss of cell viability and the rate of inactivation increases exponentially as the pressure increases. HHP inactivation seems to be multitarget in nature. Membrane is a key target, but in some cases additional damaging events occur such as:-

Extensive solute loss during pressurization,

Protein coagulation,

Key enzyme inactivation and ribosome conformational changes, together with impaired recovery mechanisms, seem also needed to kill bacteria.

The technology was first used and commercialized in 1990 in Japan. The initial products processed include juices, jellies, jams, meats, fishes etc. as reported by Augustin *et al.*, (2016). This is an emerging technology with a great future scope in food industry.

Pulse electric field processing

Pulse electric field (PEF) is one of the promising non-thermal food processing technology. It involves use of short pulses of high electric voltage (upto 5-50 kV/cm) for microseconds to milliseconds which decontaminates the food followed by aseptic packaging and refrigeration (Wouters *et al.*, 2001). The pulse electric field system is composed of three units: a treatment chamber (consist of a set of electrodes), a high voltage pulse generator, a control system for monitoring the process (Loeffler, 2006). The food is placed between the electrodes in a treatment chamber which is exposed to short pulses of high electric voltage. The two electrodes are connected to non-conductive material to prevent the electric flow from one to another. The food product experiences a force as electric field, which is responsible for the cell membrane breakdown in microorganisms and causes inactivation of microorganisms. (Fernandez-diaz, 2000) The process is majorly equipped for pasteurisation of food products including eggs, juices, milk, soups and yogurt (Bendicho, 2003).

The efficiency of PEF technology for inactivation of microbes depends largely on the microbial characteristics including type of microbe, species and strain (Macgregor, 2000). Compared to yeast cells, gram positive and gram negative bacteria are found to be more resistant to PEF technology. In like manner, bacterial and mold spores are asserted to be defiant to PEF processing (Katsuki, 2000).

The mode of action of pulse electric field mainly focuses on reduction of microbial load to produce safe quality foods. The basic mechanism of pulse electric field technology involves induction of electric field which leads to electromechanical compression. This further causes formation of pores in the microbial membrane, known as electroporation. Electroporation can be defined as the formation of pores in cells and organelles. When it ruptures membrane and causes permeability known as electropermeabilization.

Electropermiablization may be reversible or irreversible depending upon the organisational change that leads to cell death (Rowan, 2000). In general, spores are stated to more resistant to the PEF treatment than the vegetative cells (Katsuki, 2000). Bacteria and yeasts have shown morphological alterations like surface roughness, disruption of organelles, ruptures in the membrane, etc on application of pulse electric field. (Dutreux *et al.,* 2000).

Ultrasound

Ultrasound waves have a frequency that is above 16 KHz and cannot be detected by the human ear. It can be further divided into two categories: a) low energy; b) high energy. The low energy ultrasound frequency is higher than 100KHz with intensity lower than 1W/cm2. The high energy ultrasound frequency ranges 20-500 KHz at the intensity higher than 1W/cm2 (Chemat *et al.,* 2011). The commonly applied frequency for ultrasound technology by researchers ranges between 20KHz - 500 MHz (Yusaf and Al-Juboori, 2014). Ultrasonics is one of the fastest growing non-thermal food processing methods that have been devised to meet the consumer demands and provide minimum processed, high quality and healthy product (Knorr *et al.,* 2011).

Cavitation phenomenon is responsible for the lethal effects of ultrasound. In ultrasonics, electrical energy is converted to mechanical energy or vibrational energy which is passed on to the sonicated liquid system. Partial input energy is lost in the form of heat and partial can cause cavitation producing effects (O'Sullivan, 2017). The bubbles so generated as a result of cavitation implodes under an intense ultrasonic field, free radicals are generated which inactivates microbial cells. By causing grievous damage to cell wall, the acoustic cavitation phenomenon can destroy cell structure and cause impairment of functional components causing cell lysis (Jose, 2016). Ultrasonic has been applied to many liquid foods for inactivation of microbes. In a study, ultrasonic was applied to apple cider where the levels of *E. coli* O157:H7 were reduced by 5 log cfu/ml. In the same, study conducted on milk showed reduced levels of *Listeria monocytogenes* by 5 log cfu/ml. A research on ultrasound has also reported that microbes having soft and thicker capsule are found to be extremely resistant to the ultrasonic processing. (Gao and Lewis, 2014). The effectiveness of an ultrasound treatment is dependent on the type of bacteria being treated. Microorganisms (especially spores) are relatively resistant to the effects, thus extended periods of ultrasonication would be required to render a product safe. If ultrasound were to be used in any practical application, it would most likely have to be used in conjunction with pressure treatment (manosonication), heat treatment (thermosonication) or both (manothermosonication) (Piyasena *et al.,* 2003).

Irradiation

Irradiation being a non-thermal processing technology can be used to destroy the microbes and increase the shelf life of a product. It can destroy yeasts, molds and viable microorganisms (radurization) with a dosage of 0.4-10 KGy, to destroy non-spore forming food borne pathogens (radicidation) uses a dosage of 0.1-8 KGy, and to sterilize the product by killing both vegetative bacteria and spores with a dosage of 10-50 KGy (Fellows, 2000).

Irradiation preserves the food by the use of ionizing radiation (γ-rays, from electrons and X-rays). The effects of ionizing radiations are classified as direct and indirect. The direct effects are caused by the absorption of radiation energy by target molecules and indirect effects are caused by hydroxyl radicals generated from radiolysis of water inside the food. The hydroxyl radical OH• is able to react with the sugar-phosphate backbone of the DNA chain giving rise to the elimination of hydrogen atoms from the sugar. This causes the scission of the phosphate ester bonds and subsequent appearance of single strand breaks. Double strand breaks occur when two single strand breaks take place in each chain of the double helix at a close distance (Manas and Pagan, 2005). Irradiation sources are radioisotopes (cobalt-60 and cesium- 137) and machine generated (electron beams and X-rays). Vegetative cells are less resistant to irradiation than spores, whereas moulds have a susceptibility to irradiation similar to that of vegetative cells. However some fungi can be as resistant as bacterial spores (Farkas, 2006).

Biopreservation

Biopreservation or biocontrol refers to the use of natural or controlled microbiota, or its antibacterial products to extend the shelf life and enhance the safety of foods (Stiles, 1996). The biopreservation includes bacteriocins which are produced by certain microorganisms have antagonistic effect on other organisms. Deegan *et al.,* (2006) classified bacteriocins depending upon their structures as: small peptides (<10kDa; lanthionine containing; nisin, lacticin etc.), small peptides (<5kDa; non-lanthionine containing; pediocin, lactococcin etc.), large molecules (like helveticins), and circular peptides (enterocins). The mechanism of inactivation is based upon electrostatic interactions with negatively charged phosphate groups on target cell membranes which contribute to the initial binding, forming pores and killing the cells after causing lethal damage and autolysin activation to digest the cellular wall (Perez *et al.,* 2015). The established use of nisin as a preservative is found in processed cheese, various pasteurized dairy products and canned vegetables. Many other bacteriocins from lactic acid bacteria have recently been characterized. Because of potential usefulness as natural food preservatives, increased interest has been found on bacteriocins from lactic acid bacteria. Bacteriocin producing (Bac+) lactic acid bacteria (LAB) detected in retail foods indicates that the public is consuming a wide variety of Bac + LAB. This suggests a greater role for bacteriocins as biopreservatives in foods.

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How to cite this article:

Sucheta, Panvi Ahuja and Rakesh Gehlot. 2018. Microbial Behavior against Newer Methods of Food Processing and Preservation: A Review. *Int.J.Curr.Microbiol.App.Sci.* 7(03): 2833-2838. doi: https://doi.org/10.20546/ijcmas.2018.703.326