Thermal and Hurdle Approaches in Microbial Inactivation

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Thermal processing of packaged fruit and vegetable products is targeted at eliminating microbial contaminants (related to spoilage or pathogenicity) and extending shelf life using microbial inactivation or/and by reducing enzymatic activity in the food. The conventional process of thermal processing involves sterilization (canning and retorting) and pasteurization. The parameters used to design the thermal processing regime depend on the time (minutes) required to eliminate a known population of bacteria in a given food matrix under specified conditions. However, due to the effect of thermal exposure on the sensitive nutrients such as vitamins or bioactive compounds present in fruits and vegetables, alternative technologies and their combinations are required to minimize nutrient loss. The novel moderate thermal regimes aim to eliminate bacterial contaminants while retaining nutritional quality.

Keywords: fruits ; vegetables ; PEF ; PATS ; MATS

1. Introduction

Thermal processing of food can be explained as any post-harvest process that uses heat to eliminate microbial contaminants (related to spoilage or pathogenicity) and extend shelf life using either microbial inactivation or/and by reducing enzymatic or toxin activity in the food [1]. However, consumer preference for minimally processed or “fresh like” food products have attracted significant research and development on mild to moderate thermal processing techniques. However, a hurdle approach that includes both thermal and non-thermal processing techniques has indicated the potential to increase food safety by microbial inactivation while reducing loss in nutritional and sensory attributes [2]. Fruits and vegetables are considered processed if they are cut/packaged in any form that is ready to eat by consumers. However, as soon as the fruit is cut or peeled, the possibility of microbial contamination from the surface or during handling increases [3]. These challenges have led to the use of processing techniques to minimize microbial contaminations that otherwise lead to spoilage and in some cases food poisoning. Some of the common foodborne pathogens and spoilage-related microbial contaminants are listed in **Table 1**.

Table 1. Common contaminants of fruits and vegetables.

Microbial Contaminants (Bacterial/Viral/Fungal)	Relevance	Food Safety/Shelf Life-Based Concerns	Reference
Salmonella spp. (serovar Typhimurium, Montevideo, Javiana, Anatum, Enteritidis, Infantis, Stanley, Newport)	Foodborne pathogen resulting in self-limiting gastroenteritis in humans. Multidrug resistance is well known	Has been reported as a common cause of food poisoning in many countries; fresh produce can be contaminated anytime from harvest to packaging	[4][5]
E. coli O157:H7	Foodborne pathogen resulting in haemorrhagic colitis, bloody diarrhoea hemolytic uremic syndrome and death	Cross-contamination from meat during the preparation of ready-to-eat (RTE) products has been reported. Multiplication and growth of *E. coli* on fresh produce are reported at 12–25 °C	[6][7]
Campylobacter jejuni	A foodborne pathogen that causes gastroenteritis	Outbreaks associated with fresh salads have been reported. Although cross-contamination has been reported as one of the causes, many sources of contamination remain unidentified	[8][9]

Microbial Contaminants (Bacterial/Viral/Fungal)	Relevance	Food Safety/Shelf Life-Based Concerns	Reference
Listeria monocytogenes	Food poisoning resulting in mild gastroenteritis to severe blood and/or central nervous system infections with limited reports on abortion in pregnant women	*L. monocytogenes* is a contaminant of fresh produce and can also be prevalent in RTE and minimally processed meals	[10]
Aeromonas spp.	Food poisoning leading to gastroenteritis	Due to their ubiquitous nature, they contaminate the vegetables and fruits via fresh and salt water, either during harvest or post-harvest handling	[11]
Pseudomonas spp.	Opportunistic pathogens are known to be capable of producing pathogenicity factors (toxins, effector proteins, proteases, elastases and pigments) that might affect the immune system. Otherwise associated with spoilage	Mishandling during harvest or post-harvest leads to cross-contamination from Pseudomonas coming from the soil, fertilizers, manure or water used for irrigation	[12]
Hepatitis A virus	The causative agent of hepatitis A leads to mild to moderate symptoms and fatality in some cases. Additionally known to cause frequent endemics in developing countries	Fruits and vegetables can be cross-contaminated if irrigated with water/solutions that contain faecal remains.	[13]
Norovirus	Associated with foodborne outbreaks and usually referred to as stomach flu. It leads to diarrhoea, vomiting, nausea and stomach pain	Usually, cross-contamination during handling and packaging and also due to exposure to faecal cross-contaminants.	[14]
Mycotoxins: *Aspergillus* spp., *Penicillium* spp. and *Alternaria* spp.	Associated with food poisoning and spoilage and significant loss of the harvest products	Post-harvest contamination by *Aspergillus* spp., *Penicillium* spp. and *Alternaria* spp. causes toxin production as part of their secondary metabolites and in some cases leads to spoilage such as citrus brown spots by *Alternaria alternata*.	[15]

2. Conventional Thermal Processing Regimes of Fruits and Vegetables

Conventional thermal processing of fruits and vegetables can be divided into two major classes: pasteurization and sterilization. The key difference is based on the temperature and time of processing, according to which sterilization aims to remove all the bacterial contaminants including spores, whereas, in the case of pasteurization, spores might not be inactivated. While sterilization is the preferred regime for a long-term shelf life of food products, especially without the need to be stored under refrigerated conditions, pasteurization has the limitation of a limited shelf life. These applications enable the manufacturers of vegetable and fruit products to decide on the processing regimes that are fit for their purpose.

2.1. Sterilization of Fruits and Vegetables

Conventional thermal sterilization is explained by the United States Food and Drug Administration (FDA, Silver Spring, MD, USA) as any process using heat either alone or in combination with technologies that can lead to the inactivation of microorganisms including mesophiles and thermophiles to ensure that spoilage and food poisoning is eradicated [16][17]. The conventional method for evaluating the efficiency of thermal processing is dependent on the thermal value/lethality value or sterilization value F_0 (F-value), which is then defined as the time (minutes) required to eliminate a known population of the resistant bacterial population in a given food under specified conditions [18][19]. It is also usually calculated as 12 D, which is the time needed for a 12 log reduction of thermally-resistant mesophiles, most commonly, *Clostridium botulinum* spores. *Clostridium sporogenes* have been used as the biological indicator for evaluating the microbiological efficiency of sterilization processes [20] due to their high thermal resistance and absence of any toxic genes, unlike *C. botulinum*. Thermal resistance is measured using decimal reduction time or the D value, which can be defined as the time required at any specific temperature to achieve inactivation equivalent to 1 log CFU/mL [21][22]. D values for *C. sporogenes* at 121 °C have been reported to be 0.5 min in phosphate buffer (pH 7.0) [23]. The thermal resistance of bacterial spores can vary significantly based on many factors. For example, the environmental conditions pertaining to both the spore induction and spore destruction process, the water activity and moisture content of the food being treated, and the pH, salt content and methods being used for D value assessments [24][25][26][27]. Therefore, based on the D value of the biological indicator spores in a specific type of food product, their F_0 values are estimated. The F_0 values for vegetable and fruit products could therefore vary significantly.

2.2. Pasteurization of Fruits and Vegetables

Pasteurization was originally invented by French scientist Louis Pasteur, who invented the process of heating liquids at a temperature of about 55 °C for a short-defined time to eliminate bacterial contaminants [28]. With time, pasteurization became a common process in the dairy industry, and milk pasteurization can be either slow or fast. A slow process uses temperature-time combinations of 63 to 65 °C for over 30 min or 75 °C for 8 to 10 min. On the other hand, fast/rapid pasteurization uses a time-temperature combination of 85 to 90 °C or for up to 15 s [29]. Vegetables would in general be considered low acid foods and, therefore, need an efficient treatment to inactivate pathogens such as *L. monocytogenes*, which are pathogens of concern. *L. monocytogenes* has been reported to be present in either raw or minimally processed vegetables on multiple occasions, and the route of contamination is not completely known but is presumed to be soil, faeces or water [30][31][32][33]. Most of the research published has included pasteurization as a comparative standard method to see the efficiency of non-thermal technologies. For example, a study by Kathiravan, et al. [34] reported the effect of various combinations of time and temperature for pasteurization on bioactive components as well as the inactivation of native microflora in beetroot juice. The results indicated that pasteurization at 96 °C for a total heating time of 720 s resulted in the maximum retention of bioactive compounds such as betacyanin and betaxanthin while inactivating the native microflora [34]. It has been reported that juices (for example, cantaloupe juice and watermelon juice) can result in cross-protection to heat due to acid stress, thereby increasing the D values of foodborne pathogens such as *Salmonella*, *E. coli* O157:H7 and *L. monocytogenes* [35]. Another study reported similar heat resistance in *E. coli* O157:H7 E0139 in acid-adapted apple cider and orange juice, thereby resulting in an up to two times increase in D_{52} °C values [36]. Therefore, it can be concluded that the time and temperature combination of pasteurization could be dependent on the fruit and vegetable matrix, including characteristics such as moisture content and pH, and the intrinsic resistance of the bacterial species being targeted. While bacterial spore formers are known to have higher D values as compared to the non-spore forming vegetative bacterial strains, there is a significant difference in the D values among different strains of same bacterial species in various food products.

3. Alternative Approaches Involving Moderate Thermal Treatment and Hurdle Approaches for Fruits and Vegetables

The complex challenge of ensuring food safety, along with an attempt to preserve the maximum fresh-like attributes of fruits and vegetable products, has led food manufacturing companies to invest in research associated with mild to moderate thermal interventions that could be combined with non-thermal techniques to deliver similar lethality to that of pasteurization or, in some cases, sterilization. While there is no single alternative to thermal technologies/sterilization, using a combination of more than one technique, such as irradiation + heat, pressure + heat, electroporation + heat and microwave processing + heat, has recently gained significant attention due to their promising potential.

3.1. Pulsed Electric Field (PEF) Treatment and Thermal Processing

PEF treatment of food involves the dispatch of short pulses of short and high voltage to achieve electric field strengths of 15–35 kV/cm at specific energies (50–700 kJ/kg) through the food to induce the formation of pores in the outer membrane of microbial cells [37]. The application of field strengths between the electrodes leads to the formation of transmembrane potential differences over the cellular membrane, thereby leading to pore formation, which could be either reversible or irreversible [38][39]. When this potential difference exceeds a critical value, pore formation occurs in the membrane of the cells. Although PEF is considered a non-thermal technology for the extraction of bioactive food components from fruit and vegetable products, microbial inactivation has only been reported to be successful with the use of moderate temperatures (<50 °C) [40][41]. PEF treatment has many benefits over using conventional pasteurization or sterilization. For example, the use of moderate heating minimizes the loss of organoleptic properties, as well as prevents denaturation of the heat-sensitive vitamins and bioactive compounds in fruits and vegetables. However, the use of PEF for the inactivation of bacterial spores has not been successful unless the overall treatment (pre or post or during) of the system reaches more than 80 °C [42][43].

There are a few limitations that have prevented PEF being extensively used in the fruit and vegetable industries. The first limitation is around the high cost and energy requirements for the generation of the high-voltage pulses that are required to deliver sufficient power to process products in large quantities as well as in a continuous application [37]. For microbial inactivation, that is equivalent, when compared to pasteurization, to the treatment either having to employ high electric field strengths between 25 and 35 kV/cm for a longer treatment time or to use a pretreatment with mild to moderate heat, which can further increase the energy inputs and, hence, the overall cost. However, PEF has been successful in improving the extraction of phenolic compounds in fruit mass (juice or wine) and in controlling spoilage microorganisms as long as the initial bacterial load is not high [37][44][45]. However, further research on using hurdles to reduce the cost of PEF processing to achieve 5–6 Log CFU/mL of non-spore-forming bacterial populations is required.

3.2. Pressure-Assisted Thermal Processing (PATP)

PATP is a food processing method that combines the effect of pressure (600 to 900 MPa) and heat (90 to 121 °C) to inactivate bacterial pathogens in food with a reduced effect on heat-sensitive nutrients [46][47]. In comparison to the conventional heating (retorting or pasteurization) process, PATP is known to reduce the processing time due to the mechanism of adiabatic compression due to applied pressure.

Pressure-assisted thermal sterilization (PATS) is a type of PATP, which is also accepted by the Food and Drug Administration (FDA, Silver Spring, MD, USA) in the U.S as a thermal sterilization regime for shelf-stable low acid food products ((pH > 4.6) [48]. PATS uses a temperature over 100 °C and high pressure above 600 MPa to inactivate bacterial spore formers in food and generate a shelf-stable product with maximum retention of the organoleptic properties and nutrients [49]. These terms have been interchangeably used in the literature to indicate the combined use of thermal processing and high-pressure processing. The mechanism of action during bacterial inactivation by PATS is a synergistic combination of high temperature and high pressure to generate an adiabatic compression heat, which in turn affects the cellular architecture of the bacterial cell [47]. This further leads to functional damages in the cell such as an increase in cell membrane permeability, alerted structure and confirmations of the organelles modifications in the biochemical reactions, and, ultimately, cell death [50]. The alteration of the cell membrane structure through damage to proteins and the phospholipids bilayer, and therefore loss of the integral composition, has been known to be the major cause of cell death [51]. This process is accelerated by the high temperature, which alters the structural conformation of the proteins and lipids involved in the cellular structures or functions [52].

PATP has already been commercialized and is used for a processing treatment to deliver lethality equivalent to the pasteurization of fruit and vegetable soups, juices and purees. However, there exists a challenge of achieving the compression heating that is able to hold the required pressure to complete the treatment in an insulated setting so that the heat and pressure can simultaneously be used for achieving maximum microbial inactivation in a minimum time [53][54]. In addition, the cost of achieving a high pressurization rate, using a vessel material with low heat-transfer properties such as polyoxymethylene (POM) or polyether ether ketone (PEEK), and installing an internal intensifier system to prevent heat losses is another of the challenges faced by many food industries [55].

3.3. Microwave-Assisted Thermal Sterilization (MATS)

MATS technology employs a combination of both thermal (convection) and microwave energy (conduction) to sterilize food in polymeric packages to ensure microbial inactivation that is considered equivalent to thermal sterilization; however, with reduced loss of sensitive nutrients unlike thermal sterilization [16][56]. MATS has also been approved by the Food and Drug Administration (FDA, Silver Spring, MD, USA) for the sterilization of homogeneous and non-homogeneous foods [56]. In this process, the food (in polymeric packages) is kept submerged in hot water at 121 °C and treated using microwaves (915 MHz) under pressurized hot water to achieve the desired F_0 to eliminate bacterial contaminants [56]. This technology is relatively new and is mostly used for ready-to-eat food products that have vegetable and fruit portions.

The benefit of MATS over thermal sterilization is its reduced processing time, which can thereby reduce nutrient loss. Microbial inactivation for commercial sterilization aims at an F_0 of 3 (**Table 1**) or more [57].

Microwave sterilization offers several benefits over conventional methods; however, there are a few challenges yet to overcome. Since water is not the heating medium in this process of microwave sterilization, the temperature remains stable at a preset point, and therefore any over-processing or increase in the final temperature of the processed product can be reduced. However, the process of microwave-based heating is largely dependent on the dielectric property of food, especially the dielectric constant and dielectric loss factor, which determines the behaviour and interaction of food with electromagnetic fields [58][59]. Dielectric properties are dependent on the moisture, salt and fat content of the food product [60] and further on the temperature and frequency used in the treatment [56]. The challenge of non-uniform temperature distribution in conventional household microwave ovens has been overcome by the microwave sterilization regime to a great extent [61].

4. Summary

Thermal sterilization and pasteurization of fruit and vegetable products offer the safest options for microbial food safety. However, they also lead to significant losses in nutrients such as Vitamin C and bioactive compounds such as carotene, which makes the food shelf-stable but also less nutritious. For the purpose of achieving the target of sterilization as well as nutrient retention, novel technologies have now been combined with thermal treatment to achieve maximum lethal value in the minimum time of exposure. The hurdle approach or a combination of more than one technique to ensure microbial inactivation could contribute toward a medium to longer shelf life and nutrient retention.

While novel technologies including PEF and PATP have long been investigated against conventional processing, MATS remains in its early research stage. Their industrial implementation is challenging due to more than one reason. For example, the design of the instrumentation might significantly differ from one laboratory/manufacturer to another, therefore making the direct comparison of results very difficult. As these novel technologies are currently being majorly investigated as a research tool in pilot-scale laboratories, their production costs, as well as their feasibility for integration in line with existing techniques, are either higher than conventional thermal technologies or are unclear. Furthermore, if these technologies replace conventional methods, the overall impact on the environment, the economy, energy consumption and food wastage might be linked to acceptance by consumers. Therefore, further research on using the novel processing techniques at an industrial scale followed by investigations on the impact of their usage in the social, economical and financial areas will increase their sustainable applications in the food industries.

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