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ABSTRACT

Increasing consumer desire for high quality ready-to-eat foods makes thermal pasteurization important to both food producers and researchers. To be in compliance with the Food Safety Modernization Act (FSMA), food companies seek regulatory and scientific guidelines to ensure that their products are safe. Clearly understanding the regulations for chilled or frozen foods is of fundamental importance to the design of thermal pasteurization processes for vegetables that meet food safety requirements. This article provides an overview of the current regulations and guidelines for pasteurization in the U.S. and in Europe for control of bacterial pathogens. Poorly understood viral pathogens, in terms of their survival in thermal treatments, are an increasing concern for both food safety regulators and scientists. New data on heat resistance of viruses in different foods are summarized. Food quality attributes are sensitive to thermal degradation. A review of thermal kinetics of inactivation of quality-related enzymes in vegetables and the effects of thermal pasteurization on vegetable quality is presented. The review also discusses shelf-life of thermally pasteurized vegetables.

Introduction

Vegetables are important components for a balanced and healthy diet; they provide essential vitamins, minerals and dietary fiber for our bodies. A range of phytochemicals commonly found in vegetables, such as flavonoids, phenols, and carotenoids, prevent nutritional deficiencies and reduce the risk for various types of cancer, heart disease, diabetes, diverticulosis, stroke, hypertension, birth defects, cataracts, and obesity (Scheerens, 2001; Van Duyn, 1999). However, vegetables are highly perishable and need appropriate preservation technologies to prolong shelf life while maintaining nutritional and sensory qualities.

Emerging alternative technologies are being adopted in addition to traditional thermal processing, thus the term "pasteurization" has been broadened. The requisite scientific parameters for designing a thermal pasteurization process have become more important and need to be clear. Two recently published reviews have provided insights into designing pasteurization processes using 2 particular pathogens in foods as references, namely, nonproteolytic *Clostridium botulinum* spores and *Salmonella* (Silva and Gibbs, 2010, 2012). The reviews by Aamir et al. (2013) and Ling et al. (2015) cover the common and special kinetic models to describe major degradation of food quality attributes during thermal processing. The food industry worldwide has shown strong interest in novel thermal pasteurization processes that can satisfy the increased consumer desire for high quality ready-to-eat meals while

meeting food safety standards. One of the major aims of this review is to highlight the regulations or guidelines associated with food pasteurization by governmental agencies in the U.S. and Europe, and critical factors for designing a pasteurization process accordingly. Current knowledge of food safety regulators and scientists on emerging foodborne pathogens are reviewed, particularly the thermal resistance of several human norovirus surrogates. We will also provide an overview of recent findings on how thermal pasteurization affects vegetable quality, including color, texture, carotenoids, phenolics, antioxidant activity, vitamins and other attributes. In addition, enzyme inactivation and storage conditions which are closely related to the quality of pasteurized vegetables are also discussed here. Technical information provided in this review is related to the thermal processing of fresh and high moisture foods and vegetables.

Development of pasteurization concept

The word "pasteurization" was originally named after the French scientist Louis Pasteur, who invented the process of heating liquids (wine and beer) at a relatively mild temperature (about 55°C) for a short time to prevent spoilage (Silva et al., 2014; Wilbey, 2014). This quick heat method was known as pasteurization and was later applied to milk to kill pathogenic microbes and reduce the number of spoilage organisms. Thus, traditional pasteurization refers to heat

treatment of food (usually below 100°C) to destroy micro-organisms of public health significance. Pasteurization processes used in the industry do not kill all micro-organisms in foods; they only target pertinent pathogens and reduce spoilage organisms that may grow during storage and distribution (Silva and Gibbs, 2010).

Nowadays pasteurization has been widely accepted as an effective preservation method for killing pathogens in food products, with minimal loss of desired food quality. New technologies that can satisfy the goals of pasteurization have grown rapidly in recent years. The development of these emerging technologies calls for a broadening of the definition of pasteurization. However, there does not appear to be a universally accepted definition for pasteurization. In the United States, the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) has determined the requisite scientific parameters for establishing equivalent alternative methods of pasteurization, and defined pasteurization as "Any process, treatment, or combination thereof that is applied to food to reduce the most resistant microorganism(s) of public health significance to a level that is not likely to present a public health risk under normal conditions of distribution and storage" (NACMCF, 2006). This definition allows application of a broad range of technologies (one or in combination) to different foods for pasteurization, and includes thermal (steam and hot water heating, ohmic heating, microwave heating, infrared processing, etc.) and nonthermal technologies (high pressure processing, ultraviolet radiation, irradiation, pulsed electric field, chemical treatments, ultrasound, filtration, high voltage arc discharge, etc.). In addition to processing method, the following considerations should be included in developing a pasteurization process:

(1) Determining the most resistant microorganism of public health concern that is likely to survive the selected pasteurization processes;
(2) Assessing and validating the required level of inactivation of the target microorganism, to make sure it is "not likely to present a public health risk";
(3) Evaluating appropriate distribution and storage temperature and shelf life;
(4) Considering the impact of the food matrix on pathogen survival;
(5) Defining the specific equipment and operating parameters for the proposed pasteurization process.

Kinetic models and parameters used in design of thermal pasteurization processes

Proper design of industrial thermal pasteurization processes requires knowledge of target microorganism population changes with time at elevated temperatures. It is also desirable to understand thermal impact on important quality attributes. Kinetic models have been widely used to quantify the inactivation of microorganisms and enzymes as well as quality changes in foods caused by thermal processes (Ling et al., 2015; Steinfeld et al., 1998; Van Boekel, 2008). A good starting point for development of kinetic models is the general rate law:

$$-\frac{dC}{dt} = kC^n, \tag{1}$$

where t is the reaction time, k is the rate constant, n is the order of reaction, and C is the quantity of a chemical compound, quality attribute, enzyme activity or microbial population. Integrating Eq. (1) over time t yields:

$$For\ n = 1, C = C_0\ e^{-kt}, \tag{2}$$
$$For\ n \neq 1,\ C^{1-n} = C_0^{1-n} + (n-1)kt. \tag{3}$$

C_0 is the initial quantity. The Arrhenius equation is frequently used to describe the temperature effect on the rate constant k of a reaction:

$$k = Ae^{-\frac{E_a}{RT}}, \tag{4}$$

where A is a pre-exponential factor, E_a is activation energy (J/mol), R is the universal gas constant (8.314 J/(mol K)), and T is the absolute temperature (K).

The Arrhenius equation can be alternatively expressed as:

$$k = k_{ref}\ e^{-\frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)} \tag{5}$$

where T_{ref} represents the reference temperature (K) and k_{ref} is the reaction rate at the reference temperature.

Two other kinetic parameters, D-and z-values are often used to describe thermal resistance of microorganisms. D-value is the time required at a certain temperature for a one-log reduction (change) in the microorganism population, enzyme activity or quality index; while z-value is the change in temperature required for a 10-fold reduction of the D-value, representing the sensitivity of a reaction to temperature changes.

Thermal kinetic parameters (such as $n,\ k$ and E_a) in the above equations can be obtained by fitting the models with the experimental data using either one-step or two-step regression methods (Ling et al., 2015). Reaction kinetics in food systems under isothermal conditions (that is at constant temperatures) generally follows a zero, first-or second-order reaction. However, simple reaction models are not always adequate to describe the changes in microorganisms, enzymes or quality in foods. Alternative models such as biphasic first-order, fraction conversion, and Weibull models can provide a better fit (Bozkurt et al., 2014a,b,d; Morales-Blancas et al., 2002).

Kinetic parameters are usually obtained from experiments under isothermal conditions. However, industrial thermal processes are dynamic, in which product temperature changes with time. In order to apply the kinetic data obtained from an isotherm test to predict enzyme inactivation or quality losses during a dynamic thermal process, one can combine the Arrhenius equation with the kinetic model to obtain an integrated equation. This integrated equation can be used to estimate the overall influence of a dynamic thermal process on the activity of an enzyme or quality attributes in foods. Taking the 1st order reaction as an example and substituting alternative Arrhenius

equation Eq. (5) to Eq. (1) ($n = 1$ for the 1st order model) yields:

$$-\frac{dC}{dt} = k_{ref}\left[e^{-\frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)}\right]C. \qquad (6)$$

Integrating both sides of Eq. (6), it becomes:

$$ln\,\frac{C(t)}{C_0} = -\int_0^t k_{ref}\left[e^{-\frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)}\right]dt, \qquad (7)$$

where C_0 is the initial quantity for enzyme activity or food quality. The residual enzyme or quality attributes in foods can be calculated by numerical integration of Eq. (7) using the temperature-time history $T(t)$ at a representative location in the food. The representative location should be the least heated spot (cold spot) for enzyme inactivation, the hottest spot (hot spot) for quality degradation. Quality losses can also be calculated for each location and summarized over the entire food volume to assess the overall quality losses.

For food safety, the concept of equivalent time at a reference temperature is used to reflect the cumulative effect of industrial thermal processes. Commercial pasteurization processes are designed to ensure that the accumulated lethal effect at the cold spot in products exceeds a minimum time at a reference temperature specified in regulatory guidance for targeted food pathogens. The equivalent lethal time of a pasteurization process, known as pasteurization value and represented by F, can be calculated by the following equation (FDA, 2011b; Silva and Gibbs, 2010):

$$F = \int_0^t 10^{\frac{T - T_{ref}}{z}}dt\,, \qquad (8)$$

where T (°C) is the temperature measured at the cold spot (the area that receives the lowest thermal energy) at time t during a process, T_{ref} is the reference temperature, and z characterizes the sensitivity of thermal resistance of the target bacteria to temperature changes. The reference temperature for pasteurization is usually selected as 70 or 90°C (ECFF, 2006).

Pathogens of concern for thermal pasteurization

Determining the most resistant microorganism of public health concern for a pasteurization process is of paramount importance. Table 1 lists the primary pathogens of concern and their general growth conditions (ECEF, 2006). Based on Table 1, the optimum refrigerated storage temperature is ≤5°C (41°F) to eliminate the possibility of growth of most pathogens. However, as much as 10% of product temperatures in consumer refrigerators in the United States are above 7.2°C (45°F); and temperatures above 10°C (50°F) for the shelf life of the product would be considered as gross abuse for most refrigerated foods (Audits International, 1999; NACMCF, 2006). Considering the normal distribution and storage temperatures for pasteurized foods with some temperature margin,

pathogens with a minimum growth temperature lower than 7.2°C (45°F) should be selected as potential pathogens in process design and be included in the hazard analyses. *Listeria monocytogenes, Bacillus cereus,* nonproteolytic *Clostridium botulinum, Escherichia coli* O157:H7, *Salmonella, Staphylococcus aureus, Vibrio parahaemolyticus* and *Yersinia enterocolitica* all have minimum growth temperatures of less than 7.2°C (Table 1). The temperatures to cause one-log reduction of those psychrotrophic microorganisms within 1 min are summarized in Table 2. Spore-forming bacteria *C. botulinum* (nonproteolytic type B) and *B. cereus,* which might cause outbreaks in low-acid under-pasteurized foods, exhibited the highest heat resistance (highest *D*-values); a temperature less than 85°C is not adequate to yield even a one-log reduction after several minutes of processing time. But within 1 min at 70°C was sufficient to achieve a one-log reduction of all the other vegetative bacteria, such as *L. monocytogenes, E. coli, Salmonella* spp., and *C. burnetii.* Conversely a few seconds at 60°C is enough to have 90% of *Aeromonas hydrophila* and yeast, which have the lowest heat resistance, inactivated. The thermal resistance of viral pathogens is not included in this table, but is discussed below in Section "Seafood."

Regulations and guidelines for thermal pasteurization in the United States

The Food Safety Modernization Act (FSMA, 2011) requires that preventive controls such as pasteurization be implemented to significantly minimize or prevent the hazards identified in a hazard analysis. Preventive controls will need to be validated. Under the Federal Food Drug and Cosmetic Act (FFDCA, Title 21 section 403(h), 1938), a food shall be deemed to be misbranded as pasteurized unless it is subject to a "safe process," prescribed as pasteurization in a regulation, that is reasonably likely to destroy organisms of public health significance, is at least as effective as the process specified by regulation and is effective throughout the shelf-life of the product when stored under conditions of normal and moderate abuse. Under FFDCA section 403(h), the manufacturer must submit a notification to FDA with data showing effectiveness of the process before labeling a product as "pasteurized."

Table 1. Commonly accepted growth boundaries of pathogenic microorganisms (ECEF 2006).

Microorganism	Min temp (°C)	Min pH	Min a_w	Aerobic/anaerobic
L. monocytogens	−0.4	4.3	0.92	Facultative
B. cereus	4	4.5	0.93	Facultative
Campylobacter jejuni	32	4.9	0.99	Microaerophilic
C. botulinum (Mesophilic/proteolytic)	10–12	4.6	0.93	Anaerobic
C. botulinum (Psychrotrophic/nonproteolytic)	3.3	5.0	0.97 (5% NaCl)	Anaerobic
C. perfringens	12	5.5–5.8	0.935	Anaerobic
E. coli	7–8	4.4	0.95	Facultative
E. coli O157:H7	6.5	4.5	0.95	Facultative
Salmonella	6	4.0	0.94	Facultative
Staphylococcus aureus	5.2	4.5	0.86	Facultative
V. cholera	10	5.0	0.97	Facultative
V. parahaemolyticus	5	4.8	0.94	Facultative
Y. enterocolitica	−1.3	4.2	0.96	Facultative

Table 2. D-values of less than 1 min (0.1–1 min) for psychotropic microbes under pasteurization temperatures (■ spore-forming pathogens; ▨ nonspore forming pathogens; □ nonspore forming spoilage microbes; from Silva and Gibbs (2010) with modification).

Psychrotropic microbes: D-values of < 60 sec at									
55°C	60°C	65°C	70°C	75°C	80°C	85°C	90°C	95°C	>95°C
	Listeria monocytogenes								Bacillus cereus
Escherichia coli						Clostridium botulinum (nonproteolytic type B)			
Salmonella spp.									
	Yersinia enterocolitica								
Aeromonas hydrophila									
	Mycobacterium avium		Coxiella burnetii						
Lactic acid bacteria									
Yeast									

References in the table include: Ahmed et al. (1995), Bolton et al. (2000), Buduamoako et al., (1992), Cerf & Condron (2006), De-Angelis et al. (2004), Fernández et al. (2001), Franz & vonHoly (1996), Gaze & Brown (1990), Jin et al. (2008), Juneja et al. (1997 & 2007), Juneja & Marmer (1999), Jung & Beuchat (2000), Keswani & Frank (1998), Huang et al. (1992), Miettinen et al. (2005), Murphy et al. (2004a, b), Oteiza et al. (2003), Pearce et al. (2001), Selby et al. (2006), Schuman et al. (1997), Schuman & Sheldon (1997), Thomas et al. (1966), Toora et al. (1992).

At the present, there is no specific guideline available for commercial pasteurization of vegetables in the U.S., because the presence of pertinent bacteria is dependent upon food characteristics, composition (e.g., pH, a_w, salt concentration), and pre-and post-harvest environments; and thermal resistance of bacteria may also vary with different processing technologies. However, there are regulations and standards in place specifically for thermal pasteurization of milk, seafood, egg, and juice products (Table 3) that were promulgated by the responsible governmental authority, either the U.S. Department of Agriculture (USDA) or the U.S. Food and Drug Administration (FDA). It is beneficial to review standards for some of these products.

Milk

Currently, processing of milk is governed by the FDA Pasteurized Milk Ordinance (PMO), and is based on 2 fundamental principles: (1) every particle must be heated to a specified minimum temperature for a specified time and (2) equipment is properly designed and operated. The first federal standard for milk pasteurization was established in 1924, requiring a 61.7°C/30 min process, targeting *Mycobacterium tuberculosis* (Meanwell, 1927). In 1956, *Coxiella burnetii* was recognized as the most resistant bacteria of concern, and the current minimum pasteurization time and temperature combinations (63°C/30 min or 72°C/15 s) were established. Later, Enright (1961) demonstrated a more rigorous pasteurization treatment was needed for 3 milk products: cream, chocolate milk and ice cream mix (Table 3).

Seafood

The pasteurization of seafood is governed by the FDA "Fish and fisheries products hazards and control guidance" (FDA, 2011b). FDA considers a 6D (meaning 6 log reduction) process for *C. botulinum* (type E and nonproteolytic types B and F) to be generally suitable for pasteurized seafood products. In designing a thermal process, a minimum cumulative total lethality of $F_{90°C}$ (i.e., equivalent accumulated time at 90°C = 10 min) is adequate for pasteurized fish and fishery products (Table 3). For blue crabmeat, the National Blue Crab Industry Pasteurization and Alternative Thermal Processing Standards requires a process of $F_{85°C} = 31$ min, which exceeds a 12-log reduction of *C. botulinum* type E spores. Products like Dungeness crabmeat require more rigorous heating ($F_{90°C} = 57$ min) because it contains certain substances (e.g., lysozyme) that may enable the pathogen to recover more easily after heat damage (FDA, 2011b).

Egg and egg products

Under USDA Food Safety and Inspection Service (FSIS) regulations 9 CFR 590 (CFR, 2012a) the term pasteurize is defined as the "subjecting of each particle of egg products to heat or other treatments to destroy harmful viable microorganisms." Further this regulation stipulates a "Salmonella negative product" with specific time/temperature requirements given for liquid egg products. FDA regulations 21 CFR 118 (CFR, 2009) stipulate a "…process that achieves at least a 5-log destruction of *Salmonella* for shell eggs, or the processing of egg products in accordance with the Egg Products Inspection Act." Specific processing conditions for eggs and egg products are listed in Table 3.

Juice

The pasteurization of juice is regulated by the FDA regulation 21 CFR 120, Hazard Analysis and Critical Control Point (HACCP) systems (CFR, 2011). A process with a minimum 5-log reduction of the most resistant microorganism of public health significance identified as the pertinent pathogen under HACCP plan is required. The target microorganisms are dependent on the juice product and process, including *E. coli* O157:H7, *Salmonella, Cryptosporidium parvum* or *C. botulinum.* For acidic juices (pH < 4.6), *E.coli* O157:H7, *Salmonella*, and *Cryptosporidium parvum* may occur and cause serious foodborne illness outbreaks; while for low-acid juices such as carrot juice, *C. botulinum* may be present and produce toxins, and therefore becomes the pathogen hazard of concern (CDC, 2006; FDA, 2004).

In summary, the definition of pasteurization in the United States is broad. Regulations and guidelines associated with the pathogens of concern and processing conditions for pasteurization are specific, depending on the particular product, process conditions and packaging systems. There is no "one size fits all" approach to achieving microbiological safety for pasteurization of foods in the United States.

Table 3. Summary of regulations and guidelines for thermal pasteurization of foods (milk, seafood, egg and juice products) in the United States.

	Related regulations & guidelines in the U.S.	References
Category I: Milk	The pasteurization of milk is governed by FDA Pasteurized Milk Ordinance. The target microorganism originally was *Mycobacterium tuberculosis*is (1924), now it is *Coxiella burnetii* (since 1956). A process to eliminate 100, 000 guinea pig infectious doses is needed.	FDA, 2011a

Examples	Target Bacteria	Processing requirements	References
Milk, 1956-present	*C. burnetii*	63°C (145°F) for 30 min for batch process; 72°C (161°F) for 15 s for HTST	Enright et al., 1957
Cream; Chocolate milk	*C. burnetii*	66°C (150°F) for 30 min for batch process; 75°C (166°F) for 15 s for HTST	Enright, 1961
Ice cream mix	*C. burnetii*	69°C(155°F) for 30 min for batch process, 80°C (175°F) for 25 s for HTST	Enright, 1961

	Related regulations& guidelines in the U.S.	References
Category II: Seafood	Pasteurization of seafood is governed by FDA (Fish and fisheries products hazards and control guidance). FDA considers a 6D process for target *C. botulinum* (type E and nonproteolytic types B and F) to be generally suitable for pasteurized seafood products.	FDA, 2011b

Examples	Target bacteria	Processing requirements	References
Fish and fishery products generally (e.g., surimi-based products, soups or sauces)	*C. botulinum* type E and nonproteolytic types B and F	$F_{90°C} = 10$ min, z value is 7°C for temperatures less than 90°C, 10°C for temperatures above 90°C.	FDA, 2011b
Blue crabmeat	*C. botulinum* type E and nonproteolytic types B and F	$F_{85°C} = 31$ min, Z value is 9°C.	FDA, 2011b
Dungeness crabmeat	*C. botulinum* type E and nonproteolytic types B and F	$F_{90°C} = 57$ min, Z value is 8.6°C.	FDA, 2011b

	Related regulations& guidelines in the U.S.	References
Category III: Egg products	The current processing of egg products is governed by FSIS regulations 9 CFR 590. A process achieving at least a 5-log reduction of *Salmonella* is required..	CFR, 2012a

Examples	Products	Processing requirements (Min temp & Min holding time)	References
Liquid eggs	Albumen (w/o use of chemicals)	56.7°C (134°F) for 3.5 min or 55.6 (132°F) for 6.2 min	CFR, 2012b
	Whole egg	60°C (140°F) for 3.5 min	CFR, 2012b
	Whole egg blends (<2% added nonegg ingredients); sugar whole egg (2–12% sugar added); and plain yolk	61.1°C (142°F) for 3.5 min or 60°C (140°F) for 6.2 min	CFR, 2012b
	Fortified whole egg and blends (24–38% egg solids, 2–12% added nonegg ingredients)	62.2°C (144°F) for 3.5 min or 61.1°C (142°F) for 6.2 min	CFR, 2012b
	Salt whole egg (≥2% salt added); sugar yolk (≥ 2% sugar added); and salt yolk (2–12% salt added)	63.3°C (146°F) for 3.5 min or 62.2°C (144°F) for 6.2 min	CFR, 2012b
Dried egg whites	Spray-dried albumen	54.4°C (130°F) for 7 days	CFR, 2012c
	Pan-dried albumen	51.7°C (125°F) for 5 days	CFR, 2012c

	Related regulations& guidelines in the U.S.	References
Category IV: Juice[1]	The pasteurization of juice is governed by FDA (2011. 21 CFR 120.24). A process of 5-log r eduction of most resistant microorganism of public health significance under HACCP plan is required. The target bacteria are dependent on the juice product and process, including *E. coli* O157: H7, *Salmonella*, *Cryptosporidium parvum* or *C. botulinum*.	CFR, 2011; FDA, 2004

Examples	Target bacteria	References
Acidic juice (pH≤ 4.6)	*E.coli* O157:H7, *Salmonella*, and *Cryptosporidium parvum*	FDA, 2004
Low-acid juices (pH > 4.6)	*C. botulinum*	FDA, 2004

[1]Juice is defined by FDA as the aqueous liquid expressed or extracted from one or more fruits or vegetables, or concentrates of such liquids or purees.

Regulations and guidelines for thermal pasteurization in Europe

Refrigerated (or chilled) ready-to-eat meals have long been popular in Europe. The European Chilled Food Federation provides guidance for producing chilled foods in Europe (ECFF, 2006). ECFF defines chilled food as "foods that for reasons of safety and/or quality rely on storage at refrigeration temperatures throughout their entire shelf-life." According to ECFF recommendations, the common practice for heat-treated chilled food is to aim for a 6 log reduction of either (Table 4):

(1) *L. monocytogenes* (this treatment will control other vegetative pathogens).
(2) Cold growing *C. botulinum* (this treatment will not control other spore-forming pathogens such as *B. cereus*).

Table 4. Guidelines for pre-packaged chilled foods (pasteurized foods) in Europe (CSIRO, 2010; ECFF, 2006).

Products	Target bacteria	Processing requirements	Shelf-life
Heat-treated chilled foods[1]	*L. monocytogenes*	6D reduction, common practice of $F_{70°C} = 2.0$ min is considered suitable.	≤ 10 days at 5°C
	Nonproteolytic C. botulinum	6D reduction, common practice of $F_{90°C} = 10.0$ min is universally accepted	Up to 6 weeks at 5°C

[1]Chilled food: foods that for reasons of safety and/or quality rely on storage at refrigeration temperatures throughout their entire shelf life.

L. monocytogenes is the most heat-resistant vegetative pathogen while Type B is the most heat resistant form of nonproteolytic *C. botulinum*. It is generally accepted that a mild pasteurization of low-acid food ($F_{70°C} = 2.0$ min) achieving 6 log reduction of *L. monocytogenes* is suitable for a shelf life of maximally 10 days at 5°C. A severe pasteurization process of $F_{90°C} = 10.0$ min aiming at a 6D process inactivation of nonproteolytic *C. botulinum* allows a product shelf life of up to 6 weeks at 5°C (ECFF, 2006; CSIRO Food and Nutritional Sciences, 2010).

The European Parliament and the Council of the European Union regulation on the hygiene of foodstuffs (EC, 2004) states that for heat treated foods in hermetically sealed containers, any heat treatment process used for an unprocessed product should conform to an internationally recognized standard (e.g., pasteurization) and raise every particle of the product treated to a given temperature for a given period of time.

Apart from the above processing parameters suggested by EFEC for heat-treated chilled foods, commercial production of safe chilled foods should also include the formulation and preservation factors. Variability in the finished product (e.g., pH, a_w) and processing conditions, appropriate hygiene, storage conditions, and usage instructions are important considerations for safety control and in designing the thermal process and assigning shelf-life.

Viral pathogens

Though they are the common cause of foodborne diseases, pathogenic viruses have received far less attention by regulators and in food process design as compared to bacterial pathogens. Foodborne viruses typically are stable outside the host, surviving reasonably well in adverse conditions. Virus transmission can occur by consuming foods that have been contaminated by infected food handlers, during the production process or by the food handling environment. Some viruses associated with food diseases include the human noroviruses (formerly named Norwalk-like viruses), hepatitis A virus (HAV), hepatitis E virus (HEV), rotaviruses, sapoviruses, Aichi virus, astroviruses, adenoviruses, parvoviruses and other small round viruses (Hirneisen et al., 2010; Horm & D'Souza, 2011; Koopmans & Duizer, 2004; Sair et al., 2002). Of these, the noroviruses which cause gastroenteritis, and HAV which causes hepatitis, are currently recognized as the most important 2 foodborne viruses, in terms of the total number of outbreaks and people affected in the Western world. Based on the Centers for Disease Control and Prevention estimates of foodborne illness in the United States, norovirus ranks as the top pathogen contributing to domestically acquired food illnesses, the second resulting in hospitalizations (next to *Salmonella*) and the fourth resulting in death (CDC, 2011). Due to the inability to culture the human norovirus *in vitro*, knowledge about the biology and data on survival of this virus is still limited. No international standard

methods exist for viral analysis of foods, and prevalence studies are limited. The lack of quantitative data on viral load makes the establishment of microbiological criteria for viruses in foods difficult (EFSA, 2011). Current European Food Safety Authority (EFSA) recommendations focus controls on preventive measures to avoid viral contamination rather than trying to inactivate these viruses from food (EFSA, 2011). However, most of the current food hygiene guidelines are optimized for prevention of bacterial infections, and may not be effective against viruses (Koopmans and Duizer, 2004).

Heating appears to be one of the most efficient means for the inactivation of norovirus and hepatitis A & E, despite attempts to use acidification, high pressure processing, chemical disinfection and UV irradiation (Deboosere et al., 2010; Johne et al., 2016). Murine norovirus, feline calicivirus, HAV, canine calcivirus and coliphage MS2 have been used as human norovirus surrogates in various food matrices for survival studies at temperatures relevant to thermal pasteurization. The heat tolerance and survival data of human norovirus surrogates and HAV are summarized in Table 5. These results show that thermal treatment at 60°C for between 1 and 3 min could lead to a one-log reduction of murine norovirus in some tested foods (spinach, raspberry, mussel, milk and water). It also took less than several minutes at 60°C to kill one log of feline calicivirus surrogates based on most reported data (Cannon et al., 2006; Croci et al., 2012). For HAV, most authors found that several minutes of heating at 60°C were sufficient to achieve a one-log decrease in infectivity titer. However, Harlow et al. (2011) reported the *D*-value at 60°C of HAV in mussel to be 109 min; and Gibson and Schwab (2011) reported that the *D*-value at 60°C of HAV in phosphate-buffered saline was 74.6 min. The *z*-values of those viruses fell in the range of 10–20°C, as compared to ∼10°C for bacterial spores (e.g., *C. botulinum*) and ∼7°C for vegetative bacterial pathogens (e.g., *L. monocytogenes*) (see Table 6).

Thermal inactivation of viruses might be attributed to the changes in the capsid of the virus particle. When the temperature reaches higher than 56–60°C, the capsid protein of the virus can be denatured and broken down, which accelerates the inactivation rate of the virus (Croci et al., 1999, 2012; Bozkurt et al., 2013, 2014d). Higher fat and protein content in the heating medium might increase the heat resistance of the virus due to their protective roles (Bidawid et al., 2000; Croci et al., 2012). Other physical and chemical properties of the food matrix (pH, salt and sucrose concentration) may also affect the thermal resistance of the norovirus surrogates (Deboosere et al., 2004; Seo et al., 2012). No ideal human norovirus surrogates have been found so far that would serve all food matrices. For example, canine calicivirus, coliphage MS2 and feline calicivirus are sensitive to low pH while human norovirus is resistant to low pH (pH 2.7 for 3 h at room temperature) (Dolin et al., 1972; Duizer et al., 2004; Cannon et al., 2006; Hewitt

Table 5. Thermal inactivation data associated with foodborne viruses and their surrogates.

	Medium	Temperature range	D or t_D values (min), or $\log_{10}$ reduction in infectivity titer	Z value (°C)	Ref
Human viruses					
HAV (Hepatitis A virus)	Blue mussel homogenate	50–72°C	$D_{50°C} = 54.2$, $D_{56°C} = 9.32$, $D_{60°C} = 3.25$, and $D_{65°C} = 2.16$, and $D_{72°C} = 1.07$ min (1st order model); $t_D = 37.9, 10.4, 7.73, 6.73$ and 1.57 at 50, 56, 60, 65, and 72°C (Weibull model).	15.9[a] (Weibull models); 13.0 (1st order models)	Bozkurt et al., 2014a
HAV	Buffered medium	50–72°C	$D_{50°C} = 56.2$, $D_{56°C} = 8.40$, $D_{60°C} = 2.67$, $D_{65°C} = 1.73$, and $D_{72°C} = 0.88$ min (1st order model); $t_D = 39.9, 11.1, 4.76, 2.56$ and 1.03 min at 50, 56, 60, 65, and 72°C (Weibull model).	14.5 (Weibull models); 12.5 (1st order models)	Bozkurt et al.,2014b
HAV	Clam	50–72°C	$D_{50°C} = 47.4$, $D_{56°C} = 9.74$, $D_{60°C} = 6.13$, $D_{65°C} = 2.20$, and $D_{72°C} = 1.55$ min (1st order model); $t_D = 64.4, 12.6, 5.04, 2.20$ and 1.25 min at 50, 56, 60, 65, and 72°C (Weibull model).	13.0 (Weibull models); 14.8 (1st order models)	Bozkurt et al.,2015b
HAV	Homogenized milk (3.5% fat)	65–85°C	1 log reduction (71°C /0.18 min); 5 log reduction (80°C /0.68 min); (Two-phase negative exponential model	—	Bidawid et al., 2000
HAV	Milk	63 and 72°C	$D_{63°C} = 1.1$; $D_{72°C} \leq 0.3$	—	Hewitt et al., 2009
HAV	Mussel	60–100°C	5 log reduction TCID_{50}[b] ml^{-1} (100°C /2min); 4 log reduction TCID_{50} ml^{-1} (80°C /10 min)	—	Croci et al., 1999
HAV	Mussel	60–75°C	$D_{60°C} = 109$; $D_{65°C} = 72$; $D_{70°C} = 17$; $D_{75°C} = 7$	12.0	Harlow et al., 2011
HAV	Mussels au gratin	Cooking water temperature 98°C	<4.6 log reduction TCID_{50} ml^{-1} (5 min of cooking)	—	Croci et al., 2005
HAV	Mussels hors-d'oeuvre	Cooking water Temperature 98°C	<4.6 log reduction TCID_{50} ml^{-1} (9 min cooking, mussel internal temperature maintained at 71–76°C after the first 3 min)	—	Croci et al., 2005
HAV	Mussels in tomato sauce	Cooking water temperature 98°C	4.6 log reduction TCID_{50} ml^{-1} (8 min of cooking)	—	Croci et al., 2005
HAV	Phosphate buffered saline	37–70°C	$D_{50°C} = 385$; $D_{60°C} = 74.6$; $D_{70°C} = 3.84$	—	Gibson and Schwab, 2011
HAV	Skim milk (0% fat)	65–85°C	1 log reduction (71°C /0.16 min); 5 log reduction (80°C /0.59 min); (Two-phase negative exponential model)	—	Bidawid et al., 2000
HAV	Sodium phosphate buffer	N/A	—	8.40	Harlow et al., 2011
HAV	Spinach	50–72°C	$D_{50°C} = 34.4$, $D_{56°C} = 8.43$, $D_{60°C} = 4.55$, $D_{65°C} = 2.30$, and $D_{72°C} = 0.91$ min (1st order model)	13.9 (1st order models)	Bozkurt et al., 2015c
HAV	Strawberry mashes	80–90°C	$D_{80°C} = 1.22$, $D_{85°C} = 0.96$, $D_{90°C} = 0.32$ (28° Brix); $D_{80°C} = 8.94$, $D_{85°C} = 4.98$, $D_{90°C} = 3.00$ (52° Brix).	21.4 (28° Brix); 21.1 (52° Brix).	Deboosere et al., 2004
HAV	Synthetic medium (sucrose & Ca)	80–90°C	$D_{80°C} = 1.73$, $D_{85°C} = 0.80$, $D_{90°C} = 0.22$ (28° Brix); $D_{80°C} = 12.2$, $D_{85°C} = 6.28$, $D_{90°C} = 2.87$ (52° Brix).	11.3 (28° Brix); 15.9 (52° Brix).	Deboosere et al., 2004
HAV	Table cream (18% fat)	65–85°C	1 log reduction (71°C /0.52 min); 5 log reduction (80°C /1.24 min); (Two-phase negative exponential model	—	Bidawid et al., 2000
HAV	Turkey deli meat	50–72°C	$D_{50°C} = 42.0$, $D_{56°C} = 20.6$, $D_{60°C} = 5.9$, $D_{65°C} = 2.3$, and $D_{72°C} = 1.0$ min (1st order model); $t_D = 25.9, 7.0, 1.7, 0.6$ and 0.6 at 50, 56, 60, 65, and 72°C (Weibull model).	13.4 (Weibull models); 12.8 (1st order models)	Bozkurt et al., 2015a
HAV	Water	63 and 72°C	$D_{63°C} = 0.6$; $D_{72°C} \leq 0.3$	—	Hewitt et al., 2009
Human Norovirus	Spiked mussels	60 and 80°C	less than 1 log RT-PCR units ml^{-1} reduction at all temperature-time combinations (up to 15 min)	—	Croci et al., 2012
Human Norovirus	Phosphatase-buffered saline	60 and 80°C	3 log RT-PCR units ml^{-1} reduction (80°C/6 min)	—	Croci et al., 2012

Human virus surrogates	Matrix	Temperature	Reduction / D-values	z-value	Reference
Canine calicivirus	Modified Eagle's medium	4–100°C	3 D reduction (71.3°C/1 min)	—	Duizer et al., 2004
Coliphage MS2	Phosphate-buffered saline	24–85°C	$D_{50°C} = 307$ (NaCl 0.3%); $D_{50°C} = 322$ (pH 7) (Weibull models)	—	Seo et al., 2012
Murine norovirus	Blue mussel homogenate	50–72°C	$D_{50°C} = 20.2$, $D_{56°C} = 6.12$, $D_{60°C} = 2.64$, $D_{65°C} = 0.41$, and $D_{72°C} = 0.18$ min (1st order model)	9.91 (Weibull models); 11.6 (1st order models)	Bozkurt et al., 2014d
Murine norovirus	Buffered medium	50–72°C	$t_D = 19.8$, 11.4, 3.01, 0.42 and 0.15 min at 50, 56, 60, 65, and 72°C (Weibull model). $D_{50°C} = 36.3$, $D_{56°C} = 3.74$, $D_{60°C} = 1.09$, $D_{65°C} = 0.77$, and $D_{72°C} = 0.25$ min (1st order model)	9.16 (Weibull models); 9.32 (1st order models)	Bozkurt et al., 2014b
Murine norovirus 1	Dulbecco's Modified Eagle Medium	56 and 73°C	$t_D = 26.8$, 2.34, 0.68, 0.39 and 0.09 min at 50, 56, 60, 65, and 72°C (Weibull model).	—	Tuladhar et al., 2012
Murine norovirus	Dulbecco's Modified Eagle Medium	24–85°C	1 $\log_{10}$ infectivity reduction (56°C /4.21 min) 1 $\log_{10}$ infectivity reduction (73°C /1.06 min) (Weibull models)	—	Seo et al, 2012
Murine norovirus	Milk	63 and 72°C	3 log reduction (50°C/3h); 4 log reduction (60°C /10min); 4 log reduction (70°C/2.5 min); $D_{50°C} = 54.4$ (NaCl 0.3%); $D_{50°C} = 123$ (pH 7) (Weibull models)	—	Hewitt et al., 2009
Murine norovirus	Modified eagle medium (buffer solution)	50–72°C	$D_{63°C} = 0.7$; $D_{72°C} = 0.5$ $D_{50°C} = 34.5$, $D_{56°C} = 3.65$, $D_{60°C} = 0.57$, $D_{65°C} = 0.30$, and $D_{72°C} = 0.15$ min (1st order model)	9.19 (Weibull models) **9.31 (1st order models)[a]	Bozkurt et al., 2013
Murine norovirus 1	Modified eagle medium	56–72°C	$t_D = 28.3$, 3.62, 0.83, 0.37 and 0.11 min at 50, 56, 60, 65, and 72°C (Weibull model). $D_{56°C} = 3.47$; $D_{63°C} = 0.435$; $D_{72°C} = 0.166$	—	Cannon et al., 2006
Murine norovirus	Phosphate buffered saline	37–60°C	$D_{37°C} = 769$; $D_{50°C} = 106$; $D_{60°C} = 13.7$	—	Gibson and Schwab, 2011
Murine norovirus 1	Spinach	50–72°C	$D_{50°C} = 14.6$, $D_{56°C} = 3.29$, $D_{60°C} = 0.98$, $D_{65°C} = 0.40$, and $D_{72°C} = 0.16$ min (1st order model); $t_D = 15.3$, 4.09, 1.11, 0.47 and 0.22 min at 50, 56, 60, 65, and 72°C (Weibull model).	11.7 (Weibull models); 11.0 (1 st order models)	Bozkurt et al., 2014c
Murine norovirus 1	Raspberry puree	65°C /30s or 75°C / 15s	1.86 log reduction (65°C /30s); 2.81 log reduction (75°C /15s)	—	Baert et al., 2008
Murine norovirus 1	1% stool	56 and 73°C	1 $\log_{10}$ infectivity reduction (56°C /3.20 min) 1 $\log_{10}$ infectivity reduction (73°C /0.49 min)(Weibull models)	—	Tuladhar et al., 2012
Murine norovirus	Turkey deli meat	50–72°C	$D_{50°C} = 21.0$, $D_{56°C} = 7.3$, $D_{60°C} = 2.7$, $D_{65°C} = 0.9$, and $D_{72°C} = 0.2$ min (1st order model).	11.0 (Weibull models); 10.9 (1st order models)	Bozkurt et al., 2015a
Murine norovirus	Water	63 and 72°C	$t_D = 17.8$, 6.7, 2.8, 0.9, and 0.3 at 50, 56, 60, 65, and 72°C (Weibull model). $D_{63°C} = 0.9$; $D_{72°C} \leq 0.3$	—	Hewitt et al., 2009
Feline calicivirus	Basal Eagle's Medium	56 and 70°C	5–7 log reduction (56°C/60 min) 3 log reduction (70°C/1 min)	—	Doultree et al., 1999
Feline calicivirus	Blue mussel homogenate	50–72°C	$D_{50°C} = 5.20$, $D_{56°C} = 3.33$, $D_{60°C} = 0.77$, $D_{65°C} = 0.33$, and $D_{72°C} = 0.07$ min (1st order model).	12.4 (Weibull models); 11.4 (1st order models)	Bozkurt et al., 2014d
Feline calicivirus	Buffered medium	50–72°C	$t_D = 4.03$, 3.13, 0.67, 0.31 and 0.08 min at 50, 56, 60, 65, and 72°C (Weibull model). $D_{50°C} = 20.0$, $D_{56°C} = 6.37$, $D_{60°C} = 0.94$, $D_{65°C} = 0.72$, and $D_{72°C} = 0.21$ min (1st order model); $t_D = 13.3$, 4.05, 0.40, 0.35 and 0.10 min at 50, 56, 60, 65, and 72°C (Weibull model).	9.66 (Weibull models); 9.36 (1st order models)	Bozkurt et al., 2014b
Feline calicivirus	Cell culture medium or water	50–75°C	1 log reduction (50°C /15 min); 6 log reduction (70°C /90s)	—	Buckow et al., 2008
Feline calicivirus	Modified eagle medium (buffer solution)	50–72°C	$D_{50°C} = 20.2$, $D_{56°C} = 6.36$, $D_{60°C} = 0.56$, $D_{65°C} = 0.32$, and $D_{72°C} = 0.11$min (1st order model); $t_D = 13.9$, 4.04, 0.37, 0.34 and 0.06 at 50, 56, 60, 65, and 72°C (Weibull model).	9.31 (Weibull models); 9.36 (1st order models)	Bozkurt et al., 2013

Table 5. (Continued)

Medium	Temperature range	D or t_D values (min), or $\log_{10}$ reduction in infectivity titer	Z value (°C)	Ref
Feline calicivirus				
Modified eagle medium	56–72°C	$D_{56°C} = 6.72$; $D_{63°C} = 0.406$; $D_{72°C} = 0.118$	—	Cannon et al., 2006
Modified Eagle's medium	4–100°C	3 D reduction (71.3°C/1 min)	—	Duizer et al., 2004
Phosphatase-buffered saline	60 and 80°C	4 log reduction (60°C/3 min) by cell culture assay	—	Croci et al., 2012
Phosphate buffered saline	37–60°C	$D_{37°C} = 599$; $D_{50°C} = 50.6$; $D_{60°C} = 14.1$	—	Gibson & Schwab, 2011
Spiked mussels	60 and 80°C	2 log reduction (60°C/15 min) by cell culture assay	—	Croci et al., 2012
Spinach	50–72°C	$D_{50°C} = 17.4$, $D_{56°C} = 5.83$, $D_{60°C} = 0.78$, $D_{65°C} = 0.27$, and $D_{72°C} = 0.15$ min (1st order model); $t_D = 20.7$, 6.17, 0.91, 0.35 and 0.27 min at 50, 56, 60, 65, and 72°C (Weibull model).	10.9 (Weibull models); 9.89 (1st order models)	Bozkurt et al., 2014c
Turkey deli meat	50–72°C	$D_{50°C} = 9.9$, $D_{56°C} = 3.0$, $D_{60°C} = 0.8$, $D_{65°C} = 0.4$, and $D_{72°C} = 0.1$ min (1st order model); $t_D = 11.9$, 3.0, 0.8, 0.5 and 0.1 min at 50, 56, 60, 65, and 72°C (Weibull model).	11.3(Weibull models); 11.9 (1st order models)	Bozkurt et al., 2015a

[a]z-value for a Weibull model was defined as the change in temperature (°C) required to cause a 90% change in the t_D-value of a population (Bozkurt et al., 2013).

[b]TCID$_{50}$: 50% Tissue Culture Infectious Dose.

Table 6. Temperature-time combinations for "6D process" of some typical bacterial pathogens and viruses under pasteurization.

Target microorganism	Internal product temperature(°C)	Time for 6D process(min)	Lethal rate	Z value(°C)	Reference
Listeria monocytogenes	65	9.3	0.215	7.5	ECFF, 2006
	67	5.0	0.398		
	70	2.0	1.000		
Nonproteolytic	90	10.0	1.000	7.0 (T < 90°C)	ECFF, 2006
Clostridium botulinum type B	93	5.0	2.000	10.0 (T > 90°C)	
	97	2.0	5.010		
Hepatitis A virus	65	13.2	—	14.8	Bozkurt et al., 2015b
	72	9.3	—		
Hepatitis E virus	70	3	—	—	Johne et al., 2016

et al., 2009; Seo et al., 2012). More information on human norovirus (e.g., natural persistence in foods) is needed to refine the regulatory standards and monitoring approaches, and also to develop safe pasteurized foods.

The processing time required to achieve a 6-log reduction of the target pathogen with a typical process time from practical process development view point (in the range of 2–15 min full exposure) for representative pathogens is summarized in Table 6. The most heat-resistant vegetative pathogen, the most heat resistant form of nonproteolytic *C. botulinum* (*C. botulinum* Type B), and the most heat resistant viral pathogen (HAV) from the literature are included in Table 6. Thermal stability of HEV (a popular foodborne virus in Asia, Europe and Africa) is also included. As can be seen, a pasteurization process developed for 6-log reduction of *L. monocytogenes* does not achieve 6-log reductions of HAV or HEV.

Enzyme inactivation in thermal pasteurization

Thermal pasteurization of vegetables aiming to inactivate pathogens may not completely inactivate endogenous enzymes. The presence of residual endogenous enzymes in processed vegetable products may cause quality loss during storage. Therefore, together with possible growth of spoilage bacteria which in general are more thermally resistant than vegetative pathogenic bacteria, enzyme activity may also considerably shorten the shelf life of the final product. The principal enzyme responsible for a specific quality loss is product-dependent. Major enzymes related to vegetable quality attributes (texture, color and flavor) are listed in Table 7. It can be seen that some enzymes play roles in multiple quality attributes of vegetable products. For example peroxidase (POD) catalyzes the oxidation of phenolics resulting in browning (a color effect), it may also be involved in the oxidative cross-linking of cell wall polymers contributing to the texture changes of vegetables; lipoxygenase (LOX) causes off-flavor through oxidation of polyunsaturated fatty acids, it also catalyzes bleaching or oxidation of carotenoids resulting in the color changes of vegetables. Texture loss through pectin depolymerization can be catalyzed by polygalacturonase (PG), and color loss through browning by POD or polyphenol oxidase (PPO).

Table 8 summarizes published thermal kinetic parameters of several quality-associated enzymes in vegetables. The most frequently studied enzymes are pectin methylesterase (PME), PG, POD, and LOX. Little has been systematically reported on the

Table 7. Major enzymes related to the quality of raw and processed vegetables.

Category	Enzymes	Main effects	Ref
Texture- related enzymes	Pectin methylesterase (PME)	a. Catalyzes the de-esterification of pectin to create binding sites for divalent cations on the polygalacturonic acid backbone of pectin to form cross-links between pectin chains (a firming effect); b. demethoxylated pectin can also be a substrate for PG depolymerization (a softening effect); c. causes cloud loss in juices	Anthon & Barrett, 2002; Terefe et al., 2014; Van Buggenhout, 2009
	Polygalacturonase (PG)	Catalyzes the cleavage of polygalacturonic acid, resulting in pectin depolymerization (softening effect)	
	Peroxidase(POD)	Involved in the oxidative cross-linking of cell wall polysaccharides	
Color-related enzymes	Polyphenol oxidase (PPO)	Acts on phenols in the presence of oxygen, catalyzes browning	Terefe et al., 2014; Zang et al., 2013
	POD	Catalyzes the oxidation of phenolics in the presence of hydrogen peroxide resulting in browning	
	Anthocyanase	Catalyzes the hydrolysis of anthocyanins	
	Chlorophyllase	Catalyzes the degradation of chlorophyll, causes the loss of green color	
	Alliinase	Hydrolyzes the nonprotein amino acid (allicin), involved in the discoloration of processed garlic products	
	Lipoxygenase (LOX)	Causes the co-oxidation of carotenoids in the presence of free fatty acids, affects the color intensity of foods	
Off-flavor-related enzymes	LOX	Catalyzes the oxidation of polyunsaturated fatty acids, produces volatile off-flavor compounds	Terefe et al., 2014
	Hydroperoxidase lyase (HPL)	One of the key enzyme in the "LOX pathway" for producing volatile compounds, the high concentration of which results in off-flavor	
	Cystine lyase	Cleaves cystine producing ammonia, responsible for off flavor and off aroma in broccoli and cauliflower	

Table 8. Thermal kinetic parameters of quality-related enzymes in vegetables.

Enzyme	Source	Heating medium	Temp (°C)	Kinetic model	Kinetic parameters				Ref
					$k(min^{-1})$	E_a (KJ/mol)	D (min)	Z (°C)	
PG	Carrot	Carrot juice	75–84	1st order	$k_{80°C} = 1.45 \times 10^{-4}$	411	$D_{81.2°C} = 5$	—	Anthon & Barrett, 2002
PG	Tomato	Tomato juice	55–70	Fraction conversion	$k_{55°C} = 6.20 \times 10^{-6}$, $k_{60°C} = 1.33 \times 10^{-5}$, $k_{65°C} = 5.92 \times 10^{-5}$, $k_{70°C} = 2.17 \times 10^{-4}$	228	—	—	Fachin et al., 2003
PME	Black carrot	Tris-buffer, pH 7.5	50–65	1st order	$k_{55°C} = 4.00 \times 10^{-2}$, $k_{60°C} = 1.33 \times 10^{-1}$, $k_{65°C} = 3.37 \times 10^{-1}$	197	$D_{55°C} = 57.7$; $D_{60°C} = 17.3$ $D_{65°C} = 6.8$	2.16	Ünal & Bellur, 2009
PME	Carrot	Carrot juice	50–60	1st order	—	—	$D_{50°C} = 361$; $D_{52°C} = 147$; $D_{54°C} = 66.6$; $D_{56°C} = 34.8$; $D_{58°C} = 10.3$; $D_{60°C} = 7.32$	5.73	Balogh et al., 2004
PME	Carrot	Carrot juice	62–72	1st order	$k_{65°C, L} = 1.82 \times 10^{-4}$, $k_{70°C, R} = 1.90 \times 10^{-4}$	510 (L); 635 (R)	$D_{65.7°C} = 5$ (L); $D_{70.5°C} = 5$ (R)	—	Anthon & Barrett, 2002
PME	Carrot	Carrot tissue	66–74	1st order	—	—	$D_{66°C} = 648$; $D_{68°C} = 192$; $D_{70°C} = 64.8$; $D_{71°C} = 50.9$ $D_{73°C} = 18.3$; $D_{74°C} = 5.24$	4.13	Balogh et al., 2004
PME	Carrot	Citrate buffer, pH 6.0	50–60	1st order	—	—	$D_{50°C} = 284$; $D_{52°C} = 122$; $D_{54°C} = 47.7$; $D_{56°C} = 24.9$; $D_{58°C} = 10.1$; $D_{60°C} = 3.59$	5.37	Balogh et al., 2004
PME	Carrot	Tris-buffer pH 7.0	48–60	Fractional conversion	$k_{48°C} = 1.10 \times 10^{-2}$, $k_{51°C} = 3.75 \times 10^{-2}$, $k_{54°C} = 7.01 \times 10^{-2}$, $k_{57°C} = 1.69 \times 10^{-1}$, $k_{60°C} = 6.81 \times 10^{-1}$	289	—	—	Ly-Nguyen et al., 2002
PME	Carrot	Tris-HCl buffer, pH 7.0	48–60	Fractional conversion	$k_{48°C} = 2.01 \times 10^{-2}$, $k_{51°C} = 5.86 \times 10^{-2}$, $k_{54°C} = 1.33 \times 10^{-1}$, $k_{57°C} = 3.29 \times 10^{-1}$, $k_{60°C} = 8.68 \times 10^{-1}$	275	—	—	Ly-Nguyen et al., 2003
PME	Carrot	Tris-HCl buffer, pH 7.0	48–60	1st order	$k_{60°C} = 8.04 \times 10^{-2}$, $k_{62°C} = 9.97 \times 10^{-2}$, $k_{64°C} = 2.62 \times 10^{-1}$, $k_{66°C} = 4.75 \times 10^{-1}$	291	—	—	Ly-Nguyen et al., 2003
PME	Carrot	Tris-buffer, pH 7.0	54–81	1st order	$k_{54°C} = 4.47 \times 10^{-2}$, $k_{57°C} = 1.50 \times 10^{-1}$, $k_{60°C} = 4.50 \times 10^{-1}$; $k_{63°C} = 1.22$	336	—	—	Espachs-Barroso et al., 2006
PME	Cucumber	Tris-buffer, pH 6.7	55–72	—	—	—	$D_{55°C} = 166$; $D_{60°C} = 21.2$ $D_{65°C} = 2.72$	5.61	Guiavarc'h et al., 2003
PME	Potato	Potato	60–72	1st order	$k_{65°C, L} = 9.42 \times 10^{-4}$;	493 (L);	$D_{69°C} = 5$ (L);	—	Anthon & Barrett, 2002

Enzyme	Source	Medium	Temperature (°C)	Kinetic model	k	Ea	D-value	z	References
PME	Tomato	Citrate buffer, pH 4.5	62–77	1st order	$k_{70°C, R} = 1.23×10^{-4}$	759 (R); —	$D_{70°C} = 5\ (R)$	5.01	Guiavarc'h et al., 2003
PME	Tomato	Tris-buffer, pH 7.0	54-81	1st order	$k_{63°C} = 8.30×10^{-2}, k_{65°C} = 1.62×10^{-1}; k_{67°C} = 3.07×10^{-1}; k_{69°C} = 8.80×10^{-1}$	369	$D_{62°C} = 151; D_{65°C} = 39.5; D_{68°C} = 9.34; D_{70°C} = 3.94$	—	Espachs-Barroso et al., 2006
POD	Broccoli (florets)	Phosphate buffer, pH 6.5	70–95	Biphasic 1st order	$k_{70°C, R} = 4.00×10^{-5}; k_{70°C, L} = 4.17×10^{-4}, k_{95°C, R} = 1.61×10^{-4}, k_{95°C, L} = 2.40×10^{-3}$	75.0 (L); 58.0 (R)	—	—	Morales-Blancas et al., 2002
POD	Butternut Squash	Butternut squash slices	60–90	Biphasic, 1st order	$k_{65°C, L} = 2.49×10^{1};$	14.0 (L)	—	—	Agüero et al., 2008
POD	Carrot	Carrot juice	70–84	1st order	$k_{65°C, R} = 7.29×10^{-2}$	15.8 (R)	—	—	Anthon & Barrett, 2002
POD	Carrot	Carrot slices	70–90	1st order	$k_{80°C} = 2.08×10^{-4}$; $k_{80°C} = 4.00×10^{-1}$	480; 151.4	$D_{80.3°C} = 5$	—	Gonçalves et al., 2010
POD	Carrot (cortex)	Phosphate buffer, pH 6.5	70–95	Biphasic 1st order	$k_{70°C, R} = 2.10×10^{-6}, k_{70°C, L} = 7.43×10^{-4}, k_{95°C, R} = 7.70×10^{-6}, k_{95°C, L} = 4.80×10^{-3}$	95.0 (L); 86.0 (R)	—	—	Morales-Blancas et al., 2002
POD	Carrot (core)	Phosphate buffer, pH 6.5	70–95	Biphasic 1st order	$k_{70°C, R} = 1.10×10^{-6}, k_{70°C, L} = 1.54×10^{-3}, k_{95°C, R} = 3.30×10^{-6}, k_{95°C, L} = 5.33×10^{-3}$	97.0 (L); 83.0 (R)	—	—	Morales-Blancas et al., 2002
POD	Green asparagus	Green asparagus	70–90	1st order	—	15.5(bud); 20.7(upper); 23.9 (mid); 22.8 (butt)	—	—	Zheng & Lu, 2011
POD	Green asparagus (tip)	Phosphate buffer, pH 6.5	70–95	Biphasic 1st order	$k_{70°C, R} = 4.60×10^{-6}, k_{70°C, L} = 9.58×10^{-4}, k_{95°C, R} = 1.32×10^{-5}, k_{95°C, L} = 4.73×10^{-3}$	67 (L); 43 (R)	—	—	Morales-Blancas et al., 2002
POD	Green asparagus (stem)	Phosphate buffer, pH 6.5	70–95	Biphasic 1st order	$k_{70°C, R} = 1.14×10^{-5}, k_{70°C, L} = 7.48×10^{-4}, k_{95°C, R} = 3.84×10^{-5}, k_{95°C, L} = 2.48×10^{-3}$	61 (L); 53 (R)	—	—	Morales-Blancas et al., 2002
POD	Potato	Potato homogenate	67–85	1st order	$k_{80°C} = 5.60×10^{-4}$	478	$D_{83.2°C} = 5$	—	Anthon & Barrett, 2002
POD	Tomato	Tomato slurry	63–67	1st order	$k_{63°C} = 3.32×10^{-2}, k_{65°C} = 1.59×10^{-1}; k_{67°C} = 6.21×10^{-1};$	149	$D_{63°C} = 69.4; D_{65°C} = 14.5; D_{67°C} = 3.7$	—	Ercan and Soysal, 2011

Table 8. (*Continued*)

Enzyme	Source	Heating medium	Temp (°C)	Kinetic model	Kinetic parameters				Ref
					k(min⁻¹)	E_a (KJ/mol)	D (min)	Z (°C)	
LOX	Broccoli (florets)	Phosphate buffer, pH 6.5	70–95	Biphasic 1st order	$k_{70°C,\,R} = 1.15×10^{-6}$, $k_{70°C,\,L} = 2.15×10^{-4}$, $k_{95°C,\,R} = 4.50×10^{-6}$, $k_{95°C,\,L} = 9.10×10^{-4}$	61 (L); 55 (R)	—	—	Morales-Blancas et al., 2002
LOX	Green asparagus (tip)	Phosphate buffer, pH 6.5	70–95	Biphasic 1st order	$k_{80°C,\,R} = 4.57×10^{-6}$, $k_{80°C,\,L} = 1.05×10^{-3}$, $k_{95°C,\,R} = 1.15×10^{-5}$, $k_{95°C,\,L} = 3.01×10^{-3}$	76 (L); 65 (R)	—	—	Morales-Blancas et al., 2002
LOX	Green asparagus (stem)	Phosphate buffer, pH 6.5	70–95	Biphasic 1st order	$k_{80°C,\,R} = 1.15×10^{-5}$, $k_{80°C,\,L} = 5.83×10^{-4}$, $k_{95°C,\,R} = 2.30×10^{-5}$, $k_{95°C,\,L} = 1.75×10^{-3}$	78 (L); 56 (R)	—	—	Morales-Blancas et al., 2002
LOX	Green bean	Extract solution	55–70	1st order	$k_{55°C,\,L} = 4.21×10^{-2}$, $k_{55°C,\,R} = 5.00×10^{-3}$, $k_{65°C,\,L} = 1.72$;	331 (L); 188 (55–63°C, R); 515 (63–70°C, R)	—	—	Indrawati et al., 1999
LOX	Green pea	Green peas juice	60–72	1st order	$k_{65°C,\,R} = 4.80×10^{-2}$, $k_{65°C} = 8.72×10^{-2}$, $k_{70°C} = 8.02×10^{-1}$	541	—	—	Indrawati et al., 2001
LOX	Green pea	Intact green peas	60–72	1st order	$k_{65°C} = 1.79×10^{-2}$, $k_{70°C} = 6.42×10^{-1}$	584	—	—	Indrawati et al., 2001
LOX	Tomato	Tomato dices	80–98	Two-fraction 1st order	$k_{80°C,\,L} = 3.00×10^{-2}$, $k_{80°C,\,R} = 4.50×10^{-1}$, $k_{98°C,\,L} = 2.60×10^{-1}$, $k_{98°C,\,R} = 2.21$	137 (L); 99.7(R)	$D_{80°C,\,L} = 86.9$; $D_{80°C,\,R} = 143$; $D_{98°C,\,L} = 8.9$; $D_{98°C,\,R} = 34.5$	18.2 (L); 28.7 (R)	Anese & Sovrano, 2006
LOX	Tomato	Tomato extract	80–98	Two-fraction 1st order	$k_{80°C,\,L} = 1.90×10^{-1}$; $k_{80°C,\,R} = 1.13$; $k_{90°C,\,L} = 7.60×10^{-1}$; $k_{90°C,\,R} = 2.00$	147 (L); 60.8 (R)	$D_{80°C,\,L} = 12.2$; $D_{80°C,\,R} = 115$; $D_{90°C,\,L} = 3.0$; $D_{90°C,\,R} = 37.3$	16.5 (L); 20.6 (R)	Anese & Sovrano, 2006
LOX	Tomato	Tomato homogenate	50–65	1st order (multiple isoforms)	Isoform1: $k_{50°C} = 1.07×10^{-4}$; $k_{65°C} = 1.67×10^{-2}$, Isoform2: $k_{50°C} = 1.50×10^{-5}$, $k_{65°C} = 4.16×10^{-3}$, Isoform3: $k_{50°C} = 1.67×10^{-7}$, $k_{65°C} = 5.50×10^{-4}$	308 (isoform1); 336 (isoform2); 349 (isoform3)	—	—	Anthon & Barrett, 2003

PG: polygalacturonase; PME: pectin methylesterase; POD: peroxidase; LOX: lipoxygenase; L: heat-labile fraction/form; R: heat-resistant fraction/form.

thermal kinetic parameters of anthocyanase, chlorophyllase, alliinase, or cystine lyase in vegetables. The literature shows that thermal inactivation of PG and PME in vegetables (carrot, tomato and potato) follows a first-order or modified first order (fractional conversion) reaction model. For the inactivation of POD, researchers found that this enzyme system consisted of 2 isozymes with different thermal stability (a heat-labile and a heat-resistant fraction), therefore they applied a biphasic first order model to describe the overall behaviors of this enzyme. This biphasic first order model has been successfully applied to broccoli, butter nut squash, carrot and green asparagus (Morales-Blancas et al., 2002; Agüero et al., 2008). However, other researchers used a monophasic first-order to reflect the kinetic inactivation of POD and also demonstrated a good fit of this model in carrot, green asparagus, potato and tomato (Anthon & Barrett, 2002; Ercan and Soysal, 2011; Gonçalves et al., 2010; Zheng & Lu, 2011).

In terms of the thermal inactivation kinetics of LOX in vegetables, either a biphasic or monophasic first order model was adopted (Anese & Sovrano, 2006; Anthon & Barrett, 2003; Morales-Blancas et al., 2002; Indrawati et al., 2001). Enzymes may have isozymes with different stabilities; and the sequential inactivation pathways may involve intermediate forms with reduced activity, all of which make the enzyme inactivation process complicated. Therefore, a suitable kinetic model should be chosen based on a sufficient understanding of the enzyme (and isozymes) present in a particular food, and all the factors that may affect its activities to accurately predict the enzyme inactivation under thermal processing. The z-values of most reported enzymes listed in Table 8 were below 10°C, except for z-values between 17 and 29°C reported by Anese & Sovrano (2006) for LOX in tomatoes (dices and extract). Based on the publications mentioned above, the required processing time in the range of minutes (<5 min) to achieve 90% inactivation of some quality-related enzymes in vegetables is shown in Table 9. The temperature and time needed to inactivate 90% of the quality-related enzymes depends on the food matrix. Differences in the raw materials and heating medium of an enzyme may result in a wide range of thermal stability. The reported thermal resistance of LOX varied the most, probably due to its different isozymes in addition to the differences in food sources and heating mediums.

Blanching

Blanching with hot water or steam is commonly used as a pre-treatment prior to further processing (canning, freezing, dehydrating etc.) of many vegetables. It is a relatively mild form of heat treatment applied to fresh foods in order to release entrapped air and to inactivate enzymes causing product quality deterioration (Teixeira, 2014). Typical blanch temperatures and times may be in the range of 190°F (88°C) to 212°F (100°C) for from 1 to 10 min (Barrett, 1994). Low-temperature blanching (generally 50–70°C) prior to high temperature processing has also been studied in a number of vegetables in recent decades (Anthon and Barrett, 2006; Stanley et al., 1995). According to Table 2, several minutes of heating at 70°C may be sufficient for a 6-log reduction in *L. monocytogenes*, which is the most heat-resistant vegetative pathogen. Although the heat treatment applied in blanching has not traditionally been included in microbial inactivation kinetic studies, there are currently research efforts to quantify these and begin to account for them in thermal process applications. Thermal pasteurization of vegetables may or may not involve the blanching step, depending on the specific vegetable products and shelf-life expectations. Additional information about applying blanching prior to vegetable pasteurization, which was obtained from the literature, can be found in Table 10, where both blanching and pasteurization conditions have been specified.
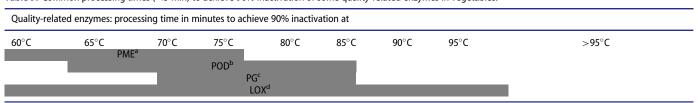
Effect of thermal pasteurization on vegetable quality

Maintaining fresh-like quality is another major goal for vegetable pasteurization, besides microorganism inactivation out of safety concerns. During the thermal pasteurization process, the heat applied to destroy the pathogens also inactivates the endogenous enzymes to limit the quality deterioration of vegetables during processing and storage. The retention of product quality is one factor for determining the shelf-life of pasteurized foods, and also is one main concern for consumer acceptance of processed vegetables. Many papers have reported the impact of thermal pasteurization on the quality of vegetables, including color, texture, carotenoids, phenolics, antioxidant activity, vitamins, and other nutritional attributes. Some of these are summarized in Table 10.

Color

Color plays a vital role in consumer acceptance of a vegetable product, and because we see things first with our eyes before we eat them, it is one of the most important characteristics of vegetables. This visual appeal comes mainly from pigments such as chlorophylls, anthocyanins, and carotenoids including lycopene, which provide health and nutrient benefits. The visual color of vegetables can be numerically expressed by color models. The CIE model is the most commonly used and its 3 color values L^*(lightness), a^* (redness: green to red) and b^*

Table 9. Common processing times (<5 min) to achieve 90% inactivation of some quality-related enzymes in vegetables.

Quality-related enzymes: processing time in minutes to achieve 90% inactivation at

60°C	65°C	70°C	75°C	80°C	85°C	90°C	95°C	>95°C

Adapted from a Anthon & Barrett (2002), Balogh et al. (2004), Espachs-Barroso et al. (2006), Guiavarc'h et al. (2003), Ly-Nguyen et al.(2002), Ünal & Bellur (2009); b Anthon & Barrett (2002), Ercan & Soysal (2011); Zheng & Lu (2011); c Anthon & Barrett (2002), Fachin et al. (2003); d Anese & Sovrano (2006), Anthon & Barrett (2002, 2003), Indrawati et al. (1999 & 2001), Morales-Blancas et al. (2002).

Table 10. Effects of thermal pasteurization on the quality of vegetables .

Quality parameters	Commodity/ products	Thermal technology	Blanching conditions	Pasteurization conditions	Kinetic parameters	Storage conditions	Enzyme studied	Microbial test	Ref
Antioxidant capacity	Carrot juice	MW heating or traditional heat	—	MW: flow rats 90–287 mL/min at 540–900 W; Traditional heat: 100°C/10 min	—	4°C/ 4 mo	PME	—	Rayman & Baysal, 2011
Antioxidant activity	Gazpacho (a cold vegetable soup)	Tubular heat-exchanger in hot water bath	—	90°C / 1 min	—	—	—	—	Elez-Martínez & Martín-Belloso, 2007
Antioxidant activity	Onion by-products (juice, paste and bagasse)	Steam	—	100°C/ 11–17 min	—	—	—	—	Roldán et al., 2008
Antioxidant capacity	Pumpkin	Thermostatic bath	Boiling water/ 90 s	85°C/ 5 min	—	4°C/ 60 d	—	Total aerobic bacteria, yeast & molds	Zhou et al., 2014
Antioxidant activity	Red sweet pepper	Water bath	—	70°C / 10 min	—	—	—	—	HernándezCarrión et al., 2014
Antioxidant capacity	Tomato juice	Heat exchanger coil in hot water bath	—	90°C/ 30 or 60 s	$k_{4°C} = 1.37 \times 10^{-2}$ d^{-1} (after 90°C /60s heating); $k_{4°C} = 1.22 \times 10^{-2}$ d^{-1} (after 90°C /30s heating); 1st order model.	4°C/ 91 d	—	—	Odriozola-Serrano et al., 2008
Carotenoids	Carrot juice	MW heating or traditional heat	—	MW: flow rats 90–287 mL/min at 540–900 W; traditional heat: 100°C/10 min	—	4°C/ 4 mo	—	—	Rayman & Baysal, 2011
Carotenoids	Carrot pieces	Steam	40°C/ 30 min	$F_{70°C}^{10} = 2$min; $F_{90°C}^{10} = 10$min	—	—	—	—	Vervoort et al., 2012
Carotenoids	Red sweet pepper	Water bath	—	70°C/10 min	—	—	—	—	Hernández-Carrión et al., 2014
Carotenoids	Tomato juice	Water bath	—	90°C/ 30 or 60 s	—	4±1°C/ 56 d	—	—	Odriozola-Serrano et al., 2009
β-carotene	Carrot pieces	Steam	60°C water/ 40 min	Actual $F_{70°C} = 1.85$ min or $F_{90°C} = 9.67$ min	—	—	—	—	Lemmens et al., 2013
β-carotene	Carrot puree	Steel tubes in water bath	—	$F_{90°C} = 10$min	—	—	—	—	Knockaert et al., 2012
Color	Asparagus	Water bath	—	70–98°C	Ea = 13.1 kcal/mol, $k_{84°C} = 6.60 \times 10^{-3}$ min^{-1},1st order model	—	—	—	Lau et al., 2000
Color	Broccoli (florets & stems)	Continuous microwave	95°C water/ 30 s	3.5 kW/ 4 min, surface temp. of vegetable packs 75–80°C.	—	30°C/ 60 d	—	Visual signs of spoilage, bacterial and yeast growth	Koskiniemi et al., 2013
Color	Carrot pieces	Steam	40°C/ 30 min	$F_{70°C}^{10} = 2$ min; $F_{90°C}^{10} = 10$ min	—	—	—	—	Vervoort et al., 2012

Quality attribute	Food product	Heating method	Blanching/pretreatment	Heating condition	Kinetic parameters	Storage condition	Enzyme	Microbial indicator	Reference
Color	Cucumber juice	Heat exchanger	—	85°C/15 s		4 ± 2°C/50d	LOX	Total aerobic bacteria	Zhao et al., 2013
Color	Pickled garlic	Hot water bath	90°C water/15 min	90°C/5.5 min		27 ± 2°C/4mo	—	*Enterobacteriaceae*, lactic acid bacteria & yeast	Rejano et al., 1997
Color	Pumpkin	Thermostatic bath	Boiling water/90 s	85°C/5 min		4°C/60 d	—	Total aerobic bacteria, yeast & molds	Zhou et al., 2014
Color	Red bell pepper	Continuous microwave	95°C water/30 s	3.5 kW/4 min, surface temp. of vegetable packs 75–80°C.		30°C/60 d	—	Visual signs of spoilage, bacterial and yeast growth	Koskiniemi et al., 2013
Color	Soybean sprouts	Heat (no information on the heating media)	100°C steam/8 min	$F_{90°C} = 10$ min; $F_{70°C} = 2$ min		3°C/36 d; 10°C/24 d	—	Aerobic, anaerobic & psychrophilic bacterial counts	Koo et al., 2008
Color	Spinach	Oil bath	—	65–90°C	a: zero-order; b: zero-order for summer and 1st order for the rest seasons	—	—	—	Aamir et al., 2014
Color	Sweatpotato	Continuous microwave	95°C water/30 s	3.5 kW/4 min, surface temp. of vegetable packs 75–80°C.		30°C/60 d	—	Visual signs of spoilage, bacterial and yeast growth	Koskiniemi et al., 2013
Color	Tomato juice	Water bath	—	90°C/30 or 60 s		4 ± 1°C/56 d	—	—	Odriozola-Serrano et al., 2009
Color	Vegetable juice	Oil bath	Boiling water/3 min	80–100°C/0–60 s	$k_{80°C} = 1.03 \times 10^{-2}$; $k_{90°C} = 1.33 \times 10^{-2}$; $k_{100°C} = 1.75 \times 10^{-2}$ (L value, s^{-1}); $k_{80°C} = 1.14 \times 10^{-2}$; $k_{90°C} = 1.81 \times 10^{-2}$; $k_{100°C} = 2.80 \times 10^{-2}$ (a value, s^{-1}); 1st order model.			—	—	Loong and Goh, 2004
Lycopene	Tomato juice	Heat exchanger coil in hot water bath	—	90°C/30–60 s	$k_{4°C} = 1.77 \times 10^{-2}$ d^{-1} (after 90°C/60s heating); $k_{4°C} = 2.27 \times 10^{-2}$ d^{-1} (after 90°C/30s heating); 1st order model.	4°C/91 d	—	—	Odriozola-Serrano et al., 2008

Table 10. (Continued)

Quality parameters	Commodity/products	Thermal technology	Blanching conditions	Pasteurization conditions	Kinetic parameters	Storage conditions	Enzyme studied	Microbial test	Ref
Phenolics	Carrot juice	MW heating or traditional heat	—	MW: flow rats 90–287 mL/min at 540–900 W; Traditional heat: 100°C/ 10 min	—	4°C/ 4 mo	—	—	Rayman & Baysal, 2011
Phenolics	Onion by-products	Steam	—	100°C/ 11–17 min	—	—	—	—	Roldán et al., 2008
Phenolics	Tomato juice	Heat exchanger coil in hot water bath	—	90°C/ 30 or 60 s	—	4°C/ 91 d	—	—	Odriozola-Serrano et al., 2008
Phenolics	Tomato juice	Water bath	—	90°C/ 30 or 60 s	—	4±1°C/ 56 d	—	—	Odriozola-Serrano et al., 2009
Phenols (total)	Pumpkin	Thermostatic bath	Boiling water/ 90 s	85°C/ 5 min	—	4°C/ 60 d	—	Total aerobic bacteria, yeast & molds	Zhou et al., 2014
Quercetin	Onion by-products	Steam	—	100°C/ 11–17 min	—	—	—	—	Roldán et al., 2008
Quercetin	Yellow "banana" pepper	Heat (no information on the heating media)	—	74°C/ 10 min	—	23°C/ 124 d	—	—	Lee & Howard, 1999
Quercetin	Tomato juice	Water bath	—	90°C/ 30 or 60 s	—	4±1°C/ 56 d	—	—	Odriozola-Serrano et al., 2009
Texture	Asparagus	Water bath	—	70–98°C	$E_a = 24$ kcal/mol; $k_{84°C} = 1.60 \times 10^{-2} min^{-1}$ (butt); $k_{84°C} = 2.70 \times 10^{-2} min^{-1}$ (bud); 1st order model	—	—	—	Lau et al., 2000
Texture	Asparagus	Microwave or hot water	—	88°C/ 10 s	—	—	POD (final products)	—	Lau & Tang, 2002
Texture	Broccoli	Continuous microwave	95°C water/ 30 s	3.5 kW/ 4 min, surface temp. of vegetable packs 75–80°C.	—	30°C/ 60 d	—	Visual signs of spoilage, bacterial and yeast growth	Koskiniemi et al., 2013
Texture	Carrot dices	Oil bath	—	80–110°C	$E_a = 140$ kJ/mol in distilled water; 2nd order model	—	—	—	Peng et al., 2014
Texture (Total pectin contents)	Carrot juice	MW heating or traditional heat	—	MW: flow rats 90–287 mL/min at 540–900 W; traditional heat: 100°C/ 10 min	—	4°C/ 4 mo	PME	—	Rayman & Baysal, 2011
Texture	Carrot pieces	Steam	40°C/ 30 min	$F_{70°C}^{10} = 2min$ or $F_{90°C}^{10} = 10min$	—	—	PME & POD (final product)	—	Vervoort et al., 2012
Texture	Carrot pieces	Steam	60°C water/ 40 min	Actual $F_{70°C} = 1.85$ min or $F_{90°C} = 9.67$ min	—	—	—	—	Lemmens et al., 2013
Texture	Jalapeño pepper rings	Water (preheating), Steam (heating)	50°C/ 0–60 min	75°C/ 5 min	—	23°C/ 5 mo	—	—	Howard et al., 1997
Texture	Pickled garlic	Hot water bath	90°C water/ 15 min	90°C/ 5.5 min	—	27±2°C/ 4 mo	—	*Enterobacteriaceae*, lactic acid bacteria & yeast	Réjano et al., 1997

Attribute	Food	Equipment	Pretreatment	Treatment conditions	Kinetics	Storage conditions	Microbial analysis	Reference
Texture	Pumpkin	Thermostatic bath	Boiling water/90 s	85°C/5 min	—	4°C/60 d	Total aerobic bacteria, yeast & molds	Zhou et al., 2014
Texture	Red bell pepper	Continuous microwave	95°C water/30 s	3.5 kW/4 min, surface temp. of vegetable packs 75–80°C	—	30°C/60 d	Visual signs of spoilage, bacterial and yeast growth	Koskiniemi et al., 2013
Texture	Red sweet pepper	Water bath	—	70°C/10 min	—	—	—	Hernández-Carrión et al., 2014 Koo
Texture	Soybean sprouts	Heat (no information on the heating media)	100°C steam/8 min	$F_{90°C}$ = 10 min or $F_{70°C}$ = 2 min	—	3°C/36 d; 10°C/24 d	Aerobic, anaerobic & psychrophilic bacterial counts	Koo et al., 2008
Texture	Sweet potato	Continuous microwave	95°C water/30 s	3.5 kW/4 min, surface temp. of vegetable packs 75–80°C	—	30°C/60 d	Visual signs of spoilage, bacterial and yeast growth	Koskiniemi et al., 2013
Texture	Yellow "banana" pepper	Heat (no information on the heating media)	—	74°C/10 min	—	23°C/124 d	—	Lee & Howard, 1999
Vitamin C	Gazpacho (a cold vegetable soup)	Tubular heat-exchanger in hot water bath	—	90°C/1 min	—	—	—	Elez-Martínez & Martín-Belloso, 2007
Vitamin C	Orange-carrot juice	Plate heat exchanger	—	98°C/21 s	$k_{10°C} = -9.63\times10^{-2}\,d^{-1}$; $k_{2°C} = -4.66\times10^{-2}\,d^{-1}$; 1st order model.	2°C/70 d; 10°C/59 d	—	Torregrosa et al., 2006
Vitamin C	Pumpkin	Thermostatic bath	Boiling water/90 s	85°C/5 min	—	4°C/60 d	Total aerobic bacteria, yeast & molds	Zhou et al., 2014
Vitamin C	Soybean sprouts	Heat (no information on the heating media)	100°C steam/8 min	$F_{90°C}$ = 10 min; $F_{70°C}$ = 2 min	—	3°C/36 d; 10°C/24 d	Aerobic, anaerobic & psychrophilic bacterial counts	Koo et al., 2008
Vitamin C	Yellow "banana" pepper	Heat (no information on the heating media)	—	74°C/10 min	—	23°C/124 d	—	Lee & Howard, 1999
Vitamin C	Tomato juice	Heat exchanger coil in hot water bath	—	90°C/30 or 60 s	$k_{4°C} = 3.04\times10^{-2}\,d^{-1}$ (after 90°C/60s heating); $k_{4°C} = 3.75\times10^{-2}\,d^{-1}$ (after 90°C/30s heating); 1st order model.	4°C/91 d	—	Odriozola-Serrano et al., 2008
Vitamin D	Vegetable juice	Plate heat exchanger	—	90°C/15 s	—	—	—	Barba et al., 2012
Vitamin E	Vegetable juice	Plate heat exchanger	—	90°C/15 s	—	—	—	Barba et al., 2012

(yellowness: blue to yellow) can be used individually or in combination in the form of hue ($h_{ab} = \arctan\frac{b^*}{a^*}$), chroma ($C_{ab}^* = \sqrt{a^{*2} + b^{*2}}$), or total color difference value ($\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$).

Publications related to color changes of vegetables by thermal pasteurization are listed in Table 10. Most authors found that color degradation in vegetables by thermal pasteurization depends mainly on the heat intensity, duration, media, compounds responsible for color, and storage time. For example, Koskiniemi et al. (2013) pasteurized 3 vegetables (broccoli, red bell pepper and sweet potato), and found that the green color of broccoli florets changed the most while the sweet potato color was stable over the course of processing. Kinetic models have been developed to predict the changes of vegetable colors during the pasteurization process. The first order kinetic model was successfully applied to describe the color degradation in asparagus at temperatures ranging from 70 to 98°C, and in a mixed vegetable juice (butterhead lettuce, celery, parsley, apple concentrate and kalamansi lime) between 80 and 100°C (Lau et al., 2000; Loong and Goh, 2004). Both studies referred to a decrease in green color, which closely correlated with reduction of chlorophyll content in the products. Aamir et al. (2014) indicated that the color changes in spinach leaves followed a zero-or first-order kinetic model under pasteurization conditions, depending on the color parameters measured and the harvest season of the spinach. Among previous studies, 6 included the study of color changes during the storage period. The discoloration of pressurized vegetables and vegetable products can occur due to enzymatic or nonenzymatic browning. The desired color values of most pasteurized vegetables decreased during the storage period, which varied from 36 to 120 days in these studies (Koo et al., 2008; Koskiniemi et al., 2013; Rejano et al., 1997; Zhao et al., 2013; Zhou et al., 2014). Only one study by Koskiniemi et al. (2013) reported that the color of broccoli florets did not change during an extended storage or even during thermal processing. The reason was that addition of acid during the equilibration process had a profound effect on the broccoli color, converting chlorophyll (green) into pheophytin (olive green); processing and storage did not produce further changes in the measured color component. The conversion of chlorophyll to pheophytin and pheophorbide can be accelerated when heating green vegetables under lower pH (acidic) conditions, which results in the discoloration of green vegetables during processing (Andrés-Bello et al., 2013).

Carotenoids

Carotenoids are one of the predominant organic pigments present in vegetables, and include α- and β-carotenes (yellow/orange), lycopene (red/orange), xanthophyll (yellow), lutein, and zeaxanthin (green/yellow). They are also one of the important bioactive compounds in carrots, and can act as antioxidants to reduce the risk of developing degenerative diseases. For example, α- and β-carotenes, vitamin A precursors responsible for the orange color in vegetables such as carrot and sweet potato, are also important for vision. Lycopene is considered a potential antioxidant and cancer-preventing agent, responsible for the red color in tomatoes. Lycopene, α- and β-carotenes

may undergo isomerization, oxidation and other chemical changes during thermal processing and storage due to their highly unsaturated structure (Rodriguez-Amaya and Kimura, 2004; Shi et al., 2003).

The effect of thermal pasteurization on the carotenoids in vegetables depends on the heat intensity and the properties of the products. Total carotenoids found in vegetables are relatively stable to mild pasteurization. Vervoort et al. (2012) heated carrot pieces under conditions from mild pasteurization ($F_{70°C} = 2$ min) to severe pasteurization ($F_{90°C} = 10$ min) and found no considerable differences occurred in total carotenoid content after processing, or their individual α- or β-carotene concentration. The authors attributed the stability of carotenes to the protective food matrix, which preserves them from degradation during pasteurization; the applied pasteurization conditions were not severe enough to cause a notable isomerization and/or oxidation of the carotenoids in carrots. Similar results were obtained by Lemmens et al. (2013), for β-carotene content in carrots. However, Odriozola-Serrano et al. (2009) observed an increase of total carotenoid content, lycopene and β-carotene after pasteurization of tomato juice at 90°C for 30 or 60 s. One explanation given was that the homogenization and heat treatment conditions disrupted cell membranes and protein-carotenoid complexes, increasing the extractability of the carotenoids. The carotenoid content of vegetables was reported as decreasing after pasteurization in some studies. Rayman and Baysal (2011) reported a decrease in total carotenoid content of carrot juice after pasteurization at 100°C for 10 min, and Hernández-Carrión et al. (2014) observed a significant reduction of carotenoids in red sweet pepper after pasteurization at 70°C for 10 min. However, the latter study reported the carotenoid content in red sweet pepper on a wet weight basis, which reduces the reliability of their results.

Carotenoids in processed vegetables in storage may also undergo oxidation and isomerization of the all-trans form to the *cis* forms, causing changes in their levels in the final products. A decreasing trend of total carotenoid content and lycopene content in pasteurized tomato juice was observed by Odriozola-Serrano et al. (2008, 2009) during 3 months storage at 4°C. The authors explained that the decrease in lycopene content throughout storage may be due to the oxygen available in the headspace of the container. In contrast, Rayman and Baysal (2011) reported an increase in the carotenoid content in carrot juice after 3 months of storage at 4°C, and attributed it to possible isomerization of β-carotene.

Thermal pasteurization may also influence the bioaccessibility of carotenoids of vegetables. The bioaccessibility refers to the fraction of a nutrient that is released from its food matrix during digestion and made accessible for absorption into the intestinal mucosa. The food matrix is one of the important factors that relates to the bioaccessibility of carotenoids, and may be changed by heat through cell wall softening. Several studies have shown an increased bioaccessibility of β-carotene in carrots and carrot products by thermal pasteurization (Knockaert et al., 2012; Lemmens et al., 2013). Higher β-carotene bioaccessibility is normally associated with intense thermal processes.

Texture

The texture of processed vegetables is another marketable characteristic for customers. Mechanisms that contribute to texture loss during heating of vegetables generally include turgor loss due to the breakdown of cellular membranes, and cell wall degradation and disassembly resulting from enzymatic and nonenzymatic transformations in pectin structure and composition (Anthon et al., 2005; Greve et al., 1994a, b; Peng et al., 2014; Sila et al., 2008). However, not all enzyme-catalyzed reactions to pectin reduce the texture of processed vegetables. For example, the de-esterification of pectin by PME during low temperature blanching contributes to the firming of vegetable tissues. For mild pasteurization in which the processing temperature is lower than 80°C, vegetable tissue softening due to pectin depolymerization by nonenzymatic degradation via β-elimination is negligible due to the relatively high temperature ($T > 80°C$) and pH levels (pH > 4.5) required for this reaction to take place (Sila et al., 2008).

Texture characteristics of vegetables can be evaluated by sensory and instrumental methods. Sensory evaluation offers the opportunity to obtain a complete analysis of the textural properties of a food as perceived by the human sense, while instrumental measurements are more convenient, less expensive, and tend to provide consistent values when used by different people (Bourne, 1982; Abbott, 2004). Evaluations of vegetable texture in the publications listed in Table 10 were all assessed by instrumental methods, although the specific equipment and method were product-dependent. Most authors applied force-compression tests by using a texture analyzer, only one study by Koo et al. (2008) used a rheometer to measure the texture of soybean sprouts.

Most authors observed a decreased texture in processed vegetables in comparison to the raw materials. A first order reaction model is usually applied to predict the softening of vegetables under pasteurization temperatures (Lau et al., 2000). Processed vegetables may also lose their texture during the storage period, and sometimes this loss in storage may exceed the texture loss during the production process. For example, Koskiniemi et al. (2013) pasteurized sweet potato, red bell pepper and broccoli in a continuous microwave system (3.5 kW) for 4 min at 75°C and held the products in insulating molds for 30 min. They found that the texture of red bell pepper was reduced by half after processing, and was almost completely lost at the end of a 60-day storage period at 30°C. A similar phenomenon of major texture loss occurring during the storage period was observed in the other 2 commodities. The authors stated that one possible reason was the addition of NaCl and citric acid, which may have resulted in tissue softening during the storage time. Besides the presence of these compounds in the media, residual enzyme activity in processed products may also degrade texture through reactions occurring during storage. Rayman and Baysal (2011) investigated residual PME activities and pectin content in pasteurized carrot juice during a 4-month storage at 4°C, but no information on the correlation between the 2 were provided in that study. Schultz et al. (2014) studied the effects of adding PME to pasteurized carrot juice, quantified the juice texture via cloud stability and particle size, and correlated texture with the levels of PME addition during juice

storage (56 days/ 5°C). The authors found that all levels of PME addition (0.15–1.0 U/g) resulted in clarification, and higher amounts had a modest effect in causing more rapid clarification, due to a faster increase in particle size.

Phenolics and antioxidant activity

Phenolics are important phytochemicals that function as bioactive compounds in vegetables. Most researchers have reported phenolics in relation to their antioxidant activity. Effects of thermal pasteurization on the total phenolics in vegetables have been associated with the properties of the food material, package and storage conditions. A reduction in the total phenolic content of pumpkin and carrot juice in pasteurization and post-pasteurization storage at 4°C up to 3 or 4 months was reported by Rayman and Baysal (2011) and Zhou et al. (2014). In contrast, Odriozola-Serrano et al. (2008) did not find significant changes in the total phenolic content between pasteurized and fresh tomato juice (90°C for 30/60 s), and noticed good maintenance of the phenolic compounds during storage. This might be due to the inactivation of the enzymes responsible for their degradation. However, the same authors later reported a decrease in the phenolic concentration of pasteurized tomato juice during storage using the same processing and storage conditions (Odriozola-Serrano et al., 2009). The authors hypothesized that the degradation of phenolic compounds during storage was associated with residual activity of peroxidase, but no enzyme activity assay was carried out to support this assumption. None of the papers that associated phenolic changes in vegetables during storage examined the related enzyme activities (Table 10).

Quercetin is one of the numerous flavonoids commonly found in vegetables. It is known that flavonoids are relatively labile to degradation due to their hydroxyl and ketone groups, and also because of the unsaturated double bonds. Most studies reported a decrease in quercetin content in pasteurized vegetables (Lee and Howard, 1999; Odriozola-Serrano et al., 2009). The changes in quercetin are also associated with the specific processing method. Roldán et al. (2008) evaluated the total quercetin content in pasteurized onion by-products and their frozen products, and found a lower value in all the pasteurized products compared to their corresponding frozen products, but higher quercetin values than the sterilized onion products. With regard to the quercetin changes during storage, stable levels in yellow banana pepper during storage (23°C /4 months) were reported by Lee and Howard (1999), while a decrease in pasteurized tomato juice (23°C /4 months) was observed by Odriozola-Serrano et al. (2009).

Antioxidant capacity is associated with the levels of some bioactive compounds in foods, such as phenolics, vitamin C, and lycopene. Generally, antioxidant activity is reduced in pasteurized vegetables, and this depletion is associated with heat intensity (Rayman and Baysal, 2011). However, Odriozola-Serrano (2008) demonstrated no changes in the antioxidant activity between fresh and pasteurized tomato juice, and explained this by the formation of novel compounds such as products from the Maillard reaction which have antioxidant activity. The authors reported that the antioxidant capacity of pasteurized tomato juice decreased with storage time and attributed this to the losses of vitamin C and lycopene.

Vitamins

Vegetables are great sources of various essential vitamins. Vitamin C (ascorbic acid) is one of the numerous vitamins in vegetables. However, vitamin C is readily changed or broken down in the presence of oxygen and light, and high temperature will accelerate this degradation process. Due to its thermolability, vitamin C in vegetables is often used as an indicator for the loss of other vitamins and thermolabile nutrients in studies that evaluate the influence of thermal processing on food qualities. Thermal degradation of vitamin C in foods is generally reported as a first-order kinetic reaction, both during thermal treatment and storage (Odriozola-Serrano et al., 2008; Torregrosa et al., 2006). Large losses of vitamin C usually occur at the beginning of the thermal treatment, and high temperatures accelerate the degradation process (Elez-Martínez and Martín-Belloso, 2007; Koo et al., 2008; Torregrosa et al., 2006). For example, thermal pasteurization of gazpacho (a cold vegetable soup) at 90°C for 1 min reduced the vitamin C level to 79.2% of its initial value (Elez-Martínez and Martín-Belloso, 2007). Other vitamins, such as vitamin E and D, have also been reported to decrease in vegetable beverages after pasteurization (Barba, 2012).

Most authors have reported reductions in vitamin C during storage following thermal treatment. The lower level depended on storage conditions, such as temperature, oxygen content, light and packaging conditions. The fist-order kinetic model has been used to describe the degradation of vitamin C in storage (Odriozola-Serrano et al., 2008). Conceivably, its degradation rate during normal storage is generally lower than during thermal processing. For example, the content of vitamin C decreased by 19.2% in thermally treated pumpkin (85°C for 5 min), and only additional 12.1% loss after 2 months storage at 4°C (Zhou et al., 2014). Low storage temperatures usually slow down ascorbic acid degradation rate (Torregrosa et al., 2006). However, Koo et al. (2008) reported that a decreased level of ascorbic acid in cook-chilled packaged sprouts stored at 3°C was much like those stored at 10°C, which may be due to a relatively low level of this nutrient after thermal treatment.

Other components

Changes in other nutrients or relevant quality aspects, such as sugars, dry matter content, fatty acids, isothiocyanates and furfural have been reported in vegetables during thermal treatments and storage (Vervoort et al., 2012; Zhou et al., 2014), this information is not included in Table 10. Vervoort et al. (2012) reported a significant reduction in dry matter content, furfural, and sugar concentration (glucose, fructose and sucrose) of carrot pieces during pasteurization, and found that increasing the processing intensity from a level of $F_{70°C} = 2$ min to $F_{90°C} = 10$ min did not cause any further significant changes in those values. However, the study by Zhou et al. (2014) showed that pasteurization did not significantly change the sugar content (sucrose, glucose and fructose) in pumpkin after thermal treatment (85°C/ 5 min) and during storage (60 days/4°C).

Storage and shelf-life of pasteurized vegetables

Storage is an important component for the entire food chain to ensure the safety and quality of foods from the field to the table. Many storage conditions, such as storage temperature, relative humidity, air circulation, ventilation, packaging, stacking, volatile substances, and hygiene may affect the quality of processed products. For pasteurized foods, refrigerated storage is normally required to extend the shelf-life of the products. Refrigeration slows down the growth of spoilage microorganisms and the rate of chemical changes in foods, therefore it reduces the food deterioration rate. Table 10 summarizes the published papers on pasteurized vegetables related to storage and enzyme studies. Few studies investigated the residual enzyme activity after processing. It is also notable that storage temperatures and time in the works cited varied from product to product. For most of the pasteurized vegetables, a storage temperature of 3–5°C was used. For some pickled, high acid vegetables, the storage temperature was much higher (23–30°C) because of the low pH of the products. The storage times reported in these publications varied from 21 days to 5 months, depending on the property of the products and the storage temperature. Aside from pH as mentioned above, other factors such as reduced water activity, preservatives, or a combination thereof can be used to control pathogen growth or spoilage during storage and affect the storage life of the products.

With regard to the shelf-life of pasteurized ready-to-eat foods in general and vegetables in particular, there does not appear to be a universally accepted standard for all products. Torregrose et al. (2006) calculated the shelf-life of pasteurized orange-carrot juice as the time taken for the ascorbic acid concentration to reduce by 50% (Table 10). Most authors do not provide reasons for the storage conditions they selected for their products. Shelf life is defined as "the period of time for which a product remains safe and meets its quality specifications under expected storage and use conditions" (ECFF, 2006). Based on the ECFF (2006), the manufacturer is responsible for determining the shelf life and must take into account microbiological safety and stability, physical characteristics and organoleptic quality. Microbiological safety and stability should always be a priority for determining the shelf life when the acceptable shelf life for either physical condition or organoleptic quality exceeds that for microbial safety. The product shelf life is influenced by a number of factors, including raw material quality, product formulation (pH, a_w), hygiene during manufacturing, scheduled heat or other preservation treatments, cooling methods applied to products, type of package, storage temperature and relevant hurdles (CAC, 1999). When determining the shelf life of products, the Codex Alimentarius Commission (CAC) suggests taking into consideration the potential for temperature abuse which may occur during manufacture, storage, distribution, sale, and handling by the consumer. For example, fluid milk is most often held at marginal refrigeration temperatures of 6.1–7.2°C (43–45°F) instead of the ideal holding temperatures (≤ 3.3°C) to determine the potential shelf-life (Murphy, 2009). The authors believed these marginal refrigeration temperatures allow defects and sanitation deficiencies to become more evident. The FDA requires control measures for storage under normal and moderate abuse

conditions (FDA, 2011b), for example, the recommended incubation temperature for inoculated pack studies with nonproteolytic *C. botulinum* is 10°C (50°F) (NACMCF, 1992). Therefore, for a food producer determining the safe shelf-life for pasteurized ready-to-eat foods and vegetables, the following information needs to be collected based on ECFF recommendations:

(1) Review relevant scientific information containing the characteristics of pathogens;
(2) Use predictive modeling programs (e.g., ComBase, USFA Pathogen Modeling Program or Growth Predictor) to estimate the growth of pathogens under the storage conditions;
(3) Conduct a challenge test with the relevant pathogens where predictive modeling on its own does not give sufficient confidence to set a safe shelf life;
(4) Collect historical data for similar products;
(5) Conduct storage trials, either by storing products at predetermined temperatures during specific time periods considering actual chill chain performance under HACCP or testing the product at minimum 3 time points for the relevant indicator and spoilage microorganisms as well as pathogens identified by HACCP.

The information above focuses on a safe shelf life. Desired quality should also be considered when determining the shelf-life of pasteurized products. This information also provides insights for researchers to conduct storage study for pasteurized products.

Conclusions and future trends

To design a thermal pasteurization process for ready-to-eat meals including vegetable products that could meet the regulations and guidelines of governmental food agencies, it is of paramount importance to fully understand regulatory requirements and select the pathogens of concern that could survive the process and storage conditions. Determination of the target pathogens for a pasteurization process according to the U.S. regulations is product and process dependent; there is no "one size fits all" approach. Despite the differences in the specific requirements for target pathogens for pasteurized foods in the United States and Europe, safety storage conditions throughout product shelf-life are common concerns. Consideration of moderate temperature abuse during storage and distribution should be included when determining the target pathogens for the process design.

Viral pathogens are an emerging concern for foods, and progress has been made in characterizing the environmental resistance (especially thermal resistance) of some human norovirus surrogates. Based on our current knowledge, norovirus or HAV may be able to exist in some low temperature processed foods. Therefore, to develop a pasteurization process, it is suggested that the presence of viral pathogens be taken into consideration in addition to the common pathogens, particularly in fruit and vegetables, shellfish, deli meats and ready-to-eat foods.

After addressing the safety concern for a pasteurized product, attention should be given to the product quality changes due to the storage and distribution of the food. Thermal kinetic data of quality-associated enzymes and quality parameters in vegetables are available in the literature, and can be used for the pasteurization process optimization. To develop a pasteurized vegetable product using a new technology, storage trials should be carried out at normal distribution and storage conditions to verify the pasteurization process and ensure safe and quality-acceptable products within their maximum shelf-life.

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