

Influence of Water Activity on Thermal Resistance of Microorganisms in Low-Moisture Foods: A Review

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Abstract: A number of recent outbreaks related to pathogens in low-moisture foods have created urgency for studies to understand the possible causes and identify potential treatments to improve low-moisture food safety. Thermal processing holds the potential to eliminate pathogens such as *Salmonella* in low-moisture foods. Water activity (a_w) has been recognized as one of the primary factors influencing the thermal resistance of pathogens in low-moisture foods. But most of the reported studies relate thermal resistance of pathogens to a_w of low-moisture foods at room temperature. Water activity is a thermodynamic property that varies significantly with temperature and the direction of variation is dependent on the product component. Accurate methods to determine a_w at elevated temperatures are needed in related research activities and industrial operations. Adequate design of commercial thermal treatments to control target pathogens in low-moisture products requires knowledge on how a_w values change in different foods at elevated temperatures. This paper presents an overview of the factors influencing the thermal resistance of pathogens in low-moisture foods. This review focuses on understanding the influence of water activity and its variation at thermal processing temperature on thermal resistance of pathogens in different low-moisture matrices. It also discusses the research needs to relate thermal resistance of foodborne pathogens to a_w value in those foods at elevated temperatures.

Keywords: low-moisture food safety, *Salmonella*, thermal processing, thermal resistance, water activity, water mobility

Introduction

Food safety is a global concern. About 1 out of 6 individuals in North America suffer from foodborne illnesses which is equivalent to about 48 million cases every year and costs billions of dollars to the food processing industry due to food product recalls (CDC 2011). Recently, recalls and foodborne illnesses associated with low-moisture foods (with water activity, $a_w < 0.6$) such as dry nuts, peanut butter, spices, and pet foods have drawn great attention from the public, industry, and research communities (Cavallaro and others 2011; Sheth and others 2011). Several food pathogens such as *Salmonella* (in spices, dry nuts, chocolate, peanut butter, and so on), *Cronobacter sakazakii* (in powdered infant formula), *Bacillus cereus* (in rice cereal), *Clostridium botulinum* (in honey), *Staphylococcus aureus* (in salami), certain viruses (hepatitis A virus in semi-dried tomatoes), and mycotoxigenic molds (in dried fruits) have been reported to survive (but may not grow) in/on low-moisture foods and environments for extended periods of time

(Beuchat and others 2011). The majority of these outbreaks related to low-moisture foods have been caused by *Salmonella* species and only a very small number of *Salmonella* cells are required to cause disease (Beuchat and others 2011; Cavallaro and others 2011). The serotypes of *Salmonella* commonly associated with these outbreaks are Enteritidis and Typhimurium (Beuchat and others 2011).

The Food Safety Modernization Act (FSMA) as enforced by the U.S. Food and Drug Administration (FDA) was signed in to law in 2011. According to the FDA website, the FSMA preventive controls for human food rule are now final (FDA 2015). FSMA focuses on preventing contamination rather than responding to contamination. Once implemented, food industries should develop preventive controls for target pathogens in all foods (FDA 2015). In the case of low-moisture foods, preventive controls may include additional processing and validation of these processing methods in order to ensure safety. In low-moisture food processing, there is currently a lack of tools and methods for process validation. Key parameters determining validation of pasteurization processes include selected processing and product factors. Identification of the suitable surrogate microorganism is also necessary for developing validation protocols of processing techniques for low-moisture foods.

This review focuses on key product factors related to the development and validation of thermal processing of low-moisture foods and presents the factors influencing thermal resistance of pathogens in low-moisture foods, emphasizing the influence of

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water activity, which is probably the most important factor. Elevated temperatures (>60 °C) during thermal processing may sharply change the characteristics of a food matrix and microorganisms in low-moisture foods. Thus, the variation in water activity at these thermal processing temperatures and its influence on thermal resistance of pathogens are discussed. Furthermore, knowledge gaps and future research needs are identified with regard to the development of thermal processing methods to improve low-moisture food safety.

Processing technologies to control pathogens in low-moisture foods

The safety of low-moisture food products may be improved by controlling the pathogens during food manufacturing and by preventing recontamination and cross-contamination of the final products (Beuchat and others 2011). However, treatments may be necessary in order to eliminate the pathogens in a contaminated low-moisture product. Several thermal and nonthermal pasteurization technologies, such as steam (Chang and others 2010), moist and dry air (Jeong and others 2009), radiofrequency (Kim and others 2012; Ha and others 2013), X-ray (Jeong and others 2012), electron beam (Black and Jaczynski 2008; Hvizdzak and others 2010), propylene oxide (Danyluk and others 2005), and use of plant extracts (Kotzekidou and others 2008) have been reported to improve low-moisture food safety. The application of these technologies may be dependent on structural characteristics (size shape), chemical composition, and nature of the low-moisture foods (*powders* such as ground spices, flour, infant formula; *pastes* such as peanut butter; or *large particulates* such as nuts). There is no single technology available which is suitable to treat different kinds of low-moisture food products. Thermal processing technologies using dry heat, wet heat, or electromagnetic radiations can effectively reduce pathogens in low-moisture foods. Dry heat treatments include superheated steam, hot air exposure, baking, roasting etc. Controlled condensation steam and moist air impingement use wet heat to control pathogens in low-moisture products such as nuts and powders (Grasso and others 2014). Radiofrequency, infrared, and microwave radiation may also be effective in heating low-moisture foods more quickly compared to conventional technologies. More information regarding the types of thermal processing techniques used to improve the safety of low-moisture foods is given in Table 1. The food processing industry is interested in the potential of existing food processing technologies also, where heat is used as a medium to manufacture low-moisture foods safe (such as drying, baking, extrusion, and frying). Insufficient understanding of the main processing factors involved in choosing technologies to treat different low-moisture foods may risk the safety of these foods. Further understanding of the critical factors associated with the development of new pasteurization technologies for low-moisture foods is essential.

Factors Influencing the Thermal Resistance of Pathogens in Low-Moisture Foods

In order to develop adequate thermal processing technologies, information on thermal resistance of the target pathogens in low-moisture foods should be available. Several product-related factors (water activity, water mobility, type, and nature and composition of product) and process-related factors (relative humidity and mode of heating) may influence the thermal resistance of pathogens in low-moisture foods. Elements related to the microorganisms, such as strain, growth conditions, and age and number of bacterial cells, may also influence thermal resistance. The following subsections

focus on the main product-related factors associated with thermal resistance of pathogens in low-moisture foods.

Water activity

Water activity is considered as one of the most important parameters in food preservation of dehydrated products. The water activity concept is more than 100 years old. Schloesing first reported the relationship between water content and equilibrium relative humidity at a certain temperature or the water vapor sorption isotherm of textile fibers back in 1893 (van den Berg and Bruin 1981). G. N. Lewis introduced the concept of activity of a component in 1907, including activity of water in a system based on thermodynamic principles described earlier by J. W. Gibbs (Lewis 1907; van den Berg and Bruin 1981). The first systematic studies on the relationship between microbial growth and relative humidity was conducted in the 1920s by Walderdorff and Walter (van den Berg and Bruin 1981). Later, in the 1930s, Australian microbiologist W. J. Scott conducted experiments on microbial growth in food systems in relation to the relative humidity of the environment (Scott 1936). In 1953, Scott explained the concept of water activity in food systems, and presented the correlation between growth rate of microorganism and a_w of the growth medium (Scott 1953). Scott's research led to many developments in the fundamental understanding of the a_w concept and its applications in food processing and storage. These studies include contributions by Christian and Scott (1953), Christian (1955), Christian and Waltho (1962), Brown (1975), Chirife and others (1981), Corry (1975), Mossel (1975), Labuza (1975, 1977), Troller and Christian (1978 a,b), Chirife and Iglesias (1978), and Karel (1975, 1981, 1986). The use of food stability map was advanced by M. Karel, S. R. Tannenbaum, and T. P. Labuza (Labuza and others 1970). The next development of the a_w concept led to the formation of a scientific organization called the International Symposium on the Properties of Water (ISOPOW, www.isopow.org) in 1974, which is aimed at advancing the understanding of the properties of water in food and related biological systems.

Water activity is defined as the ratio of water vapor pressure in a food system (P_v) (Pa) to the saturation water vapor pressure (P_{vs}) (Pa) at the temperature of the food system.

$$a_w = \frac{P_v}{P_{vs}} \quad (1)$$

Water activity is a thermodynamic property, related to the fugacity, or escaping tendency, of water from food (Loncin 1988). If water vapor is considered as an ideal gas phase (which is a safe assumption in food manufacturing/industry conditions), the ratio of fugacity of water in food to that of pure liquid water (which is chosen as the reference system) is equivalent to their vapor pressure ratio (Loncin 1988). The vapor pressure of water in a food system or its components in a multicomponent food system is equivalent to the vapor pressure of water in air in thermodynamic equilibrium with the food in a closed system.

Water activity is also a measure of thermodynamic free energy or water chemical potential, which is equivalent to partial molar free energy of water in a food system. At equilibrium, the chemical potential (μ) of a system is given by (Reid 2008):

$$\mu = \mu_0 + RT \ln a_w = \mu_0 + RT \ln \frac{P_v}{P_{vs}} \quad (2)$$

Table 1—Selected studies on thermal processing techniques to control pathogens in low-moisture foods.

Authors and year	Processing technique	Processing conditions	Product	Target microorganism	Major results
Fine and Gervais (2005)	Hot air	200 to 600 °C from 0.1 to 30 s heating followed by instantaneous cooling at -80 °C	Wheat flour	<i>Bacillus subtilis</i> spores and <i>Saccharomyces cerevisiae</i> cells	<ul style="list-style-type: none"> About 5 to 8 log reduction in microbial population was obtained based on the initial water activity level
Cenkowski and others (2007)	Superheated steam	105 and 185 °C with steam velocities of 0.35, 0.65, 1.3, and 1.5 m/s	Wheat grains	<i>Fusarium</i> mycotoxin deoxynivalenol (DON) and <i>Geobacillus</i> (<i>Bacillus</i>) <i>stearothermophilus</i> ATCC 10149	<ul style="list-style-type: none"> The DON concentration reduction was up to 52% at 185 °C for 6 min processing with superheated steam The <i>D</i>-values of <i>G. stearothermophilus</i> spores processed with superheated steam ranged from 2.2 to 23.5 min for 105 to 175 °C The <i>z</i>-value for <i>G. stearothermophilus</i> spores exposed to superheated steam at 130 to 175 °C was determined to be 28.4 °C
Brandl and others (2008)	Infrared heating (3000 to 5458 W/m ²)	90 to 113 °C for 30 to 45 s	Almond kernels	<i>S. Enteritidis</i>	<ul style="list-style-type: none"> Infrared heating with immediate cooling of the product resulted in 0.63 to 1.51 log reductions in microbial population IR treatment followed by holding the kernels at warm temperature for 60 min, resulted in more than 7.5-log reduction in <i>S. Enteritidis</i> on almond kernels Macroscopic assessment (morphology, color) showed that the quality of IR-treated kernels was not significantly different from that of untreated kernels
Chang and others (2010)	Steam pasteurization	95 °C for maximum 65 s, 143 kPa	Almonds	<i>S. Enteritidis</i>	<ul style="list-style-type: none"> Steam pasteurization achieved 5-log reduction of <i>S. Enteritidis</i> within 25 s without changing the visual quality of almonds
Jeong and others (2011)	Moist-air convection heating	121 to 204 °C, 5% to 90% relative humidity, for 72 to 6344 s	Almonds	<i>E. faecium</i> strain NRRL B-2354 and <i>Salmonella</i> Enteritidis PT30	<ul style="list-style-type: none"> The mean log reductions for <i>E. faecium</i> were 0.6 log and 1.4 log lower than those for <i>S. enterica</i> Enteritidis PT30 The <i>D</i>-values for <i>E. faecium</i> on the surface of almonds subjected to moist-air heating (30% to 90% moisture by volume) were 30% larger than those for SE PT30 <i>E. faecium</i> can be used as a conservative surrogate for SE PT30 during moist-air heating
Studer and others (2013)	Aerated steam treatment	70 °C for 30 to 300 s	Alfalfa and mung bean seeds	<i>E. coli</i> O157:H7, <i>E. coli</i> O178:H12, <i>S. Weltevreden</i> , and <i>L. monocytogenes</i> Scott A	<ul style="list-style-type: none"> Populations of <i>E. coli</i> O157:H7 and <i>S. Weltevreden</i> on alfalfa and mung bean seeds could be completely eliminated by a 300-s treatment with steam at 70 °C The 300-s treatment was able to reduce the population of <i>L. monocytogenes</i> to undetectable levels The germination rate of mung beans was not affected by the 300-s treatment compared to the germination rate of untreated seeds whereas that of alfalfa seeds was significantly lower by 11.9%
Ha and others (2013)	Radiofrequency heating (27 MHz)	77 to 86 °C for maximum 90 s	Peanut butter cracker sandwich	<i>S. Typhimurium</i> and <i>E. coli</i> O157:H7	<ul style="list-style-type: none"> RF treatment resulted in 4.3 log reductions of <i>S. Typhimurium</i> and 4.4 log reductions in <i>E. coli</i> O157:H7 population, respectively in creamy peanut butter cracker sandwich RF treatment resulted in 4.6 log reductions of <i>S. Typhimurium</i> and 5.3 log reductions in <i>E. coli</i> O157:H7 population, respectively in chunky peanut butter cracker sandwich RF treatment did not influence the color and sensory characteristics peanut butter and crackers
Jeong and Kang (2014)	Radiofrequency heating (27 MHz)	90 °C for maximum of 80 s	Dried red and black pepper powder	<i>S. Typhimurium</i> and <i>E. coli</i> O157:H7	<ul style="list-style-type: none"> More than 7 log reductions of the target microorganisms were achieved during RF heating RF heating did not affect product quality Moisture content of the products decreased significantly during RF heating

where μ (J/mol) is the chemical potential of the system, that the thermodynamic activity of water, μ_o is the chemical potential of the pure material at temperature T (K), and R is the gas constant (8.314 J/mol.K). When the food and the surrounding environment reach equilibrium, the chemical potential of water in the food and the chemical potential of water vapor in the environment become the same. Thus, net transfer of water between the food and the environment becomes approximately zero (Labuza and Altunakar 2007). But dynamic changes in external factors, like temperature and pressure, during food processing and storage make true equilibrium unlikely. Most foods exist in a metastable state, which is a pseudo-equilibrium with their environment. The water activity of foods determined in their metastable state are within experimental uncertainty of true equilibrium (Chirifé and Buera 1996). Water activity is also an estimate of thermodynamically available water for various physicochemical or biological reactions. Its influence on reactant mobility during processing and storage of foods is greater than that of water content; that makes it a more useful parameter than water content to understand product quality and stability (Bassal and others 1993a,b).

Water activity and thermal resistance of microorganisms in low-moisture foods. Microorganisms in low-moisture environments are in general quite tolerant to heat. Convincing experimental results show a sharp increase in thermal resistance of microbial pathogens, such as *Salmonella*, when the a_w of a food system is reduced below 0.6 (Archer and others 1998; Bari and others 2009). The influence of a_w on thermal resistance of microorganisms in low-moisture environments was recognized in the late 1960s. Murrel and Scott (1966) reported that the thermal resistance, as explained by D -values (time required for 90% reduction in the population of a specific pathogen), of bacterial spores (*Bacillus megaterium*, *Bacillus stearothermophilus* ATCC 7953, *Clostridium botulinum* type E ATCC 9564, *C. botulinum* type B ATCC 7949, *Clostridium bifermentans*, and *Bacillus coagulans*) equilibrated to a_w values of 0.2 to 0.4 at 110 °C varied from 2 to 24 min. The thermal resistance of *Clostridium botulinum* 62A spores was reported over the a_w range of 0 to 0.9 (Alderton and others 1980). Higher thermal resistance (D -value) was observed between a_w range of 0.1 to 0.5, while the spores exhibited smaller thermal resistance at a_w between 0.7 to 0.9 (Alderton and others 1980). Goepfert and others (1970) determined the thermal resistance of selected *Salmonella* spp. and *E. coli* at a selected a_w range of 0.75 to 0.99 in sucrose, fructose, glycerol, and sorbitol solutions. The thermal resistance of the selected microorganisms increased as the a_w was reduced (Goepfert and others 1970).

The D - and z - (temperature difference required for 90% increase or decrease in D -value of a specific pathogen) values of *S. Typhimurium* in a salt solution model system with a_w of 0.3 at a temperature range of 90 to 120 °C ranged from 10.6 to 20 min and 40 °C, respectively (Akinleye 1994). The D -value and z -value of *S. Weltevreden* at a_w range of 0.5 to 0.6 at approximately 70 °C were 80 min and approximately 30 °C, respectively, which are relatively high (Archer and others 1998). Doyle and Mazzota (2000) showed that the D -value of *S. Typhimurium* at 68.3 °C in a sucrose solution at initial a_w of 0.98 was 0.12 min, but if the a_w decreased to 0.83, the D -value jumped to 40.2 min (300-fold increase). The thermal resistance of *S. cerevisiae* and *L. plantarum* in wheat flour and skim milk was studied at different a_w levels ranging from 0.1 to 0.7 (measured at room temperature) at 150 and 200 °C (Laroche and others 2005). The highest thermal resistance of *Saccharomyces cerevisiae* and *Lactobacillus plantarum* in wheat flour was observed at a_w levels of 0.40 and 0.35,

respectively, while in skim milk, maximum thermal resistance was observed at 0.3 a_w (Laroche and others 2005). The D -value of *S. Enteritidis* PT30 in almond flour was reported to be 0.42 min at 68 °C and 0.95 a_w , but increased to 15.2 min (36 times) at 70 °C when water activity was reduced to 0.60 a_w (Villa-Rojas and others 2013). Hence, low-moisture foods may need to be heat-treated for long periods of time in order to achieve adequate reduction in pathogen populations (5 log), which is challenging since heat may impact the quality of the product. Table 2 presents some of the reported studies of thermal resistance of selected pathogens and a_w range in specific low-moisture foods. Most of the studies reported a_w values of low-moisture foods at room temperature, although the studied food systems were treated at elevated temperatures (>60 °C) in sealed containers.

Water mobility

Water activity is a more macroscopic concept, whereas water mobility is a molecular concept, which is related to the translational, rotational, or vibrational motion of water molecules in food. Through translational motion, or self-diffusion, a water molecule can change its location in a 3-dimensional space (Schmidt 2004). The self-diffusion coefficient (D_{self}) is a measure of translational motion of water molecules, which is determined using the Stokes-Einstein relationship (Schmidt 2004). Water molecules in liquid and vapor phases exhibit translational motion, which can be measured using nuclear magnetic resonance (NMR) and magnetic resonance imaging spectroscopy (MRI) (Sun and Schmidt 1995). A water molecule can spin on its axis, resulting in rotational motion in its liquid and vapor phases but less in the solid phase (Schmidt 2004). The vibrational motion of a water molecule is an intramolecular motion, through stretching, bending, or rotation of bonds (Schmidt 2004).

In nonequilibrium systems like food systems, water molecules are mobile and it may influence the availability of water to microorganisms for growth and survival. Studies have related the growth of microorganisms to water mobility in food systems (Lavoie and others 1997; Vittadini and others 2005). The growth rate (exponential phase) of *Staphylococcus aureus* correlated better with mobile water than water activity or water content in brain heart infusion (BHI) broth media with glycerol, NaCl, and raffinose as solutes, as presented in NMR signal intensity data determined using ^{17}O NMR (Lavoie and others 1997). A few studies have focused on the influence of water mobility on thermal resistance and survival of microorganisms in low-moisture foods at elevated temperatures. Farakos and others (2013) reported that water mobility did not influence the survival of *Salmonella* in whey protein powder during storage at selected temperatures ranging from 21 to 80 °C. Lian and others (2015) studied the influence of water mobility on the survival of *Salmonella enterica* by altering the tertiary structure to change the water-protein interaction in skim milk powder using ultra-high pressure. They stored the inoculated skim milk powder with a_w values of 0.33, 0.53, and 0.81 at 37 °C for 60 d. The influence of a_w was greater while water mobility influenced little at low water activities. But water mobility exerted a greater influence on the survival of *Salmonella* in skim milk powder at a_w of 0.81 during storage. The above studies show that thermal resistance of microorganisms was better related to water activity than water mobility in low-moisture foods. Growth of microorganisms was better correlated with water mobility.

Table 2—Thermal resistance of microorganisms in selected low-moisture foods influenced by water activity. Water activity values were measured at room temperature unless stated otherwise.

Authors and year	Title	Product	Target microorganism	Water activity and temperature range	Major results thermal resistance data (<i>D</i> - and <i>z</i> -values)
Murrel and Scott (1966)	The heat resistance of bacterial spores at various water activities	Selected growth medium	<i>Bacillus megaterium</i> , <i>B. stearothermophilus</i> ATCC 7953, <i>Clostridium botulinum</i> type EATCC 9564, <i>C. botulinum</i> type B ATCC 7949, <i>C. bifementans</i> , <i>B. coagulans</i>	0.11 to 0.80; 70 to 120 °C	<ul style="list-style-type: none"> Heat resistance was maximum at a_w values of about 0.2 to 0.4, the maximum <i>D</i> values at 110 °C varying from about 2 to 24 h At a_w less than 0.2, the heat resistance decreased At a_w greater than 0.4 the resistance of selected microorganism decreased considerably
Goepfert and others (1970)	Relation of the heat resistance of salmonellae to the water activity of the environment	0.01 M phosphate buffer at pH 6.9 ± 0.1. with sucrose and glycerol added to change a_w	Selected <i>Salmonella</i> and <i>E. coli</i> strains	0.87 to 0.99 using sucrose and 0.75, 0.90, and 0.99 a_w by glycerol, 57 °C	<ul style="list-style-type: none"> Heat resistance of the organisms increased as the a_w of the heating medium was reduced The type of solute used to control a_w affected the thermal resistance of selected microorganisms
Sumner and others (1991)	Heat resistance of <i>Salmonella typhimurium</i> and <i>Listeria monocytogenes</i> in sucrose solution of various water activities	Sucrose solution	<i>S. Typhimurium</i> and <i>L. monocytogenes</i>	0.83 to 0.99 temperatures ranging from 65.6 to 76.7 °C for <i>S. typhimurium</i> and 4 temperatures ranging from 60 to 68.3 °C for <i>L. monocytogenes</i>	<ul style="list-style-type: none"> Heat resistance of selected microorganisms increased with decrease in a_w The decrease in <i>D</i>-values of <i>Salmonella</i> was greater than that of <i>Listeria</i>
Mattick and others (2001)	Effect of Challenge Temperature and Solute Type on Heat Tolerance of <i>Salmonella</i> Serovars at Low Water Activity	Tryptone soy broth	<i>Salmonella Typhimurium</i> DT104	0.65 to 0.90; 55 to 80 °C	<ul style="list-style-type: none"> <i>Salmonella</i> was heat sensitive at a_w of 0.65 when the temperature was low (55 to 60 °C)
Beuchat and Scouten (2002)	Combined effects of water activity, temperature and chemical treatments on the survival of <i>Salmonella</i> and <i>Escherichia coli</i> O157:H7 on alfalfa seeds	Alfalfa seeds	<i>Salmonella</i> and <i>E. coli</i> O157:H7	0.15 to 0.54; 50, 60, 70, and 80 °C	<ul style="list-style-type: none"> The rate of inactivation of <i>Salmonella</i> and <i>E. coli</i> O157:H7 on alfalfa seeds was increased at higher a_w and temperature
Laroche and others (2005)	Water activity affects heat resistance of microorganisms in food powders	Wheat flour and skim milk	<i>Saccharomyces cerevisiae</i> and <i>Lactobacillus plantarum</i>	Initial a_w of 0.1 to 0.7 and a_w was monitored during drying, 150 and 200 °C	<ul style="list-style-type: none"> Maximum viability for <i>L. plantarum</i> and <i>S. cerevisiae</i> was observed at a_w values of 0.35 and 0.4, respectively, in wheat flour Maximum viability for <i>L. plantarum</i> and <i>S. cerevisiae</i> was observed at a_w values of 0.2 to 0.5 and 0.3 to 0.5, respectively, in skim milk powder
Villa-Rojas and others (2013).	Thermal inactivation of <i>Salmonella</i> Enteritidis PT 30 in almond kernels as influenced by water activity	Almond kernels	<i>Salmonella</i> Enteritidis PT 30	70, 73, 76, and 80 °C at a a_w of 0.601; 62, 65, 68, and 71 °C at a a_w of 0.72; 59, 62, 65, and 68 °C at a_w of 0.888; 56, 60, 64, and 68 °C at an a_w of 0.946	<ul style="list-style-type: none"> A nonlinear thermal inactivation behavior of <i>Salmonella</i> was observed Small increase in a_w reduced the thermal inactivation time
Farakos and others (2013)	Modeling the influence of temperature, water activity and water mobility on the persistence of <i>Salmonella</i> in low-moisture foods	Whey protein powder	<i>Salmonella Typhimurium</i> , <i>S. Tennessee</i> , <i>S. Agona</i> , and <i>S. Montevideo</i>	0.19 and 0.54; 21, 36, 50, 60, 70, and 80 °C	<ul style="list-style-type: none"> Survival of <i>Salmonella</i> serovars were significantly influenced by a_w with increase in survival at low a_w
He and others (2013)	Increased water activity reduces the thermal resistance of <i>Salmonella enterica</i> in peanut butter	Peanut butter	<i>Salmonella Typhimurium</i> , <i>S. Tennessee</i> , <i>S. Enteritidis</i> , 3-serotype cocktail	0.2, 0.4, 0.6, and 0.8, 90 and 126 °C	<ul style="list-style-type: none"> Water activity increases significantly reduced the thermal resistance of selected microorganisms at 90 °C

Physical structure and composition of food products

Food systems are structurally complex. Thermal resistance of *Salmonella* was influenced by variation in physical structure (whole beef muscle compared with ground beef compared with beef puree) (Mogollón and others 2009). The variation in physical structure, such as foods with small particulates (for example, wheat flour, ground spices), foods with large particulates (tree nuts), or pastes (peanut butter), may result in different thermal resistance values for pathogens in low-moisture foods.

The chemical composition of food components present in food systems may also influence the thermal resistance of microorganisms (such as carbohydrate- or protein- or fat-rich food systems). The presence of solutes, such as glycerol, sucrose, and sodium chloride, has been reported to influence the thermal resistance of microorganisms, probably attributed to the reduction in a_w of the media (Moats and others 1971; Gibson 1973; Hsieh and others 1975). At equivalent water activities, sucrose might protect *Salmonella* better than sodium chloride and glucose-fructose in tryptic soy broth during thermal treatments at selected temperatures ranging from 55 to 72 °C at water activities of 0.75, 0.80, and 0.90 (Mattick and others 2001). In those studies, the media were adjusted to the selected a_w values at 25 °C, not at treatment temperatures. It may be presumed that a difference exists in the relationship between a_w and thermal resistance in actual practice as a_w may vary at the treatment temperatures.

A number of studies have focused on the protective effect of lipid materials on microorganisms during heat treatments (Ababouch and Busta 1987; Ma and others 2009; Shigemoto and others 2010; Li and others 2014). Ma and others (2009) observed unusually greater heat resistance of *Salmonella* strains in peanut butter with a_w of 0.45 in comparison to many high-moisture foods such as ground beef but with higher a_w , attributed to the high fat content (approximately 53%) and low a_w of peanut butter. The D -values of *Salmonella* strains at 71 °C ranged from 26.5 to 30.6 min while at 90 °C, the D -values ranged from 8.6 to 13.4 min (Ma and others 2009). Kataoka and others (2014) reported greater survival of selected *Salmonella* strains and *Enterococcus faecium* at lower a_w (0.3 compared with 0.6) in a peanut butter formulation stored for 12 mo at 20 °C after heat treatment at 75 °C for 25 to 50 min. However, a greater fat content (56% compared with 47%) in peanut butter formulation did not influence the survival of the selected bacteria, so that >47% may offer no further protective effect of fat on the survivability of bacterial cells (Kataoka and others 2014). Ababouch and Busta (1987) observed greater thermal resistance (higher D - and z -values) of *Bacillus cereus*, *Clostridium botulinum*, and *C. sporogenes* spores suspended in oil (olive oil and commercial oil containing rapeseed oil and soy oil) compared to aqueous buffer (pH of 7.2). This was attributed to the reduction in a_w during thermal treatments in the presence of lipid materials. They determined a_w values of oils at 25 °C and thermal treatment temperatures (110 to 128.5 °C) using equations reported by Loncin (1955) and Hilder (1971). The spores survived better in olive oil compared to commercial oil, even though the water activity was slightly higher for olive oil, showing that oil composition may also directly contribute to the thermal resistance of microorganisms (Ababouch and Busta 1987). Furthermore, the significantly greater z -values of the spores presented in the oils compared to those in aqueous buffer, suggesting that, the inactivation mechanism of the spores in these matrices may be different (Ababouch and Busta 1987). Shigemoto and others (2010) reported that *Bacillus subtilis* spores survived better at higher soybean

oil content in an oil–water emulsion system. However, they observed a higher death rate in the initial phase. The spores in the oil phase may have a lower death rate in comparison to those in aqueous phase, suggesting that location of spores in the emulsion system may be important and related to the thermal resistance (Shigemoto and others 2010). The thermal resistance of spores may be affected by change in the location of spores during heating from aqueous phase to oil phase. This may be attributed to the separation of liquid phases and increase in hydrophobicity of spores during heating (Shigemoto and others 2010). Additional knowledge on the influence of microenvironment on thermal resistance of pathogens is necessary, which will be helpful in designing thermal processing techniques for low-moisture foods.

Microbiological factors

The thermal resistance of microorganisms may be influenced by type of species, growth conditions, such as log phase or stationary phase, presence of other microflora, growth medium, presence of calcium, magnesium, iron or fatty acids in the medium, growth temperature, and so on (Doyle and Mazzotta 2000). More details regarding the summary of microbiological factors can be found in Doyle and Mazzotta (2000) and Sugiyama (1951). For an example of the influence of type of organisms, the reported D -values at 55 °C of *S. Senftenberg* and *S. Bedford*, grown to stationary phase and survivors recovered on peptone-Lemco agar with oxalated horse blood after 48 h at 37 °C, were 36.2 and 18.8 min, respectively (Baird-Parker and others 1970). Fatty acids in the medium increased the thermal resistance of *C. botulinum* spores, and greater thermal resistance was observed in the presence of fatty acids with longer chains (Sugiyama 1951). Exposure of bacterial cultures to environmental stress prior to thermal treatments can induce higher resistance in bacterial cells to external stresses (Mattick and others 2000, 2001; Ma and others 2009). Successive bacterial culture transfer in laboratories may also increase the heat tolerance of bacteria (Ma and others 2009).

Possible mechanisms to explain higher thermal resistance of bacteria in low-moisture conditions

Elevated temperatures cause protein unfolding and denaturation, ribosomal damage, and enzyme inactivation, resulting in inactivation of microorganisms (Mackey and others 1991; Nguyen and others 2006). Experimental evidence supports that the principal cause of thermal inactivation of bacterial cells under high-moisture conditions is irreversible destabilization of ribosomes, specifically 30S and 50S ribosomal subunits (Mackey and others 1991; Lee and Kaletunc 2002). Bacterial spores exhibit higher thermal resistance than vegetative bacterial cells, attributed to the lower flexibility of bacterial protein structure (Potts 1994; Sunde and others 2009). With regard to thermal resistance, bacterial spores are less sensitive to temperature change as reflected by their higher z -values (approximately 10 °C) (Stumbo 1973) compared to vegetative bacterial cells (approximately 5 °C) in high-moisture foods, also due to the lower molecular flexibility of protein structures. Vegetative bacterial cells even in low-moisture environments may have much lower water content than bacterial spores, and they exhibit similar thermal resistance and z -values compared to bacterial spores. For example, the z -value of *S. Enteritidis* PT 30 in almond kernel flour was 8.28 °C while the z -value of *S. Seftenberg* in milk chocolate was 18 °C (Tomlins and Ordal 1976; Villa-Rojas and others 2013). It may be hypothesized that desiccation of bacterial cells sharply reduces molecular mobility and help stabilize ribosomal units against irreversible

damage due to thermal energy in low-moisture environments. Further studies on the influence of molecular flexibility of protein components of bacterial cells on their thermal resistance in low-moisture environments is highly desirable to elucidate their thermal inactivation mechanisms.

A number of other mechanisms associated with increased resistance of microorganisms at low-water activities have been studied. The mechanisms associated with long-term survival of microorganisms in low-moisture foods may include accumulation of osmoprotectant molecules (betaine (N,N,N-trimethyl glycine), proline for example), filamentation, σ^E and σ^S regulated genes, viable but nonculturable state of bacterial cells, and biofilm formation (Finn and others 2013). Under stress conditions, such as low-water activity and high temperature environments, microorganisms may express specific genes such as *rpoS* in order to respond to the adverse condition rapidly (Mattick and others 2000). The survival strategies of microorganisms in low-moisture environments during thermal treatments should be further investigated in order to learn the mechanisms behind their greater thermal resistance.

Knowledge gaps

Additional research is needed to better understand the effect of water activity and bacterial protein mobility variation, and microbial mechanisms to adjust to the thermal stress, in order to develop thermal process protocols to eliminate target pathogens in specific low-moisture foods. Thermal treatments for pasteurization of bulk materials are often dynamic processes; there may be significant variation in a_w depending on the chemical composition of food matrices and the process conditions. The microorganisms in a food matrix also achieve the same water activity of foods as they are in thermodynamic equilibrium with the foods. Investigations into the effectiveness of pasteurization for low-moisture products require knowledge of the water activities of the food matrices at temperatures used during the process (60 to 140 °C). Published studies have related heat resistance (*D*- and *z*-values) of *Salmonella* to water activities of food matrices at room temperature but not that of the treatment temperature (Table 2). Most of these studies failed to report the changes in water activity of food samples when heated from room temperature to the treatment temperature, because available instruments usually do not measure a_w at temperatures higher than 60 °C. Consequently, there is little knowledge about how a_w of low-moisture foods of different compositions at elevated temperatures might affect microbial inactivation. The following sections of this review present the importance of a_w variation during thermal processing and its influence on thermal resistance of microorganisms in low-moisture foods.

Thermal resistance of pathogens and water activity variation in low-moisture foods at elevated temperatures. Only a few very early studies related thermal resistance of pathogens with water activity variation in low-moisture foods at elevated temperatures. Murrel and Scott (1966) used 5 methods to relate thermal inactivation kinetics with water activity of bacterial spores (*Bacillus megaterium*, *B. stearothermophilus* ATCC 7953, and *Clostridium botulinum* type E ATCC 9564). In their experiments, the spores were equilibrated with LiCl or NaOH or H₂SO₄ solutions and treated at elevated temperatures for specific periods and cooled rapidly in ice water (Murrel and Scott 1966). The thermal resistance observed at 0.2 to 0.4 a_w was up to 10,000 times greater than the thermal resistance observed close to 1.0 a_w (Murrel and Scott 1966).

Loncin (1955) and Senhaji (1977) reported that a_w of oils decreases with increasing treatment temperatures in comparison to room temperature (approximately 23 °C). The water activity of oils can be calculated (Loncin 1955):

$$\frac{C_0}{a_w = C_s} = \frac{\text{concentration of water in oil}}{\text{concentration of water in oil at saturation level}} \quad (3)$$

The a_w of oils decreases as temperatures are elevated, because C_s increases, with temperature increase, while C_0 remains the same. The C_s values can be predicted using the following equation reported by Hilder (1971):

$$\ln C_s = 7.118 - \frac{1222}{T} + 1.459 \ln T \quad (4)$$

where C_s and T are expressed in molar fraction and K , respectively.

Based on the above equations, Ababouch and Busta (1987) calculated a_w values for olive oil and commercial oil at 110 to 128.5 °C, and related thermal resistance of bacterial spores suspended in olive oil and commercial oil to a_w at these temperatures. The greater thermal resistance of spores in oil compared to buffer was attributed to reduction in a_w of oils at the thermal treatment temperatures (Ababouch and Busta 1987).

The thermal resistance of food pathogens such as *Salmonella* in low-moisture foods may also be related to the modified water activity of those foods at the treatment temperatures. Thus it is critical to understand how water activity values of food systems change temperature.

The water activity is determined at a thermodynamic equilibrium state where a uniform constant temperature is required to achieve it (Murrel and Scott 1966). A complete water vapor equilibrium between the food and surrounding environment is hardly possible under dynamic thermal processing conditions, considering the general treatment time periods (Murrel and Scott 1966). This may be one of the main reasons why the water activity variation was not considered in previous studies. For low-moisture foods, thermal treatments (with temperature range of 60 to 90 °C) require several minutes to hours to achieve 3 to 5 log reduction in microbial population in closed containers. Hence, one could assume a pseudo-equilibrium between low-moisture food and surroundings in sealed containers during thermal treatments. This makes it possible to consider the modified water activity at that elevated temperature, instead of initial water activity of the food at room temperature, in designing effective thermal treatments. At elevated temperatures, water migration inside a food system or the surrounding environment is most likely through water vapor diffusion as liquid water to vapor conversion increases and hence water vapor pressure increases. The diffusion coefficient of water vapor in air is in the order of 10^{-5} m²/s, which is about 10,000 times more than the liquid water diffusion coefficient (in the order of 10^{-9} m²/s). This suggests that water activity equilibration in food may be quick during heat treatments. The determination of equilibration time required for the target microorganisms in a low-moisture food to adjust to the modified temperature and relative humidity during thermal processing will explain the importance of water activity determination at elevated temperatures.

Water Activity of Foods at Elevated Temperatures State of water in foods

Water inside foods and other biological materials can be bound or free water (Pakowski and others 2007). Bound water is tightly

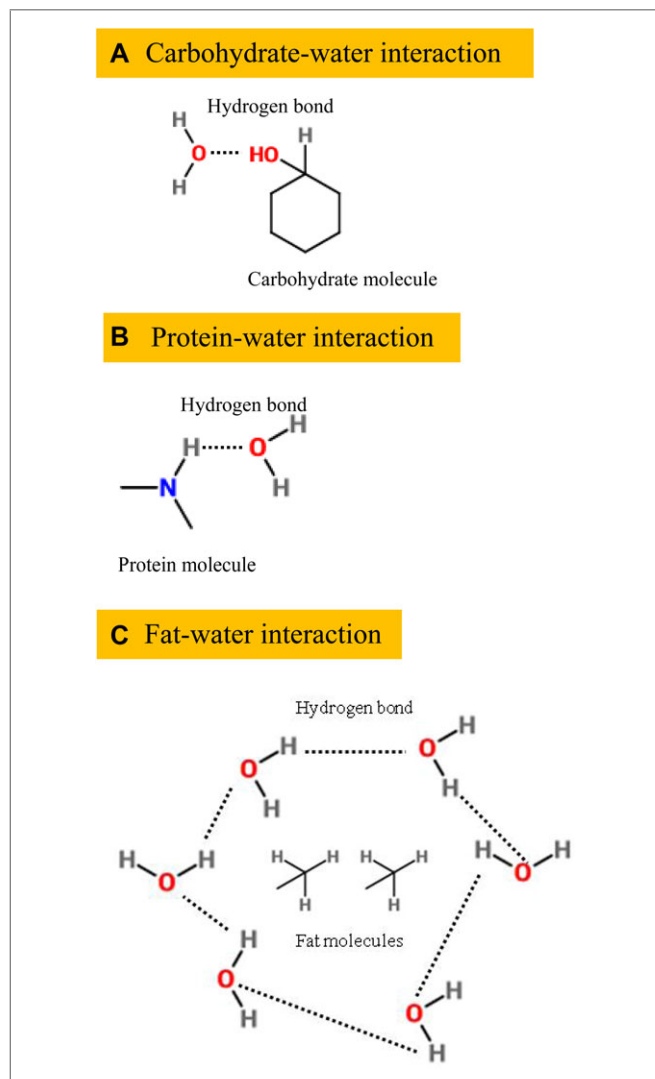


Figure 1—Schematic diagram showing interaction of water molecules with carbohydrates, proteins, and fats (Iglesias and Chirife 1977; Shang and others 1995; Sun 2002; Khuwijitjaru and others 2002; Pakowski and others 2007).

hydrogen-bonded to hydroxyl groups of food components and may not be easily available for physicochemical reactions. On the other hand, the free water exists in liquid form and is loosely attached to solid matrices by capillary forces (Pakowski and others 2007). The free water molecules are available for reactions in food matrices.

Water molecules are polar, with electrons shared unequally between hydrogen and oxygen atoms. Water molecules interact with food macromolecules through hydrophilic or hydrophobic interactions (Figure 1). Hydrophilic substances like carbohydrates interact and dissolve in water by forming hydrogen bonds with water molecules, resulting in hydration of macromolecules (Figure 1). In protein-rich foods, water molecules also interact with hydrophilic sites of protein structures (Iglesias and Chirife 1977).

Nonpolar compounds such as fats and oils, on the other hand, do not interact or form hydrogen bonds with water molecules. Mixing water with hydrophobic substances results in hydrophobic hydration, which is a thermodynamically unfavorable event with positive free energy change ($\Delta G > 0$) as the entropy is decreased

(Fennema 1999). Water molecules may re-arrange around nonpolar fat molecules to form a more orderly structure along with a decrease in entropy of the system. Surrounded fat molecules may also aggregate together to reduce the interfacial surface area or minimize their association with water, resulting in a more thermodynamically favorable hydrophobic interaction (Fennema 1999) (Figure 1). More about hydrophobic interactions of water is detailed by Fennema (1999).

Water activity of a food system above 100 °C

In an open system, the partial water vapor pressure and partial air pressure in a water vapor-air mixture is equal to the ambient pressure. That is,

$$P_{\text{ambient}} = P_{\text{air}} + P_v \quad (5)$$

The above equation can be written as:

$$\frac{P_{\text{ambient}}}{P_{vs}} = \frac{P_{\text{air}}}{P_{vs}} + \frac{P_v}{P_{vs}} \quad (6)$$

where P_{vs} is saturated water vapor pressure at product temperature. Substituting Eq. 1 into the above equation yields:

$$\frac{P_{\text{ambient}}}{P_{vs}} = \frac{P_{\text{air}}}{P_{vs}} + a_w \quad (7)$$

In an ambient environment, P_{air} has a positive value. Thus,

$$\frac{P_{\text{ambient}}}{P_{vs}} > a_w \quad (8)$$

The above equation shows the maximum water activity of a food system when treated at elevated temperature in an open system. For example, at sea level, $P_{\text{ambient}} = 101.8$ kPa. When foods are heated to 120 °C, the saturated water vapor pressure ($P_{vs120\text{C}}$) is 201.8 kPa. Thus, at equilibrium the a_w of the food system is:

$$a_w < \frac{101.3}{201.8} = 0.5 \quad (9)$$

Based in this relationship, the equilibrium a_w of a food system in an open environment ranges from 1.0 to 0.5, at temperatures between 100–120 °C while, it reduces below 0.5 at temperatures greater than 120 °C. This relationship should be carefully considered for designing thermal processing of low-moisture foods when using a temperature beyond 100 °C. For instance, many of the low-moisture foods like spices are heated using air or steam at about 120 °C to inactivate pathogens such as *Salmonella*. If the a_w of the food is high, the food will lose water to reach the a_w of less than 0.5 at the treatment temperature. The pressure should be maintained greater than atmospheric pressure to achieve greater relative humidity of the surrounding environment and, hence, the water activity of a food system.

Influence of food components on water activity variation with temperature

For carbohydrates and protein-rich foods, it is well known that increasing temperature increases their water activity values when water contents remain same (Figure 2). This is possibly due to the structural changes in carbohydrate and protein molecules at elevated temperatures, affecting their interaction with water and a_w -water content equilibrium (Iglesias and Chirife 1977). At elevated temperatures, those biomaterials may become less hygroscopic, attributed to the reduced number of active water-binding

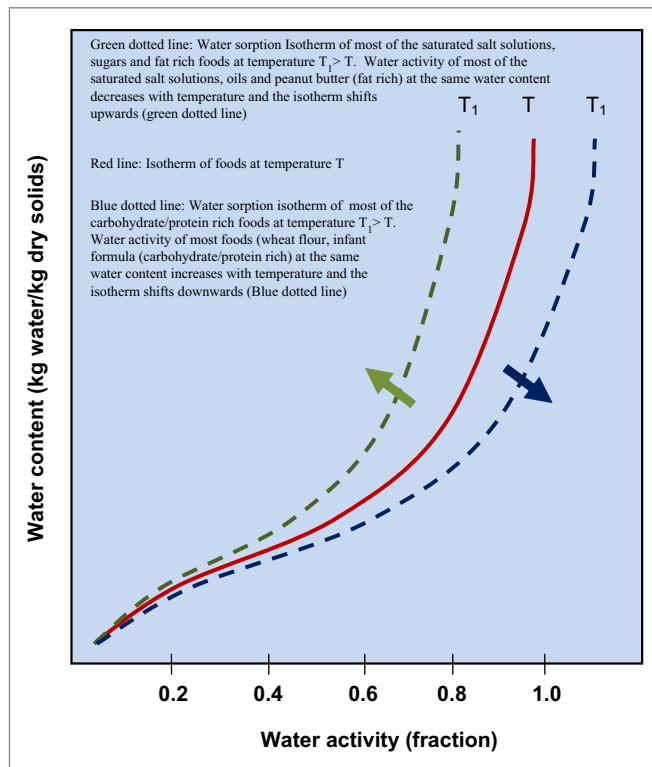


Figure 2—Schematic diagram presenting temperature influence on sorption isotherm of different food macromolecules.

sites and greater energy levels of unstable water molecules (Sun 2002; Pakowski and others 2007). Molecular simulation experiments have revealed that for liquid water at ambient conditions, the radial distribution function $[g(r)]$ exhibits a peak around 2.8 Å for oxygen–oxygen $g(r)$ and a peak around 1.9 Å for hydrogen–oxygen $g(r)$ designating the presence of strong hydrogen–bonding between water molecules (Harvey and Friend 2004). The radial distribution function for hydrogen–oxygen $[g(r)]$ peaks is significantly reduced at elevated temperatures, weakening the hydrogen bonds among water molecules with biomolecules and between water molecules (Harvey and Friend 2004). Thus, fewer binding sites and reduced attractive forces, more water has sufficient energy to escape from liquid phase in food into the vapor phase, increasing the a_w of carbohydrates and protein-rich foods at elevated temperatures (Palipane and Driscoll 1993).

However, a_w may decrease with increased temperature in hydrophobic materials such as oils (Senhaji 1977) (Figure 2). We developed sorption isotherms (equilibrium water activity–water content relationship of a food system at a specific temperature) of peanut butter at specific temperatures and observed that the water activity of peanut butter indeed decreased with temperature at fixed water contents (Figure 3) (unpublished data). Similar observations were reported for other fat-rich foods such as peanut oil, oleic acid (Loncin and others 1968), olive oil, commercial oil containing rapeseed oil, and soy oil (Ababouch and Busta 1987). This behavior may be attributed to an increase in the solubility of nonpolar solids, such as fats in water at elevated temperatures (Khuwijtjaru and others 2002).

Water activity measurement devices

Chilled-mirror dew point sensors and capacitance hygrometers are generally used to measure water activity of foods.

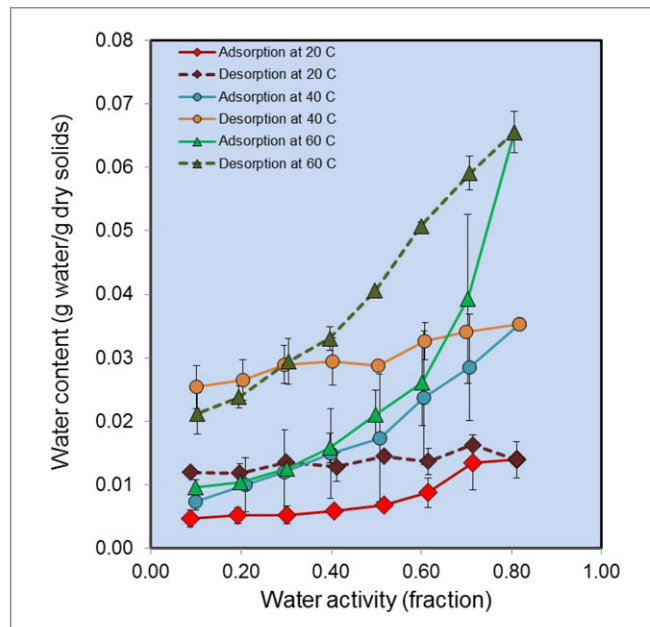


Figure 3—Sorption isotherms of peanut butter at 20, 40, and 60 °C determined using a vapor sorption analyzer (Decagon Devices, Inc.).

Chilled-mirror dew point instrument measurements are accurate ($\pm 0.003 a_w$), fast (measurement within 5 min), and are the primary means of a_w determinations (Fontana, 2007). With this method, a food sample is equilibrated in a sealed chamber where a mirror, an optical sensor, and a fan are located. The mirror is cooled thermoelectrically until dew forms on the chilled-mirror. The dew point temperature is measured by registering the mirror temperature when the dew formation is detected using the optical reflectance sensor (Fontana 2007). The relative humidity of the headspace is computed as the ratio of saturated water vapor pressure corresponding to the dew point temperature to the saturation vapor pressure at the original sample temperature:

$$RH = \frac{P_{vs}(T_d)}{P_{vs}(T_s)} \times 100 \quad (10)$$

where P_{vs} is the saturation water vapor pressure, T_d is the dew point temperature, and T_s is the sample temperature. P_{vs} is calculated from the following equation:

$$P_{vs} = 0.611 \exp\left(\frac{17.502 T}{240.97 + T}\right) \quad (11)$$

where T is the temperature in °C. When the sample and headspace air are in equilibrium, the relative humidity of the headspace gives the water activity of the sample (Fontana 2007). If volatiles such as ethanol are present in a sample, they can impact the water activity results. The volatiles will cause artificially high readings as they co-condense on the chilled-mirror changing the dew point temperature.

Capacitance hygrometers are less accurate, but the measurement is not much affected by volatiles as in the chilled mirror (Fontana 2007). This instrument consists of a dielectric hygroscopic polymer film and charged plates. When the sample is in thermodynamic equilibrium with the air headspace, the sensor measures the capacitance of the polymer film, which is a function of the a_w of the sample. Regular calibration is required to

achieve accurate measurement. The accuracy of the hygrometer is also dependent on the difference between the sensor temperature and the sample temperature. If volatiles are present, they can be also absorbed by the capacitance sensor, which can alter its calibration. The above commercially available instruments measure water activities of foods at a temperature range of 20 to 60 °C. Consequently, methods are needed to make direct measurements of water activity and develop moisture sorption isotherms at temperatures above 60 °C.

Literature Data of Water Activity of Materials at Elevated Temperatures

Sorption isotherms were generated in the past with custom-built instruments that are not available commercially or readily accessible to most laboratories. Results of those studies that measure a_w in biological materials at temperatures above 60 °C using custom-built instruments are summarized in Table 3. For most of these studies, a sample was placed inside a high-pressure chamber and exposed to elevated temperatures and pressures (Figure 4). The relative humidity inside the chamber was varied by changing the chamber pressure to achieve different water activities and water contents. The water content was measured by monitoring the changes in weight of the sample (Figure 4). For example, in the studies of Bassal and others (1993a,b) water activities of microcrystalline cellulose, potato starch, cake dough, and lactose were measured at temperatures of 100 to 130 °C. This was done by measuring the steam vapor pressure inside an equilibrium chamber with a sample. The process consisted of creating a pure steam atmosphere inside a chamber with the sample at elevated temperatures (>100 °C). Then the pressure inside the chamber was changed using a pressure regulator and a vacuum pump. The weight change in the sample was monitored and the moisture content of the sample was determined at equilibrium at a specific temperature and pressure condition. The water activity was determined as the ratio of vapor pressure inside the chamber and saturated vapor pressure at the selected temperature.

The food materials studied by Bassal and others (1993a,b) were primarily rich in carbohydrates. They observed that an increase in temperature increased the a_w at a given moisture content for these food materials. For instance, the a_w of microcrystalline cellulose increased from 0.3 to approximately 0.7 when the temperature was increased from 25 to 100 °C. Also, the a_w of potato starch increased considerably from 0.3 to approximately 0.8 when the temperature was increased from 20 to 100 °C. However, the influence of temperature on a_w was minimal at higher temperature (Bassal and others 1993a, b). They observed structural changes in potato starch due to gelatinization and in cake dough possibly due to starch gelatinization, protein denaturation, and sugar crystallization at 100, 115, and 130 °C during the a_w measurement. At temperatures above 100 °C, the maximum water activity values observed for the selected materials followed predictions by Eq. 7 and 8. For example, the maximum water activity value of microcrystalline cellulose during desorption at 132 °C was approximately 0.35 at atmospheric pressure. Experiments in selected foods were conducted at a pressure greater than 1 bar in order to achieve water activities above 0.5 and the corresponding water contents (Bassal and others 1993a). The desorption isotherm of lactose was similar to that of a typical crystalline material with 3 distinct zones based on its water sorption properties (Bassal and others 1993a,b).

The water activity of wood and bark chips was determined at elevated temperatures by measuring the pressure inside an equilib-

rium chamber and saturated vapor pressure of condensing steam at 140 and 160 °C (Bjork and Rasmuson 1995). An electrically heated steam generator and a throttle valve were used to produce steam and to control the pressure inside a sample testing box, respectively. The pressure inside the chamber was varied by flowing different quantities of steam inside the chamber. The saturated vapor pressure was determined from the condensation temperature of the steam. The water activity was determined as the ratio of the saturated pressure at the temperature and saturated pressure at the superheated temperature. The equilibrium sample moisture content of the sample was determined at required pressure levels. The water activity at a given equilibrium water content of materials at elevated temperatures may be dependent on the partial pressure of the superheated steam used to achieve different water activities and the type of material as reported by Bjork and Rasmuson (1995). In particular, they observed that the temperature has less effect on a_w and equilibrium moisture content of spruce and aspen as their water activities at given equilibrium water contents at 140 and 160 °C were similar.

Lenth and Kamke (2001) developed desorption isotherms of wood samples (yellow poplar, loblolly pine, and aspen) at 50 and 160 °C using a system similar to that mentioned above (Figure 4). The wood samples were kept inside a pressurized vessel and the mass of the sample was monitored at different relative humidity levels created by changing the pressure from saturation to atmospheric pressure at constant temperature conditions. The weight changes in the sample at different relative humidity levels were monitored using an electronic balance to determine the equilibrium water content. Initially, the internal pressure of the vessel was raised to 587 kPa at 160 °C to achieve a relative humidity level of 95%. At this point, most of the air inside the chamber is replaced by water vapor. The pressure inside the chamber is increased above atmospheric pressure to avoid boiling of water at high temperatures. Then pressure was gradually reduced to atmospheric pressure to attain different relative humidity levels. The lower values of relative humidity of the air and corresponding water activity values of the samples were thus obtained by essentially a drying process. Alternatively, Lenth and Kamke (2001) determined that the a_w values of wood samples (aspen, yellow poplar, and loblolly pine) at 160 °C were greater than those at 50 °C. For instance, the a_w of juvenile and mature aspen wood at 50 °C at a constant equilibrium water content of 5% was approximately 0.3, which increased to approximately 0.45 in mature and approximately 0.6 in juvenile aspen wood (Lenth and Kamke 2001). Further, they observed a drastic increase in equilibrium water contents for water activities above 0.5 for these wood samples. Mass loss of wood due to thermal degradation at 160 °C was observed, specifically above 0.5 a_w . They reported that the sorption isotherms of materials at elevated temperatures may cross over those at lower temperatures at certain water activities, which may be attributed to the increased adsorption and softening due to glass transitions in the sample (Lenth and Kamke 2001).

Kuboijima and others (2003) reported a similar instrument as in Figure 4 to measure the equilibrium moisture content of green wood at different relative humidity values and elevated temperatures. The technique required a hermetically sealed pressure vessel and a humidifier to produce steam. They set temperature and relative humidity to 107 to 160 °C and 75% to 99% RH, respectively, through superheating control. The weight of the sample at each relative humidity value, with different total pressure inside the chamber, was monitored and the moisture content at

Table 3–Water activity determination of materials above 100 °C

Authors and year	Title	Product and temperature range	Technique	Major results
Bassal and others (1993a)	Measurement of water activity above 100 °C	Microcrystalline cellulose and natural potato starch	Manometric and dynamic sorption method	<ul style="list-style-type: none"> Desorption isobar of microcrystalline cellulose and potato starch were developed GAB model, which accounted the effect of temperature was used
Bassal and others (1993b)	Sorption isotherm of food materials above 100 °C	Microcrystalline cellulose (MCC) potato starch cake dough lactose, Temperature range: 100 to 140 °C		<ul style="list-style-type: none"> Desorption isotherms and isobars of the selected products were determined The influence of temperature on the sorption isotherm is small at elevated temperature
Kubojima and others (2003)	Moisture content of green wood in high-temperature water vapor	Green sitka spruce (wood), Temperature range: 105 to 160 °C	Changing the relative humidity by varying the pressure inside a hermetically sealed pressure chamber containing superheated steam and measuring the weight of the sample at equilibrium for water content determination	<ul style="list-style-type: none"> Temperature-relative humidity-pressure-equilibrium moisture content values in the range of 105 to 160 °C, 75% to 99% RH and 0.02 to 0.39 MPa were determined
Bjork and Rasmuson, (1995)	Moisture equilibrium of wood and bark chips in superheated steam	Chips of spruce and aspen, Temperature range: 140 and 160 °C	Measuring the saturation pressure using a testing box and saturation pressure of at the superheated temperature	<ul style="list-style-type: none"> A weak dependence of temperature on the sorption isotherms at elevated temperatures was observed The Dent model was used to simulate the experimental sorption data
Lenth and Kamke (2001)	Equilibrium moisture content of wood in high temperature pressurized environments	Yellow-poplar, loblolly pine, and aspen, Temperature range: 50 and 160 °C	Changing the relative humidity by varying the total pressure inside pressure chamber containing and measuring the weight of the sample at equilibrium for water content	<ul style="list-style-type: none"> The desorption isotherms at 160 °C were significantly lower than the those at 50 °C denoting reduced a_w at higher temperature The results show that it would be inaccurate to extrapolate a_w values at elevated temperatures from low temperature data
Pearson and others (2012)	Equilibrium moisture content of radiata pine at elevated temperature and pressure reveals measurement challenges	Radiata pine (<i>Pinus radiata</i> D. Don)	Changing the relative humidity by varying the total pressure inside pressure chamber containing and measuring the weight of the sample at equilibrium for water content	<ul style="list-style-type: none"> There was a change in sorption properties of wood when the temperature and moisture were above glass transition temperature of lignin

equilibrium was determined using a cantilever-type load cell with strain gauges placed in the pressure chamber. They observed a decrease in equilibrium water content of green sitka spruce at a constant equilibrium relative humidity when temperature was increased. For instance, at an equilibrium relative humidity of 95%, the equilibrium water content decreased from 13.2% to 5.1% when temperature was increased from 107 to 150 °C.

Gruszkiewicz and others (2005) used a static gravimetric approach to develop water adsorption/desorption isotherms. They determined the vapor pressure as a function of molality and equilibrium water content of selected synthetic and natural porous solids, such as controlled-pore glass, activated carbon fiber monoliths, natural zeolites, pillared clay, and geothermal reservoir rocks, at a range of temperatures between 105 and to 250 °C. They used an isopiestic apparatus to systematically change the vapor pressure from vacuum to saturation vapor pressure at a specific temperature by injecting or removing water in the chamber to develop adsorption/desorption isotherms. The sample mass was monitored to determine the equilibrium water content of samples during adsorption/desorption using an electromagnetic balance. They did not observe any temperature dependence on the

adsorption isotherm. However, a small decrease in equilibrium water content (increase in water activity) with temperature during desorption was observed. The difference between adsorption and desorption isotherms (hysteresis) of rock samples decreased when temperature increased.

Pakowski and others (2007) developed sorption isotherms of willow *Salix viminalis* for a range of temperatures up to 85 °C using a water bath and keeping the samples inside a closed container with selected supersaturated salt solutions with different relative humidity values in a water bath. This seems like a logical approach, but the challenge is that the relative humidity values of the specific supersaturated salt solutions may vary significantly with temperature. The only way to determine the correct water activity of the saturated slurries at higher temperatures would be to measure the water activity or predict the effect, which comes back to the original challenge of high-temperature water activity testing. They also developed sorption isobars of willow *S. viminalis* in a superheated steam environment. The wood sample was kept at a superheated steam temperature by heating with steam produced by a boiler. The sample weight was monitored at different steam temperatures and the equilibrium water content was determined.

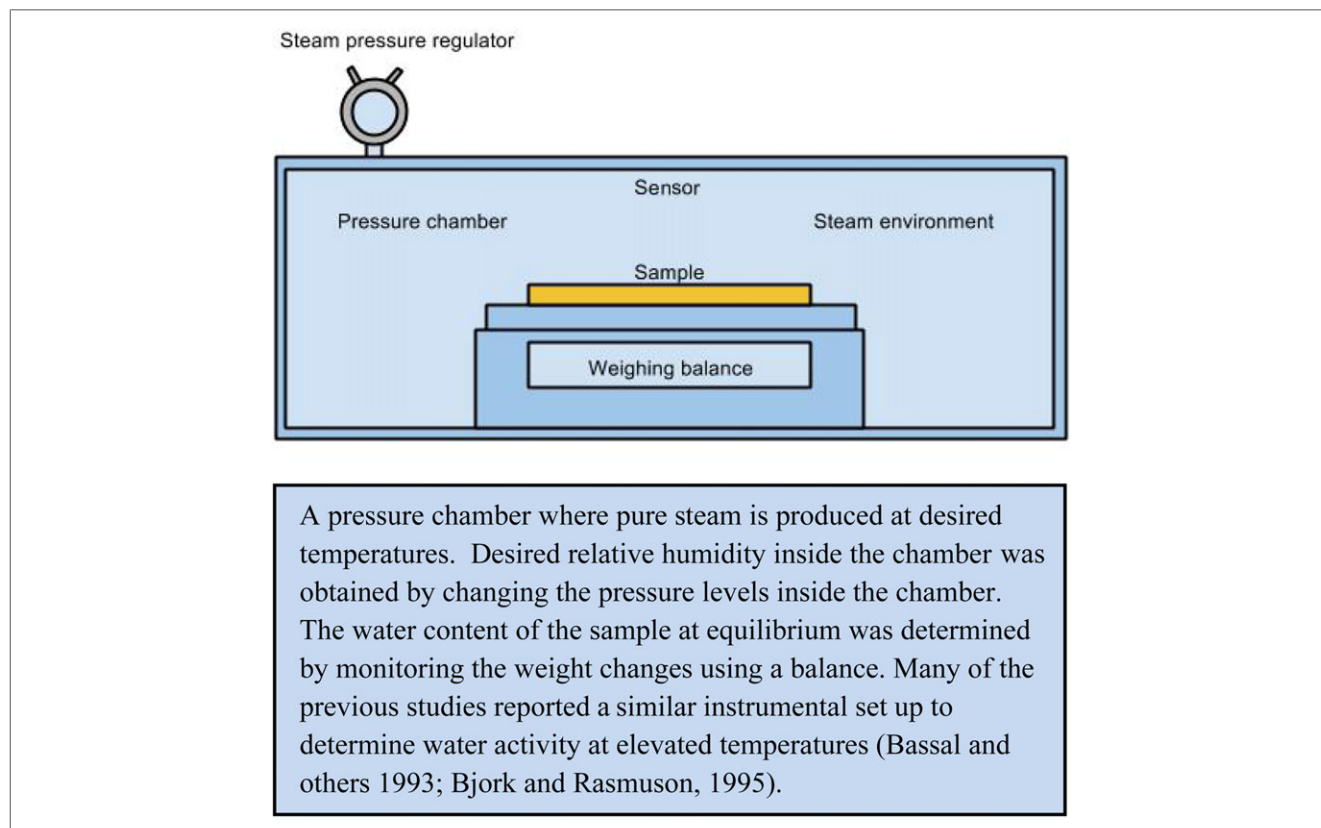


Figure 4—General principle of a_w measurement apparatuses developed in the past.

Pakowski and others (2011) used an instrumental set-up that included a superheated chamber containing the sample. The weight change of the sample was then traced to develop sorption isobars of lignite. Temperatures up to 200 °C at pressure values of 0.101, 1.0, and 2.5 MPa were used (Pakowski and others 2011). They observed a significant increase in water activity of lignite when temperature was increased, attributed to decreased hygroscopicity of materials at elevated temperatures.

Adsorption isotherms of casein, wheat starch, potato starch, apple-pectin, and microcrystalline cellulose were generated at 40, 60, and 80 °C using a sorption apparatus (Bandyopadhyay and others 1980). The apparatus consisted of a pre-saturator and saturator that created the desired humidity inside a sorption chamber that could be electrically heated to a desired temperature (Figure 5). After equilibration, the water content of the samples was determined to develop the adsorption isotherms. These selected food components are rich in either carbohydrates or proteins, and their isotherms at elevated temperatures exhibited an increase in a_w with an increase in temperature at the same water content, attributed to their lower hygroscopicity at higher temperatures (Bandyopadhyay and others 1980). The water-binding capacity of cellulose was reported lowest at all water activities at the elevated temperatures among the selected food components (Bandyopadhyay and others 1980). In contrast, the a_w of pure sugars such as glucose and fructose decreased as temperature increased from 30 to 80 °C, attributed to the structural changes at 80 °C, resulting in increased hygroscopicity and solubility (Audu and others 1978). This implies that water activity change due to temperature is greatly dependent on the nature of food ingredients. It is also possible that pure components may show a different

behavior compared to multicomponent food systems (Fontana and others 2007).

Equilibrium moisture content values of radiata pine at elevated temperature and pressure levels were reported by Pearson and others (2012). They used a pressure chamber coupled with an elevated pressure high temperature (EPHT) cylinder to achieve selected sample temperatures and pressures of between 70 and 150 °C and 0% to 100 % RH (using a high-temperature dew point sensor). The equilibrium moisture content during the sorption experiments was determined by the relative extension of a spring attached to the sample using a linear variable differential transformer (LVDT) measurement system.

We developed a sealed thermal cell which could be used to measure water activity of samples at high temperature (Figure 6) (Syamaladevi and others 2015). A humidity/temperature sensor (HIH8120-021-001 from Honeywell Sensing and Control) able to withstand 125 °C was sealed inside the cell to measure the equilibrium RH of the headspace above the food sample. The a_w of the food sample is equivalent to the RH of the headspace in equilibrium with it, and this was taken as the food a_w . The sample holder was placed in a constant temperature chamber at the desired temperature. The water vapor pressure (P_v) can be calculated from water vapor concentration (C , kg/m³) from the following equation:

$$P_v = \frac{CR(T + 273.15)}{m_r} \quad (12)$$

where T is the temperature in °C, m_r is the molecular weight of water (kg/mol) = 0.018 and $R = 8.314$ J/mol. K. The a_w of

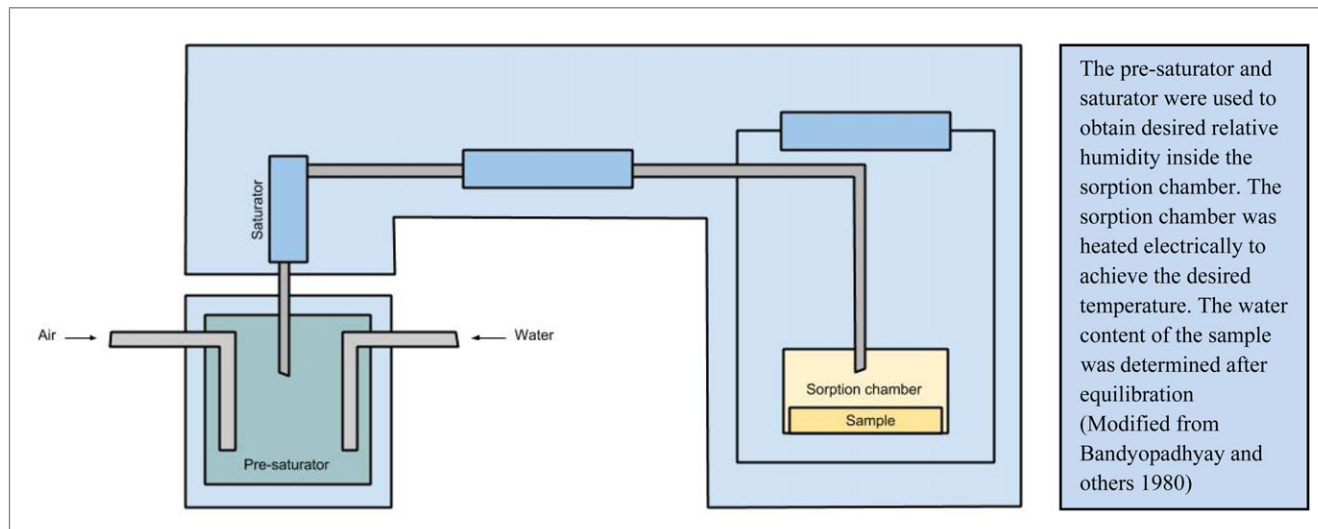


Figure 5—Instrumental apparatus developed by Bandyopadhyay and others (1980) to determine a_w of foods at high temperatures.

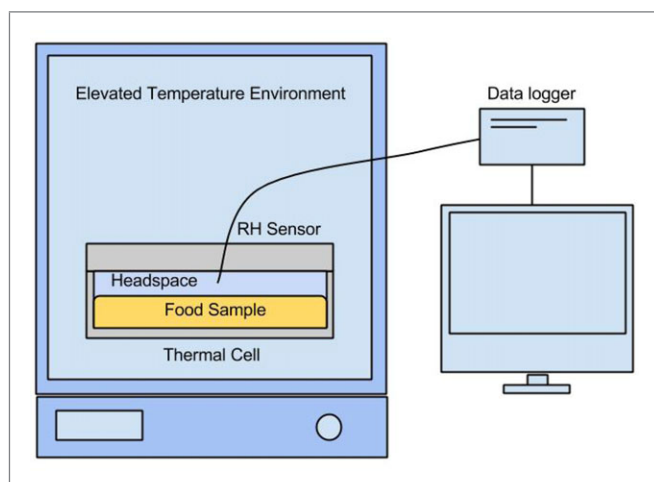


Figure 6—Water activity measurement by thermal cell with RH sensor developed at Washington State University in collaboration with Decagon Devices, Inc.

food/relative humidity (RH) of air and water vapor pressure are related by:

$$a_w = RH = \frac{P_v}{P_{vs}} \times 100 \quad (13)$$

where P_{vs} is the saturation vapor pressure, which can be determined at a temperature using Eq. 11.

Limitations of Water Activity Measurement Methods at Elevated Temperatures

Most of the previous studies noted that lengthy exposure of samples to elevated temperature may result in systematic errors in equilibrium water content determinations (Lenth and Kamke 2001; Pakowski and others 2007). This may be attributed to mass loss at elevated temperatures, chemical modification, and the resulting decrease in hygroscopicity (Pearson and others 2012). It is interesting to note that previous studies determined the water activities at elevated temperatures of mostly wood materials. Sorption isotherms and water activity measurements at elevated temperatures are required to design drying processes, such as air-drying or

superheated steam-drying of wood materials. Even though these drying methods are also used in the food industry, and sorption isotherms are necessary to predict the drying time or design drying processes, studies on isotherm generation for various foods at elevated temperatures are limited.

Many of the previous studies on water activity determinations at elevated temperatures analyzed pure water vapor systems with superheat control systems to achieve relative humidity values close to 100% at elevated temperatures (Pearson and others 2012). Condensation can occur at any part of the instrumental set-up when dry bulb temperature equals the dew point temperature at 100% RH. This condensed water can induce errors in the measured quantity of equilibrium moisture content (Pearson and others 2012). Furthermore, the target temperature and humidity should be carefully controlled since small variation in temperature results in large errors in humidity. It may also lead to erroneous results during water activity measurement at a given equilibrium moisture content (Pearson and others 2012). The thermal degradation of samples is also reported to be greater when they are exposed to higher relative humidity at elevated temperatures during experiments. Thus, it is desirable to minimize equilibration time at elevated temperatures (Lenth and Kamke 2001).

Mathematical Modeling of Sorption Isotherms at Elevated Temperatures

At a fixed temperature, a unique relationship exists between water activity and water content for a food system depending on its composition. This relationship is referred as an isotherm. Several empirical and semi-empirical models have been proposed to describe water sorption isotherms at different temperatures for various foods. Semi-empirical models such as Brunauer, Emmett and Teller (BET) or the Guggenheim, Anderson and de Boer (GAB) models could provide theoretical understanding on the influence of temperature on water activity of most foods. The GAB equation fitted well with isotherms of a variety of foods including fruits, vegetables, meat, milk products, starchy foods, nuts and oilseeds, coffee, tea, and spices (Lomauro and others 1985a,b). BET and GAB models are based on the assumption of multilayered adsorption with no lateral interactions (Quirijns and others 2005). The BET isotherm predicts water contents of a material with water activity values between 0.05 and 0.45, while

the GAB model is capable of accurate water content prediction for water activity values up to 0.9 (Rahman 1995). The GAB model is expressed as:

$$\frac{X}{X_m} = \frac{CKa_w}{(1 - Ka_w)(1 - Ka_w + CKa_w)} \quad (14)$$

where X is the water content (dry basis) of the material, X_m is the monolayer water content (dry basis), C and K are parameters based on the multilayer adsorption of water. The parameter C is a measure of strength of binding water to the primary binding sites of the food; a higher value for C indicates greater strength of water binding and larger enthalpy difference between the monolayer and multilayer water molecules (Quirijns and others 2005). The parameter K is often called the correction factor, which has a more entropic than enthalpic contribution (Quirijns and others 2005). The parameters C and K are expressed as:

$$C = C_o \exp\left(\frac{\Delta H_C}{RT}\right) \quad (15)$$

$$K = K_o \exp\left(\frac{\Delta H_K}{RT}\right) \quad (16)$$

where ΔH_C is generally positive and indicates the difference in enthalpy between monolayer and multilayer sorption. ΔH_K is generally negative and is the difference between the heat of condensation of water and the heat of sorption of the multimolecular layer (Quirijns and others 2005). In most cases, the monolayer water content (X_m) is considered as a constant, but a similar expression to describe the temperature dependence of X_m can be presented (Quirijns and others 2005):

$$X_m = X_{m_o} \exp\left(\frac{\Delta H_X}{RT}\right) \quad (17)$$

Incorporating the temperature-dependence of the parameters X_m , C , and K to the GAB model will allow us to predict the sorption isotherms of foods at elevated temperatures. Several other semi-empirical models such as modified Henderson equation (Henderson 1952), modified Halsey equation (Halsey 1948; Chirife and Iglesias 1978), modified Oswin equation (Oswin 1946; Chen 1988), and Chung-Pfost equation (Chung and Pfost 1967) were also used to describe sorption phenomena of food materials at elevated temperatures. A detailed review of the models to describe the water sorption phenomena can be found in Quirijns and others (2005). Equation 15 to 17 may not fit well for oil-rich foods (such as peanut butter, almond butter, and emulsions). In addition, previous research on isotherms was limited to temperatures less than 60 °C, because of lack of water activity measurement instruments for higher temperatures. More research is needed to understand the direction of water activity variation and usefulness of the above-mentioned equations in describing water sorption isotherms of those foods.

Extrapolation of current theoretical models that do not consider any state and phase transitions at high temperatures will give inaccurate results (Bassal and others 1993a,b). State and phase transitions (glass transition, vaporization, changes in crystalline form) and physicochemical changes (protein denaturation, lipid oxidation, and so on) may affect water-macromolecules interaction and, therefore, a_w of foods at elevated temperatures (Lenth

and Kamke 2001). So, any model that intends to predict a_w at elevated temperatures must consider state and phase transitions and physicochemical changes (Bassal and others 1993a). The influence of those changes in the product should be incorporated into the mathematical model to predict the water activity at elevated temperatures which may be oftentimes product-specific. So, it is necessary to conduct experiments to determine water activity at elevated temperatures in various food matrices.

Future Research

Water activity changes in different food systems at elevated temperatures

Water activity changes at elevated temperatures are unique to each food component (Figure 2). Local microenvironments created by multiple components in a food system may influence survival and thermal inactivation of microorganisms during processing and storage, which may be related to the water availability at/during thermal processing (Hills and others 1996). Water activity change during thermal processing may be directly associated with the water availability to microorganisms, which is governed by the state of water molecules and their interaction with food macromolecules in specific food systems at selected temperatures. Further studies on macroscopic and molecular aspects of water availability by understanding the water activity and protein mobility changes at elevated temperatures used in thermal processing are required.

Greater survival of microorganisms has been reported in fat-rich versus carbohydrate- or protein-rich foods during thermal processing (Senhaji 1977; Li and others 2014). There appears to be a direct link between the decrease in water activity during thermal processing and associated increase in the thermal resistance of microorganisms in fat-rich foods. Li and others (2014) studied *Salmonella* inactivation in 2 model systems consisting of peanut butter and nonfat dry milk powder with identical composition. In one system, *Salmonella* was initially inoculated into peanut butter before mixing with nonfat dry milk powder while in another, *Salmonella* was inoculated into nonfat dry milk powder before mixing with peanut butter. The survival of *Salmonella* was greater in the model system where *Salmonella* was initially inoculated into peanut butter suggesting that the local microenvironments had an impact on survival (Li and others 2014). It is necessary to understand water redistribution in the local microenvironment during thermal processing, which may influence the survival of *Salmonella*. Additional studies are required to understand the influence of temperature on hydrophilic and hydrophobic interactions between water and food macromolecules such as carbohydrates, proteins, and fats. This may explain the underlying reasons of opposite trends in water activity variation in foods rich in fats and other food components.

Thermal process calculations for low-moisture foods

Water activity may be added as a new dimension along with temperature to determine the thermal resistance of pathogens in low-moisture foods. The thermal resistance of a target pathogen (or D -value, a the time in minutes required to destroy 1 log cycle (90%) of the target pathogen) in specific low-moisture foods at different initial water activities may be experimentally determined and relationship can be established (Equation 18):

$$D = f(T, a_{w(T, X_w)}) \quad (18)$$

It is important to establish a relationship (Eq. 19) between variation in water activity at specific water contents with process temperatures in order to develop the thermal process calculation for low-moisture foods:

$$a_w = f(T, X_w) \quad (19)$$

Based on Bigelow's thermo-bacteriological approach (1921) (Eq. 20), Mafart and Leguerinel (1998) proposed a secondary model (Eq. 21) to assess the influence of temperature, pH, and a_w on the heat resistance of *Bacillus cereus* spores where a linear relationship between $\log D$ of and $(1 - a_w)$ was observed (Gaillard and others 1998).

$$D_T = D_{T_{ref}} \times 10^{\frac{(T - T_{ref})}{z}} \quad (20)$$

$$\log \frac{D}{D_{ref}} = \frac{-(T - T_{ref})}{z_T} - \frac{(a_w - 1)}{z_{a_w}} - \frac{(pH - pH_{ref})^2}{z_{pH}^2} \quad (21)$$

Villa-Rojas and others (2013) used the following simplified version of the above model (Eq. 22) by ignoring the pH component as pH did not change in the low-moisture products:

$$\log \frac{D}{D_{ref}} = \frac{-(T - T_{ref})}{z_T} - \frac{(a_w - 1)}{z_{a_w}} \quad (22)$$

where the temperature (Z_T) or water activity change (Z_{a_w}) required to change the D -value of the target pathogen by a factor of 10 (90%) in specific low-moisture foods may be determined using the following relationships (Eq. 23 and 24).

$$Z_T = \frac{T_2 - T_1}{\log D_1 - \log D_2} \quad (23)$$

$$Z_{a_w} = \frac{a_{w2} - a_{w1}}{\log D_1 - \log D_2} \quad (24)$$

The relationship between Z_T with water activity of food and Z_{a_w} with treatment temperature may be established for a target microorganism in selected food systems. Mafart and Leguerinel (1998) modified the lethal rate (L) and F -value concept by incorporating the effect of pH as below:

$$L = 10^{\left[\frac{(T - T_{ref})}{z} \right] + \left[\frac{(pH - pH_{ref})^2}{z_{pH}} \right]} \quad (25)$$

$$F = \int_0^t 10^{\left[\frac{(T - T_{ref})}{z} \right] + \left[\frac{(pH - pH_{ref})^2}{z_{pH}} \right]} dt \quad (26)$$

A great deal of work is required to extend the lethal rate and F -value concepts in the pasteurization of low-moisture foods by incorporating water activity variation at elevated temperatures during thermal processing. In order to accomplish this objective, appropriate equations (similar to Eq. 25 and 26) using target pathogens in specific low-moisture foods during thermal processing need to be developed and validated.

Conclusions

Recent outbreaks related to low-moisture foods have generated much interest in research communities and industries in finding

solutions to these problems. The food industry is interested in thermal processing technologies to reduce pathogens in low-moisture foods. However, improved knowledge of the factors influencing thermal resistance of pathogens is necessary to develop and implement adequate processing protocols. These factors may include water activity, protein mobility, composition of food matrices, and characteristics of target microorganisms. Previous research has identified the importance of water activity, which may be the most important factor influencing the thermal resistance of pathogens in low-moisture foods. Further understanding of the microenvironments in a food matrix and their influence on water activity during thermal processing is required. The dependence of water activity on temperature is essential and must be considered when developing protocols and equations relevant to multiple thermal processing technologies. Close collaboration between research institutions and the food industry and support from government organizations will ensure the positive outcomes needed to overcome this challenge.

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References

- Ababouch L, Busta FF. 1987. Effect of thermal treatments in oils on bacterial spore survival. *J Appl Bacteriol* 62(6):491-502.
- Alderton G, Chen JK, Ito KA. 1980. Heat resistance of the chemical resistance forms of *Clostridium botulinum* 62A spores over the water activity range 0 to 0.9. *Appl Environ Microbiol* 40(3):511-5.
- Akinleye IO. 1994. The effect of water activity on the heat resistance of *Salmonella* Typhimurium [PhD thesis]. University of Reading, Reading, UK.
- Archer J, Jarvis ET, Bird J, Gaze JE. 1998. Heat resistance of *Salmonella* Weltevreden in low-moisture environments. *J Food Prot* 61(8):969-73.
- Audu TOK, Loncin M, Weisser H. 1978. Sorption isotherms of sugars. *LWT - Food Sci Technol* 11(1):31-4.
- Baird-Parker AC, Boothroyd M, Jones E. 1970. The effect of water activity on the heat resistance of heat sensitive and heat resistant strains of *Salmonellae*. *J Appl Bacteriol* 33(3):515-22.
- Bandyopadhyay S, Weisser H, Loncin M. 1980. Water-adsorption isotherms of foods at high-temperatures. *Food Sci Technol-Lebensm-Wiss Technol* 13(4):182-5.
- Bari ML, Nei D, Sotome I, Nishina I, Isobe S, Kawamoto S. 2009. Effectiveness of sanitizers, dry heat, hot water, and gas catalytic infrared heat treatments to inactivate *Salmonella* on almonds. *Foodborne Pathog Dis* 6(8):953-58.
- Bassal A, Vasseur J, Lebert A. 1993a. Measurement of water activity above 100 °C. *J Food Sci* 58(2):449-52.
- Bassal A, Vasseur J, Loncin M. 1993b. Sorption isotherms of food materials above 100 °C. *Food Sci Technol-Lebensm-Wiss Technol* 26(6):505-11.
- Bell LN. 2008. Moisture effects on food's chemical stability. In: Barbosa-Cánovas GV, Fontana AJF, Schmidt SJ, Labuza TP, editors. *Water activity in foods*. Oxford: Blackwell Publishing Ltd. p 173-98.
- Beuchat LR, Scouten AJ. 2002. Combined effects of water activity, temperature and chemical treatments on the survival of *Salmonella* and *Escherichia coli* O157:H7 on alfalfa seeds. *J Appl Microbiol* 92(3):382-95.
- Beuchat LR, Komitopoulou E, Beckers H, Betts RP, Bourdichon F, Joosten HM, Fanning S, Kuile B. 2011. Persistence and survival of pathogens in dry food processing environments. Available from: <http://www.ilsa.org/Europe/Documents/Persistence%20and%20survival%20report.pdf>. Accessed 2015 September 15.
- Bigelow WD. 1921. The logarithmic nature of thermal death time curves. *J Infect Dis* 29:528-36.
- Björk H, Rasmuson A. 1995. Moisture equilibrium of wood and bark chips in superheated steam. *Fuel* 74(12):1887-90.

- Black JL, Jaczynski J. 2008. Effect of water activity on the inactivation kinetics of *Escherichia coli* O157:H7 by electron beam in ground beef, chicken breast meat, and trout fillets. *Intl J Food Sci Technol* 43(4):579–86.
- Brandl MT, Pan Z, Huynh S, Zhu Y, McHugh TH. 2008. Reduction of *Salmonella enteritidis* population sizes on almond kernels with infrared heat. *J Food Prot* 71(5):897–902.
- Brown AD. 1975. Microbial water relations. effects of solute concentration on the respiratory activity of sugar-tolerant and non-tolerant yeasts. *J Gen Microbiol* 86(2):241–9.
- Cavallaro E, Date K, Medus C, Meyer S, Miller B, Kim C, Nowicki S, Cosgrove S, Sweat D, Phan Q, Flint J, Daly ER, Adams J, Hyytiä-Trees E, Gerner-Smidt P, Hoekstra RM, Schwensen C, Langer A, Sodha SV, Rogers MC, Angulo FJ, Tauxe RV, Williams IT, Behravesh CB. 2011. *Salmonella typhimurium* infections associated with peanut products. *N Engl J Med* 365(7):601–10.
- CDC. 2011. CDC estimates of foodborne illness in the United States. Available from: http://www.cdc.gov/foodborneburden/PDFs/FACTSHEET_A_FINDINGS_updated4-13.pdf. Accessed 2015 September 23.
- Cenkowski S, Pronyk C, Zmidzinska D, Muir WE. 2007. Decontamination of food products with superheated steam. *J Food Eng* 83(1):68–75.
- Chang S-S, Han AR, Reyes-De-Corcuera JI, Powers JR, Kang D-H. 2010. Evaluation of steam pasteurization in controlling *Salmonella* serotype Enteritidis on raw almond surfaces. *Lett Appl Microbiol* 50(4):393–8.
- Chen C. 1988. A study of equilibrium relative humidity for yellow-dent corn kernels [PhD thesis]. University of Minnesota, St Paul, Minn., U.S.A.
- Chirife J, Buera MD. 1996. Water activity, water glass dynamics, and the control of microbiological growth in foods. *Crit Rev Food Sci Nutr* 36(5):465–513.
- Chirife J, Iglesias HA. 1978. Equations for fitting water sorption isotherms of foods. Part I – a review. *J Food Technol* 13:159–74.
- Chirife J, Fontan CF, Scorza OC. 1981. The intracellular water activity of bacteria in relation to the water activity of the growth medium. *J Appl Bacteriol* 50(3):475–9.
- Christian JHB. 1955. The water relations of growth and respiration of *Salmonella oranienburg* at 30 °C. *Aus J Bio Sci* 8:490–97.
- Christian JHB, Scott WJ. 1953. Water relations of *Salmonella* at 30 °C. *Aus J Bio Sci* 6:565–73.
- Christian JHB, Waltho JA. 1962. The water relations of staphylococci and micrococci. *J App Bacteriol* 25:369–77.
- Chung DS, Pfost HB. 1967. Adsorption and desorption of water vapor by cereal grains and their products. Part II: development of the general isotherm equation. *Trans ASAE* 10:549–57.
- Corry JEL. 1975. The effect of water activity on the heat resistance of bacteria. In: Duckworth RB, editor. *Water relations of food*. London, UK: Academic Press. p 325–37.
- Danylyuk MD, Uesugi AR, Harris LJ. 2005. Survival of *Salmonella* Enteritidis PT 30 on inoculated almonds after commercial fumigation with propylene oxide. *J Food Prot* 68(8):1613–22.
- Doyle ME, Mazzotta AS. 2000. Review of studies on the thermal resistance of *Salmonellae*. *J Food Prot* 63(6):779–95.
- Farakos SMS, Frank JF, Schaffner DW. 2013. Modeling the influence of temperature, water activity and water mobility on the persistence of *Salmonella* in low-moisture foods. *Intl J Food Microbiol* 166(2):280–93.
- Fennema OR. 1999. *Food chemistry*. 3rd ed. Boca Raton, Fla., U.S.A.: CRC Press.
- Fine F, Gervais P. 2005. A new high-temperature short-time process for microbial decontamination of seeds and food powders. *Powder Technol* 157(1–3):108–13.
- Finn S, Condell O, McClure P, Amézquita A, Fanning S. 2013. Mechanisms of survival, responses and sources of *Salmonella* in low-moisture environments. *Front Microbiol* 4:331.
- Fontana AJ. 2007. Measurement of water activity, moisture sorption isotherms, and moisture content of foods. In: Barbosa-Cánovas GV, Fontana AJF, Schmidt SJ, Labuza TP, editors. *Water activity in foods*. Oxford, UK: Blackwell Publishing Ltd. p 155–71.
- Food and Drug Administration. 2015. FSMA final rule for preventive controls for human food. Available from: <http://www.fda.gov/Food/GuidanceRegulation/FSMA/ucm334115.htm>. Accessed 2015 September 21.
- Gaillard S, Leguerinel I, Mafart P. 1998. Model for combined effects of temperature, pH and water activity on thermal inactivation of *Bacillus cereus* spores. *J Food Sci* 63(5):887–9.
- Gibson B. 1973. The effect of high sugar concentrations on the heat resistance of vegetative microorganisms. *J Appl Bacteriol* 36(3):365–76.
- Goepfert JM, Iskander IK, Amundson CH. 1970. Relation of the heat resistance of *Salmonellae* to the water activity of the environment. *Appl Microbiol* 19(3):429–33.
- Grasso EM, Stam CN, Anderson NM, Krishnamurthy K. 2014. Heat and steam treatments. In: Gurtler JB, Doyle MP, Kornacki JL, editors. *The microbiological safety of low water activity foods and spices*. New York, N.Y., U.S.A.: Springer. p 403–424.
- Gruszkiewicz MS, Simonson JM, Burchell TD, Cole DR. 2005. Water adsorption and desorption on microporous solids at elevated temperature. *J Therm Anal Calorim* 81(3):609–15.
- Ha J-W, Kim S-Y, Ryu S-R, Kang D-H. 2013. Inactivation of *Salmonella enterica* serovar Typhimurium and *Escherichia coli* O157:H7 in peanut butter cracker sandwiches by radio-frequency heating. *Food Microbiol* 34(1):145–50.
- Halsey G. 1948. Physical adsorption on non-uniform surfaces. *J Chem Phys* 16(10):931–37.
- Harvey AH, Friend DG. 2004. Physical properties of water. In: Palmer DA, Fernández-Prini R, Harvey AH, editors. *Aqueous systems at elevated temperatures and pressures*, London, UK: Academic Press. p 1–27.
- Henderson SM. 1952. A basic concept of equilibrium moisture. *Agric Eng* 33:29–32.
- Hilder MH. 1971. The solubility of water in edible oils and fats above 100 °C. *J Am Oil Chem Soc* 48(6):296–8.
- Hills BP, Manning CE, Ridge Y, Brocklehurst T. 1996. NMR water relaxation, water activity and bacterial survival in porous media. *J Sci Food Agric* 71(2):185–94.
- Hsieh F, Acott K, Elizondo H, Labuza TP. 1975. The effect of water activity on the heat resistance of vegetative cells in the intermediate moisture range. *LWT - Food Sci Technol* 8:78–81.
- Hvizdzak AL, Beamer S, Jaczynski J, Matak KE. 2010. Use of electron beam radiation for the reduction of *Salmonella enterica* serovars Typhimurium and Tennessee in peanut butter. *J Food Prot* 73(2):353–7.
- Iglesias HA, Chirife J. 1977. Effect of heating in the dried state on the moisture sorption isotherm of beef. *LWT - Food Sci Technol* 10(5):249–50.
- Jeong S, Marks BP, Orta-Ramirez A. 2009. Thermal inactivation kinetics for *Salmonella enteritidis* PT30 on almonds subjected to moist-air convection heating. *J Food Prot* 72(8):1602–9.
- Jeong S, Marks BP, Ryser ET. 2011. Quantifying the performance of *Pediococcus* sp. (NRRL B-2354: *Enterococcus faecium*) as a nonpathogenic surrogate for *Salmonella* Enteritidis PT30 during moist-air convection heating of almonds. *J Food Prot* 74(4):603–9.
- Jeong S, Marks BP, Ryser ET, Harte JB. 2012. The effect of X-ray irradiation on *Salmonella* inactivation and sensory quality of almonds and walnuts as a function of water activity. *Intl J Food Microbiol* 153(3):365–71.
- Jeong S-G, Kang D-H. 2014. Influence of moisture content on inactivation of *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium in powdered red and black pepper spices by radio-frequency heating. *Intl J Food Microbiol* 176:15–22.
- Karel M. 1975. Fundamentals of dehydration processes. In: Spicer A, editor. *Advances in preconcentration and dehydration of foods*. New York, N.Y., U.S.A.: Applied Science Publications. p 45–94.
- Karel M. 1986. Control of lipid oxidation in dried foods. In: MacCarthy D, editor. *Concentration and drying of foods*, London, UK: Elsevier Applied Science Publishers. p 37–51.
- Karel M, Yong S. 1981. Autoxidation-initiated reactions in foods. In: Rockland LB, Stewart GB, editors. *Water activity: influences on food quality*, New York, N.Y., U.S.A.: Academic Press.
- Kataoka A, Enache E, Black DG, Elliott PH, Napier CD, et al. 2014. Survival of *Salmonella* Tennessee, *Salmonella* Typhimurium DT104, and *Enterococcus faecium* in peanut paste formulations at two different levels of water activity and fat. *J Food Prot* 77(8):1252–9.
- Khuwijitjaru P, Adachi S, Matsuno R. 2002. Solubility of saturated fatty acids in water at elevated temperatures. *Biosci Biotechnol Biochem* 66(8):1723–6.

- Kim S-Y, Sagong H-G, Choi SH, Ryu S, Kang D-H. 2012. Radio-frequency heating to inactivate *Salmonella* Typhimurium and *Escherichia coli* O157:H7 on black and red pepper spice. *Intl J Food Microbiol* 153(1-2):171-5.
- Kotzekidou P, Giannakidis P, Boulamatsis A. 2008. Antimicrobial activity of some plant extracts and essential oils against foodborne pathogens in vitro and on the fate of inoculated pathogens in chocolate. *LWT - Food Sci and Technol* 41(1):119-27.
- Kubojima Y, Suzuki Y, Tonosaki M, Ishikawa A. 2003. Moisture content of green wood in high temperature water vapor. *Holzforchung* 57(6):634-8.
- Labuza TP. 1975. Oxidative changes in foods at low and intermediate moisture levels. In: Duckworth RB, editor. *Water relations of foods*, New York, N.Y., U.S.A.: Academic Press. p 455-74.
- Labuza TP. 1977. The properties of water in relationship to water binding in foods: a review. *J Food Proc Pres* 1(2):167-90.
- Labuza TP, Tannenbaum SR, Karel M. 1970. Water content and stability of low moisture and intermediate moisture foods. *Food Technol* 24:543-50.
- Labuza TP, Altunakar L. 2007. Water activity prediction and moisture sorption isotherms. In: Barbosa-Cánovas GV, Fontana AJF, Schmidt SJ, Labuza TP, editors. *Water activity in foods*. Oxford: Blackwell Publishing Ltd. p 109-54.
- Laroche C, Fine F, Gervais P. 2005. Water activity affects heat resistance of microorganisms in food powders. *Intl J Food Microbiol* 97(3):307-15.
- Lavoie JP, Labbe RG, Chinachoti P. 1997. Growth of *Staphylococcus aureus* as related to ^{17}O NMR water mobility and water activity. *J Food Sci* 62(4):861-6.
- Lee J, Kaletunc G. 2002. Evaluation of the heat inactivation of *Escherichia coli* and *Lactobacillus plantarum* by differential scanning calorimetry. *Appl Environ Microbiol* 68(11):5379-86.
- Lenth CA, Kamke FA. 2001. Equilibrium moisture content of wood in high-temperature pressurized environments. *Wood Fiber Sci* 33(1):104-18.
- Lewis GN. 1907. Outlines of a new system of thermodynamic chemistry. *Proc Am Acad Arts Sci* 43(7):259-93.
- Lian F, Zhao W, Yang R, Tang Y, Katiyo W. 2015. Survival of *Salmonella* enteric in skim milk powder with different water activity and water mobility. *Food Control* 47:1-6.
- Li H, Fu X, Bima Y, Koontz J, Megalis C, et al. 2014. Effect of the local microenvironment on survival and thermal inactivation of *Salmonella* in low- and intermediate-moisture multi-ingredient foods. *J Food Prot* 77(1):67-74.
- Lomauro CJ, Bakshi AS, Labuza TP. 1985a. Evaluation of food moisture sorption isotherm equations. Part I. Fruit, vegetable and meat products. *LWT - Food Sci Technol* 18:111-7.
- Lomauro CJ, Bakshi AS, Labuza TP. 1985b. Evaluation of food moisture sorption isotherm equations. Part II. Milk, coffee, tea, nuts, oilseeds, spices and starchy foods. *LWT - Food Sci Technol* 18:118-24.
- Loncin M. 1955. The solubility of water in fats and oils and the vapor pressure of the dissolved water (in German). *Fette Seifen Anstrichmittel* 57:413-5.
- Loncin M. 1988. Activity of water and its importance in pre-concentration and drying of foods. In: Bruin S, editor. *Preconcentration and drying of food materials*. London, UK: Elsevier. p 15-34.
- Loncin M, Bimbenet JJ, Lenges J. 1968. Influence of the activity of water on the spoilage of foodstuffs. *Intl J Food Sci Technol* 3(2):131-42.
- Mackey BM, Miles CA, Parsons SE, Seymour DA. 1991. Thermal denaturation of whole cells and cell components of *Escherichia coli* examined by differential scanning calorimetry. *J Gen Microbiol* 137:2361-74.
- Mafart P, Leguerinel I. 1998. Modeling combined effects of temperature and pH on heat resistance of spores by a linear-bigeelow equation. *J Food Sci* 63(1):6-8.
- Ma L, Zhang G, Gerner-Smidt P, Mantripragada V, Ezeoke I, Doyle MP. 2009. Thermal inactivation of *Salmonella* in peanut butter. *J Food Prot* 72(8):1596-601.
- Mattick KL, Jørgensen F, Legan JD, Cole MB, Porter J, et al. 2000. Survival and filamentation of *Salmonella enterica* serovar enteritidis PT4 and *Salmonella enterica* serovar typhimurium DT104 at low water activity. *Appl Environ Microbiol* 66(4):1274-9.
- Mattick KL, Jørgensen F, Wang P, Pound J, Vandeven MH, et al. 2001. Effect of challenge temperature and solute type on heat tolerance of *Salmonella* serovars at low water activity. *Appl Environ Microbiol* 67(9):4128-36.
- Moats WA, Dabbah R, Edwards VM. 1971. Survival of *Salmonella anatum* heated in various media. *Appl Microbiol* 21(3):476-81.
- Mogollón MA, Marks BP, Booren AM, Orta-Ramirez A, Ryser ET. 2009. Effect of beef product physical structure on *Salmonella* thermal inactivation. *J Food Sci* 74(7):M347-51.
- Mossel DAA. 1975. Water and microorganisms in foods—a synthesis. In: Duckworth RB, editor. *Water relations of food*. London, UK: Academic Press. p 347-61.
- Murrell WG, Scott WJ. 1966. The heat resistance of bacterial spores at various water activities. *J Gen Microbiol* 43(3):411-25.
- Nguyen HTT, Corry JEL, Miles CA. 2006. Heat resistance and mechanism of heat inactivation in thermophilic *Campylobacters*. *Appl Environ Microbiol* 72:908-13.
- Oswin CR. 1946. The kinetic of package life. III—the isotherm. *J Chem Ind* 65:419-21.
- Pakowski Z, Krupinska B, Adamski R. 2007. Prediction of sorption equilibrium both in air and superheated steam drying of energetic variety of willow *Salix viminalis* in a wide temperature range. *Fuel* 86(12-13):1749-57.
- Pakowski Z, Adamski R, Kokocińska M, Kwapisz S. 2011. Generalized desorption equilibrium equation of lignite in a wide temperature and moisture content range. *Fuel* 90(11):3330-35.
- Palipane KB, Driscoll RH. 1993. Moisture sorption characteristics of in-shell macadamia nuts. *J Food Eng* 18(1):63-76.
- Pearson H, Gabbias B, Ormarsson S. 2012. Equilibrium moisture content of radiata pine at elevated temperature and pressure reveals measurement challenges. *J Mater Sci* 48(1):332-41.
- Potts M. 1994. Desiccation tolerance of prokaryotes. *Microbiol Rev* 58(4):755-805.
- Quirijns EJ, van Bostel AJ, van Loon WK, van Straten G. 2005. Sorption isotherms, GAB parameters and isosteric heat of sorption. *J Sci Food Agric* 85(11):1805-14.
- Rahman MS. 1995. *Food properties handbook*. Boca Raton, Fla., U.S.A.: CRC Press.
- Reid DS. 2008. Water activity: fundamentals and relationships. In: Barbosa-Cánovas GV, Fontana AJF, Schmidt SJ, Labuza TP, editors. *Water activity in foods*. Oxford: Blackwell Publishing Ltd. p 15-28.
- Schmidt SJ. 2004. Water and solids mobility in foods. *Adv Food Nutr Res* 48:1-101.
- Scott WJ. 1936. The growth of microorganisms on Ox muscle. I. The influence of water content of substrate on rate of growth at -1 °C. *J Council Sci Ind Res Aust* 9:177-82.
- Scott WJ. 1953. Water relations of *Staphylococcus aureus* at 30 °C. *Aust J Biol Sci* 6:549-64.
- Senhaji AF. 1977. The protective effect of fat on the heat resistance of bacteria (II). *Intl J Food Sci Technol* 12(3):217-30.
- Shang S, Horne R, Ramey H. 1995. Water vapor adsorption on geothermal reservoir rocks. *Geothermics* 24(4):523-40.
- Sheth AN, Hoekstra M, Patel N, Ewald G, Lord C, Clarke C, Villamil E, Nicksich K, Bopp C, Nguyen TA, Zink D, Lynch M. 2011. A national outbreak of *Salmonella* serotype Tennessee infections from contaminated peanut butter: a new food vehicle for salmonellosis in the United States. *Clin Infect Dis* 53(4):356-62.
- Shigemoto M, Nakagawa K, Sakamoto JJ, Tsuchidoi T. 2010. Thermal death of *Bacillus subtilis* spores in oil-water systems. *Biocontrol Sci* 15(1):27-31.
- Studer P, Heller WE, Hummerjohann J, Drissner D. 2013. Evaluation of aerated steam treatment of alfalfa and mung bean seeds to eliminate high levels of *Escherichia coli* O157:H7 and O178:H12, *Salmonella enterica*, and *Listeria monocytogenes*. *Appl Environ Microbiol* 79(15):4613-19.
- Stumbo CR. 1973. *Thermobacteriology in food processing*. 2nd Ed. New York: Academic Press.
- Sugiyama H. 1951. Studies on factors affecting the heat resistance of spores of *Clostridium botulinum*. *J Bacteriol* 62(1):81-96.
- Sumner SS, Sandros TM, Harmon MC, Scott VN, Bernard DT. 1991. Heat resistance of *Salmonella typhimurium* and *Listeria monocytogenes* in sucrose solutions of various water activities. *J Food Sci* 56(6):1741-3.
- Sun WQ. 2002. Methods for the study of water relations under desiccation stress. In: Black M, Pritchard HW, editors. *Desiccation and survival of in plants*. Wallingford: CABI Publishing. p 47-92.
- Sun X, Schmidt SJ. 1995. Probing water relations in foods using magnetic resonance techniques. In: Webb GA, Belton PS, McCarthy MJ, editors. *Annual reports on NMR spectroscopy*. New York, N.Y., U.S.A.: Academic Press. p 239-73.

- Sunde EP, Setlow P, Hederstedt L, Halle B. 2009. The physical state of water in bacterial spores. *Proc Nat Acad Sci* 106(46):19334–9.
- Syamaladevi RM, Tadapaneni RK, Xu J, Villa-Rojas R, Tang J, Carter B, Sablani S, Marks B. 2015. Water activity change at elevated temperatures and thermal resistance of *Salmonella* in All Purpose Flour and Peanut Butter. *Food Res Int* (In Press).
- Tapia MS, Alzamora SM, Chirife J. 2007. Effects of water activity (a_w) on microbial stability: as a hurdle in food preservation. In: Barbosa-Cánovas GV, Fontana AJF, Schmidt SJ, Labuza TP, editors. *Water activity in foods*. Oxford: Blackwell Publishing Ltd. p 239–55.
- Tomlins RH, Ordal ZJ. 1976. Thermal injury and inactivation in vegetative bacteria. In: Skinner FA, Hugo WB, editors. *Inhibition and inactivation of vegetative microbes*. London, UK: Academic Press. p 153–90.
- Troller JA, Christian JHB. 1978a. Enzyme reactions and nonenzymatic browning. In: Troller JA, Christian JHB, editors. *Water activity and food*. New York, N.Y., U.S.A.: Academic Press. p 48–68.
- Troller JA, Christian JHB. 1978b. Lipid oxidation, changes in texture, color, and nutritional quality. In: Troller JA, Christian JHB, editors. *Water activity and food*. New York, N.Y., U.S.A.: Academic Press. p 69–85.
- van den Berg C, Bruin B. 1981. Water activity and its estimation in food systems: theoretical aspects. In: Rockland LB, Stewart GB, editors. *Water activity: influences on food quality*, New York, N.Y., U.S.A.: Academic Press. p 1–62.
- Villa-Rojas R, Tang J, Wang S, Gao M, Kang D-H, Mah J-H, Gray P, Sosa-Morales ME, Lopez-Malo A. 2013. Thermal inactivation of *Salmonella enteritidis* PT 30 in almond kernels as influenced by water activity. *J Food Prot* 76(1):26–32.
- Vittadini E, Chinachoti P, Lavoie JP, Pham X. 2005. Correlation of microbial response in model food systems with physico-chemical and “mobility” descriptors of the media. *Innov Food Sci Emerg Technol* 6(1):21–8.
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