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

Roopesh M. Syamaladevi, Juming Tang, Rossana Villa-Rojas, Shyam Sablani, Brady Carter, and Gaylon Campbell

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*<sub>w</sub>) 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*<sub>w</sub> 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*<sub>w</sub> 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*<sub>w</sub> 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*<sub>w</sub> 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*<sub>w</sub> < 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 into 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...
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; Hvizdak 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 aw of the growth medium (Scott 1953). Scott's research led to many developments in the fundamental understanding of the aw concept and its applications in food processing and storage. These studies include contributions by Christian and Scott (1953), Christian (1955), Christian and Walho (1962), Brown (1975), Chirife and others (1981), Corry (1975), Mossel (1973), 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 aw 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 (Pw) (Pa) to the saturation water vapor pressure (Pw0) (Pa) at the temperature of the food system.

\[ \alpha_w = \frac{P_w}{P_{w0}} \]  

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):

\[ \mu = \mu_0 + RT \ln \alpha_w = \mu_0 + RT \ln \frac{P_w}{P_{w0}} \]
Table 1–Selected studies on thermal processing techniques to control pathogens in low-moisture foods.

<table>
<thead>
<tr>
<th>Authors and year</th>
<th>Processing technique</th>
<th>Processing conditions</th>
<th>Product</th>
<th>Target microorganism</th>
<th>Major results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fine and Gervais (2005)</td>
<td>Hot air</td>
<td>200 to 600 °C from 0.1 to 30 s heating followed by instantaneous cooling at −80 °C</td>
<td>Wheat flour</td>
<td>Bacillus subtilis spores and Saccharomyces cerevisiae cells</td>
<td>• About 5 to 8 log reduction in microbial population was obtained based on the initial water activity level</td>
</tr>
<tr>
<td>Cenkowski and others (2007)</td>
<td>Superheated steam</td>
<td>105 and 185 °C with steam velocities of 0.35, 0.65, 1.3, and 1.5 m/s</td>
<td>Wheat grains</td>
<td>Fusarium mycotoxin deoxynivalenol (DON) and Geobacillus (Bacillus) stearothermophilus ATCC 10149</td>
<td>• The DON concentration reduction was up to 52% at 185 °C for 6 min processing with superheated steam</td>
</tr>
<tr>
<td>Ha and others (2013) &amp; Jeong and Kang (2014)</td>
<td>Radiofrequency heating (27 MHz)</td>
<td>77 to 86 °C for maximum 90 s &amp; 90 °C for maximum of 80 s</td>
<td>Peanut butter cracker sandwich &amp; Dried red and black pepper powder</td>
<td>S. Typhimuroidum and E. coli O157:H7</td>
<td>• RF treatment resulted in 4.3 log reductions of S. Typhimuroidum and 4.4 log reductions in E. coli O157:H7 population, respectively in creamy peanut butter cracker sandwich &amp; RF treatment resulted in 4.6 log reductions of S. Typhimuroidum and 5.3 log reductions in E. coli O157:H7 population, respectively in chunky peanut butter cracker sandwich &amp; RF treatment did not influence the color and sensory characteristics peanut butter and crackers &amp; More than 7 log reductions of the target microorganisms were achieved during RF heating RF heating did not affect product quality &amp; Moisture content of the products decreased significantly during RF heating</td>
</tr>
</tbody>
</table>

& Brandl and others (2008) | Infrared heating (3000 to 5458 W/m²) | 90 to 113 °C for 30 to 45 s | Almond kernels | S. Enteritidis | • Infrared heating with immediate cooling of the product resulted in 0.63 to 1.51 log reductions in microbial population |
| Chang and others (2010) | Steam pasteurization | 95 °C for maximum 65 s, 143 kPa | Almonds | S. Enteritidis | • The mean log reductions for E. faecium were 0.6 log and 1.4 log lower than those for S. enterica Enteritidis PT30 |
| Jeong and others (2011) | Moist-air convection heating | 121 to 204 °C, 5% to 90% relative humidity, for 72 to 6344 s | Almonds & E. faecium strain NRRL B-2354 and Salmonella Enteritidis PT30 | • The D-values for E. faecium on the surface of almonds subjected to moist-air heating (30% to 90% moisture by volume) were 30% larger than those for SE PT30 & E. faecium 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 | E. coli O157:H7, E. coli O178:H12, S. Weltevreden, and L. monocytogenes Scott A | • Populations of E. coli O157:H7 and S. Weltevreden on alfalfa and mung bean seeds could be completely eliminated by a 300-s treatment with steam at 70 °C |
| brandl and others (2008) | Radiofrequency heating (27 MHz) | 77 to 86 °C for maximum 90 s | Peanut butter cracker sandwich | S. Typhimuroidum and E. coli O157:H7 | • RF treatment resulted in 4.3 log reductions of S. Typhimuroidum and 4.4 log reductions in E. coli O157:H7 population, respectively in creamy peanut butter cracker sandwich |
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where $\mu$ (J/mol) is the chemical potential of the system, that the thermodynamic activity of water, $\mu_w$, 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 (Chirife and Buera 1996). Water activity is also an estimate of thermodynamically available water for various physicochemical or biological reactions. Its influence on reagent 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*, *B. stearothermophilus* ATCC 7953, *Clostridium botulinum* type E ATCC 9564, *C. botulinum* type B ATCC 7949, *Clostridiun bifermantans*, 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 $D$- 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 Mazotta (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 12.1 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 ($D_{s,d}$) 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 $^{2}$H 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.

<table>
<thead>
<tr>
<th>Authors and year</th>
<th>Title</th>
<th>Product</th>
<th>Target microorganism</th>
<th>Water activity and temperature range</th>
<th>Major results thermal resistance data (D- and z-values)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Murrel and Scott (1966)</td>
<td>The heat resistance of bacterial spores at various water activities</td>
<td>Selected growth medium</td>
<td>Bacillus megaterium, B. stearothermophilus ATCC 7953, Clostridium botulinum type EATCC 9564, C. botulinum type B ATCC 7949, C. biferegmentans, B. coagulans</td>
<td>0.11 to 0.80; 70 to 120 °C</td>
<td>Heat resistance was maximum at ( a_w ) values of about 0.2 to 0.4, the maximum value at 110 °C varying from about 2 to 24 h. At lower values of 0.2, the heat resistance was significantly reduced. At higher values of 0.4, the resistance of the microorganism decreased considerably.</td>
</tr>
<tr>
<td>Goepfert and others (1970)</td>
<td>Relation of the heat resistance of salmonellae to the water activity of the environment</td>
<td>0.01 M phosphate buffer at pH 6.9 ± 0.1 with sucrose and glycerol added to change ( a_w )</td>
<td>Selected Salmonella and E. coli strains</td>
<td>0.87 to 0.99 using sucrose and 0.75, 0.90, and 0.99 ( a_w ) by glycerol, 57 °C</td>
<td>Heat resistance of the organisms increased as the ( a_w ) of the heating menstruum was reduced. The type of solute used to control ( a_w ) affected the thermal resistance of selected microorganisms.</td>
</tr>
<tr>
<td>Sumner and others (1991)</td>
<td>Heat resistance of Salmonella typhimurium and Listeria monocytogenes in sucrose solution of various water activities</td>
<td>Sucrose solution</td>
<td>S. Typhimurium and L. monocytogenes</td>
<td>0.83 to 0.99 temperatures ranging from 65.6 to 76.7 °C for S. typhimurium and 4 temperatures ranging from 60 to 68.3 °C for L. monocytogenes</td>
<td>Heat resistance of selected microorganisms increased with decrease in ( a_w ). The decrease in D-values of Salmonella was greater than that of Listeria.</td>
</tr>
<tr>
<td>Mattick and others (2001)</td>
<td>Effect of Challenge Temperature and Solute Type on Heat Tolerance of Salmonella Serovars at Low Water Activity</td>
<td>Tryptone soy broth</td>
<td>Salmonella Typhimurium DT104</td>
<td>0.65 to 0.90; 55 to 80 °C</td>
<td>Salmonella was heat sensitive at ( a_w ) of 0.65 when the temperature was low (55 to 60 °C).</td>
</tr>
<tr>
<td>Beuchat and Scouten (2002)</td>
<td>Combined effects of water activity, temperature and chemical treatments on the survival of Salmonella and Escherichia coli O157:H7 on alfalfa seeds</td>
<td>Alfalfa seeds</td>
<td>Salmonella and E. coli O157:H7</td>
<td>0.15 to 0.54; 50, 60, 70, and 80 °C</td>
<td>The rate of inactivation of Salmonella and E. coli O157:H7 on alfalfa seeds was increased at higher ( a_w ) and temperature.</td>
</tr>
<tr>
<td>Laroche and others (2005)</td>
<td>Water activity affects heat resistance of microorganisms in food powders</td>
<td>Wheat flour and skim milk</td>
<td>Saccharomyces cerevisiae and Lactobacillus plantarum</td>
<td>Initial ( a_w ) of 0.1 to 0.7 and ( a_w ) was monitored during drying, 150 and 200 °C</td>
<td>Maximum viability for L. plantarum and S. cerevisiae was observed at ( a_w ) values of 0.35 and 0.4, respectively, in wheat flour. Maximum viability for L. plantarum and S. cerevisiae was observed at ( a_w ) of 0.4 to 0.5 and 0.3 to 0.5, respectively, in skim milk powder.</td>
</tr>
<tr>
<td>Villa-Rojas and others (2013)</td>
<td>Thermal inactivation of Salmonella Enteritidis PT 30 in almond kernels as influenced by water activity</td>
<td>Almond kernels</td>
<td>Salmonella Enteritidis PT 30</td>
<td>70, 73, 76, and 80 °C at ( a_w ) of 0.601; 62.65, 68, and 71 °C at ( a_w ) of 0.72; 59.62, 65, and 68 °C at ( a_w ) of 0.888; 56.60, 64, and 68 °C at ( a_w ) of 0.946</td>
<td>A nonlinear thermal inactivation behavior of Salmonella was observed. Small increase in ( a_w ) reduced the thermal inactivation time.</td>
</tr>
<tr>
<td>Farakos and others (2013)</td>
<td>Modeling the influence of temperature, water activity and water mobility on the persistence of Salmonella in low-moisture foods</td>
<td>Whey protein powder</td>
<td>Salmonella Typhimurium, S. Tennessee, S. Agona, and S. Montevideo</td>
<td>0.19 and 0.54; 21, 36, 50, 60, 70, and 80 °C</td>
<td>Survival of Salmonella serovars were significantly influenced by ( a_w ) with increase in survival at low ( a_w ).</td>
</tr>
<tr>
<td>He and others (2013)</td>
<td>Increased water activity reduces the thermal resistance of Salmonella enterica in peanut butter</td>
<td>Peanut butter</td>
<td>Salmonella Typhimurium, S. Tennessee, S. Enteritidis, 3-serotype cocktail</td>
<td>0.2, 0.4, 0.6, and 0.8, 90 and 126 °C</td>
<td>Water activity increases significantly reduced the thermal resistance of selected microorganisms at 90 °C.</td>
</tr>
</tbody>
</table>
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 glycercol, 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. spongenes* 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 osmolates 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. Senftenberg* 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, $\sigma^-$ and $\sigma^+$ 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 spo$\Sigma$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. steathermophilus* ATCC 7953, and *Clostridium botulinum* type E ATCC 9564). In their experiments, the spores were equilibrated with LiCl or NaOH or H$_2$SO$_4$ 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):

$$a_w = \frac{C_o}{C_s}$$

concentration of water in oil

$$C_s = \text{concentration of water in oil at saturation level}$$

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

$$\ln C_s = 7.118 - \frac{1222}{T} + 1.459 \ln T$$

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 $10^{-5}$ m$^2$/s, which is about 10,000 times more than the liquid water diffusion coefficient (in the order of $10^{-9}$ m$^2$/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
Influence of water activity... 

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).

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,

\[
P_{\text{ambient}} = P_{\text{air}} + P_v
\]

The above equation can be written as:

\[
\frac{P_{\text{ambient}}}{P_v} = \frac{P_{\text{air}}}{P_v} + a_w
\]

where \(P_v\) is saturated water vapor pressure at product temperature.

Substituting Eq. 1 into the above equation yields:

\[
\frac{P_{\text{ambient}}}{P_v} = \frac{P_{\text{air}}}{P_v} + a_w
\]

In an ambient environment, \(P_{\text{air}}\) has a positive value. Thus,

\[
\frac{P_{\text{ambient}}}{P_v} > a_w
\]

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, \(P_{\text{ambient}} = 101.8 \text{ kPa}\). When foods are heated to 120 °C, the saturated water vapor pressure (\(P_{v_{120^\circ C}}\)) is 201.8 kPa. Thus, at equilibrium the \(a_w\) of the food system is:

\[
a_w < \frac{101.3}{201.8} = 0.5
\]

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 hydroscopic, attributed to the reduced number of active water–binding
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). 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 (Khuwijitjaru and others 2002).

**Water activity measurement devices**

Chilled-mirror dew point sensors and capacitance hygrometers are generally used to measure water activity of foods. Chilled-mirror dew point instrument measurements are accurate (±0.003 $a_w$), fast (measurement within 5 min), and are the primary means of $a_w$ determinations (Fontana 2007). 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:

$$RH = \frac{P_v(T_d)}{P_v(T)} \times 100$$

where $P_v$ is the saturation water vapor pressure, $T_d$ is the dew point temperature, and $T$ is the sample temperature. $P_v$ is calculated from the following equation:

$$P_v = 0.611 \exp\left(\frac{17.502}{T + 240.97}\right)$$

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 hydroscopic 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 equilibrium 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
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.

Gruszkiwicz 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 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.

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

<table>
<thead>
<tr>
<th>Authors and year</th>
<th>Title</th>
<th>Product and temperature range</th>
<th>Technique</th>
<th>Major results</th>
</tr>
</thead>
</table>
| Bassal and others (1993a) | Measurement of water activity above 100 °C | Microcrystalline cellulose and natural potato starch | Manometric and dynamic sorption method | • 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 | 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 | • Desorption isotherms and isobars of the selected products were determined  
• The influence of temperature on the sorption isotherm is small at elevated temperature  
• 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 |
| 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 | • 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 | • 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 | • The desorption isotherms at 160 °C were significantly lower than the those at 50 °C denoting reduced $e_w$ at higher temperature  
• The results show that it would be inaccurate to extrapolate $e_w$ 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 (Pinus radiata 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 | • There was a change in sorption properties of wood when the temperature and moisture were above glass transition temperature of lignin |
Influence of water activity...

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 \( (C, \text{ kg/m}^3) \) from the following equation:

\[
P_v = \frac{CR(T+273.15)}{m_r}
\]  

(12)

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

$$a_w = RH = \frac{P_v}{P_{vs}} \times 100$$  \hspace{1cm} (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

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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:

\[
\frac{X}{X_\infty} = \frac{CK_{a_w}}{(1 - K_{a_w}) (1 - K_{a_w} + CK_{a_w})}
\]

(14)

where \( X \) is the water content (dry basis) of the material, \( X_\infty \) 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:

\[
C = C_o \exp \left( \frac{\Delta H_C}{RT} \right)
\]

(15)

\[
K = K_o \exp \left( \frac{\Delta H_K}{RT} \right)
\]

(16)

where \( \Delta H_C \) is generally positive and indicates the difference in enthalpy between monolayer and multilayer sorption. \( \Delta H_K \) 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 (\( X_\infty \)) is considered as a constant, but a similar expression to describe the temperature dependence of \( X_\infty \) can be presented (Quirijns and others 2005):

\[
X_\infty = X_{\infty_0} \exp \left( \frac{\Delta H_{K0}}{RT} \right)
\]

(17)

Incorporating the temperature-dependence of the parameters \( X_\infty \), \( C \), 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, 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):

\[
D = f \left( T, a_w(T, X_{\infty}) \right)
\]

(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:

$$a_w = f(T, X_w)$$  \hspace{1cm} (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).

$$D_T = D_{T_{ref}} \times 10^{\frac{(T-T_{ref})}{Z_T}}$$ \hspace{1cm} (20)

$$\log \frac{D}{D_{ref}^{c/f}} = - \frac{(T - T_{ref})}{z_T} - \frac{(a_w - 1)}{z_{a_w}} - \frac{(pH - pH_{ref})^2}{z_{pH}^2}$$ \hspace{1cm} (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:

$$\log \frac{D}{D_{cf}^{c/f}} = - \frac{(T - T_{cf})}{z_T} - \frac{(a_w - 1)}{z_{a_w}}$$ \hspace{1cm} (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).

$$Z_T = \frac{T_{T} - T_{T_{ref}}}{\log D_T - \log D_2}$$ \hspace{1cm} (23)

$$Z_{a_w} = \frac{a_{w2} - a_{w1}}{\log D_{a_w} - \log D_2}$$ \hspace{1cm} (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:

$$L = 10L \left[ \frac{(T-T_{ref})}{Z_T} \right]^2 + \left[ \frac{(pH-pH_{ref})}{z_{pH}} \right]^2 \hspace{1cm} (25)$$

$$F = \int_0^t 10L \left[ \frac{(T-T_{ref})}{Z_T} \right]^2 + \left[ \frac{(pH-pH_{ref})}{z_{pH}} \right]^2 \hspace{1cm} (26)$$

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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