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

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Outline

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.

Key words: *Water activity, Salmonella, low-moisture food safety, thermal resistance, water mobility, thermal processing*

1. Introduction

Food safety is a global concern. About one out of six people in North America suffer from foodborne illnesses which is equivalent to about 48 million cases every year and costs billions of dollar to food processing industry due to food recalls and outbreaks (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 etc.), *Cronobacter* (in powdered infant formula), *Bacillus* (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). 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) by Food and Drug Administration (FDA) was signed to law in the United States of America 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 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 the key product factors related to the development and validation of thermal processing of low-moisture foods. The review presents the factors influencing the thermal resistance of pathogens in low-moisture foods, emphasizing on the influence of water activity, which is probably the most important factor. Elevated temperatures (>60°C) during thermal processing which may sharply change the characteristics of food 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. Further, the knowledge gaps and future research needs are identified with regard to the development of thermal processing methods to improve low-moisture food safety.

1.1 Processing to Control Pathogens in Low-Moisture Foods

The safety of low-moisture food products may be improved by controlling the pathogens during production 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 product 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

118 others 2012), electron beam (Black and Jaczynski 2008; Hvizdzak and others 2010), propylene
119 oxide (Danyluk and others 2005), plant extracts (Kotzekidou and others 2008) have been
120 reported to improve low-moisture food safety. The application of these technologies may be
121 dependent on the structural characteristics (size, shape, and nature) of the low-moisture foods
122 (i.e. *powders* such as ground spices, flour, infant formula or *pastes* such as peanut butter or
123 *large particulates* such as nuts). Adaptation of existing pasteurization technologies, such as
124 steam, radiofrequency, and irradiation, faces many challenges. There is no unique technology
125 available which is suitable to treat different kinds of low-moisture food products. Further, food
126 processing industries are interested in the potential of traditional food processing technologies
127 where heat is used as a medium to develop low-moisture foods (such as drying, baking,
128 extrusion, and frying) on the inactivation of pathogens in final product. Insufficient
129 understanding on the main processing factors involved in choosing technologies to treat
130 different low-moisture foods may lead to food safety issues and poor dissemination of potential
131 new and traditional technologies. Further understanding of the critical factors associated with
132 the development of pasteurization technologies for low-moisture foods is essential.
133
134 **1.2 Thermal Processing for Low-moisture Foods**
135 Thermal processing has the potential to eliminate pathogens in low-moisture foods. Thermal
136 processing technologies using dry heat, wet heat or electromagnetic radiations can be effective in
137 reducing pathogens in low-moisture foods. The dry heat treatments include hot air treatment,
138 baking, roasting. Controlled condensation steam and moist air impingement use wet heat to
139 control pathogens in low-moisture products such as nuts and powders (Grasso and others 2014).
140 Radiofrequency, infrared, and microwave radiation may also be effective in heating low-

141 moisture foods quickly compared to conventional technologies and reducing the pathogens in
142 them. More information regarding the types of thermal processing techniques used to improve
143 the safety of low-moisture foods is given in Table 1.
144
145 **2. Factors Influencing the Thermal Resistance of Pathogens in Low-Moisture Foods**
146 In order to develop adequate thermal processing technologies, information on thermal resistance
147 of the target pathogens in selected low-moisture foods should be available. Several product
148 related (eg. water activity, water mobility, type, nature and composition of product) and process
149 related factors (eg. relative humidity, mode of heating) may influence the thermal resistance of
150 pathogens in low-moisture foods. Elements related with the microorganisms such as strain,
151 growth conditions, age and number of cells heated will also influence the thermal resistance. The
152 following subsections focus on the main product related factors associated with thermal
153 resistance of pathogens in low-moisture foods.
154
155 **2.1 Water Activity**
156 Water activity is considered as one of the most important parameters in food preservation. The
157 water activity concept is more than 100 years old in research. Schloesing first reported the
158 relationship between water content and equilibrium relative humidity at a certain temperature or
159 the water vapor sorption isotherm of textile fibers back in 1893 (van den Berg and Bruin 1981).
160 G. N. Lewis introduced the concept of activity of a component in 1907, including activity of
161 water in a system based on thermodynamic principles described earlier by J. W. Gibbs (Lewis
162 1907; van den Berg and Bruin 1981). The first systematic studies on the relationship between
163 microbial growth and relative humidity was conducted in the 1920s by Walderdorff and Walter

(van den Berg and Bruin 1981). Later in 1930s, Australian microbiologist W. J. Scott conducted experiments on microbial growth in food systems in relation to 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 fundamental understanding of a_w concept and its applications in food processing and storage. These studies include 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 (1978a&b), Chirife and Iglesias (1978), Karel (1974, 1981, 1986). The use of food stability map was developed by M. Karel, S. R. Tannenbaum and T. P. Labuza (Labuza and others 1970). The development of a_w of concept led to the formation of a scientific organization called International Symposium on the Properties of Water (ISOPOW, www.isopow.org) in 1974, which is aimed at progressing 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) to the saturation water vapor pressure (P_{vs}) 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 in 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):

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

where, μ (J/mol) is the chemical potential of the system, i.e., 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 surrounding environment reaches equilibrium, the chemical potential of water in the food and the chemical potential of water vapor in the environment become same. Thus, the net transfer of water between the food and 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 that is a pseudo-equilibrium during storage. 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 reactant mobility during processing and storage of foods is greater than that of water content; that makes it more useful parameter than water content to understand product quality and stability (Bassal and others 1993a&b).

2.1.1 Water activity and Thermal Resistance of Microorganisms in Low-Moisture Foods

208 Microorganisms in low-moisture environments are in general more tolerant to heat. Convincing
209 experiment results show a sharp increase in thermal resistance of microbial pathogens such as
210 *Salmonella*, when the a_w of a food system was reduced below 0.6 (Archer and others 1998, Bari
211 and others 2009). The influence of a_w on thermal resistance of microorganisms in low-moisture
212 environments was recognized in late 1960s. Murrel and Scott (1966) reported that the thermal
213 resistance, as explained by D-values (time required for 90% reduction in the population of a
214 specific pathogen), of bacterial spores (*Bacillus megaterium*, *B. stearothermophilus* ATCC 7953,
215 *Clostridium botulinum* type E ATCC 9564, *C. botulinum* type B ATCC 7949, C. *bgermentans*,
216 *B. coagulans*) equilibrated to a_w values of 0.2-0.4 at 110°C varied from 2 to 24 min. The thermal
217 resistance of *Clostridium botulinum* 62A spores was reported over a_w range of 0 to 0.9 (Alderton
218 and others 1980). A higher value of thermal resistance was observed between a_w range of 0.1
219 and 0.5 while the spores exhibited smaller thermal resistance at a_w of 0 and between 0.7 and 0.9
220 (Alderton and others 1980). Goepfert and others (1970) determined the thermal resistance of
221 selected *Salmonella* spp. and *E. coli* at selected a_w range of 0.75 to 0.99 in sucrose, fructose,
222 glycerol and sorbitol solutions. The thermal resistance of the selected microorganisms increased
223 as the a_w was reduced, however, a direct association between a_w and thermal resistance was not
224 established (Goepfert and others 1970).

225 The D- and z (Temperature difference required for 90% reduction in D-value of a specific
226 pathogen) values of *S*. Typhimurium in salt solution model system with a_w of 0.3 at temperature
227 range of 90-120°C ranged from 10.6-20 min and 40°C, respectively (Akinleye 1994). The D-
228 value and z value (temperature change required for 90% reduction in D-value) of *S*. Weltevreden
229 at a_w range of 0.5-0.6 at ~70°C were 80 min and ~30°C, respectively, which are relatively high
230 (Archer and others 1998). Doyle and Mazzota (2000) showed that the D-values of *S*.

231 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
232 decreased to 0.83, the D-value jumped to 40.2 min (300 times). The thermal resistance of
233 *Saccharomyces cerevisiae* and *Lactobacillus plantarum* in wheat flour and skim milk was
234 studied at different a_w levels ranging from 0.1 to 0.7 at 150 and 200ºC (Laroche and others
235 2005). The highest thermal resistance of *Saccharomyces cerevisiae* and *Lactobacillus plantarum*
236 in wheat flour was observed at the a_w levels of 0.40 and 0.35, respectively while in skim milk,
237 maximum thermal resistance was observed at 0.3 a_w (Laroche and others 2005). The D-value of
238 *S*. Enteritidis PT30 in almond flour was reported to be 0.42 min at 68°C and 0.95 a_w, but
239 increased to 15.2 min (36 times) at 70°C when water activity was reduced to 0.60 a_w (Villa-Rojas
240 and others 2013). Hence, low-moisture foods may be heat treated for long periods of time in
241 order to achieve adequate reduction in pathogen population (5 log) which is challenging, as long
242 heat treatments may impact the quality of the product. Table 2 presents some of the reported
243 studies of thermal resistance of selected pathogens and a_w range in specific low-moisture foods.
244 Most of the reported studies determined a_w values of low-moisture foods at room temperature
245 although the studied food systems were treated at elevated temperatures (>60ºC) in sealed
246 containers.
247

248 **2.2 Water Mobility**

249 Water activity is a more macroscopic concept while water mobility is a molecular perception,
250 which is related to the translational, rotational or vibrational motion of water molecules in food.
251 Through translational motion or self-diffusion, a water molecule can change its location in a
252 three dimensional space (Schmidt 2004). The self-diffusion coefficient (D_{self}) is a measure of
253 translational motion of water molecules, which is determined using Stokes-Einstein relationship

(Schmidt 2004). Water molecules in liquid and vapor phases exhibit translational motion, which can me 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 solid phase (Schmidt 2004). The vibrational motion of a water molecule is an intramolecular motion, which can be through stretching, bending and 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 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* was 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). Only a few studies have focused on the influence 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 days. The influence of a_w was greater while water mobility influenced little at low water activities but water mobility influenced greater 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

277 better related to water activity than water mobility in low-moisture foods though growth of
278 microorganisms was better correlated with water mobility.

279

280

281 **2.3 Physical Structure and Composition of Food Products**

282 Food systems are structurally complex. Survival of microorganisms in low-moisture foods
283 during thermal processing may be influenced by type, nature and composition of food systems.
284 For instance, thermal resistance of *Salmonella* was influenced by variation in physical structure
285 (whole beef muscle vs ground beef vs beef puree) (Mogollón and others 2009). The variation in
286 physical structure [such as foods with small particulates (eg. food powders such as wheat flour,
287 ground spices), foods with large particulates (eg. tree nuts such as almonds), or paste (eg. peanut
288 butter)] may result in different thermal resistance values for pathogens in low-moisture foods.

289 The type of food components present in food systems may also influence the thermal
290 resistance of microorganism (eg. Carbohydrate- vs protein- vs fat-rich food systems). The
291 presence of solutes such as glycerol, sucrose and sodium chloride was reported to influence the
292 thermal resistance of microorganisms, probably attributed to the reduction in a_w of the media
293 (Gibson 1973; Hsieh and others 1975; Moats and others 1971). At equivalent water activities,
294 sucrose might protect *Salmonella* strains better than sodium chloride and glucose-fructose in
295 tryptic soy broth during thermal treatments at selected temperatures ranging from 55 to 72°C at
296 water activities of 0.75, 0.80 and 0.90 (Mattick and others 2001). The a_w of the media was
297 adjusted to the selected a_w values at 25°C not treatment temperatures. It may be presumed a

298 difference in the relationship between a_w and thermal resistance in actual situation as a_w may
299 vary at the treatment temperatures.

300 A number of studies have focused on protective effect of lipid materials on microorganisms
301 during thermal treatments (Ma and others 2009; Ababouch and Busta 1987; Shigemoto and
302 others 2010; Li and others 2014). Ma and others (2009) observed unusually greater heat
303 resistance of *Salmonella* strains in peanut butter with a_w of 0.45 in comparison to many high-
304 moisture foods such as ground beef but with higher a_w, attributed to the high fat content (~53%)
305 and low a_w of peanut butter. The D-values of *Salmonella* strains at 71°C ranged from 26.5 to
306 30.6 min while at 90°C, the D-values ranged from 8.6-13.4 min (Ma and others 2009). Kataoka
307 and others (2014) reported greater survival of selected *Salmonella* strains and *Enterococcus*
308 *faecium* at lower a_w (0.3 Vs 0.6) in peanut butter formulation stored for 12 months at 20°C after
309 heat treatment at 75°C for 25-50 min. However, a greater fat content (56% Vs 47%) in peanut
310 butter formulation did not influence the survival of the selected bacterial strains, that >47% may
311 have maximized the protective effect of fat on the survivability of bacterial cells (Kataoka and
312 others 2014). Ababouch and Busta (1987) observed greater thermal resistance (higher D- and z
313 values) of *Bacillus cereus*, *C. botulinum*, and *C. sporegenes* spores suspended in oil (olive oil
314 and commercial oil containing rapeseed oil and soy oil) compared to aqueous buffer (pH of 7.2).
315 This was attributed to the reduction in a_w during thermal treatments in the presence of lipid
316 materials. They determined a_w values of oils at 25°C and thermal treatment temperatures (110 to
317 128.5°C) using equations reported in Loncin (1955) and Hilder (1971). The spores survived
318 better in olive oil compared to commercial oil even though the water activity was slightly higher
319 for olive oil, showing that oil composition may also directly contribute to the thermal resistance
320 of microorganisms (Ababouch and Busta 1987). Further, the significantly greater z values of the

321 spores in the selected oils compared to aqueous buffer, suggesting that the inactivation
322 mechanism of the spores in these matrices may be different (Ababouch and Busta 1987).
323 Shigemoto and others (2010) reported that *B. subtilis* spores survived better at higher soybean oil
324 content in an oil-water emulsion system. However, they observed a higher death rate in the initial
325 phase. The spores in the oil phase may have a lower death rate in comparison to those in aqueous
326 phase, suggesting that location of spores in the emulsion system may be important related to the
327 thermal resistance (Shigemoto and others 2010). The thermal resistance of spores may be
328 affected by change in the location of spores during heating from aqueous phase to oil phase. This
329 may be attributed to the separation of liquid phases and increase in hydrophobicity of spores
330 during heating (Shigemoto and others 2010). Additional knowledge on the influence of
331 microenvironment on thermal resistance of pathogens is necessary, which will help design
332 thermal processing techniques for low-moisture foods.

333

334 **2.4 Microbiological Factors**

335 The thermal resistance of microorganisms may be influenced by type of strain, growth conditions
336 such as log phase vs stationary phase, presence of other microflora, growth media, presence of
337 calcium, magnesium and iron or fatty acids in the media, growth temperature etc. (Doyle and
338 Mazzotta 2000). More details regarding the summary of microbiological factors can be found in
339 Doyle and Mazzotta (2000) and Sugiyama (1951). For an example to the influence of type of
340 strains, the reported D-values at 55°C of *S.* Senftenberg and *S.* Bedford grown in stationary phase
341 and the survivors recovered on peptone-Lemco agar with oxalated horse blood after 48 h at 37°C
342 were 36.2 and 18.8 min, respectively (Baird-Parker and others 1970). Fatty acids in the media
343 increased the thermal resistance of *C. botulinum* spores, 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).

A number of mechanisms associated with increased resistance of microorganism at low-water activities were studied in the past. 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 etc.), filamentation, σ^E and σ^S regulated genes, viable but nonculturable state of bacterial cells, and biofilm formation etc (Finn and others 2013). The exposure of microorganisms to low-water activity environments may enhance their resistance to other stresses such as heat (Finn and others 2013). The greater thermal resistance of microorganisms in low-moisture foods may be attributed to reduced water molecule vibrations during heating as their water content is decreased (Tapia and others 2007). Under stress conditions like 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 continuously investigated in order to further learn the mechanisms behind their greater thermal resistance.

2.5 Knowledge Gaps

Additional research is needed to better understand the effect of water activity and water mobility variation in low-moisture foods, physical structure, composition, changes in interaction between

food components during thermal processes 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. This review focuses on the effect of a_w and its variation during thermal processes on the thermal resistance of microorganisms in low-moisture foods. Thermal processes such as pasteurization for bulk materials are often dynamic processes; there may be significant variation in a_w depending on the nature of food matrices and the process conditions. The microorganisms in food matrix also achieve 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 (60-140^{o}C). Published studies have related heat resistance (D and z values) of *Salmonella* to water activities of food matrices at room temperature and not that of the treatment temperature (Table 2). Most of these studies ignored the changes in water activity of food samples when heated from room temperature to the treatment temperature, since available instruments usually do not measure a_w at temperatures higher than 60^{o}C. Consequently, there is little knowledge about how a_w of low-moisture foods with different major components at elevated temperatures might affect microbial inactivation. 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.

2.5.1 Thermal Resistance of Pathogens and Water Activity Variation in Low-Moisture Foods at Elevated Temperatures

Few studies related thermal resistance of pathogens with water activity variation in low-moisture foods at elevated temperatures. Murrel and Scott (1966) used five methods to relate thermal

390 inactivation kinetics with water activity of bacterial spores (*Bacillus megaterium* (Knaysi, Strain
391 cl), *B. stearothermophilus* ATCC 7953, and *Clostridium botulinum* type E ATCC 9564),
392 considering the variation in water activity at elevated temperatures. In their experiments, the
393 spores were equilibrated with LiCl, or NaOH or H_2SO_4 solutions and treated at elevated
394 temperatures for specific periods of time and cooled rapidly in ice water (Murrel and Scott
395 1966). The thermal resistance of spores increased initially with increasing a_w from 0.2 to reach a
396 maximum thermal resistance at 0.4 a_w and decreased steadily from 0.4 to 1.0 a_w. The thermal
397 resistance observed at 0.2 to 0.4 a_w was up to 10,000 times greater than the thermal resistance
398 observed close to 1.0 a_w (Murrel and Scott 1966).

399 Loncin (1955) and Senhaji (1977) reported that a_w of oils which are rich in lipid materials
400 decreases with increasing treatment temperatures in comparison to room temperature (~23ºC).
401 The water activity of oils can be calculated as (Loncin 1955):

402 $$\frac{C_0}{C_s} = \frac{\text{Concentration of water in oil}}{\text{Same concentration at saturation level}} \quad (3)$$

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

406 $$\ln C_s = 7.118 - \frac{1222}{T} + 1.459 \ln T \quad (4)$$

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

408 Based on the above equations, Ababouch and Busta (1987) calculated a_w values for olive oil
409 and commercial oil at thermal treatment temperatures (110 to 128.5ºC), and relate thermal
410 resistance of bacterial spores suspended in olive oil and commercial oil to a_w at elevated
411 temperatures. The greater thermal resistance of spores in oil compared to buffer was attributed to

412 reduction in a_w of oils at the thermal treatment temperatures, and the presence of lipid materials
413 (Ababouch and Busta 1987).

414 Hence, the thermal resistance of food pathogens such as *Salmonella* in low-moisture foods
415 should be related with the modified water activity of those foods at the treatment temperature.
416 However, the water activity is determined at a thermodynamic equilibrium state where a uniform
417 constant temperature is required to achieve it (Murrel and Scott 1966). A complete water vapor
418 equilibrium between the food and surrounding environment is hardly possible during dynamic
419 thermal processing conditions, considering the general treatment time periods (Murrel and Scott
420 1966). This may be one of the main reasons why the water activity variation was not considered
421 in the previous studies. For low-moisture foods, thermal treatments (with temperature range of
422 60-90^{o}C) require several minutes to hours to achieve 3-5 log reduction in microbial population in
423 closed containers. Hence, one could assume a pseudo-equilibrium between low-moisture food
424 and surrounding in sealed containers during thermal treatments. This makes it possible to
425 consider the modified water activity at that elevated temperature, instead of initial water activity
426 of the food, in designing effective thermal treatments. At elevated temperatures, water diffusion
427 inside a food system or the surrounding environment is most likely through water vapor diffusion
428 as liquid water to vapor conversion increases and hence water vapor pressure increases. The
429 diffusion coefficient of water vapor in air is 2.5×10^{-5} m^2/s which is much faster than liquid water
430 diffusion coefficient ($\sim10^4$ times more). This suggests that water activity equilibration in food
431 may be quick during heat treatments. The determination of equilibration time required for the
432 target microorganisms in a low-moisture food to adjust to the modified temperature and relative
433 humidity during thermal processing will explain the importance of water activity determination
434 at elevated temperatures.

435

436 **3. Water Activity of Foods at Elevated Temperatures**

437 **3.1 State of Water in Foods**

438 The water inside foods and other biological materials can be bound or free water (Pakowski and
439 others 2007). Bound water is tightly hydrogen bonded to hydroxyl groups of food components
440 and may not be easily available for physicochemical reactions. On the other hand, the free water
441 exists in liquid form and is loosely attached by capillary forces with unrestricted movement or
442 orientation (Pakowski and others 2007). The unrestricted water molecules are more available for
443 reactions in food matrices.

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

450 Nonpolar compounds such as fats and oils, on the other hand, do not interact or form
451 hydrogen bonds with water molecules. Mixing water with hydrophobic substances results in
452 hydrophobic hydration, which is a thermodynamically unfavorable event with positive free
453 energy change ($\Delta G>0$) as the entropy is decreased (Fennema 1999). Water molecules may re-
454 arrange around nonpolar fat molecules to form more orderly structure along with a decrease in
455 entropy of the system. Surrounded fat molecules may also aggregate together to reduce the
456 interfacial surface area or minimize their association with water, resulting in a more

457 thermodynamically favorable hydrophobic interaction (Fennema 1999) (Figure 1). More about
458 hydrophobic interactions of water is detailed by Fennema (1999).

459

460 **3.2 Water Activity of a Food System above 100°C**

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

463 $$P_{ambient} = P_{air} + P_v \quad (5)$$

464 The above equation can be written as:

465 $$\frac{P_{ambient}}{P_{vs}} = \frac{P_{air}}{P_{vs}} + \frac{P_v}{P_{vs}} \quad (6)$$

466 Where, P_{vs} is saturated water vapor pressure at product temperature. Substituting equation (1)
467 into the above equation yields:

468 $$\frac{P_{ambient}}{P_{vs}} = \frac{P_{air}}{P_{vs}} + a_w \quad (7)$$

469

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

471 $$\frac{P_{ambient}}{P_{vs}} > a_w \quad (8)$$

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

476 $$a_w < \frac{101.3}{201.8} = 0.5 \quad (9)$$

477 This concept has to be carefully considered for designing thermal processing of low-moisture
478 foods when using temperature beyond 100°C. For instance, many of the low-moisture foods like
479 spices are heated using air or steam at ~120°C to inactivate pathogens such as *Salmonella*. If the
480 a_w of the food is high, food will lose water to reach the a_w of less than 0.5 at the treatment
481 temperature. The pressure should be maintained greater than atmospheric pressure to achieve
482 greater relative humidity of the surrounding environment and hence the water activity of a food
483 system.

484

485 **3.3 Influence of Food Components on Water Activity Variation with Temperature**

486 For carbohydrates and protein rich foods, it is well known that increasing temperature increases
487 their water activities a_w (Figure 2). This is possibly due to the structural changes in carbohydrate
488 and protein molecules at elevated temperatures, affecting its interaction with water and a_w-water
489 content equilibrium (Iglesias and Chirife 1977). At elevated temperatures, those biomaterials
490 may become less hygroscopic, attributed to the reduced number of active water binding sites and
491 greater energy levels of unstable water molecules (Sun 2002; Pakowski and others 2007).
492 Molecular simulation experiments revealed that for liquid water at ambient conditions, the radial
493 distribution function [*g(r)*] exhibits a peak around 2.8 Å for oxygen-oxygen *g(r)* and a peak
494 around 1.9 Å for hydrogen-oxygen *g(r)* designating the presence of strong hydrogen bonding
495 between water molecules (Harvey and Friend 2004). The radial distribution function for
496 hydrogen-oxygen [*g(r)*] peaks is significantly reduced at elevated temperatures, weakening the
497 hydrogen bonds among water molecules with biomolecules and between water molecules

498 (Harvey and Friend 2004). Thus, fewer binding sites and reduced attractive forces, more water
499 has sufficient energy to escape from liquid phase in food into the vapor phase, increasing the a_w
500 of carbohydrates and protein rich foods at elevated temperatures (Palipane and Driscoll 1993).

501 However, a_w may decrease with increased temperature in hydrophobic materials such as oils
502 (Senhaji 1977) (Figure 2). Authors developed sorption isotherms (equilibrium water activity-
503 water content relationship of a food system at a specific temperature) of peanut butter at specific
504 temperatures and observed that the water activity of peanut butter indeed decreased with
505 temperature at fixed water contents (Figure 3) (unpublished data). Similar observations were
506 reported for other fat rich foods such as peanut oil, and oleic acid (Loncin and others 1968), olive
507 oil, commercial oil containing rapeseed oil and soy oil (Ababouch and Busta 1987). This
508 behavior may be attributed to an increase in the solubility of nonpolar solids, such as, fats in
509 water at elevated temperatures (Khuwijitjaru and others 2002). Some reported studies on wood
510 materials also observed a decrease in a_w with an increase in temperature (Ishikawa and others
511 2004; Lenth and Kamke 2001; Kubojima and others 2003). Shang and others (1995) reported
512 that the increase in water content at elevated temperatures may be attributed to temperature
513 dependent chemical adsorption.

514

515 **3.4 Water Activity Measurement Devices**

516 Chilled-mirror dew point sensors and capacitance hygrometers are generally used to measure
517 water activity of foods. Chilled-mirror dew point instruments are accurate (±0.003 a_w), fast
518 (measurement within 5 min), and are the primary means of a_w determination (Fontana, 2007).
519 With this method, a food sample is equilibrated in a sealed chamber where a mirror, optical

sensor, and a fan are located. The mirror is cooled thermoelectrically until dew forms on a 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 ^{o}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 (±0.015 a_w), 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. New methods should be developed for rapid and accurate measurement of a_w in foods at temperatures >60°C.

4. Literature Data of Water Activity of Materials at Elevated Temperatures

Commercially available instruments measure water activities of foods at a temperature range of 20-60°C. Estimating a_w by extrapolation of a_w data of foods above 60°C using theoretical models may not give accurate results due to state and phase transitions (Bassal and others 1993a&b). Consequently, methods are needed to make direct measurements of water activity and moisture sorption isotherms at elevated temperatures (>60°C). However, sorption isotherms at temperatures above 60°C were generated in the past with custom built instruments that are not available commercially or readily accessible to most laboratories.

The studies that measured a_w in biological materials at temperatures above 60°C 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 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

566 weight change in the sample was monitored and the moisture content of the sample was
567 determined at equilibrium at a specific temperature and pressure condition. The water activity
568 was determined as the ratio of vapor pressure inside the chamber and saturated vapor pressure at
569 the selected temperature.

570 The studied food materials by Bassal and others (1993a&b) were mainly rich in
571 carbohydrates. They observed an increase in temperature increased the a_w at a given moisture
572 content for these food materials. For instance, the a_w of microcrystalline cellulose increased from
573 0.3 to ~0.7 when temperature was increased from 25 to 100°C. Also, the a_w of potato starch
574 increased considerably from 0.3 to ~0.8 when temperature was increased from 20 to 100°C.
575 However, the influence of temperature on a_w was minimal at higher temperature (Bassal and
576 others 1993a&b). They observed structural changes in potato starch due to gelatinization and in
577 cake dough possibly due to starch gelatinization, protein denaturation, and sugar crystallization
578 at 100, 115 and 130°C during the a_w measurement. At temperatures above 100°C, the maximum
579 water activity values observed for the selected materials followed predictions by equations 7-8.
580 For example, the maximum water activity value of microcrysalline cellulose during desorption at
581 132°C was ~0.35 at atmospheric pressure. Experiments were conducted at pressure greater than 1
582 bar in order to achieve water activities above 0.5 and the corresponding water contents in
583 selected foods (Bassal and others 1993a). The desorption isotherm of lactose was similar to that
584 of a typical crystalline material with three distinct zones based on its water sorption properties
585 (Bassal and others 1993a&b).

586 The water activity of wood and bark chips was determined at elevated temperatures by
587 measuring the pressure inside an equilibrium chamber and saturated vapor pressure of
588 condensing steam at 140 and 160°C (Bjork and Rasmuson 1995). An electrically heated steam

589 generator and a throttle valve were used to produce steam and control pressure inside a sample
590 testing box, respectively. The pressure inside the chamber was varied by flowing different
591 quantities of steam inside the chamber. The saturated vapor pressure was determined from the
592 condensation temperature of the steam. The water activity was determined as the ratio of the
593 saturated pressure at the temperature and saturated pressure at the superheated temperature. The
594 equilibrium sample moisture content of the sample was determined at required pressure levels.
595 The water activity at a given equilibrium water content of materials at elevated temperatures may
596 be dependent on the partial pressure of the super-heated steam used to achieve different water
597 activities and the type of material as reported by Bjork and Rasmuson (1995). In particular, they
598 observed that the temperature has less effect on a_w and equilibrium moisture content of spruce
599 and aspen as their water activities at given equilibrium water contents at 140 and 160^{o}C were
600 similar.

601 Lenth and Kamke (2001) developed desorption isotherms of wood samples (yellow-poplar,
602 loblolly pine, and aspen) at 50°C and 160°C using a system similar to that mentioned above
603 (Figure 4). The wood samples were kept inside a pressurized vessel and the mass of the sample
604 was monitored at different relative humidity levels created by changing the pressure from
605 saturation to atmospheric pressure at constant temperature conditions. The weight changes in the
606 sample at different relative humidity levels were monitored using an electronic balance to
607 determine the equilibrium water content. Initially, the internal pressure of the vessel was raised
608 to 587 kPa at 160^{o}C to achieve a relative humidity level of 95%. At this point, most of the air
609 inside the chamber is replaced by water vapor. The pressure inside the chamber is increased
610 above atmospheric pressure to avoid boiling of water at high temperatures. Then pressure was
611 gradually reduced to atmospheric pressure to attain different relative humidity levels. The lower

612 values of relative humidity of the air and corresponding water activity values of the samples
613 were thus obtained by essentially a drying process. Alternatively, Lenth and Kamke (2001)
614 determined that the a_w values of wood samples (aspen, yellow-poplar, and loblolly-pine) at
615 160°C were greater than those at 50°C. For instance, the a_w of juvenile and mature aspen wood at
616 50°C at a constant equilibrium water content of 5% was ~0.3, which increased to ~0.45 in mature
617 and ~0.6 in juvenile aspen wood (Lenth and Kamke 2001). Further, they observed a drastic
618 increase in equilibrium water contents for water activities above 0.5 for these wood samples.
619 Mass loss of wood due to thermal degradation at 160°C was observed, specifically above 0.5 a_w,
620 which may be exhibited by food materials. They reported that the sorption isotherms of materials
621 at elevated temperatures may cross over those at lower temperatures at certain water activities
622 attributed to the increased adsorption and softening due to glass transitions in the sample (Lenth
623 and Kamke 2001).

624 Kubojima and others (2003) reported a similar instrument as in Figure 4 to measure the
625 equilibrium moisture content of green wood at different relative humidity values and elevated
626 temperatures. The technique required a hermetically sealed pressure vessel and a humidifier to
627 produce steam. The set temperature and relative humidity to 107 to 160 °C and 75–99% RH,
628 respectively, through superheating control. The weight of the sample at each relative humidity
629 value with different total pressure inside the chamber was monitored and the moisture content at
630 equilibrium was determined using a cantilever-type load cell with strain gauges placed in the
631 pressure chamber. They observed a decrease in equilibrium water content of green sitka spruce at
632 a constant equilibrium relative humidity when temperature was increased. For instance, at an
633 equilibrium relative humidity of 95%, the equilibrium water content decreased from 13.2 to 5.1%
634 when temperature was increased from 107 to 150°C.

635 Gruszkiewicz and others (2005) used a static gravimetric approach to develop water
636 adsorption/desorption isotherms. They determined the vapor pressure as a function of molality
637 and equilibrium water content of selected synthetic and natural porous solids such as controlled
638 pore glass, activated carbon fiber monoliths, natural zeolites, pillared clay, and geothermal
639 reservoir rocks at a range of temperatures between 105 and to 250°C. They used an isopiestic
640 apparatus to systematically change the vapor pressure from vacuum to saturation vapor pressure
641 at a specific temperature by injecting or removing water in the chamber to develop
642 adsorption/desorption isotherms. The sample mass was monitored to determine the equilibrium
643 water content of samples during adsorption/desorption using an electromagnetic balance. They
644 did not observe any temperature dependence on adsorption isotherm. However, a small decrease
645 in equilibrium water content (increase in water activity) with temperature during desorption was
646 observed. The difference between adsorption and desorption isotherms (hysteresis) of rock
647 samples decreased when temperature increased.
648 Pakowski and others (2007) developed sorption isotherms of willow *Salix viminalis* for a
649 range of temperatures up to 85ºC using a water bath and keeping the samples inside a closed
650 container with selected super saturated salt solutions with different relative humidity values in a
651 water bath. This seems like a logical approach, but the challenge is that the relative humidity
652 values of the specific super saturated salt solutions may vary significantly with temperatures. The
653 only way to determine the correct water activity of the saturated slurries at higher temperatures
654 would be to measure the water activity or predict the effect, which comes back to the original
655 challenge of high temperature water activity testing. They also developed sorption isobars of
656 willow *Salix viminalis* in a super heated steam environment. The wood sample was kept at a
657 super heated steam temperature by heating with steam produced by a boiler. The sample weight

658 was monitored at different steam temperatures and the equilibrium water content was
659 determined. Pakowski and others (2011) used an instrumental set up that included a super heated
660 chamber containing the sample. The weight change of the sample is then traced to develop
661 sorption isobars of lignite. Temperatures up to 200°C at pressure values of 0.101, 1.0 and 2.5
662 MPa were used (Pakowski and others 2011). They observed a significant increase in water
663 activity of lignite when temperature was increased, attributed to decreased hygroscopicity of
664 materials at elevated temperatures.

665 A sorption apparatus developed adsorption isotherms of casein, wheat starch, potato starch,
666 apple-pectin, and microcrystalline cellulose were generated at 40, 60 and 80°C (Bandyopadhyay
667 and others 1980). The apparatus consisted of a pre-saturator and saturator that created the desired
668 humidity inside a sorption chamber that could be electrically heated to a desired temperature
669 (Figure 5). After equilibration, the water content of the samples was determined to develop the
670 adsorption isotherms. These selected food components are rich in either carbohydrates or
671 proteins, and their isotherms at elevated temperatures exhibited an increase in a_w with increase in
672 temperature at the same water content, attributed to their lower hygroscopicity at higher
673 temperatures (Bandyopadhyay and others 1980). The water binding capacity of cellulose was
674 reported lowest at all the water activities at the elevated temperatures among the selected food
675 components (Bandyopadhyay and others 1980). In contrast, the a_w of pure sugars such as
676 glucose and fructose decreased as temperature increased from 30 to 80°C, attributed to the
677 structural changes at 80°C, resulting in increased hygroscopicity and solubility (Audu and others
678 1978). This implies that water activity change due to temperature is greatly dependent on the
679 nature of food ingredients. It is also possible that pure components may show a different
680 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 (2013). They used a pressure chamber coupled with an Elevated Pressure High Temperature (EPHT) cylinder to achieve selected sample temperatures and pressures between 70–150ºC and 0–100 % 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.

Authors developed a sealed chamber 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 125ºC was sealed inside the chamber 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/m^3) from the following equation:

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

where T is the temperature in ºC, m_r is the molecular weight of water (kg/mol) = 0.018 and R = 8.314 J/mol. K. The a_w of 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 equation 11.

5. 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 determination (Lenth and Kamke 2001; Pakowski and others). This may be attributed to mass loss at elevated temperatures, chemical modification and the resulting decrease in hygroscopicity (Pearson and others 2013). It is interesting to note that the 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 determination at elevated temperatures analyzed pure water vapor systems with super heat control systems to achieve relative humidity values close to 100% at elevated temperatures (Pearson and others 2013). Condensation can happen 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 2013). Further, the target temperature and humidity should be carefully controlled since small variation in temperature results in large errors in humidity and hence water activity values (Pearson and others 2013). In most of the previous studies, the samples were exposed to elevated temperatures for long periods of time. This could result in chemical modification and structural changes in the samples, affecting their hygroscopicity and other properties. It may also lead to erroneous results during water activity

727 measurement at a given equilibrium moisture content (Pearson and others 2013). The thermal
728 degradation of samples is also reported to be greater when they are exposed to higher relative
729 humidity at elevated temperatures during experiments. Thus, it is desirable to minimize
730 equilibration time at elevated temperatures (Lenth and Kamke 2001).

731

732 **6. Mathematical Modeling of Sorption Isotherms at Elevated Temperatures**

733 At a fixed temperature, a unique relationship exists between water activity and water content for
734 a food system depending on its composition. This relationship is referred as an isotherm. Several
735 empirical and semi-empirical models have been proposed to describe water sorption isotherms at
736 different temperatures for various foods. Semi-empirical models such as Brunauer, Emmett and
737 Teller (BET) or the Guggenheim, Anderson and de Boer (GAB) models could provide
738 theoretical understanding on the influence of temperature on water activity of most foods. GAB
739 equation fitted well with isotherms of variety of foods including fruits, vegetables, meat, milk
740 products, starchy foods, nuts and oilseeds, coffee, tea and spices (Lomauro and others 1985a,
741 1985b). BET and GAB models are based on the assumption of multilayered adsorption with no
742 lateral interactions (Quirijns and others 2005). The BET isotherm predicts water contents of a
743 material with water activity values between 0.05 and 0.45, while GAB model is capable of
744 accurate water content prediction for water activity values up to 0.9 (Rahman 1995). The GAB
745 model is expressed as:

746 $$\frac{X}{X_m} = \frac{CKa_w}{(1 - Ka_w)(1 - Ka_w + CKa_w)} \qquad (14)$$

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 binding of water 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:

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

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

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).

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

Incorporating the temperature dependence of the parameters X_m, C and K to GAB model will allow one to predict the sorption isotherms of foods at elevated temperatures. Several other semi-empirical models such as modified Henderson equation (Henderson 1952), modified Halsey

766 equation (Chirife & Iglesias 1978; Halsey 1948), modified Oswin equation (Chen 1988; Oswin
767 1946), Chung–Pfost equation (Chung & Pfost 1967) were also used to describe sorption
768 phenomena of food materials at elevated temperatures. A detailed review of the models to
769 describe the water sorption phenomena can be found in Quirijns and others (2005). Equations
770 15-17 may not fit well for oil rich foods (such as peanut butter, almond butter, emulsions etc.). In
771 addition, previous research on isotherms are limited to temperatures less than 60°C, because of
772 lack of water activity measurement instruments for higher temperatures. More research is needed
773 to understand the direction of water activity variation and usefulness of abovementioned
774 equations in describing water sorption isotherms of those foods.

775 Extrapolation of current theoretical models that don't consider any state and phase transitions
776 at high temperatures will give inaccurate results (Bassal and others 1993a&b). State and phase
777 transitions (glass transition, vaporization, changes in crystalline form) and physicochemical
778 changes (protein denaturation, lipid oxidation etc.) may affect water-macromolecules interaction
779 and therefore a_w of foods at elevated temperatures (Lenth and Kamke 2001). So any model that
780 intends to predict a_w at elevated temperatures must consider state and phase transitions and
781 physicochemical changes (Bassal and others 1993a). The influence of those changes in the
782 product should be incorporated to the mathematical model to predict the water activity at
783 elevated temperatures which may be often times product specific. So, it is necessary to conduct
784 experiments to determine water activity at elevated temperatures in various food matrices.

785

786 **7. Future Research**

787 **7.1 Effect of Microenvironment on Water Activity Variation at Elevated Temperatures**

788 Water activity changes at elevated temperatures is unique to each food component (Figure 7).
789 Local microenvironments created by multiple components in a food system may influence
790 survival and thermal inactivation of microorganisms during processing and storage, which may
791 be related to the water availability at/during the thermal processing (Hills and others 1996).
792 Water activity and water mobility variation during the thermal processing may be directly
793 associated to the water availability to microorganisms, which is governed by the state of water
794 molecules and their interaction with food macromolecules in specific food systems at selected
795 temperatures. Further studies on macroscopic and molecular aspects of water availability by
796 understanding the water activity and water mobility variation at elevated temperatures used in
797 thermal processing are required.

798 Greater survival of microorganisms have been reported in fat rich foods in comparison to that
799 in carbohydrate or protein rich foods during thermal processing (Senhaji 1977; Li and others
800 2014). There appears to be a direct link between the decrease in water activity during thermal
801 processing and associated increase in the thermal resistance of microorganisms in fat rich foods.
802 Li and others (2014) studied *Salmonella* inactivation in two model systems consisting of peanut
803 butter and nonfat dry milk powder with identical composition. In one system, *Salmonella* was
804 initially inoculated to peanut butter before mixing with nonfat dry milk powder, while in another,
805 *Salmonella* was inoculated in nonfat dry milk powder before mixing with peanut butter. The
806 survival of *Salmonella* was greater in the model system where *Salmonella* was initially in
807 inoculated in peanut butter suggesting that the local microenvironments had an impact on
808 survival (Li and others 2014). It is necessary to understand water redistribution in local
809 microenvironment during thermal processing, which may influence the survival of *Salmonella*.
810 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.

7.2 Development of Equations Relevant to Thermal Processing of 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 target pathogen (or D-values i.e. the time in minutes required to destroy one 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).

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

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

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

Based on Bigelow's thermo-bacteriological approach (1921) (Equation 20), Mafart and Leguerinel (1998) proposed a secondary model (Equation 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).

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

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

833 Villa-Rojas and others (2013) used the following simplified version of the above model
834 (Equation 22) by ignoring the pH component as pH was not changed in the low-moisture
835 products.

836 $$\log\frac{D}{D_{ref}}=\frac{-\left(T-T_{ref}\right)}{z_T}-\frac{\left(a_w-1\right)}{z_{a_w}} \quad (22)$$

837

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

841 $$Z_T=\frac{T_2-T_1}{\log D_1-\log D_2} \quad (23)$$

842

843 $$Z_{a_w}=\frac{a_{w2}-a_{w1}}{\log D_1-\log D_2} \quad (24)$$

844 The relationship between Z_T with water activity of food and Z_{aw} with treatment temperature may
845 established for target microorganism in selected food systems (Figure 8). Mafart and Leguerinel
846 (1998) modified the lethal rate (L) and F-value concept by incorporating the effect of pH as
847 below.

848 $$L=10^{\left[\frac{\left(T-T_{ref}\right)}{z}\right]+\left[\frac{\left(pH-pH_{ref}\right)}{z_{pH}}\right]^2} \quad (25)$$

849 $$F=\int_0^t 10^{\left[\frac{\left(T-T_{ref}\right)}{z}\right]+\left[\frac{\left(pH-pH_{ref}\right)}{z_{pH}}\right]^2}dt \quad (26)$$

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

Conclusions

Recent outbreaks related to low-moisture foods have generated a great interest in research communities and industries in finding solutions to these issues. 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, water mobility, composition of food matrices and characteristics of target microorganism. Previous research presents the importance of water activity, which may be the most important factor influence 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 to be considered to develop protocols and equations relevant to multiple thermal processing technologies. Close collaboration between research institutions and food industry and support from government organizations will ensure positive outcomes to overcome this challenge.

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Figure Titles

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

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

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

Figure 4 General principle of a_w measurement set ups developed in the past

Figure 5 Instrument set up 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

Figure 7 Schematic diagram showing log D against a_w relationship for a pathogen at elevated temperatures. Significant change in a_w can happen at those temperatures compared to the initial a_w depending on the type of food ingredients

Figure 8 Relationship between Z_T with water activity of food and Z_{aw} with treatment temperature of a food system

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1222 Table 1. Selected studies on thermal processing techniques to control pathogens in low-moisture
1223 foods
1224

Authors and year	Processing technique	Processing conditions	Product	Target microorganism	Major results
Fine and Gervais (2005)	Hot air	200-600°C from 0.1-30 s heating followed by instantaneous cooling at -80°C	Wheat flour	*Bacillus subtilis* spores and *Saccharomyces cerevisiae* cells	• 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	Fusarium mycotoxin deoxynivalenol (DON) and *Geobacillus (Bacillus) stearothermophilus* ATCC 10149	• The DON concentration reduction was up to 52% at 185°C for 6 min processing with superheated steam • The D-values of *G. stearothermophilus* spores processed with superheated steam ranged from 2.2-23.5 min for 105-175°C • The Z-value for *G. stearothermophilus* spores exposed to superheated steam at 130–175°C was determined to be 28.4°C.
Brandl and others (2008)	Infrared heating (3000-5458 W/m^2)	90-113°C for 30-45 sec	Almond kernels	*S.* Enteritidis	• Infrared heating with immediate cooling of the product resulted in 0.63-1.51 log reductions in microbial population • IR treatment followed by holding of the kernels at warm temperature for 60 mm, resulted in more than 7.5-log reduction in *S.* Enteritidis 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 sec, 143 kPa	Almonds	*S.* Enteritidis	• Steam pasteurization achieved 5-log reduction of *S.* Enteritidis with 25 s without deteriorating the

					visual quality of almonds
Jeong and others (2011)	Moist-air convection heating	121-204°C, 5-90% relative humidity, for 72-6344 s	Almonds	*E. faecium* strain NRRL B-2354 and *Salmonella* Enteritidis PT30	• The mean log reductions for E. faecium were 0.6 log and 1.4 log lower than those for *S.enterica* 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 30-300 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 • The 300-s treatment was able to diminish the population of *L.* • *monocytogenes* 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)	77-86°C for maximum 90 sec	Peanut butter cracker sandwich	*S.* Typhimurium and *E.* Coli O157:H7	• RF treatment resulted in 4.29 log reductions of *S.* Typhimurium and 4.39 log reductions in *E. coli* O157:H7 population, respectively in creamy peanut butter cracker sandwich • RF treatment resulted in 4.55 log reductions of *S.* Typhimurium and 5.32 log reductions in *E. coli* 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 sec	Dried red and black pepper powder	*S*. Typhimurium and *E*. Coli O157:H7	• 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

Table 2. Thermal resistance of microorganisms in selected low-moisture foods influenced by water activity

Authors and year	**Title**	**Product**	**Target microorganism**	**Water activity and temperature range**	**Major results Thermal resistance data (D- and z values)**
Murrel and Scott (1966)	The heat resistance of bacterial spores at various water activities	Selected growth medium	*Bacillus megaterium*, *B. stearothermophilus* ATCC 7953, *Clostridium botulinum* type E ATCC 9564, *C. botulinum* type B ATCC 7949, C. *bifegermentans, B. coagulans*	0.11-0.80 70-120°C	• Heat resistance was maximum at a_w values of about 0.2-0.4, the maximum *D* values at 110°C varying from about 2 to 24 hr. • 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	Selected *Salmonella* and *E. coli* strains	0.87-0.99 using sucrose and 0.75, 0.90 and 0.99 a_w by glycerol 57°C	• Heat resistance of the organisms increased as the a_w of the heating menstruum was reduced. • The type of solute

		glycerol added to change a_w			used to control a_w affected the thermal resistance of selected microorganisms
Sumner and others (1991)	Heat resistance of *Salmonella typhimurium* and *Listeria monocytogenes* in sucrose solution of various water activities	Sucrose solution	*S.* Typhimurium and *L. monocytogenes*	0.83-0.98 Five temperatures ranging from 65.6 to 76.7°C for *S. typhimurium* and four temperatures ranging from 60 to 68.3°C for *L. monocytogenes*	• Heat resistance of selected microorganisms increased with decrease in aw • The decrease in D-values of *Salmonella* was greater than that of *Listeria*
Mattick and others (2001)	Effect of Challenge Temperature and Solute Type on Heat Tolerance of *Salmonella* Serovars at Low Water Activity	Tryptone soy broth	*Salmonella* Typhimurium DT104	0.65-0.90 55-80°C	Salmonella was heat sensitive at aw of 0.65 when the temperature was low (55-60°C) while reverse was observed when temperature was higher than 60°C
Beuchat and	Combined effects of water activity, temperature and chemical treatments on the survival of Salmonella and Escherichia coli O157:H7 on alfalfa seeds	Alfalfa seeds	*Salmonella* and *E.coli* O157:H7	0.15-0.54 50, 60, 70 and 80°C	• The rate of inactivation of *Salmonella* and *E. coli* 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	*Saccharomyces cerevisiae* and *Lactobacillus plantarum*	Initial a_w of 0.1 to 0.7 and a_w was monitored during drying 150 and 200°C	• 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 values of 0.2-0.5 and 0.3-0.5, respectively in skim milk powder.
Villa-Rojas and others (2013).	Thermal Inactivation of Salmonella Enteritidis PT 30 in Almond Kernels as Influenced by	Almond kernels	*Salmonella* 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	• A nonlinear thermal inactivation behavior of *Salmonella* was observed • Small increase in a_w reduced the thermal inactivation time at a

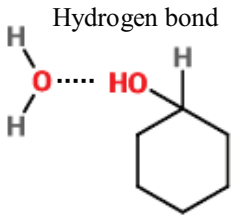
	Water Activity			68°C at a_w of 0.888 56, 60, 64, and 68°C at an aw of 0.946	specific treatment temperature meaning higher thermal resistance of Salmonella at lower a_w
Farakos and others (2013)	Modeling the influence of temperature, water activity and water mobility on the persistence of Salmonella in low-moisture foods	Whey protein powder	*Salmonella* Typhimurium, *Salmonella* Tennessee, *Salmonella* Agona, and *Salmonella* Montevideo	0.19 and 0.54 21, 36, 50, 60, 70 and 80°C	Survival of *Salmonella* serovars were significantly influenced by a_w with increase in survival with decrease in a_w
He and others (2013)	Increased Water Activity Reduces the Thermal Resistance of *Salmonella enterica* in Peanut Butter	Peanut butter	*Salmonella* Typhimurium, *Salmonella* Tennessee, *Salmonella* Enteritidis Three-serotype cocktail	0.2, 0.4, 0.6, and 0.8 90 and 126°C	• Water activity increase significantly reduced the thermal resistance of selected microorganisms at 90°C • The difference in thermal resistance values of selected microorganisms was less at 126°C compared to that at 90°C

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	• 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: 100-140°C		• Desorption isotherms and isobars of the selected products were determined • The influence of temperature on the sorption isotherm is small at elevated temperature

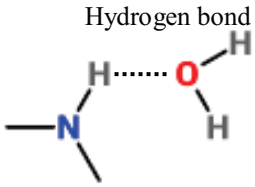
Kubojima and others (2003)	Moisture content of green wood in high temperature water vapor	Green sitka spruce (wood) Temperature range: 105-160°C	Changing the relative humidity by varying the pressure inside a hermetically sealed pressure chamber containing superheated steam and measuring the weight of the sample at equilibrium for water content determination	• Temperature-Relative humidity-Pressure-Equilibrium moisture content values in the range of 105-160°C, 75-99% RH and 0.02-0.39 MPa were determined experimentally
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
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 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 Dent model was used to simulate the experimental sorption data • The desorption isotherms at 160°C were significantly lower than the those at 50°C denoting reduced a_w at higher temperature • The results shows that it would be inaccurate to just extrapolate a_w values at elevated temperatures from low temperature data • There was a change in sorption properties of wood when the temperature and moisture were above glass transition temperature of lignin
Pearson and others (2013)	Equilibrium moisture content of radiata pine at elevated temperature and pressure reveals measurement challenges	Radiata pine (*Pinus radiata* D. Don)		

A. Carbohydrate-water interaction



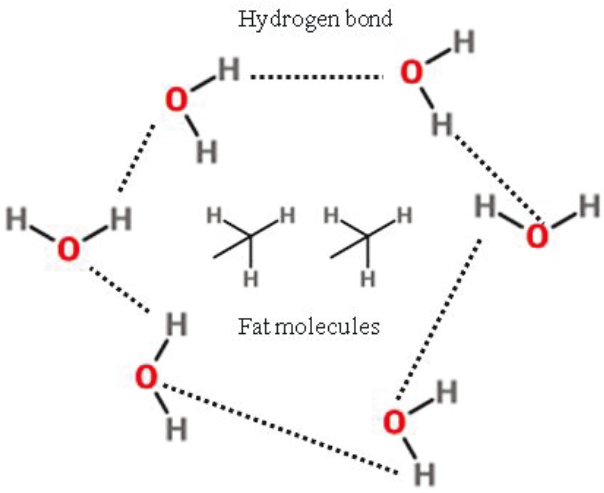
Carbohydrate molecule

B. Protein-water interaction



Protein molecule

C. Fat-water interaction



Elevated temperatures may disrupt the hydrogen bonds between water and hydrophilic molecules such as (A) carbohydrates and (B) proteins (hydrophilic side), resulting in more number of free water molecules which may increase the water activity (C) Hydrophobic interaction between water and fat. Water molecules aggregate together while fat molecules are combined at the center forming a cage like structure. It is hypothesized that greater interaction between water and fat molecules may happen at elevated temperatures due to their higher solubility in water, resulting in a decrease in water activity

Figure 1

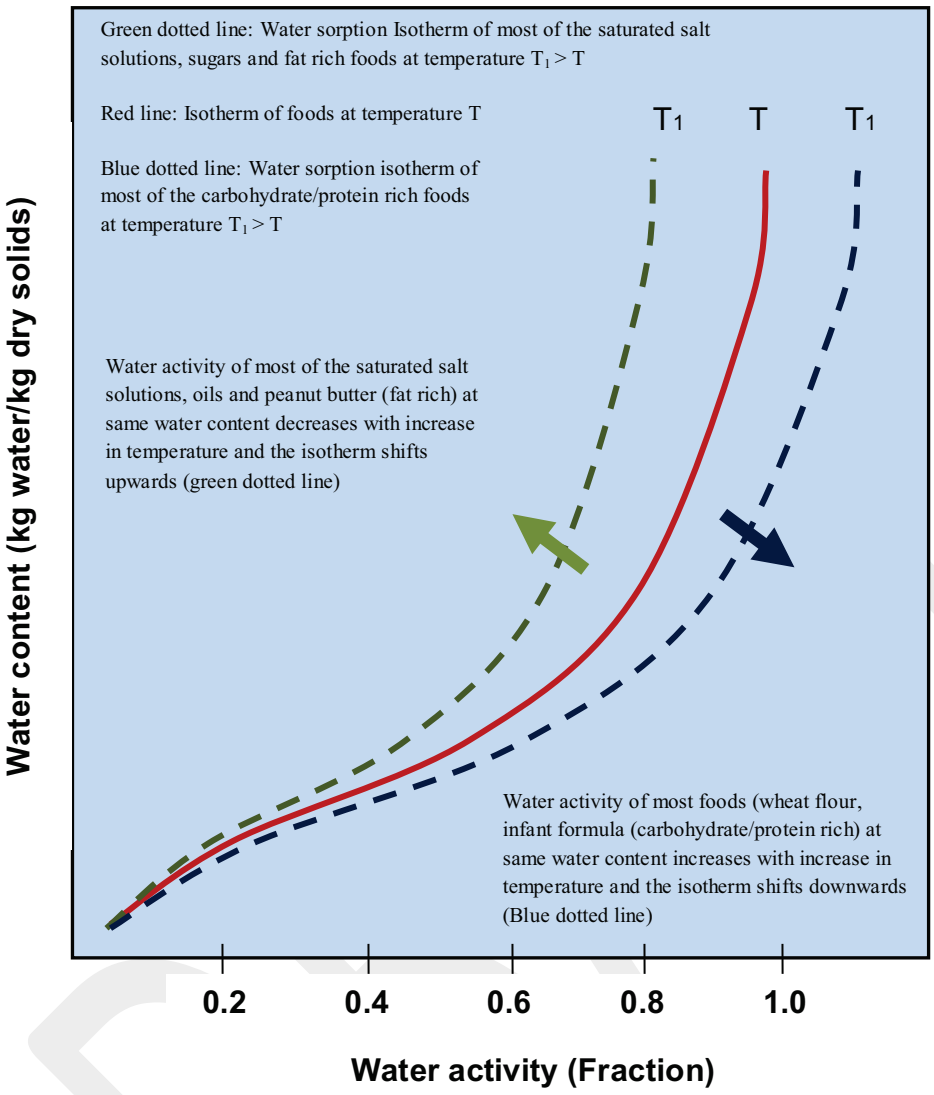

Green dotted line: Water sorption Isotherm of most of the saturated salt solutions, sugars and fat rich foods at temperature $T_1 > T$
Red line: Isotherm of foods at temperature T
Blue dotted line: Water sorption isotherm of most of the carbohydrate/protein rich foods at temperature $T_1 > T$
T_1
T
T_1
Water activity of most of the saturated salt solutions, oils and peanut butter (fat rich) at same water content decreases with increase in temperature and the isotherm shifts upwards (green dotted line)
Water activity of most foods (wheat flour, infant formula (carbohydrate/protein rich) at same water content increases with increase in temperature and the isotherm shifts downwards (Blue dotted line)
Water content (kg water/kg dry solids)
0.2
0.4
0.6
0.8
1.0
Water activity (Fraction)

Figure 2

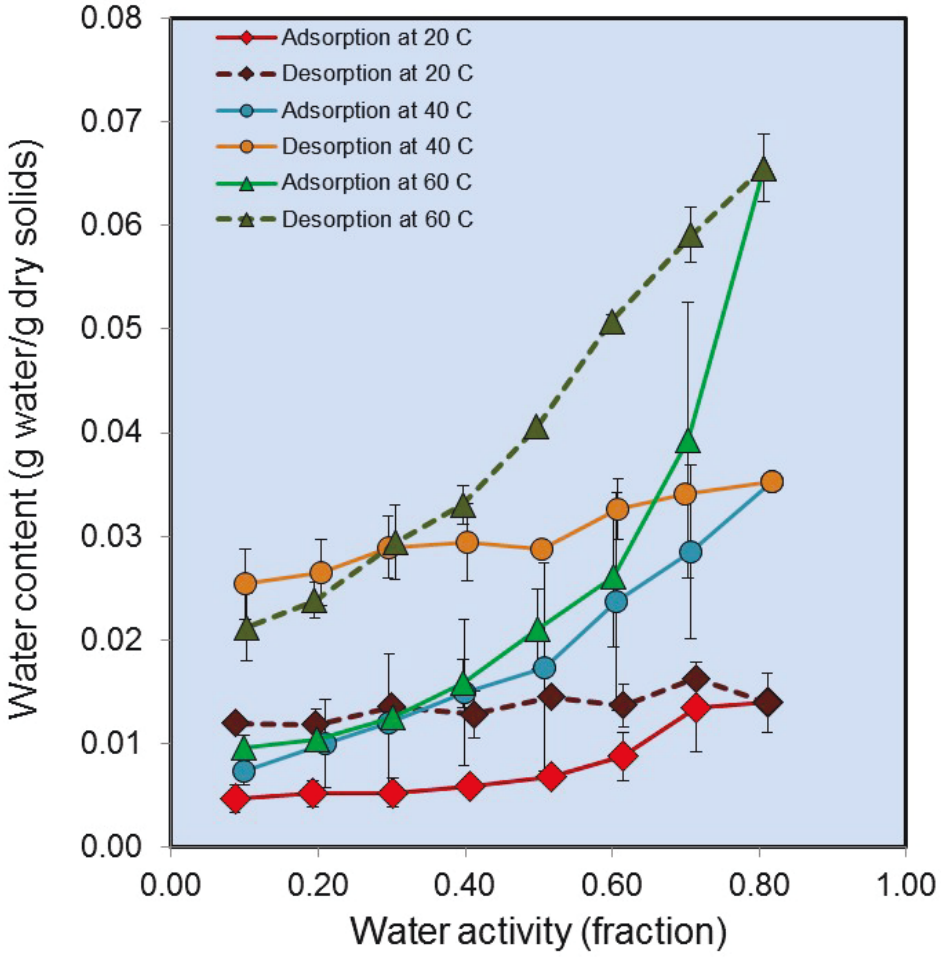
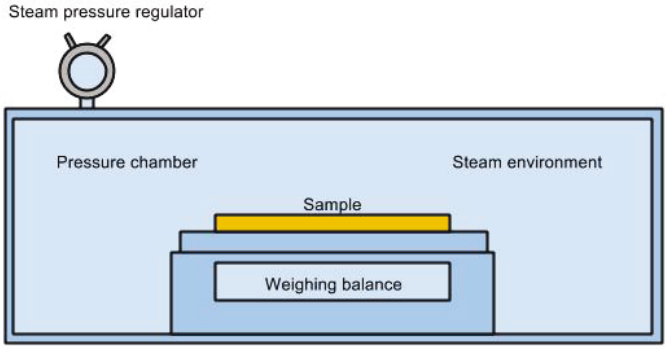
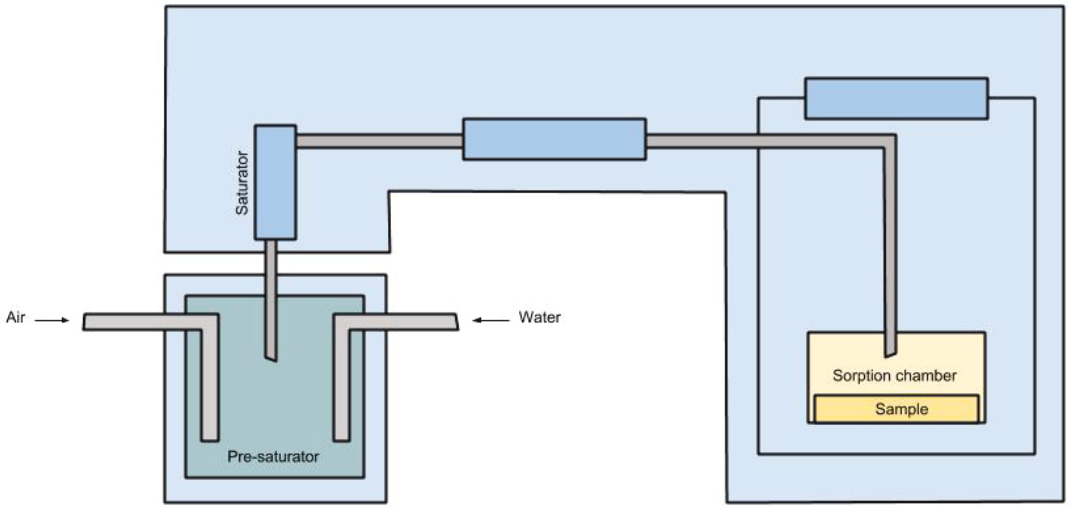

Adsorption at 20 C
Desorption at 20 C
Adsorption at 40 C
Desorption at 40 C
Adsorption at 60 C
Desorption at 60 C
Water content (g water/g dry solids)
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.00
0.20
0.40
0.60
0.80
1.00
Water activity (fraction)

Figure 3



A pressure chamber where pure steam is produced at desired temperatures. Desired relative humidity inside the chamber is obtained by changing the pressure levels inside the chamber. The water content of the sample at equilibrium was determined by monitoring the weight changes using a balance. Many of the previous studies reported similar instrumental set up to determine water activity at elevated temperatures (Bassal and others 1993; Bjork and Rasmuson, 1995).

Figure 4



The pre-saturator and saturator were used to obtain desired relative humidity inside the sorption chamber. The sorption chamber is heated electrically to achieve the desired temperature. The water content of the sample is determined after equilibration (Modified from Bandyopadhyay and others 1980)

Figure 5

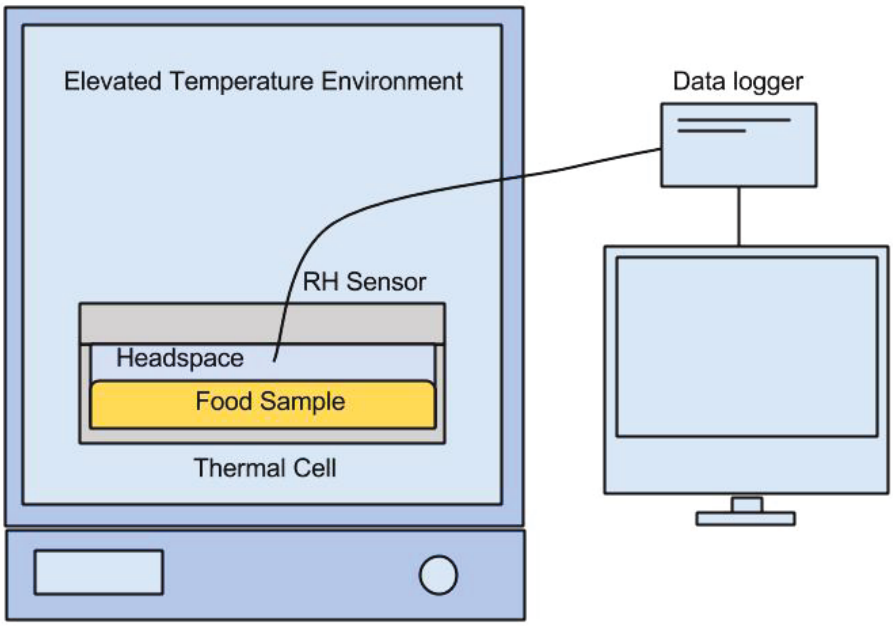

Elevated Temperature Environment
Data logger
RH Sensor
Headspace
Food Sample
Thermal Cell

Figure 6

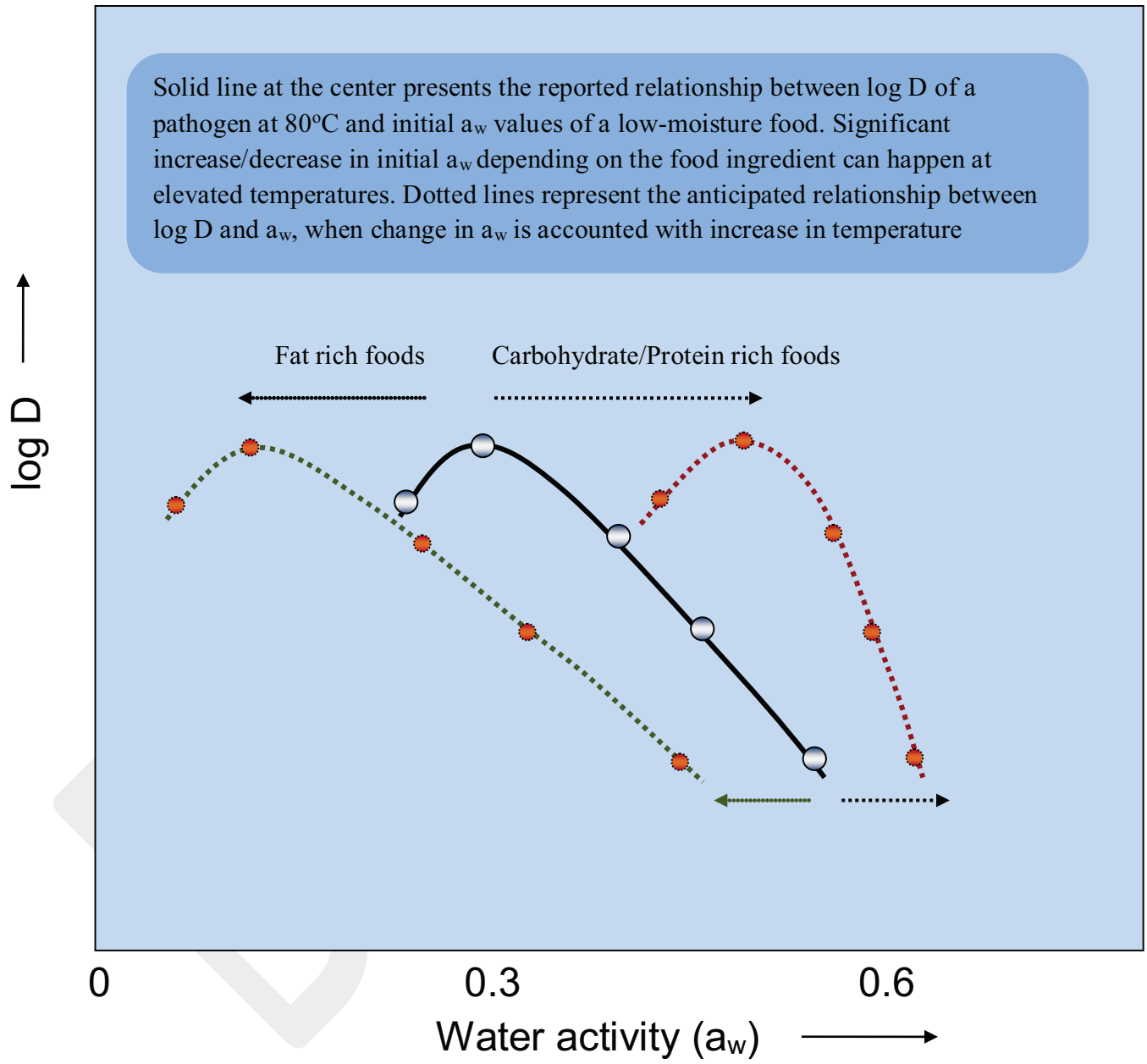

Solid line at the center presents the reported relationship between log D of a pathogen at 80°C and initial a_w values of a low-moisture food. Significant increase/decrease in initial a_w depending on the food ingredient can happen at elevated temperatures. Dotted lines represent the anticipated relationship between log D and a_w, when change in a_w is accounted with increase in temperature
Fat rich foods
Carbohydrate/Protein rich foods
log D
0
0.3
0.6
Water activity (a_w)

Figure 7

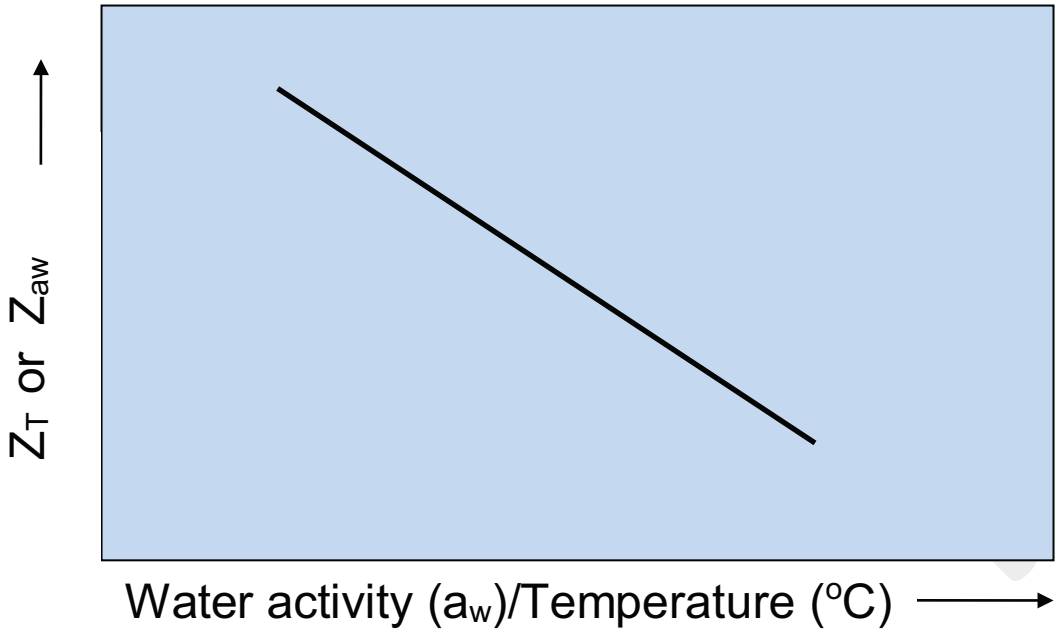


Figure 8