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Influence of water activity on inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* in peanut butter by microwave heating

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ABSTRACT

This study evaluated the efficacy of a 915 MHz microwave with 3 different electric power levels to inactivate three pathogens in peanut butter with different a_w. Peanut butter inoculated with *Escherichia coli* O157:H7, *Salmonella enterica* serovar Typhimurium and *Listeria monocytogenes* (0.3, 0.4, and 0.5 a_w) were treated with a 915 MHz microwave with 2, 4, and 6 kW for up to 5 min. Six kW 915 MHz microwave treatment for 5 min reduced these three pathogens by 1.97 to >5.17 log CFU/g. Four kW 915 MHz microwave processing for 5 min reduced these pathogens by 0.41–1.98 log CFU/g. Two kW microwave heating did not inactivate pathogens in peanut butter. Weibull and Log-Linear + Shoulder models were used to describe the survival curves of three pathogens because they exhibited shouldering behavior. T_d and T_{5d} values were calculated based on the Weibull and Log-Linear + Shoulder models. T_d values of the three pathogens were similar to D-values of *Salmonella* subjected to conventional heating at 90 °C but T_{5d} values were much shorter than those of conventional heating at 90 °C. Generally, increased a_w resulted in shorter T_{5d} values of pathogens, but not shorter T_d values. The results of this study can be used to optimize microwave heating pasteurization system of peanut butter.

1. Introduction

Escherichia coli O157:H7 is an increasingly common cause of illness, including bloody diarrhea and hemolytic uremic syndrome (Besser et al., 1999). *Salmonella enterica* serovar Typhimurium is the most commonly isolated *Salmonella* serotype and causes non-typhoidal salmonellosis which has a symptom of self-limiting gastroenteritis (Boyle et al., 2007). *Listeria monocytogenes* is a one of the most widespread gram-positive bacteria which causes abortion, stillbirth, neonatal sepsis, meningitis, sepsis, and gastroenteritis (Salamina et al., 1996). Robertson et al. (2016) reported that there were 163 outbreaks from 2007 to 2012, in the United States, comprising 89 *E. coli*, 56 *Salmonella*, and 11 *L. monocytogenes* cases. The 4132 cases of illnesses were reported due to 163 outbreaks, *Salmonella* comprised 68%, STEC comprised 26% of illnesses, and *L. monocytogenes* showed the highest fatality (38%). Low a_w foods have usually been considered safe regarding foodborne pathogens because the optimum a_w for growth of these pathogens is over 0.95. But, unfortunately, there have been several outbreaks due to low a_w foods contaminated with *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*. In 1998, there was an outbreak caused by *E. coli* O157:H7 in southern Ontario, Canada. The major source of contamination was dry fermented Genoa salami (Williams et al., 2000). There was a large outbreak in Norway and Finland due to *S.* Typhimurium-contaminated chocolate in 1987. Because of this outbreak 361 people were infected with *S.* Typhimurium and many young children developed acute hemorrhagic diarrhea (Kapperud et al., 1990). Also, a *L. monocytogenes* infection outbreak in Finland was traced to contaminated butter (Lyytikäinen et al., 2000).

Peanut butter has low a_w which disrupts the growth of foodborne pathogens, and generally, is pasteurized by conventional

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heating at 70–75 °C (Ha et al., 2013). But, unfortunately, multistate outbreaks caused by peanut butter have been reported. In 2007 in the USA, there was a large outbreak of salmonellosis due to consumption of *Salmonella enterica* serovar Tennessee-contaminated peanut butter. Because of this outbreak, 425 people were infected with *S.* Tennessee and 71 people were hospitalized (CDC, 2007). In 2008–2009, a multistate outbreak of *S.* Typhimurium infections linked to peanut butter occurred. This outbreak affected 714 people from 46 states of the USA; 24% of whom required hospitalization and resulted in 9 deaths (CDC, 2010). Also, in 2012 in the USA, 42 cases of *Salmonella* Bredeney infections linked to peanut butter consumption were reported (CDC, 2012). And also, there have been several studies which confirmed that conventional heating is not sufficient to inactivate *Salmonella* in peanut butter. Ma et al. (2009) reported that conventional thermal treatment at 71 °C for 50 min reduced *Salmonella* Tennessee by just 2 log CFU/g. He et al. (2011) also reported that thermal treatment at 72 °C for 60 min reduced *Salmonella enterica* by less than 2 log CFU/g in peanut butter with 0.4 a_w.

Microwave heating is a form of dielectric heating which is used industrially for the processing of food and also used domestically for cooking or thawing of food. Microwave irradiation produces efficient volumetric heating by utilizing the ability of microwave which can penetrate the material directly without any need of intermediate heat transfer medium (Zhu et al., 2007). Microwave heating is greatly affected by water in food because of the dipolar nature of water. When an electric field is applied to water, the dipolar water molecules try to realign in the direction of the electric field. This million times per second realignment due to the high frequency of microwaves cause internal friction of water molecules resulting in the volumetric heating of food. Because of this reason, microbial inactivation of food by microwave heating is focused on foods which have moisture contents higher than 50%, such as, milk (Choi et al., 1993), juice (Cañumir et al., 2002), meat (Shamis et al., 2008) and poultry (Pucciarelli and Benassi, 2005).

Water is one of the key factors which control the effect of microwave heating. And also, the a_w of food affects the heat resistance of microorganisms in food. Goepfert et al. (1970) reported that reduced a_w increased the D-value of salmonellae. But there has been no study investigating the effect of a_w of foods on microwave heating and inactivation of pathogens in foods by microwave heating treatment. Recently, we reported that microwave heating is effective for the pasteurization of peanut butter (Song and Kang, 2016). Therefore, in this study we evaluated the effect of a_w on inactivation of three foodborne pathogens (*E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*) in peanut butter by microwave heating and obtained the inactivation kinetics of the three pathogens.

2. Materials and methods

2.1. Bacterial strains and cell suspension

Strains of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890), *S.* Typhimurium (ATCC 19585, ATCC 19115, DT 104), and *L. monocytogenes* (ATCC 15313, ATCC 19111, ATCC 19115) were obtained from the bacteria culture collection of Seoul National University (Seoul, Republic of Korea) for this study. Stock cultures were kept frozen at −80 °C in 0.7 ml of Tryptic Soy Broth (TSB; Difco, BD, Sparks, MD) and 0.3 ml of sterile 50% (V/V) glycerol. Working cultures were streaked onto Tryptic Soy Agar (TSA; Difco, BD), incubated at 37 °C for 24 h and stored at 4 °C. Each strain of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* was cultured in 5 ml TSB at 37 °C for 24 h, harvested by centrifugation at 4000g for 20 min at 4 °C and washed three times with sterile distilled water. The final pellets were resuspended in sterile distilled water, corresponding to approximately 10^8–10^9 CFU/ml. Mixed culture cocktails were prepared by blending together equal volumes of each test strain.

2.2. Sample preparation and inoculation

Experiments were performed using commercially processed creamy peanut butter. The peanut butter used for this study was purchased at a local grocery store (Seoul, Republic of Korea) and stored at room temperature (22 ± 1 °C). Twenty-five g of peanut butter samples were aseptically placed in sterile 100 ml Pyrex beakers. For inoculation, 0.2 ml of culture was inoculated into the sample and thoroughly mixed for 1 min with a sterile spoon to ensure even distribution of the pathogens. A_w of inoculated peanut butter was 0.30. To increase peanut butter a_w, a select volume of sterile distilled water was mixed into the inoculated peanut butter samples to adjust the a_w to 0.4 and 0.5. Generally peanut butter has water activity range from 0.20 to 0.33 (Burnett et al., 2000). But some studies used peanut butter with a_w 0.4 to 0.5 (Ha et al., 2013; Ma et al., 2009; Shachar and Yaron, 2006) so we used these a_w (0.3, 0.4, and 0.5). Uniform distribution of inoculum was confirmed by similar log CFU counts (log 5–6 CFU/g) obtained from 1 g subsamples of inoculated peanut butter taken from three randomly selected locations and appropriate tenfold serial dilutions (method described in section 2.4 *Bacterial enumeration*) spread-plated onto Sorbitol MacConkey agar (SMAC; Difco), Xylose Lysine Desoxycholate agar (XLD; Difco), and Oxford Agar Base with antimicrobial supplement (OAB; MB Cell) for enumeration of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. After inoculation, we removed excess peanut butter to obtain 25 g of inoculated sample because total sample weight was increased due to inoculation and adjustment of a_w. Water activity of inoculated peanut butter was measured with an Aqualab model 4TE a_w meter (Decagon Devices, Pullman, WA).

2.3. Microwave heating treatment

Microwave treatment was performed in a previously described apparatus (Sung and Kang, 2014). The beaker containing 25 g of peanut butter sample was located at the center of the turntable. For temperature measurements, the geometric center temperature of a non-inoculated sample adjusted to 0.3, 0.4, and 0.5 a_w in a beaker was measured by a fiber optic sensor (FOT-L; FISO Technologies Inc., Quebec, Canada) connected to a signal conditioner (TMI-4; FISO Technologies Inc., Quebec, Canada). For the inactivation study, 25 g of peanut butter with different a_w was placed in a 100 ml Pyrex beaker. An inoculated sample-filled beaker was subjected to microwave heating at 3 different power levels (2, 4, and 6 kW) for up to 5 min.

2.4. Bacterial enumeration

After microwave heating treatment, 25 g of sample was mixed with 25 ml of 0.2% peptone water (PW). Then, the sample and 0.2% PW mixture was diluted in 200 ml of sterile 0.2% PW and homogenized for 2 min in a stomacher (EASY MIX, AES Chemunex, Rennes, France). After homogenization, 1 ml aliquots of homogenized samples were 10-fold serially diluted in 9 ml of sterile 0.2% PW, and 0.1 ml of sample or diluent was spread-plated onto SMAC, XLD, and OAB for enumeration of the three pathogens. Where low populations of surviving cells were anticipated, 1 ml aliquots of the original homogenate were equally distributed between four plates and spread-plated. All plates were incubated at 37 °C for 24 h and colonies were counted.