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Survival of *Salmonella* and *Enterococcus faecium* in high fructose corn syrup and honey at room temperature (22 °C)

Jaza Alshammari [a,1], Nitin Dhowlaghar [b,1], Yucen Xie [a], Jie Xu [a,c], Juming Tang [a,*], Shyam Sablani [a], Mei-Jun Zhu [b,**]

[a] Department of Biological Systems Engineering,Washington State University, Pullman, WA, USA
[b] School of Food Science, Washington State University, Pullman, WA, USA
[c] Center for Nanotechnology and Nanotoxicology, Harvard T. H. Chan School of Public Health, Harvard University, 665 Huntington Avenue, Boston, MA, 02115, USA

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ABSTRACT

Salmonellosis has been frequently associated with the consumption of high-sugar, low-moisture foods. Honey and high fructose corn syrup (HFCS) are widely used liquid sugars that are added as humectants in low-moisture foods. The objective of this study was to determine the ability of *Salmonella* and its presumable surrogate, *Enterococcus faecium* NRRL B-2354, to survive in honey and HFCS during storage at room temperature (~22 °C). Using freeze-dried and lawn grown bacteria, the survival of *Salmonella* and *E. faecium* in honey and HFCS was determined. Regardless of the inoculation methods, more than 5 $\log_{10}$ reductions were observed in both bacteria in honey and HFCS after 21-days of storage at 22 °C. The pathogens and surrogate in honey and HFCS fell below the detectable limit after 28-days of storage. Thus, the tested bacteria are not able to survive in honey and HFCS over one-month storage at room temperature. The similar level of bacterial reduction in honey and HFCS in storage suggests that the main cause was not the commonly perceived antimicrobial agents in honey. In addition to high acidity of these liquid sugars, the extremely high osmotic pressure is likely the main reason for bacterial death in honey and HFCS during storage. The data provided useful information in developing effective microbial-safe strategies to be incorporated in the preparation or storage of low-moisture food and ingredients.

1. Introduction

Foodborne disease is one of the biggest public health concerns globally. In the United States, *Salmonella* causes around 11% of foodborne diseases annually (Scallan et al., 2011). *Salmonella* outbreaks were frequently associated with high-moisture poultry and meats, such as eggs, poultry and beef (CDC, 2018, 2020; Tauxe, 1991), as well as vegetables and fruits, such as onions and peaches (CDC, 2020a & b). Additionally, there are increasing outbreaks caused by *Salmonella* associated with low-moisture foods, such as spices, nuts, cereal, coconut, peanut butter, milk powder, and dried fruits (CDC, 2008, 2009, 2016). In a low water activity (a_w) environment, *Salmonella* cannot grow or multiply, but it can survive for a long time and cause safety issues for human beings. For example, *Salmonella* were detected in dried fruits, including dried cranberries, raisins, and strawberries after 42 days; and in date paste after 126 days of storage under ambient conditions (Beuchat & Mann, 2014; Podolak, Enache, Stone, Black, & Elliott, 2010).

Low-moisture food products containing high concentrations of sugar have also been associated with salmonellosis outbreaks. These foods include chocolate bars (60% sugar content) (Eun et al., 2019; Werber et al., 2005), halva and high sugar, sesame seed-based product (Brockmann, 2001; De Jong et al., 2001), and honey smacks cereal (30–50% of sugar content) (USDA-FDA, 2019). For some *Salmonella* serotypes such as *Salmonella* serovars Eastbourne, Napoli and Typhimurium isolated from chocolate, a very low infection dose ($<10^1$–10^2 CFU/g) of bacteria counts in low-moisture products was enough to cause salmonellosis infections (EU, 2014).

Liquid sugars such as high fructose corn syrup (HFCS) and honey are added as an ingredient for sugars in most of the low-moisture foods. HFCS is a fructose-glucose liquid sweetener which is used as an alternative to sucrose, due to its low cost and desired physical and functional attributes to food and beverage applications, including sweetness, flavor

* Corresponding author. Biological Systems Engineering, Washington State University,P.O Box 646120, Pullman, WA, 99164-6120, USA.
** Corresponding author. School of Food Science, Washington State University, P.O Box 646376, Pullman, WA, 99164-6120, USA.
E-mail addresses: jtang@wsu.edu, jtang@mail.wsu.edu (J. Tang), meijun.zhu@wsu.edu (M.-J. Zhu).
[1] Contributed equally.

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enhancement, color and flavor development, and osmotic stability (White, 2014). HFCS is as generally recognized as safe (GRAS), primarily due to the addition of enzymes during preparation that were affirmed as GRAS (FDA, 2017) . The sugar composition (i.e. glucose to fructose ratio) is nearly the same as that of honey, invert sugar, or sucrose which were previously declared as GRAS (Stavanja et al., 2006; USFDA, 1996). HFCS is used extensively in baked goods, canned fruits, jams and jellies, chocolate syrups and many other processed foods (Hanover & White, 1993).

Honey, a naturally sweet substance, is consumed as a healthy food ingredient and applied toward the treatment of a broad spectrum of diseases (Ajibola, Chamunorwa, & Erlwanger, 2012). Honey is known to be a complex product with its main ingredients being fructose and glucose and its minor components including vitamins, minerals, amino acids, organic acids, enzymes, and polyphenols (Ćelechovská & Vorlova, 2001). Honey is known for its antimicrobial activities against various types of bacteria (Rao, Krishnan, Salleh, & Gan, 2016; Snowdon & Cliver, 1996). It is also well known that yeasts and bacterial spores survive in honey and are able to withstand the acidity and concentrated sugar (Snowdon & Cliver, 1996). Those contaminants may come from primary sources such as pollen, honeybees' digestive tracts, dirt, dust, air, and flowers, or secondary sources of contamination, such as humans, equipment, and containers (Olaitan, Adeleke, & Ola, 2007). Currently, information on the survival of *Salmonella* in HFCS and honey alone are limited. Therefore, this study aimed to (1) determine the survival of *Salmonella* and *Enterococcus faecium* NRRL B-2354 in HFCS and honey stored at ambient temperature (~22 °C), (2) verify the *E. faecium* is an appropriate surrogate strain of *Salmonella* in these liquid sugars, and (3) study the influence of osmotic pressure on survivability of *Salmonella* and *E. faecium*.

2. Materials and methods

2.1. Sample preparation

Organic raw honey (Great Value Brand) was purchased from a local Walmart store (Pullman, WA). High fructose corn syrup (HFCS-55) containing 55% fructose & 45% glucose was obtained from Golden barrel (Honey brook, PA). The a_w of honey and syrup was measured at 23 °C with an Aquameter (Aqualab Series 3, Decagon Devices, Inc., Pullman, WA). The moisture content of samples was measured on wet basis according to an AOAC method, using a gravimetric method with an ADP-31 vacuum oven (Yamato 116 Scientific, Inc., Santa Clara, CA) set at 70 °C with a vacuum pressure of 0.08 MPa for 24 h. The pH, moisture content, density, and sugar content (on wet basis) were determined according to the International Honey Commission (Stefan, 1984). The viscosity of the samples was measured using a Discovery Hybrid Rheometer HR-3 (159 Lukens Drive, New Castle, DE). All samples were measured in triplicate.

2.2. Determination of osmotic pressure of HFCS and honey

Osmotic pressure is the hydrostatic pressure exterted across a semi-permiable membrane due to osmosis. The osmotic pressure in a solution of low solute concentration can be determined using Eq. (1) (Foster & Spector, 1995; Spector & Kenyon, 2012):

$$\pi = iMRT \quad (1)$$

where,

π = Osmotic pressure (atm)
i Van't Hoff's factor (this is the number of ions that will form when a solute is dissolved in water).
M = Osmolarity or osmotic concentration (mol/L)
R = Gas constant (0.08206 L atm. mol^{-1}. K^{-1})
T = Temperature in Kelvin (K).

Osmolarity is the number of osmoles of solute per liter of solution. It is expressed as mOsmol/L (Erstad, 2003). Osmolarity was determined either experimentally or calculated by using Eq. (2) as described below.

$$M = \frac{n}{V} \quad (2)$$

where,

M = Osmolarity (mol/L)
n = number of moles of solute (mole)
v = volume of the solution in liters

Table 2 lists: (a) Density (g/L), (b) Concentration of solute (g), (c) Estimated molecular weight (g/mole), (d) Calculated volume of solution (v), (e) Calculated number of moles of solute (n), and (f) Calculated molarity for 100% honey and HFCS.

The experimental osmolarity was determined using the osmometer analyzer (Osmette S model 4002 Precision Systems, INC, Natick, MA) (Ali, Alqarni, Owayss, Hassan, & Smith, 2017; Erstad, 2003). Due to the narrow measurement range (0–2.000 mOsmol/L) of this instrument and high osmolarities of honey and HFCS, samples were diluted with distilled water to 5, 10, 15, 20, 25, and 30%. The diluted samples were then used to measure the osmolarity. A correlation between sample concentrations and the measured osmolarities was developed, and the osmolarity for 100% honey and HFCS were extrapolated (Fig. 1). Finally, the osmotic pressure was determined using Van't Hoff's equation (Eq. (1)).

2.3. Bacterial strains

Three *Salmonella* strains, (*S.* Enteritidis PT30, *S.* Tennessee K4643, & *S.* Agona 447967), were used in this study to prepare a three-strain cocktail. *S.* Enteritidis PT30 was obtained from Dr. Linda Harris (University of California, Davis). *S.* Tennessee K4643 and *S.* Agona 447967 were kindly gifted by Dr. Nathan Anderson (USDA, Greater Chicago, Illinois). *E. faecium* NRRL B-2354 strain was obtained from the USDA Agricultural Research Service (USDA-ARS) from Peoria, Illinois. All the strains were stored in a stock solution of trypticase soy broth supplemented with 0.6% (w/v) yeast extract (TSBYE) (Hardy Diagnostics, Santa Maria, CA) and 20% glycerol at −80 °C until use.

2.4. Culture and inoculum preparation

2.4.1. Dry inoculation method

For dry inoculation method, a loop of culture stock of each *Salmonella* strain and *E. faecium* was transferred to 9 mL of TSBYE and incubated at 37 °C for 24 h. Three mL of each culture were transferred to 30 mL of TSBYE and incubated at 37 °C for 24 h. Then, 4 mL of these previous cultures were transferred to 400 mL of TSBYE in a conical flask and placed in an incubated shaker (Thermo Scientific ™ MaxQ4000 Benchtop Orbital Shakers, Marietta, OH, USA) with a constant shaking speed at 230 rpm at 37 °C for 24 h. The cultured bacteria was transferred to centrifuge tubes and then centrifuged at 6000×*g* for 15 min at 4 °C (Centrifuge 5810 R®, Eppendorf North America, Hauppauge, NY). The pellets were washed three times and re-suspended in 2.0 mL of sterilized distilled water before use. For freeze-drying, a 250 μL sample of the prepared suspension was transferred into a sterile 1.5 mL Snaplock Microtube, pre-frozen immediately in liquid nitrogen and transferred to a freeze-drying system (Labconco Corporation, Kansas City, MO, USA) where it was frozen dried at −90 °C for 48 h (Xu et al., 2018).

Liquid honey at room temperature was highly viscous. It was difficult to thoroughly mix bacterial culture with honey samples. Thus, before inoculation, 20 g of liquid honey was transferred to 50 mL beaker, heated at 45 °C for 5 min in a convection oven (Yamato Scientific

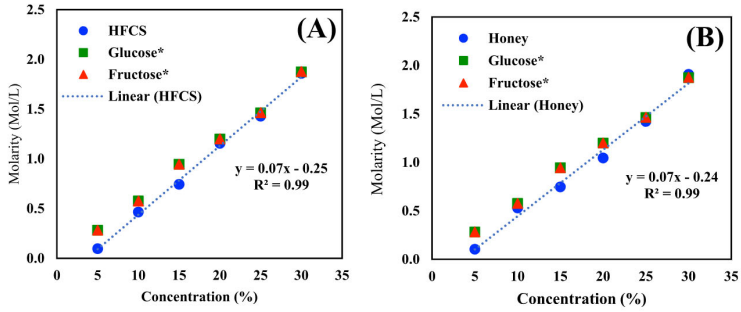

Fig. 1. Experimental molarity at different concentrations of high fructos corn syrup (HFCS) (A) and honey (B) at 22 °C.
*The green and red dots are the molarity of glucose and fructose adopted from (Lide, 2004). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

America Inc., CA, USA), mixed with 100 mg of freeze-dried bacteria, and then cooled immediately to room temperature (22 °C). For HFCS, a 100 mg of freeze-dried bacteria was added to a 20 g of HFCS without heating. The inoculated samples were vortexed (Fisher Scientific, Standard Vortex Mixer, USA) for at least 1 min to allow for sufficient mixing. In our preliminary study, we found pre-heating honey at 45 °C for 10 min and further addition of freeze dried bacteria did not reduce the population when enumerated immediately when inoculated honey reach to 22 °C. Also, studies indicated *Salmonella* when subjected to sublethal heat treatments at 45–48 °C for 30 min did not reduce its population (Bunning, Crawford, Tierney, & Peeler, 1990; Mackey & Derrick, 1986). The initial bacterial populations were quantified immediately following inoculation into honey and HFCS.

2.4.2. Lawn inoculation method

For the lawn inoculation method, cultures were regrown twice by successively sub-culturing in TSBYE at 37 °C for 24 h. Three hundred microliters of each strain were plated onto sterile tryptic soy agar with 0.6% yeast extract (TSAYE) (Hardy Diagnostics, Santa Maria, CA) in a 100 × 15 mm plate and incubated at 37 °C for 24 h. The bacterial lawn was collected from TSAYE using a plastic hockey-stick spreader and flooding with 5 mL of 1 × phosphate-buffered saline buffer (PBS), and then centrifuged at 8000×*g* at 4 °C for 15 min (Centrifuge 5810 R®, Eppendorf North America, Hauppauge, NY). The resulting pellets were re-suspended in sterile PBS to achieve $\sim 10^{9-10}$ CFU/mL, then combined in an equal volume to obtain the *Salmonella* cocktail. In addition to the three-cocktail strains, this inoculation method was also used to compare the survival of *S.* Enteritidis PT-30 and *E. faecium* in honey and HFCS.

One-hundred grams of honey or HFCS were placed in a 250 mL DURAN® brand glass bottle with a magnetic stirrer and 1 mL each of either the 3-strain *Salmonella* cocktail, *S.* Enteritidis PT30, or *E. faecium* was added to the samples and stirred for 3 min to achieve $\sim 10^{10}$ CFU/mL. These inoculated samples were stirred until sufficiently mixed, and the initial bacterial populations were immediately quantified.

2.5. Survival of bacteria during storage

To determine the survival of bacteria in honey or HFCS sample, 1 mL of inoculated samples were added to 9.0 mL of sterile PBS. Samples were repeatedly diluted this way to obtain a series of 10-fold serial diluted. The appropriate dilutions were spread plated in duplicate on TSAYE plates followed by incubation at 37 °C for 48 h for enumeration. The survival testing of bacteria was performed weekly for up to four weeks.

2.6. Statistical analysis

The survivability of *E. faecium* and *Salmonella* in honey and HFCS for both inoculation methods were analyzed with one-way ANOVA with a confidence interval of 95% ($\alpha = 0.05$) using Minitab software (version 19.2, Minitab, LLC, PA). Three independent experiments were performed. Each experiment had two Duran bottles-where two subsamples were serially diluted and plated in duplicates. Results were represented as Mean ± standard deviation.

3. Results

3.1. Physicochemical properties

The comparison of physicochemical properties of honey and HFCS are listed in Table 1. There were no significant differences ($P > 0.05$) between honey and HFCS in terms of moisture content, water activity (a_w), density, and sugar (°Brix). However, the pH and viscosity of HFCS was lower than the honey samples (Table 1).

3.1.1. Molarity and osmotic pressure

The calculated molarity and osmotic pressure of the 100% honey and HFCS are presented in Table 2. Honey is a complex food system that contains varying amounts of compositions, which varies with the sources that bees collected. Ninety-five percent of these molecules are dispersed with water and carbohydrates, and the rest are associated with proteins and other chemicals (Bogdanov, 2009). The carbohydrates in honey are mostly found in the form of fructose (~38%) and glucose (~32%). Because fructose and glucose are the main compounds in honey, and both having the same molecular weight (180.16 g/mol).

Table 1
Moisture content, pH, viscosity, a_w, density, sugar, and osmotic pressure of honey and HFCS at room temperature (~22 °C).

Physicochemical property	Honey	HFCS
Moisture Content (%)	15.3 ± 0.4[a]	14.9 ± 0.1[a]
pH	3.8 ± 0.1[a]	3.4 ± 0.1[b]
Dynamic Viscosity (N.s.m^{-2})	8.67 ± 0.39[a]	2.00 ± 0.1[b]
Water Activity (a_w)[a]	0.55 ± 0.08[a]	0.55 ± 0.03[a]
Density (g/ml)	1.40 ± 0.02[a]	1.38 ± 0.01[a]
Sugar (%)	83.1 ± 0.3[a]	82.4 ± 0.7[a]

[a-b] Mean within a row in different letters mean significantly different ($P < 0.05$).
[a] Water activity was measured at room temperature. Mean ± standard deviation. HFCS: High fructose corn syrup. Moisture and sugar contents are on wet basis.

Table 2
Calculation of molarity and osmotic pressure for honey and HFCS at (~22 °C).

	100% honey	100% HFCS
Approximate molecular-weight (g/mol)	−182	−185
Density (g/ml)	1.40	1.38
Concentration of sugar (g)	83.1	82.4
Solution (g)	100	100
Volume of solution (L)[a]	v= (100/1.40) *(1/1000) = 0.071	v= (100/1.38) *(1/1000) = 0.072
Number of moles of sugar (mole)[b]	n= (83.1/182) = 0.46	n= (82.4/185) = 0.45
Molarity (mol/L)[c]	M= (0.46/0.071) = 6.5	M= (0.45/0.072) = 6.3
Osmotic pressure (atm)[d]	157.4	152.5

[a] $V = \frac{Solution\ (g)}{Density\ (g/ml)}$

[b] $n = \frac{solute\ concentration\ (g)}{Molecular\ weight\ (g/mol)}$

[c] Calculated per Equation (2).
[d] Calculated per Equation (1).

Therefore, it is assumed that the molecular weight of honey as −182 g/mol based on their respective proportions, and in comparison, the molecular weight of HFCS as provided by the company was 185 g/mol (Marshall, Goff, & Hartel, 2012). The calculated molarity for honey and HFCS are 6.5 M and 6.3 M, respectively. The osmotic pressure for 100% honey and HFCS were 157.4 atm and 152.5 atm, respectively (Table 2).

The measured molarity and osmotic pressure of the diluted honey and HFCS are shown in Table 3. The molarity for 100% honey and HFCS, calculated from the linear equation Fig. 1, was 6.6 M and 6.7 M, and the osmotic pressure was 160.2 atm (16.3 MPa) and 161.5 atm (16.4 MPa), respectively. These values of molarity and osmotic pressure are comparable to the calculated and experimental results (Fig. 2).

The molarity of glucose solutions with a concentration up to 60% and fructose solution of a concentration up to 48% were previous reported (Lide, 2004); and these data are comparable with our experimental data for the diluted honey and HFCS with concentrations up to 30% (Fig. 1). It should be noted that the Van't Hoff osmotic pressure equation (Eq. (1)) is only accurate for diluted solutions. When applying it to highly concentrated solutions, the results would be inaccurate. According to the theoretical equation derived by (Chaplin, 2011; Huang & Xie, 2012), the actual osmotic pressures should be 3 times higher than the calculated using the Van't Hoff's equation. Thus, the true osmotic pressure in the pure honey and HFCS samples should be more than 3 times of the calculated value of 160 atm.

3.2. Survival of bacteria using the lawn inoculation method

The survival of *Salmonella* cocktail, *S.* Enteritidis PT30 and *E. faecium* prepared by lawn-based inoculum in honey and HFCS during stored at 22 °C for 4 weeks is shown in Table 5. The initial population of *S.* Enteritidis PT30 and *E. faecium* in honey was ~10.0 $\log_{10}$ CFU/mL. After one-week of storage, there was a reduction of 2.8 and 1.8 $\log_{10}$ CFU/mL for *S.* Enteritidis PT30 and *E. faecium*, respectively. The final log reduction (after 3 weeks of storage) was 7.4 and 6.5 $\log_{10}$ CFU/mL, respectively. However, the survivability of *E. faecium* was greater (6.6 $\log_{10}$ CFU/ml) when compared to *Salmonella* PT30 in honey after four weeks of storage. The initial population of *S.* Enteritidis PT30 and *E. faecium* in HFCS was 10.1 and 9.4 $\log_{10}$ CFU/mL, respectively. After one week of the storage, both bacterial populations reduced by ~2.7 $\log_{10}$ CFU/mL. *E. faecium* counts were reduced by 6.2 $\log_{10}$ CFU/mL after a three-week storage period and was beyond the detectable level (2 $\log_{10}$ CFU/mL) after four-weeks of storage (Table 4). However, *S.* Enteritidis PT30 cells were not even detectable after three weeks of storage.

The survival of the 3-strain *Salmonella* cocktail in honey and HFCS showed a similar trend as that of *S.* Enteritidis PT30 (Table 4). After one week of storage, the counts from *Salmonella* cocktail were reduced by 3.1 and 2.1 $\log_{10}$ CFU/mL in honey and HFCS, respectively. The number of *Salmonella* cocktail was reduced by −6.0 $\log_{10}$ CFU/mL after 3 week storage at 22 °C, and was not detectable after 4 weeks.

3.3. Survival of bacteria using dry inoculation method

The survival of *S.* Enteritidis PT30 and *E. faecium* prepared by dry inoculation method in honey and HFCS during storage at room temperature (22 °C) are presented in Table 5. The initial population of freeze-dried *S.* Enteritidis PT30 and *E. faecium* in honey was −9.6 $\log_{10}$ CFU/mL. There was 2-3 $\log_{10}$ CFU/mL reduction during the first week of storage, and ~5.0 or 7.0 $\log_{10}$ CFU/mL reduction of *E. faecium* or *S.* Enteritidis PT30 after three weeks of storage at 22 °C in honey (Table 5). The survivability of *E. faecium* was greater in honey after 28 days of storage compared to *S.* Enteritidis PT30 which were not detectable. Similarly, in HFCS, from initial ~9.9 $\log_{10}$ CFU/mL-the population of *E. faecium* and *S.* Enteritidis PT30 was reduced by > 5.0 $\log_{10}$ CFU/mL after a 3-week storage at 22 °C and either bacteria were not detectable after 28-days of storage at 22 °C (Table 5).

Table 3
Experimental osmotic pressure of diluted honey and HFCS at (~22 °C).

Concentration	Osmotic pressure	Osmotic pressure
(%)	(atm)	(atm)
5	2.5 ± 0.1	2.3 ± 0.2
10	12.8 ± 0.2	11.3 ± 0.1
15	18.1 ± 0.2	18.0 ± 0.2
20	25.3 ± 0.8	28.0 ± 0.5
25	34.5 ± 3.3	34.6 ± 0.1
30	46.2 ± 0.4	45.0 ± 1.3
100[a]	160.2 ± 4.6	161.5 ± 5.4

Mean ± standard deviation (n = 3).
[a] The osmotic pressure calculated from the extrapolated equations in (Fig. 1).

4. Discussion

Sugar plays an important role in maintaining the functional property of foods, by providing an essential carbohydrate source, increasing the food's sweetness, and enhancing its flavors. It is also helpful for flavor balance, color formation, bulkiness and texture maintenance, fermentation and preservation (CFIA, 2018). Due to their hygroscopic nature, sugars can easily dissolve in water by forming hydrogen bonds with water molecules, which helps in preserving and extending the shelf-life of food products (Syamaladevi et al., 2016). High-sugar products such as fruit preserves, syrups, confections, and dried fruits are not generally thought to pose a microbiological hazard. It is hypothesized that high concentrations of sugars exert an osmotic shock, which is not suitable for the growth of most microorganisms or causes cell death (Peña-Meléndez, Perry, & Yousef, 2014).

The results from this study suggest that *Salmonella* and *E. faecium* were not able to survive in honey after 28 days of storage at 22 °C, regardless of the inoculation methods. In support of our finding, Tysset and Durand (1973) reported a 9-log reduction of *S.* Enteritidis PT30 in honey stored at 18–20 °C for 34 days. Many studies speculate that honey has an antibacterial effect, mainly caused by total phenolic compounds such as methyl syringate (Al-Waili, Salom, Al-Ghamdi, & Ansari, 2012; Almasaudi et al., 2017). It was also suggested that the presence of amino acids, phenol antioxidants, antibiotic-rich proteins, as well as kynurenic acid contribute to the antibacterial effect of honey products (Beretta, Gelmini, Lodi, Piazzalunga, & Facino, 2010; G Vallianou, 2014). However, other researchers postulated that the bacterial inhibition of honey was due to the non-peroxide and osmotic effect (Al Somal, Coley, Molan, & Hancock, 1994). In our study, the reduction of *S.* Enteritidis PT30 and other *Salmonella* serovars in HFCS was similar to those in honey. The

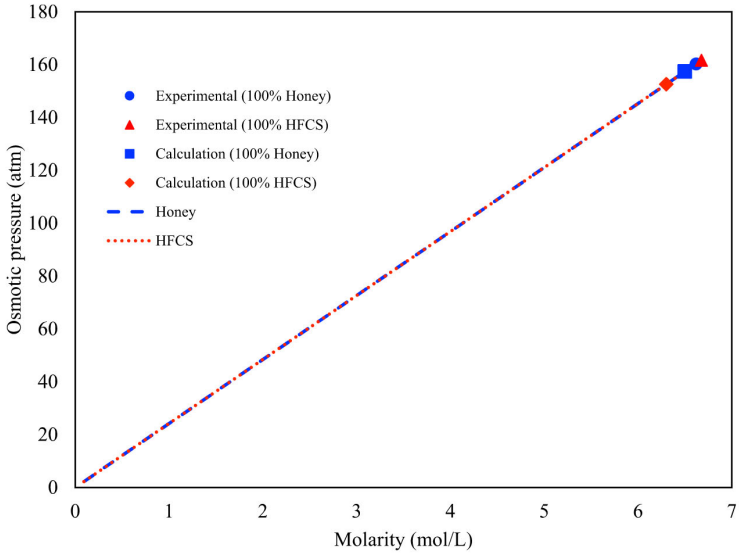

Fig. 2. The calculated osmotic pressure from calculated and experimental molarity data for honey and high fructose corn syrup (HFCS).

Table 4
Survival counts of *Salmonella* and *E. faecium* in honey and HFCS using lawn inoculation method.

Days	Honey (Log_{10} CFU/mL)			HFCS (Log_{10} CFU/mL)		
	E. faecium	*S.* Enteritidis PT-30	*Salmonella* cocktail	E. faecium	*S.* Enteritidis PT-30	*Salmonella* cocktail
0	10.4 ± 0.1	10.4 ± 0.1	10.1 ± 1.3	9.4 ± 0.2	10.1 ± 0.5	9.5 ± 0.5
7	8.6 ± 0.2	7.6 ± 0.1	7.0 ± 0.1	6.7 ± 0.1	7.4 ± 0.1	7.4 ± 0.2
14	7.0 ± 0.1	4.0 ± 0.1	5.7 ± 0.6	5.2 ± 0.3	3.3 ± 0.1	5.5 ± 0.1
21	5.0 ± 0.0	3.0 ± 0.2	3.5 ± 0.1	3.2 ± 0.1	[a]ND	3.5 ± 0.0
28	3.8 ± 0.2	[a]ND	[a]ND	[a]ND	[a]ND	[a]ND

[a] ND: Not-detectable. Mean ± standard deviation, averaged from three independent studies, 3 replicates per treatment at each sampling day within each independent study. HFCS: high fructose corn syrup.

Table 5
Survival counts of *S.* Enteritidis PT-30 and *E. faecium* in honey and high fructose corn syrup using freeze-dried inoculation method.

Days	Honey (Log_{10} CFU/mL)		HFCS (Log_{10} CFU/mL)	
	E. faecium	*S.* Enteritidis PT30	E. faecium	*S.* Enteritidis PT30
0	9.6 ± 0.1	9.6 ± 0.0	9.9 ± 0.1	9.9 ± 0.1
7	7.6 ± 0.2	6.7 ± 0.2	7.8 ± 0.0	6.9 ± 0.2
14	6.0 ± 0.0	4.5 ± 0.1	6.0 ± 0.0	5.0 ± 0.2
21	4.5 ± 0.0	2.6 ± 0.9	3.8 ± 0.1	4.3 ± 0.0
28	2.6 ± 0.7	[a]ND	[a]ND	[a]ND

[a] ND: Not-detectable. Mean ± standard deviation, averaged from three independent studies, 3 replicates per treatment at each sampling day within each independent study. HFCS: high fructose corn syrup.

bacterial populations in honey and HFCS were reduced by more than a 5-log reduction after 21–28 days storage at 22 °C. HFCS and honey have a similar concentration of sugar (around 83%) with a ratio 1.2:1 of fructose and glucose, respectively, but HFCS does not have notable antimicrobials. This suggests that the reduction of *Salmonella* in honey might not be due to antimicrobial agents in honey, but have been mainly caused by the high osmotic pressure. Studies have shown that short time (~10 min) high pressure processing (HPP) ranging 300–500 MPa resulted in complete reduction of *S.* Enteritidis population on chicken fillets, beef (Argyri, Papadopoulou, Nisiotou, Tassou, & Chorianopoulos, 2018; Rodrigues et al., 2016; Tananuwong, Chitsakun, & Tattiyakul, 2012) and ~3.3 log reduction in almonds (Goodridge, Willford, & Kalchayanand, 2006). Also HPP validation of *E. faecium* in strawberry juice producing 5-log reduction (Yildiz, Pokhrel, Unluturk, & Barbosa-Cánovas, 2019). In our study, the estimated osmotic pressures in honey and HFCS are about one order of magnitude smaller than that used in HPP processing. It is likely that in the presence of high osmotic pressure (i.e., 50 MPa) in honey and HFCS, the vegetative bacterial cells might gradually lost their viability during 28 days of storage. It can also be postulated that in addition to high osmotic pressure, low pH of HFCS and honey (pH 3.4 and 3.8 respectively) contributed for gradual reduction of high density of bacteria during 4-week of storage. The bactericidal effect of honey and sugar was significantly reduced when increasing the pH 3.4 to 7.0 (Kwakman et al., 2010). The hygroscopic nature of honey and HFCS can draw the moisture out of the environment

of the bacteria and cause cell death. High osmotic pressure causes ribosomal changes and protein denaturation in the bacteria (Abe, 2007).

Salmonella can survive in a dry product for an extended duration of time. For example, *Salmonella* was found to survive for 52–61 days in intermediate moisture foods such as brioche (a_w 0.88) at 20 °C (Kapetanakou et al., 2019). In a previous study conducted by Beuchat and co-workers on the survival of *Salmonella* in granulated sucrose, regardless of inoculation level (2.2 or 5.2 $\log_{10}$ CFU/g), wet or dry inoculation, a_w 0.54 or 0.24, and storage temperature 5 or 24 °C, *Salmonella* was able to survive over 52 weeks of storage (Larry R Beuchat, Mann, Kelly, & Ortega, 2017). In our study, *Salmonella* was completely inhibited during 4 weeks of storage in honey and HFCS with a_w of 0.55. This suggests that a_w is not the main factor that had caused inactivation of *Salmonella* in liquid sugars. In addition, the observed similar level of reduction of *Salmonella* between honey and HFCS indicate that the perceived antimicrobials in honey were not the main cause for microbial reduction; whereas, osmotic pressure and high acidity among these sugars exerted in a similar fashion. On the other hand, inoculation methodology has a great impact in the reproducibility and survival of bacteria in low a_w food studies. Different inoculation methods have been used to inoculate various dry foods-carriers such as sand or talc, use of a dry or wet bacterial inoculum in order to represent the route of contamination. Our study showed neither inoculation method (i.e. wet or dry) nor *Salmonella* strain had an impact on the survival of *Salmonella* or *E. faecium* in honey or HFCS.

In this study, with few exceptions, the survival of *E. faecium* in both honey and HFCS was similar to *Salmonella* either with lawn grown or dry inoculation (Tables 4 and 5). This suggests that *E. faecium* can be considered as an appropriate surrogate for determining the survivability of *Salmonella* in liquid sugars. *E. faecium* was shown as a suitable surrogate in determining survival and thermal resistance of different low a_w food such as date paste (Ozturk et al., 2019), toasted oat cereals (Deen & Diez-Gonzalez, 2019), coconut (Dhowlaghar, Zhu, & Ballom, 2019), wheat flour (Xu et al., 2019) and cocoa powder (Tsai et al., 2019).

5. Conclusion

Salmonella and *E. faecium* die off in honey and HFCS stored at 22 °C for 28 days, regardless of the inoculation method. Besides the antimicrobial compounds existed in honey, the high osmotic pressure in high sugar products determined in this study is likely another main killing factor responsible for the observed bacterial reduction. These results indicate that honey and HFCS are fairly safe as compared to other low-moisture foods or sugars. More systematic studies are still needed to investigate the survival of other pathogenic foodborne bacteria in high concentration liquid sugar products.

Author Contributions

Jaza Alshammari and Nitin Dhowlaghar prepared the first draft of the manuscript, designed the experiments, collected data, and analyzed data. and assisted with preparation of the manuscript. Yucen Xie contributed in preparation of the experiments and in editing. Juming Tang and Meijun Zhu supervised the project, contributed in interpreting results and editing the manuscript. Also, Jie Xu, Shyam Sablani, and contributed in editing the manuscript. Authors declare no competing interests in this study.

Declaration of competing interest

The authors declare there is no conflict of interest in this research.

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