Effect of water activity and storage of tahini on the viability of stressed *Salmonella* serovars

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**Abstract**

Tahini (sesame seed paste) is a low water activity product that has been involved in several salmonellosis outbreaks. The objectives of the study were to examine over a year the impact of using and storage temperature of tahini on the viability of *Salmonella* serovars previously stressed by drying or heat exposure. Tahini samples adjusted to aw values of 0.17, 0.35 and 0.50 were inoculated with a mixed culture containing 10^9-10^10 CFU/g of 4 serovars of unstressed, desiccation- or heat-stressed *Salmonella* and stored for up to 12 months at 10 and 25 °C. Generally, viability of stressed or unstressed *Salmonella* decreased as the storage temperature and time increased or the aw of tahini decreased. The survival of stressed or unstressed *Salmonella* in all samples decreased during storage for up to 12 months by ca. 6.0 and 3.3 log10 CFU/g, respectively. Exposing *Salmonella* to heat stress had no significant effect on survival in tahini, while desiccation stress significantly decreased survival during storage, especially at 25 °C in low aw tahini.

**Keywords:** tahini safety; water activity; *Salmonella*; bacterial stresses; storage.

**Practical Application:** This study indicates that *Salmonella* serovars can survive in tahini for as long as 12 months. Survival of *Salmonella* in tahini decreased as storage time and temperature increased and as the aw of tahini decreased.

1 **Introduction**

Tahini or sesame seed pastries are consumed widely in the Middle East and Eastern Mediterranean countries as a dip, salad dressing or as a main ingredient in many ready-to-eat foods such as halva and hummus (Osaili & Al-Nabulsi, 2016). Tahini contains 57-65% fat, 23-27% protein, 6.4-9% carbohydrate and 1% moisture (Abu-Jdayil et al., 2002) and has a low water activity (aw) of 0.16 with a shelf life at room temperature of 1 year (Osaili et al., 2016). Tahini can pose a microbial risk to consumer health as it has been involved in several salmonellosis outbreaks. In 2002 and 2003, imported tahini contaminated with *Salmonella* Montevideo was responsible for three outbreaks in New Zealand and Australia (Unicomb et al., 2005). In 2011, hummus and tahini contaminated with *S.* Bovismorbificans were associated with a multistate outbreak in the US (Centers for Disease Control and Prevention, 2012). Also, in 2013 an illness outbreak linked to *Salmonella* serovar contamination of tahini was documented in the US (Centers for Disease Control and Prevention, 2013). Recently, another two salmonellosis outbreaks linked to tahini which infected a total of 11 persons were reported in the US in 2018 and 2019 (Centers for Disease Control and Prevention, 2018, 2019). The undercooked meat and meat products, particularly poultry products have been known as a major source of *Salmonella* serotypes; however, other food products such as fresh produce, dairy products, and low aw foods have been found to be contaminated with different *Salmonella* spp. (Beuchat et al., 2013; Chávez-Martínez et al., 2019; Cruz et al., 2019; Cunha-Neto et al., 2019; Mendonça et al., 2019). *Salmonella* can be eliminated from foods by pasteurization of high aw foods such as liquid milk or juices (Beuchat et al., 2013) or using the microwave processing of low aw foods such as infant milk (Portela et al., 2019), which indicates the susceptibility of *Salmonella* to heat.

The basic operation in tahini production involves roasting of sesame seeds to enhance the color, flavor and texture as well as improve its palatability. The optimum roasting conditions to obtain the best texture and color of tahini ranges from 155 to 170 °C for 40 to 60 min (Kahyaoglu & Kaya, 2006). According to Torlak et al. (2013), *Salmonella* did not survive roasting of sesame seeds at 110, 130, and 150 °C. However, Zhang et al. (2017) reported survival of *Salmonella* on sesame seeds increased as aw decreased during roasting. Thus, the presence of *Salmonella* in tahini might be due to the ability of *Salmonella* to survive the roasting process if the seeds are at a low aw at the beginning of the roasting process (Zhang et al., 2017), or their presence may be due to post-heat treatment contamination from equipment, workers or the processing environment (World Health Organization, 2008; Torlak et al., 2013).

*Salmonella* have the ability to survive in low aw products for long periods (Burnett et al., 2000; Farakos et al., 2013; Osaili et al., 2017). Their survival is influenced by food composition, temperature, as well as aw (Beuchat et al., 2013; Farakos et al., 2013; Osaili et al., 2017). Farakos et al. (2013)
modeled the influence of storage temperature (21 to 80 °C), \( a_w \) (0.19 to 0.54) and water mobility on the survival of Salmonella in low moisture food products. The researchers reported that \( a_w \) affected the viability of Salmonella significantly, since Salmonella survival at 21 °C was greater at lower \( a_w \) levels (0.16 and 0.26) in comparison with higher \( a_w \) levels (0.34 to 0.53). A study conducted by Burnett et al. (2000) on products similar to tahini found that Salmonella had the ability to survive during storage in peanut butters and peanut butter spreads. The study also found that populations of 5.7 log\(_{10}\) CFU/g decreased after 6 months at 21 and 5 °C by 4.1-4.5 log\(_{10}\) CFU/g and 2.9-4.3 log\(_{10}\) CFU/g, respectively, depending on the formula composition. In a recent study, Osaili et al. (2017) reported that Salmonella serovar survival in halva decreased as storage temperature and time increased. After 1 year at 10 and 25 °C, Salmonella numbers decreased by 2.7, and 5.2 log\(_{10}\) CFU/g, respectively.

Before contaminating food, microorganisms might be exposed to one or more environmental stresses, including desiccation, starvation, heat, cold, or different types of chemical agents in processing plant environments (Osaili et al., 2008a, b). Exposure of a microorganism to a stress may cross-protect the microbe against naïve stresses, and therefore boost its survival (Bunning et al., 1990; Abee & Wouters, 1999). Exposing Salmonella serovars to heat or desiccation stress prior to inoculation extended their viability in halva during storage (Osaili et al., 2017).

As mentioned, \( a_w \) value and temperature during storage interact to influence the viability of Salmonella in low \( a_w \) food products. However, no studies were found on the effect of \( a_w \) and storage temperature on the viability of Salmonella serovars in tahini, even though this microbe may behave differently if producers manipulate tahini \( a_w \) and storage temperature. Thus, the objective of the current study was to examine the impact of increasing tahini \( a_w \) from 0.17 to 0.35 or 0.50 and decreasing temperature from 25 °C to 10 °C on the survival of inoculated Salmonella serovars. In addition, the current study assessed the effect of heat and desiccation stresses on the viability of Salmonella serovars in tahini during storage for one year.

2 Materials and methods

2.1 Tahini samples

Tahini samples of one brand with no additives were obtained from a local grocery store in Irbid, Jordan. The purchased samples were checked for the presence of Salmonella using an ISO method (International Organization for Standardization, 2002) and were found Salmonella free. The total mesophilic aerobic bacteria of tahini samples were determined by adding 10 g of tahini to 90 mL peptone water (Oxoid, Hampshire, UK) followed by vigorous mixing and serial 10-fold serial dilution. Thereafter, 1 mL of the appropriate dilution was mixed with molten, tempered Plate Count Agar (Oxoid) using the pour plate technique and solidified plates were incubated at 35 °C for 48 h.

2.2 Proximate analysis of Tahini

AOAC methods (Association of Official Analytical Chemists, 1984) were used to determine carbohydrate, protein, fat, moisture and ash contents of tahini. The analyses were performed on triplicate samples and the average value of each component was determined.

2.3 Water activity (\( a_w \))

The \( a_w \) of tahini was determined at room temperature (21 ± 1 °C) using an \( a_w \) meter (HygroLab, Rotronic Inst. Corp, Huntington, NY, US). The original \( a_w \) value of the tahini samples was 0.17 ± 0.01. Then other samples of tahini were prepared and the \( a_w \) values were adjusted to 0.35 ± 0.01 and 0.50 ± 0.01 by mixing tahini samples with sterile distilled water.

2.4 Culture preparation

Four serovars of Salmonella used in this study were previously isolated from commercial tahini from the local market (S. Cubana T123, S. Aberdeen T108, S. Typhimurium T069, and S. Paratyphi A T193) and were obtained from the Jordanian Food and Drug Administration. Fresh cultures were prepared from the frozen state by streaking a loopful of each culture onto selective medium (Xylose Lysine Deoxycholate XLD agar) (Oxoid). The plates were incubated for 24 h at 37 °C. One typical colony from each isolate was grown individually in Tryptic Soy Broth (Oxoid) followed by 24 h incubation at 37 °C. Before beginning the experiments, three consecutive transfers were conducted to obtain an active culture to be used in the trials.

2.5 Preparation of Salmonella serovar suspensions

A 10 mL broth sample of freshly cultivated Salmonella was centrifuged at 4000 g for 15 min and the supernatant was discarded. The resulting pellets were mixed with 0.25 mL of sterile buffered peptone water (BPW). The suspension was mixed vigorously by means of a vortex mixer. The individually prepared suspensions of each Salmonella serovar were combined together to form a mixture of 4 Salmonella serovars with a final concentration of approximately10⁹ CFU/mL.

2.6 Preparation of desiccation and heat-stressed Salmonella suspensions

The mixture of Salmonella serovars was exposed to desiccation or heat stresses. Stresses were applied according to protocols published previously (Gruzdev et al., 2011; Osaili et al., 2016) with slight modifications to obtain a treatment condition that would result in no more than 1 log\(_{10}\) reduction in bacterial numbers. Desiccated cells were prepared by dividing 1 mL of fresh Salmonella mixture into 40 portions of 25 µL which were placed into a sterile Petri dish held in biosafety cabinet without a lid for 4 h. Thereafter, the desiccated culture was rehydrated by adding 1 mL of BPW to the plate and the liquefied, desiccated culture was gently shaken and collected in a sterile test tube. For the preparation of heat-stressed Salmonella, a 15 mL screw-cap test tube containing 9 mL of 0.1M, pH 6.8 potassium phosphate buffer that had been heated to 50 °C in a shaking water bath was used. One mL of the Salmonella mixture was added to the buffer and kept for 10 min at 50 °C. Thereafter, the tube was removed and cooled instantly using running tap water. Then the
mixture was centrifuged for 15 min at 4000g. The supernatant was discarded and the pellet was remixed with 1 mL of BPW.

2.7 Tahini inoculation

Tahini samples (100 g with \( a_w \) levels of 0.17, 0.35 and 0.50) were inoculated with 0.2 mL of a freshly stressed or unstressed *Salmonella* serovar mixture to achieve an inoculation level of \( 10^6 \) to \( 10^7 \) CFU/g. The inoculation procedure was tested and found to elicit no change in the \( a_w \) value of the samples.

2.8 Storage of the samples

After thoroughly mixing tahini samples with the cultures, 10 g sub-samples were placed in sterile Stomacher bags. The bags were massaged manually to expel air, followed by heat sealing and storing at 10 or 25 °C for up to 12 months.

2.9 Bacterial enumeration

Tahini samples inoculated with a stressed or unstressed *Salmonella* mixture were sampled at 0, 1 and 2 weeks, and monthly for 12 months. All samples were serially diluted in 0.1% peptone water. The thin agar layer method was used to recover *Salmonella* survivors (Osaili et al., 2010). In this method, an appropriate dilution was spread-plated onto XLD overlaid with a thin layer of Tryptic Soy Agar (Oxoid) in duplicate to recover injured cells. The plates were incubated aerobically for 24 to 48 h at 37 °C.

2.10 Statistical analysis

All trials completed during this study were conducted in triplicate. Effects of stress type, storage temperature, time and \( a_w \) on the survival of *Salmonella* in tahini were evaluated using SPSS version 19.0 (2009; Chicago, IL, US). To compare effects of the two temperatures a t-test was performed. The effects of \( a_w \) stress type and storage time were examined using one-way ANOVA. To determine the difference between variables, the Duncan post-hoc test was performed. For statistical significance the cut-off level was based on a \( P \)-value of < 0.05.

3 Results

3.1 Proximate analysis and total plate count of Tahini

Fat, protein, carbohydrate, ash and moisture contents of tahini samples were 58.12 ± 0.71%, 24.86 ± 0.17%, 13.52 ± 0.86%, 3.7% ± 0.0, and 0.04 ± 0.01%, respectively. The total bacterial plate count in tahini was 2.7 ± 0.2 log10 CFU/g.

3.2 Effect of storage temperature and time on the viability of stressed and unstressed *Salmonella* in Tahini

The survival of unstressed, and desiccation or heat-stressed *Salmonella* serovars in tahini with \( a_w \) of 0.17, 0.35 and 0.50 stored for up to 12 months at 10 and 25 °C is shown in Figures 1-3. Unstressed cells of *Salmonella* were able to survive well in tahini during storage at 10 and 25 °C for at least 12 and 9 months, respectively, regardless of \( a_w \) level. The survival of both stressed and unstressed *Salmonella* in tahini decreased as the storage temperature, time and \( a_w \) of tahini samples increased. Storage temperature had a significant (\( P < 0.05 \)) effect on the viability of stressed and unstressed *Salmonella* in tahini regardless of storage time and \( a_w \) value. At the same \( a_w \) level, the viability of *Salmonella* in samples stored at 10 °C was significantly (\( P < 0.05 \)) higher than when stored at 25 °C. A significantly greater reduction in the number of unstressed *Salmonella* survivors was noticed after 14 d storage at 25 °C compared to 10°C in samples with \( a_w \) values of 0.17 and 0.35 but not at 0.50. Yet, a significantly greater reduction was noticed at an \( a_w \) of 0.5 after 2 months storage at 25 °C compared to 10 °C. For desiccation-stressed cells (Figure 2), the impact of temperature on the survival of *Salmonella* was also influenced by \( a_w \). At 25 °C the extent of reduction was generally more pronounced than at 10 °C. The magnitude of reduction in *Salmonella* numbers at 25 °C was significantly (\( P < 0.05 \)) greater from 2 weeks until 10 months of storage than at 10 °C when the \( a_w \) was 0.17 and 0.35. Also, the reduction was more pronounced during the initial storage period (2 weeks to 1 month) at an \( a_w \) of 0.17 and 0.35 at the higher storage temperature.

The reduction in unstressed and stressed *Salmonella* survival was significant throughout storage irrespective of temperature and \( a_w \) value of the samples. After 12 months at 10 °C, the viability of unstressed *Salmonella* in samples with \( a_w \) values of 0.17, 0.37 and 0.50 decreased by 3.1, 3.1, and 2.3 log10 CFU/g, respectively, and became undetectable (<10 CFU/g) in samples stored at 25 °C. After 1 and 3 months at 25 °C, the number of desiccation or heat-stressed cells dropped by 2.5-3.3 and 3.2-3.7 log10 CFU/g.

![Figure 1](https://example.com/figure1.png) Survival (log10 CFU/g) of unstressed (control) *Salmonella* spp. in tahini samples with different \( a_w \) values (0.17, 0.35 and 0.50) during storage at (a) 10 °C and (b) 25 °C for up to 12 months. Values are represented as a mean of three replications ± standard deviation. Detection limit < 10 CFU/g.
Survival of Salmonella in tahini

respectively, compared with their initial numbers. However, the reduction of stressed cells was significantly lower in tahini stored at 10 °C for the corresponding storage durations.

3.3 Effect of aw on the viability of stressed and unstressed Salmonella in Tahini

Survival of stressed or unstressed Salmonella serovars in samples with higher aw was greater than in those with lower aw during storage at 10 °C. The order of the aw effect on Salmonella reduction in samples was 0.17 > 0.35 > 0.50 (Figures 1-3). In general, the effect of aw on the survival of stressed and unstressed Salmonella in tahini was more pronounced at 25 °C, in particular after 5 months of storage. Generally, there were no significant differences among the survival of unstressed Salmonella in samples with aw values of 0.17, 0.35 and 0.50 during the first 3 months of storage at either 10 or 25 °C. However, during 3-7 months storage at 10 °C, desiccation-stressed Salmonella showed a greater ability to survive at an aw of 0.50 than at 0.17. In comparison, heat-stressed Salmonella cells exhibited greater survival at an aw of 0.50 than at 0.17 only during the first 4 months of storage at 10 °C.

3.4 Effect of desiccation and heat stresses on the viability of Salmonella in Tahini

There were significant differences (P < 0.05) between the survival of stressed Salmonella and unstressed cells during storage of tahini with different aw values at 10 and 25 °C for up to 12 months (Figures 1-3). At all aw levels, the survival of desiccation-stressed Salmonella was lower than that of unstressed or heat-stressed Salmonella cells. It was noted that survival of unstressed Salmonella decreased below the detection limit after 12, 11, and 10 months of storage at 25 °C with aw values of 0.17, 0.35 and 0.50, respectively. Desiccation-stressed Salmonella reached undetectable levels after 7, 7, and 9 months of storage at 25 °C in tahini with aw values of 0.50, 0.35 and 0.17, respectively. For heat-stressed Salmonella, no cells were detectable after 8, 9, and 12 months storage at 25 °C in tahini with a similar order of aw values.

4 Discussion

The observations made during the present study are in agreement with other reports. Osaili et al. (2017) reported that Salmonella survived in halva during storage for 1 year at 10 and 25 °C and that their populations decreased significantly as storage temperature and time increased. Holliday et al. (2003) indicated that Salmonella serovars were able to survive well in fat spreads, and the reduction in the numbers was greater at 21 °C compared to 4 or 10 °C. Similarly, Kotzekidou (1998) found that Salmonella survived better in halva after 8 months at 4 °C compared to room temperature storage. In addition, Burnett et al. (2000) reported Salmonella survived better in peanut butter when stored at 5 °C compared to 21 °C. They pointed out
that the initial 5.7 log_{10} CFU/g of Salmonella mixed culture in commercial peanut butter and peanut butter spreads decreased by 4.1-4.5 log_{10} CFU/g or 2.9-4.3 log_{10} CFU/g after storage for 6 months at 21 or 5 °C, respectively. Also, it was reported that the reduction in S. Typhimurium and E. coli O157:H7 numbers was greater at 37 °C than at 10 °C during storage of tahini for 28 d (Al-Nabulusi et al., 2014, 2020). Kilonzono-Nthenge et al. (2009) reported that Salmonella and E. coli O157:H7 survived better in peanut butter stored at 25 °C than in that stored at 4 °C for the same period. Similarly, the number of Salmonella viable in a mixed culture decreased during 4 months storage of tahini at 4 and 22 °C by 3.3 and 4.5 log_{10} CFU/g, respectively (Torlak et al., 2013). However, other reports revealed that Salmonella numbers in low a_w products either did not change during storage or were not affected by storage temperature. Zhang et al. (2017) indicated that Salmonella can survive with no change in numbers in tahini during refrigerated storage for 4 months. Additionally, no significant differences were noticed between the levels of S. Tennessee in 4 of 5 commercial brands of peanut butter stored at 4 or 22 °C. The S. Tennessee viability dropped by 0.15 to 0.65 or 0.34 to 1.29 log_{10} CFU/g in samples stored 14 d at 4 or 22 °C, respectively. In another study, the initial population of Salmonella inoculated on the shell of walnuts was reduced by 0.29 log_{10} CFU per nut after the first 8 d storage at 10°C and an RH of 65% after drying, but no change in the population was noted after 3 months of storage (Frelka et al., 2016). Salmonella possess strategies to survive long times in a desiccated state. These survival strategies may include the accumulation of osmoprotectant molecules/metabolites, filamentation of cells, and switching to a metabolically dormant state (Finn et al., 2013). The variation between these and the results presented in the current study may have been due to serovar differences, variation in culture cultivation procedures, product composition or the variability in the storage time of the products that was mostly compatible with anticipated shelf-life of the product. In the studies by Zhang et al. (2017) and Frelka et al. (2016), bacteria used were taken from solid media and were sessile cells, while in the present study bacteria used were planktonic in nature, grown in liquid media. In addition, tahini samples used in the Zhang et al. (2017) study were prepared by grinding sesame seeds with olive oil in a food processor while commercial tahini samples were used in the present study.

In the current study, the reduction in Salmonella viability was more evident at 25 °C than 10 °C. This might be explained by the observation that the fluidity of the cell membrane is enhanced as temperature increases, and this in turn, increases its permeability to phenolic compounds that abundantly exist in tahini and can exert an inhibitory effect against Salmonella (Al-Nabulusi & Holley, 2006). Tahini is a colloidal suspension of lipid and water. It is possible that Salmonella in tahini would have a tendency to collect or localize within or around the water phase. In a study conducted by Burnett et al. (2000) it was stated that the survival of Salmonella in peanut butter is probably influenced by the droplet size of water and lipid distributed in the products, and droplet size and aggregation may increase throughout lengthy storage. A similar situation may happen in tahini where Salmonella viability is expected to differ in the primary stages of storage compared to later stages; depending on emulsion stability and water droplet sizes in tahini. However, additional studies are needed to explain the relationships among emulsion stability, droplet size and the survival of pathogens in an emulsion like tahini.

Water, a major constituent of food, is a vital element in growth/inactivation of microorganisms (Podolak et al., 2010). The cell-water interaction is described as a_w. In contrast to the present work, some studies have shown that reduced a_w protects against the decrease of Salmonella serovars in low moisture food products (Archer et al., 1998; Doyle & Mazzotta, 2000; Beuchat & Scouten, 2002; Keller et al., 2013; Gradl et al., 2015). Gradl et al. (2015) and Keller et al. (2013) reported that Salmonella viability decreased during 25 and 37 °C storage of ground ginger and black pepper when a_w was about 0.86, however, viability remained relatively stable when the a_w of samples was < 0.40. Similarly, Salmonella numbers decreased during 25 and 37 °C storage on alfalfa seeds with an a_w of 0.21 to 0.60, but not when stored at 5 °C, as the a_w in seeds was increased (Beuchat & Scouten, 2002). However, in agreement with the findings in the present study, Jung & Beuchat (1999) stated that Salmonella strains survived in whole egg white and yolk powders with a_w adjusted to 0.29-0.37 and 0.51-0.61 at 13 and 37 °C during 8 weeks. The survival of different strains of Salmonella was greater at a_w 0.51-0.61 than at 0.29-0.37. As noted earlier, differences between published results and those presented here may have arisen from differences in tested serovars, differences in their cultivation before inoculation, differences in tahini formulations, product a_w values, test temperatures and intervals. In addition, sesame seeds contain the phytochemicals: lignan, sesamolin, sesamin, sesamol and methylene dioxyphenyl compounds (Kähkönen et al., 1999). A study on sesame by Mahendra Kumar & Singh (2015) indicated that sesamol and lignans have antioxidant and antimicrobial effects, and roasted sesame oil contains a higher amount of sesamol. They also pointed out that when the concentrations of lignans and sesamol were increased, the viability of Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus cereus decreased. Therefore, it is anticipated that a low a_w of 0.17 and phenolic compounds will probably act synergistically to cause reductions of Salmonella serovars. Nonetheless, further investigation of the impact of low a_w and the presence of antimicrobials in tahini is needed to confirm their effect upon the survival of Salmonella in tahini.

In the current study, unstressed Salmonella were capable of surviving better under different a_w and storage temperatures compared to desiccation and heat-stressed Salmonella. On the contrary, Osaili et al. (2017) found that the survival of desiccation and heat-stressed Salmonella in halva samples was slightly greater (P ≥ 0.05) than that of unstressed cells stored at 10 and 25 °C. It is worth noting that when stressed or unstressed Salmonella are inoculated into tahini they are exposed to additional desiccation stress in the product. In the current study, heat stress may cross-protect Salmonella against a low a_w environment when stored in tahini with an a_w of 0.17 at 25 °C. In contrast to heat stress, desiccation stress reduced Salmonella viability as no cells were detected after 9 months. Salmonella might have been exposed to multiple stresses not only during preparation of desiccation-stressed cells, but also from the product itself during storage. In addition, microbial
viability under desiccated conditions is mainly affected by the osmotic stress resistance of the microbe. Increased osmotic pressure by reducing a_w affects microbial survival by inducing shrinkage and plasmolysis, inhibits DNA replication, nutrient uptake of bacteria and cell growth (Csonka, 1989).

5 Conclusion

The present study indicates that unstressed or desiccation or heat-stressed Salmonella can survive in tahini with different a_w values for the entire 12 month product shelf-life when kept at 10 °C. Storage temperature has a remarkable effect on the survival of Salmonella in tahini. Unstressed Salmonella possessed better survival compared to desiccation and heat-stressed Salmonella at the same storage temperatures. Additionally, the survival of unstressed and stressed Salmonella was greater at 10 °C compared to 25 °C. A higher tahini a_w of 0.50 resulted in greater Salmonella survival compared to when its a_w was low (0.17 or 0.35), particularly at 10 °C.

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