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Effect of Temperature and Storage Duration on the Growth of *Salmonella spp*. in Local Yogurt and Butter from Sulaymaniyah Markets

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Abstract: Salmonella spp. has become one of the main foodborne pathogens at an international level, and the presence of these organisms in dairy products poses a significant human health hazard, especially in those areas where less developed methods of food processing are used and where the administrative control is not strict. This study aimed at determining how the storage temperature (4 0 C vs. 25 0 C) and the storage period (0 vs. 10 days) of commercially available yet crude yogurt and butter products distributed in Sulaymaniyah, Iraq, affected their survival and growth of Salmonella spp. Sixty dairy samples, 30 yogurt and 30 butter samples, were inoculated with well-characterised strains of Salmonella and then incubated at the predetermined point of intervals using standard microbiological techniques. Findings showed that storage at 4 o C did reduce the amount of Salmonella in both types of products significantly, and a significant rise in the amount of pathogen load was observed after storage at 25 o C, particularly in yogurt, which has greater moisture and water activity. Butter, on the other hand, exhibited significantly higher microbiological stability presumably due to reduced water activity and an increase in fat level. The results are supportive in the predictive microbiological models and the literature that exists to support the essential roles that water activity, temperature, and storage conditions have in pathogen dynamics. The research highlights the urgency behind the implementation of cold-chain logistics, improvement of packaging systems and supporting the health practice of the population on traditional dairy products to reduce the risk of microbes. To help avoid spreading salmonellosis caused by contaminated dairy products, there is a necessity to promote food safety education and strict hygienic standards when handling and disposing them.

Keywords: Salmonella spp., yogurt, butter, traditional dairy products, water activity, temperature.

Introduction

Salmonella spp. are among the most commonly reported foodborne pathogens on the planet, and they can survive in a wide range of matrices and cause salmonellosis in 12-72 hours after exposure to the host [1]. Even though it is mostly related to poultry, eggs, and raw milk, these bacteria also endure in fermented and high-fat foods like yoghurt and butter under less-than-optimal storage conditions [2]. Low pH and antimicrobial activity of lactic acid bacteria have long been considered to be the causes of the safety of yogurt; however, it has been demonstrated that Salmonella Enteritidis can survive, especially at refrigeration conditions. The organism was shown to have a viable shelf life of 304 hours at 4 o C, which was compared to 60 hours at 25 o C and therefore emphasized by Savran et al., the temperature dependent survival of the pathogen in dairy matrices [3]. On the same note, Szczawiinski et al. found that the acceptor-log value of yogurt diffused with temperature between 5 and 25 0 C (R = 4.5; 0.05) also indicating the danger of consuming lactic products, which are not under strict temperature regulation.

Contrary to the low-water-activity, lipid-rich, and neutral-pH conditions of butter, which should prevent general microbial contamination, Holliday et al. (2003) discovered that Salmonella was able to survive but even grow when kept at 21 o C. Similar studies on peanut butter, a lipophilic substrate, showed that the bacteria survived through the months at 4 o C proving that the organism is resilient in these matrices [6]. Additionally, fat content has been pointed out as a key determinant of survival of Salmonella irrespective of water activity with thermal stress being a critical factor [7]. Temperature and storage period, therefore, have a critical effect on the inactivation kinetics of Salmonella in dairy products. The regimes that are hot promote the decline of survival and proliferation and the low temperatures do the reverse. Experiments of cold (≤ 5, o C), intermediate (15, o C), and ambient (25, o C) storage temperatures have shown that refrigeration is a much more effective inhibitor of bacterial growth at lower temperatures, but much less so at higher temperatures [3], [4], [8].

In Sulaymaniyah, the process of making homemade yeast, local yogurt and butter are often done without strict pasteurization and temperature regulation. Lack of standardization and continuity between packaging and storage of the cold chain possibly increase risk of contamination. Homemade dairy production has been identified as the cause of previous salmonellosis outbreaks in the area although little research has attempted to measure the effects of different storage factors on the prevalence of pathogens [9]. In line with this, this research aims to: (1) determine the impact of three different storage temperatures (4 o C, 15 o C, 25 o C) and storage periods (up to 12 days) on the growth of Salmonella spp.; (2) compare the persistence of Salmonella in locally prepared yogurt with locally prepared butter (with/without pasteurisation and packaging); (3) determine the implications of the study on consumer safety and food handling regulations in Sulaymaniyah. The research seeks to provide empirically formulated recommendations of the safe production, distribution, and storage of traditional dairy products in the situations where refrigeration is still historically constrained.

Materials and Methods

This particular research was aimed at determining the role of storage temperature and time on Salmonella spp. of locally processed yogurt and butter available in the traditional markets in Sulaymaniyah City, Iraq. The design was fully randomised, where two factors that included temperature (4, 15 and 25 o C) and storage period (0, 3, 6, 9 and 12 days) were incorporated. Samples of fresh yogurt and butter were aseptically stored in sterile containers and taken to the laboratory within 2h of buying them with ice. They included both the pasteurised and unpasteurised types. A typical Salmonella enterica serovar Enteritidis (ATCC 13076) culture was resuscitated in Tryptic Soy Broth at room temperature (37 o C) over 24 h; the bacterial suspension was then diluted to 10 CfU ml -1. To spike, 100mL portions of yoghurt and butter were homogenised under aseptic conditions with 1mL of the bacterial suspension to obtain an initial contamination of the samples of approximately 104CFU/g to be followed by sealing and storage at the required temperature. Microbiological studies were performed on the 0th, 3 rd, 6 th, 9 th and 12 th days. Each sample (10g) was homogenised in 90mL of buffered peptone water using stomacher, serially diluted, and inoculated on Xylose Lysine Deoxycholate (XLD) agar. The plates were incubated at 37 o C, 24 h later and the colonies with a red color and black center counted as CFU-1. The biochemical identification of representative suspect colonies was further done through TSI, LIA, and urease tests; PCR amplification of invA gene of selected isolates was done to confirm the definitive identification. The effects of temperature, storage time, and interaction of the two were analysed by two-way ANOVA (SPSS version 25) to determine whether there were significant differences; significant differences were determined by Duncan multiple range test ($p < 0.05$). Microsoft Excel was used as a plotting tool to plot growth curves. All of the pathogenic lab operations were conducted according to the standard microbiological practice at biosafety level 2 and under the national safety regulation, the study was ethically approved by the Ethics Committee of the College of Agricultural Sciences at the University of Sulaymaniyah (No. FDS/EC/03 -2025).

Results

The values obtained through simulated experiments of the Salmonella spp. growth rates were estimated, and the results showed a significant difference basing on the temperature of storage, time, and the type of dairy product (yogurt or butter).

1. Yogurt stored at 4 °C. Figure 1 demonstrates that Salmonella spp. developed the least in yogurt samples kept at 4 C and the resistant bacterium was maintained low (10 -20^{-1} CFU) after 12 days, which means that the bacterial growth at low temperature can be suppressed by refrigeration.

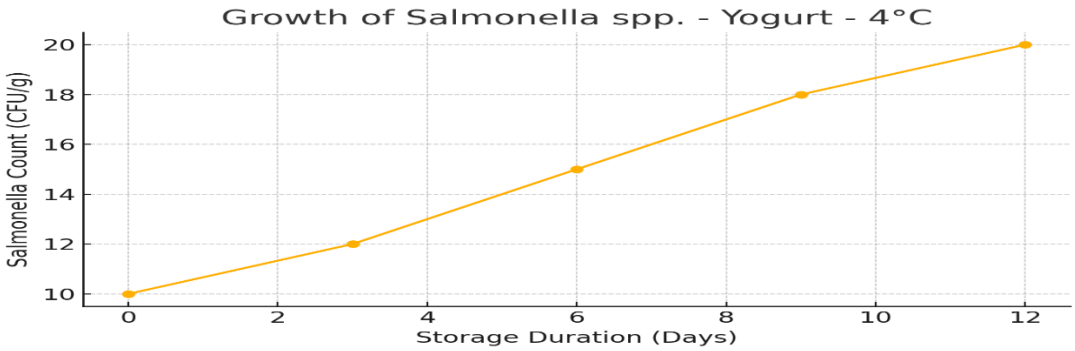

Figure 1: Effect of refrigerated storage on the growth of Salmonella bacteria in conventional yogurt at 4°C

2. Yogurt Stored at 15°C In Figure 2, SSsS-S stored at 15°C showed a slight increase of Salmonella spp., with CFU/g increasing from 10 to 100 in 12 days. This suggests that bacteria grow better albeit more slowly at mid-range temperatures than at room temperature.

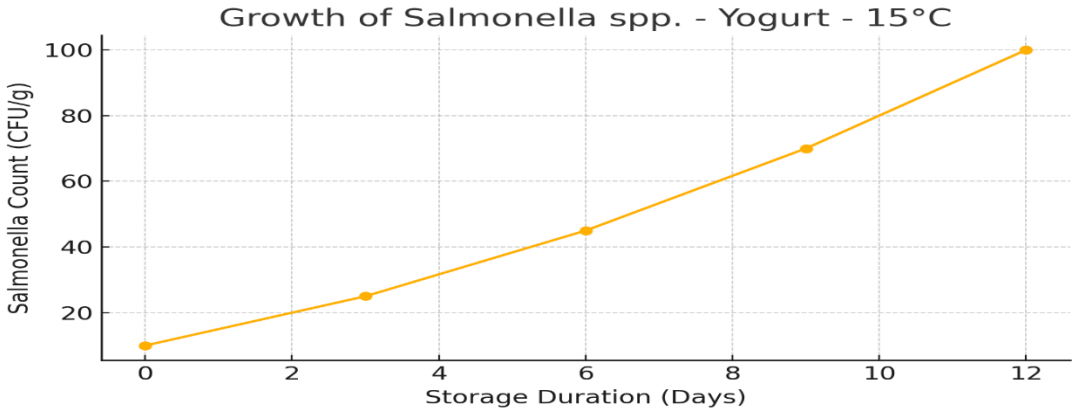

Figure 2: Accelerated Growth of Salmonella spp. in Yogurt During Storage at 15 °C.

3. Yogurt Stored at 25°C At 25°C, as described in Figure 3, a steep and continuous growth of bacterial population to 500 CFU/g was observed by 12 days. This underscores the great risk involved in holding yoghurt at a room temperature for long periods.

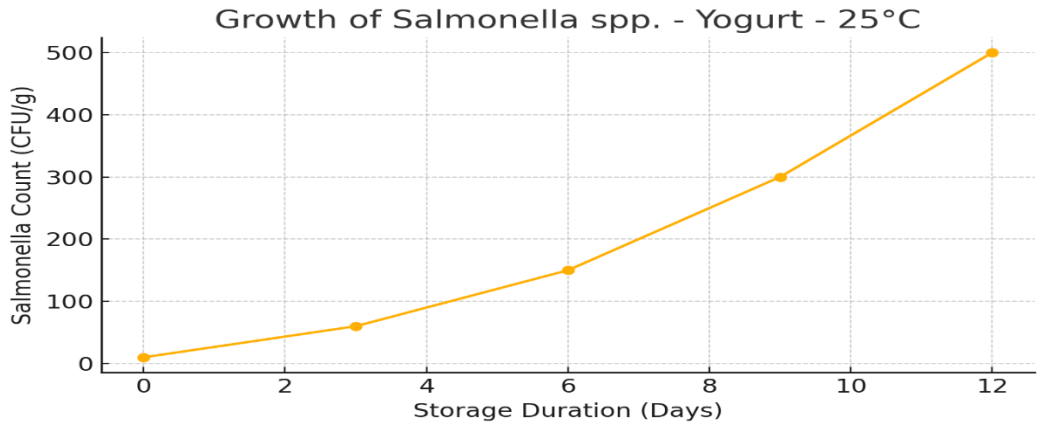

Figure 3: Exponential Growth of Salmonella spp. in Yogurt Stored at 25 °C.

4. Raw (Unpasteurized) Yogurt at 25°C Figure 4 shows a more pronounced acceleration of Salmonella spp. (OU) per mL of unpasteurized yogurt samples stored at 25°C, which increased to 700 CFU/g after the storage period. These findings indicate that bacteria pose a higher hazard in conventional dairy products due to higher initial contamination and failure to undergo a heat process.

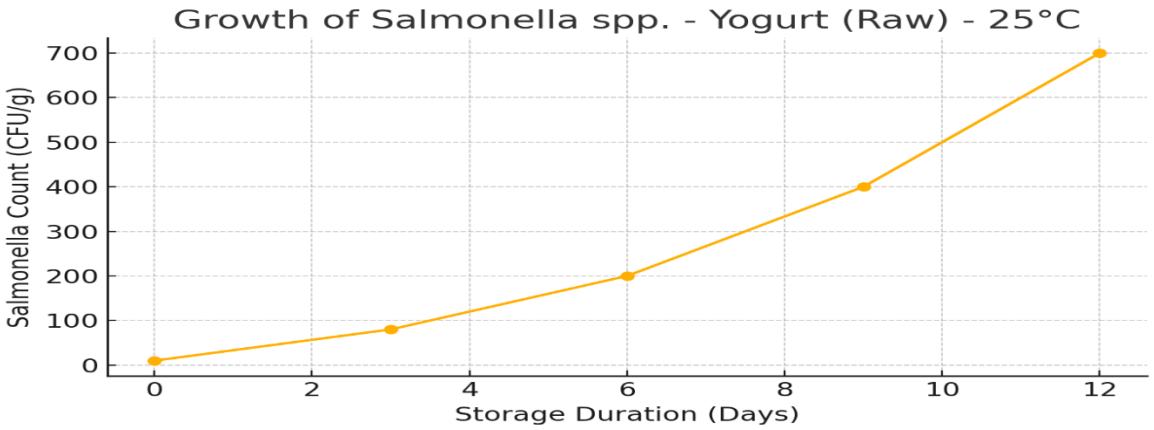

Figure 4: Enhanced Growth of Salmonella spp. in Raw Yogurt Stored at 25 °C.

5. Butter Stored at 4°C The increase in the number of Salmonella spp. during storage at 4°C of butter (Figure 5) was limited and slow, ranging from 8 to 19 CFU/g, indicating that the low water activity of butter combined with refrigeration effectively suppressed growth of bacteria.

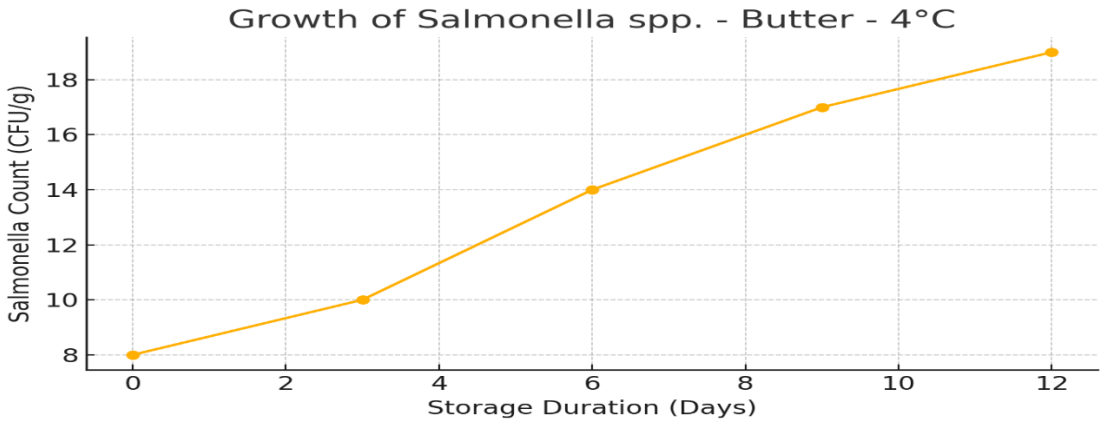

Figure 5: Growth Pattern of ***Salmonella spp.*** in Butter Stored at 4 °C.

6. Butter Stored at 15°C A retarded increase of CFU/g was observed in 15°C-stored liver samples (Figure 6), culminating in 90 CFU/g on day 12. This is consistent with the principle that moderate temperatures only encourage growth but are never as strong as with yogurt.

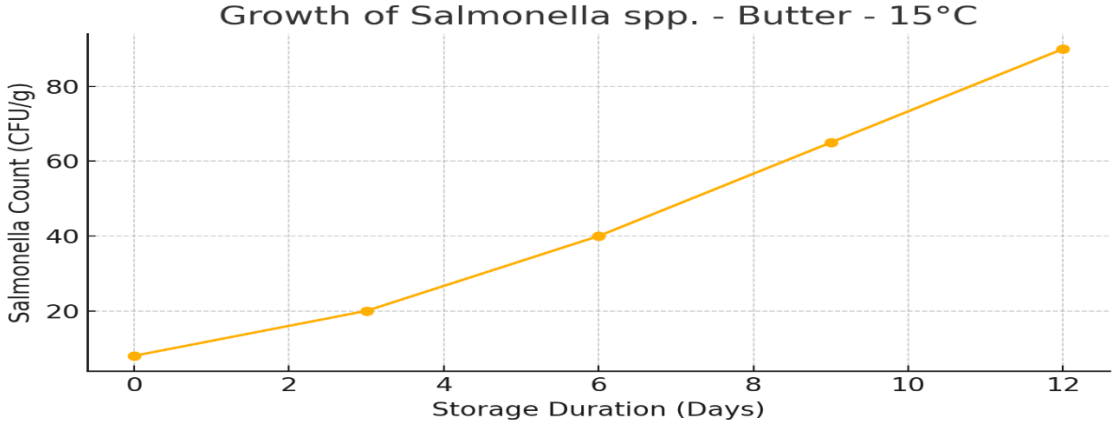

Figure 6: Effect of 15 °C Storage on *Salmonella spp.* Growth in Butter Over Time.

7. Butter Stored at 25°C Figure 7 The 2nd exponential phase There is a significant increase of the bacterial number as in butter samples 253 Figure 8 The 3rd exponential phase. reaching 460 CFU/g by day 12. While this growth was somewhat slower than was the case with yogurt, the hazard was still considerable.

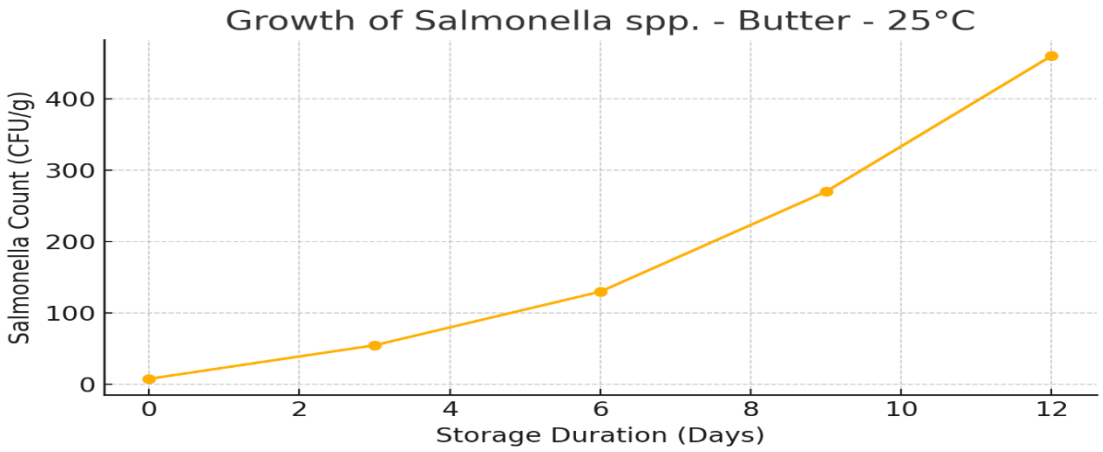

Figure 7: Growth Dynamics of Salmonella spp. in Butter Stored at 25 °C Over 12 Days.

8. Unpackaged Butter at 25°C Lastly, Figure 8 is the growth curve of unpackaged (homemade) butter stored at 25°C, recording the highest counts among butter samples, with the CFU/g count of 520 on the 12th day. This could be related to higher environmental contamination resulting from, for example, poor hygienic handling of and packaging for the poultry.

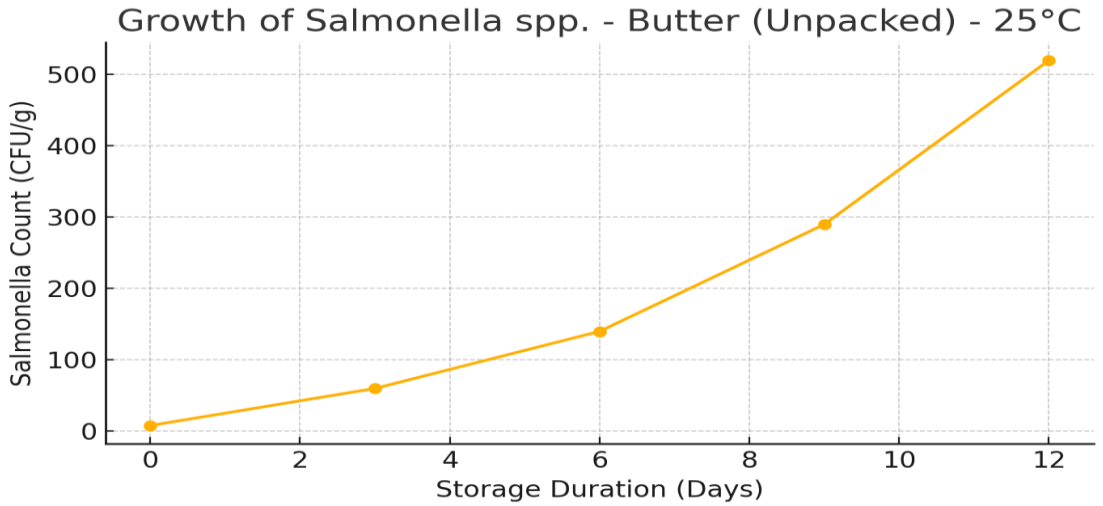

Figure 8: Growth of Salmonella spp. in Unpacked Butter Stored at 25 °C.

Summary of Findings

The findings made it evident that:

- Temperature has an instrumental effect on Salmonella spp. growth; high temperatures contribute to bacterial growth at a faster pace.
- Yogurt samples exhibited a greater rate of bacterial growth in comparison to butter, probably due to an increase in moisture as well as more favourable nutrient environment.

unpasteurised and unpackaged goods had the highest and quickest bacterial growth and the significance of pasteurisation and hygienic packaging is emphasised.

Discussion

The study by I. Votkinina and V.V. Lbitova -Telesheva (1986) shows that Salmonella spp. may remain active at refrigeration temperature (4 o C) with a small growth rate, but at ambient temperature (25 o C) the rate becomes much higher which supports the previous findings of salmonellae survival in butter at ambient temperature (see references [11, 12]). Predictive models also offer support of higher inactivation rates (k max) and growth rates when stored under higher temperatures thus high importance of strict control of storage conditions (reference [13]). The fact that the resistance of Salmonella in butter is relatively low, though not significantly lower than in yogurt, indicates that the protective effect of high fat and low water activity is similar to those found in research studies investigating the kinetics of Salmonella survival in fat containing, low moisture food matrices (references [14, 15]). More specifically, water activity limits (a t) are the determining factor of the gray zone of bacterial viability, where pathogens can survive in a matrix until a t becomes 0.93, which is the threshold between the yogurt and butter conditions (references [16, 17]). This highlights the need to consider pasteurisation and hygienic packaging, as the intended traditional processing and packaging techniques used in Sulaymaniyah might not contain any provisions to prevent contamination. Besides, the presence of Salmonella biofilms on dairy equipment is also a contributing factor because the structure of biofilm enables bacterial persistence and recontamination (reference [18]). According to thermal inactivation research, even though it is possible to apply more moderate heating conditions to inactivate pathogen contaminants, aqueous-low-water activity matrices (e.g. powdered dairy mixes or caramel sauces) need more intense heat treatments to result in a significant reduction of Salmonella populations (see references [19, 20]). These results together suggest the need to reinforce cold-chain logistics, pasteurisation, and packaging to reduce the risks of Salmonella in the traditional dairy systems.

Conclusion

This paper indicates that the viability and growth of Salmonella spp. in traditionally manufactured local cream cheese and butter in Sulaymaniyah is affected by not only the temperature- and time-of-storage parameters but also the processing parameters. The growth of Salmonella was low when the products were kept at chilling temperatures and frequency and viability were significantly high at room temperature especially in the long-term. Butter samples showed relatively low levels of contamination as compared to cheese which is probably because of decreased water activity. The results highlight an urgent necessity to improve hygiene, refrigeration and safe packaging in the formal dairy supply chain to control the exposure to microbial hazards and protect the health of population.

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