Development And Characterization Of Lemon And Ginger Juice Based Probiotic Beverage

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Abstract

Focused on the development of a lemon and ginger probiotic beverage with addition of inoculum level ranged 8 to 10 per cent and the incubation time 10 hrs. The probiotic sample prepared with 10 % LAB strains (Lactioplantibacillus plantarum and Lactobacillus delbrueckii) was found to be organoleptically more acceptable than other samples prepared with 8% inoculum.According to the results of the storage study's organoleptic evaluation, the product's taste and flavour can be preserved for one month in refrigerator storage (4^0C), considering the high viable cell count (10^9cfu/ml) even after 4 weeks of storage, as well as the technological and economic viability.It supports the viability of using lemon and ginger juice for commercial purposes. Further results of chemical analysis after storage periods TSS concentration w was found to be decreased from a starting value14 to 13.6^0 Brix. Further changes in pH, acidity and ascorbic acid were found to be 3.6, 3.5, 3.4, 3.2, 3.1, 1.2, 1.5, 2.0, 2.1, 2.5 and 20.10, 21.05, 19.00, 19.25, 18.64 respectively. Microbial analysis results show that the prepared beverage has no harmful traces of mold, yeast or coliform bacteria. To conclude, developing lemon and ginger juice-based probiotic beverages was safe for four weeks.

Keywords: Lemon and Ginger juice, Lactobacillus delbrueckii, Lactioplantibacillus plantarum.

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INTRODUCTION

Functional foods that contain probiotic microorganisms have been developed in response to consumer demand for foods that promote health while preventing diseases. Fermented dairy products are excellent vehicles for delivering probiotics, but due to the high prevalence of lactose intolerance, their high fat and cholesterol content, and the rising popularity of vegetarian diets, consumers are looking for alternatives. The potential for probiotic bacteria added in non-dairy items such fruits, vegetables, and cereals has thus been thoroughly researched(Aspri et al., 2020).

Research scholars have been inspired to create new functional foods items, such as foods with probiotics bacteria, as a result of consumers' rising interest in consuming meals thatpromote health. Probiotics are typically included in fermented foods and dairy products. However, different food matrices have been investigated as potential carriers for these microbes due to the rising number of people with lactose sensitivity and/or vegans (Gomes et al., 2021).

The popularity ofprobiotic drinks made with fruit has grown among consumers in recent years. Probiotic culture use in fruit-based beverages and beverage producers are interested in creating new or different products in the market (Prado et al., 2008).

Fruit drinks with probiotic modifications are also useful for creating health-improving products, especially for peoplewho have a dairy allergy(Sheehan et al., 2007). Probiotic added in fruit juices can be viewed as a new category of functional products because the

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bacteria that are introduced to them create a variety of bioactive compound.because of delivery advantageous health qualities from both sources, such combinations could enhance the nutritive attributes of fruit juices and bring about health advantages fruit juices and probiotic(Petric & Putnik, 2020).

Fruit juices and other drinks in the same category provide an excellent probiotic delivery vehicle. Fruit juices are healthier when probiotics are added since fruits are naturally abundant in important macro- and microelements. There are many obstacles to be addressed, such as probiotics' ability to survive and their impact on sensory qualities (Patel A.R, 2017).A highly functional synbiotic product is created by combining healthy lactic acid bacteria (probiotic) and nutrient rich fruit juices (prebiotics).

Mixing the fruit with other fruits can reduce its acidity and using cutting edge food processing technologies can increase cell viability (Mustafaa et al., 2016).Recently, beverages prepared from fruits, vegetables, cereals, and soybeans have been proposed as new products containing probiotic strains; fruit juices in particular have been identified as a distinctive and ideal probiotic medium due to their incorporation of essential nutrients. Additionally, they are typically described as foods that are healthful and suitable for all age groups (Luckow & Delahunty, 2004).

The demand for non-dairy probiotic products with their nature as healthy alternatives to dairy probiotic foods match the demand for non-dairy probiotic foods alternativesto dairy probiotic foods, it is imperative to develop new, economical, and technological matrices. Although there is a lot of potential for fruit juices to be used as probiotic goods, there aren't many studies on how they're made or produced (Reddy, 2015).

Both the creation of food and the maintenance of good health depend on lactic acid bacteria (LAB).The discovery of the numerous potential health advantages linked to these species is generating growing interest. The numbers of bacteria present in the gastrointestinal system affects the activities of LAB, which are species and strain-specific. Consumers are particularly concerned about processed foods and artificial preservatives. The use of LAB in products or processing is recognised as a natural method of food preservation and health promotion. This study sought to analyse recent research on the probiotic LAB's function in food preservation, gastrointestinal tract immunomodulation, and health benefits (Quintoet al., 2014).

Probiotic microorganisms, which are prevalent in fermented foods, also aid in digestion and the creation of vitamins and other nutrients, prevent and treat diarrhoea and suppress the growth of food spoilage organisms(Kavitha, M. B., & Kiruthika, 2020). The nutritional and therapeutic benefits of citrus are well known all over the world. Traditional medicine uses the plants that make up the medicine. The literature that is currently available does not indicate any negative effect(Chaturvedi Dev et al., 2016).

Lemon

In the *Rutaceae* family, lemon is a significant therapeutic plant. The main reason it is grown is for the alkaloids in it, which have anticancer qualities. Lemon's leaves stem, root, and flower have been demonstrated to have antibacterial properties against a clinically significant bacterial strain when used in crude extract form(Kawaii et al., 2000).

The term "lemon" is derived from old French. There are numerous additional names for lemon fruits. The key components of the chemical composition include flavonoids, acids, caffeine, pectin, and minerals. Lemon is antibacterial, antifungal, anti-inflammatory, anti-cancer, depurative, and antiscorbutic, among other properties(Qudah et.al, 2018).

Lime is a common ingredient in sorbets, beverages, pickles, jams, jellies, nibbles, candies, sugar-boiled confectionaries, and culinary dishes all over the world. Lime comes in a wide range of varieties and is especially common in tropical and Mediterranean climate(Mohanapriya et al., 2013).

Citrus fruit juices have long been prized for their healthy, antioxidant and nutritive qualities. Citrus fruits and juice also offers

many other established health advantages due to their abundance in vitamins, minerals and antioxidants. Lemon juice has the potential to increase chemical properties including antioxidant activity and antibacterial properties. a useful non-dairy food item(Hashemi et al., 2017).

Ginger

Ginger(*Zingiber officinale*) is a very effective in gallstone therapy and aids in the proper release of bile. It lowers cholesterol and is thought to be helpful in cardiovascular treatment. Additionally, it is said to lessen the risk of arthritis(Moghaddasi & Kashani, 2012). Due to their nutritional benefits and high energy content, consumers prefer to consume beverages with ginger as an ingredient. Ginger beverages have been effective for supplying proper nutrition and health advantages, which aids to improve consumer health and the socioeconomic status of the area(Ahsan et al., 2021).

Materials and Methods

Lemon, ginger,sugar and glass bottles were procured from a nearby market. The chemicals, processing equipment, and analytical tools were obtained from School of Science Department of Microbiology, Sandip University Nashik.

Isolated bacterial culture

Culture *Lactioplantibacillusplantarum* and *Lactobacillus delbruckii* were isolated and identified by using 16SRNA sequencing then stored on slants at 4ºC at School of Science Department of Microbiology, Sandip University Nashik.

Making starter cultures

With a few adjustments, the approach reported by(Thakur & Sharma, 2017) was used to prepared the starter culture. Probiotic organisms like *Lactioplantibacillusplantarum*and *Lactobacillus delbruckii* were cultivated separately in MRS broth for 48 hours at 37^{0}C. The cells were then harvested by centrifugation the cultured MRS broth at 4000 rpm for 10 minutes.The remaining MRS media was removed from the collected biomass by twice washing it in sterile saline solution. Consequently, inoculum was made shown in **fig 1.**

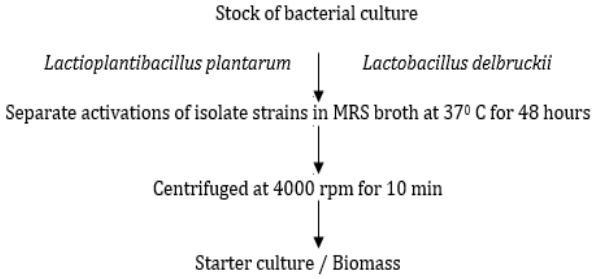

Figure 1 Production of starter culture

Lemon and ginger juice preparation

Lemon and ginger were procured from local market in Nashik, Maharashtra. Lemon and ginger juice was prepared by usingjuicer and mixerthen juice pasteurized to 80^{0}C for 10 minutes followed by blending at various concentrations shown in figure2, 3and table 1. The prepared beverage's total soluble solids were maintained at 14^0 Brix and refrigerated at 4^{0}C.

Extraction of lemon juice

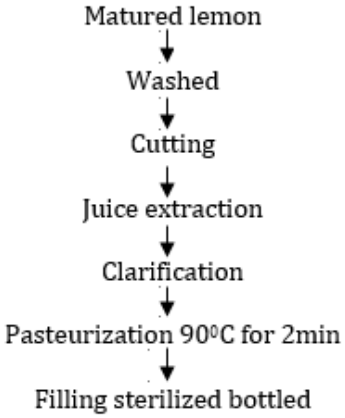

Figure2 Extraction of lemon juice

Extraction of ginger juice

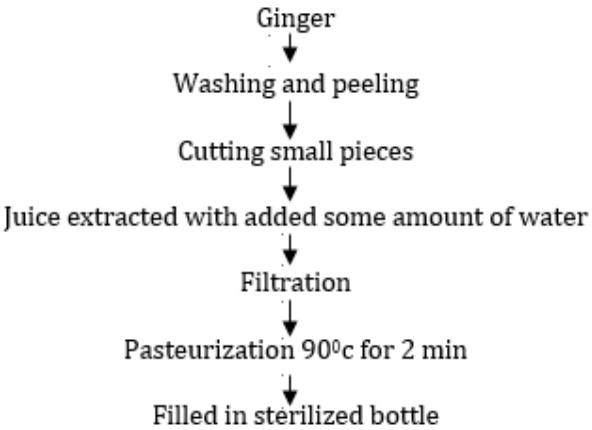

Figure 3 Extraction of ginger juice

Preparation of different concentration of lemon and ginger juice

Standardization of lemon and ginger juice ratio based on sensory evaluation. Different concentration of juice blend was prepared, on the basis of sensory evaluation score, shows in **Table 1.**

Table 1different concentrationlemon and ginger juice

Ingredients	**A**	**B**	**C**
Lemon juice	95 ml	90ml	85ml
Ginger juice	5ml	10ml	15ml

Standardization of TSS content in blended lemon and ginger juice

Lemon and ginger juice's initial TSS was not very appetizing as organoleptically. So beverage samples with different TSS values were maintained through sensory evaluation.TSS variations ranging from12 to 14^0 Brix; were used to create blended lemon and ginger juice samples, as shown in **Table 2**.

Table 2 Standardization of TSS contentin lemon and ginger juice

Sample	TSS
A	12
B	13
C	14

Standardization of probiotic lemon and ginger juice preparation

Lemon and ginger juice was prepared differently depending on the amount of inoculum level and incubation time. Blended juice was allowed to incubate in order to standardize the preparation of probiotic beverage. Equal quantities of the starter cultures *Lactioplantibacillusplantarum* and Lactobacillus *delbruckii* were employed. The inoculum level was between 6 and 10 percent and the incubation period varied from 6 to 10 hours

Table 4 Standardized Parameter for the Preparation of Probiotic blended lemon and ginger juice

Parameter	**Standardization**
Lemon juice	85ml
Ginger juice	15ml
L.P	5%
L.D	5%
Fermentation time	10hrs

Table 10 Preparation of probiotic blended lemon and ginger beverage were developed in variations with respect to inoculum quantity and incubation period

Juice Sample	Inoculum%	Incubation time(h)
T1	6	6
T2	8	6
T3	10	6
B1	6	8
B2	8	8
B3	10	8
C1	6	10
C2	8	10
C3	10	10

Each value is the average of three determinations L.P. – *Lactioplantibacillusplantarum*, L.D.– Lactobacillus delbruckii. T1B1C1 (6%) =3% L.P. + 3% L.D., Incubation time (6h); T2B2C2(8%) = 4% L.P. + 4% L.B.Incubationtime (8h); T3B3C3 (10%) = 5% L.P. + 5% L.D. Incubationtime (10h).

Production of probiotic beverage

The probiotication studies were carried out in 200ml glass bottles containing 100ml of pasteurized blended juices after standardizing the inoculum quantity and incubation period based on organoleptic analysis. To prepare the juice was mixed with the starter culture at a 10% inoculum level (5% of*Lactioplantibacillusplantarum* and 5% *Lactobacillus delbruckii*each), and the mixture was then incubated for 10 hours at 37°C shows as**figure 4.**The probiotic beverage was stored at 4^0C for further storage studies.

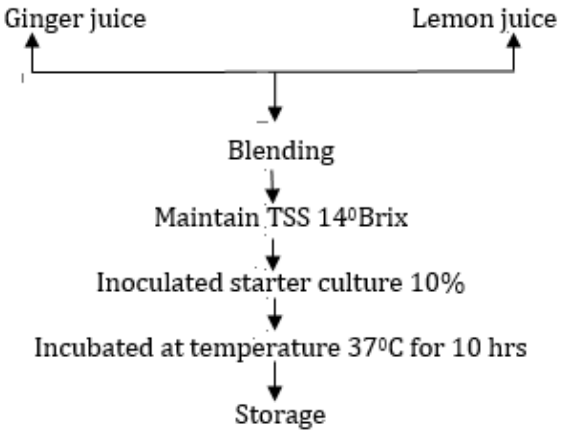

Figure 4 Production of lemon ginger probotic beverage

Sensory analysis

The sensory evaluation was carried out by a panel of judges with semi-trained; samples

were assessed on a 9-point Hedonic scale.Here a taste panel made up of five people selected from the School of Science Sandip University staff and student determined the beverage's sensory qualities. The panellists were tasted with rating the samples according to their appearance, aroma, taste, flavour, and overall acceptability using a composite score system stated in **Table 4 and 5**(Yamagata Sugawara, 2014).

Sensory assessment of samples with changes in Total soluble solids

The information in Table 8 showed that the samples made with different TSSs had taste and flavour ratings that ranged from 7.5 to 8.5. Sample C (8.5) had the highest rating for general acceptance, which was determined to be considerably higher than

Chemical characterization of developed probiotic beverage

Total soluble solids: The sugar concentrations of the probiotic beverage were determined by using hand Refractometer(William et al., 2005)**.**

PH: After calibrating the pH meter with buffer solutions with pH values of 4.0 and 9.2, then pH was measured. Readings were taken after the pH electrode was dipped into the sample(William et al., 2005)**.**

Titratable Acidity: It was possible to estimate the sample's titratable acidity by exposing a known aliquot to an N/10 NaOH solution while using phenolphthalein as an indicator. The proportion of lactic acid is used to calculate and express the titratable acidity, as will be detailed below.

$$\textbf{Tritable acidity (\%)} = \frac{\text{Volume of NaOH consumed for titration} \times \text{Normality of NaOH equivalent weight of acid} \times 100}{\text{Volume of the sample taken}}$$

Ascorbic acid: Using the titration method and 2, 6-dichlorophenol indophenols, the ascorbic acid concentrations of the samples were determined.

Storage stability of the finished probiotic beverage

All of the probiotic juices that were chosen were kept in a refrigerator, at intervals of 7 days, the various probiotic juices were examined for their pH, acidity, sensory evaluation, viable count, and microbial count of survival for up to 28 days of storage shown in **Table 6.**

Microbial examination of probiotic beverage

The ideal protocol for quality assessment is a microbial investigation, which is carried out in quality analysis of It is a must in a probiotic product, though. Coliform count, yeast and mold count, and total plate count were all examined.

Total plate count

Microbial analysis was carried out to determine the probiotic beverage's total plate count (TPC) on nutritional agar media (1966). Plate count agar medium was produced and sterilized for 15 minutes at pressure (15 psi). Up to eight times of serial dilutions were prepared, and microbe counts of 10^{-7}, 10^{-8}, and 10^{-9} dilutions were examined. 15-20mL of medium was added to a petriplate with 1ml of the diluting.The plates were incubated at 37^0C for 48 hours. The plates and colonies were counted using a colony counter(Cappuccino & Sherman, 1996)

Total bacterial count
(CFU/mL)=no .of colonies x dilution factor/mL of aliquot

Yeast and mold count Microbial

The pour plate method was used to calculate the yeast and mold counts. The PDA media was prepared and sterilized at pressure (15 psi) for 15 minutes. The sample was diluted up to eight times, and 1 mL of each aliquot was utilized for plating. Results were recorded in CFU/mL after plates were incubated for 48–72 hours at37^0C. Yeast and mold count was examined every week (Cappuccino & Sherman, 1996) as results shows in **Table 10.**

Coliform test

It is essential to check for contamination becausethe indicator bacteria of feces-related water contamination are coliform, particularly E. coli. It was used for testing because the coliform red, pink, or purple colonies on VRB

agar. The pour-plate method was used to add tempered VRB agar to 1mL aliquots that were appropriately placed in duplicate plates. After allowing the agar to settle, a second layer of around 5 mL of VRB agar was added. Plates were turned over and incubated for 24 hours at 37^0C. Presumptive Coliforms are reported as red colonies with a precipitation zone around them in CFU/mL(Cappuccino & Sherman, 1996) as results shows in **Table 10.**

Result and Discussion

Production of lemon ginger probiotic beverage

All probiotic beverages sample A, B, and C sample of probiotic beverage was prepared. But based on sensoryanalysis results found to be sample C most acceptable than other sample.

Sensory assessment of probiotic beverage with changes in Total soluble solids

The information in Table 5 showed that the samples made with different TSS levels scored between 8.4 and 8.9 for appearance, taste, and scent. Sample C3 (8.9) received the highest rating for general acceptability and was determined to be superior to samples T1 and B2.

Sensory analysis of probiotic beverage with varying inoculum levels and incubation times

The information in Table 6 demonstrates that the T2, T3, and C1 scores are all equal. Sample C3 is determined to have the highest mean taste rating (8.9), making it more palatable (fig. 4).

Samples B and C's overall sensory score (acceptance) while being stored

According to the information in Table 7, sample C had a higher overall acceptability score than sample B after a four-week storage period(Panghal et al., 2017) (Fig. 4).During the storage periods sensory evaluation of total three sample T, B.and Canalyzed according to their taste, aroma, appearanceand overall acceptability using a composite score system and final selection of beverage on basis of score obtained. All three sampleswere analyzed at weeklyintervals. Results revealed that there was little difference in the sensory aspects of all three samples when they were tasted just after incubation.

Chemical analysis of (sample C) beverage after storage

During storage periods of 4 week developed probiotic beverage,TSS concentration was observed to have decreased from a initial value of 14 to 14.6^0 Brix .Then changes in pH, acidity and ascorbic acid during storage of refrigerated storage periods was found to be 3.6, 3.5, 3.4, 3.2 and 3.1 ,1.2,1.5,2.0,2.1,2.5,and 20.10,21.05,19.00,19.25,18.64 respectively.Following the production of probiotic beverages, an isolated lactic acid bacterialculture may have used carbohydrates and created a small quantity of organic acid, reducing the pH of the product during storage shows in **Table 8**.

Viability of developed probiotic beverage during storage

On the basis of sensory analysis C sample was selected for analyze viability condition.The viability was checked second week of storage periods.The quantity of probiotic bacterial viability increased in first and second week from3.9 x 10^9 to 5.5 x 10^9. However, after three and four weeks of storage at 4^0C, the probiotic bacteria's viable numbers decreased.. Despite a decline in viable count, it was still higher than the therapeutic minimum dose of 10^8cfu/ml and above the acceptable level shows in **Table 9**. Same results reported that,(Ahmed et al., 2013),and(Rathod PS, 2017).

Microbial testing of probiotic beverage

The probiotic beverage was prepared under sanitary and hygienic conditions, which ensured that it was free of coliform and E. coli and that it would remain fresh for the whole four-week storage duration at refrigerator temperature (4^0C). The steady decrease in the yeast and mold population is attributed, in part, to the rise in acidity that followed storage, as shown by the findings from Table 10.

Discussion

It may be concluded from (Hashemi et al., 2017) that citric acid, total phenolic compounds, and sugar levels were dramatically decreased in fermented sweet lemon juice, demonstrating L. Plantarum ability to consume

higher amounts of the substrate in comparison to other strains observed. After the sweet lemon juice underwent fermentation and lactic acid production, the ascorbic acid content did not, however, change appreciably right away. After 36 hours of fermentation, L. plantarum cell counts were 8.52 log 0.34 log CFU/mL; however, after 28 days at 4 C, they had decreased to an acceptable level of 7.14 log 0.21 log CFU/mL. The chemical characteristics, such as antioxidant activity and antibacterial capabilities, of sweet lemon juice during fermentation may be enhanced.(Pandey et al., 2019) reported a decline in pH of probiotic beverage from whey and pineapple juice after 20 days period of storage (Biswas et al., 2016). Similar observations were recorded by (Islam et al., 2015) who carried out the analysis on mixed fruit juice from orange and pineapple.

Adding probiotic starter culture to a beverage lowered its pH while simultaneously raising its titratable acidity, according to(Martín-Diana et al., 2003), The pH of the product may have been lowered during storage as a result of a LAB culture using carbohydrates to produce a small amount of organic acid.

Table 5 Sensory evaluation with variation in TSS

Juice sample	Appearance	Taste	Aroma	Overall acceptability
Control	6.940	7.040	6.920	6.800
B	7.060	7.340	7.340	7.460
C	**8.440**	**8.600**	**8.700**	**8.920**
SE(M)	0.089	0.160	0.153	0.133
CD	0.277	0.500	0.476	0.414
SE(d)	0.126	0.227	0.216	0.188
CV	2.663	4.683	4.463	3.846

Table Sensory analysis of probiotic juice samples with various inoculum concentrations and incubation periods

Juice sample	Appearance	Taste	Aroma	Overall acceptability
Control	6.840	7.020	7.020	6.840
B	7.020	7.200	7.300	7.180
C	**8.520**	**8.560**	**8.620**	**8.900**
SE(M)	0.112	0.110	0.117	0.108
CD	0.348	0.342	0.363	0.337
SE(d)	0.158	0.155	0.165	0.153
CV	3.347	3.232	3.410	3.161

Table 7 Sensory score of Sample B and C after storage periods

Juice sample	Appearance	Taste	Aroma	Overall acceptability
Control	7.060	7.140	6.860	6.960
B	7.200	7.320	7.320	7.480
C	**8.620**	**8.640**	**8.680**	**8.960**
SE(M)	0.145	0.152	0.143	0.172
CD	0.451	0.475	0.446	0.535
SE(d)	0.205	0.215	0.202	0.243
CV	4.249	4.423	4.198	4.927

Table 8 Chemical analysis of probiotic beverage during storage

week	(TSS^{0}Brix)	pH	% Acidity	Ascorbic acid
0	14.0	3.6	1.2	20.10
1	14.1	3.5	1.5	21.05
2	13.3	3.4	2.0	19.00
3	13.7	3.2	2.1	19.25
4	13.6	3.1	2.5	18.64

Table9 Viability of probiotic beverage

Week	Viable count (CFU / mL) of probiotic bacteria
0	$1.2x10^8$
1	$3.9x10^9$
2	$5.5x10^9$
3	$2.4x10^9$
4	$1.3x10^9$

Table 10 Microbial evaluation of probiotic beverage

Sr.No.	Properties	Results
1	TPC (CFU / ml)	8.8×10^9
2	Yeast mold count (CFU / ml)	Nil
3	Coliform count (CFU / ml)	Nil

Conclusion

The results of the current experiment indicated that the 10% LAB strain probiotic beverage "C" sample (Lactioplantibacillus plantarum and Lactobacillus delbrueckii) was determined to be more consumable organoleptically than other beverage preparations. It also concluded that these lemon ginger probiotic beverages may be consumed without flavour or taste changing after four weeks of storage. 10^9CFU/mL of probiotic cultures, which are beneficial for preserving the health of the gastrointestinal system, were discovered in the beverage according to the microbiological analysis. Additionally, there was no evidence of coliform bacteria, yeast, mold, or other potentially harmful microorganisms in the prepared beverage, indicating that only healthy bacteria were present.

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Conflict of Interest

Authors claim they have no conflicts of interest.

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