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Comparative study on rheological and physiochemical properties of PEF (Pulsed electric field) treated rice and black gram mixed batter during fermentation

Praveen Kumar Dubey, Dr. Ashish Rawson, Ananda Kumar Sugumar and N Baskaran

Abstract
The aim of this study was to examine the rheological and physicochemical characteristics of rice and black gram batter following exposure to a 20 kV/cm pulsed electric field (PEF) with a constant pulse width of 10 µs at 30±2 °C for 0, 6, 12, and 24 hours. PEF treatment significantly increased pH, color freshness, bulk density, and conductivity value in fermented batter compared to the untreated batter. However, the PEF treated sample's water activity showed no significant changes. Apparent viscosity was found lesser in PEF treated batter in comparison to untreated batter at 30 °C as the increase in shear rate. The results of this study imply that PEF treatment at 20 kV/cm may boost bioactivity and bioavailability due to increased values for the batter's pH, bulk density, and conductivity, which demonstrate that the batter's properties do not significantly change until 24 hours at 30 °C.

Keywords: Apparent viscosity, pulsed electric field, batter, color, conductivity

1. Introduction

Rheological property is an important factor in the production of rice and black gramme batter-based foods (Ronda *et al*., 2011) [22]. Since raw materials can have a considerable impact on rheology. Rheological properties are essential for quantitatively evaluating batter characteristics including spreadability and firmness (Bhattacharya and Bhat, 1997) [5]. It is a method of analysis that provides a fundamental insight of how food is structurally organised and is required for heat transmission to fluid food (Ahmed *et al*., 2007) [1]. These traits have a considerable impact on the sensory attributes and customer acceptability of the end product. For the automation of the process in the production setting, rheological information is essential (Manickavasagan *et al*. 2013; Nagaraju and Manohar, 2000) [15, 18].

Cereals are generally low in lysine but high in sulfur-containing amino acids, whereas legumes are low in methionine and cysteine but high in lysine. One of the high-carbohydrate staple foods grown in more than 100 nations is rice (Rani *et al*., 2019) [21]. Easy to digest, hypoallergenic, and containing gluten-free protein is rice starch. Due to its higher GI, rice starch is essential for providing sports and marathon runners with quick energy (Bai *et al*., 2021) [3]. In comparison, black gram is high in protein, fibre, and vitamin B. Therefore, the fermented batter made from cereals and legumes can make up for the shortage.

On the Indian subcontinent, the main crops are rice and black gram. Rice-black gram batter is often utilized in numerous South-East Asian countries' traditional convenience foods (TCFs) (Bhattacharya and Bhat, 1997) [5]. Black gram can be replaced by chickpea, soybean, or green gram. Rice can be replaced with wheat or maize to make Indian dhokla, jalebi, idli, dosa, and so on (Ramakrishnan 1979; Steinkraus 1996) [20, 24]. Rice and black gram are traditionally soaked and pounded in a mortar and pestle while adding water to formulate a batter with the correct consistency. Salt is added to the batter in a tiny proportion. *Leuconostoc mesenteroides* and *Streptococcus faecalis*, which are naturally present in grains, legumes, and utensils and proliferate quickly, overwhelm the initial contaminants and take control of the fermentation while the batter ferments overnight. The batter is made anaerobically by these bacteria, which also leaven the finished product by generating lactic acid (>1.0%) and carbon dioxide. Several factors include the impact of raw materials, the temperature impact of fermentation and processing, and the role of microorganisms in nutritional and biochemical changes.

As a processing factor, the pulsed electric field is a non-thermal process and has the potential over thermal processing.

PEF developed a region in the cell membrane to deactivate and release intercellular and intracellular media (Lebovka *et al*., 2011) [13].Nutrient, color, and flavor loss are more prevalent during thermal processing and chilling, however, PEF can prevent these issues. PEF has little impact on the volatile freshness and nutritional value of food goods, including vitamin C, terpinolene, -phellandrene, -fenchyl alcohol, and -terpineol in orange juices (Lee *et al*., 2021) [14]. To meet these requirements, PEF performs better than conventional pasteurization, such as chemical and biological preservation. The pulse electric field uses short pulses of less than a second between two electrodes to inactivate microorganisms and enzymes with minimal impact on food quality. As a result, in order to address the demand for ready-to-cook cereal-legumes-based foods, an attempt was made to explore the physiochemical and rheological behaviour of PEF treated and untreated batter produced of parboiled rice and decorticated black gramme blends during their fermentation period, as well as to generate the basic data required for batter automation utilising PEF treated non-thermal procedure.

2. Material and Method

2.1 Materials

Parboiled rice variety IR20, decorticated black gramme (*Phaseolus mungo* L.) variety Aduthurai 3 (ADT3), iodized salt from a neighbourhood grocery store, and phenolphthalein indicator, NaOH from Himedia, Tiruchirapalli, India were all purchased. The other chemicals used in this study were all of analytical-reagent quality (Merck, Sigma, Chennai, Tamil Nadu, India).

2.2 Batter preparation

The batter was prepared using the method proposed by Iyer and Ananthanarayan in 2008. A 3:1 (g/g) ratio of parboiled rice and decorticated black gram was weighed and taken. Each was rinsed individually with distilled water. Rice and black gram were soaked separately in distilled water at a 1:4 (g/l) ratio for 4 hours. When making a fine paste (Ultra Fastgrind, 2 litres, 150 W, India), rice and black gram are both drained and processed separately with a 1:1 water ratio. Then, 0.9% iodized salt and a batter consisting of rice and black gram paste are added. The batter was then allowed to ferment in 100 ml closed glass jars at 30±2 °C for various fermentation durations (0, 6, 12, and 24 h).

2.3 PEF machine

Pulsed Electric Field treatment can be done in either a continuous or stationary mode using monopolar square wave pulses. PEF Generator and transformer assembled treatment unit make up a newly developed PEF system (Sureview Instruments LLP, India). The pulsed strength of a PEF generator ranges from 1 kV/cm to 40 kV/cm. A supply current, an energy storage component, a switch, and a switch for setting a pulsed parameter (single, multiple, and continuous) make up the PEF generator. There are single, multiple, and continuous processes built into the PEF system. It includes the four properties of pulse width, pulse off time, pulse number, and pulse duration.

PEF was carried out in a static mode chamber that contained 250 ml of fermented batter. The experiment's parameters were a steady medium electric field intensity of 20 kV/cm, a pulse width of 10 µs, a pulse off-time of 20 ms, a number of pulses of 1499, and a treatment duration of 30 seconds. A total of eight samples of batter were taken for physiochemical as well as rheological parameters analysis based on certain time intervals. Four batters received PEF treatment, while the remaining four were untreated control batters.

2.4 pH and conductivity

The pH was determined in accordance with Dubey *et al*. (2019), with each sample measured in triplicate (n=3). A sample of 10 g of PEF treated and untreated rice and black gram batter at 30±2 °C was mixed with 100 ml of distilled water, vortexed for 2 minutes, and the pH was then measured using a pH meter (model- PH-3813, Analytical technologies limited, India).

By Akin and Evrendilek (2009) [2], a conductivity meter was used to measure the sample's conductivity (model-conductivity-3813, Analytical technologies limited, India). The results of the samples' conductivity were presented in µS/cm and mS/cm.

2.5 Percentage titrable acidity

The prepared PEF treated and untreated rice and black gram batter (3g) was diluted with distilled water (20 ml) and titrated using 0.1 (N) NaOH and phenolphthalein as an indicator (Gaikwad *et al*. 2020). The following formula was used to compute the total acidity of the batter at intervals of 0, 6, 12, and 24 hours:

$$\text{Titratable acidity (\%)} = \frac{\text{The equivalent weight of acid} \times \text{N of NaOH} \times \text{Titratable value}}{\text{Weight of sample} \times 1000} \times 100$$

[4].

2.6 Water activity

According to Preethi *et al*. (2020) [19], the water activity of PEF treated and untreated rice and black gram batter at a certain time interval was measured in a triplicate (n=3) by transferring 3g batter into the small size water activity plastic cup and inserted into a water activity meter (AQUALAB 4TE (Pullman, Washington (USA)).

2.7 Bulk density

The PEF treated and untreated batter rice and black gram batter (100 mL) was measured in a measuring cylinder, and its weight was calculated using an analytical balance (BL220H, Shimadzu Corporation, Philippines) to estimate the bulk density (ratio of mass by volume) of the sample and expressed as $g.cm^{-3}$ (Balasubramanian & Viswanathan, 2007)

2.8 Gas formation in the Aluminium pouch

Gas formation of PEF treated and untreated rice and black gram batter of 100 ml was filled into 200 ml aluminum pouches, sealed, and placed at 30±2 °C for 24 hrs to check the volume of level gas formation at the different time intervals between the treated and untreated batter.

2.9 Optical properties

The color value of the prepared PEF treated and the untreated batter was assessed using a USA-made Hunter Lab colorimeter, model CFEX-0925 (Preethi *et al*. 2020) [19]. Before analysis, the Hunter Lab is first standardized using a black and white plate. The value in terms of L*, which is lightness in the range of 0 to 100 (indicating black to white), a*, which denotes +a* to -a* (redness to greenness), and b*, which denotes +b* to -b*, are then displayed to Hunter Lab (yellowness to blueness). According to Manzoor *et al*. (2019) [16], the color value measurement was assessed by averaging each triplicate form of treated and untreated fermented batter individually using the formula in Equation 2.

$$\Delta E = \sqrt{(\Delta l)^2 + (\Delta a)^2 + (\Delta b)^2}$$

2.10 Rheological assessment
The rheological behavior of rice and black gram batter was investigated using steady-state shear measurements of the material supply (Shanthamma *et al*. 2021) [23]. Rheometer (MCR 52 series, Anton Paar Co. Ltd., Austria) measurements were performed using parallel plates with a distance of 100mm between them and a diameter of 25 mm. The steady-state measurements were carried out in the 0.1 to 100 s-1 shear rate range at 30 °C and 50 °C. The collected data were fitted with a Herschel-Bulkley model to evaluate the rheological properties of the material supply (Eq. 3).

$$\sigma = \sigma_\gamma + K\gamma^n \quad \text{Eq 3}$$

Where σ is shear stress in Pa, σ_γ is deformation rate or shear rate in s^{-1}, K is the consistency coefficient in $Pa.s^n$, and n is the dimensionless flow behavior index.

2.11 Statistical Analysis
According to Duque *et al*. (2020) [7], Minitab 20 Statistical software was used to carry out all of the statistical analyses. Results were statistically analyzed as mean standard error for three different measurements. One-way ANOVA was used to analyze the data sets of PEF-treated and untreated batter samples at various time intervals, and the post-hoc Tuckey's Honestly Significant Difference (HSD) approach was chosen to determine the mean significant difference and 95% confidence level.

3. Result and Discussion
3.1 Bulk density, pH, titratable acidity (TA)
The pH of the rice and black gram in the PEF-treated and untreated batter ranged from 6.2 to 4.5 (Table 1), placing it in the same range as milk, soda, and rainfall. The pH value is dropping, which means that the environment is becoming more acidic. This is mostly caused by the expansion of S. faecalis, which also releases carbon dioxide to leaven the batter and lactic acid to lower pH. The amount of soluble nutrients rises when black gram is soaked in water, promoting the growth of lactic acid bacteria. The bulk density and titrable acidity, however, were found to be between 1.007 to 0.57 and 0.65 to 0.23, respectively. Similarly, according to Balasubramanian & Viswanathan (2007) [4], the rice and black gram batter pH approximately ranged between 5.9 to 4.1, bulk density 0.94 to 0.59 and titratable acidity 0.4 to 0.9 at the time between 0 hr to 24 hrs.
However, compared to the control sample, PEF treated at 20kV/cm on rice and black gram batter at 30±2 °C exhibits only little or no significant changes in pH, bulk density, and titratable acidity (Table 1). Similar pH and acidity results were investigated by Morales-de La Pena *et al*. (2010) [17] in a fruit juice-soymilk beverage treated with high-intensity PEF (35 kV/cm, 4 μs bipolar pulses, treatment period was 800 or 1,400 μs, and frequency 200 Hz). Similar outcomes were noted by Manzoor *et al*. (2019) [16] on almond milk, which revealed a non-significant difference in bulk density, pH, and titratable acidity at pulse strengths of 7, 14, 21, and 28 kV/cm, bipolar square-wave, the pulse width of 40 μs, frequency of 1 kHz, and treatment time of 200 μs. Therefore, PEF does not affect bulk density, pH, and titratable acidity on the batter of rice and black gram.

3.2 Water activity and conductivity
Water activity is often used as a significant indicator of product degradation. Table 1 depicts water activity for rice and black gram batter between specific time periods at 30±2 °C for untreated and PEF treated (20 kV/cm) materials. It was found that there is no significant difference between PEF treated and untreated sample, at 12 hrs water activity value of treated and untreated sample shows slight changes. Cropotova *et al*. (2021) [6] found that pulsed strengths of 0.3 and 0.6 kV/cm had no influence on the water activity of sea bass when compared to the control sample.
The conductivity of rice and black gram batter demonstrated a substantial difference between untreated and PEF treated batter samples at specific time intervals at 30±2 °C, ranging from 159.4 μS to 2.87 mS. The conductivity results in soymilk-tofu heated by ohmic resistance at 140, 150, and 160 V were comparable. This result is consistent with results by Manzoor *et al*. (2019) [16], who observed that almond milk's conductivity increased at pulse strengths of 7, 14, 21, and 28 kV/cm. The increase in nutrients brought on by PEF treatments may be responsible for the increase in conductivity. The conductivity of the fluid increased with the rise in the electric field intensities at essentially the same rates regardless of the method of mixing (Park, Ryu, Kim, & Kang, 2021) [14]. Hence, the conductivity of PEF-treated rice and black gram batter increased compared to the untreated batter.

3.3 Optical properties
Color assessment is one of the most important factors in establishing product quality. Color coordinates of PEF treated and untreated batter were acquired to examine the impact of PEF on quality immediately after treatment (0 hrs), 6 hrs, 12 hrs, and 24 hrs after PEF application. The colour of the batter altered as a result of PEF treatment and fermentation intervals. The batter of untreated rice and black gram had lightness L* of 89.62 to 90.15, redness a* of -0.2 to -0.43, and yellowness b* of 10.33 to 11.31 (Table 1). Whereas, PEF treated at 20 kV/cm on rice and black gram batter shows L* value 89.27 to 90.09, redness a* value -0.17 to -0.61, and yellowness b* value 10.85 to 10.45. Gua *et al*. (2014) studied the color of pomegranates treated at PEF strengths of 35 and 38 kV/cm, 281 s at 55 °C, and discovered that the L* value was somewhat lower, a* value was slightly higher, and the b* value was unchanged, indicating that the pomegranate juice's freshness was still there. Color difference (ΔE) values of untreated 6 hrs sample is highest (1.27) difference among all sample, whereas treated sample at 6 hrs shows little lesser (1.021) than untreated 6 hrs, which might belong to the rate of fermentation in the batter. Therefore, the total color difference (ΔE) varies from 0.032 to 1.27 at different PEF intensities as well as different time intervals, which is considered as clear and visible. The color shift generated by PEF could be related to electroporation and the release of intracellular material (Grimi, Mamouni, Lebovka, Vorobiev, & Vaxelaire, 2010, Wiktor *et al*. 2015) [10, 26]. Our results regarding significant changes happened in color obtained after the PEF treatment

which probably happened due to a reduction in the fermentation rate at different intervals of time, and makes more fresh in comparison to a* value of untreated batter.

3.4 Rheological assessment
Rheological measurements can provide significant information on the flow behaviour of material supply, which is an important aspect of untreated and PEF-treated rice and black gram batter. Rheological characteristics such as K, n, and σ_γ of fermented batters at a shear rate of 20-100 s^{-1} for a fitted model of equation 3 include apparent viscosity (η) and nonlinear correlation coefficient (R^2 = 0.9). The viscosity versus shear rate measurements of these batter samples is the best fit by Herschel Bulkley's model. The flow curves show how apparent viscosity changes with applied shear rate (Figure 1). All samples of untreated and PEF-treated batter at 30 °C and 50 °C exhibit shear-thinning behaviour, which is explained by the fact that apparent viscosity reduced as shear rate rose. The batter solution contains smaller particles as a result, this fermented batter exhibits non-Newtonian behavior ($n < 1$) and does not exert as much of a barrier to shear aggregation on PEF-treated samples. Mostly, the apparent viscosity of PEF treatment of 20kV/cm at 30 °C shows lower viscosity than untreated batter sample whereas, at 50 °C the apparent viscosity of PEF treated batter is quite lesser than the untreated sample. This result outcome that PEF treated batter sample apparent viscosity is more thin-skinned than untreated batter sample while increasing the shear rate. Similar findings were noted, according to Manzoor *et al.* (2019) [16] and Theagarajan *et al.* (2020) [25], in which rice starch, exhibited non-Newtonian pseudoplastic behavior as a result of a decrease in apparent viscosity and an increase in shear rate while remaining constant. Hence, treated PEF of 20 kV/cm rice and black gram batter at 30 °C and 50 °C express more sensitivity to apparent viscosity compared to untreated batter samples as an increase in the shear rate.

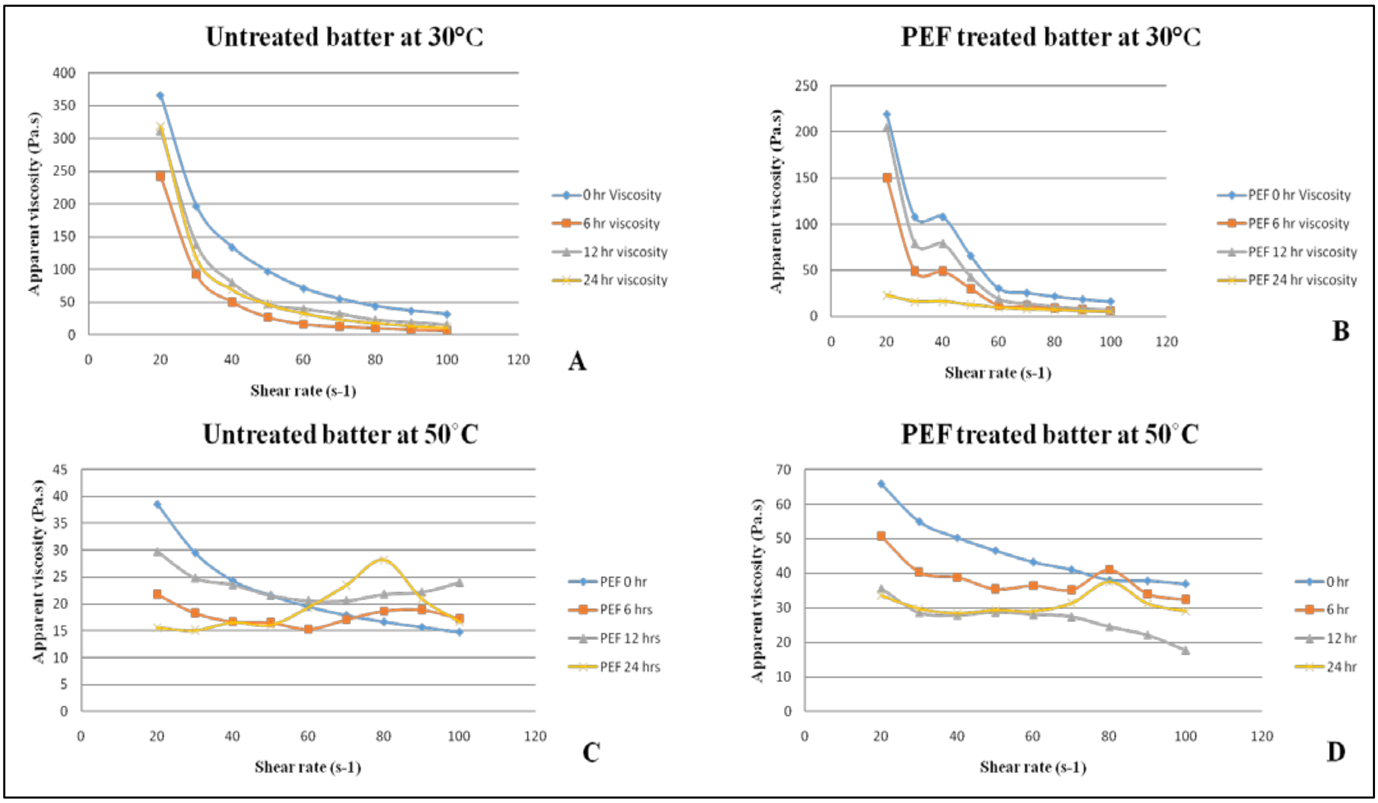

Fig 1: Rheological behavior: A- rheological property of untreated rice and black gram batter at 30 °C at different interval of time, B- rheological property of PEF treated rice and black gram batter at 30 °C at different interval of time, C- rheological property of untreated rice and black gram batter at 50 °C at different interval of time, D- rheological property of PEF treated rice and black gram batter at 50 °C at different interval of time

Table 1: Physiochemical properties of PEF treated and untreated rice and black gram batter at certain interval of time.

Sample	Color				Water activity	Conductivity	pH	%TA	Bulk density (g/cm^3)
	L	*a*	*b*	ΔE					
Control	89.62±0.00^{c}	-0.2±00^{a}	10.33±0.00^{e}	0.00	1.006±0.001^{ab}	171.07±4.03^{bc} µS	6.2±0.1^{a}	0.23±0.02	1.0076±0.01^{ab}
Untreated 6 hrs	90.56±0.02^{a}	-0.38±0.04^{b}	10.59±0.13^{cd}	1.27	1.008±0.00176^{ab}	146.13±0.71^{d} µS	5.47±0.06^{c}	0.43±0.01	0.84±0.03^{b}
Untreated 12 hrs	90.15±0.12^{b}	-0.43±0.04^{bc}	10.66±0.07^{bcd}	0.779	1.005±0.001^{ab}	159.40±3.26^{cd} µS	4.86±0.06^{e}	0.63±0.00	0.52±0.01^{e}
Untreated 24 hrs	89.73±0.14^{c}	-0.36±0.06^{b}	11.31±0.11^{a}	1.108	1.009±0.0018^{a}	198.91±2.61^{a} µS	4.5±0.1^{f}	0.73±0.04	0.57±0.01^{de}
PEF treated	89.27±0.02^{d}	-0.177±0.01^{a}	10.85±0.03^{b}	0.032	1.008±0.001^{ab}	2.18±0.04^{a} mS	6.07±0.15^{a}	0.27±0.00	1.02±0.03^{a}
PEF treated 6 hrs	90.09±0.08^{b}	-0.52±0.02^{cd}	10.88±0.12^{b}	1.021	1.008±0.003^{ab}	178±2.85^{bc} µS	5.73±0.06^{b}	0.33±0.03	0.963±0.02^{c}
PEF treated 12 hrs	89.66±0.04^{c}	-0.61±0.03^{e}	10.45±0.08^{de}	0.47	1.004±0.001^{b}	2.59±0.05^{a} mS	5.1±0.1^{de}	0.56±0.02	0.58±0.01^{d}
PEF treated 24 hrs	89.63±0.06^{c}	-0.53±0.04^{de}	10.79±0.02^{bc}	0.72	1.005±0.001^{ab}	2.76±0.11^{a} mS	5.23±0.06^{cd}	0.65±0.05	0.607±0.01^{d}

All values are mean of triplicate determination (n = 3)±standard deviation, PEF represent Pulsed Electric Field at 20 kV/cm. Each different superscript letters indicate significant differences at $P < 0.05$ where, $^{a} > ^{b} > ^{c} > ^{d} > ^{e}$

4. Conclusion

Findings of this study show that PEF treatment of 20 kV/cm of rice and black gram batter at 30 °C with 0 hrs, 6hrs, 12hrs, and 24 hrs time intervals could decrease to fermentation process with different physicochemical (color, water activity, pH, titratable acidity, bulk density, and conductivity) and rheological properties in compared to untreated batter sample. The pH and water activity decrement of PEF treated batter was found lesser than the untreated sample at certain intervals of time. Mostly, conductivity and bulk density of treated batter showed higher values among untreated batter at each time interval. L*, a*, b* optical properties of PEF treated batter showed more freshness than an untreated batter at different intervals of time. Whereas, the rheological assessment showed more decrement in apparent viscosity of PEF treated batter at 30 °C and higher at 50 °C in comparison to untreated batter sample as increasing shear rate. All the positive changes happened in color and rheological assessment is might due to an increase in bioavailability of batter through the principle of pulsed electric field's electro-poration and electro-permeabilization of granules of treated batter. All result indicates that PEF at 20 kV/cm reduce the fermentation process as well as apparent viscosity of rice and black gram batter and shows more color freshness and pH in comparison to the untreated batter.

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