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Functional Coloring: Utilizing Aronia melanocarpa Extract as a Health-Promoting Replacement for Artificial Colorants in Confectionery

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ABSTRACT: Matching the general trend of replacing synthetic additives with healthier natural products, the present research studies the effects of different concentrations of chokeberry extract which substitute carmoisine dye in jelly candies. Also, the colour and antioxidant properties of the aforementioned extract and their changes at various pH and in presence of different mineral salts from foods are analysed. The phenolic content of the extract was determined using HPLC and spectrophotometric methods. A high concentration of polyphenols was found in the chokeberry extract, of which around 97% were flavonoids. Catechin, epicatechin, ferulic acid and its methyl esther, protocatechuic, gallic and para-hydroxybenzoic acids were the major phenolics identified in the extract. The total antiox- idant activity decreased in acidic media, while close-to- neutral and alkaline pH values did not exhibit any effect on this parameter. Furthermore, the green/red colour parame- ter, the chroma and the hue angle were enhanced in the most acidic media (pH 2.3 and 3.5). From the studied salts, $CaCl_2$ and KNO_3 had the most significant effects on colour. The chokeberry extract proved to be suitable as replace- ment of carmoisine dye in jelly candies, as the physico- chemical and microbiological properties comply with the regulated requirements. More than that, the extract improved the antioxidant and sensory properties of jellies in all studied concentrations and the best total sensoryscore was obtained for 1.5% extract. After 5 and 50 days of storage, the microbiological properties were improved in candies prepared with aronia extracts compared to car- moisine, as the total viable count registered important diminutions.

Keywords: Aronia, Carmoisine, Antioxidant, CIELab colour parameters, Total viable count, Storage

I. INTRODUCTION

Many studies proved that certain synthetic food additives may cause immediate or long-term health problems, when consumed for long periods of time (Borzelleca and Hallagan 1988). The ingestion of artificial dyes such as tartrazine and erythrosine combined with a diet rich in trans-fats, nitrites, nitrates and reduced intake of fibres is related to malignancies, specifically in the oesophagus, breast, rectum, stomach, and ovaries (Polonio and Peres 2009). Carmoisine (also named azorubine, or E 122) may induce allergic reactions, intensifying of asthma symptoms, intolerance in people sensitive to salicylates, children hyperactivity and is sus- bladder cancer (Ghendov-Mosanu et al. 2016). On the other hand, jelly candies are multi-coloured sweets with gelatinous structure, especially popular among children, the production of which involves the use of various colouring additives to obtain an attractive appearance (Tamer et al. 2013). The replacement of the synthetic food dyes with colourings of natural origin is therefore a current concern.

The use of natural ingredients as food additives is becoming more and more popular across the food industry due to their health properties. However, the choice of natural food colorants on the market is limited, this being caused by the insufficient exploitation of renewable natural resources and by-products of food processing. Due to the mentioned trend, the producers of colorants are continu- ously extending their product portfolio. Thus, the necessity of food dyes and antioxidants of natural origin for the food industry is increasing the demand of natural plant extracts. Many studies have shown that wood berries are a source of natural colourants and, at the same time, candidates for dietary interventions when it comes to mitigating chronic inflammation. Recent studies place the black chokeberry (Aronia melanocarpa, also named aronia) amongst the berries with the highest antioxidant activity and polyphenol content. They contain high levels of flavonoids, the majority of which are proanthocyanidins and anthocyanins, which might have potential health benefits (Savikin et al. 2014). The bioactive value of berries results from their composition in substances such as vitamins, pro-vitamins and related compounds, minerals, phytosterols and phe- nolic compounds (Savikin et al. 2014). Thus, the use of chokeberry extract in food production will not only act as a technological aid, but will also improve the product interms of its effect on consumers' health.

Previous studies have come to the conclusion that in order to fully understand the influence of processing on the bioactive compounds, researches on different technological treatments should be performed (Tolic et al. 2015). Therefore, in the present study a hydroalcoholic extractfrom chokeberry fruits is prepared and characterized, after which the influence of pH and different salts on its prop- erties (such as antioxidant activity and colour parameters) is analysed in order to predict possible interactions in complex food matrices. Except this, the main objective wasto test the mentioned extract as a food dye in jelly candy, in order to improve this product's properties and storage period, and make it healthier. Analyses of the chokeberry's extract influence on the physicochemical, microbiological, sensory characteristics and antioxidant activity, as well as the change of jellies during storage were also performed.

II. MATERIALS AND METHODS

ABTS ($2,2^0$-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) and sinapic acid were purchased from Alfa Aesar (Germany); Folin-Ciocalteu reagent—from Merck (Ger- many); DPPH (2,2-diphenyl-1-picrylhydrazyl), (?)-cate- chin, caffeic acid, syringic acid, ferulic acid and ferulic acid methyl ester, gallic acid, protocatechuic acid, gentisic acid, para-hydroxybenzoic acid, salicylic acid, para-cou- maric acid, quercetin, meta-hydroxybenzoic acid, vanillic acid, epicatechin, cis-resveratol—Sigma (Germany, Japan, China); procyanidin B1, procyanidin B2, polydatin, hyperoside—Extrasynthese (France); trans-resveratrol—TCI Europe (Belgium). All the spectrophotometric mea- surements were made using Analytic Jena Specord 200 Plus spectrophotometer.

Extraction

The ethanolic extract was prepared from mature Aronia melanocarpa berries harvested in Moldova. The choke- berries were dried at 60.0 ± 1.0 °C to 8.05 ± 0.13% moisture content, milled to powder with the median par- ticle (d_{50}) of 140 lm, then sieved. The obtained powder was subjected to extraction in 50% hydroethanolic solvent, with solid:liquid ratio 1:10, under stirring at 60 rpm for 30 min. After the filtration of extract, the polyphenol composition and the antioxidant activity were determined, and also, the trials involving the influence of salts and pH were performed. For the use in jelly production, the extract was concentrated in a rotary evaporator at 65.0 ± 1.0 °C until the mass fraction of dry substance reached 75.0 ± 0.1%. The extract was stored in glass bottles at ± 1.0 °C, in the dark.

Characterization of chokeberry extract

Total polyphenols and total flavonoids

The content of total polyphenols was determined by the reaction with Folin-Ciocalteu reagent following the method described by Singleton and Rossi (1965). The results for total polyphenols were calculated from a calibration curve using gallic acid and expressed as equivalents of gallic acid per litre (mg GAE/L). The total content of flavonoids was determined using precipitation with formaldehyde at pH \ 0.8 (Filimon et al. 2020), followed by the reaction with Folin-Ciocalteu reagent, according to the method described by Spranger et al. (2008).

Absorbance at 280 nm

The absorbance at 280 nm (Abs 280) is a parameter which reflects the total polyphenol content and was determined according to the method described by Ribereau-Gayon et al. (2006). The results were expressed as mg equivalents of gallic acid per litre (mg GAE/L) from a calibrationcurve.

The content of anthocyanins by difference of pH

The content of total and monomeric anthocyanins was determined by pH differential method described by Giusti and Wrolstad (2001). Each sample was brought at pH 1 (with KCl/HCl buffer) and 4.5 (with CH_3COONa/HCl buffer), and at each pH, two reads of the absorbance were performed: at 520 nm and 700 nm (Giusti and Wrolstad 2001; Cristea et al. 2019). The results were calculated according to Cristea et al. (2019).

Total cinnamic acids

The content of total cinnamic acids was determined using the method described by Demir et al. (2014). The results were expressed as mg caffeic acid equivalents per litre (mg CAE/L) based on a calibration curve with standard of caffeic acid.

Total flavonols

The content of total flavonols was determined spec- trophotometrically, at 360 nm after acidification with HCl. The results were expressed as mg quercetin equivalents per litre (mg QE/L) based on a calibration curve with standard of quercetin (Demir et al. 2014).

Antioxidant activity

The antioxidant activity of the extracts was measured by two assays:

a) $ABTS^{\cdot}$ ($2,2^0$-azino-bis(3-ethylbenzothiazoline-6-sul- fonic acid) radical) scavenging activity, following the method described by Re et al. (1999). The results were expressed as mmol trolox equivalents per litre (mmol TE/L) from a calibration curve.

b) DPPH˙ (2,2-diphenyl-1-picrylhydrazyl radical) scavenging activity, according to Brand-Williams et al. (1995). The results were expressed as % of inhibition.

Colour parameters (CIEL*a*b*)
The CIEL*a*b* colour parameters were assessed according to method OIV-MA-AS2-11 (2006). The transmittance was measured every nm between 380 and 780 nm, using a1 mm optical glass cuvette and the reference was distilled water. The illuminant was D65 and the observer 10°. The luminosity (L*), the green/red parameter (a*), the blue/ yellow parameter (b*), the chroma (C*), and the hue angle (H*) were determined (OIV-MA-AS2-11 2006).

HPLC of polyphenols
The polyphenol composition was analysed with Agilent 1100 Series HPLC with LiCrospher 100 RP (18; 5 lm) in LiChroCart 250-4 guard column thermostatted at 35 °C. The injection volume was 20 lL and the run time 90 min.
The phases consisted of ultrapure water, methanol and trifluoroacetic acid as follows: A: H_2O:MeOH:TFA (98:1:1) and B: H_2O:MeOH (50:50) ? TFA (1%), with a flow rate of 1.5 mL/min. The detection was carried out at 256 nm, 280 nm, 324 nm, and 365 nm. The gradient of elution was 100% (A): for 10 min; 82% (A): 18% (B) for the next 10 min; 70% (A): 30% (B) for 10 min; 65% (A): 35% (B) for 6 min; 40% (A): 60% (B) for 15 min; 20% (A): 80% (B) for 5 min; 100% (B) for 15 min, and 100% (A) for 10 min.

Studies of factors influencing the properties of chokeberry extract
Studies on the influence of salts
The influence of some salts, often used in different food processing technologies, was studied by adding NaCl, $CaCl_2$, or KNO_3 to the extract in the concentrations: 0.001 M, 0.01 M, and 0.1 M. The extracts were kept att = 4 ± 1 °C for 12 h, and then the antioxidant activity and the CIEL*a*b* colour parameters were measured.

Studies on pH influence
The extracts were brought to some pH values found in different foods: 2.3; 3.5; 5.6; 7.3, and 8.0 using various buffers, and then stored at t = 4 °C for 12 h. Control samples were prepared by diluting the extracts with the same volumes of ethanol 50% as the ones of the buffers used for pH adjustment (Cristea et al. 2019). Afterwards, the antioxidant activity and the colour parameters were determined.

Jelly candies preparation
Sugar, sorbitol, molasses, and water (25% of the total amount of sucrose and sorbitol) were used to prepare the syrup having 78.0 ± 1.0% dry substance. A suspension was prepared using one part corn starch and three parts water and was added to the cooked syrup. Afterwards, swelled gelatine (ratio 1:2), heated citric acid and carmoisine (0.03%) or chokeberry extract were added to the mixture at 64.0 ± 1.0 °C. The obtained jelly-like mass fraction with about 70% dry matter was poured into moulds and cooled to room temperature. The candies were removed from the moulds, dried at 45.0 ± 1.0 °C for 24 h, cooled, packed, and stored in the refrigerator at 5.0 ± 1.0 °C. The samples with chokeberry extract were obtained by replacing the car- moisine synthetic dye with 1.0%, 1.5%, or 2.0% extract. The specific quantities of the manufacturing recipe were: 357.14 g sugar, 392.86 g molasses, 57.14 g gelatine, 42.86 g corn starch, 35.71 g sorbitol, 10.00 g citric acid, 104.29 g water, and either 0.3 g of carmoisine for the control sample, or 10 g, 15 g, 20 g of aronia extract for 1% aronia extract sample, 1.5% aronia extract sample, 2% aronia extract sample, respectively.

Jelly candies analysis
Physicochemical analysis
The physicochemical parameters of the jelly candy were determined following the procedures described in the Official Methods of Analysis of AOAC International (2016). The mass fraction of dry matter was quantified by refractometry. The mass fraction of reducing substances was determined by reaction with ferricyanide. The acidity was determined by titration with sodium hydroxide using phenolphthalein as indicator (Official Methods of Analysis of AOAC International 2016).

Microbiological analysis
The microbiological analysis was carried out in accordance with the rules on microbiological criteria for foodstuffs (G.D. of R.M. no. 221 2009). The total viable count (TVC) of mesophilic aerobic organotrophic bacteria was determined after incubation at 37 °C for 4–72 h.

Sensory analysis
The sensory analysis of the products was carried out following the procedure described in the standard SR ISO 6658:2017 of the International Organization for Standardization. The sensory attributes: appearance, taste, odour, colour and consistency were appreciated using the 5 points system by an expert panel formed of food technology specialists.

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In vitro antioxidant activity of the candies

The antioxidant activity was measured in vitro, in conditions of gastric digestion, by the DPPH˙ radical scavenging assay. The gastric digestion was simulated with pepsin(150 mg/100 g of product) at pH = 2.0 ± 0.1 (1.5 M HCl) and 37.0 ± 0.1 °C, stirring 2 h at 600 rpm. After simula- tion of digestion, the samples were centrifuged at 6000 rpm for 10 min, filtered and then the DPPH˙ radical scavenging activity was measured at room temperature (20 ± 1 °C), according to Brand-Williams et al. (1995).

Statistical analysis

The mean values and the standard deviations were calculated from three independent experiments. One-way ANOVA and post hoc Tukey test were used to distinguish between means and evaluate the results. The considered significance level was p B 0.05. All calculations were made using SPSS Statistics 23 and Microsoft Office Excel 2007. Mathematical modelling and informational analysis were done using MATLAB (Matlab and Statistics Toolbox Release 2015a; MathWorks, Natick, MA, USA). The informational analysis of the obtained experimental data allows the evaluation of the mutual influences of the measured parameters. It is based on two main concepts: entropy and information, and is expressed in bits, as unit of measure. The higher the values of the mutual information, the lower are the uncertainties and hence, the higher the predictions (Fellin 2005, Scafetta 2001).

III. RESULTS AND DISCUSSION

Characterization of the chokeberry extract

The results of the analysis of the phenolic composition and the antioxidant activity of the extract used for candy manufacturing are presented in Table 1.

A high content of polyphenols was found in the chokeberry extract, of which approximately 97% are fla- vonoids, but also, important quantities of cinnamic acids and flavonols. Other authors have also found high con- centrations of polyphenols, e.g. Ovaskainen et al. (2008) found the highest content of polyphenols in chokeberry in astudy in which 143 plant species were analysed (Ovas- kainen et al. 2008).

The main phenolic compounds identified in the choke- berry extract were catechin (7.8 mg/100 mL) and epicate- chin (3.95 mg/100 mL), but also ferulic acid and its methyl esther, protocatechuic and gallic acids, para- and meta-hydroxybenzoic acids, quercetin, procyanidin B2 and B1 etc. Also, high contents of total and monomeric antho- cyanins were found (Table 1). The major anthocyanins in chokeberry are glycosides of cyanidin (Kulling and Rawel 2008). According to Savikin et al. (2014), the main com- ponents of chokeberry teas are cyanidin-galactoside and cyanidin-arabinoside (concentrations above 2.3 mg/ 100 mL), while cyanidin-glucoside and cyanidin-xyloside are present in amounts lower than 1.3 mg/100 mL (Savikinet al. 2014). Other authors have also analysed different parts of the chokeberry shrub and have found that cyanidin 3-galactoside was the major anthocyanin in all three Aronia melanocarpa cultivars of the following ‘‘Moskva’’, ‘‘Hugin’’, and ‘‘Nero’’. They found minor differences in antioxidant content between the three aforementioned cultivars of Aronia melanocarpa and one cultivar of Aronia prunifolia, and were comprised between 21.2 and 35.7 DPPH IC50 (lg/mL) in the 80% ethanolic extract

Table 1 Composition,

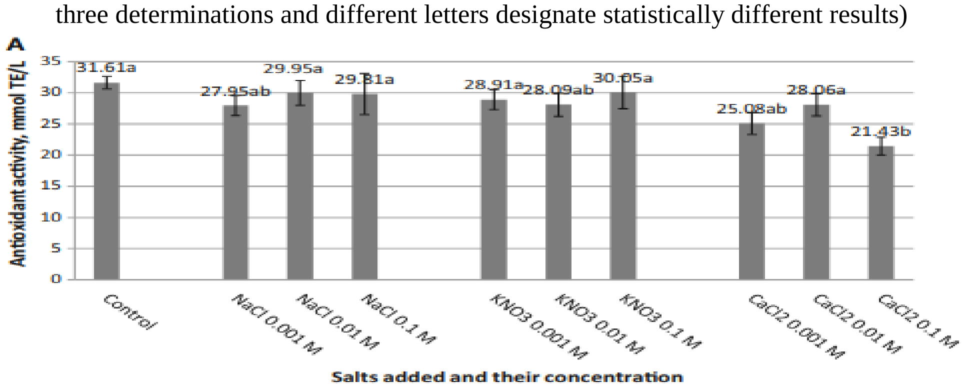
Parameter	Value
Total polyphenols (Folin-Ciocalteu) (mg GAE/L extract)	4441 ± 243
Total flavonoids (mg GAE/L extract)	4293 ± 209
Total polyphenols by Abs280 (mg GAE/L extract)	3470 ± 21
Cinnamic acids (mg CAE/L extract)	580 ± 21
Flavonols (mg QE/L extract)	501 ± 15
Total anthocyanins (mg/L extract)	102 ± 2
Monomeric anthocyanins (mg/L extract)	61 ± 2
ABTS˙ scavenging antioxidant activity (mmol TE/L extract)	31.61 ± 1.02
DPPH˙ scavenging antioxidant activity (% inhibition)	72.95 ± 0.24
Catechin (mg/100 mL)	7.8 ± 0.0
Epicatechin (mg/100 mL)	3.95 ± 0.32
Ferulic acid (mg/100 mL)	3.71 ± 2.57
Protocatechuic acid (mg/100 mL)	1.50 ± 0.07
Ferulic acid methyl ester (mg/100 mL)	1.32 ± 0.54
Gallic acid (mg/100 mL)	0.36 ± 0.05
p-hydroxybenzoic acid (mg/100 mL)	0.23 ± 0.04
Quercetin (mg/100 mL)	0.21 ± 0.11
Procyanidin B2 (mg/100 mL)	0.17 ± 0.06
m-hydroxybenzoic acid (mg/100 mL)	0.11 ± 0.01
Sinapic acid (mg/100 mL)	0.10 ± 0.01
Hyperoside (mg/100 mL)	0.10 ± 0.01
Procyanidin B1 (mg/100 mL)	0.09 ± 0.05
Vanillic acid (mg/100 mL)	0.08 ± 0.01
Chlorogenic acid (mg/100 mL)	0.08 ± 0.06
Syringic acid (mg/100 mL)	0.04 ± 0.01
p-coumaric acid (mg/100 mL)	0.04 ± 0.01
Gentisic acid (mg/100 mL)	0.01 ± 0.00
Cis-resveratrol (mg/100 mL)	0.01 ± 0.00
Trans-resveratrol (mg/100 mL)	0.008 ± 0.003
Salicylic acid	Traces
Caffeic acid (mg/100 mL)	–
Polydatine (mg/100 mL)	–

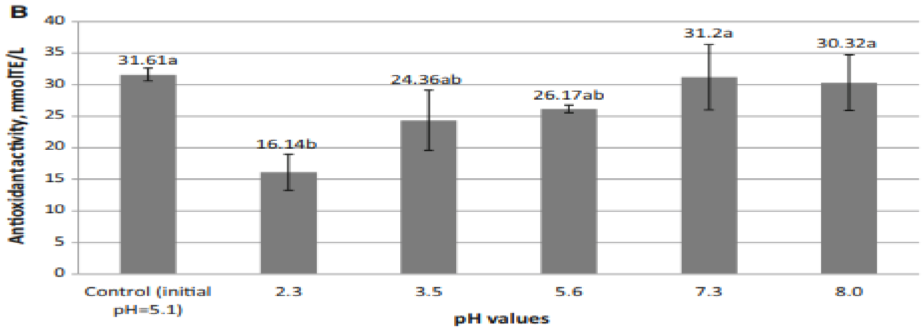
(Wangesteen et al. 2014). Same authors detected high contents of chlorogenic acid in the chokeberry decoction (5.24 mg/100 mL) and infusion (4.02 mg/100 mL) in a research covering three species of berries, namely: chokeberry, bilberry, and black currant. Also, it was shown that the antioxidant activity and the total polyphenol content depend on the cultivar, berry size, yield, and extraction solvent (Wangesteen et al. 2014).
Researchers from Croatia have evaluated the polyphenol content and the antioxidant activity of various chokeberry products, namely juices, powders, teas, capsules, and dry berries. It was found that all products contain high amount of phenolics (3002–6639 mg/L and 1494–5292 mg/100 g dry weight, depending on the product) and a lower amount of total anthocyanins (150–1228 mg/L and 141–2468 mg/ 100 g dry weight). The antioxidant activity of the examined juices and other chokeberry products was also found to be high (12.09–40.19 mmol TE/L or 58.49–191.31 mmol TE/100 g dry weight) (Tolic et al. 2015). The values found by the Croatian authors are comparable to the ones found in the present study.

The influence of different salts on the antioxidant activity and colour parameters of chokeberry extract

The pH and the ionic strength are key parameters in the gelation process, impacting greatly both the sol–gel transition and the concentration of the thickening agent. Both the antioxidant activity and the colour of the extract are influenced by pH and ionic strength variations. The cause of this phenomenon lies in the fact that anthocyanins exist in different mesomeric structures. Cation flavylium (AH?), for example, with its many resonance structures is dominant at low pH. As the pH increases, the carbinol pseudo base, which is in tautomeric equilibrium with its colorless chalcone, forms proportionally with the decrease in the AH? concentration. At pH bigger than 5, anhydrous blue quinoidal base begins to form. Each of these structures has different antioxidant capacity. The ionic strength also influences the colour of the extract due to the direct involvement of the electrolyte ions in the tautomeric balance. The sol–gel transition is also influenced by pH value and ionic strength, because the condensation process can be electrocratically stabilized, maintaining the total gel volume. In case this stabilization does not occur, the connections in the three-dimensional structure may break, causing the spontaneous release of the liquid (syneresis) (Sturza and Deseatnicova 2012). Consequently, the following two sections of this study will focus on the analysis of the influence of ionic strength and pH on the antioxidant activity and colour of chokeberry extract. The changes of the antioxidant activity after the addition of NaCl, KNO3 and CaCl2 are shown in Fig. 1a. No significant changes of the antioxidant activity were observed at the addition of sodium chloride or potassium nitrate. High concentration of CaCl2 changed the antioxidant activity by lowering its value from 31.61 mmol TE/L to 21.43 mmol TE/L, which was a statistically significant decrease. Previous studies proved that the addition of calcium chloride led to a reduction of the antioxidant activity also in the grape marc extract (Cristea et al. 2019) and this phenomenon was explained by the ability of certain flavonoids to entrap cations, which results in a change of their antioxidant properties (Jabbari and Gharib 2012).

Fig. 1 Effects of salts (a) and pH (b) on the antioxidant activity of chokeberry extract (errors bars present the standard deviation of three determinations and different letters designate statistically different results)





IV. CONCLUSION

Within the global tendency to replace the synthetic additives with natural compounds extracted from plants in order to obtain healthier foods, the present research studied the properties of an underused fruit extract and its changes in presence of different pHs and inorganic salts usu- ally present in foods, as well as the effects of the substi- tution of carmoisine dye with different concentrations of the aforementioned extract in jelly candies.

The chokeberry extract proved to have a high radical scavenging capacity and to contain important concentrations of polyphenols, of which, around 97% were flavonoids.

The pH is a factor strongly influencing the extract's properties only in the case of high acidic media. The total antioxidant activity decreases significantly at pH 2.3 and the green/red parameter, the chroma and the hue angle were enhanced. From the studied salts, $CaCl_2$ and KNO_3 had the most significant effects on colour. All colour changes of aronia extract depending on pH or salts can be useful in the production of different food, in order to adequately modify the chromatic properties.

The physicochemical and microbiological properties of aronia jelly candies comply with the regulated requirements for such products. The extract improved the antioxidant and sensory properties of jellies in all studied concentrations and the best total sensory score was obtained for 1.5% extract. After 5 and 50 days of storage, the microbiological properties were improved in the candies prepared with aronia extracts, comparing to carmoisine, as the total viable count registered important diminutions. Present study recommends the use of chokeberry extract as replacement of carmoisine dye in the production of jelly candies.

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