Effect of milk sources on whey protein and fractions of casein content

ABSTRACT:

Aim: Milk from Cow, Buffalo and goat ~~were~~ was used for the study of isolation and fractionation of casein and whey proteins.Buffalomilkandgoatmilkweresubjectedto ~~physico-chemical~~physicochemical attribute studies and a detailed protein profile namely total protein,casein and whey protein along with its various fractions of casein viz; α, β and κ-casein. Casein fractions separated were α-, β- and κ casein by urea fractionation. The aim of this study was to compare various casein fractions isolated from ~~cow's~~cows, Buffalo and Goat milk.~~t~~~~The~~The method of separating casein into its fractions is based on the solubility of the individual components in urea ~~solution, the~~solutionthe solution. The separation of casein fractions was conducted by changing the urea concentration in acid media and the yield of fractions and total protein percentages were calculated. Buffalo and goat milk~~s~~ have comparatively higher total protein and whey protein content as compared to cow milk. M~~m~~aximum whey proteins were found in ~~the~~ goat milk (20.58 %) whereas cow milk had the lowest whey protein~~low lowest contents~~ (19.29 %). Among casein fractions, α fraction (16.64%) was found maximum in Buffalo milk followed by cow (14.92 %) and goat milk~~s~~ (5.42 %). ~~Goat~~ The goatmilk ~~sample~~ showed the highest β casein (17.81 %) ~~and~~ whereas, the lowest concentration of β casein was found in cow milk (9.38%).

Study**Design:**A significant contribution to the total milk production of India comes from buffalo milk and goat milk. ~~Inspite of~~Despite that, Buffalo milk and goat milk are not being utilized for many products ~~in view~~because of their inherent problems associated in the production of *products*.

Place and Duration of Study: Karnataka Veterinary Animal and Fisheries Sciences University (KVAFSU), Dairy Science College, Hebbal, Bangalore Karnataka, India

Methodology: The Goat milk samples were collected from Sinchana Goat and S~~s~~heep farm, Marenahalli village (Bengaluru Rural Dist) and Buffalo milk was obtained from C~~e~~ountry Delight Pvt. Ltd., J. P. Nagar, Bengaluru, Karnataka. Cow milk used in this investigation was collected from SEDP, Dairy Science College, Hebbal, Bengalore. Commercially available pure Neutrase enzyme was purchased from DSM Nutritional Products India Pvt. Ltd, Bangalore. All the glassware used ~~were~~ was soaked in chromic acid solution, repeatedly washed with water, rinsed with distilled water and dried before use. For microbiological analysis dried test tubes, conical ~~flask~~flasks, pipettes were cotton plugged and sterilized in a hot air oven. The chemicals and reagents used in this study were mainly of analytical grade procured from Prince Laboratory Company Pvt.L~~l~~imited, Bangalore. The protein molecular weight markers ~~were~~ used for the electrophoretic study were procured from Bangalore Genei Pvt. Ltd.

Results: The sources of protein had a significant influence on the total casein and whey ~~proteins~~protein content of different species. ~~Highest~~ The highest quantity of total caseins (34.30g/l) and wheyprotein (8.87 g/l) were noted in buffalo milk than in cow (28.52 g/l) and Goat milk(28.45 g/l). Total protein, casein and whey protein contents were greatly affected bythesourceofmilk obtained.

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Comment [G1]: Cow milk was not fractionated?

Comment [G2]: Revise the 2nd sentence as 'Different protein was isolated from cow, buffalo and goat milk such as total protein, casein and whey protein. Furthermore, various fractions of casein such as α-, β- and κ casein was isolated by urea fractionation'.

Comment [G3]: Either remove "study design' section or merge it with methodology section.

Comment [G4]: Write the name of the 'products'.

Comment [G5]: Remove Duration of study as this a one-time study done from collected milk samples.

Conclusion: Thus this investigation has shown that both buffalo and goat milk ~~milk~~ could be ~~uninterruptedly~~undoubtedly used in the preparation of *Rasagulla*, without ~~comprismising~~compromising any of thequality characteristics, which means that the ~~utilization share of buffalo and goat milkcouldbeenhanced besides adding onto thebetternutritional profile~~share of the buffalo and goat milk can be increased in the production of various milk products without affecting the nutritional profile of that product.~~.~~

Recommendation: *Rasagulla*will be consumeduniversallybyallagegroupsglobally.Thus,the consumptionof*Rasagulla*helpsinprovidingoverall nutritional ~~requirement~~requirements. However, the study has been conducted on a pilot scale.Moreover, commercial production may ~~necessiates~~ necessitateshuge cost on enzymatic modificationofproteinsandseparationpeptidesforvalueaddition.

Keywords:*Rasagulla;Chhana; Buffalo milk; Goat milk; Milk protein; Whey Protein and Casein*

INTRODUCTION

MilkproductioninIndia~~increased~~reachedto221.0milliontonnesintheyear2021-22with a growth rate of 6.38 per cent per annum.The share of milk contribution by Cow, Buffaloand Goat to India's milk production is 51.85 per cent, 44.84 per cent and 2.93 percent,respectively.Among the species, indigenous Buffaloes have the highest share of milkproduction in India with 32.13 per cent in the fiscal year 2022, followed by cross breedcows accounting for over 29.31 per cent of the total milk production in the country[3].~~They are of great economic importance in India in production of milkand milk products~~. The richness of buffalo milk makes it highly suitable for processing ifproperprocessingtechnologies areexploited.

Buffalomilk,likecow'smilk,canbeutilizedforthemanufacturinge~~e~~ofawidevarietyof dairy products such as cream, butter, butter oil (clarified butter or ghee), UHT cream,ice cream, yoghurt and some cheeses without changing the equipment or processingstrategies.However,processingtechnologyandequipmentdesignedfor manufacturing cowmilkproduct~~manufacture~~areoftennotadequatelysuitablefor~~the~~ productionofcertaindairyproductsusingBuffalo milk due to the differences in compositional, physico-chemical, and functionalproperties.~~Buffalomilkcompositionalvariationsgreatlyaffecttheprocessingandyieldofcertaindairyproducts~~The variation in the composition of buffalo milk as compared to cow milk affects the processing and yield of various dairy products, as the machineries are standardized for cow milk.OutoftotalmilkproducedinIndiamorethan50percentofmilkisbeingutilizedforthepreparation ofdairy products.

Comment [G6]: Remove recommendation from abstract and merge it with the conclusion section in the detailed manuscript.

Comment [mt7]: Provide reference

Comment [mt8]: Provide reference

Buffalomilkisoftennotconsideredasanidealfluidforthemanufactureofseveraltypes of cheeses, milk powders, evaporated&, condensed milk, infant formulae and Chhanabased*Chhana-based*dairysweets,duetothehigherconcentrationofcalcium,protein,fatandlargersizeofcaseinmicelles,whichproducesundesirablequality,attributesthuscausingand causestexturaldefectsindairyproducts.Therefore,theconventionalprocessingtechnologiesareoftenunsuitableandcannotbeappliedirectlyforthe productionof*chhana*and*chhanabased*sweets out of buffalo milk.PatternofmilkconsumptioninIndiaindicatesthatabout6percentof milkisconvertedinto*Chhana*and*Chhana* based*Chhana-based* products[6].

Generally, cow milk is preferred for *chhana* making as it produces soft body andsmooth texture product which is highly suitable for the production of *chhana* based*chhana-based* sweets, particularly*rasagulla*.However,buffalomilkbecauseofmanyinherentdifferencesinphysico-chemical make up as compared to cow milk, poses many technological problems inthe preparation of good quality *chhana* and *rasagulla.*Hence suitable modificationsare required in buffalo milk in order to manipulate the composition which is similar to cow milk processing of buffalo milk intoproducts needs suitable modification with respect to composition similar to that of cowmilk, thuswhich ultimately makes it suitable to produce *chhana* and *chhana* based*chhana-based* sweets particularly*rasagulla.*

Goatmilkdiffersfromcoworbuffalomilkinhavingbetterdigestibility,alkalinity,buffering capacity and certain therapeutic values in medicine and human nutrition. Goatmilk is considered to be an ideal food for people suffering from cow milk allergies andother gastro intestinalgastrointestinal ailments. Feeding goat milk to infants provides significantly higherdigestibilityascomparedtocowmilk.Thechildrenfedongoatmilksurpassedthose fedon cowmilkinweightgain,skeletalmineralizationandbloodserumcontentofvitaminA,calcium,thiamin,riboflavin , niacinand hemoglobin[7].

The protein fractions such as α-casein,β-casein,k-casein,β-lactoglobulin andα-lactalbumin are similar in concentration in goat milk and cow milk.Goat milk proteins are similar to the cow milk proteins fractions such as α-casein,β-casein,k-casein,β-lactoglobulin,α-lactalbumin,buttheydifferwidelyingeneticpolymorphisms and their frequencies in the goat population. Peptides formed from theenzymatic cleavage of caseins of goat milk have greater advantages than those from cowmilk casein. Goat milk fat differs in contents of fatty acids profile significantly fromaverage cow milk fat.Goat milk has a higher content of monounsaturated fatty acids, polyunsaturatedpolyunsaturated fatty acids, medium chain fatty acids, than cow milk which are proven to bebeneficialforcardiovascular disorders.

Comment [mt9]: Provide reference

Comment [mt10]: Provide references for all the statements given in the last para of introduction.

MATERIALS AND METHODS

The materials used and methods followed in this investigation for production of protein hydrolysates and bioactive peptides from Buffalo milk and Goat milk. The Goat milk samples were collected from Sinchana Goat and sheep farm, Marenahalli village (Bengaluru Rural Dist) and Buffalo milk was obtained from Ccountry Delight Pvt. Ltd., J. P. Nagar, Bengaluru, Karnataka. Cow milk used in this investigation was collected from SEDP, Dairy Science College, Hebbal, Bengalore. Commercially available pure Neutrase enzyme was purchased from DSM Nutritional Products India Pvt. Ltd, Bangalore. All the glassware used were wassoaked in chromic acid solution, repeatedly washed with water, rinsed

with distilled water and dried before use. For microbiological analysis dried test tubes, conical ~~flask~~flasks, pipettes were cotton plugged and sterilized in hot air oven. The chemicals and reagents used in this study were mainly of analytical grade procured from Prince Laboratory Company Pvt. L~~l~~imited, Bangalore. The protein molecular weight markers ~~were~~ used for the ~~elcterophorectic~~ electrophoreticstudy ~~were~~was procured from Bangalore Genei Pvt Ltd. All the necessary reagents were prepared in distilled or double glass distilled water for all analytical purposes and freshly prepared reagents were used in the study. Standard procedures (IS 1479) 2001 were followed for analysis milk.

Isolation~~Preparation~~ofwholecasein andwheyproteins

Whole casein and whey proteins were isolated~~prepared~~ by coagulation of buffalo and goatskim milk separately at pH 4.6 using 10 per cent dilute hydrochloric acid. Then the suspension was c~~C~~ooled ~~thesuspension~~ downto room temperature and was ~~l~~eave~~l~~eft ~~it~~ for 5 min. Afterwards, it was f~~F~~ilter~~ed~~ through muslin cloth andcasein precipitate was washed 2 to 3 times with cold distilled water to remove traces ofacid.Theresultantproductwasfreezedried~~asperthemethod~~[5].Wheyproteins were separated by precipitation and filtration of whey. The protein was estimatedbyKjeldahl Method.

Fractionationofcaseinsbyureasolubilitymethod

Casein fractions were separated ~~on the basis of~~based on their differential solubility in ureasolutionas perthemethod outlined[5].

Analysisofcasein fractionsbySDS-PAGE

SDS-PAGEwascarriedouttoassessthemolecularweightrangesofcaseinfractions ~~by following the method~~[9].The following reagentswereemployed for analysis.

RESULTS AND DISCUSSION

Effectofsourceofmilkproteinonyieldofcaseinsandwheyproteins

Caseinandwheyproteinswereisolatedfromcow,buffaloandgoatmilk.Theyieldof total protein, casein, and whey protein~~'s~~ are presented in Table (1). As observed fromthe table, buffalo milk resulted in a significantly higher total protein content (42.50 g/l) followedby goat milk (35.82 g/l) and cow milk (35.76 g/l). The respective casein content of cow,buffalo and goat milk were observed to be 28.52 g/l, 34.30g/l, and 28.45 g/l. The caseinyieldwashighestinbuffalomilk(80.71%)followedbycowmilk(79.76%)andgoatmilk(79.42%).Theproteinc ontentofcow,buffaloandgoatmilkwasobservedtobe3.58,4.25and3.68percentrespectively.Thewhey~~proteins~~protein yieldwasobservedtobe7.24,8.20and7.37g/l,respectively,forcow,buffaloandgoatmilk.Wheyproteinyieldv ariedbetween19.29to20.58percentasagainst ~~yield ofcasein~~casein yield whichvariedbetween79.42to80.71 percent.

The cow, buffalo and goat milk used in this investigation were analysed for total protein, casein and whey proteins content, and the results are presented in Table (1~~.~~). It is pertinent to note that buffalo milk resulted in highest yield of total protein (42.50 g/l) as compared to cow's (35.76 g/l) and goat milk (35.82 g/l). Higher yield of total protein in buffalo milk is mainly attributed to higher initial protein content in

Comment [mt11]: Either change the description of the first para of the material method or remove the methodology section from the abstract, as both of them are similar word to word.

Comment [mt12]: Either mention the name and concentration of the reagents used or remove the sentence.

buffalo milk. The buffalo milk resulted in higher yield of casein (34.30 g/l) as compared Cow (28.52 g/l) and goat milk (28.45g/l). Buffalo milk yielded not only higher casein but also higher whey protein content. In case of buffalo milk the yield of whey protein was higher (8.20 g/l) as against cow's milk (7.24 g/l) and goat milk (7.37 g/l), these results are in agreement with the earlier workers[1,4 & 9]. The Buffalo milk possessed higher casein and whey protein content as compared to cow's milk and goat milk.

Comment [mt13]: The para is just the repetition of the data mentioned the 1st para of results and discussion. Remove references mentioned in the 2nd para and incorporate it in the 1st para.

Effectofsourceofprotein onyieldofvariousfractions ofcasein

The effect of source of milk protein on yield of various fractions of protein ispresentedin Table (2)and Fig (2).Thetotalcaseincontentofcow,buffaloandgoatmilkwasrecordedtobe27.47g/l,34.13g/l,and26.62g/l,respectively.Thetotalαcaseincontentinbuffalomilk(16.64g/l)and cow milk (14.92 g/l) were significantly higher as compared to goat milk (5.42 g/l).Goat milk had significantly higher β casein (17.85 g/l) as compared to buffalo (12.30 g/l)and cow milk (9.38 g/l,). It is observed from the table (3) that the concentration of k-casein was much higher in buffalo milk (5.19 g/l) as compared to goat milk (3.35 g/l) andcow milk (3.17 g/l). There was a significant difference in k-casein content of buffalo milk(5.19g/l)ascomparedtocow(3.17g/l)andgoatmilk(3.35g/l).Significantdifferencewasobservedin the proteincontent amongst Cow,Buffalo and Goat.

Comment [mt14]: Kindly mention the word 'significant', if the results were statistically significant. If it is statistically significant, mention the name of the statistical test used in material and methods section.

The effect of source of milk proteins on yield of various fractions of casein was studied and results are presented in Table (2). The casein obtained from various sourceswas subjected to fractionation and the yield of various fractions viz α casein, β casein and k casein were estimated. It is pertinent to note that buffalo milk resulted in higher yield of total α casein and k-casein (16.64 and 5.19 g/l) as compared to cow (14.92 and 3.17 g/l) and goat milk (5.42g/l and 3.35 g/l). This could be due to genetic inheritance of buffalo milk, which carry higher proportion of α casein and k-casein than cow and goat milk. The results are in agreement with earlier workers[8&9].Similarly, β casein content was also higher in buffalo milk (12.30 g/l) as compared to cow milk (9.38 g/l). But in goat milk β casein (17.85 g/l) content was significantly higher as compared to cow (9.38 g/l) and buffalo milk (12.30 g/l), though goat milk yielded lowest per cent of α-casein as compared to cow and buffalo milk. This may be due to variation from species specific[2& 7].

Comment [mt15]: Again, the para is just the repetition of the data mentioned the above paragraph. Remove references mentioned in the 2nd para and incorporate it in the above para.

Table1:Effect ofsourceofmilk proteinon yield ofcaseinsand wheyproteins.

Sourceo fMilk	TotalPro tein(g/l)	Protein(%)	Caseins(g/l)	Casein Yield(%)	WheyProt eins(g/l)	WheyProteinsYie ld (%)
Cow	35.76[a]	3.58[a]	28.52[a]	79.76[a]	7.24[a]	20.24[a]
Buffalo	42.50[b]	4.25[b]	34.30[b]	80.71[a]	8.20[b]	19.29[a]
Goat	35.82[a]	3.68[a]	28.45[a]	79.42[a]	7.37[ac]	20.58[a]
CD(p<0.05)	**0.57**	**0.51**	**0.55**	**0.53**	**0.53**	**0.58**

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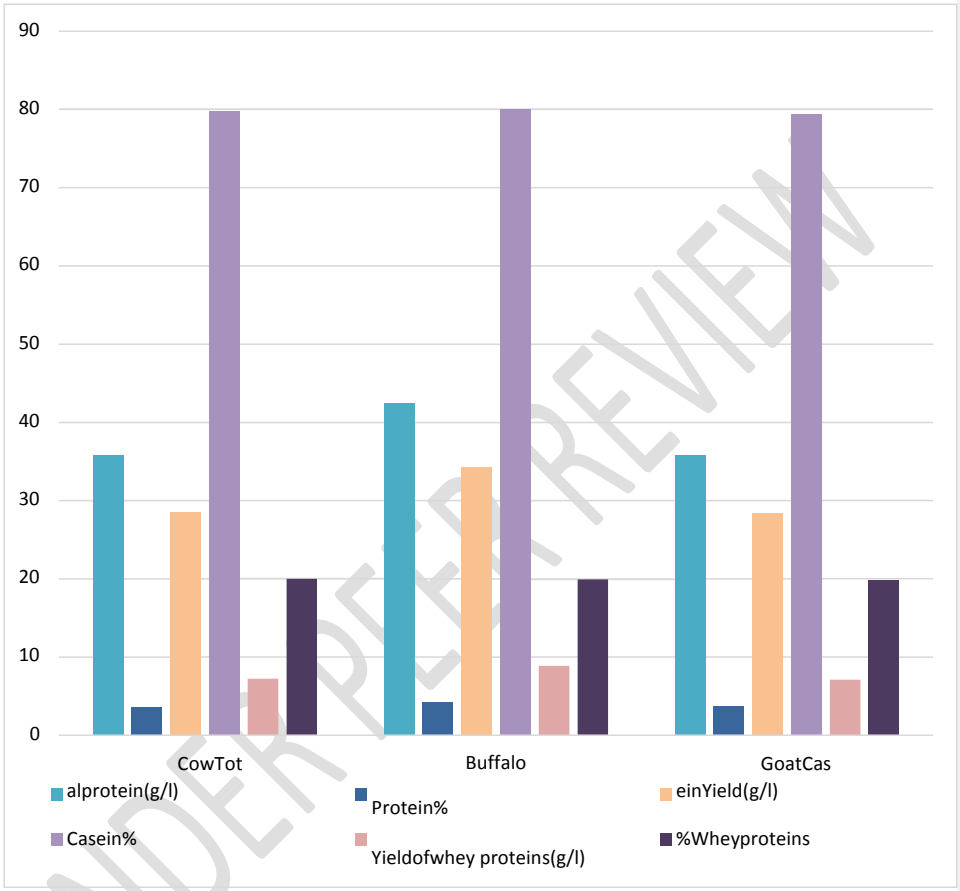

Fig.1.Effectofsourceofmilkproteinon yield ofcaseinsandwheyprotein.

Comment [mt16]: Mention this Figure in any section of result.

Table2:Effect ofsourceofproteinon yield ofvariousfractions ofcasein

Sourceof CaseinFractions	TotalCasein(g/l)	Yield(%)	Totalα-Casein (g/l)	Yield(%)	Totalβ-Casein (g/l)	Yield(%)	Totalk-Casein (g/l)	Yield(%)
Cow	27.47[a]	76.83[a]	14.92[a]	54.31[a]	9.38[a]	34.14[a]	3.17[a]	11.53[a]
Buffalo	34.13[b]	76.70[a]	16.64[b]	48.75[b]	12.30[b]	36.03[b]	5.19[b]	15.20[b]
Goat	26.62[c]	72.33[b]	5.42[c]	20.36[c]	17.85[c]	54.05[c]	3.35[ac]	20.49[c]
CD(p< 0.05)	**0.53**	**0.54**	**0.47**	**0.60**	**0.56**	**0.50**	**0.55**	**0.49**

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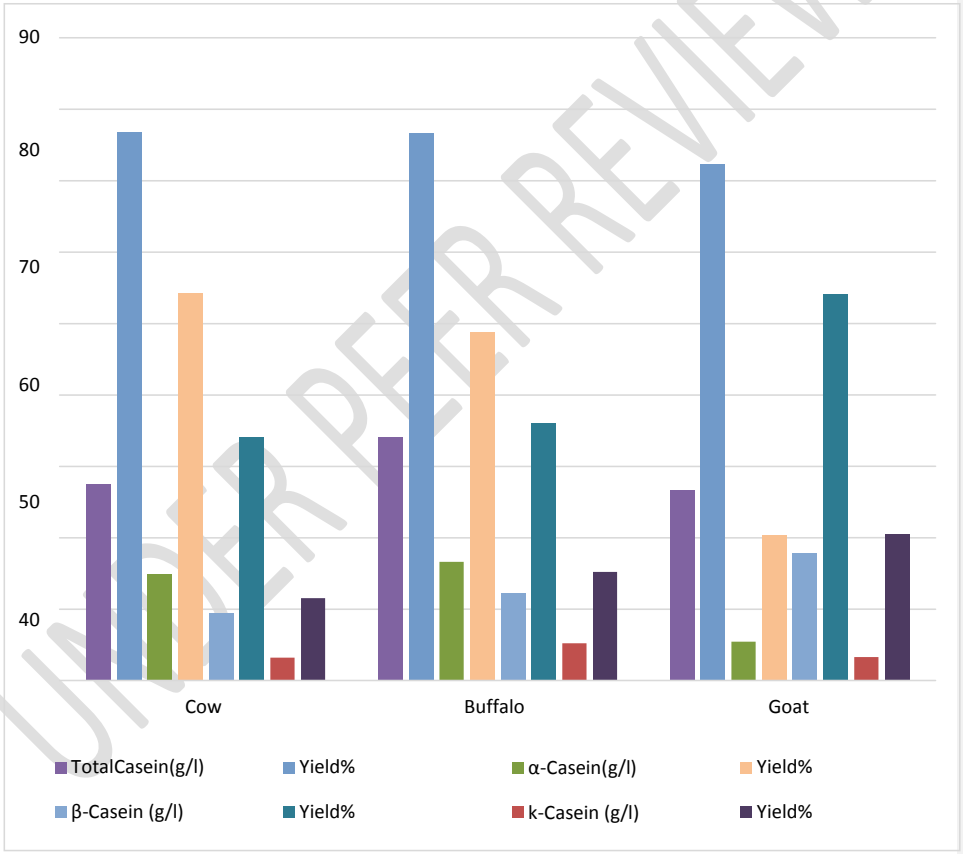

Fig.2.Effectofsourceofproteinon yield ofvarious fractionsofcasein

CONCLUSION:

Caseinobtainedfromcow,buffaloandgoatmilkwerefractionatedbyureasolubilitymethodandthefractions werequantified.Thesignificanteffectofsourceonproteinyieldandfractionsofcaseinswasobserved.Higheryieldo fα-casein(54.31%)wasobserved in cow milk than buffalo milk (48.95 %) and lower yield (20.36 %) wasfoundingoatmilk.Amongstthethreespecies,highestpercentofβcaseinwasnotedingoatmilk (54.05%)followedbybuffalo (36.03%)andcowmilk (34.14%), The highest molecular weight ~~in respect~~ of α-casein (23.82 kDa) was observed incowmilkfollowedbygoatmilk(23.61KDa)andbuffalomilk(22.74kDa).Whereasthe molecular weight of β casein in ~~respect of~~ cow milk was 24.31, and it was 23.84for buffalo milk and 23.82 for goat milk. There was no wide variation in molecularweight of k-casein irrespective of source of milk. The molecular weight of k- caseinvariedbetween 19.15 to19.38kDa.

Comment [mt17]: Mention units

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