



## Research article

## Total carotene content and quality characteristics of pumpkin flavoured buffalo milk

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## ABSTRACT

As per the World Health Organization (WHO) and Global Hunger Index, the incidence of malnutrition and Vitamin-A deficiency in preschool-age children is higher in South-East Asian countries. Therefore, this study was envisaged to formulate value-added flavoured buffalo milk and to carry out the sensory evaluation, nutritional analysis, storage characteristics and total carotene content of the developed product. Flavoured buffalo milk was prepared by incorporation of *Cucurbita pepo* (Pumpkin) pulp and ground sugar at a concentration of 15% and 10%, respectively. The formulation was found preeminent by panellists and then subjected to nutritional analysis and storage studies at room temperature following “in bottle heat treatment”. Nutritional analysis revealed that the protein, fat, total carbohydrates, total ash and moisture content of pumpkin flavoured buffalo milk were 3.07%, 5.21%, 12.63%, 0.61% and 78.48%, respectively. The storage study of flavoured buffalo milk showed a significantly ( $p < 0.05$ ) declined score in colour and appearance as well as flavour only after day 90. The overall acceptability score also declined significantly ( $p < 0.05$ ) after day 60 of storage. Though the sensory score declined gradually during the storage period, the product was liked by the panellist even till day 180. The carotene content of pumpkin flavoured buffalo milk was 1.2 mg/100 g at day 0 and it decreased significantly as storage prolongs. The nutritional components were not affected significantly during the 180 days storage period. Standard plate counts, coliform counts as well as yeast and mould counts were not detected during storage of pumpkin flavoured buffalo milk.

## 1. Introduction

According to the Global Hunger Index, most of the South-East Asian countries have high child malnutrition rate. Further, in these countries, the night blindness (caused by vitamin A deficiency) in preschool-age children (82.4%) and pregnant women (96.8%) were highest as per the survey of World Health Organization (2009). Good quality animal protein sources like milk or meat can help to reduce nutritional deficiencies and malnutrition in children and pregnant women. A major part of the buffalo population resides in Asian countries. Buffalo milk plays a key role in human nutrition, predominantly in developing countries. As compared to cow milk, buffalo milk is wealthier in almost all the main milk nutrients (El-Salam and El-Shibiny, 2011). Owing to more fat and protein, buffalo milk provides more energy than cow milk but contains a negligible quantity of carotene.

*Cucurbita pepo* (Pumpkin), traditionally known as Kolu, Kaddu or Kohala, is a cultivar of the squash plant. Fleshy shell, leaves, seeds, and even the flowers of *Cucurbita pepo* are used for edible purpose. Pumpkin is lavish in dietary fibre, carotene, minerals, vitamins, pectin (Djutin, 1991) and other bioactive substances such as phenolic compounds and terpenoids (Crozier, 2003). Kulkarni and Joshi (2014) reported high (3mg/100g) carotene content in pumpkin pulp. It was also reported that total carotenoids content in epicarp and mesocarp of various *Cucurbita pepo* cultivars ranges from 68 to 4453 mgKg<sup>-1</sup> and 35 to 371 mgKg<sup>-1</sup>, respectively (Martínez-Valdivieso et al., 2015). Carotene from fruits, tubers and pulpy vegetables, such as pumpkin is measured to be better engrossed than those from dark green leafy vegetables (DePee, 1996). Pumpkin is also utilized in various countries as an anti-inflammatory, antiviral, anti-ulcerative, antidiabetic and antioxidant in one or other form (Smith, 1997). The sulfated and phosphorylated polysaccharides

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present in pumpkin act as an excellent antioxidant (Chen and Huang, 2019a, b). The phyto-chemicals exist in pumpkin affect glucose and insulin levels in laboratory diabetic models (Perez Gutierrez, 2016). Globally, India ranks second after China in pumpkin production. Pumpkin is available worldwide at a cheap rate and has economical value (Schaffer and Paris, 2016).

At present, many researchers delve into the development of value-added milk and milk products. Hence, the present study was planned to formulate value-added pumpkin flavoured buffalo and to carry out the sensory evaluation, nutritional analysis and storage study.

## 2. Materials and methods

Buffalo milk was procured from organized dairy farms and subjected to rapid platform tests for quality evaluation. Pumpkins were procured from the local market. The pumpkin flavoured buffalo milk was prepared following the procedure of De (1980) with slight modifications (Figure 1).

### 2.1. Preparation of pumpkin flavoured buffalo milk (PFBM)

Buffalo milk was preheated and filtered at 35–40 °C followed by standardization at 6% fat and 9% SNF using skim milk powder (Himedia brand). An appropriate amount of milk was heated above pasteurization temperature (65 °C) in an iron vessel with constant stirring. Simultaneously, mesocarp of pumpkin was steam-cooked (5 min) in the cooker as per the method described by Carvalho et al. (2014). Cooked content was minced with the help of mincer. The pumpkin pulp and ground sugar at different concentrations (Table 1) were mixed with milk at 35–40 °C which is taken as different treatments (Table 1). After the standardization of concentrations, the products were filled in clean sterilized glass bottles (250 ml) followed by “in bottle heat treatment” at  $110 \pm 2$  °C for 25 (De, 1980) and stored at refrigeration temperature.

### 2.2. Sensory evaluation

Samples were evaluated in three replications for sensory characteristics on a 9-point hedonic scale (Lawless and Heymann, 1998) by a panel of six judges for nine possible treatments. Informed consent was obtained from all participants before the sensory evaluation. Sensory characteristics like colour and appearance, flavour and overall acceptability were evaluated. During the sensory evaluation, the products were presented in

**Table 1.** Different concentration of pulp and sugar given to the product (w/v).

Treatment	Cucurbita pepoPulp (%)	Ground Sugar (%)
T1	5	5
T2	5	10
T3	5	15
T4	10	5
T5	10	10
T6	10	15
T7	15	5
T8	15	10
T9	15	15

chilled (10 °C) form and panellists cleansed their mouths between samples using clean potable drinking water.

### 2.3. Nutritional analysis

The nutritional composition (protein, fat, total carbohydrates, moisture and ash content) of PFBM, raw milk and pumpkin pulp were estimated in three replications as per methods described in BIS SP: 18, Part XI (1981).

### 2.4. Storage study

The PFBM was stored at room temperature for 180 days and storage study was carried out at a fortnight interval in three replications. During storage study, the product was evaluated for the sensory characteristics (Lawless and Heymann, 1998), microbial quality (APHA Speck, 1984) and nutritional composition (BIS SP: 18, Part XI, 1981). The carotene content was estimated as per the method described by Lee and Castle (2001) with a slight modification. Briefly, the PFBM samples (2.5 ml) were homogenized with 5 ml of extracting distilled acetone and centrifuged for 5 min at 6,500 rpm at 5 °C. The acetone containing the top layer were recovered and transferred to glass tubes secluded from light and homogenized with a Pasteur pipette. After that, 1 ml of this supernatant was transferred to a 25 ml volumetric flask, and the volume was completed with acetone. The total carotenoid determination was carried out on an aliquot of the acetone extract by measuring the absorbance at 450 nm using a UV-Vis spectrophotometer (Thermo Scientific™ Evolution 201).

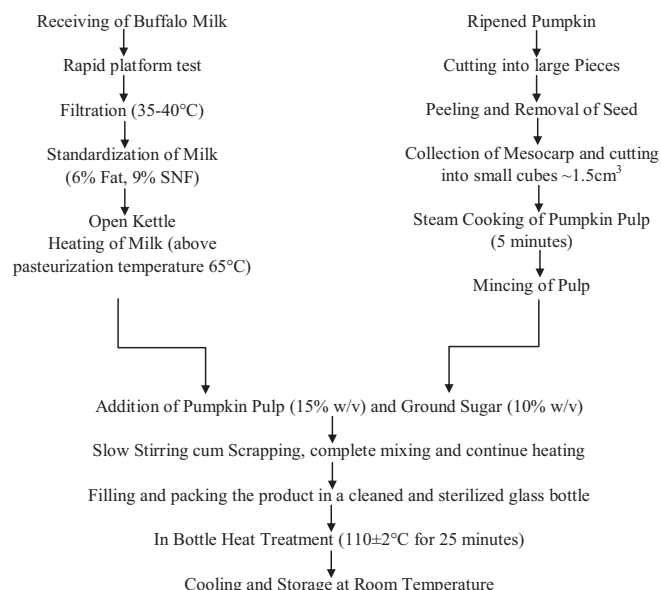
### 2.5. Statistical analysis

The data obtained from the sensory evaluation and nutritional analyses were subjected to analysis of variance according to the method described by Snedecor (2012) using IBM Statistical Package for Social Sciences (SPSS) software. The Duncan's multiple range tests for post hoc analysis was used for comparing the means of sensory scores to find the significance of the acceptability of developed product by the panellists.

## 3. Results and discussion

### 3.1. Sensory evaluation

All the sensory attributes of the Product *i.e.* colour and appearance, flavour as well as overall acceptability assume an important position in the product acceptance by consumers. The sensory score obtained during analysis is presented in Table 2. The sugar and pumpkin pulp concentrations have a significant effect on flavoured milk. The score for mean colour and appearance improves significantly ( $p < 0.05$ ) as the concentration of pumpkin pulp increase. This might be due to increase in the concentration of Beta-carotenes with higher levels of Yellow-Orange colour of pumpkin pulp. Bajwa and Mittal (2015) revealed similar



**Figure 1.** Process flow chart for preparation of pumpkin flavoured buffalo milk.

**Table 2.** Sensory analysis of flavoured buffalo milk incorporated with *Cucurbita pepo* pulp.

Sr. no.	Treatment	Colour and Appearance	Flavour	Overall Acceptability
1	T <sub>1</sub>	5.8 ± 0.2 <sup>a</sup>	5.6 ± 0.2 <sup>a</sup>	5.6 ± 0.2 <sup>a</sup>
2	T <sub>2</sub>	6.2 ± 0.2 <sup>ab</sup>	6.7 ± 0.2 <sup>bc</sup>	6.2 ± 0.3 <sup>b</sup>
3	T <sub>3</sub>	6.8 ± 0.1 <sup>bc</sup>	6.7 ± 0.2 <sup>bc</sup>	6.6 ± 0.2 <sup>bc</sup>
4	T <sub>4</sub>	6.3 ± 0.2 <sup>ab</sup>	6.1 ± 0.3 <sup>ab</sup>	6.2 ± 0.2 <sup>b</sup>
5	T <sub>5</sub>	7.2 ± 0.2 <sup>cd</sup>	8.0 ± 0.1 <sup>d</sup>	7.6 ± 0.1 <sup>c</sup>
6	T <sub>6</sub>	7.2 ± 0.2 <sup>c</sup>	6.7 ± 0.2 <sup>bc</sup>	7.2 ± 0.2 <sup>de</sup>
7	T <sub>7</sub>	7.1 ± 0.3 <sup>c</sup>	6.9 ± 0.3 <sup>c</sup>	6.8 ± 0.2 <sup>cd</sup>
8	T <sub>8</sub>	8.2 ± 0.1 <sup>e</sup>	8.1 ± 0.2 <sup>d</sup>	8.3 ± 0.1 <sup>f</sup>
9	T <sub>9</sub>	7.8 ± 0.2 <sup>de</sup>	7.2 ± 0.2 <sup>c</sup>	7.3 ± 0.1 <sup>de</sup>

Superscripts are to be read column-wise for mean comparison. Mean with different superscripts differ significantly (p < 0.05).

**Table 3.** Nutritional Analysis of pumpkin flavoured buffalo milk.

Sr. no.	Nutritional Analysis	Pumpkin Flavoured Buffalo Milk	Raw milk	Pumpkin pulp
1	Fat (Ether Extract)%	5.2 ± 0.1	6.0 ± 0.4	0.5 ± 0.1
2	Protein%	3.1 ± 0.1	3.4 ± 0.2	1.3 ± 0.2
3	Total CHO%	12.6 ± 0.4	5.8 ± 0.3	2.9 ± 0.3
4	Moisture%	78.5 ± 0.3	84.2 ± 0.4	94.7 ± 0.4
5	Total Ash%	0.6 ± 0.02	0.6 ± 0.04	0.5 ± 0.04

results in colour and appearance score of mango pulp based milk drink. The mean flavour score showed a significant (p<0.05) difference as the concentration of sugar and pumpkin pulp changes. A similar trend was also found by [Güven and Karaca \(2002\)](#) in the preparation of frozen yoghurt. They reported that an increase in sugar and fruit level improved the aroma and flavour. [Changade et al. \(2012\)](#) reported a similar trend in the effect of pumpkin pulp concentration on kheer. The mean overall acceptability score showed a significantly (p<0.05) higher score with increasing level of pumpkin pulp and sugar except for treatment T<sub>9</sub>. Values obtained over sensory analysis revealed that the colour and appearance of T<sub>9</sub> did not differ significantly (p>0.05) from T<sub>8</sub> probably because of the same concentration of pumpkin pulp in both the treatments. Similarly, the flavour score of treatments T<sub>5</sub> and T<sub>8</sub> did not vary significantly (p>0.05). This might be due to equal sugar concentration in both the treatments. As per the score obtained on sensory evaluation, the product with 10% sugar was liked preeminent by the panellists. This result was in close agreement with [Coyle et al. \(2019\)](#) who reported in a cross-sectional study across Australia, England and South Africa that total sugar content of flavoured milk ranged from 4.3 to 15.0 gm/100 ml with a mean of 9.1 ± 2.0 gm/100 ml. Moreover, [World Health Organization \(2015\)](#) published guidelines that the sugar added to foods should be <10% of their total energy intake. As per the score given in [Table 2](#), T<sub>8</sub> (15% pumpkin pulp, 10% sugar) was found to be preeminent by a panel of judges and it might be due to the combined effect of sugar and pumpkin pulp.

### 3.2. Nutritional analysis

The nutritional analysis of the PFBM, raw milk and pumpkin pulp is given in [Table 3](#). The data revealed that the protein, fat, total carbohydrates, total ash and moisture content of PFBM was 3.07%, 5.21%, 12.63%, 0.61% and 78.48%, respectively. However, the protein, fat, total carbohydrates, total ash and moisture content of raw milk was 3.4%, 6.01%, 5.84%, 0.59% and 84.16%, respectively and for pumpkin pulp it was 1.28%, 0.49%, 2.97%, 0.53% and 94.73%, respectively. A lower fat and protein content in the product as compared to raw milk might be due to the low level of fat and protein in pumpkin pulp. The data were in close

**Table 4.** Sensory evaluation of pumpkin flavored buffalo milk during storage of 180 days (6 months).

Storage days	0	15	30	45	60	75	90	105	120	135	150	165	180
Colour and Appearance	8.33 <sup>a</sup> ± 0.21	7.83 <sup>ab</sup> ± 0.17	7.67 <sup>abc</sup> ± 0.21	7.50 <sup>abcd</sup> ± 0.22	7.50 <sup>abcd</sup> ± 0.22	7.50 <sup>abcd</sup> ± 0.22	7.17 <sup>bcde</sup> ± 0.17	6.83 <sup>bcde</sup> ± 0.17	6.67 <sup>cde</sup> ± 0.21	6.50 <sup>de</sup> ± 0.22	6.50 <sup>de</sup> ± 0.22	6.33 <sup>e</sup> ± 0.21	6.33 <sup>e</sup> ± 0.21
Flavour	8.17 <sup>a</sup> ± 0.31	7.83 <sup>a</sup> ± 0.17	7.67 <sup>ab</sup> ± 0.21	7.67 <sup>ab</sup> ± 0.21	7.67 <sup>ab</sup> ± 0.21	7.33 <sup>abc</sup> ± 0.21	6.67 <sup>bcd</sup> ± 0.21	6.67 <sup>bcd</sup> ± 0.21	6.67 <sup>bcd</sup> ± 0.21	6.67 <sup>bcd</sup> ± 0.21	6.50 <sup>cd</sup> ± 0.22	6.17 <sup>d</sup> ± 0.17	6.17 <sup>d</sup> ± 0.17
Overall Acceptability	8.33 <sup>a</sup> ± 0.21	7.83 <sup>ab</sup> ± 0.17	7.83 <sup>ab</sup> ± 0.17	7.83 <sup>ab</sup> ± 0.17	7.33 <sup>bc</sup> ± 0.21	7.33 <sup>bc</sup> ± 0.21	7.17 <sup>bc</sup> ± 0.17	7.17 <sup>bc</sup> ± 0.17	6.83 <sup>c</sup> ± 0.17	6.83 <sup>c</sup> ± 0.17	6.83 <sup>c</sup> ± 0.17	6.83 <sup>c</sup> ± 0.17	6.50 <sup>c</sup> ± 0.22

Superscripts are to be read row wise for mean comparison. Mean with different superscripts differ significantly (p < 0.05).

Table 5. Evaluation of nutritional compositions and carotene content of pumpkin flavoured buffalo milk during storage of 180 days.

Storage days	0	15	30	45	60	75	90	105	120	135	150	165	180
Fat (Ether Extract)	5.21 ± 0.13	5.15 ± 0.13	5.18 ± 0.13	5.25 ± 0.20	5.31 ± 0.18	5.18 ± 0.18	5.23 ± 0.22	5.23 ± 0.18	5.22 ± 0.13	5.11 ± 0.13	5.19 ± 0.13	5.10 ± 0.15	5.28 ± 0.18
Protein	3.07 ± 0.10	3.07 ± 0.15	3.01 ± 0.10	3.12 ± 0.07	3.03 ± 0.12	3.07 ± 0.10	3.13 ± 0.10	3.05 ± 0.15	3.18 ± 0.15	3.07 ± 0.07	3.10 ± 0.12	3.04 ± 0.10	3.05 ± 0.12
Total CHO	12.63 ± 0.37	12.51 ± 0.45	12.60 ± 0.43	12.89 ± 0.53	12.85 ± 0.29	12.61 ± 0.33	12.48 ± 0.37	12.61 ± 0.45	12.63 ± 0.51	12.58 ± 0.31	12.91 ± 0.37	12.60 ± 0.43	12.63 ± 0.29
Moisture	78.48 ± 0.34	76.08 ± 0.84	78.96 ± 0.48	76.26 ± 0.93	78.55 ± 0.34	79.33 ± 0.68	78.40 ± 0.34	78.71 ± 0.93	79.96 ± 0.28	77.56 ± 0.28	76.49 ± 0.34	78.67 ± 0.80	78.20 ± 0.54
Total Ash	0.61 ± 0.02	0.60 ± 0.02	0.65 ± 0.03	0.63 ± 0.02	0.63 ± 0.03	0.61 ± 0.03	0.60 ± 0.02	0.64 ± 0.02	0.63 ± 0.03	0.63 ± 0.02	0.65 ± 0.02	0.63 ± 0.03	0.63 ± 0.02
Carotene content (mg/100gram)	1.200 <sup>a</sup> ± 0.058	0.958 <sup>b</sup> ± 0.021	0.810 <sup>bc</sup> ± 0.045	0.734 <sup>cd</sup> ± 0.032	0.668 <sup>cd</sup> ± 0.025	0.589 <sup>de</sup> ± 0.020	0.478 <sup>ef</sup> ± 0.025	0.397 <sup>fg</sup> ± 0.044	0.319 <sup>gh</sup> ± 0.035	0.274 <sup>ghi</sup> ± 0.044	0.226 <sup>ghi</sup> ± 0.047	0.190 <sup>hi</sup> ± 0.033	0.126 <sup>i</sup> ± 0.021

Superscripts are to be read row wise for mean comparison.

Mean with different superscripts differ significantly (p &lt; 0.05)).

agreement with the report of [Nadaf et al. \(2012\)](#) in the gulkand and rose petal incorporated shrikhand. They said that the protein content significantly decreased with increase in gulkand and rose petal powder quantity. Similar results were also reported in preparation of papaya pulp ([Nigam et al., 2009](#)) and apple pulp ([Kumar et al., 2011](#)) incorporated shrikhand. The increased level of total carbohydrate is due to the concentration of sugar used in product preparation. [Gambelli et al. \(1999\)](#) also revealed that the increasing level of saccharose can significantly affect the total carbohydrate content of fermented milk products.

### 3.3. Storage study

During the period of storage, PFBM was evaluated for sensory quality, nutritional compositions and microbial quality. The observations recorded are as under.

#### 3.3.1. Evaluation of sensory quality during storage study

The sensory score of PFBM obtained during storage study is presented in [Table 4](#). It was found that during storage of sterilized products for 6 months at room temperature, colour and appearance score of product decreased gradually and it was significantly (P<0.05) lower at day 90 of storage as compared to day 0. The flavour and overall acceptability scores also decreased gradually during the storage period. This might be due to non-enzymatic browning reaction during storage which leads to burnt/bitter-sweet aftertaste in milk drinks ([Mittal and Bajwa, 2014](#)). The flavour score at 90 days, differed significantly (P<0.05) from the score at day 0 of storage and the overall acceptability score was significantly (P<0.05) lower at 60 days as compared to day 0. Though the sensory score declined during storage study, the product was liked by the panellists even at the end of the whole storage period of 180 days. The results were in agreement with the report by [Shukla et al. \(2018\)](#) who reported decreased sensory score for sterilized mango based dairy beverages after storage for 75 days. Similarly, [Mittal and Bajwa \(2014\)](#) reported that the sensory score of sterilized milk drinks did not differ significantly up to 150 days of storage.

#### 3.3.2. Evaluation of nutritional composition and carotene content during storage study

The data obtained over the estimation of nutritional compositions and carotene contents is presented in [Table 5](#). Nutritional values for different parameters did not differ significantly for the product even after the storage up to 6 months. Similar results were also reported by [Yadav et al. \(2010\)](#) in whey-based banana herbal beverage and [Hassan et al. \(2015\)](#) in fruit flavoured milk-based beverage. [Mittal and Bajwa \(2014\)](#) found that the total solids content of sterilized milk drink was not affected significantly during 150 days of storage. However in this study, the carotene content of product reduced significantly (P < 0.05) as the storage period prolongs. The carotene content of sterilized PFBM was 1.2 mg/100 g at day 0 which reduced to 0.126 mg/100 g at day 180. The loss of carotene in flavoured milk might be due to the effect of “in bottle heat treatment” and storage period. This result was in close agreement with [Muntean \(2005\)](#) who also reported a loss of carotene in pumpkin juice due to heat treatment and storage period. The results were also similar to [Britton et al \(1995\)](#) who reported that heat treatment significantly affects carotenoids. The β-carotene significantly decreased during storage up to three months which might be due to the auto-oxidative degradation and non-enzymatic browning during processing and storage of pumpkin puree at high temperature ([Provesi et al., 2011](#)). Moreover, [Kulkarni and Joshi \(2014\)](#) reported that carotene content in pumpkin powder reduced to 48.71% when stored at 30 °C for 180 days. A similar decreasing score in β-carotene was reported by [Chen et al. \(1996\)](#) in carrot juice and [Lin and Chen \(2005\)](#) in tomato juice storage.

#### 3.3.3. Evaluation of microbial quality during storage study

The microbial quality was evaluated during the storage period for PFBM. The total plate count, coliform as well as yeast and mould count



was not observed during the whole storage period of 180 days. It might be due to the hygienic conditions maintained during product preparation and processing. These results were in close agreement with Shukla et al. (2018) who also reported that throughout the storage of 75 days microbial counts were not detected in sterilized mango based dairy beverages. Similarly, Mittal and Bajwa (2014) did not detect standard plate count as well as yeast and mould count up to 150 days of storage in sterilized low-calorie milk drinks.

#### 4. Conclusions

The pumpkin flavoured buffalo milk prepared by incorporation of *Cucurbita pepo* pulp and ground sugar at a concentration of 15% and 10%, respectively, was found best by panellists. During storage period of 180 days at room temperature, the sensory score of product decreased significantly only after 90 days of storage. However, at the end of whole storage period, as per score, the product was liked by a sensory panel. There was no significant change in nutritional composition except carotene content and the product was microbially safe and liked by a sensory panel up to 180 days of storage.

#### Declarations

##### Author contribution statement

Ajay S Patel: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Akshay R Bariya: Conceived and designed the experiments; Performed the experiments.

Sanjay N Ghodasara: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Jignesh A Chavda: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Shrikant S. Patil: Analyzed and interpreted the data; Wrote the paper.

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##### Competing interest statement

The authors declare no conflict of interest.

##### Additional information

No additional information is available for this paper.

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