Food Research 8 (6) : 40 - 47 (December 2024)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

RESEARCH PAPER

Effect of kefir grain concentration on rheological, microbiological, and physicochemical characteristics of bovine colostrum kefir

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Article history:
Received: 7 November 2022
Received in revised form: 22 January 2023
Accepted: 9 March 2024
Available Online: 15 November 2024

Keywords:
Colostrum,
Kefir grain,
Lactic acid bacteria,
Rheology,
Yeast

DOI:
https://doi.org/10.26656/fr.2017.8(6).558

Abstract

Bovine colostrum has high nutrition content such as protein, fat, peptide, micro-nutrients, antimicrobial components and bioactive compounds that benefit the body. Kefir is one way of diversifying food products as fermented beverage products to increase the utilization and functional properties of bovine colostrum, which has a unique taste. Production of colostrum kefir requires the right concentration of kefir grains to obtain high-quality characteristics. Evaluating the effect of kefir grain concentration on the rheological, microbiological, and physicochemical characteristics of bovine colostrum kefir was the purpose of this research. This study used one factorial design with the variation of kefir grain concentrations 10% (T1), 20% (T2), and 30% (T3) (w/v) with seven replications. Rheological analysis was conducted, followed by total lactic acid bacteria, yeast, microbes, protein, fat, ash, water content, carbohydrate, pH, total dissolved solids and alcohol content. Based on the result, different kefir grain concentrations significantly decreased total LAB, yeast, microbes, protein, ash, pH, and total dissolved solids but increased carbohydrate and water content with the addition of kefir grain concentration. Rheology behavior, fats, and alcohol content had no significant difference. Treatment of 10% of kefir grain concentration is the most suitable as it contains the highest total LAB and yeast.

1. Introduction

Kefir is a fermented beverage made from kefir grains that has an acidic flavor, a touch of alcohol and a trace bit of soda (Beirami-Serizkani *et al.,* 2021). Unlike yogurt, kefir is produced using kefir grains containing microbes such as lactic acid bacteria (LAB) and yeast. As a functional product, kefir has beneficial effects on the body. Kefir has antibacterial and antifungal properties from fermented organic acids as well as peptides and bacteriocins in kefir (Erdogan *et al.,* 2019). Kefir contains bioactive compounds that act as antihypertensive, anticancer, antidiabetic, antimicrobial, antioxidant, and anti-inflammatory, and has the effect of lowering cholesterol (Azizi *et al.,* 2021). There are several types of kefir based on the substrate used in fermentation, namely dairy kefir and water kefir. Dairy kefir can be further divided into several types, including whey kefir, optima kefir, prima kefir, super prima kefir, and colostrum kefir.

Colostrum kefir is a kefir with colostrum as the substrate for the growth of microorganisms in kefir grains. Bovine colostrum is produced after giving birth and becomes the first initial milk for calves. Colostrum contains high nutrients which are beneficial to the body. Colostrum has different contents and characteristics compared to milk. Colostrum tends to contain less lactose but more fat, protein, peptide, ash, antimicrobial components, hormones, enzymes, vitamins, and minerals and has a reddish-yellow color with higher specific gravity than milk (McGrath *et al.,* 2016). Colostrum contains highly bioactive compounds in the form of immunoglobulins, lactoferrin, and lysozyme, which can strengthen the body's immune system (Dzik *et al.,* 2017). The functional bioactive compounds in colostrum have a role in increasing the immunity of newborn calves with antimicrobial activity as protection from viral, bacterial, and fungal infections.

Bovine colostrum is rich in macro- and micro-nutrients, antimicrobial activity, and bioactive compounds. This high nutritional content of colostrum

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eISSN: 2550-2166 /
Published by Rynnye Lyan Resources

indicates that colostrum may act as a functional food. Therefore, it is necessary to increase the benefits of colostrum by diversifying food products with functional value, one of which is colostrum kefir. Colostrum kefir has the advantage of containing better bioactive and antimicrobial compounds than kefir with milk as raw material.

The production of colostrum kefir requires the right concentration of kefir grains so that the kefir product has good quality and characteristics. Therefore, the purpose of this study was to determine the effect of kefir grain concentration on the rheological, microbiological, and physicochemical characteristics of bovine colostrum kefir.

2. Materials and methods

2.1 Materials

Bovine colostrum was provided from dairy cows farm in Ungaran, Indonesia. Kefir grains were purchased from a Small Medium Enterprises Omah Kefir, Ungaran. The materials for experimental analysis, such as Anton Paar rheometer MCR302 (Anton Paar, Austria), Fat Extractor E-500 (Buchi, Switzerland), analytical balance (Ohaus, US), digital refractometer (Atago, Japan), pH meter, UV-Vis spectrophotometer, burette, Kjeldahl unit (Buchi, Switzerland), oven, desiccator, evaporating dish, furnace, Erlenmeyer, pipette bulb, graduated cylinder, beaker glass, micropipette, petri dish, Conway dish, test tube, vortex, magnetic stirrer, water bath, laminar air flow, incubator, MRSA (de Man Rogosa Sharpe Agar), SDA (Sabouraud Dextrose Agar), PCA (Plate Count Agar), 0.85% NaCl, sulfuric acid, boric acid, sodium hydroxide, hydrochloric acid, potassium dichromate, sodium carbonate, petroleum benzene, and 1% phenolphthalein indicator.

2.2 Production of kefir bovine colostrum

Colostrum kefir production refers to Windayani *et al.* (2019) with modifications. Cow colostrum was pasteurized using the low-temperature long-time method. The temperature and time of pasteurization of bovine colostrum were at 60°C for 120 mins (Elizondo-Salazar *et al*., 2010). The temperature of colostrum was then lowered to room temperature (28°C) and inoculated with the addition of starter grain kefir with 10% (T1), 20% (T2), and 30% (T3) (w/v) treatments. Colostrum kefir was put in a closed container that was sterilized and stored for 48 hrs at room temperature (28°C) for fermentation. The colostrum kefir was filtered to separate the starter grain from the colostrum kefir.

2.3 Analysis of rheology

The rheological behavior of bovine colostrum kefir was determined using Anton Paar rheometer MCR302. The measurements were equipped with PP50 (50 mm diameter parallel plate). The bovine colostrum kefir samples were poured into the lower plate, and the upper plate was lowered until the upper plate reached 1 mm gap. All the measurements were conducted with a temperature ramp test from 20 to 70°C. The storage modulus (G′), loss modulus (G′′), loss tangent (tan δ), and complex viscosity (η*) value of colostrum kefir were evaluated during the test.

2.4 Determination of proximate composition

Proximate composition was evaluated using the standard method (Association of Official Analytical Collaboration (AOAC) International, 2006) for protein, fat, water, and ash. Determination of carbohydrate content used the difference method.

2.5 Microbiology analysis

Total LAB, yeast, and total microbes were evaluated by the total plate count with the pour plate method. A sample of bovine colostrum kefir was diluted 10-fold serially in 0.85% NaCl. Samples from 10^{-5}, 10^{-6}, and 10^{-7} dilutions were pipetted 1 mL into a petri dish, then the MRSA, SDA, and PCA media were poured into the petri dish in duplicate to determine the total LAB, yeast, and total viable bacteria, respectively (Singracha *et al.,* 2017). The total viable bacteria and lactic acid bacteria were incubated under anaerobic conditions at 37°C for 24-48 hrs, while yeast was incubated aerobically at 30°C. The plates having 30–300 colonies were counted after the incubation process is completed.

2.6 Analysis of chemical properties

The pH meter is used to determine the pH value of the samples. The total dissolved solids were measured using a digital refractometer. Analysis of alcohol content is referred to (Nahak *et al.,* 2021) by using UV-Vis spectrophotometer with a color chromophore of dichromatic acid solution. The maximum wavelength of the dichromatic acid solution used was 480 nm. Determination of alcohol content is conducted by making a standard solution of 0; 0.025; 0.05; 0.075; and 0.1% concentration of ethanol from a 0.5% ethanol stock solution in aquadest. The absorbance measurement of the standard solution was incubated in a Conway dish with a solution of dichromate acid and sodium carbonate. The centre of the Conway dish was filled with 1 mL of the dichromatic acid solution, and the outer circle of Conway dish was filled with 1 mL of sodium carbonate and 1 mL of the standard solution. Conway dish was

https://doi.org/10.26656/fr.2017.8(6).558

closed with a lid given silicon grease. Incubation was carried out at 37°C for 2 hrs. Following incubation, the dichromate acid solution was pipetted and diluted in a 10-fold serial. The absorbance of the dichromate acid solution obtained from the standard solution was read using a spectrophotometer at the maximum wavelength. Regression line equation $y = -4.2128x + 0.8107$ was obtained from plotting the absorbance data and the respective standard solution concentration. The alcohol content of colostrum kefir samples was determined by diluting samples 1:4 in aquadest, then pipetted 1 mL in Conway dish next to 1 mL of sodium carbonate solution, and 1 mL of dichromatic acid solution was pipetted in the middle. Conway dish was closed and incubated, and then the dichromate acid solution was pipetted and diluted. The dichromate acid solution was measured for its absorbances using a UV-Vis spectrophotometer. The absorbances were plotted into the regression line equation to obtain the respective alcohol contents, which were multiplied four times to reverse the dilution.

2.7 Design of experiment

In this study, the experimental design used a single factor, namely variation of kefir grain concentrations 10% (T1), 20% (T2), and 30% (T3) (w/v). All three treatments of the products were analyzed with seven replications. The data obtained were analyzed statistically using SPSS 25 software. Data was analyzed with one-way ANOVA. Provided that there was a significant difference, Duncan's Multiple Range Test was performed with a significance level of 5%.

3. Results and discussion

3.1 Rheological characteristic

Figure 1 shows the dynamic mechanical spectra of colostrum kefir. The storage modulus (G′) and loss modulus (G″) value represents the amount of energy stored in a material and the amount of lost energy through viscous dissipation accompanied by deformation (Bortnowska *et al.,* 2014).

Figure 1 shows higher G′ than G″ of colostrum kefir in the temperature range of 20-70°C. A higher G′ (storage modulus) indicates more elastic solid behavior (Glibowski and Kowalska, 2012), which shows a gel-like structure (Yousefvand *et al.,* 2022). This result was

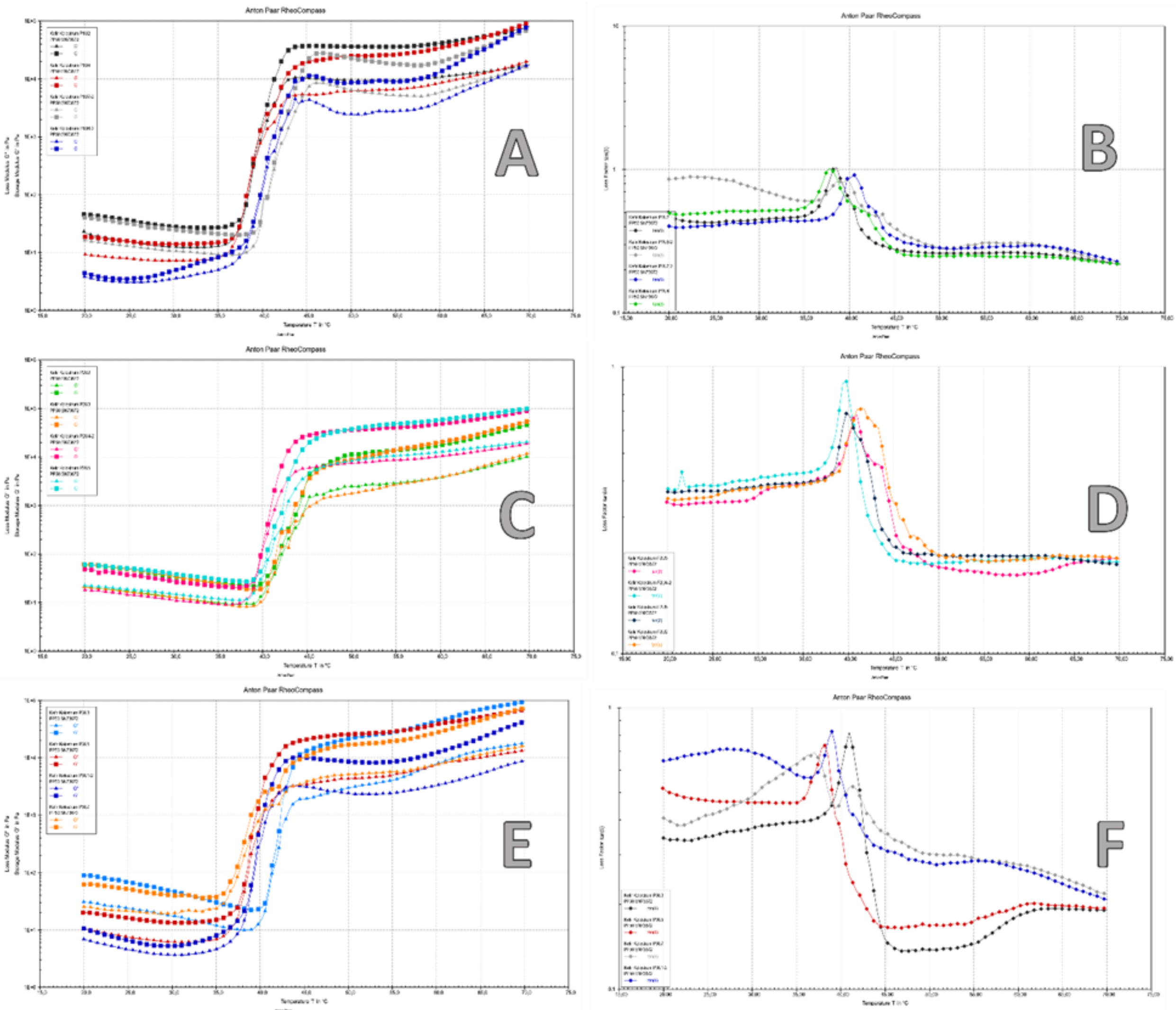

Figure 1. Two-D graphic plot showing G', G"(left) and tan δ (right) of T1 (A, B), T2 (C, D) and T3 (E, F) at a temperature range of 20-70°C.

https://doi.org/10.26656/fr.2017.8(6).558

consistent with previous studies, which reported that kefir was predominantly elastic than viscous with a higher G″ than G′ value (Yilmaz *et al.,* 2020).

Different treatments of grain concentration resulted in different onset temperatures of increasing G′ and G″, which were around 37.5°C, 39°C, and 35°C respectively, for T1, T2, and T3. The increase of both G′ and G″ was estimated as the result of the heat effect on protein interactions and arrangements (Ould Saadi *et al.,* 2020), which could form a gel (Gratzer and Beaven, 1969).

The loss tangent is the ratio of G″ to G′ value, which contrasts the lost energy due to viscous flow with the saved energy caused by elastic distortions. At the range of 20-70°C, the loss tangent curves were located below one, indicating colostrum kefir's solid-like character rather than liquid-like (Crockett *et al.,* 2011). During the abrupt rising of G′ dan G″, all loss tangent curves peaked around 37.5-42.5°C, which indicated that colostrum kefir′s viscosity increased before plummeting to even more elastic solid. A previous study about heat-induced pea protein gelation also observed the increasing pattern of G′ and G″ during heating. The succeeding steady low-loss tangent starting at around 45-50°C represented fewer flexible bonds (Liu *et al.,* 2014), which indicated the formation of a stable gel (Sun and Arntfield, 2010). The abrupt rise of G′ and G″ was considered as the event of protein denaturation, which was significantly contributed by the formation of casein and whey protein complexes with disulfide bonds (Asaduzzaman *et al.,* 2021) that were enhanced through processing with heat treatment and in acidic condition (Xu *et al.,* 2015).

3.2 Microbiological analysis

Treatments of various kefir grain concentrations in colostrum kefir significantly affect the result of the microbiology test. Table 1 shows that total LAB, yeast, and microbes decreased with the increase in kefir grain concentration. The highest total LAB, yeast, and microbes were in colostrum kefir with 10% grain concentration, while the lowest was at 30% grain kefir concentration. The higher concentration of grains used causes the total LAB to decrease caused by the accumulation of lactic acid content, increasing acidity and lowering the pH value. High levels of acidity can inhibit the growth of LAB and cause a decrease in total LAB. 26. Harun-ur-Rashid *et al.* (2007) reported the minimum pH value for the growth of LAB was 3.5-4.5, which are *Lactobacillus lactis* (pH 3.5), *Lactobacillus species D6 40-4* (pH 4.5), *Lactobacillus bulgaricus* (pH 3.0), *Lactobacillus fermentum* (pH 4.0), *Leuconostoc dextranicum* (pH 4.0), *Leuconostoc mesenteroides* (pH 4.0), *Leuconostoc lactis* (pH 4.0), *Leuconostoc raffinolactis* (pH 4.0), and *Streptococcus lactis* (pH 4.5).

The more grains added to kefir, the more colonies of microbes grow in it to compete for food. The decreased nutrient content for microbes causes competition for food sources. Bovine colostrum contains 2.5±0.7% lactose (Kehoe *et al.,* 2007), while lactose in milk is 4.6-4.8% (Ohlsson *et al.,* 2017). As the availability of lactose decreases, the source of nutrients for microbes is not supplied, causing a decrease in total LAB, yeast, and microbes. The amount of yeast in colostrum kefir tends to be higher than LAB. Yeast could grow very well in acidic environments with an ideal pH range of 4 to 6 (Anton *et al.,* 2016). The role of yeast in kefir fermentation is synthesizing metabolites, which increase the flavor and aroma of the product (Ganatsios *et al.,* 2021). LAB's metabolites cause an acidic condition supporting yeast's growth and generating ethanol and CO_2 (Setyawardani *et al.,* 2020). Furthermore, yeasts produce several compounds for the growth of bacteria, such as vitamins and amino acids.

Table 1. Total lactic acid bacteria, yeast, and microbes of colostrum kefir.

Microorganism Counts (log CFU/mL)	Kefir Grain Concentration		
	10%	20%	30%
LAB	6.07±0.11^{a}	5.9 1± 0.91^{ab}	5.75±0.30^{b}
Yeast	6.30±0.10^{a}	6.16±0.43^{b}	5.98±0.30^{c}
Total viable bacteria	6.77±0.22^{a}	6.63±0.24^{ab}	6.34±0.19^{b}

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different ($p<0.05$).

The addition of kefir grain concentration resulted in lower total microbes in colostrum kefir. This result was related to the indirect interactions with microorganisms in kefir due to extracellular metabolites from the metabolic process of kefir microbes (Nejati *et al.,* 2020). The primary fermentation products on kefir are lactate, ethanol, and acetate; each has different roles in the interactions. Yusriyah and Agustini (2014) reported that lactate as metabolites produced by *L. bulgaricus* could inhibit of growth of *Candida* in the kefir. In addition, according to Blasche *et al.* (2021), the growth of *L. lactis* was stimulated by amino acid from yeast proteolysis but inhibited by acetate and lactate. Thus, the higher microorganism in kefir resulted in more complex interactions between kefir microorganism species.

3.3 Proximate composition

Higher kefir grain concentration resulted in a decrease in protein and ash content but an increase in water and carbohydrate content (Table 2). Fewer microbes might cause less protein content to be available

https://doi.org/10.26656/fr.2017.8(6).558

in kefir with higher kefir grain concentration, as the decrease in pH level results in lower activity of bacteria (Sabokbar and Khodaiyan, 2015). The ash content in colostrum kefir was affected by the high total solids and mineral compositions (Saleh *et al.,* 2020). The ash contents ranged from 1.03 - 1.19%. The ash contents of the 30% grain colostrum kefir concentrations were significantly lower compared to the other treatments. These results indicate that the ash contents tend to decrease for each treatment. The minerals in kefir samples might be used for the viability of microorganisms. Malaka *et al.* (2020) reported that the metabolism of sugars by microorganisms used minerals as electron acceptors. Fat content had no significant difference between each concentration level. Kefir grains have little effect on the fat of colostrum kefir but tend to lower the fat content. Lactic acid bacteria were able to hydrolyze fat with lipase enzymes (Devita *et al.,* 2019) and caused a decrease in fat content.

Table 2 shows that the colostrum kefir water content ranged from 69.00 - 71.62% and carbohydrate content ranged from 3.13 – 5.45%. The moisture and carbohydrate content of the 10% grain kefir concentrations was significantly lower compared with the other treatments. The rise in carbohydrates is respective to the actions of lactic acid bacteria and yeast, which simplify lactose to compounds, such as glucose and galactose. The increase in moisture content with higher grain concentrations was suspected because kefir fermentation adsorbed more water from its environment (Melia *et al.,* 2020). The microorganism used the moisture for metabolic activities (Lim *et al.,* 2019), leading to higher moisture adsorption.

Table 2. Proximate composition of colostrum kefir.

Proximate Composition	Kefir Grain Concentration		
	10%	20%	30%
Protein (%)	20.91±1.22[a]	18.21±0.63[b]	15.67±0.52[c]
Fats (% of dry basis)	5.83±1.45[a]	5.90±1.25[a]	6.94±1.78[a]
Ash (%)	1.19±0.03[a]	1.17±0.05[a]	1.03±0.12[b]
Water content (%)	69.00±0.57[b]	70.68±1.47[a]	70.94±1.10[a]
Carbohydrate (%)	3.13±1.22[b]	3.88±1.52[b]	5.45±1.19[a]

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different ($p<0.05$).

3.3 Chemical properties

Different grain concentrations in colostrum kefir significantly affect the pH value and total dissolved solids, but the alcohol content is not significantly affected (Table 3). The pH value ranged from 4.15-4.34, and total dissolved solids ranged from 23.88 – 25.00%. Total dissolved solids tend to decrease with a higher concentration of grain. This result was similar to the literature for buffalo milk kefir (Rizqiati *et al*., 2021). The dissolved solids in milk and other dairy products are mainly lactose and minerals (Rizqiati, Nurwantoro, Susanti and Prayoga *et al*., 2021). Higher grain concentration will break down the lactose even more through microbial activities. The reduced sugar concentration will increase related to decreasing total dissolved solids (Setiawati *et al.,* 2021). Based on Table 3, the pH level of bovine colostrum kefir shows a significant decrease from 10% to 20% and 30% kefir grain concentration. This result was caused by the fermentation of microorganisms that produce acid (Yuan *et al.,* 2019) so the pH level decreased in line with the increased acid production. The percentage of alcohol content ranged from 0.11 - 0.15%. The higher grain concentrations gave an insignificantly higher alcohol concentration. The low concentrate alcohol occurred during kefir fermentation due to the yeast content in the kefir grain (Setyawardani *et al.,* 2020). Fermentation will affect the LAB to reduce the aldehyde into alcohol. This increasing alcohol concentration had the same result as other literature (Setyawardani *et al.,* 2014).

Table 3. pH value, total dissolved solid and alcohol content of colostrum kefir.

Proximate Composition	Kefir Grain Concentration		
	10%	20%	30%
pH	4.34±0.02[a]	4.18±0.03[b]	4.15±0.03[b]
Total dissolved solid (% Bx)	25.00±0.53[a]	24.06±0.27[b]	23. 88±0.61[b]
Alcohol (%)	0.11±0.06[a]	0.15±0.29[a]	0.15±0.06[a]

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different ($p<0.05$).

4. Conclusion

The treatment of different kefir grain concentrations had a significant effect on decreasing total LAB, yeast, microbes, protein, ash, pH, and total dissolved solids while increasing carbohydrate and water content with the addition of kefir grain concentration. Rheology behavior, fats, and alcohol content had no significant difference. This result indicates that 10% of kefir grain concentration is the most suitable treatment based on microbiological parameters' test results, which tend to decrease with the addition of kefir grain concentration.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors appreciated the financial support from

LPDP (Indonesia Endowment Fund for Education) and the support from PRTPP BRIN in this project.

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