



Energy, macronutrients, and sensory characteristics of gluten-and casein-free (GFCF) mocaf biscuits substituted with kidney bean and *keluih* seed flour for children with autism

Kandungan energi, zat gizi makro, dan karakteristik sensori biskuit tepung mocaf bebas gluten dan bebas kasein dengan substitusi tepung kacang merah dan tepung biji keluih untuk anak autisme

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Abstract

Autism is often associated with macronutrient deficiencies due to food sensory preferences, as well as gluten and casein allergies, which can trigger digestive disorders. In this study, gluten- and casein-free (GFCF) biscuits were developed by substituting kidney bean flour and *keluih* seed flour, followed by an evaluation of their energy content, macronutrient composition, and sensory characteristics to determine the best formulation. Four formulas were tested: F0 (100:0:0), F1 (30:52.5:17.5), F2 (30:35:35), and F3 (30:17.5:52.5). Macronutrient content was analyzed using standard laboratory methods (Kjeldahl, Soxhlet, and difference), whereas energy was calculated based on macronutrients. The hedonic test involved 50 untrained panelists, and statistical analysis was conducted using One-Way ANOVA, Kruskal-Wallis test, and a multi-attribute decision compensatory model. The biscuits contained 432–455 kcal of energy, 3–8% protein, 30–32% fat, and 32–40% carbohydrate. Significant differences were observed in energy ($P = 0,028$), protein ($P < 0,001$), fat ($P = 0,005$), and carbohydrate ($P < 0,001$), as well as sensory preferences for taste ($P < 0,001$), aroma ($P = 0,002$), and overall preference ($P = 0,017$). F1 was selected as the best formulation because of its favorable sensory score and balanced nutrient content. Kidney bean flour improved protein and sensory properties, while *keluih* seed flour increased energy and fat but lowered preference levels. This product offers practical potential as a GFCF snack to support the dietary management of children with autism.

Keywords: Autism, biscuit, kidney bean, *keluih* seed, sensory characteristics

Abstrak

Autisme sering dikaitkan dengan kekurangan makronutrien akibat preferensi sensorik terhadap makanan serta alergi gluten dan kasein yang dapat memicu gangguan pencernaan. Penelitian ini mengembangkan biskuit bebas gluten dan kasein (GFCF) dengan mensubstitusi tepung kacang merah dan tepung biji kluwih, kemudian mengevaluasi kandungan energi, komposisi makronutrien, dan karakteristik sensorinya untuk menentukan formulasi terbaik. Empat formula diuji: F0 (100:0:0), F1 (30:52,5:17,5), F2 (30:35:35), dan F3 (30:17,5:52,5). Kandungan makronutrien dianalisis menggunakan metode laboratorium standar (Kjeldahl, Soxhlet, dan difference), sedangkan energi dihitung dari makronutrien. Uji hedonik melibatkan 50 panelis tidak terlatih, dan analisis statistik dilakukan menggunakan *One Way ANOVA*, *Kruskal-Wallis*, serta penentuan formulasi terbaik melalui *multiattribute decision compensatory model*. Biskuit mengandung 432–455 kkal energi, 3–8% protein, 30–32% lemak, dan 32–40% karbohidrat. Perbedaan signifikan ditemukan pada energi ($p = 0,028$),

protein ($p<0,001$), lemak ($p=0,005$), karbohidrat ($p<0,001$), serta preferensi sensorik untuk rasa ($p<0,001$), aroma ($p=0,002$), dan keseluruhan ($p=0,017$). F1 terpilih sebagai formulasi terbaik karena nilai sensoris yang baik dan kandungan gizi yang seimbang. Tepung kacang merah meningkatkan kandungan protein dan sifat sensorik, sedangkan tepung biji kluwih meningkatkan energi dan kandungan lemak tetapi menurunkan kesukaan. Produk ini menawarkan potensi praktis sebagai kudapan GFCF untuk mendukung manajemen diet pada anak dengan autisme.

Kata Kunci: autisme, biskuit, kacang merah, biji kluwih, karakteristik sensori

Introduction

Autism spectrum disorder (ASD) is a developmental disorder that typically manifests in children around the age of three (Astuti et al., 2021; Azwar & Yanti, 2020; Iskandar & Indaryani, 2020). Individuals with autism often consume the same types of food over extended periods and have relatively limited food variety (Azwar & Yanti, 2020). Based on a previous study, the average intake of protein, fat, and carbohydrates among children with autism at the School for Students with Special Needs (SLBN) 1 Garut remains below the recommended levels (Rukmasari & Ramdhani, 2019). The eating habits of children with autism can influence their behavior (Azwar & Yanti, 2020; Rukmasari & Ramdhani, 2019).

Children with autism tend to be more sensitive to sensory characteristics, such as texture, color, taste, and aroma (Chen et al., 2022; Chistol et al., 2018; Huxham et al., 2021). They generally prefer consistent rather than mixed food textures (Baraskewich et al., 2021; Chistol et al., 2018; Huxham et al., 2021). Most studies have indicated that crunchy, smooth, dry, and soft textures are favored (Huxham et al., 2021; Strand, 2021). In addition, they are sensitive to strong aftertastes and tend to avoid foods with intense flavors. In terms of visual preference, children with autism typically avoid yellow-colored foods and prefer brown or green foods (Strand 2021).

The gluten-free casein free (GFCF) diet was designed to eliminate the consumption of gluten and casein in children with autism. When digested, these proteins form casomorphin and gluteomorphin, which may lead to behavioral disorders or hyperactivity (Astuti et al., 2021). A previous study found a significant relationship between GFCF diet implementation and behavioral improvements in children (Azwar & Yanti, 2020). However, most commercially available snacks and meals still contain gluten and casein, making it challenging to apply the GFCF diet consistently (Triningrum et al., 2020).

Biscuits are processed food products with a dry texture, typically made by baking a mixture of wheat flour or its substitutes, oil, fat, and other permitted ingredients (Badan Standarisasi Nasional, 2018). Biscuits can be prepared using flour that does not contain gluten, allowing them to be modified as a GFCF snack (Tanjung & Kusnadi, 2015). Biscuits are generally golden brown, dry, and crispy, which makes them well-suited to the food preferences of children with autism (Saputro et al., 2017).

As part of the efforts to diversify local food ingredients, mocaf (modified cassava flour) can serve as a gluten-free substitute for wheat flour. Mocafs are widely available and more affordable than wheat flour (Ihromi et al., 2017; Ihromi et al., 2018). However, its protein content is lower than that of wheat flour, making it necessary to add other ingredients to improve the nutritional quality of biscuits (Augustyn et al., 2017).

Kidney beans (*Phaseolus vulgaris* L.) are protein-rich legumes. They also serve as sources of complex carbohydrates, fiber, B vitamins (especially folic acid and vitamin B1), calcium, and phosphorus (Nurhayatun et al., 2020). The nutritional content of 100 g of kidney beans includes 314 kcal of energy, 22,1 g of protein, 1,1 g of fat, and 56,2 g of carbohydrates (Mahmud et al., 2018). Based on data from the Indonesian Ministry of Defense in 2014, annual kidney bean production has been increasing, making it more accessible and relatively affordable in the market (Direktorat Jenderal Hortikultura, 2015). A previous study used kidney bean flour as a raw material for making *pukis*, which produced the best nutritional content and sensory characteristics with a ratio of 30% kidney bean flour to 30% breadfruit flour: 10% (Chrestella et al., 2020).

In addition to kidney beans, *keluih* seeds (*Artocarpus camansi*) can be used as a raw material for making gluten-free biscuits. *Keluih* seeds were selected because their protein content was nearly equal to that of soft flour. The protein and complex carbohydrate content

in *keluih* seeds is relatively high compared to jackfruit and durian seeds, yet their utilization remains limited, and they often become waste. Therefore, *keluih* seeds could serve as an innovative ingredient for functional foods (Faradiva et al., 2022; Naz et al., 2019; Sugeng et al., 2021).

Keluih seed flour contains 345,6 kcal of energy, 64,9 g of carbohydrate, 8,8 g of protein, and 5,6 g of fat (Kusumaningrum et al., 2021). The application of *keluih* seed flour in biscuit products can increase energy, protein, and fat content but does not contribute to higher carbohydrate levels. Carbohydrates are primarily increased through the use of wheat flour, which has a higher carbohydrate content than *keluih* seed flour (Melyani et al., 2019). However, *keluih* seeds have the disadvantages of strong odor and bitter taste, making it important to select sprouted *keluih* seeds to reduce these characteristics (Muhardina et al., 2023; Nurliana & Rachmawati, 2023). The germination of *keluih* seeds increases their protein content while reducing the strong odor and bitter taste (Melyani et al., 2019).

Despite the growing interest in GFCF diets, most commercially available snacks still contain gluten and casein, which poses a challenge for children with autism. Additionally, while alternative flours have been explored for use in GFCF diets, limited research has focused on the use of kidney beans and *keluih* seeds in gluten-free biscuits. This study aims to address this gap by evaluating the potential of these locally available ingredients in formulating nutritious, affordable, and sensory-acceptable GFCF snacks for children with autism. The specific objectives of this study were to assess the effects of substituting kidney bean flour and *keluih* seed flour on the energy content, macronutrient profile, and sensory characteristics of GFCF biscuits and to determine the best formula based on these attributes. The formulation was also adjusted to meet the daily snack needs of children aged 4-6 years, in accordance with the 2019 Indonesian Recommended Nutrient Intake (AKG), which is 140 kcal, while considering typical snack consumption patterns in this age group. (Kementerian Kesehatan Republik Indonesia, 2019; Syahroni et al., 2021)

Methods

This experimental study in the field of nutrition science, focusing on functional foods, was conducted from September to November 2023

at the Nutrition Science Food Technology Laboratory, Faculty of Medicine, Universitas Diponegoro. This study employed a one-factor completely randomized design to formulate biscuits from red bean flour and *keluih* seed flour. Ethical approval was granted by the Health Research Ethics Commission of the Faculty of Medicine, Universitas Diponegoro (no. 495/EC/KEPK/FK-UNDIP/X/2023).

Table 1. Formulation of biscuit ingredients in each treatment

Biscuit ingredient	Amount of ingredient per treatment (g)			
	F0	F1	F2	F3
Mocaf	200	60	60	60
Kidney bean flour	0	105	70	35
<i>Keluih</i> seed flour	0	35	70	105
Cornstarch	15	15	15	15
Margarine	150	150	150	150
Xylitol sugar	80	80	80	80
Egg yolk	20	20	20	20
Egg white	10	10	10	10

Ingredients and Formulation

The ingredients used in the biscuit formulation were Ladang Lima mocaf, gasoline red bean flour, *keluih* seed flour, maizenaku cornstarch, Blue Band margarine, eggs, and MH Food xylitol sugar. *Keluih* seed flour was obtained from mature sprouted seeds to reduce the strong odor and bitter taste (Melyani et al., 2019). The seeds were peeled, sliced, washed, dried using a food dehydrator at 50°C for 10 h, ground using a blender, and sieved through an 80-mesh sieve to obtain a fine flour consistency.

Biscuit Processing

A preliminary study was conducted to determine the appropriate composition, targeting 140 kcal per serving, based on the Indonesian Ministry of Health guidelines (2019) for children aged 4–6 years. Biscuit production involves roasting red bean, *keluih* seed, kidney bean, and cornstarch flours. The ingredients were weighed, mixed, and kneaded with margarine, egg yolks, egg whites, and xylitol until the dough became smooth. The dough was rolled to a thickness of 2–3 mm, molded, and baked at 150°C for 15 min. After cooling, the biscuits were rebaked at 90°C for 1 h to improve their texture and crispness.

Macronutrient Analysis

The macronutrient content, including protein, fat, carbohydrate, and energy, was determined through direct laboratory analysis in duplicate, using validated methods (Afifah et al., 2022; Normilawati et al., 2019; Rahmaniar et al., 2023). Protein content was analyzed using the Kjeldahl method, fat content was analyzed using Soxhlet extraction, and carbohydrate and energy levels were measured by difference.

Sensory Evaluation

Sensory evaluation was conducted with 50 purposively selected panelists, consisting of parents from the SLB Negeri Semarang and Bina Anggita Autism Schools. The panelists were selected based on their willingness to participate, overall health, and absence of anosmia or dysgeusia. Sensory attributes such as color, aroma, taste, texture, and overall acceptability were evaluated using a 9-point

hedonic scale. To avoid bias, each panelist received biscuits with different codes.

Statistical Analysis

Statistical analyses were performed using SPSS version 25. One-way ANOVA followed by Duncan's multiple range test (DMRT) was used to assess the differences in macronutrient content. Sensory data were analyzed using the Kruskal-Wallis test, and further testing was conducted using the Mann-Whitney U test. The optimal formulation was determined through a multi-attribute decision-making method using a compensatory model (additive weighting technique), considering both nutritional content and sensory characteristics (Sullivan et al., 2019). Weights for each attribute were assigned based on their relative importance in meeting the daily nutritional needs of the children with autism.

Result and Discussion

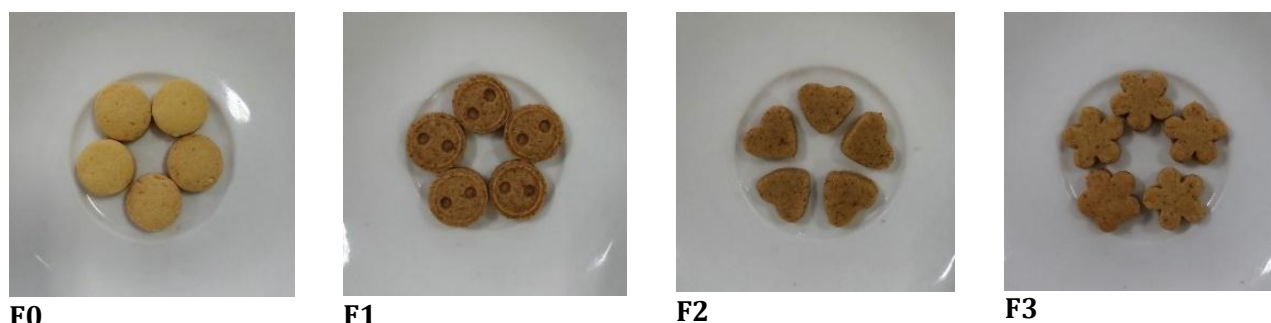


Figure 1. Biscuit results

Table 2. Results of energy and macronutrient content analysis of biscuits per 100g

Nutrient	Standard	Formulation				p-value
		F0 (95%CI)	F1 (95%CI)	F2 (95%CI)	F3 (95%CI)	
Energy	353kkal/ 100g (USDA, 2019)	455,32 ± 5,31 ^a	432,15 ± 6,04 ^b	439,32 ± 6,04 ^{ab}	452,15 ± 13,12 ^a	0,028*
Protein	Min 4,5% (BSNI, 2018)	3,11 ± 0,09 ^a	8,15 ± 0,05 ^b	7,85 ± 0,12 ^c	7,63 ± 0,21 ^c	<0,001*
Fat	16,3g/ 100g (USDA, 2019)	31,00 ± 0,50 ^a	30,06 ± 0,14 ^a	30,97 ± 0,22 ^a	32,61 ± 1,05 ^b	0,005*
Carbohydrate	44,6g/ 100g (USDA, 2019)	40,97 ± 1,10 ^a	32,24 ± 1,34 ^b	32,30 ± 1,45 ^b	32,05 ± 1,15 ^b	<0,001*

Note: *Significantly different with p-value < 0,05 on One-Way Anova

Numbers followed by different superscript letters indicate significant differences across treatments. Ratio of mocaf: kidney bean flour: keluhih seed flour = F0 (100:0:0), F1 (30:52,5:17,5), F2 (30:35:35), and F3 (30:17,5: 52,5).

Visual Characteristics of Biscuits

Figure 1 shows the visual appearance of the biscuits from each treatment (F0–F3). Noticeable color gradation was observed in the

samples. The control formula (F0), which contained only mocaf, appeared light yellow. As kidney bean flour and *keluhih* seed flour were introduced and increased in the formulations

(F1 to F3), the biscuits progressively darkened, resulting in a more brownish hue.

Energy and Macronutrient Content of Biscuits

Table 2 presents the energy and macronutrient composition of the biscuits formulated with red bean and *keluih* seed flours. Substitution significantly affected energy, protein, fat, and carbohydrate contents. Increased red bean flour enhanced protein levels but reduced energy and fat content (F0 to F3). Conversely, higher *keluih* seed flour increased energy and fat, while reducing protein content. The carbohydrate

content showed a fluctuating trend, with the highest in F0, decreasing in F1, increasing in F2, and declining again in F3.

Sensory Characteristics of Biscuits

Table 3 shows the sensory scores for color, taste, aroma, texture, and overall acceptability. Substitutions significantly influenced taste, aroma, and overall liking ($p < 0.05$) but had no significant effect on color or texture. Biscuits F0, F1, and F2 were rated as "liked" in most attributes, while F3 was "slightly liked." F0, F2, and F3 were preferred over F1 for color.

Table 3. Hedonic test analysis results of biscuits

Parameter	Formula				p-value
	F0	F1	F2	F3	
Color	6,80 ± 1,429; Like	6,46 ± 1,129; Slightly Like	6,78 ± 1,016; Like	6,54 ± 1,147; Like	0,155
Taste	6,96 ± 1,177 ^a ; Like	6,72 ± 1,144 ^{ab} ; Like	6,36 ± 1,225 ^{bc} ; Slightly Like	5,86 ± 1,414 ^c ; Slightly Like	<0,001*
Aroma	7,08 ± 0,877 ^a ; Like	6,74 ± 1,006 ^{ab} ; Like	6,42 ± 1,263 ^{bc} ; Slightly Like	6,22 ± 1,489 ^c ; Slightly Like	0,002*
Texture	6,76 ± 1,205; Like	6,62 ± 1,086; Like	6,18 ± 1,335; Slightly Like	6,34 ± 1,423; Slightly Like	0,104
Overall	6,98 ± 1,134 ^a ; Like	6,74 ± 1,065 ^{ab} ; Like	6,52 ± 0,974 ^b ; Like	6,40 ± 0,948 ^b ; Slightly Like	0,017*

Note: *Significantly different with p-value < 0,05 on One-Way Anova

Numbers followed by different superscript letters indicate significant differences across treatments. Ratio of mocaf: kidney bean flour: *keluih* seed flour = F0 (100:0:0), F1 (30:52,5:17,5), F2 (30:35:35), and F3 (30:17,5: 52,5).

Best Formulation

The optimal formulation was determined using a compensatory model (additive weighting method) that combined nutritional and sensory parameters. Each parameter score was normalized using min-max normalization, where the lowest value was assigned a score of 0, and the highest a score of 1. The intermediate values were scaled proportionally. The final score was

calculated by multiplying each normalized value by its assigned weight and summing across all parameters. Table 4 shows that F1 had the highest total score and was selected as the best formulation. F1 contained 432,15 kcal of energy, 8,15% protein, 30,06% fat, and 32,24% carbohydrates. The sensory scores for F1 were 6,46 (color), 6,72 (taste), 6,74 (aroma), 6,26 (texture), and 6,74 (overall).

Table 4. Best Formulation

Parameter	Rank	Weight	F1	F2	F3			
Energy	2	0,06	0,00	0,00	0,31	0,02	0,86	0,05
Protein	4	0,11	1,00	0,11	0,94	0,10	0,90	0,10
Fat	3	0,08	0,00	0,00	0,36	0,03	1,00	0,08
Carbohydrate	1	0,03	0,02	0,00	0,03	0,00	0,00	0,00
Color	7	0,19	0,00	0,00	0,94	0,18	0,24	0,05
Taste	6	0,17	0,78	0,13	0,45	0,08	0,00	0,00
Aroma	5	0,14	0,60	0,08	0,23	0,03	0,00	0,00
Texture	8	0,22	0,76	0,17	0,00	0,00	0,28	0,06

Substituting red bean and *keluih* seed flours significantly altered the nutritional profile of the biscuits. Visual differences were observed

in addition to these nutritional changes. Biscuits with higher proportions of red bean and *keluih* seed flour (F1–F3) appeared darker than those

of the control (F0). This color change could be due to the presence of natural pigments in the flour and the enhanced Maillard reactions that occur during baking (Lund & Ray, 2017). The F3 formulation, which contained the highest proportion of *keluih* seed flour, exhibited the highest energy and fat contents. This can be attributed to the higher fat content of *keluih* seed flour (5,6%) than that of mocaf (0,6%) and kidney bean flour (1,1%) (Kusumaningrum et al., 2021; Mahmud et al., 2018), which aligns with previous studies that reported increased fat and energy content in products enriched with *keluih* seed flour (Kusumaningrum et al., 2021; Meliyana et al., 2019). However, variations in formulation composition may yield different results (Nurliana and Rachmawati, 2023). This variability warrants further investigation to determine the most effective formulations for enhancing the nutritional value.

All formulations exceeded the USDA minimum energy and fat standards for baked products (353 kcal/100 g and 16,3% fat, respectively) (United States Department of Agriculture 2019). With an average energy contribution of 455,32 to 432,15 kcal per 100 g, a 33–35 g serving meets approximately one-third of a child's energy needs per meal (Sharlin & Edelstein, 2014). This is beneficial for children with autism who may experience reduced energy intake due to feeding difficulties (Aisyah et al., 2023). Additionally, the fat derived from these ingredients may help address potential deficiencies in essential fatty acids, such as omega-3, which support brain development and immune regulation (Esposito et al., 2021; Pancheva et al., 2023). However, the study's limitations, including the absence of long-term testing, should be acknowledged as they may impact the generalizability of these findings.

F1 had the highest protein content owing to its greater proportion of red bean flour, which contains 19,48% protein compared to 8,8% in *keluih* seed flour (Damayanti et al., 2020). This finding aligns with those of other studies on legume-based bakery products (Gunawan et al. 2021). All formulations exceeded the 4,5% protein threshold set by the SNI 2973:2018. Proteins support immune function and growth, and are often impaired in children with autism (Khotimah et al., 2021; Mustafa & Elliya, 2020).

The carbohydrate content was highest in the control (F0), which used 100% mocaf, which

is known for its higher carbohydrate content compared to the substituted flour. Although the substitution slightly reduced carbohydrate levels, these changes were minor. This observation supports previous research, indicating that increased protein and fat may lower carbohydrates (Melyani et al., 2019). F1 was the preferred formulation.

Higher proportions of *keluih* seed flour (F3) resulted in a more pronounced nutty flavor owing to lipoxygenase enzyme activity and volatile compound formation. Nevertheless, the color and texture remained acceptable across all the formulations. Color differences may be due to Maillard reactions during baking (Salsabila et al. 2019). Texture is influenced by protein and fiber in red bean flour, contributing to firmer consistency (Astuti et al., 2019; Puspita et al., 2021). F1 was selected as the best formulation using a compensatory model that integrated both nutritional and sensory parameters. Weighting was applied to each parameter based on its relevance to the dietary and behavioral needs of children with autism, with energy, protein, and fat prioritized over sensory attributes. For instance, energy was weighted at 30%, protein at 25%, fat at 20%, taste at 15%, and aroma at 10%. Each score was normalized and multiplied by its respective weight to yield the final composite score. F1 achieved the highest total score, offering a balanced nutritional profile (432,15 kcal, 8,15% protein, 30,06% fat, and 32,24% carbohydrates) along with the highest sensory ratings, making it a practical and nutrient-dense option for this target population.

However, this study had certain limitations. The sample size was relatively small (n=50), which may not fully capture the diversity of sensory preferences or reflect the broader population. The absence of long-term testing to assess the effects of these biscuits on the health and development of children with autism is a significant limitation. Additionally, the sensory evaluation may have been subject to bias because the panelists were not blinded to the product formulations. Future research should address these limitations by including larger sample sizes, incorporating long-term studies, and using blinded testing methods.

Conclusion

The substitution of kidney bean and *keluih* seed flours significantly influenced the nutritional and sensory properties of GFCF biscuits. Increasing kidney bean flour enhanced protein content and improved taste, aroma, texture, and overall acceptability, whereas increasing *Keluih* seed flour contributed to higher energy and fat levels but reduced sensory appeal. Carbohydrate levels remained relatively stable across the formulations. Based on a compensatory model that integrates nutritional and sensory parameters, formulation F1 was selected as the optimal product because of its balanced nutrient profile and favorable sensory evaluation. These findings support the potential use of GFCF biscuits in dietary management of autism, particularly to improve energy intake, essential fatty acid adequacy, and sensory acceptance. The product may also inform school-based snack programs or community-level interventions for children with autism.

This study was limited by the absence of micronutrient analysis and acceptability testing in the target population. Future studies should include comprehensive micronutrient profiling and assess the long-term acceptability and clinical relevance of the product in children with autism in practical settings.

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