

# OPTIMIZATION OF BEEF PATTIES FORMULATION WITH TEXTURED SOY PROTEIN, OKARA AND BACON USING A SIMPLEX-CENTROID MIXTURE DESIGN

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*Abstract- Large amounts of okara are currently generated by the production of soy aqueous extract and tofu. Although it show as potential application in products such as biscuits, breads, cereals, cheeses and meat products, their use is still limited. The objective of this study was to optimize the beef patty formulation with textured soy protein (TSP), okara and bacon using the simplex-centroid mixtures design. Seven formulations were developed using different proportions (0% up to 8%) of these components. After mixing and molding, samples were frozen, roasted and analyzed as to their technological and functional properties (shrinkage, yield, water retention, fat retention, water absorption index - WAI and oil absorption index - OAI). From the mathematical models and response surface the formulated product with 8% TSP had the lowest shrinkage, higher yield and moisture retention. As for the retention of fat binary mixture between TSP and okara showed better results. TSP and okara had the same effect on WAI property. However a higher contribution in the equation of OAI was show by the binary mixture of okara and bacon. The results indicated that okara is a byproduct with high a high potential application in beef patties once the technological and functional properties of patties with okara similar to those obtained with samples added only with TSP.*

**Keywords** – meat product; sub product; development, quality parameters  
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## I. INTRODUCTION

A large amount of okara are produced worldwide as a result of an increase on soybean consumption. Okara is the residue left from grinded soybeans after production tofu or soy milk. It is very susceptible to spoilage due to its high moisture content (about 80%) [7,16,23], making its immediate use in large soybean soluble extract production plants difficult. An alternative is to dry the okara to extend its shelf life [16,22].

Okara is source of carbohydrates, minerals, fibers and proteins, of adequate nutritional quality higher protein concentration and lower energy content when compared to soybeans itself [29, 34]. It does not change the taste of products, does not have gluten in their composition and increases the nutritional value of the products which adds okara [2].

Due to the low market value, it can be considered a potential source of vegetable protein for human consumption [17] by developing new products using it in the form of flour, adding thus value to a lot of products [16]. Therefore, okara can become extremely important to decreased protein deficit of the world population.

In addition to the factors such as color, flavor and texture, other criteria such as shrinkage, water loss and fat loss are also important for the consumer[32]. This study aims to optimize beef patty formulations containing textured soy protein, okara and bacon using the simplex-centroid design.

## II. MATERIALS AND METHODS

Textured soy protein (TSP), monosodium glutamate as well as other ingredients, were purchased from local establishments. Beef and bacon were acquired on the same day of production and kept refrigerated (5 ° C). The okara flour was obtained from soybean Cultivar BRS 232, donated by Brazilian Agricultural Research Corporation (EMBRAPA). Its preparation was obtained from an adaptation of Mandarino's methodology [18]. First the soybeans were cooked for 5 minutes in boiling water (soybean:water, 1:5) with subsequent draining and washing of the grains in running water at room temperature. This procedure was repeated with grains in new boiling water (soybean:water, 1:3) for another 5 minutes. After cooking, the grains together with the cooking water, were cooled to room temperature. Shortly after, grains were milled in an industrial blender for 1 minute, and subsequently filtered. The residue consisted on okara. This was centrifuged and dried in an oven with forced air circulation at 60 ° C for 10 hours [14], with subsequent grinding to obtained okara flour.

### Sample preparation

The beef's fat content was standardized in order to reduce possible texture variations associated with the presence of connective tissue. For bacon, preparation consisted of removing the skin. Both were stored in separate containers under refrigeration (5 ° C). The quantity of ingredients used for the preparation of patties is in Table 1. After preparation of raw materials, both underwent milling with 8 mm diameter disc grinder (Skymesen Make / Model PSEE-98MHD), and

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other ingredients. After suitable mixing (manual, 10 minutes), patties were molded in circular shape with a diameter of 9 cm 0.5 cm of height. The patties obtained were packed in PVC film, identified and frozen at -18 ° C. After freezing, the samples were placed in aluminum molds and roasted in an industrial gas oven for 30 minutes at a temperature of 180 ± 5 ° C. It was roasted in an average time of 15 minutes for both sides of the product, time necessary to obtain color uniformity and the internal temperature of the patties reach at least 72 ° C (temperature measured with skewer thermometer, Incoterm brand, model TD-01) in the center of the product.

TABLE I Formulations and their ingredients

Formulations							
Ingredient	F1	F2	F3	F4	F5	F6	F7
Oregano	0,02	0,02	0,02	0,02	0,02	0,02	0,02
Garlic powder	0,10	0,10	0,10	0,10	0,10	0,10	0,10
Condiment for burger *	2,67	2,67	2,67	2,67	2,67	2,67	2,67
Monosodium glutamate	1,00	1,00	1,00	1,00	1,00	1,00	1,00
Water	5,00	5,00	5,00	5,00	5,00	5,00	5,00
Beef	76,21	76,21	76,21	76,21	76,21	76,21	76,21
Bacon <sup>1</sup>	7,00	7,00	15,00	7,00	11,00	11,00	9,67
TSP <sup>2</sup>	8,00	0	0	4,00	4,00	0	2,66
Okara <sup>3</sup>	0	8,00	0	4,00	0	4,00	2,66
Total (g)	100	100	100	100	100	100	100

\* Composition: Prepared Condiment (salt, maltodextrin, sugar, pepper powder, onion powder, antioxidant sodium erythorbate, annatto coloring and paprika oleoresin, anti-wetting agent silicon dioxide, flavoring and stabilizer.

1, 2 e 3 – Independent variables

**Formulation development of beef patties using TSP, okara and bacon using simplex-centroid mixture design**

Seven formulations with Okara, TSP and bacon were developed as shown in Figure 1. The formulation was optimized using simplex centroid design [28] with three different components [ $X_1$  = TSP (%),  $X_2$  = Okara ( %) and  $X_3$  = bacon (%)], totalizing 7 trials. The content of each independent variable added varied between 0% and 8% (Figure 1). In Figure 1, points 1, 2 and 3 (triangle vertices) corresponds to TSP, okara and bacon respectively. Points 4, 5 and 6 corresponds to binary mixture of two ingredients. Point 7 (center of the triangle) is the ternary mixture of the 3 ingredients.

For the standard formulation it was considered the formulation added only with TSP (F1, Table 1), since it is common to use this component in patties already commercialized in Brazil. All formulations were individually prepared in the same way, in order not to obtain variations in the process. For all formulations the other components were held constant.

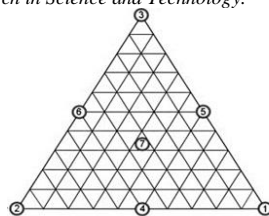


Fig. 1 Simplex-centroid mixture design

Tests(t)	Mixture proportion (%)		
	TSP ( $X_1$ )	Okara ( $X_2$ )	Bacon ( $X_3$ )
F1	8	0	0
F2	0	8	0
F3	0	0	8
F4	4	4	0
F5	4	0	4
F6	0	4	4
F7	2,67	2,67	2,66

(1) Random tests;  $x_1 + x_2 + x_3 = 1$

**Study and optimization of response surface methodology**

The quality of fit of the experimental model was verified by analysis of variance (ANOVA) by the coefficient of determination ( $R^2$ ). All calculations and constructions of graphs presented here were obtained from Statistica 10.0 program.

The seven different formulations generated were evaluated using the following response functions:  $y_1$  = Shrinkage,  $y_2$  = yield,  $y_3$  = moisture retention,  $y_4$  = retention fat,  $y_5$  = water absorption index and  $y_6$  = oil absorption index. The response functions were analyzed using the Statistica 10.0. The canonical model [28] (Equation 1) was adjusted to the experimental data and the linear, quadratic and cubic models have been tested for their respective regression coefficients.

$$y = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{123} x_1 x_2 x_3 \quad (1)$$

where  $y$  is the observed response function,  $\beta_1, \beta_2, \beta_3, \beta_{12}, \beta_{13}, \beta_{23}$  and  $\beta_{123}$  are the estimated regression coefficients  $x_1, x_2$  and  $x_3$  are coded levels added the dependent variable and  $1 > x_i > 0$  and  $\Sigma = 1.0$ .

**Physical and functional characterization of beef patties**

Shrinkage (%) -  $y_1$  [19, 32] and yield -  $y_2$  [12, 19] were measured in accordance with the equations below:

$$y_1 = [(raw\ thickness - cooked\ thickness) + (raw\ diameter - cooked\ diameter) / (raw\ thickness + raw\ diameter)] \times 100 \quad (2)$$

$$y_2 = (cooked\ weight / raw\ weight) \times 100 \quad (3)$$

Moisture retention (%) -  $y_3$  and fat retention (%) -  $y_4$  [12], were calculated with the following equations:

$$y_3 = (\text{percent yield} \times \text{percent moisture in cooked samples}) / 100 \quad (4)$$

$$y_4 = [(\text{cooked weight} \times \text{percent fat in cooked sample}) / (\text{raw weight} \times \text{percent fat in raw sample})] \times 100 \quad (5)$$

The values of water absorption index (WAI) and oil absorption index (OAI) were also obtained [27].

**III. RESULTS AND DISCUSSION**

**Optimized formulation of beef patties with okara, textured soy protein and bacon**

For the dependent variable shrinkage (response function  $y_1$ ), only the linear terms were significant. However, the quadratic terms were considered due to the better quality of adjustment obtained. For the coefficients of equation ( $y_1$ , Table 3), the variable  $X_3$  (bacon) showed the greatest contribution to the shrinkage of the samples. The variables  $X_2$  (okara) and  $X_1$  (TSP) had a lower contribution.

In other works, the behavior was similar. The reduction of 15 % of fat in meatballs added with corn flour also decreased significantly shrinkage of the product and the highest shrinkage was observed in sample containing only the addition of fat [26]. In another work, the authors found similar results when they studied the effects of interaction of different proportions of fat (15-30%), water (10-20%) and TSP (3-9%) in the same type of product, encountering the greatest shrinkage in samples containing the largest amount of fat (30%) and which the addition of TSP shrinkage value also decreased [32]. Analyzing the response corresponding surface (Figure 2a), it is observed that there is a reduction in shrinkage with increased amounts of TSP. Okara, when used in fermented chicken patties with *Lactobacillus acidophilus*, reduced the shrinkage values, result similar to this work. The authors attributed this effect to a likely okara's ability to increase the water retention in the product, possibly due to its high protein concentration [6]. The increasing amounts of isolated soy protein (0.5 to 3%) in pork burgers also resulted in lower observed shrinkage ( $p < 0.05$ ) [3].

In meat products, proteins determine the yield [15], structure and its quality [20]. For the yield of burgers (response function  $y_2$ ), linear and quadratic terms were significant. However, the quadratic terms were considered due to better model fit. For the coefficients of the equation  $y_2$ , the variable  $X_1$  (TSP) exerted greater contribution followed by the variable  $X_2$  (okara). After analyzing the response surface (Figure 2b), an increase was observed with the addition of TSP ( $X_1$ ) and okara ( $X_2$ ) binary mixture. The values ranged from 58.29% to 76.78%, in the range of studied parameters (Table 2) and are lower than the values found by Yildiz-Turp and Meltem [35] (76.1 and 79.6% %), which studied the effect of adding plum purée in beef burgers with low fat content.

When evaluating the effect of different concentrations of fresh okara in beef burgers with low fat, it was observed that, the greater the amount of okara, the lower the cooking losses, with a consequent increase in performance and reduction of product cost [31]. This high performance is due to the water retaining capacity of soy protein, which can also be considered in the present study. In another case, the addition of isolated soy protein in larger quantities than 2% significantly increased ( $p < 0.05$ ) the yield of pork burgers [3].

The values for moisture retention ranged between 29.98% and 43.13% (Table 2). For moisture retention (response function  $y_3$ ) of beef patties, linear and quadratic terms were significant, but only the quadratic terms were considered due to better model fit. From the coefficients of the equation ( $y_3$ ), the variable  $X_1$  (TSP) had greater contribution in the equation. Analyzing the response surface (Figure 2c) one can see the trend of increased moisture retention with the addition of TSP. This behavior is very important since during their cooking, meat proteins denature causing a decrease in water holding capacity, and shrinkage of the network proteins. This shrinkage exerts a mechanical force between the water and the fibers. In the presence of these pressure gradients, the excess water is expelled to the surface of the meat. Similar results were found by Velioglu et al [32] in which water loss during cooking is reduced by increased amounts of TSP. The authors associated this behavior to the high capacity of water

absorption of this component, resulting in a firmer texture. The addition of the protein enhances the moisture retention of the product [1] and is the major responsible for moisture retention in meats [21]. The addition of okara also resulted in higher moisture retention during cooking because its ability to hold water. The addition of fresh okara in this type of product clearly showed a higher moisture content during cooking and the water, that had not connected to proteins, was lost during cooking [31].

**TABLE 2 Simplex-centroid mixture design and their response functions: effect of different levels of TSP, okara and bacon on the quality parameters of beef burger patties.**

Response function <sup>1</sup>						
Tests	$y_1^{(2)}$	$y_2^{(3)}$	$y_3^{(4)}$	$y_4^{(5)}$	$y_5^{(6)}$	$y_6^{(7)}$
1	15,36±0,36	76,78±0,72	43,13±0,65	95,97±3,23	1,61±0,06	1,62±0,11
2	17,45±2,29	72,88±1,14	37,54±1,36	80,31±6,26	1,61±0,14	1,36±0,05
3	24,50±1,52	58,29±1,55	30,44±2,61	58,36±1,84	1,44±0,17	1,32±0,06
4	16,79±1,42	69,18±1,79	34,11±3,11	132,61±2,37	1,80±0,05	1,46±0,17
5	19,10±1,91	66,83±1,17	35,95±0,63	85,81±1,50	1,41±0,12	1,31±0,05
6	22,37±0,73	65,11±2,22	34,71±1,18	75,20±2,57	1,32±0,07	2,12±0,31
7	23,29±0,57	61,19±1,46	29,98±0,71	95,21±2,46	1,37±0,04	2,09±0,09

<sup>1</sup>Mean ± standard deviation.

<sup>2</sup> $y_1$  = shrinkage (%), <sup>3</sup> $y_2$  = yield (%), <sup>4</sup> $y_3$  = moisture retention (%), <sup>5</sup> $y_4$  = oil retention (%), <sup>6</sup> $y_5$  = water absorption index, raw sample, <sup>7</sup> $y_6$  = oil absorption index, raw sample.

**TABLE 3 Regression coefficients and analysis of variance of mathematic models<sup>1</sup>**

Response function <sup>2</sup>						
Coefficients	$y_1$	$y_2$	$y_3$	$y_4$	$y_5$	$y_6$
Linear						
$\beta_1$	15,66*	73,92*	40,08*	112,34*	1,63*	1,56*
$\beta_2$	18,64*	70,11*	35,11*	95,57*	1,59*	1,68*
$\beta_3$	25,21*	57,50*	30,17*	59,29*	1,30*	1,59*
R <sup>2</sup>	0,79	0,74	0,51	0,57	0,46	0,01
Lack of fit (p)	0,045*	0,065	0,24	0,18	0,29	0,98
Quadratic						
$\beta_1$	15,10	77,13*	43,42*	96,60*	1,62*	1,59
$\beta_2$	17,19	73,23*	37,83	80,94	1,62*	1,33
$\beta_3$	24,24	58,64*	30,73	58,99	1,45*	1,29
$\beta_{12}$	6,67	-29,54	-30,68	165,31	0,59	0,41
$\beta_{13}$	1,81	-9,76	-9,12	22,01	-0,63	-0,10
$\beta_{23}$	10,71	-8,84	-2,90	10,89	-0,99	3,66
R <sup>2</sup>	0,89	0,94	0,90	0,98	0,94	0,87
Lack of fit (p)	0,84	0,61	0,53	0,25	0,40	0,44
Cubic						
$\beta_{123}$	---	---	---	---	---	---

<sup>1</sup>  $y = \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_{12}x_1x_2 + \beta_{13}x_1x_3 + \beta_{23}x_2x_3 + \beta_{123}x_1x_2x_3$   
 $x_1 = \text{TSP}, x_2 = \text{Okara}, x_3 = \text{bacon}$

$y_1$  = shrinkage (%),  $y_2$  = yield (%),  $y_3$  = moisture retention (%),  $y_4$  = oil retention (%),  $y_5$  = water absorption index, raw sample,  $y_6$  = oil absorption index, raw sample.  
\*Significant at 5%.

For the variable fat retention (response function  $y_4$ ), the values found were between 58.36% and 132.61%, in the

range of the parameters studied (Table 2). This dependent variable ( $y_4$ ) showed significant linear and quadratic terms, but only the quadratic terms were considered due to the better

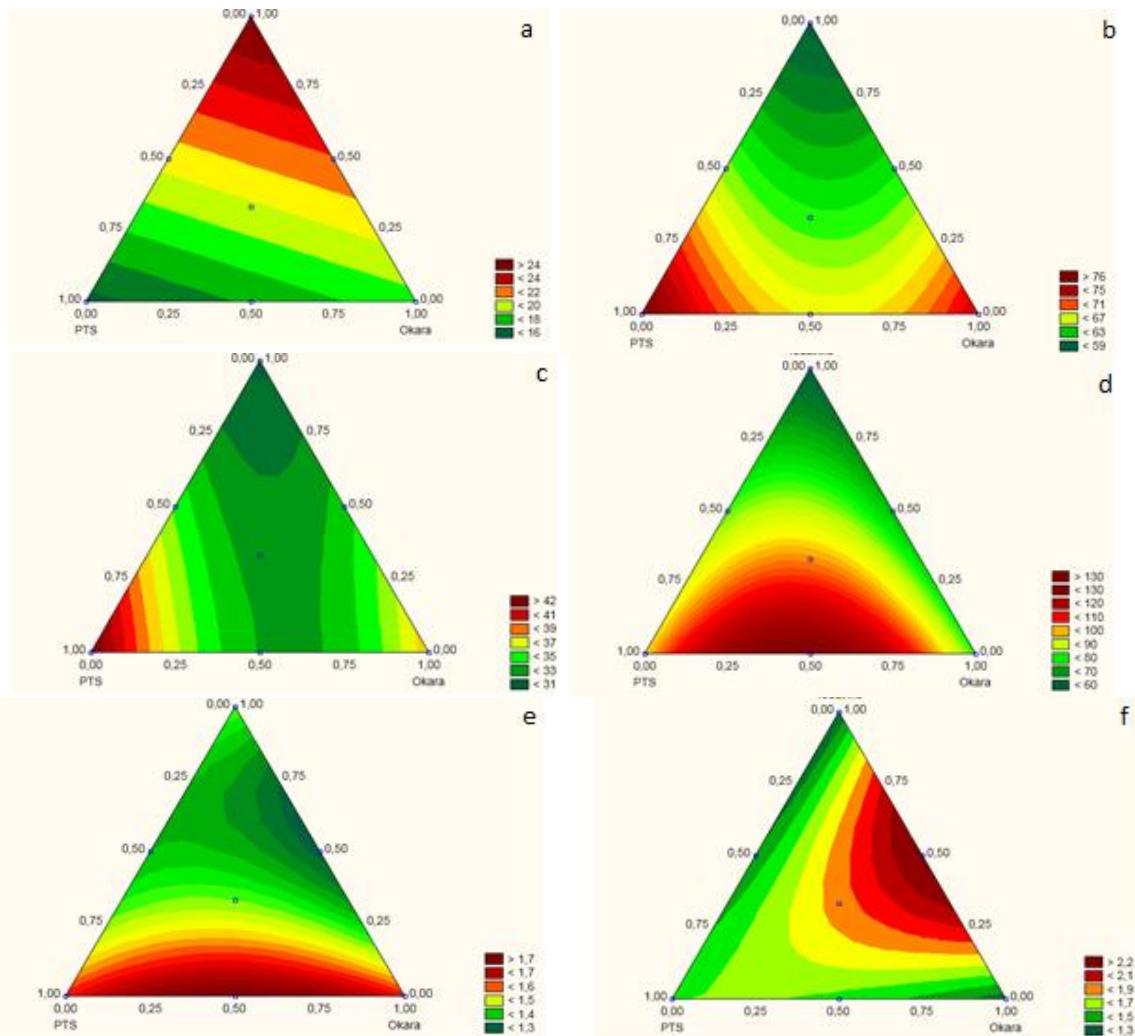


Figure 2 Response surfaces for  $y_1$  Shrinkage (a),  $y_2$  Yield (b),  $y_3$  Moisture retention (%) (c),  $y_4$  Oil retention (d),  $y_5$  water absorption index (e) and  $y_6$  oil absorption index (f) for a mixture of TSP, okara and bacon.

model fit. By analyzing the response surface (Figure 2d) we observed a trend of increased fat retention for binary mixture TSP ( $X_1$ ) and okara ( $X_2$ ). The quadratic coefficient  $\beta_{12}$  response function  $y_4$  (fat retention) was positive, confirming that the binary combination of TSP ( $X_1$ ) and okara ( $X_2$ ) leads to increased fat retention in this type of product. The same result was observed in formulation 4, wherein the assay containing a binary mixture of TSP and okara, presented a greater amount of fat retention compared to the other formulations (Table 2, trial 4,  $y_4$  response function). The addition of protein enhances the retention of fat in products [1, 15]. The same happened with the addition of TSP, the most important factor to minimize the loss of fat during cooking [32]. However, analyzing the response surface (Figure 2d), fat retention had a decreasing tendency with the

addition of larger amounts of fat ( $X_3$ ). Analyzing the coefficients of the response function  $y_4$ , this fact is confirmed since the obtained coefficients have the smallest contribution in equation ( $\beta_{13}$  and  $\beta_{23}$ , Table 3). In meatballs this behavior was similar, there was an increase on fat retention with a decrease of fat levels in the formulation [26]. With increasing fat content in the formulation, the free distance between the fat globules decreases, leading to coalescence and, therefore consequent extravasation of the product [30].

The WAI may be related to the capacity of water absorption of the proteins, or protein interaction with water. Thus, their degree of interaction is related to the texture, viscosity, gelling and emulsifying capacity [8]. For the response function  $y_5$  (WAI), linear and quadratic terms were significant, but because of its contribution to the

model adjustment, the quadratic terms were considered. For the coefficients of the equation  $y_5$ , the variables  $X_1$  (TSP) and  $X_2$  (okara) demonstrated the same behavior followed by variable  $X_3$  (bacon). Among the intrinsic factors affecting the dietary protein binding capacity with water, there is amino acid composition, protein conformation and polarity of the surface. Polar amino groups are the key for the interactions between protein and water [11].

Analyzing the response surface (Figure 2.e) an increase in this property is noted with an increase of components  $X_1$  (TSP) and  $X_2$  (okara). The variable  $X_3$  (bacon) had less influence on water absorption, because the addition of this variable ( $X_3$ ) on formulations reduced the proportion of polar amino acids present, reducing the products water absorption capacity.

The oil absorbing capacity is a determinant factor for the retention of flavor in food [13].

For response function OAI ( $y_6$ ) only linear terms were significant, but due to better model fit, quadratic terms were considered. For the equation coefficients  $y_6$ , the binary mixture with  $X_2$  (okara) and  $X_3$  (bacon) showed greater influence in the equation compared to the variables  $X_1$  (TSP),  $X_2$  (okara) and  $X_3$  (bacon). By observing the response surface (Figure 2f), this fact can be verified. The oil absorbing mechanism was explained as a physical restraint which occurs by capillary action. Additionally, the hydrophobicity of these proteins also plays an important role, since its association with the nonpolar proportions of the existing amino acid side chains from the surface of the protein molecules, thus favors consequently an increase on oils absorption [10].

#### IV. CONCLUSIONS

The addition of different concentrations of TSP, okara and bacon, had significantly influenced on the quality parameters of beef patties. TSP and okara in higher concentrations resulted in less shrinkage and higher yield of the samples. The addition of TSP provided higher moisture and fat retention, whereas higher concentrations of bacon resulted in a lower amount of fat in the final product. For water absorption index, both okara and TSP contributed positively in the product and the oil absorbing rate was positively affected by binary mixture of bacon and okara. Therefore we conclude that the hamburgers added with 8% okara flour showed satisfactory physical and technological characteristics, and okara presents a high potential as a substitute for TSP in beef patties.

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