The effect of addition of biostarter "bekasam" on the quality and characteristics of fermented catfish (*Pangasius* sp.) sausage

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Abstract. Fermented fish sausage is one of the processed fish meat products that utilizes the role of Lactic Acid Bacterias (LABs). To accelerate the fermentation process of sausages, it is necessary to add a starter culture in the form of a fermented fish substrate (*bekasam*) which contains a lot of lactic acid bacterias. This research was conducted using an experimental method and arranged using a Randomized Block Design (RBD). The treatments applied were differences in the concentration of *bekasam* added, as much as 0%, 2%, 4%, and 6% of the weight of the fish meat, then fermented at room temperature for 6 days. The results showed that the treatment of adding *bekasam* substrate with a concentration of 4-6% produced the best fermented catfish sausage, which was mostly preferred by panelists. The best fermented sausages produced was characterized with attractive appearance, uniform, consistent, and bright, smell fermented product aroma, a spicy but no fishy aroma, a delicious taste, quite sour as fermented tasted, with quite soft and compact texture. The fermented sausages have pH value 5.00, Aw value 0.77, total acid content 0.66%, TVB-N value 12.31 mgN/100 gs and total LABs obtained 6.9 x 10^6 cfu/g.

1 Introduction

Fermented sausage processing is carried out to prevent sausage fermentation while adding flavour and improving the texture of the sausage to make it more compact and preferred by consumers. Fermented sausage is one of the processed meat products that applies the role of modern technology by utilizing bacteria with or without the help of starter bacteria [1]. Traditionally, the manufacture of fermented sausages is carried out spontaneously, namely traditional processing with simple work procedures. Spontaneous fermentation products often produce non-uniform end products, due to the lack of control over the growth of fermentative microbes during the fermentation process. To accelerate the fermentation process and to obtain the best sausage fermentation results, the right biostarter is needed, according to the characteristics of the best fermented sausage desired, namely by adding a starter culture. Starter culture or biostarter contains microorganisms that play a role in making

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fermented products [2-4]. Furthermore, [5] explained that the addition of a starter can accelerate the breakdown of complex compounds into simpler compounds so that it has an impact on the process of forming a specific fermentation flavour. Biostarter can be obtained from lactic acid bacteria (LAB) starter culture, from back slopping, or from substrates derived from similar or other fermentation products suspected of containing LAB.

Fish fermentation substrate is a basic ingredient containing lactic acid bacteria that can be mixed in making fermented fish sausages, for example, *bekasam* substrate from patin fish. *Bekasam* is a processed fish product that is fermented spontaneously with salt and a mixture of rice [6-7]. The addition of salt and carbohydrates in making *bekasam* will stimulate the growth of lactic acid bacteria in *bekasam*, especially *bekasam* processed from patin fish [8-10]. LAB will break down starch into simple compounds, such as lactic acid, acetic acid, propionic acid and ethyl alcohol, which function as flavourings and preservatives in bekasam [8, 11-12].

The *bekasam* substrate contains biostarter that can be added to the manufacture of fermented sausages. The addition of biostarter contained in bekasam aims to accelerate the sausage fermentation process, improve product quality, produce final products with a greater and more uniform amount of LAB, and play a role in preserving meat so that it has a long shelf life [13]. In addition, secondary metabolites produced by LAB play an [14]. Research on the chemical characterization of fermented sausages using fermented mackerel biostarter obtained the best treatment of 4%, by producing the breakdown of complex compounds into simpler compounds.

Temperature, humidity and fermentation time play an important role in regulating the growth and population of microorganisms produced in the final product. Storage or fermentation at room temperature between 25 ^{o}C to 30 ^{o}C is considered the optimal temperature that can accelerate the growth of microorganisms during the fermentation process [15-16]. According to [17], sausage fermentation is carried out between temperatures of 18-45 ^{o}C with air humidity reaching 80-95%. Conversely, fermentation at low temperatures of 8 - 10 ^{o}C can inhibit the growth of microorganisms and slow down the fermentation process. Generally, sausages to be fermented are left or fermented for a period of 18 - 24 hours in order to optimize the fermentation process [16,18]. According to [20], the fermentation time usually takes up to 18-48 hours with a good fermentation temperature at room temperature of 30-37 ^{o}C. Based on research by [14], the best fermentation time treatment for sausages using *peda* fermented fish biostarter is 6 days. In this treatment, the sausages produced showed characteristics with a total LAB of 1.5 x 10^4 cfu/g, pH of 4.1, total acids of 3.9%, and N-Amino of 7.1 mgN/100 gs. Information on the factors that influence the process of making catfish sausage is still very limited, especially information on the addition of *bekasam* which aims to accelerate the fermentation process. Therefore, research on the effect of adding *bekasam* substrate on the quality characteristics of catfish sausage fermented at room temperature is important to be explored.

2 Methods

2.1. Materials

The materials used in making fermented catfish sausage are catfish (*Pangasius* sp) measuring around 300 gs per fish obtained from the traditional market in Pekanbaru in amount 15 kg, edible and halal collagen casings, complementary spices such as: table salt, ginger, pepper, garlic coriander, and *bekasam* substrate.

The chemicals used for proximate analysis and calculation of total acid: aquadest, 0.1 N NaOH (Sodium hydroxide) 50%, H_2SO_4 (Sulfuric acid), H_2BO_3 (Boric acid) 2%, 0.1 N HCl

(Hydrochloric acid), 1 g of Cu-catalyst, chloroform solvent, PP indicator, while the chemicals used for determining total BAL: MRSA media (Man Ragosa and Sharpe Agar) and MRSB (Man Ragosa and Sharpe Broth), Nutrient Agar, and Potato Dextrose Agar.

2.2 Methods

This study used an exploratory experimental method, namely by processing catfish sausages with the addition of different concentration of *bekasam* substrates during fermentation at room temperature. The research design used was a randomized block design (RBD) with 4 levels of treatment, namely without the addition of *bekasam* substrate (B0), the addition of *bekasam* substrate 2% (B2), 4% (B4), and 6% (B6). Furthermore, the fermented sausage was observed and its quality was tested during the fermentation process for 2, 4, 6 days.

The parameters measured in this study were: sensory evaluation and consumer preference for their appearance, colour, texture and taste, the value of pH, Aw, total lactic acid, and TVB-N and total LABs.

The test results were calculated with an analyse of variance (ANOVA) by using the Excell programme.

2.2.1 Preparation of fish bekasam substrate

The first stage was making of *bekasam* begins with weeding and removing the contents of the stomach and gills, then washing it until clean. Next, cut the fish meat into several parts and wash it again until clean with running water. After that, the fish is drained for 10 minutes. The next stage is salting. The fish and table salt as much as 20% of the weight of the fish was mixed until evenly distributed, then let it sit for 5 hours. The next stage is the separation of salt from fish, by taking only the fish, while the remaining salt in the jar is thrown away. Fish that have been salted before were then mixed with carbohydrates (rice) as much as 50% of the weight of the fish. Then, the fish that has been mixed with salt and carbohydrate sources were put into a jar and closed tightly, and then fermented for 7 days.

2.2.2 Procedure for processing fermented fish sausage [14]

Firstly, the fresh catfish was cleaned using running water, then the meat of the fish was gotten and separated from the head and the bones. Then, the fish is washed clean to remove the blood that sticks, then grind the fish meat that has been weighed by adding ice cubes and salt in it. Then, all the sausage dough spices (garlic, ginger, pepper and coriander) was grinded. Then, the ground spices were mixed into the meat dough, and added with 10 % of tapioca flour based on the catfish meat, and then stired until evenly distributed. After that, *bekasam* substrate were added into the dough in each treatment, as much as: 0%, 4%, 6%, 8% from the fish meat weight, and stired until evenly mixed. Then they were continued with filling the sausage dough into the casing. After that, the conditioning process by letting them for 24 hours at room temperature. After the next day, the sausages were steamed for 30 minutes at 40 ^{o}C. After that, they were fermented at room temperature for 2, 4, and 6 days, to be observed.

3. Results and Discussions

3.1 Bekasam Substrate

The making of fermented sausages in this study used catfish (*Pangasius* sp) as the raw material, as well as the raw material for making *bekasam*. The use of *bekasam* from catfish as a starter in making fermented sausages was made using carbohydrate sources of rice and salt, which were then fermented for 7 days at room temperature in the dark [9]. The bekasam image can be seen in Figure 1 below.

Fig. 1. Catfish meat that was added with rice and salt (a) and *bekasam* substrate produced (b)

The *bekasam* from catfish produced had a pH value of 4.3 with a distinctive taste and aroma that smelled of lactic acid, and a soft and tender texture.

3.1. Sensory Evaluation of Fermented Fish Sausage

Sensory evaluation is known as sensory assessment, which is a method of assessment by utilizing human senses in observing the appearance, texture, aroma and taste of a product as seen at Figure 2.

Fig. 2. Fermented catfish sausage added with catfish *bekasam* substrate.

The sensory evaluation the foodstuff was based on the assessment of consumer preference as the hedonic value and the quality of the product. Hedonic assessment was an assessment of the appearance, aroma, taste and texture of fermented catfish sausage added with different amount of the substrate catfish *bekasam*. Thirty non trained panelists were asked to give a score according to the impression they get. The results of the panelist assessment for the hedonic value can be seen in Table 1.

Table 1. Hedonic value of fermented catfish sausage

Category	**Addition of catfish *bekasam***			
	0% (B0)	**2% (B2)**	**4% (B4)**	**6% (B6)**
Appearance	7.1 ± 0.1^{a}	7.2 ± 0.1^{b}	7.3 ± 0.1^{c}	7.3 ± 0.1^{c}

Category	Addition of catfish *bekasam*			
	0% (B0)	2% (B2)	4% (B4)	6% (B6)
Aroma	7.2±0.1[a]	7.2±0.1[a]	7.3±0.1[b]	7.3±0.0 [b]
Flavor	7.2±0.1[a]	7.2±0.1[a]	7.3±0.1[b]	7.3±0.1[b]
Texture	7.2±0.1[a]	7.3±0.0 [b]	7.3±0.0 [b]	7.4±0.1[c]

Note: Numbers followed by different letters in the same row mean significantly different ($p<0.05$)

The value of organoleptic quality was assessment on the characteristic of appearance, aroma, taste and texture of fermented catfish sausage added with different amount of the substrate catfish *bekasam*. Twenty trained panelists were asked to give a score according to the organoleptic characteristic. The description of organoleptic characteristics of fermented sausage with the addition of *bekasam* substrate fermented at room temperature for 6 days can be seen on Table 2 below.

Table 2. Description of organoleptic characteristics of fermented sausage

Addition of catfish *bekasam*	Organoleptic characteristics			
	Appearance	Aroma	Taste	Texture
0% (B0)	Quite interesting, color is not uniform and pale	Typical fermentation aroma is quite real, quite strong fishy smell, less pronounced spice aroma is not tasty	Pronounced spice aroma is not tasty, sour taste is slightly felt meat fish	Catfish meat is not tender, not dense, and not compact
2% (B2)	Quite interesting, color is not uniform, and rather pale	Fermentation aroma is not real, slightly fishy smell, spice aroma is felt	Slightly tasty, sour taste, and slightly felt	Catfish meat is not tender, not dense, and compact
4% (B4)	Attractive, uniform color, consistent, and not pale	Typical fermentation aroma is quite real, no fishy smell, spice aroma is felt	Attractive, Slightly tasty, quite sour	Catfish meat is not felt quite tender, dense, and compact
6% (B6)	Attractive, uniform color, consistent, bright and not pale	Typical fermentation aroma is quite real, no fishy smell, spice aroma is felt	Attractive, Slightly tasty, quite sour	Meat is not felt catfish is too soft, very dense, and very compact.

The appearance characteristics of fermented patin fish sausage at the beginning of production have a dominant sausage color with the color of patin fish meat (grayish white), slightly watery and very soft. The color of the sausage during fermentation changes at each level of treatment, namely from white to slightly brownish. These changes can be caused by the Maillard reaction that occurs during the fermentation process and the effect of cooking (boiling) the sausage after a 24-hour fermentation period. The Maillard reaction is a non-enzymatic reaction involving reducing sugar compounds (carbonyl groups) and amino groups from patin fish protein, which produces melanoidin compounds. These melanoidin compounds are what give the product a slightly bright brown color [21].

The color changes that occur in fermented catfish sausage can also be caused by enzymatic hydrolysis [22] and lipid oxidation contained in *bekasam*. *Bekasam* contains lipid compounds that can be oxidized during the fermentation and ripening process of the sausage. The result of lipid oxidation is to produce brown compounds that affect the color of the sausage's appearance. During the fermentation process, lactic acid bacteria will produce lactic acid which lowers the pH of the sausage, the decrease in pH will cause protein denaturation

[23], which will cause color changes. Color changes in meat can also be caused by the binding of solutions by meat tissue [24].

Consumer acceptance of the aroma value of fermented sausage with the addition of *bekasam* substrate at room temperature for 6 days in the valued of 7.3. The highest panelists' preference for the aroma of fermented catfish sausage was produced by the addition of the highest *bekasam* substrate (6%) but not significantly different to the addition of 4% *bekasam* substrat. The duration of fermentation can affect the aroma produced in the final product. This is in accordance with research [25-26] the longer the fermentation, the sourer the aroma developed. This is due to the activity of acidic compounds formed from the results of LAB fermentation. Changes in the aroma of the product produced during fermentation can also be caused by lactic acid and other organic acids formed from the process of glucose breakdown by LAB anaerobically and can also be caused by the content of compounds produced from the process of fat and protein degradation [27]. The longer the fermentation, the alcohol content and acidity will also increase, which makes the aroma of fermented sausages more pungent [28]. The compounds that form the aroma during fermentation are the results of glucose hydrolysis and alcohol oxidation which have volatile properties [29].

The stronger the distinctive aroma of fermentation produced, the higher the consumer preferences, especially consumer acceptance of the aroma value of the sausages. *Bekasam* substrate contains fermentation compounds that provide a distinctive and strong aroma such as organic acids, alcohol and other volatile components due to the activity of lactic acid bacteria [30] as well as other aromas caused by spices added to the sausage dough. The distinctive aroma of fermentation can reduce th fishy aroma of catfish, this is in accordance to [30], stating that fermentation not only removes the fishy aroma from raw materials but the length of the fermentation process will also produce a very strong distinctive aroma of fermentation.

The taste value of fermented catfish sausage with the addition of the highest *bekasam* substrate (6%) with an average value of 7.3 with a fermentation period of 6 days. The best treatment gived a real sour taste, delicious, specific fish sausage taste and a little spicy. According to the research by [9], the sour taste produced during the fermentation process is caused by the activity of lactic acid bacteria that produce lactic acid which forms a sour taste in fermented products. The sour taste is caused by the increase in acid during the fermentation process, therefore, decrease the pH value and increase the total acid [31-33] In general, the sour taste produced during fermentation is related to the organic acids formed [11]. The sour taste produced in sausages can be influenced by the sour taste contained in *bekasam.*

Consumer acceptance to the texture of fermented sausage with the addition of *bekasam* substrate at room temperature for 2, 3 and 6 days ranged from 7.2 to 7.4. Based on the results of the research that has been done, the level of panelists' preference for the texture quality value of fermented catfish sausage with the addition of the highest *bekasam* substrate (6%) Fermented catfish sausage that was fermented for 24 hours at room temperature produced a soft, chewy, not too hard, dense and compact catfish sausage texture. Changes in the texture of fermented sausage can occur due to biochemical changes during the fermentation process. LAB in meat fermentation role to produce the lactic acid, and other metabolite products such as acetic acid and bacteriocin. In addition, the ingredients used in the manufacturing process also work in changing the texture of the fermented sausage produced, such as soy protein isolate [34]. The fermentation time of fermented catfish sausage after fermentation and the fermentation time on the 2nd day significantly affected the texture of the sausage on the 4th and 6th day of fermentation. It can be concluded that fermented catfish sausage has good texture stability during the fermentation process. [35] stated that fish sausages that have commercial potential are fish sausages that have a soft and chewy texture.

During the fermentation process, *bekasam* substrate as a starter can produce a distinctive value of appearance, taste, texture and aroma of sausages. The addition of a starter using

bekasam substrate can play a role in improving the texture and taste of fermented sausages [36-37]. The protein structure of fermented sausages is responsible for the formation of gels that can affect the proper texture of sausages after salting, cutting and fermentation [38]. During cooking, the protein structure of fermented sausages is denatured by organic acids such as lactic acid, and the water content gradually decreases. The fermentation process occurs under controlled temperature and humidity conditions, resulting in acidification and dehydration that contribute to the firmness, compactness, and acidity of fermented sausages.

3.3 Physico-chemistry Characteristic of Fermented Fish Sausage

This research also presents data on the results of measuring pH values, water activity, total acid and TVB-N to describe the physico-chemical characteristics of fermented fish sausage, as seen in Table 3 below.

Table 3. Physico-chemistry characteristic of fermented fish sausage

Characteristic	Addition of catfish *bekasam*			
	0% (B0)	2% (B2)	4% (B4)	6% (B6)
pH	5.20±1.00^{a}	5.10±1.04^{a}	5.04±1.03^{ab}	5.00±1.04^{b}
Aw	0.76±0.04^{a}	0.72±0.05^{b}	0.71±0.05^{c}	0.70±0.18^{d}
Total Acid (%)	0.42 ±0.08^{a}	0.49±0.10^{b}	0.59±0.12^{c}	0.66±0.12^{d}
TVB-N (mgN/100 g)	20.49 ±4.98^{a}	17.71±3.82^{a}	14.06^{b}±4.40^{b}	12.31±3.23^{c}

Note: Numbers followed by different letters in the same row mean significantly different ($p<0.05$)

Table 4 shows that the pH of catfish sausage without the addition of *bekasam* substrate (B0) is significantly different from fermented catfish sausage with the addition of *bekasam* substrate in treatments (B2, B4 and B6). During fermentation period 6 days, the pH of the sausage decreased up to pH 5,00 for catfish sausage with the addition of *bekasam* substrate 6%. The pH value continued to decrease on the first day to 6th day of fermentation, this is thought to be because, during the fermentation process, the pH value of fish sausage will decrease by around 5.2 to 4.2 [41]. The decrease in pH value can be caused by the breakdown of carbohydrates into lactic acid compounds which are acidic [11]. The decrease in pH value in fermented sausages will have an impact on the growth of pathogenic microbes, this is in accordance with the results of research by [42], which states that the role of lactic acid bacteria has the potential to extend the shelf life of a product and suppress the growth of bacteria that cause decay. A decrease in pH value in fermented catfish sausages can cause muscle protein to be able to touch isoelectric which can cause denaturation and loss of water retention ability [43].

The decrease in pH value occurs along with the concentration of the starter used, the more lactic acid and other organic acids are produced by starter activity [44]. The decrease in pH in fermented sausages is caused by the concentration of *bekasam* substrate used, which involves BAL activity which produces lactic acid and fatty acids. According to [45], the decrease in pH caused by BAL activity can hydrolyze the sugar content in the ingredients into lactic acid and other organic acids.

The total acid value of fermented catfish sausage with the addition of *bekasam* substrate fermented at room temperature has the highest value in treatment B6, which is 0.64%, and significantly different with a fermentation period of 6 days. The total acid value of fermented sausage with the addition of *bekasam* substrate fermented at room temperature has the highest total acid value in treatment B6 with a fermentation period of 6 days. High total acid will cause a decrease in the pH value of fermented sausages [8], [46], [47] This can be caused by

lactic acid bacteria that break down glucose into pyruvate compounds which will then undergo reduction by $NADH_2$ into lactic acid, which then along with increasing lactic acid and will be followed by an increase in H^+ [46].

The total acid content will increase with increasing fermentation time, will cause a higher increase in total acid [48], [46] this will affect the taste of the fermented catfish sausage produced, namely, the taste is increasingly sour [16]. The results of the data analysis showed that the B6 treatment had a higher concentration of *bekasam* substrate compared to other treatments. Likewise, the fermentation time of fermented catfish sausages, the highest value was on the 6th day. This indicates that the longer the fermentation process, the greater the total amount of acid in fermented catfish sausage.

Based on Table 3, the total amount of TVB-N of fermented catfish sausage with the addition of *bekasam* substrate fermented at room temperature has the highest value in treatment B0, which is 20.49 (mgN/100 gs), with a fermentation period of 2 days. Meanwhile, the lowest value is in treatment B6, with a value of 12.31 (mgN/100 gs) with a fermentation period of 6 days.

In the fermentation period, fermented catfish sausage with the addition of *bekasam* substrate fermented in the 2 days had a significant effect on the fermentation period on the 4th and 6th days. Meanwhile, the fermentation period on the 4th day did not have a significant effect on the fermentation period on the 6th day. The longer the fermentation of catfish sausage, the lower the TVB-N value produced, this is due to the activity of the enzymes produced during fermentation, so that the process of breaking down proteins into simpler compounds and amino acids is faster, so that it does not result in the formation of volatile bases [49].

The threshold value of TVB-N in a fishery product that is suitable for consumption is if the TVB-N value produced is 30 mgN/100 gs [50]. Fermented catfish sausage with the addition of *bekasam* substrate fermented at room temperature, has a TVB-N content that is still suitable for consumption based on the threshold limit that has been completed. The treatment of adding *bekasam* substrate to fermented catfish sausage fermented at room temperature can slow down the increase in TVB-N drastically and slow down the decay process. In addition, the decrease in TVBN value in fermented catfish sausage can also be caused by sausage dough formulation ingredients that contain antibacterial compounds that can play an important role in inhibiting bacterial growth during the fermentation process.

3.4 Total LAB of Fermented Fish Sausage

The total value of lactic acid bacterias (LABs) in catfish sausage with the addition of *bekasam* substrate fermented at room temperature can be seen in Table 4.

Table 4. Total LAB of fermented fish sausage (CFU/g)

Fermentation Time	Addition of catfish *bekasam*				Mean
	0% (B0)	2% (B2)	4% (B4)	6% (B6)	
2 days	2.7×10^6	3.7×10^6	4.7×10^6	5.7×10^6	4.2×10^{6a}
4 days	3.4×10^6	4.4×10^6	6.3×10^6	7.5×10^6	5.4×10^{6b}
6 days	4.5×10^6	5.2×10^6	7.5×10^6	9.9×10^6	8.0×10^{6c}
Mean	3.5×10^{6a}	4.5×10^{6a}	6.2×10^{6b}	6.8×10^{6b}	

Note: Numbers followed by different letters in the same row or the same column mean significantly different ($p<0.05$)

Based on Table 4 above, the total value of lactic acid bacteria in fermented catfish sausage with the addition of 6% *bekasam* substrate and fermented at room temperature has the

highest, which is 6.8 x10^6 (CFU/g), with a fermentation period of day 6th. Meanwhile, the lowest value is shown in the addition of 0% *bekasam* substrate with a value mean of 3.5 x 10^6 (CFU/g).

The number of lactic acid bacteria produced in fermented catfish sausages continued to increase from the beginning to the 6th day of fermentation. Furthermore, the use of *bekasam* substrate as a biostarter also had a significant effect on the growth of lactic acid bacteria produced during sausage fermentation. The addition of carbohydrates to fermented catfish sausages can also support the growth of LAB. Lactic acid bacteria contained in *bekasam* substrates with the addition of rice have a high number of lactic acid bacteria [51]. The length of fermentation time can affect the number of lactic acid bacteria produced. The more amount of LAB produced. [5] stated that the length of fermentation can affect the increase in the number of bacteria produced. The increase in total lactic acid bacteria affects the decrease pH value. The increase in total lactic acid bacteria produced is related to the nutrients in the medium. The number of lactic acid bacteria produced is still within the SNI limit, which states that the minimum requirement for a good total lactic acid bacteria value is 10^6 Cfu/mL. The requirement for a probiotic product containing a total of lactic acid bacteria that are still alive when consumed is > 10^6 Cfu/mL. Based on the results of the study, fermented catfish sausage with the addition of different *bekasam* substrates, and fermented at room temperature has met the standards.

4 Conclusion

The use of *bekasam* substrate with different concentrations is significantly affected to the sensory quality characteristics of fermented catfish (*Pangasius* sp) sausage for 6 days fermentation. The best quality of fermented catfish sausage is produced by addition of *bekasam* substrate 6% but not significantly different to those added with *bekasam* substrate 4%. The product is the most preferred by panelists, with the characteristic of attractive in appearance, uniform, consistent, bright, and not pale colors, a very real fermentation aroma, no fishy odor and a spicy aroma, a good taste that feels quite sour and does not taste of catfish meat, and a texture that is quite soft, dense and compact. The chemical and microbiological characteristics of fermented sausage with the addition of *bekasam* substrate has the pH value of 5.00, Aw value of 0.77, a total acid value of 0.66%, a TVB-N value of 12.31 mgN/100 gs and the total LABs number in fermented catfish sausage obtained at 6.9 x 10^6 CFU/g.

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