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SEMI-SOLID STATE AND LIQUID STATE FERMENTATION TECHNOLOGY INCREASE BIOCHEMICAL NUTRITION VALUES OF BAKU TU BANGGAI YAMS FLUOR (DISCOREA GLABRA ROXB)

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Abstract

Banggai yam is a typical plant of Banggai Regency, Central Sulawesi, Indonesia which has the potential to be used as a raw material for gluten-free flour with high nutritional value. Efforts to increase the potential utilization of Banggai yam flour are through the fermentation process. This study aims to determine the nutritional value and sensory quality of the Baku Tu' (*Dioscorea glabra* Roxb) Banggai yam flour using semi-solid state and liquid state fermentations and sensory analysis. The independent variable used was fermentation time which consisted of 5 levels of 24, 48, 72, 96, and 120 hours. Nutrition values of fermented flour were determined by lipid, fibre, protein, moisture, ash, and proximate carbohydrate contents analysis. Results showed that fermented flour has lower lipid, but higher fibre and protein content at 48 h fermentation time. Organoleptic test showed that prolonged fermentation decreases the hedonic scale of Banggai yam flour. Our study suggests that a 48-hour semi-solid fermentation of Baku Tu Banggai yam is the best condition for producing flour that is suitable for a healthy diet.

Keywords: Banggai yams, Fermentation technology, Nutrients, Organoleptic.

1. Introduction

Fermentation is the breakdown of complex compounds into simpler compounds so that they are easily digested, absorbed, and utilized by the body [1]. To date, besides natural fermentation [2], two types of fermentation technology are usually used in plants or fruits, i.e., liquid or submerged fermentation and semi-solid fermentation [3-5]. Liquid fermentation involves the utilization of microorganisms in an aqueous growth medium, while in semi-solid fermentation, the microorganisms are utilized on a solid medium in a lack of flowing water. Furthermore, fermentation technology is one of the good alternative ways to increase the nutritional value of plant-based food [6]. It also promotes benefits to health [7]. There are plenty of plant-based foods have been fermented to improve their nutritional quality, such as soya beans, maize [8], cassava [3], sweet potato [9], cabbage [10], and even Moringa [6, 11].

Important nutrients like proteins, lipids, fibres, and total carbohydrates tend to change in terms of amount after the fermentation process. For instance, fermented cassava flour increases protein contents and decreases its total carbohydrate [3], fermented sweet potato flour decreases fat content [9], and decreases fibre content in fermented maize flour [12].

Banggai yam (Discorea Spp) is a local sweet potato that is widely cultivated in the Banggai Islands, Central Sulawesi. People consume it as a staple food ingredient [13]. Banggai yam belongs to the Dioscoreaceae family which has more than 600 species, 10 of which are cultivated as food and medicine [14]. Discorea has great potential as a source of calories because it contains 73.1% carbohydrates, 1.85% sugar, 17.2% starch, 3.3% minerals, 0.3% fat, and 11.95% protein [15]. Yam can also be used as a food product with high nutritional value as a wheat flour replacement [16]. Little information is found about the fermented Banggai Yam, particularly Baku tu (Discorea glabra Roxb) as shown in Fig. 1.

This research aimed to study the better fermentation technology between liquid and semi-solid fermentation in producing higher quality Banggai yam flour and to evaluate its sensory quality.

Fig. 1. Baku Tu Banggai yam (Discorea glabra Roxb).

2. Materials and Method

Materials used and methods performed are described in the following sub-headings.

2.1. Material

Banggai yam Baku Tu' (Discorea glabra Roxb), commercial yeast (Fermipan), aquadest, H_2SO_4, NaOH, biuret reagent, BSA, and n-Hexane. Unless otherwise stated, all materials were in pharmaceutical grade.

2.2. Preparation and fermentation of Banggai yam

Banggai yams were prepared before being fermented and characterized. Changes of nutritional values were measured before and after fermentation.

2.2.1. Liquid fermentation [17]

The yam was peeled, washed, and then cut into chips by slicing using a slicer with a size of 1.5-3 mm. As much as the composition of 5 g of commercial yeast: 1000 g of yams were prepared. The fermentation process was carried out by submerging the commercial yeast: yams in sterilized water (1:1) for 12, 24, 36, 48, and 72 hours. Next, fermented yam was hot dried in an oven at an initial temperature of 50 ℃ for 8 hours and continued at 60 ℃ for 10 hours. The dried fermented yam was milled to become flour and then sieved with a mesh size of 80.

2.2.2. Semi-solid fermentation [18]

Firstly, peeled, cleaned, and chipped (1.5-3 mm) yams were soaked in sterilized water for 2 h, and then dried. Secondly, 5 g of commercial yeast was added and mixed evenly with 1000 g of dried yam, then fermented in a plastic jar with holes for 24, 48, and 72 at room temperature. Next, fermented yams were hot dried in an oven at 60 °C for 24 h and then sieved with a size of 80 mesh.

2.3. Lipid content analyses [19]

Two-gram flour sample was inserted in filter paper and then put into a pre-weighted flask and connected to a Soxhlet extractor. Next, n-hexane as a solvent was poured from the top into the extraction chamber. The flask was then heated at 40°C with an electric heater for 6 hours. Following the extraction process, the extracted lipid was concentrated using a rotary evaporator. The flask was then dried in an oven at 105 °C for 1 h and reweighted. Lipid content is determined by the formula:

$$\boldsymbol{lipid\ content}\ (\%) = \frac{\boldsymbol{Wflask+extract-Wempty\ flask}}{\boldsymbol{Wsample}} \times \mathbf{100}\% \tag{1}$$

2.4. Fiber content analyses [19]

One g of flour sample was inserted in a flask then added 50 ml of 0.325 N H_2SO_4, was heated for 30 minutes at 100°C. Next, let cool down and add 50 ml of 1.25 N NaOH, and reheat for 30 minutes. Samples then were filtered using pre-weighted Whatman filter paper 41. The filter paper was rinsed successively with boiled aquadest, 25 ml of 0.325 N H_2SO_4, then boiled aquadest, and lastly with 95% ethanol. The rinsed filtered paper was then dried in an oven at 105°C for one hour, drying was carried out to a constant weight. Crude fiber content is calculated by the following formula:

$$\boldsymbol{fiber\ content}\ (\%) = \frac{\boldsymbol{Wfinal\ filter\ paper-Winitial\ filter\ paper}}{\boldsymbol{Wsample}} \times \mathbf{100}\% \tag{2}$$

2.5. Protein content analyses [20]

The 0.3-gram flour sample was added with 1 ml aquadest, mixed then 5 ml of 1 M NaOH and 4 mL of aquadest were added and mixed well. The solution was heated at 90 ℃ for 15 minutes, let cool in a room temperature, adjusted to 25 mL with aquadest in a volumetric flask, then centrifuged at 300 rpm, 4°C, for 20 minutes.

One mL Supernatant was collected and mixed with 3 mL Biuret reagent, homogenized, and incubated for 30 minutes at room temperature. Then the absorbance of the sample was measured with a spectrophotometer at a wavelength of 540 nm. The results of the absorbance of the sample solution are interpolated in the equation y = bx + a so that the protein concentration of the sample solution is obtained. Bovine Serum Albumin was used as the standard protein.

2.6. Moisture content analyses [19]

One gram flour sample was put into a pre-weighted and preheated crucible with a cover and then dried in an oven for 6 h at 105 °C without cover. Next, the crucible was covered and then put in a desiccator until dried completely, then weighed. Moisture content was calculated with the following formula:

$$\boldsymbol{moisture\ content}\ (\%) = \frac{\boldsymbol{M1-M2}}{\boldsymbol{M1-M0}} \times \boldsymbol{100}\%$$

(3)

2.7. Ash content analyses [19]

Five-gram dried flour sample was weighed in a pre-weighted and pre-heated crucible then inserted into a furnace and heated at 600 °C for 7 h. Next, it was allowed to cool in a desiccator and re-weighted. Ash content was calculated with the following formula:

$$\boldsymbol{ash\ content}\ (\%) = \frac{\boldsymbol{Wash\ sample+crucible-Wcrucible}}{\boldsymbol{Wsample}} \times \boldsymbol{100}\%$$

(4)

2.8. Total carbohydrate proximate analyses [19]

Carbohydrate content was carried out by difference, namely the result of reducing 100% with water content, ash content, protein content, and fat content so that the carbohydrate content depended on the reduction factor. This is because carbohydrates greatly affect other nutrients. Carbohydrate content can be calculated using the formula:

$$\%Carbohydrate = 100\% - (\%ash + \%water + \%fat + \%protein) \tag{5}$$

2.9. Organoleptic analyses [6]

The organoleptic analyses were carried out by asking for responses from the panelists about likes and dislikes where the level of preference is called the hedonic scale. To determine the panelist's level of preference for Banggai yam fermented flour sensory tests were carried out on 30 panelists. The attributes proposed consisted of color, aroma, and texture. Each panelist was given a sample and was rated according to a scale of 1-5. The panelist's assessment is written in the form of a hedonic scale of 1-5 (1 = really dislike, 2 = dislike, 3 = quite like, 4 = like, and 5 = really like).

3. Results and Discussion

In general, both fermentation technologies alter fat, protein, fiber, and ash content. Changing of textures and colors are observed as well.

3.1. Fermentation decreases the fat content in Banggai yam flour

Lipids in food consist of fat and oil. Fat is solid at room temperature, while oil is in liquid form. Compared to oil, fat has a higher amount of SFAs which determine its structure [21]. Fermentation may alter lipid composition. Solid-state fermentation of cassava products decreases lipid content when fermented with mixed culture, but it was increased by natural fermentation [3]. Our result, both fermentation types produced Banggai yam flour with significantly lower lipid content. Liquid fermentation yielded a slightly lower amount of lipid than semi-solid fermentation (Fig. 2(a)). The reduction of lipid content was due to its breakdown by lipase enzyme, and the use of simple fatty acids as the energy source for metabolism in microorganisms [22]. Low-lipid content can be beneficial, it can slow the emergence of rancidity due to fat oxidation and increase water content, so that the condition of the food is not easily damaged, both physically and nutritionally [23]. Lipid content can affect the quality, shelf life, and characteristics of the food. Statistical analysis using paired t-tests indicated that there was a significant difference between semi-solid and liquid fermentation ($P < 0.05$). Furthermore, before fermentation has a very statistically significant difference with after fermentation ($P < 0.003$).

3.2. Fermentation increases fibre content in Banggai yam flour

Our results showed that the crude fibre of Banggai yam flour increased after fermentation. It slightly decreased in lag and early 24-hour phase, then increased following longer fermentation time (Fig. 2(b)). Paired t-test analyses indicated that liquid and semi-solid were significantly different ($P<0.05$). The increasing fibre probably is the soluble fibre which is elevated due to the breaking down of the Banggai yam cell wall. Commercial yeast which contains *Saccharomyces cereviceace* releases enzymes that digest cell walls and hydrolyses polysaccharides into simple sugars during fermentation resulting softer texture [24]. It was reported that fermented regular potato has an increased amount of fibre due to the complex formation of polysaccharides, proteins, and lipids [25]. The higher fibre content in food gives extra benefits for our health as it helps reduce the risk of type-2 diabetes, cardiovascular disease, and cancers, and is recommended as an adjunctive medical nutritional therapy for CKD [26].

3.3. Optimum fermentation time for higher protein content in Banggai yam flour

Next, we performed the spectrophotometry method combined with the Biuret method to reveal the effect of both fermentations on protein content. The colour change is a result of the complex formation between the Cu^{2+} and N-peptide bond. In alkaline conditions, Cu^{2+} ions from the biuret reagent will react with polypeptides in proteins to form violet complex compounds [20]. It was then measured its absorbance under the predetermined wavelength.

There was a significant increase in protein content after fermentation indicating fermentation time affects the protein content of the fermented Banggai yam flour (Fig. 2(c)) of protein content (one-way ANOVA, $P<0.05$). The optimum fermentation time to produce the highest protein content for both fermentation types was 72 hours, yielding 14.2% and 16.9 % for liquid and semi-solid fermentation, respectively, however, no significant difference between the two methods. Prolonged fermentation (96 h and 120 h) exhibited a decrease in total protein content, but still higher than unfermented ones (0 h). During fermentation, commercial yeast which contains *Saccharomyces cerevisiae* releases enzymes that digest complex carbohydrates and proteins, utilizing carbon as the main source of energy to grow, and produce simple sugars and amino acids. At prolonged fermentation, yeast will use amino acids as a source of nitrogen to support growth and produce biomass [6]. Our result is similar to other fermentation of cassava, moringa seeds, maize, and yam which were reported to have increasing protein content [3, 6, 8, 9].

3.4. Higher ash content in semi-solid fermentation

Fermentation increases ash content by degradation of antinutrients in Banggai yam. Ash in foods commonly contains minerals and inorganic substances, i.e., calcium, magnesium, potassium, phosphorous, and traces like iron, manganese, and zinc [12]. The result showed in Fig. 2(d) that semi-solid fermentation increased the amount of ash compared to liquid one (insignificantly different, P=0.49). In liquid fermentation, yams were washed and dried after fermentation, which is probably the cause of the lower ash content. However, both techniques showed the highest ash content at 48 h fermentation. The increase in ash content after fermentation was probably due to the degradation of antinutrients by *Saccharomyces cerevisiae* from commercial yeast.

There are three possible reasons for higher ash content in semi-solid fermented Baku Tu Banggai yam: 1) Fermentation increases the bioavailability of minerals and traces due to the degradation of phytic acids and oxalates which form complexes with minerals, resulting in their free form and availability [27]. 2) Fermentation enzymes like phytase and amylase loosen the complex matrix by digesting phytates, and starches contained in foods. 3) It provides optimum pH conditions for the degradative enzyme to work, particularly phytate degradation [28].

3.5. Moisture content is comparable in both fermentation types

As shown in Fig. 2(e), moisture content remains comparable among liquid or semisolid fermented Banggai Yam suggesting fermentation does not affect the storage life of the product (Paired t-test, P=0.11). Moisture level indicating vulnerability of product from microorganism exposure. The higher the moisture content, the higher the chance of getting microbial exposure. Lower moisture enhances stability in storage due to preventing microbial growth and leads to increasing self-life. The moisture content level is a key factor in determining how the three-phase structure changes, which affects water retention, permeability, and thermal conductivity [3].

3.6. Total proximate carbohydrate

The purpose of proximate carbohydrate determination in fermented food is to assess the nutritional value of the food and to understand how the fermentation process affects the carbohydrate content. Carbohydrates are a macronutrient that provides energy for the body. They are also important for gut health and other bodily functions. Fermentation is a process in which microorganisms break down food into simpler compounds. This process can change the carbohydrate content of food in several ways. For example, fermentation can convert complex carbohydrates into simpler carbohydrates that are more easily digested [29]. The functional carbohydrate of fermented Banggai yam flour varied from 75.8% - 84.8 % for liquid fermentation and 72.0% - 84.4% for semi-solid fermentation (Paired t-test, P<0.05). Those values are exclusive differences of ash, moisture, crude fat, and crude protein contents. Fermentation time of 48 and 72 hours decreases 10 % of carbohydrate content but increases the protein content significantly (Fig. 2(c) and (e)).

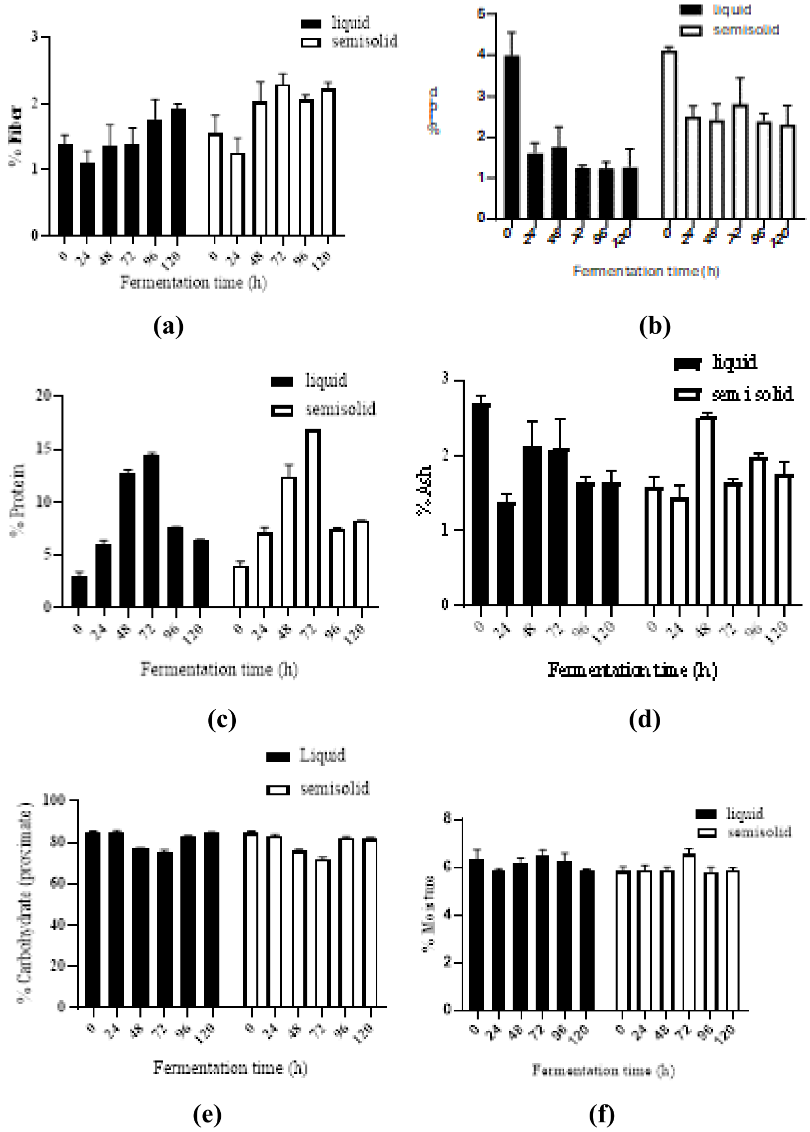


Fig. 2. Effect of liquid and semi-solid fermentation on lipid (a), fibre (b), protein (c), ash (d), proximate carbohydrate (e), and moisture (f) contents of Baku Tu Banggai Yam.

3.7. Organoleptic properties of fermented Banggai yam flour

A sensory evaluation was performed to assess the quality of fermented Banggai yam flour. A group of 30 trained panellists evaluated the texture, aroma, and colour of the product. Food texture is a complex property that is influenced by the material's structure, which can be characterized by three main elements: mechanical properties (hardness, elasticity), geometric properties (particle size, distribution), and mouthfeel (oily, moisture) [6, 30]. The hedonic scale of liquid fermentation was comparable to up to 48 h fermentation, then decreased following extensive fermentation time. Concurrently, the semi-solid fermentation exhibited a comparable hedonic scale of all fermentation times (Fig. 3(left)), and the paired t-test indicated that liquid and semisolid fermentation were significantly different ($P<0.05$). In liquid fermentation, pprolonged fermentation can have a significant effect on flour texture, making it softer and more extensible. This is due to the action of enzymes produced by utilized commercial yeast. These enzymes break down gluten proteins, making them more susceptible to proteolysis by other enzymes and acids. This softer texture in prolonged fermentation flour (72 – 96 h) was disliked by the panellists.

Aroma testing was considered an important parameter in this evaluation, as it can quickly provide information about product quality and acceptability, as well as detect spoilage [30, 31]. Both liquid and semi-solid fermentation affect the aroma of the fermented Banggai yam flour. The panellists quite liked the fermented Banggai yam flour up to 48 h fermentation time (hedonic value of 3.7 and 3.3 for liquid and semisolid fermentation, respectively). Prolonged fermentation decreases the preference for the product with a hedonic value of 1.5 (Fig. 3(right)). In extensive fermentation, yeasts produce a variety of volatile compounds that contribute to the characteristic sour and yeasty aromas resulting in a more complex and strong flavour flour.

Food quality is determined by a combination of factors, including aroma, texture, and colour. However, visual appearance and colour are the most important factors influencing consumer acceptance, and they can also provide insights into chemical changes that have occurred in the food. The panellist preferred a white and brighter colour observed in 0 – 24 h fermentation time compared to a brownish and darker colour as a result of extended fermentation time (Figs. 3(middle) and 4). Prolonged fermentation led to the formation of Maillard reaction products, which contributed to the browning of the flour crust. The enzymes produced by yeast can break down carotenoids, which are pigments that give flour its yellow colour [30, 31].

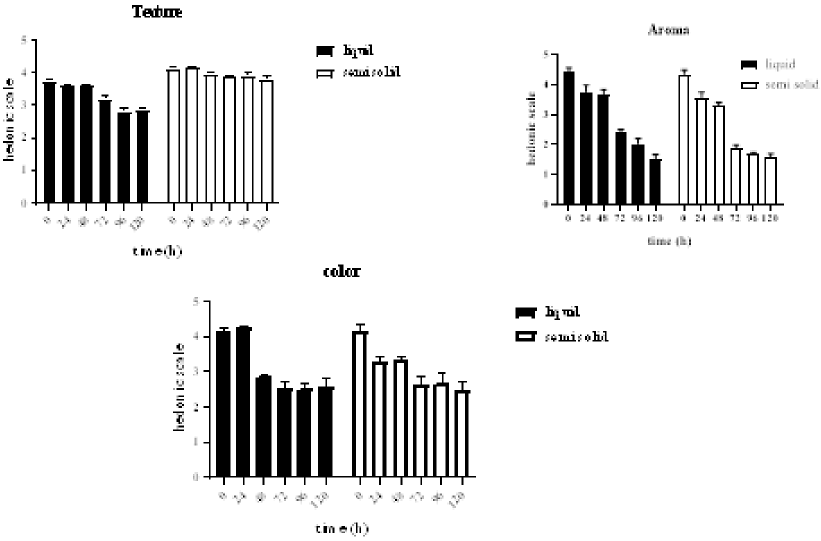
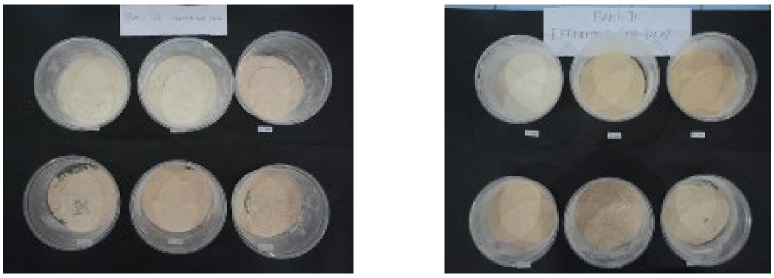


Fig. 3. The hedonic scale of fermented Banggai yam flour: texture (left), aroma (right), colour (middle).

In general, prolonged fermentation time (72 hours or even more extended to 4 and 5 days), may lead to negative effects on foods as it decreases total protein content, increases fibre content, softens the textures, and darkens the colour of food [32]. Although, these changes were caused by the breakdown of proteins and carbohydrates by microorganisms in extensive fermentation time, however, the exact mechanism responsible for these differences is still not fully understood.



(a) **(b)**

Fig. 4. Colour changes of Banggai yam flour after liquid (a) and semisolid (b) fermentation (Upper containers from left to right: 0 h, 24 h, 48 h. Lower containers from left to right: 72 h, 96 h, 120 h).

4. Conclusions

Research has been performed on the effect of liquid and semi-solid fermentation on Banggai yam flour and how it changes the nutritional value and sensory quality. Several conclusions are given below.

- Liquid and semi-solid fermentation increased the nutrition values of Banggai yam flour (Baku Tu) or Discorea glabra Roxb. Semi-solid fermentation provided better impact with higher fat, total fibre, protein, and ash contents compared to liquid fermentation. Both fermentation technologies lowered total fat content and increased total fibre and protein content which are essential for a healthy diet.

- In terms of sensory quality, fermentation times of up to 48 hours for both liquid and semi-solid were still likable by the panellists. Prolonged fermentation decreases the nutritional values and hedonic scale of Baku Tu Banggai yam flour.

Nomenclatures

M_0	Weight of crucible and its cover, g
M_1	Weight of crucible, cover, and fresh sample, g
M_2	Weight of crucible, cover, and dried sample, g
$W_{ashsample+crucible}$	Weight of ash sample and crucible after analyses, g
$W_{crucible}$	Weight of crucible before analyses, g
$W_{initialfilterpaper}$	Weight of initial filter paper before analyses, g
$W_{finalfilterpaper}$	Weight of final filter paper after analyses, g
W_{sample}	Weight of sample, g
$W_{empty\ flask}$	Weight of empty flask before analyses, g
$W_{flask+extract}$	Weight of flask and extract after analyses, g

Abbreviations

ANOVA	Analysis of Variance
BSA	Bovine Serum Albumin
CKD	Chronic Kidney Disease
SFAs	Saturated Fatty Acids

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