An Analysis of Soluble and Insoluble Fiber Content of Various Modified Corn Flours Using Fermentation Followed by Pregelatinization – Systematic Literature

An Analysis of Soluble and Insoluble Fiber Content of Various Modified Corn Flours Using Fermentation Followed by Pregelatinization – Systematic Literature

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Abstract. This study was conducted to determine the effect of engineering on the fermentation process combined with pregelatinization in the production of modified high-fiber corn flour. Modified corn flour was produced from BISI-18 corn that had been previously ground and treated with spontaneous and controlled fermentation. The spontaneous fermentation method used control (A1), while the controlled fermentation method used single cultures of *Lactobacillus fabifermentans* (A2) and *Aspergillus* sp. (A3) as well as mixed cultures of *Lactobacillus fabifermentans* and *Aspergillus* sp. at a ratio of 1:3 (A4). Microaerophilic fermentation was carried out for 24 hours at room temperature. All the treated fermented corn flours were dried and pregelatinized at 80 °C for 15 minutes and dried using a room dryer at ± 50 °C for 48 hours. This study used a randomized block design with four treatments, each consisting of 3 groups. The observation variables included insoluble dietary fiber, soluble dietary fiber, and total dietary fiber. Data were analyzed using Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) at a significant level of 0.05. The results showed that the fermentation process affected the insoluble dietary fiber and soluble dietary fiber content of modified corn flour but did not affect the total dietary fiber content. It was also found that the best modified corn flour was produced with the addition of a single culture of *Aspergillus* sp., containing 9.36% insoluble dietary fiber, 6.26% soluble dietary fiber, and 15.62% total dietary fiber.

Keywords: *Lactobacillus fabifermentans*; *Aspergillus* sp.*; Modified Corn Flours; Soluble Dietary Fiber; Insoluble Dietary Fiber*

1. Introduction

Fiber is part of a plant that the human body cannot absorb. Fiber is beneficial for human health. For instance, it maintains weight or keeps from obesity (overweight), controls diabetes, maintains normal blood cholesterol, and prevents digestive tract diseases, colon cancer, and cardiovascular disease. The fiber in food is divided into crude fiber and dietary fiber. Dietary

fiber is further divided into soluble dietary fiber and insoluble dietary fiber. Soluble dietary fiber is fiber that can be dissolved in hot water; this causes the soluble dietary fiber to easily pass through the small intestine and be fermented by microbes in the large intestine. Meanwhile, insoluble dietary fiber is fiber that cannot be dissolved in water. According to [1], insoluble dietary fiber cannot form gel when passing through the small intestine and is hard to be fermented by microbes in the human large intestine.

Corn is one of the most widely grown crops in Indonesia. Corn production in 2017 increased to 27.95 million tons from 23.18 million tons in 2016 [2]. Corn is one type of agricultural product that contains a lot of fiber. Complex carbohydrates in corn kernels, especially in the pericarp and tip cap, are also found in the endosperm cell wall. A small amount is located in the institutional cell wall.

The characteristics of flour are related to flour quality, in which case the factors of flour can affect the quality of the resulting product. The attributes of corn flour are not the same as those of wheat flour, so the use of corn flour in the manufacture of processed food products is minimal. Modifications to corn flour need to be made. They involve various methods: chemical modification (fermentation), physical modification (pregelatinization), and a combination of both (fermentation on pregelatinization). Fermentation breaks down organic material into simpler compounds using enzymatic reactions and complex organic catalysts produced by bacteria, fungi, and yeasts. Pregelatinization is physical modification of starch using the heating method at temperatures above the gelatinization temperature range of starch [3].

Modified corn flour is expected to have better characteristics than ordinary corn flour. Therefore, this study aimed to analyze the effect of the modified method (fermentation and pregelatinization) on the dietary fiber content of corn flour.

2. Methodology

The experimental design used in this study was a randomized block design. The influencing factor in this design was the variation of the microbial cultures used in the fermentation, followed by pregelatinization, repeated three times. The data obtained were analyzed using ANOVA. If there were differences between treatments, the analysis would be followed by Duncan's Multiple Range Test (DMRT).

The variables observed included insoluble dietary fiber, soluble dietary fiber, and total dietary fiber [4]. This research was conducted at the Agricultural Technology Education Laboratory, Faculty of Engineering, Universitas Negeri Makassar, and the Chemical Laboratory, Department of Chemical Engineering, Politeknik Negeri Ujung Pandang, Makassar.

Tools and Materials

The equipment used in this research was disc mill, autoclave, laminar airflow, incubator, water bath, oven, vacuum pump, scales, desiccator, dropper pipette, stirring rod, bulb pipette, Bunsen burner, test tube, Beaker glass, hotplate, Erlenmeyer flask, volume pipette, micropipette, inoculum needle (ose), pH meter, gloves, biuret, analytical balance, and thermometer.

The materials used in this study were BISI-18 corn obtained from Bantaeng Regency, *L. fabifermentans* culture and *Aspergillus* sp. pure isolate from the spontaneous fermentation of BISI-16 corn flour [5], PDA medium, MRSB medium, aluminum foil, plastic wrap, plastic labels, cotton, 96% ethanol, Termamyl enzymes, amyloglucosidase, pepsin, HCl, NaOH, acetone, hexane, phosphate buffer, and Whatman filter paper.

Stages of Preparation [6]
Corn Flour Making

The corn used went through the grinding process and separation of the husk and the kernels. Then, the corn kernels were soaked for ± 48 hours, drained, and milled using a machine. The milled flour was dried in a drying room for 48 hours. The resulting flour was then sieved using an 80 mesh sieve.

Refreshing *Lactobacillus fabifermentans* Culture
Cultures of *L. fabifermentans* were refreshed on MRSB medium. Restoring the cultures on the MRSB medium was done by taking them from the old MRSB medium using a one ml pipette aseptically and then transferring them to the new MRSB medium. The new MRSB medium was then incubated for two days at 30 °C.

Refreshing *Aspergillus* sp. Culture
Pure cultures of *Aspergillus* sp. were refreshed on PDA medium. Reviving the cultures on PDA medium was done by taking them from the old PDA medium as much as one inoculum needle aseptically then scraping them on the new PDA medium. The PDA agar medium was then incubated for 120 hours at 30 °C.

Implementation in Corn Flour [6]
Making *L. fabifermentans* starter and *Aspergillus* sp. starter
Fifty grams of corn flour and 100 ml of distilled water were mixed at a ratio of 1:2, then the *L. fabifermentans* cultures (previously incubated for two days) was added. *Aspergillus* sp. cultures were aseptically added after five days of incubation. Next, the corn flour solution was fermented for two days.

Starter Application
The starter cultures of *L. fabifermentans* and *Aspergillus* sp. were aseptically pipetted at 10 ml and then put into 500 g of corn flour. One thousand ml of distilled water was added to the flour at a water-to-flour ratio of 1:2. The mixed starter cultures of *L. fabifermentans* and *Aspergillus* sp. were applied at a ratio of 1:3. The corn flour solution that had been added to the starter was then fermented for 24 hours. Next, the corn flour was filtered and dried in a drying room for 48 hours at 50 °C. The corn flour that had been dried and mashed using a blender was then sieved using an 80 mesh sieve.

Flour Pregelatinization
The corn flour obtained from the fermentation process was then modified again utilizing pregelatinization. Pregelatinization was done by adding distilled water at 30% of the amount of the flour (150 ml) in an aluminum container and then by stirring until a homogeneous state was achieved. The container that already contained corn flour was then heated in a steaming pan at 80 °C for 15 minutes, during which stirring was constantly performed. Next, the corn flower was removed from the steamer pan and dried again for 48 hours in a room dryer. After that, the corn flour was dried for 48 hours. The corn flour was ground and then sieved again using an 80 mesh sieve.

3. Results and Discussion

Insoluble Dietary Fiber

Insoluble dietary fiber is a type of fiber that cannot be dissolved in water. This type of fiber cannot form gel when passing through the small intestine and is very difficult to ferment by the human colon microflora [7]. In general, insoluble dietary fiber is resistant to microbial degradation so that only a small portion is fermented [8]. Insoluble dietary fiber increases the volume of feces and shortens the transit time of wastes in the large intestine. Therefore, dietary fiber, especially insoluble fiber, is commonly used to treat digestive tract disorders, such as constipation, diverticular disease, and irritable bowel syndrome [9].

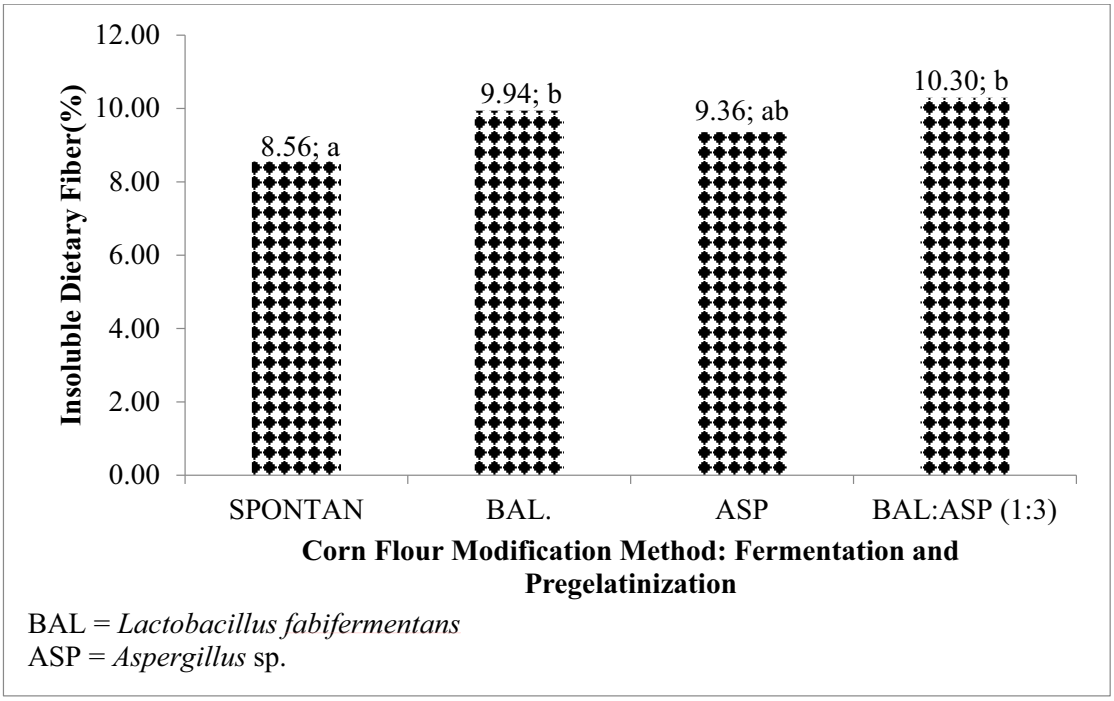

Figure 1. Insoluble Dietary Fiber Content

The content of water-insoluble dietary fiber in cellulose, hemicellulose, and lignin is relatively higher in vegetables, wheat, cereals, and beans [10]. The insoluble dietary fiber content of modified corn flours made by fermentation and pregelatinization from various treatments can be seen in Figure 1.

The results of the analysis of the parameters of insoluble dietary fiber showed that the values of all controlled fermentation treatments with the addition of microbial cultures were higher than the value derived from the spontaneous fermentation treatment. The highest value of insoluble dietary fiber was obtained from the controlled fermentation treatment with a mixture of cultures of *L. fabifermentans* and *Aspergillus* sp. at a ratio of 1:3 (10.30%), and the lowest value was obtained from the spontaneous fermentation treatment (8.56%).

The results of Duncan's test analysis on the value of insoluble dietary fiber showed that the fermentation treatment followed by pregelatinization had a very significant effect on the content of insoluble dietary fiber in modified corn flour. The lowest range of insoluble dietary fiber was produced by the corn flour made with the spontaneous fermentation treatment (8.56%), while

the highest content of insoluble dietary fiber was found in the corn flour made with the fermentation treatment with the addition of a single culture of *L. fabifermentans* and mixed cultures of L. *fabifermentans* and *Aspergillus* sp. (1:3) (9.94% and 10.30%, respectively). This is thought to be due to *L. fabifermentans* being less able to decompose insoluble dietary fiber because these bacteria are less than optimal in producing cellulase enzymes.

L. fabifermentans bacteria can cause the pH of the fermented liquid to be very low or very acidic during the fermentation process. Fermentation liquid that is too acidic can cause inhibition of microbial growth of moulds, yeasts, bacteria, and indigenous cellulolytic LAB. Thus, it is suspected that the amount of cellulase enzyme produced is less than those which were produced with the spontaneous fermentation treatment and the treatment with the addition of a single culture of *Aspergillus* sp. According to [11], the lactic acid produced will reduce the pH value of the growth environment and cause a sour taste. This acidic nature also inhibits the growth of several other types of microorganisms. In addition, a very acidic pH can also affect the activity of cellulase, in which case cellulase can work optimally at an alkaline pH. [12] stated that the optimum pH of cellulase activity is pH 8.

The lowest insoluble dietary fiber content was obtained from the spontaneous fermentation treatment. This could happen because the microbes were more diverse in the spontaneous fermentation process. More insoluble dietary fiber was hydrolyzed due to the cellulase enzyme produced by indigenous microbes that grew during fermentation. The microbes involved in the spontaneous fermentation of BISI-18 corn were not only from the lactic acid bacteria group. Moulds, yeasts, and other bacterial groups were also involved [13].

The fermentation treatment with the addition of a single culture of *Aspergillus* sp. also produced low levels of insoluble dietary fiber. This was because *Aspergillus* sp. can produce cellulase that functions to hydrolyze insoluble dietary fibers such as cellulose and hemicellulose. [14] stated that most *Aspergillus* sp. bacteria have various enzymes, such as cellulase, amylase, glucoamylase, lipase, and protease.

Modification with pregelatinization after fermentation did not significantly affect the content of insoluble dietary fiber in modified corn flour. The temperature used during the pregelatinization process could not degrade insoluble dietary fiber. Hemicellulose is degraded by heat in the temperature range of 220–315 °C, with the fastest decomposition occurring at a temperature of around 270 °C. Cellulose is degraded in the temperature range of 315–400 °C, with the most immediate decomposition occurring at a temperature of about 350 °C. Meanwhile, lignin is slowly degraded in the temperature range of 160–900 °C [15].

Soluble Dietary Fiber

Soluble dietary fiber can be dissolved in hot water, so it can pass through the small intestine easily and is fermented by the sizeable intestinal microflora. Most soluble dietary fiber can be fermented rapidly by microbes [8]. The analysis results showed a difference in soluble dietary fiber content between the spontaneous and controlled fermentation treatments with the addition of single cultures and mixed cultures (Figure 2). The results of Duncan's test analysis on the value of soluble dietary fiber showed that the fermentation treatment followed by pregelatinization had a very significant effect on the soluble dietary fiber content of modified corn flour. The lowest soluble dietary fiber content was produced by corn flour made with the fermentation treatment with the addition of mixed cultures of *L. fabifermentans* and *Aspergillus* sp. at a ratio of 1:3 (4.76%), while the highest soluble dietary fiber content was found in the corn flour made with the single culture treatment of *Aspergillus* sp. (6.26%).

Dietary fiber is part of plant cell walls that cannot be hydrolyzed or digested by human digestive enzymes, including hemicellulose, cellulose, lignin, pectin, and -glucan. Dietary fiber which provides soluble dietary fiber includes pectin and -glucan. Pectin in available in corn at 28.7 (high methoxyl pectins), 34.8 (low methoxyl pectins), and 36.5 (protopectin) [16].

The highest soluble dietary fiber content was obtained from the fermentation treatment with the addition of a single culture of *Aspergillus* sp. This was because *Aspergillus* sp. can produce pectinase enzymes that can hydrolyze water-insoluble protopectin into water-soluble pectin. As a result, the more *Aspergillus* sp. bacteria growing during the fermentation process the more the amount of soluble dietary fiber in corn flour. [17] stated that *Aspergillus* and *Rhizopus* species are productive microbial strains of the type of moulds that produce commercial pectinase enzymes with high pectinolytic activity on an industrial scale.

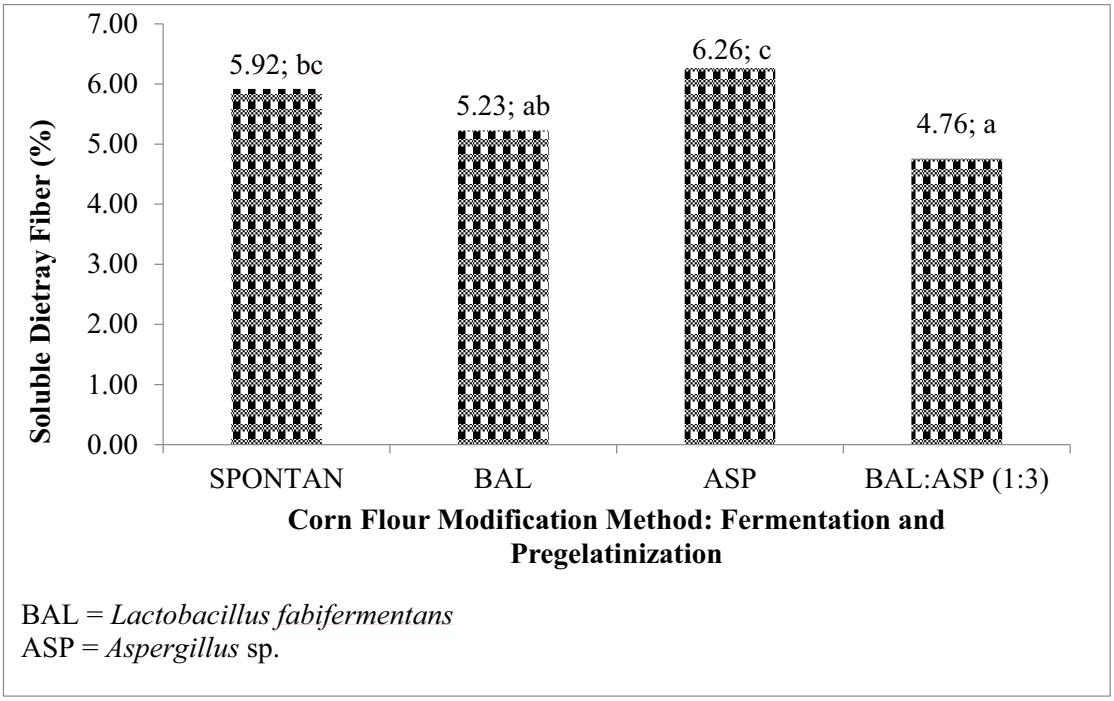

Figure 2. Soluble Dietary Fiber Content

The lowest soluble dietary fiber content was produced in the fermentation treatment with mixed cultures of *L. fabifermentans* and *Aspergillus* sp. (1:3). This was because *L. fabifermentans* is less able to hydrolyze insoluble dietary fiber into soluble dietary fiber. In addition, *L. fabifermentans* can also produce very acidic pH in the fermentation process. An acidic pH value in the fermentation process can inhibit the growth of indigenous microbes. It can also affect the performance of pectinase, which will work optimally at pH 5. [18] stated that the optimum activity of pectinase by *A. niger* was at pH 5, at a temperature of 40 °C, and for a fermentation time period of 96 hours, with pectinase concentration of 7.99 g/mL and activity of 20.14 units.

Pectinase is an enzyme used in the degradation of pectin molecules [19]. Pectinase enzymes are divided into three major groups: enzymes that carry out de-esterification (pectinesterase), enzymes that carry out depolymerization (hydrolases and lyases), and protopectinase. The de-

esterification enzyme cleaves the ester bond between the carboxyl group of the polygalacturonic acid unit and the methyl group. Depolymerization enzymes cleave 1,4 glycosidic bonds in pectin compounds, while protopectinase is a pectinase enzyme that dissolves protopectin [20].

The pregelatinization treatment after fermentation did not significantly affect the soluble dietary fiber content of modified corn flour due to the low temperature used during the pregelatinization process. The pectin compounds contained in corn flour had not been hydrolyzed optimally. The high temperature during the pregelatinization process would increase the amount of pectin produced; the higher the temperature given, the higher the rate of the protopectin hydrolysis into pectin [21].

Total Dietary Fiber

Dietary fiber is the main component of the cell walls of such plants as fruit plants, vegetable plants, cereal plants, and various tuber plants. Features of dietary fiber include indigestible polysaccharides that consist of insoluble dietary fiber (IDF) and soluble dietary fiber (SDF), such as cellulose, hemicellulose, oligosaccharides, pectin, gums, and waxes [22]. The analysis results showed a difference in total dietary fiber content between the spontaneous and controlled fermentation treatments with the addition of single cultures and mixed cultures (Figure 3). The controlled fermentation treatment with the addition of diverse cultures of *L. fabifermentans* and *Aspergillus* sp. at a ratio of 1:3 had the lowest total dietary fiber content (15.06%), while the controlled fermentation treatment with the addition of a single culture of *Aspergillus* sp. had the highest total dietary fiber content (15.62%).

The analysis of variance on the value of total dietary fiber showed that the fermentation treatment followed by pregelatinization did not have any significant effect on the total dietary fiber content of modified corn flour. The total dietary fiber of modified corn flour ranged from 14.48% to 15.62%. This shows that modified corn flour has high enough dietary fiber content to be used as a source of dietary fiber. The WHO (World Health Organization) recommends a fiber intake of 25–30 grams per day.

Total dietary fiber (TDF) consists of soluble dietary fiber (SDF) and insoluble dietary fiber (IDF). The nature of this solubility greatly determines the physiological effect of fiber on processes in digestion and metabolism of nutrients [23]. IDF is defined as dietary fiber that is insoluble in hot and cold water. Sources of IDF are cellulose, lignin, mostly hemicellulose, small amounts of chitin, plant wax, and sometimes insoluble pectate compounds. Meanwhile, SDF is dietary fiber soluble in warm or hot water and precipitable by water mixed with four parts of ethanol. Sources of SDF include gum, pectin, and some soluble hemicellulose found in plant cell walls [24].

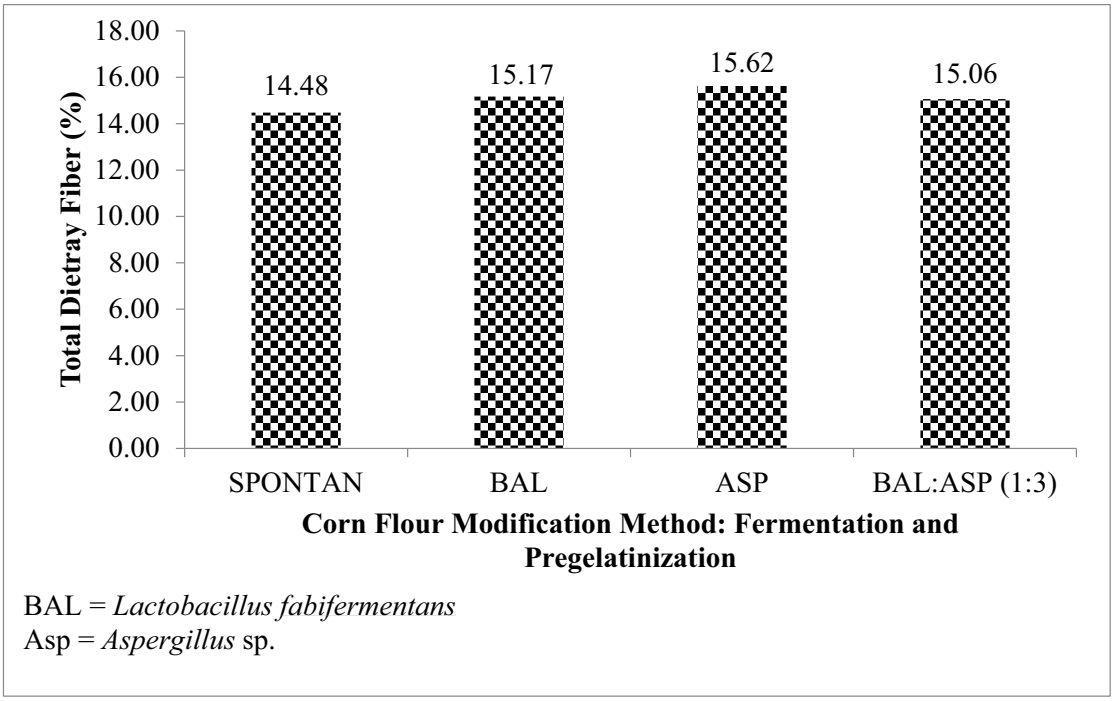

Figure 3. Total Dietary Fiber Content

4. Conclusion

Variations in the combination method of fermentation followed by pregelatinization affected the dietary fiber content of corn flour. In this study, the best treatment was one with the addition of a single culture of *Aspergillus* sp., with 9.36% insoluble dietary fiber content, 6.26% soluble dietary fiber content, and 15.62% total dietary fiber content.

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