Characteristic of Modified Tannia Flour: Study of Starter Type and Fermentation Time

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Abstract. This research analyzes the type of microbial starter and fermentation time on the characteristics of Modified Tannia Flour (MOTIF) with randomized design— nine treatments and three replications. The treatments were F (*Lactobacillus bulgaricus* bacteria, lactic acid bacteria and Bimo-CF Starter) and P (fermentation time 24 h, 36 h and 48 h). Observed variables being resistant starch, swilling power and water-soluble index and color test (l, a, b). The research results showed that the best treatment was the starter type *Lactobaccilus bulgaricus* with 36 h of fermentation producing a content of 10.50 %, ash content of 1.64 %, resistant starch content of 24.21 % and swelling power of 35.38 %. The use of a starter type of lactic acid bacteria with a fermentation time of 36 h has characteristics of flour with a water content of 13.41 %, ash content of 1.70 %, resistant starch content of 23.93 % and swelling power of 34.99 %, while using Bimo-CF starter with a fermentation time of 36 h has characteristics of flour with a water content of 17.22 %, ash content of 1.51 %, resistant starch content of 24.36 % and swelling power of 35.44 %.

Keywords: Bimo-CF, improve fluor quality, motif, *Xanthosoma sagittifolium* [(L.) Schott].

1 Introduction

The taro plant is a tuber plant that has long been known and used by Indonesian people [1]. Taro plants in Indonesia consist of several genera, namely the genus *Xanthosoma sagittifolium* (L.) Schott, the genus *Colocasia esculenta* (L.) Schott (taro Bogor) and the genus *Colocasia gigantea* (Blume ex Hassk.) Hook.f. (taro Padang). Kimpul taro (*X. sagittifolium*) is known to have a greater starch content, reaching 77.90 % compared to *C. gigantea*, which is only 70.99 % [2]. The taro plant is a type of intercrop that has several advantages, such as being easy to grow in all places, both in tropical and subtropical areas, easy to cultivate because it does not have special growing conditions. Intercropping is a popular production system in small and marginal holdings in developing countries [3] and

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easy maintenance. However, as a food crop that is cheap and easy to breed, taro is still not cultivated in Indonesia but is only used as an intercrop. Variations in the use of taro are also limited to fried tubers, boiled tubers, and animal feed. This is what makes taro plants have low economic value so that farmers in Indonesia are less interested in cultivating them. Meanwhile, if studied more deeply, taro has great potential to be used as a food product that can be used more widely.

To improve the physical and chemical properties of taro flour, a modification process is needed, one of which is through a fermentation process. Fermentation is a process that helps break down large organic molecules via the action of microorganisms into simpler ones [4]. The yield of Resistant Starch (RS) in the multigrain flour reaches 77.42 %. Combining heat - moisture treatment causes protein denaturation, starch gelatinization, and flour clumps in MF, facilitating starch recrystallization and interactions among short-chain starch, monomeric proteins, and lipids [5]. Resistant Starch (RS) is one of the nutritional contents of taro which has a role in the human digestive system. In addition, consuming large amounts of resistant starch cannot cause constipation and flatulence because it can bind and maintain water content in feces [6]. Resistant Starch (RS) plays a key role in providing metabolic and colonic health benefits. In particular, RS type III (RS3) is of great interest because of its thermal stability and its preserved nutritional functionality [7].

Overall, the type of bacteria and fermentation time can influence the quality of modified flour to produce resistant starch, swelling power and water-soluble index. This research aims to determine the type of starter microbe and fermentation time on the chemical and physical characteristics of Modified Tannia Flour (MOTIF).

2 Materials and methods

Research will be carried out at the Microbiology Laboratory and Agricultural Product Process Engineering Laboratory, University of Tribhuwana Tunggadewi Malang (S 7°55'49.1232" E 112°35'54.096") and the Nutrition Laboratory, Department of Public Health Nutrition, Airlangga University (S 7°16'3.1656" E 112°46'59.0592"), starting in February to July 2023.

2.1 Materials

The tools needed for this research are manual scales, knife, basin, 2 mm grater, drying oven (Binder ED53 572 °F, USA), 80 mesh sieve, blender, digital scales, autoclave (Hirayama HVE 50, Japan), glass beaker, erlenmeyer, stirring rod, cotton roll, parchment paper, aluminum foil, rubber bands, ossicle needles, bunsen lamps, spirits, encases, incubators (Memmert INE500, Germany) and refrigerators (Sharp SJ-236 MG-GB/GR, Japan). The tools used for chemical analysis are moisture analyser (OHAUS MB25, USA), incubator (WTB Binder, Germany), furnance (Ney Vulcan A550, USA), water bath Kjeldhal flasks, texture analyzers, pipettes, UV-Vis spectrophotometers, centrifuge tubes, petri dishes, weighing bottles and desiccators.

The ingredients used in making Modified Tannia Flour are Kimpul taro obtained from the Malang traditional market (coordinate: S 7°59'10.4748" and E 112°38'0.474"), Bimo-CF starter is a seed for fermentation in the process of making biologically modified cassava flour obtained from Balitbangtan Agricultural Gene Bank (Agricultural Research and Development Agency - *Badan Penelitian dan Pengembangan Pertanian* – Republic of Indonesia) Bogor, the starter consists of a carrier material and active ingredients of lactic acid bacteria, lactic acid bacteria starter, pure culture of *Lactobacillus bulgaricus* [(Orla-Jensen 1919) Rogosa & Hansen 1971 Weiss *et al.* 1984] baker, 70 % alcohol, distilled water, and nutrient broth.

2.2 Methods

This research uses a randomized nested design with two factors that do not interact with each other. The factors are (i) Factor 1 starter type, consists of three levels: S1: *Lactobacillus bulgaricus*, S2: Lactic Acid Bacteria (LAB) starter, S3: Bimo-CF starter; (ii) Factor 2 fermentation time, consisting of three levels: F1: 24 h, F2: 36 h, F3: 48 h.

Table 1. Combination of research treatments.

Number	Treatmen code	Treatment
1	S1F1	*Lactobacillus bulgaricus* 24 h
2	S1F2	*Lactobacillus bulgaricus* 48 h
3	S1F3	*Lactobacillus bulgaricus* 36 h
4	S2F1	Lactic acid bacteria 24 h
5	S2F2	Lactic acid bacteria 36 h
6	S2F3	Lactic acid bacteria 48 h
7	S3F1	Bimo-CF starter 24 h
8	S3F2	Bimo-CF starter 36 h
9	S3F3	Bimo-CF starter 48 h

From these two research factors, nine treatment combinations will be obtained with repetition three times so that 27 research samples will be obtained.

2.2.1 Determination of resistant starch

Analysis of resistant starch refers to the procedure [8], a total of 1 g of modified taro flour was dispersed into 20 mL of 0.1 M sodium acetate buffer; pH 5.2 heated in a water bath for 30 min. Starch dispersion was cooled at 37 °C, mixed with a 5 mL enzyme solution consisting of pancreatine extract and AMG amyloglucosidase, then incubated in a water bath at 37 °C. Pancreatine extract was obtained from: 3 g of pancreatine suspended in 20 mL of distillate deionized water, steered for 10 min at room temperature, and centrifuge. After that it is heated in a water bath with a water temperature of 100 °C for 10 min and then cooled at room temperature. The sample was then diluted with the addition of 10 mL of aqueous and measured using a spectrophotometer at a wavelength of 550 nm. Aqueous is used as blanks. A standard curve is created using a standard glucose solution with a 5 000 mg L^{-1} glucose solution as the parent solution. The working solution used as standard is 500 mg L^{-1}, 1 000 mg L^{-1}, 1 500 mg L^{-1}, 2 000 mg L^{-1}, 2 500 mg L^{-1}, 3 000 mg L^{-1}.

$$(\%\ \text{Resistant Starch} = \text{A} \times \text{FP} \times 100 \times 0.9\ \text{SW}) \quad (1)$$

Where,
A : Sample absorbance
S : Slopeslope of standard curve
FP : Dilution factor
W : Gram sample weight

2.2.2 Measurement of sweeling power and water-soluble index

Analysis of measurement of sweeling power and WSI refers to the procedure [9], Modified Tannia Flour was weighed (100 mg) and placed in a screw-cap test tube (known as an empty

weight). Distilled water (10 mL) added to test tube. Modified Tannia Flour and distilled water were mixed using vortex mixer for 10 s. Then, it was incubated in a water bath (85 °C) for 30 min while stirring occasionally. Then, it was cooled in ice water to room temperature. The solution was centrifuged at 2 000 rad s^{-1} for 30 min. The supernatant liquid was transferred into a cup that had been weighed and then it was heated in an oven to a constant weight (W1). The precipitate was left in the test tube weighed (Ws)15. The calculation of swelling power and water-soluble index values were in Equation (2) and Equation (3).

$$\mathrm{SP} = \frac{\mathrm{Ws}}{(0.1 \times (100\ \% - \mathrm{WSI})} \left(\frac{\mathrm{g}}{\mathrm{g}}\right) \tag{2}$$

$$\mathrm{WSI} = \frac{\mathrm{W1}}{0.1} \times 100\ \% \tag{3}$$

2.2.3 Color characteristics

This analysis using hunterlab colorFlex EZ spectrophotometer [10]. Color testing using a color system Hunter L* (white), a* (red), b* (yellow). Chromameter first calibrated with color standards white on the tool. The results white degree analysis is L*, a*, b* values. Total degree measurement the base color is used as white standard.

Data obtained from research results such as the results of analysis of proximate composition, resistant starch content, sweeling power and water-soluble index and color characteristics will be analyzed using the ANOVA (Analysis of Variance) method which aims to increase the accuracy of the research results. If the results of the ANOVA analysis are significantly different, it will be continued with the least significant difference test (LSD) at the 5 % level. However, if the results of data analysis show a very significant difference, then the LSD test will be continued with a 1 % level [11]. The color of the flour has an important role because it will influence the derivative products produced. This research aims to compare the color of modified taro flour resulting from several types of bacteria and the fermentation time. The color of the modified flours was determined with a colorimeter (Minolta, CR300, Tokyo, Japan) using the granular solids device and expressed as Hunter L, a, and b color values. Color values were recorded as L, darkness/lightness (0, black; 100, white); a (−a, greenness; +a, redness); and b (−b, blueness; +b, yellowness) [10].

2.2.4 Statistical Analysis

Data from analysis of the physical and chemical properties of modified flour were tabulated in Microsoft Excel and then carried out through ANOVA [12, 13].

3 Results and discussion

3.1 Resistant starch total

The combination of bacterial starter type and fermentation time on the resistant starch content of Modified Tannia Flour is presented in Figure 1.

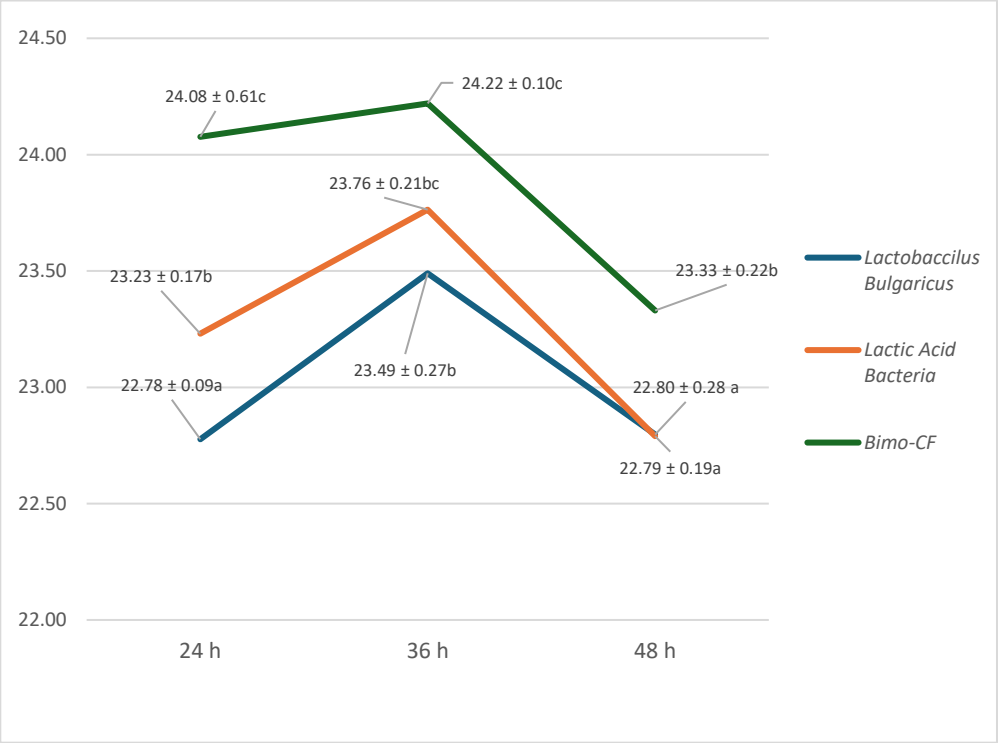

Fig. 1. Resistant starch levels of Modified Tannia Flour

Figure 1 shows the highest increase in resistant starch levels in Bimo-CF fermentation with a fermentation time of 36 h (24.22 %), then Bimo-CF fermentation 24 h (24.08 %) and lactic acid bacteria fermentation 36 h (23.76 %). ANOVA calculations showed significant difference ($P < 0.05$). The type of bacteria and fermentation time do not affect the level of resistant starch in Modified Tannia Flour. The resistant starch content of Modified Tannia Flour is higher when compared to modified cassava (*Manihot esculenta* Crantz) flour [14], sago starch and red bean (*Phaseolus vulgaris* L.) flour [15] but lower when compared to modified sorghum (*Sorghum bicolor* L.) resistant starch [16]. In the fermentation treatment, there was an increase in resistant starch levels, but the value was not significantly different when compared to the control treatment. This is due to the hydrolysis of natural resistant starch in cassava, namely resistant starch type 1 (RS1) and resistant starch type 2 (RS2) by the enzyme's amylase and pullulanase produced by lactic acid bacteria [17].

The amount of lactic acid produced by the three bacteria is relatively low so it does not have an influence on the formation of RS because the linearization of amylopectin by lactic acid is not optimal.The increase is influenced by the fermentation time with an optimum time of 36 h, while 48 h causes a significant decrease because the amylose fraction comes out of the starch granules and is dispersed in water.

3.2 Sweeling power and water-soluble index

The combination of bacterial starter type and fermentation time on the sweeling power content of Modified Tannia Flour is presented in Figure 2.

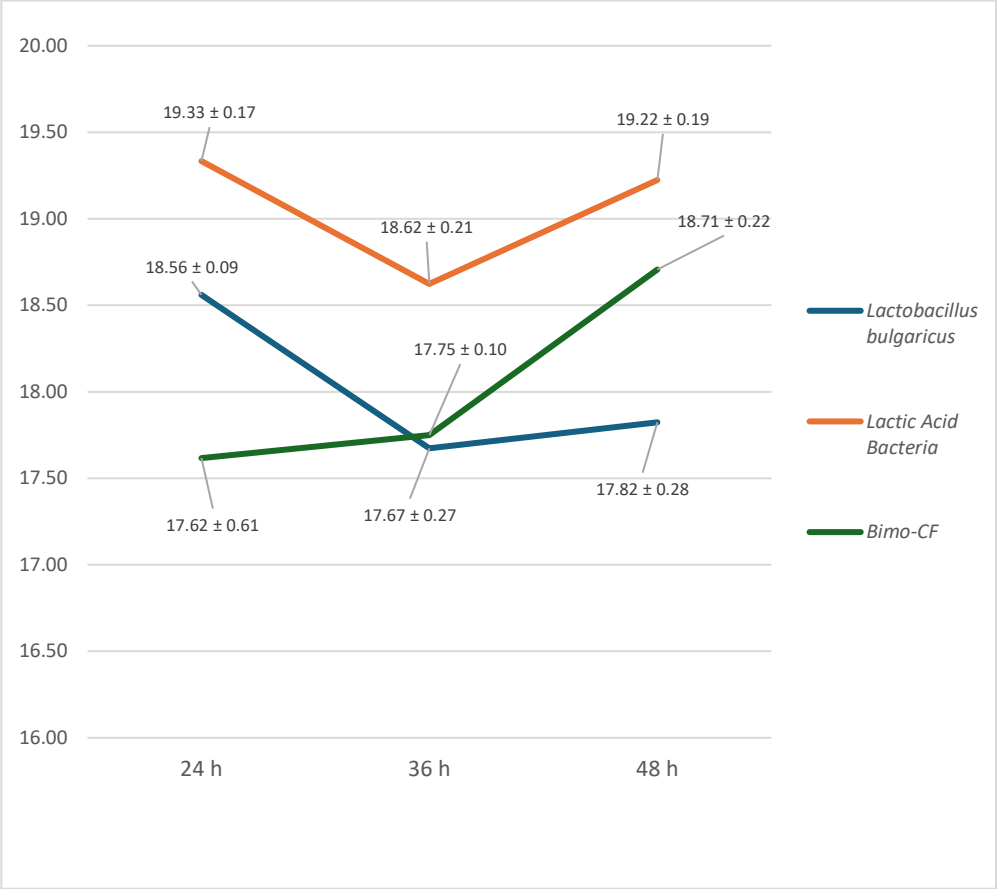

Fig. 2. Sweeling power of Modified Tannia Flour

Swelling power and solubility index of the flour and starch were determined as previously reported [18]. The sweeling power of Modified Tannia Flour values ranged from 17.62 % to 19.33 % (Figure 2). The higest value (19.33 %) of Modified Tannia Flour was found in lactic acid bacteria with 24 h fermentation, while the lowest value (17.62 %) of Modified Tannia Flour was found Bimo-CF starter with 24 h fermentation. ANOVA calculations showed no significant difference ($P < 0.05$). The type of bacteria and fermentation time do not affect the level of sweeling power in Modified Tannia Flour.

The swelling strength of modified taro starch is higher due to the low level of intermolecular association and lower amylose content compared to cassava flour 13.80 % [18]. Factors that influence starch solubility are inter-associative forces in amorphous and crystalline starch domains, and the presence of other components (phosphorus, etc.) [19].

The sweeling power of Modified Tannia Flour is higher when compared to modified bangka sago (*Metroxylon sago* Rottb) starch [20], cassava and sweet potatoes flour [21] but lower when compared to modified sweeling power [9]. Modified Tannia Flour have high sweeling power due to their amylopectin in compairing with cassava flour, sweet potatoes and bangka sago flour. The combination of bacterial starter type and fermentation time on the Water-Soluble Index (WSI) of Modified Tannia Flour is presented in Figure 3.

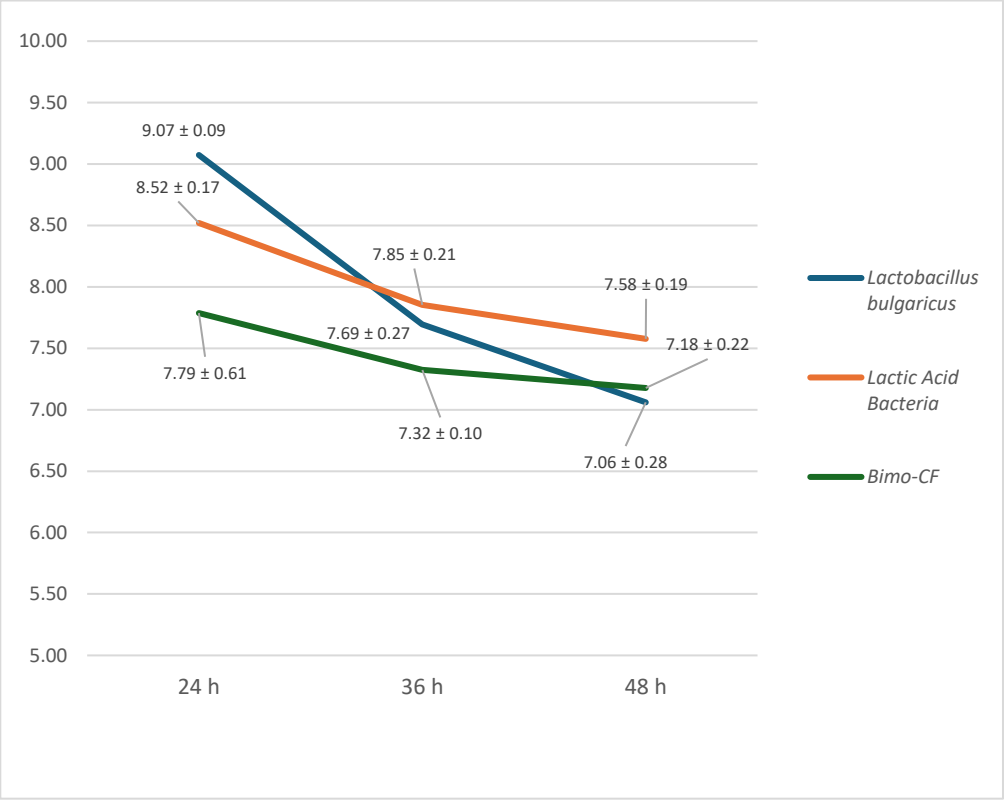

Fig. 3. Water-soluble Index (WSI) of Modified Tannia Flour

The Water-soluble index of Modified Tannia Flour values ranged from 7.06 % to 9.07 % (Figure 3). The higest value (9.07 %) of Modified Tannia Flour was found in *Lactobacillus bulgaricus* bacteria with 24 h fermentation, while the lowest value (17.62 %) of Modified Tannia Flour was found *Lactobacillus bulgaricus* starter with 36 h fermentation. ANOVA calculations showed no significant difference ($P < 0.05$). The type of bacteria and fermentation time do not affect the level of water-soluble index in Modified Tannia Flour.

The water-soluble index of Modified Tannia Flour is higher when compared to modified cassava and sweet potatoes flour [21] but lower when compared to modified Sweeling power and bangka sago satrch [20]. The fermentation process of starch with limited moisture content caused the gelatinization of starch. The starch garanules sweeled up and broken. Some changes in starch like starch granules became hydrated and swell [19]. The WSI was determined with the amount of dried solids recovered by evaporating the supernatant from the water absorption test and the result was expressed as a percentage of dry solids in the 2.5 g of sample [15].

3.3 Color test (L*, a*, b*)

The combination of bacterial starter type and fermentation time on the color test (L*, a*, b*) of Modified Tannia Flour is presented in Table 2.

The results of the color analysis showed the highest average value (88.31) of Modified Tannia Flour was found in Bimo-CF bacteria with a fermentation time of 36 h. Meanwhile, the lowest average value (85.14) was found in lactic acid bacteria with a fermentation time of 48 h. The results of the a* color analysis showed the highest average value (2.31) was

found in *Lactobacillus bulgaricus* bacteria with a fermentation time of 24 h. Meanwhile, the lowest average value (0.35) was found in lactic acid bacteria with a fermentation time of 36 h. The results of the b* color analysis showed the highest average value (8.27) of Modified Tannia Flour was found in Bimo-CF bacteria with 48 h fermentation. Meanwhile, the lowest average value (6.51) was found in lactid acid bacteria with fermentation time of 48 h. Based on the results of the color test variance analysis for both the L* a* and b* components, the results showed "not significantly different" for both the starter type treatment and the length of fermentation nested in the starter type, so it can be concluded that the starter type treatment and fermentation time have no effect to the color test [23].

Table 2. Color test (L*, a*, b*) of Modified Tannia Flour .

Treatment		Color		
		L*(brightness)	a* (red)	b* (yellow)
Lactobacillus bulgaricus	24 h	85.73	2.31	8.18
	36 h	86.72	1.26	7.92
	48 h	85.91	0.48	8.08
Lactic acid bacteria	24 h	84.86	0.43	6.52
	36 h	85.65	0.35	7.74
	48 h	85.14	0.79	6.51
Bimo-CF	24 h	87.93	0.79	7.35
	36 h	88.31	1.33	7.98
	48 h	87.53	0.69	8.27

3.4 The best treatment is based on the parameters of resistant starch, sweeling power, WSI and color index

The combination of bacterial starter type and fermentation time on best treatment is presented in Table 3.

Table 3. Combination of bacterial starter type and fermentation time on best treatment.

Treatment		Value						NH total
		Resistant starch	Sweeling power	Water-soluble index	L* color	a* color	b* color	
Lactobacillus bulgaricus	24 h	0.17	0.11	0.16	0.15	0.14	0.00	0.77
	36 h(i)	0.08	0.16	0.07	0.16	0.13	0.12	0.81
	48 h	0.00	0.00	0.00	0.00	0.00	0.09	0.09
Lactic acid bacteria	24 h	0.17	0.00	0.03	0.00	0.00	0.12	0.43
	36 h(ii)	0.12	0.16	0.00	0.16	0.05	0.00	0.51
	48 h	0.00	0.06	0.16	0.00	0.14	0.08	0.43
Bimo-CF	24 h	0.17	0.00	0.02	0.00	0.00	0.12	0.42
	36 h(iii)	0.07	0.16	0.16	0.11	0.03	0.00	0.51
	48 h	0.00	0.00	0.00	0.16	0.14	0.00	0.39

From the results of the analysis of variance for each parameter which resistant starch, sweeling power, WSI and color index, the best treatment analysis was carried out using a nested design where the highest NH (yield value). Determining the best treatment uses the effectiveness index and Effectiveness Assessment which refers to Nurhamidah *et al.* [24] with several steps, namely, determining parameter weights (BP) and normal weights (BN), determining the average of the worst and best values, determining the effectiveness value (NE) and determining the result value (NH). Table 3 shows the results of calculating the Yield Value (NH) from the parameters resistant starch, sweeling power, WSI and color index.

From Table 3 it can be seen that the highest yield value indicates the best treatment, namely: (i) The starter type *Lactobacillus bulgaricus* had the best treatment with a fermentation time of 36 h with the highest total value of 0.81. (ii) The starter type of Lactic Acid Bacteria (LAB) was the best treatment with a fermentation time of 36 h with a total value of 0.51 (iii) The Bimo-CF starter type was the best treatment with a fermentation time of 36 h with a total value of 0.51.

4 Conclusion

The resistant starch total value of Modified Tannia Flour was 22.78 % to 24.22 %. Type of microbial starter and fermentation time had a significant effect on resistant stach. Whereas a type of microbial and fermentation time had not significant on sweeling power, water-soluble index and color test. The best treatment of bacteria *Lactobacillus bulgaricus* with fermentation time 36 h.

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