

3. RESULTS

3.1. The pH of Black Soybean Natto

Fermentation was carried out with different times and a different ratio of co-cultures. Before fermentation processes pH of black soybean was 6.48 ± 0.07 . pH value of Black Soybean Natto showed in Table 2 and Figure 2.

Table 3. pH of Black Soybean Natto

Ratio Co-cultures	Fermentation Time		
	24 h	36 h	48 h
Y0	7.45 ± 0.04^{a1}	7.62 ± 0.01^{b1}	7.81 ± 0.03^{c1}
Y1	$7.38 \pm 0,02^{a2}$	7.68 ± 0.02^{b2}	7.88 ± 0.02^{c2}
Y2	7.39 ± 0.02^{a3}	7.68 ± 0.02^{b3}	7.96 ± 0.02^{c3}
Y3	7.45 ± 0.02^{a4}	7.70 ± 0.02^{b4}	8.04 ± 0.03^{c4}

Information:

1. *Y0: indicate Black Soybean natto with 100% starter, Y1: 75% starter + 25% Yeast, Y2: 50% starter + 50% Yeast, Y3: 25% starter + 75% Yeast.
2. Data show the mean \pm standard deviation (n=6)
3. ^{a-c} means in the same row (fermentation time) followed by different letters: there was a significantly different at 95% ($p < 0.05$) confidence level based on Two-way ANOVA
4. ¹⁻⁴ means in the same column (ratio of co-cultures) followed by different numbers: there was a significantly different at 95% ($p < 0.05$) confidence level based on Two-way ANOVA

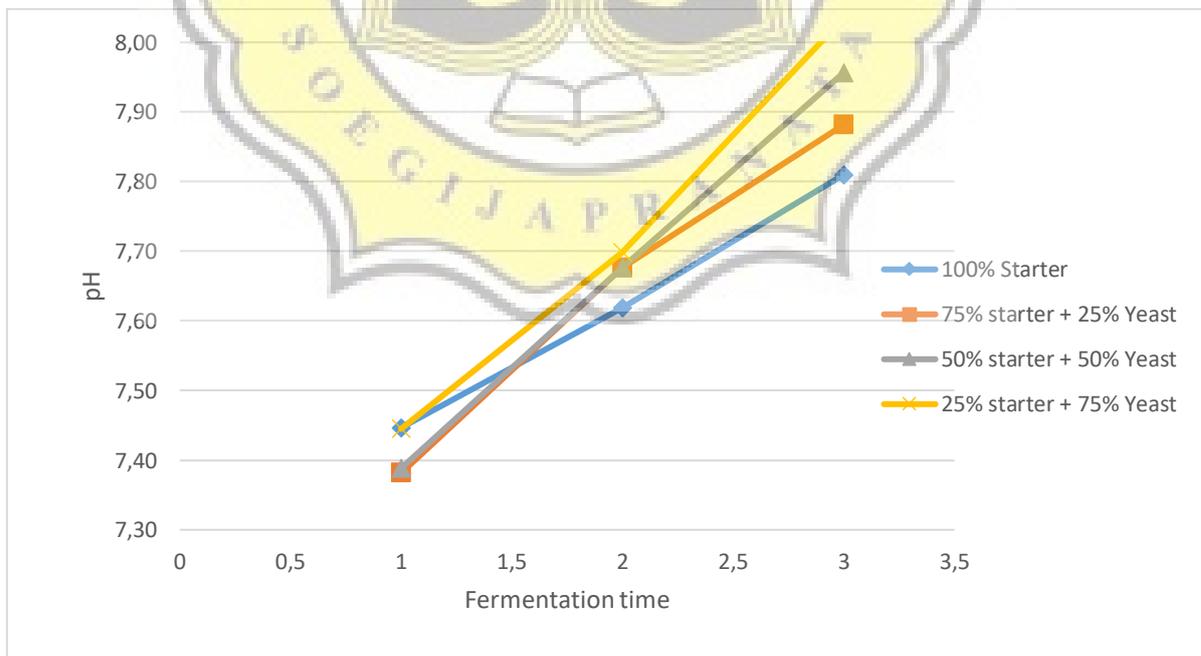


Figure 3. pH of Black Soybean Natto

Information:

*T1 indicates Black Soybean natto with 24 hours fermentation time, T2: 36 hours, T3: 48 hours.

The pH value of Black Soybean natto was shown in Table 2 and Figure 2. It can be seen that the fermentation processes at different times and a different ratio of co-culture shown a significantly different in pH value of black soybean natto. The pH of steamed black soybean without fermentation was 6.48 ± 0.07 . The highest pH value was 8.04 ± 0.03 with fermentation time at 48 hours and the ratio co-cultures 25% starter + 75% Yeast. While the lowest pH value was 7.38 ± 0.02 with fermentation time at 24 hours and the ratio co-cultures 75% starter + 25% Yeast. Overall, the pH value was increased along with the longer of fermentation time and the pH value with fermentation time at 48 hours higher than pH value with fermentation time at 24 hours and 36 hours at all different ratio.

3.2. The Lightness of Steamed Beans and Black Soybean Natto

Fermentation was carried out with different times and a different ratio of co-cultures. Before fermentation the whole bean was soaked for 16 hours with the color of L value was 45.31 ± 0.97 and after steaming L value was 50.66 ± 1.06 . The color (L value) of steamed bean and black soybean natto showed in table 3.

Table 4. Color (L value) of Black Soybean Natto

Ratio Co-cultures	Fermentation Time		
	24 h	36 h	48 h
Y0	45.61 ± 0.98^{c3}	43.23 ± 0.88^{b3}	38.81 ± 1.67^{a3}
Y1	42.97 ± 1.95^{c2}	38.54 ± 1.10^{b2}	37.06 ± 1.19^{a2}
Y2	42.86 ± 1.12^{c2}	37.53 ± 1.70^{b2}	37.00 ± 1.66^{a2}
Y3	40.57 ± 0.67^{c1}	36.22 ± 1.29^{b1}	35.07 ± 1.85^{a1}

Information:

1. *Y0: indicate Black Soybean natto with 100% starter, Y1: 75% starter + 25% Yeast, Y2: 50% starter + 50% Yeast, Y3: 25% starter + 75% Yeast.
2. Data show the mean \pm standard deviation (n=6)
3. ^{a-c} means in the same row (fermentation time) followed by different letters: there was a significantly different at 95% ($p < 0.05$) confidence level based on Two-way ANOVA
4. ¹⁻³ means in the same column (ratio of co-cultures) followed by different numbers: there was a significantly different at 95% ($p < 0.05$) confidence level based on Two-way ANOVA

The color (L value) of black soybean natto was shown in table 3. L value means brightness. It can be seen that after steaming the L value lower than after soaking. The L value black soybean natto was significantly different between fermentation time 24 hours until 48 hours. However, L value of black soybean natto with ratio co-cultures

75% starter + 25% Yeast and 50% starter + 50% Yeast not significantly different. The highest L value was 45.61 ± 0.98 with fermentation time at 24 hours and the ratio co-cultures 100% starter. The lowest L value was 35.07 ± 1.85 with fermentation time at 48 hours and the ratio co-cultures 25% starter + 75% Yeast. Overall, L value was decreased along with the longer of fermentation time and the L value with fermentation time at 24 hours higher than L value with fermentation time at 36 hours and 48 hours at all different ratio.

3.3. Antioxidant Activity of Black Soybean Natto using DPPH Radical Scavenging Activity

Antioxidant activity of black soybean natto was measured by DPPH radical scavenging activity method. Before fermentation processes, antioxidant activities of black soybean was 34.54 ± 0.76 . The antioxidant activity of black soybean natto shown in Table 4 and Figure 3.

Table 5. Antioxidant Activity of Black Soybean Natto (%)

Ratio Co-cultures	Fermentation Time		
	24 h	36 h	48 h
Y0	24.24 ± 0.92^{a1}	28.83 ± 0.94^{b1}	32.00 ± 1.54^{c1}
Y1	27.69 ± 3.39^{a2}	32.13 ± 2.41^{b2}	34.21 ± 1.46^{c2}
Y2	26.36 ± 2.13^{a1}	31.23 ± 2.24^{b1}	31.45 ± 2.36^{c1}
Y3	29.24 ± 3.56^{a12}	30.04 ± 2.87^{b12}	30.47 ± 2.40^{c12}

Information:

1. *Y0: indicate Black Soybean natto with 100% starter, Y1: 75% starter + 25% Yeast, Y2: 50% starter + 50% Yeast, Y3: 25% starter + 75% Yeast.
2. Data show the mean \pm standard deviation (n=6)
3. ^{a-c} means in the same row (fermentation time) followed by different letters: there was a significantly different at 95% ($p < 0.05$) confidence level based on Two-way ANOVA
4. ¹⁻² means in the same column (ratio of co-cultures) followed by different numbers: there was a significantly different at 95% ($p < 0.05$) confidence level based on Two-way ANOVA

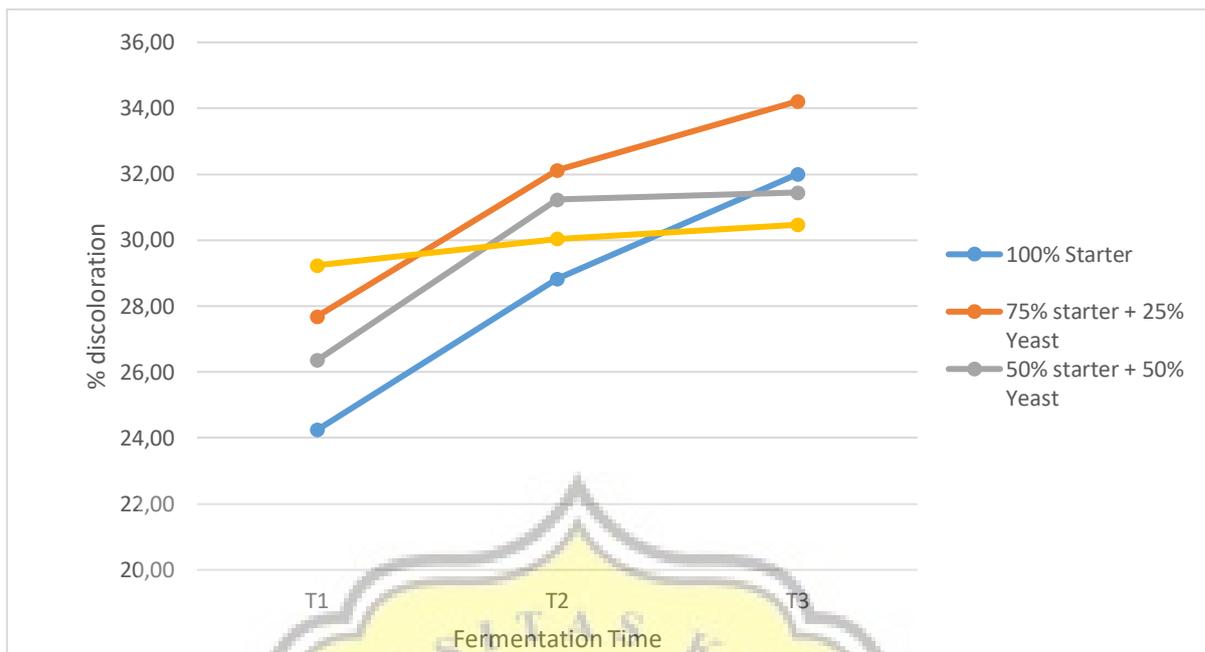


Figure 4. Antioxidant Activity of Black Soybean Natto

Information:

*T1 indicates Black Soybean natto with 24 hours fermentation time, T2: 36 hours, T3: 48 hours.

The antioxidant activity of Black Soybean natto was shown in Table 4 and Figure 3. It can be seen that the antioxidant activity black soybean natto was significantly different between fermentation time 24 hours until 48 hours. However, the antioxidant activity of black soybean natto with ratio co-cultures 100% starter, 25% starter + 75% Yeast, and 50% starter + 50% Yeast not showed a significantly different. Antioxidant activity of black soybean natto with ratio co-cultures between 75% starter + 25% Yeast and 25% starter + 75% Yeast also not showed significantly difference but, black soybean natto with ratio co-cultures 75% starter + 25% Yeast showed significantly difference with black soybean natto with ratio co-cultures 100% starter and 50% starter + 50% Yeast. The highest antioxidant activity was 34.21 ± 1.46 with fermentation time at 48 hours and ratio co-cultures 75% starter + 25% Yeast. While the lowest antioxidant activity was 24.24 ± 0.92 with fermentation time at 24 hours and the ratio co-cultures 100% starter. Overall, the antioxidant activity was increased along with the longer of fermentation time and the antioxidant activity with fermentation time at 48 hours higher than antioxidant activity with fermentation time at 24 hours and 36 hours at all different ratio.

3.4. Total Phenolic Content of Black Soybean Natto

Fermentation was carried out with different times and a different ratio of co-cultures. Before fermentation processes Total Phenolic Content of Black Soybean was 85.24 ± 1.92 . Total phenolic content of Black Soybean Natto presented the result as $\mu\text{g GAE/g}$ shown in Table 5 and Figure 4.

Table 5. Total Phenolic Content of Black Soybean Natto (%)

Ratio Co-cultures	Fermentation Time		
	24 h	36 h	48 h
Y0	59.48 ± 0.99^{a3}	70.50 ± 0.60^{b3}	79.86 ± 0.58^{c3}
Y1	54.94 ± 1.11^{a1}	61.08 ± 0.92^{b1}	68.94 ± 2.04^{c1}
Y2	61.70 ± 1.91^{a2}	66.99 ± 0.99^{b2}	75.96 ± 1.30^{c2}
Y3	51.76 ± 3.34^{a1}	60.12 ± 3.27^{b1}	74.19 ± 2.55^{c1}

Information:

- *Y0: indicate Black Soybean natto with 100% starter, Y1: 75% starter + 25% Yeast, Y2: 50% starter + 50% Yeast, Y3: 25% starter + 75% Yeast.
- Data show the mean \pm standard deviation (n=6)
- ^{a-c} means in the same row (fermentation time) followed by different letters: there was a significantly different at 95% ($p < 0.05$) confidence level based on Two-way ANOVA
- ¹⁻³ means in the same column (ratio of co-cultures) followed by different numbers: there was a significantly different at 95% ($p < 0.05$) confidence level based on Two-way ANOVA

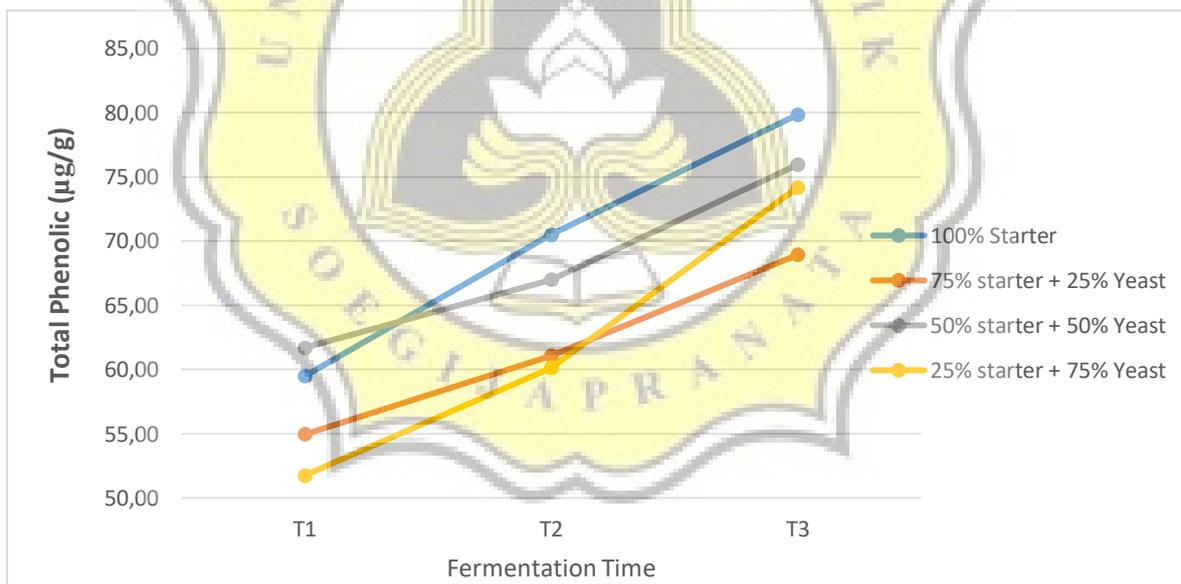


Figure 5. Total Phenolic Content of Black Soybean Natto

Information:

*T1 indicates Black Soybean natto with 24 hours fermentation time, T2: 36 hours, T3: 48 hours.

The antioxidant activity of Black Soybean natto was shown in Table 5 and Figure 4. It can be seen that the total phenolic content black soybean natto was significantly different between fermentation time 24 hours until 48 hours. However, the total

phenolic content of black soybean natto with ratio co-cultures 75% starter + 25% and 25% starter + 75% Yeast not showed a significantly different. The highest total phenolic content was 79.86 ± 0.58 with fermentation time at 48 hours and ratio co-cultures 100% starter. While the lowest antioxidant activity was 51.76 ± 3.34 with fermentation time at 24 hours and ratio co-cultures 25% starter + 75% Yeast. Overall, the total phenolic content was increased along with the longer of fermentation time and the total phenolic content with fermentation time at 48 hours higher than antioxidant activity with fermentation time at 24 hours and 36 hours at all different ratio.

3.5. Analysis of Sensory

Analysis sensory done by comparing Black Soybean natto with the commercial natto “Goku Kotsubu Okame” and evaluate the drawing situation and smell of natto based on the table of sensory evaluation standard (Table 1.) and also evaluate the alcohol smell. Analysis of sensory black soybean natto shown in table 6

Table 6. Sensory Analysis of Black Soybean Natto

Item	Control	Y0	Y1	Y2	Y3
Drawing	28.07±2.28	13.87±4.41	10.00±4.00	14.53±6.32	10.40±5.68
Smell	18.47±2.13	9.33±5.12	10.33±5.34	9.33±4.27	8.93±4.82
Total	46.53	23.20	20.33	23.87	19.3
Alcohol smell	No	No	Yes	No	Yes

*Y0: indicate Black Soybean natto with 100% starter, Y1: 75% starter + 25% Yeast, Y2: 50% starter + 50% Yeast, Y3: 25% starter + 75% Yeast.

Analysis sensory of black soybean natto was shown in table 6. Sensory carried out by fifteen people untrained panelist. Black soybean natto with fermentation time 24 hours compare with commercial natto. The highest score in the sensory analysis is commercial natto with a score of 46.53. The lowest score in the sensory analysis was 19.3 with ratio co-cultures 25% starter + 75% Yeast.