

Research Article

Study on the Improvement of Natto-production Process

¹Ying-Ying Ma, ¹Qing-Bin Liu, ²Hua-Wei Yang, ¹Yong-Yao Tang and ¹Jian-Gang Yang

¹College of Bioengineering, Sichuan University of Science and Engineering,

²Agricultural Sciences Institute of Zigong, Zigong 643000, China

Abstract: To improve the Natto flavor, in this experiment, different ratios between black soybean and soybean as raw materials were used to explore the suitable fermentation conditions for Natto producing. The results of orthogonal test show that the best condition is: the ratio of black soybean to soybean is 1:4, boiling for 30 min, *Bacillus natto* inoculation amount 10%, fermentation temperature 38°C and fermentation time 13 h.

Keywords: Fermentation, improvement, Natto, nattokinase, producing craft

INTRODUCTION

Natto is a kind of functional food with rich nutrition, which is made from the fermentation of boiled soybeans by inoculated *Natto bacillus*. Natto is rich in nutrients such as various organic acids and oligosaccharides, which can be easily absorbed and utilized by the human and it contains a large number of physiologically active substances, such as Nattokinase, vitamin K, vitamin E, superoxide dismutase, soy isoflavones, saponin, tocopherol and B vitamins (wherein its vitamin B2 content is more than 6 times higher than that of the steamed or boiled soybeans) (Ma, 2007); therefore, after being processed into Natto, the nutrient value of soybeans is multiplied. In the course of Natto's fermentation, the Nattokinase is generated, which is a kind of protein kinase of *hay bacillus*. Sumi Hiroyuki, a Japanese cardiovascular specialist-doctor, found that Nattokinase has the strong function of dissolving thrombus in the study and experiment of dissolving thrombus medicines, which can reduce blood viscosity, strengthen blood circulation and increase the blood vessel elasticity (Wei *et al.*, 2007). So for a long time, Natto has been popular among Japanese consumers. It has been reported as edible and medicinal food and even the unique Nattokinase was made into capsules for thrombosis patients.

China has rich soybean resources, non-genetically modified small soybean used for making Natto has higher yield in Northeast China, so we should actively explore the development of soybean products and promote Natto, a kind of richly nutritious health care product. It is not accepted by part of Chinese because of the ammonia smell of Natto, so it is necessary to improve its taste in the case of keeping the nutrients

invariable and make it more suitable for Chinese. It was found that Natto produced by fermentation from soybean has more Nattokinases and ammoniacal smell is heavier, but the Natto produced from black bean as the raw material has less Nattokinases and ammoniacal smell is light with good taste. If black bean and yellow bean could be mixed in certain ratio and then ferment to explore an optimal fermentation condition to produce and to improve the taste, a Natto product suitable for Chinese could be developed.

MATERIALS AND METHODS

Experiment materials: Black beans (purchased from the market), soybeans (a new soybean variety X73 provided by the Agricultural Science Institute of Zigong) and purebred *Bacillus natto*.

The manufacturing craft of Natto: Weigh Accurately five fractions fifty gram soybeans, clean two to three times, clean and decorticate after soaking twelve hours, all of these are put respectively into five stainless steel boxes which are sterilized, steam and boil under the high-pressure 121°C, inoculate seed solution when the natural cooling reaches to the indoor temperature, cover the sterile gauze after mixing equably, train respectively in the constant temperature incubator, take out and put them into the 4° freezer to be ripe 24 h.

Culture of bacillus Natto seed solution: Make BPY culture medium, use inoculating loop to carry *Bacillus natto* inoculation to nutrient solution under aseptic conditions and set rotating speed 150 r/min, temperature 37°C and culture for 16 h through shaking method.

Corresponding Author: Jian-Gang Yang, College of Bioengineering, Sichuan University of Science and Engineering, Zigong 643000, China

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Table 1: The level of experimental factors and comparison table

Classification	Black bean: soybean	Inoculation amount (%)	Culture temperature (°C)
1	1:3	9	37
2	1:4	10	38
3	1:5	11	39

Table 2: The sensory review standard of Natto (Zhang *et al.*, 2012)

Item	Scoring standard	Score
Drawing situation	With much mucus, many drawings and good viscosity	20~30
	With more mucus and drawings and ordinary viscosity	10~20
	With less mucus and few or no drawings	0~10
Color and taste (30)	With golden color and melted taste	20~30
	Dark color with a crisp and soft taste	10~20
	With brown or dark brown color, dry and hard taste	0~10
Odor (30)	Have specific bean flavor of fermented natto with unobvious ammonia odor	30~40
	Natto's fragrance is relatively light with acceptable ammonia smell	15~30
	Poor aroma and strong ammonia odor	0~15

Single-factor experiment: Respectively select ferment time, culture temperature, Natto strain's inoculation amount, stewing time, black bean: yellow bean and beans' peel removed or not, six single factors' influence on Natto's quality to carry on research, each factor selects five gradient variance.

Orthogonal experiments: Experiment with three factors and three levels were selected according to the experiment results of the univariate analysis (Table 1).

Sensory assessment indicators: Sensory assessment adopts three indicators: drawing situation, color and taste and smell. Seven persons are to carry out the sensory assessment on a scale where the full mark is 100 and the lowest mark is 0. Then the average mark is taken. The ammonia taste generated by Natto during fermentation has an impact on the popularity of this healthy food in our country, so we weight higher on smell (Table 2).

Detection and analysis methods: Measurement of protein content in Natto: the Kjeldahl method (Wang, 2012). Measurement of bacteria content in Natto: agar plate counting method (Huang *et al.*, 2011). Measurement of Nattokinase content in Natto: Flion-phenol method (Zhao, 2003; Lowry *et al.*, 2011).

RESULTS AND DISCUSSION

The effect of fermentation time on the quality of Natto: Figure 1 shows that the quality of Natto changes evidently with the increase of fermentation time. After it reaches the best incubation time, the quality of Natto declines little by little. From the morphological characteristics, we can see that the satins of Natto and its mucus decrease gradually and the colour changes from golden yellow to dark yellow, while the ammonia odor gradually becomes thick and bitter taste appears. Comprehensively assessing the result, the optimal fermentation time of 13 h is chosen.

The effect of fermentation temperature on Natto's quality: Figure 2 shows that the sensory score of Natto rises gradually and then declines with the fermentation

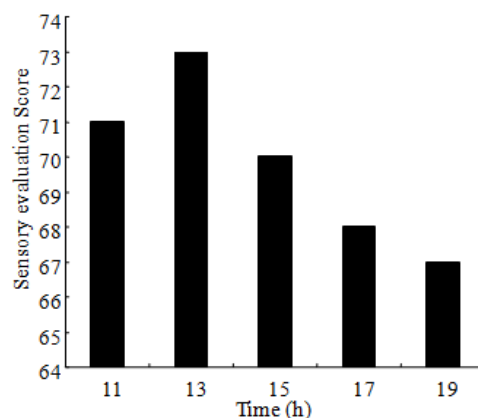


Fig. 1: Effect of fermentation on Natto quality by sensory evaluation

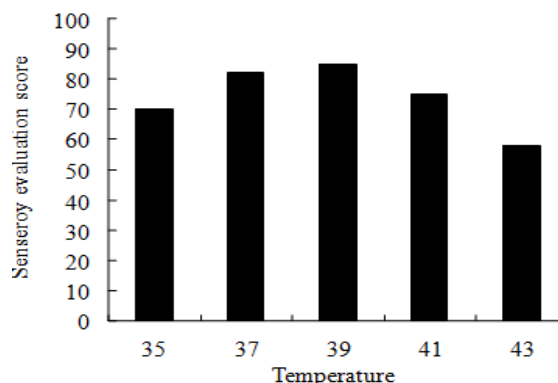


Fig. 2: Effect of fermentation temperature on the quality of Natto

temperature increasing, with the peak value of 82.5 points at 39°C. At this moment, the grain of Natto are in full sizes, with crisp and soft texture and moist, but it has long drawing, large amount of mucus, a strong stickiness and relatively weak ammonia odor. When the temperature goes gradually up to 43°C, the color of Natto is dark with bad texture and strong ammoniacal odor. The consolidated sensory assessment result is that Natto's optimal fermentation temperature ranges from 37 to 39°C.

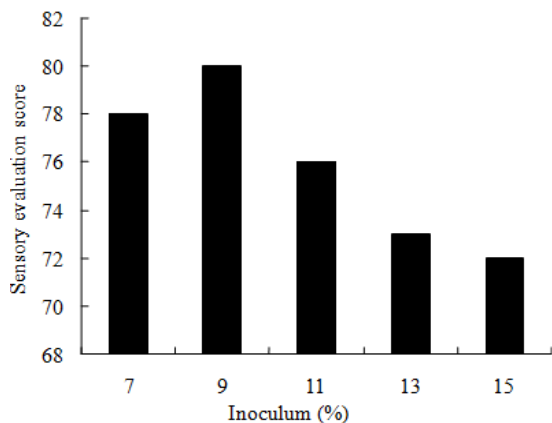


Fig. 3: Effect of inoculum of bacillus on the quality of Natto

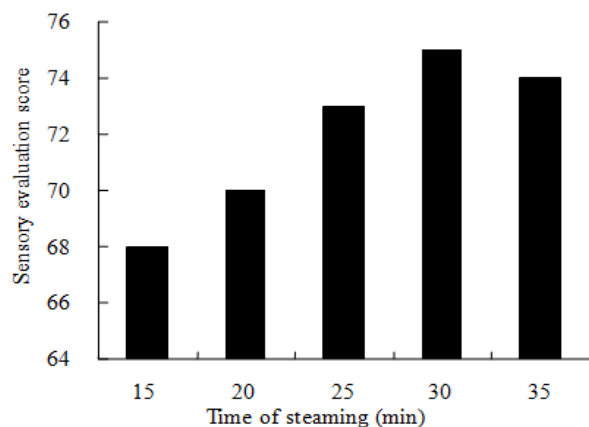


Fig. 4: Effect of steaming time of beans on the quality of Natto

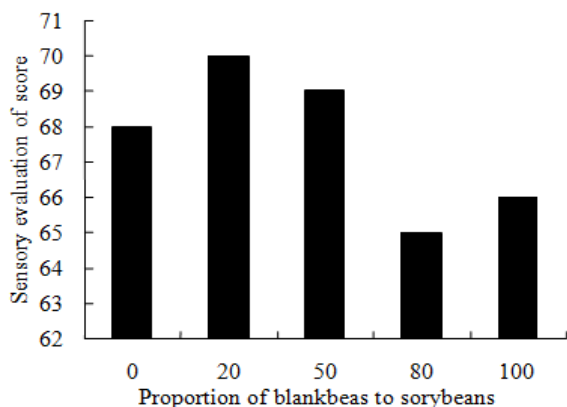


Fig. 5: Effect of proportion of black beans to soybeans on the quality of Natto

The effects of inoculation amount of bacillus Natto on Natto's quality: In Fig. 3, the results showed that the influence of inoculation quantity on the quality of Natto is significant. With too little inoculation amount, the sticky substance on Natto's surface is quite less and drawing is easily strain, the Natto products have some

Table 3: Effects of peeled and not peeled of beans on the Natto quality

Experiment	Peeling factors	Sensory evaluation score (score)
1	Peeled black beans	63.0
2	Black beans without peeling	53.4
3	Peeled soybeans	77.0
4	Soybeans without peeling	73.4

beany flavor. When increasing the inoculation amount, the surface of Natto is dry, drawing decrease, its color goes dark yellow with strong ammonia odor and bitter taste grows. According to the assessment result of the sensory assessment indicator, the optimal inoculation amount should be 9%.

Effect of cooking time on quality of Natto: As seen in Fig. 4, the cooking time has certain influence on the quality of the Natto. As the cooking time increases, the Natto tastes tenderer and the color is golden yellow. When the cooking time reaches 30 min, the taste is the best. Continue increasing the cooking time, the texture of Natto goes worse and ammonia odor grows, which affects Natto's sensory assessment. The findings of overall sensory assessment are that, the best cooking time is 30 min.

The effects of peeled and not peeled of raw beans on the Natto quality: It can be seen from Table 3, black soybean without peeling can produce Natto products with no ammonia odor and nice tender, but nearly no draw bench grow, worse stickiness and low scores in sensory assessment. Natto products fermented from peeled black soybean contain a small amount of drawing with crisp and soft texture and improved quality. Soybean without peeling produce Natto that has soft texture, moisture content, much mucus, strong in viscous, but the Natto product has ammonia smell, which affects the quality; Natto fermented from peeled soybeans tastes crisp and soft, with much mucus, strong viscosity and large amounts of drawings and slight odor of ammonia. It is shown in the overall sensory assessment findings that choosing peeling process craft after soaking black soybean and soybean can help Natto ferment and improve Natto's quality.

The effect of the ratio of black soybean to soybean on the quality of Natto: As it can be seen from Fig. 5, when the black soya beans account for 20% (namely, the ratio of black soya beans to soybeans is 1:4), the sensory evaluation is the highest. At this moment, the Natto tastes soft, is moist, much mucus, strong viscosity and high content of drawing, namely, Nattokinase, so the quality of Natto is the best. With the increasing scale of black soybean, the drawbench of Natto gradually decrease and less mucus, but well texture. The findings of consolidated sensory assessment are that the best scale of black soybean and soybean is 1:4.

Orthogonal test: According to observations of the single factor experiment, it can be seen that more

Table 4: Factors and levels

Grouping	Level	Fermentation temperature A (°C)	Inoculum concentration B (%)	The proportion of black beans and soybeans C
The first group	Experiment 1	37	9	1:3
	Experiment 2	37	10	1:5
	Experiment 3	37	11	1:4
The second group	Experiment 4	38	10	1:4
	Experiment 5	38	11	1:3
	Experiment 6	38	9	1:5
The third group	Experiment 7	39	11	1:5
	Experiment 8	39	9	1:4
	Experiment 9	39	10	1:3

Table 5: Results of L₉ (3³) orthogonal experiments and range analysis

Row	1	2	3	4
Level	A	B	C	Sensory evaluation score (score)
1	1	1	1	63.92
2	1	2	3	65.20
3	1	3	2	65.54
4	2	2	2	65.69
5	2	3	1	64.95
6	2	1	3	65.19
7	3	3	3	63.06
8	3	1	2	64.42
9	3	2	1	63.97
The mean K1	64.887	64.510	64.280	-
Mean value K2	65.277	64.953	65.217	-
Mean value K3	63.817	64.517	64.483	-
Range (R)	1.460	0.443	0.937	-
Primary and secondary factors	A	C	B	-
The optimal level	A2	B2	C2	-

drawings were produced when the seed peels of soybeans were removed. In particular, without removing seed peels, the Natto made from black soya beans produced nearly no drawing. And the quality of the Natto produced under the condition of 30-min cooking and 13-h cultivation was significantly better than that of other groups. Therefore, in determining the optimal production process of compound Natto, an orthogonal experiment with three levels or three factors, which are fermentation temperature, inoculation amount and the proportion of black beans and soybeans, was conducted and its combinations is shown in Table 4.

Nine experiments were divided into 3 groups as showed in Table 3 and 2. Considering limited lab equipment, the 3 groups would be done at 3 times with the same interval. Take the first group as an example: 3 sets of 50 g black soybean and soybean with the ratio of 1:3, 1:4 and 1:5 were weighed out accurately. All beans in each set were cleaned, soaked for 12 h and peeled, then placed in three stainless plates respectively, steamed for 30 min under high pressure and 121°C, cooled down to the room temperature naturally, inoculated with the seed solutions of 4.5 mL (9%), 5.0 mL (10%) and 5.5 mL (11%), respectively in sterile environment. After mixing, the plates were covered with disinfected gauzes and then placed in a thermostatic incubator of 37°C for incubation. After 13 h, the plates were taken out and put into a refrigerator of 4°C for 24 h for after-ripening. The results are shown in Table 5.

It can be concluded from Table 5 that the optimal craft for the composite beans to ferment Natto is A2B2C2, which is fermentation temperature of 38 degree Celsius, inoculation amount of 10%, the ratio of black soya bean to soybean of 1:4. This configuration was evaluated by 7 persons and the selected Natto products had features of weak ammoniacal smell, long drawings and rich mucus. They were of the best quality and scored the highest mark of 65.69 points as shown in the orthogonal experiment table, higher than any other combination. From Table 5, it can also be concluded that the primary factor that affects the fermentation of composite Natto is the fermentation temperature, the secondary factor is the ratio of black bean to yellow bean and then follows the inoculum concentration. Analyze the relationship between the factors of experiments and the levels from this, the range of the overall mark of composite Natto fermentation indicates that the optimal condition of Natto production obtained from this orthogonal experiment is the fermentation temperature of 38°C, the inoculum concentration of 10% and the ratio of black bean to yellow bean of 1:4.

Protein content, bacillus Natto and nattokinase activity in produced Natto: From Table 6, it can be seen that different fermentation conditions have significant effects on both the content of bacillus Natto and the activity of Nattokinase. The content of bacillus Natto in Natto products is positively correlated with the activity of Nattokinase. When the Natto bacteria

Table 6: The protein content, bacillus Natto content and nattokinase activity in produced Natto

Experiment	Protein content of Natto (g/100 g)	Bacteria content of Natto (cfu/g)	Nattokinase activity (U/g)
1	17.02	3.9×10^9	1957.32
2	16.82	3.6×10^9	1937.09
3	16.98	4.1×10^9	2099.80
4	16.89	3.7×10^9	1944.33
5	16.92	3.9×10^9	1957.99
6	17.07	4.0×10^9	2008.97
7	16.82	3.8×10^9	2052.11
8	16.80	3.7×10^9	1932.78
9	16.88	3.7×10^9	1947.51

content is higher in Natto products, Nattokinase activity is also higher, such as the third group; when the Natto bacteria content is lower in Natto products, Nattokinase activity is also lower, such as the eighth group.

CONCLUSION

On the basis of the single factor assay, orthogonal test and sensory evaluation, the following conclusions were drawn.

The best production process of excellent Natto is: the ratio of peeled black soya bean and peeled soybean is 1:4; the steaming and boiling time is 30 min; the inoculation amount is 10%; the fermentation temperature is 38°C; and the fermentation time is 13 h.

It can be seen that the protein content of fermented Natto is increased by 4%, compared with that of steamed and boiled beans.

Different processing condition of fermentation has significant effect on the content of Natto bacteria. The order of magnitude of content of Natto bacteria in each gram of Natto products measured in the experiment reaches 109 cfu/g, suggesting the Natto bacteria strain has higher activity.

The bacillus Natto content in Natto products through different fermentation technologies positively correlates with Nattokinase activity. When bacillus Natto content is relatively higher, the Nattokinase activity is more active; When the Bacillus subtilis content is relatively low, Nattokinase activity is also relatively low.

Natto prepared in the research is golden brown, whole-grained with profuse mucus, plentiful and long drawings, soft taste and acceptable ammonia smell. At the same time, it is found that this kind of Natto contains high content of Bacillus subtilis Natto, with

high Nattokinase activity and high content of protein, which is a health food in line with the modern concept of healthy eating.

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