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Optimization and preservation of Natto manufacturing technique

Ying-ying Ma, Qing-bin Liu, Xiao-qing Zhou and Jian-gang Yang*

College of Bioengineering, Sichuan University of Science and Engineering, Zigong, China

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ABSTRACT

Natto is a kind of food taking soybean as raw material and generated by bacillus natto fermentation. Natto is especially applicable to the old and children due to high nutritive value and healthcare function. With time going on, natto will produce odorous ammonia flavor with its color deepening and bitter taste coming out which has a strong impace on product's taste and appearance because of short guarantee period. The pape mainly start with process optimization and preservation to determine optimal experiment condition for natto manufacturing technique by single factor experiment and orthogonal experiment. On this condition, conduct preservation experiment by adding corrosion remover sodium benzoate and conduct sensory evaluation and measure natto kinase activity and natto total plate count through regarding 10 days as a period and study influence of sodium benzoate on natto guarantee period. Results display that optimal manufacturing technique condition is 39℃ culture temperature, 13h fermentation time and 11% inoculum size and when additive amount of sodium benzoate is 0.25g/kg, natto guarantee period can be lengthened.

Keywords: Natto; Process optimization; Preservation

INTRODUCTION

Natto is a kind of traditional Japanese fermented food with soybean as its raw material, generated by bacillus natto fermentation which has speical flavor and stickness and is similar to traditional food fermented blank bean in China, but natto is generated after pure strain fermentation with higher healthcare function. In term of appearance, natto is the soybean granular food with thin hoar-frost whose color is golden with crisp and limp taste and long viscin thread will generated when being lifted up with chopsticks. Natto has high nutritive value with content of contained amino acid, calcium, iron, Vitamin B2, Vitamin K higher than that of steamed and cooked soybean, especially content of Vitamin B more than 6 times than that of steamed and cooked soybean and natto also has various healthcare functions such as dissolving thrombus, preventing osteoporosis, anticancer and antioxidant. In recent years, some research and development institutions in China have started to dip into natto deep processing with some achievements gained. At present, dozens of domestic enterprises engaging in research and development and production of products related to natto lay the foundation of further development for natto food industry[1]. However, due to short guarantee period of natto, with time going on, quality variation will happen to natto which will generate ammoniacal odour with bitter taste aggravated, thus most poeple can not accept which is the material cause of natto failing to be popularized on a large scale in our country. Therefore, the experiment topic focuses on process optimization and preservation, and determine optimal manufacturing conditions by single factor experiment and orthogonal experiment, and then under this condition, natto is produced, and after cold storage and postripeness, add corrosion remover sodium benzoate to study actual additive effect on lengthening natto guarantee period and effective way of preserving natto in order to promote natto commercialization and lay theoretical foundation of natto entering into Chinese people life and improving health level of Chinese people.

MATERIALS AND METHODS

Experimental instrument
Primary instrument: constant temperature and humidity incubator LHP-250, Changzhou Putian Instrument Manufacture Co., Ltd.; portable stainless steel pressure steam sterilizing pot YX280A, Shanghai Sanshen Medical Equipment Co., Ltd.; sterility inoculation hood WJ-JZX, Jinan Jiekang Purification Equipment Factory, etc.

Experimental materials and reagent
Primary materials and reagent: soybean (gongqiu soybean 6128-5), purebred natto bacteria, sodium benzoate, etc.

Natto manufacturing technique
Weigh and choose quantitative soybeans, wash them for 2-3 times, then wash and drain them off after soaking for 20h, put them into stainless steel plate being sterilized, cook them under high pressure at 121℃ for 30min, and add seed solution after cooling naturally to indoor temperature, cover moist gauze being sterilized after homogeneous mixing, put into constant temperature and humidity incubator for culture and conduct postripeness for 24h at 4℃ after being taken out.

Single factor experiment
Select fermentation time, culture temperature, natto bacteria quantity, bean removing the peel and not removing the peel to study natto quality being influenced by above four single factors with five variables chosen for each factor.

Orthogonal experiment
Select factor have a greater effect on natto fermentation through single factor experiment, and then determine orthogonal experiment factor level table.

Sensory index evaluation standard
Set natto sensory index evaluation standard according to rawing, color, taste and smell, among them, natto quality choiceness and content and activity of natto kinase are directly reflected by drawing; taste is an important basis of judging natto acceptability, so for score setting, above two items account for alarger proportion, special standard for evaluation refers to bibliography [2] and conduct alteration as shown in Table 1.

Table 1 The sensory evaluation standard of Natto

Item	Evaluation Standard	Score
Drawing (30)	Long drawing, lots of mucus and good viscidity	20~30
	Longer drawing, more mucus and general viscidity	10~20
	Short drawing, less mucus	0~10
Color (20)	Brilliant yellow	10~20
	Dim color	5~10
	Brown or dark brown	0~5
Taste (30)	Being limp and moist with spicy soybean flavour and not being bitter	20~30
	Being moist with slight bitter taste	10~20
	Being hard and dry with obvious bitter taste	0~10
Smell (20)	With special and strong natto perfume and no ammoniacal smell	10~20
	Tasteless perfum with slight ammoniacal smell	5~10
	With no ferfume and strong ammoniacal smell	0~5

Preservation experiment
Select adding corrosion remover sodium benzoate and additive amount, because according to GB2760-2011[3], maximum level of sodium benzoate is 0.5 g/kg, set additive amount to be 0, 0.25 g/kg and 0.5 g/kg for experiment.

Detection analysis method
Activity of natto kinase is determined by using Flion-phenol method[4].

Determination of natto aerobic bacterial is counted by using agar plate count [5].

RESULTS AND DISCUSSION

1 Effect of fermentation temperature on natto fermentation
Accurately weigh and chosse five portions of 50g soybeans, soak for 20h after washing, drain off moisture, respectively put them into five stainless steel plates being sterilized, cook for 30min in high pressure sterilizing pot at 121℃, add 11% seed solution after taking out and cooling down to indoor temperature, cover moist gauze being

sterilized after homogeneous mixing, put them into constant temperature and humidity incubator for 13h culture at 35℃, 37℃, 39℃, 41℃ and 43℃, and conduct postripeness for 24h at 4℃. The result is as shown in Fig. 1.

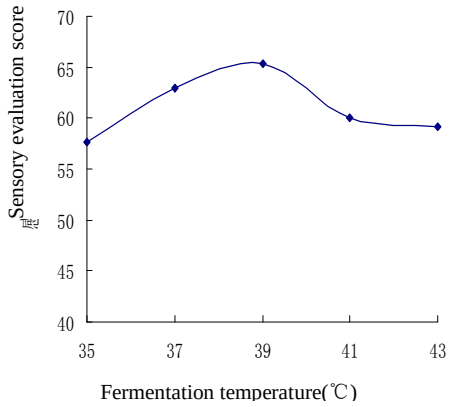

Fig.1 Effect of fermentation temperature on natto quality

It can be found out from Fig. 1 that with fermentation temperature increase, distinct change has happened to natto quality showing trend of firstly increasing and then decreasing. Among them, highest score comes out at 39℃ with optimal natto quality, long drawing, a lot of mucus, being limp and moist, no bitter taste and ammoniacal smell. When temperature rises, natto quality worsens with dull yellow color and bitter taste and strong ammoniacal smell. By synthesizing sensory evaluation results, optimal fermentation temperature interval to be chosen is 37℃-39℃.

2 Effect of fermentation time on natto fermentation

Accurately weigh and chosse five portions of 50g soybeans, soak for 20h after washing, drain off moisture, respectively put them into five stainless steel plates being sterilized, cook for 30min in high pressure sterilizing pot at 121℃, cover moist gauze being sterilized after taking out, cooling down to indoor temperature and homogeneous mixing, conduct culture at 39℃ for 11h, 12h, 13h, 14h, 15h and conduct postripeness for 24h at 4℃. The result is as shown in Fig. 2.

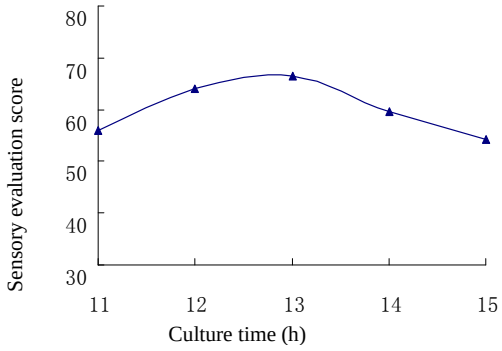

Fig.2 Effect of fermentation time on natto quality

It can be found out from Fig. 2 that with fermentation time increase, distinct change has happened to natto quality. Among them, optimal fermentation time is 13h. At the moment, Natto is with best fermentation, long drawing, no ammoniacal smell and bitter taste, limp and moist taste. With time going on, natto continues to conduct fermentation with color turning from golden yellow to brown little by little and ammoniacal smell aggravated and being bitterer. By synthesizing sensory evaluation results, optimal fermentation time interval to be chosen is 12h~14h.

3 Effect of the inoculating scale on natto fermentation

Accurately weigh and chosse five portions of 50g soybeans, soak for 20h after washing, drain off moisture, respectively put them into five stainless steel plates being sterilized, cook for 30min in high pressure sterilizing pot at 121℃, add 7%, 9%, 11%, 13% and 15% seed solutions after taking out and cooling down to indoor

temperature, cover moist gauze being sterilized after homogeneous mixing, put them into constant temperature and humidity incubator for 13h culture at 39℃, and conduct postripeness for 24h at 4℃. The result is as shown in Fig. 3.

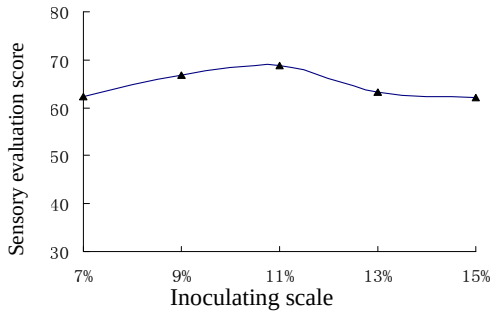

Fig.3 Effect of the inoculating scale on natto quality

It can be found out from Fig. 3 that inoculating scale has a great effect on natto quality. When there is a smaller inoculating scale, natto has insufficient fermentation with less viscous matters and almost no drawing; when there is a larger inoculating scale, in case of other same conditions, natto turns to be dully yellow with stronger ammoniacal smell and bitter taste aggravated. By synthesizing sensory evaluation results, optimal inoculating scale interval to be chosen is 9%~11%.

4 Orthogonal experiment result

In accordance with single factor experiment, major factors influencing natto fermentation are culture temperature of natto bacteria, inoculating scale and fermentation time. And its optimal condition ranges are culture temperature (37~39℃), natto inoculating scale (9%~11%) and fermentation time (12~14h). Do experiment with three factors and three levels[6] on culture temperature, inoculating scale and fermentation time. Specific experimental method is as shown in Table 3.

Table 2 Factors and levels of the orthogonal test

Grouping	Level	Fermentation Temperature A(℃)	Inoculating Scale B (%)	Culture time C(h)
Fisrt group	Experiment 1	37	9	12
	Experiment 2	37	10	13
	Experiment 3	37	11	14
Second group	Experiment 4	38	10	14
	Experiment 5	38	11	12
	Experiment 6	38	9	13
Third group	Experiment 7	39	11	13
	Experiment 8	39	9	14
	Experiment 9	39	10	12

Table 3 Results and analysis of orthogonal test

Experiment	A	B	C	Score
1	1	1	1	63.46
2	1	2	2	64.37
3	1	3	3	65.84
4	2	2	3	65.47
5	2	3	1	64.59
6	2	1	2	65.25
7	3	3	2	68.03
8	3	1	3	65.42
9	3	2	1	66.93
Mean value k_1	64.56	64.71	64.99	—
Mean value k_2	65.10	65.59	66.67	—
Mean value k_3	66.79	66.15	65.57	—
Range(R)	2.23	1.44	1.68	—
Main factors→secondary facot	A	C	B	—
Optimal scheme	A_3	B_3	C_2	—

Conduct specific arrangement on orthogonal experiment as shwon Tab. 2, and do experiment in form of three groups according to different temperatures due to limited constant temperature and humidity incubators in the lab. Take first group as example: accurately weigh and select three portions of 50g soybeans, soak for 20h after washing, drain off

moisture, respectively put them into five stainless steel plates being sterilized, cook for 30min in high pressure sterilizing pot at 121℃, add 9%, 10% and 11% seed solutions after taking out and cooling down to indoor temperature, cover moist gauze being sterilized after homogeneous mixing, put them into constant temperature and humidity incubator for 12h, 13h and 14h culture at 37℃, and then take them out and conduct postripeness for 24h in the refrigerator at 4℃. And then conduct sensory evaluation and intuitive analysis to determine optimal manufacturing condition. The orthogonal experiment result is as shown in Table 3.

It can be found out from Table 3 that optimal manufacturing condition of natto fermentation is $A_3B_3C_2$ with 39℃ fermentation temperature, 11% inoculating scale and 13h culture time. As for experimental results, invite 8 students to conduct sensory evaluation on finished natto products and calculate mean value with above data obtained, among them, $A_3B_3C_2$ has highest score with highest quality natto which has long drawing, no ammonia smell, limp taste and slight bitter taste. Range $R_A>R_C>R_B$ is gained according to intuitive analysis, and the larger rang is, the greater influence on experimental results the level of that factor will have. Thus primary and secondary sequence of each factor affecting natto fermentation is from A (fermentation temperature) to B (fermentation time) to C (inoculating scale).

5 Preservation experiment result

Natto preservation experiment takes optimal experiment group determined by orthogonal experiment as culture condition, and weigh and select three portions of 200g soybeans, soak for 20h after washing, drain off moisture, respectively put them into stainless steel plates being sterilized, cook for 30min in high pressure sterilizing pot at 121℃, add 11% seed solutions after taking out and cooling down to indoor temperature, cover moist gauze being sterilized after homogeneous mixing, put them into constant temperature and humidity incubator for 13h culture at 39℃ and conduct postripeness for 24h at 4℃ And then add corrosion remover sodium benzoate as per 0, 0.25g/kg and 0.5g/kg dose, add according to proportion of sodium benzoate: water =1:50 and conduct homogeneous mixing with natto, conduct low temperature preservation at 4℃, conduct sensory evaluation, determine nattokinase activity and natto aerobic bacterial count by regarding 2, 4, 6, 8 and 10 days as the period and study effect of corrosion remover sodium benzoate on natto guarantee period by analyzing results.

6 Sensory evaluation results

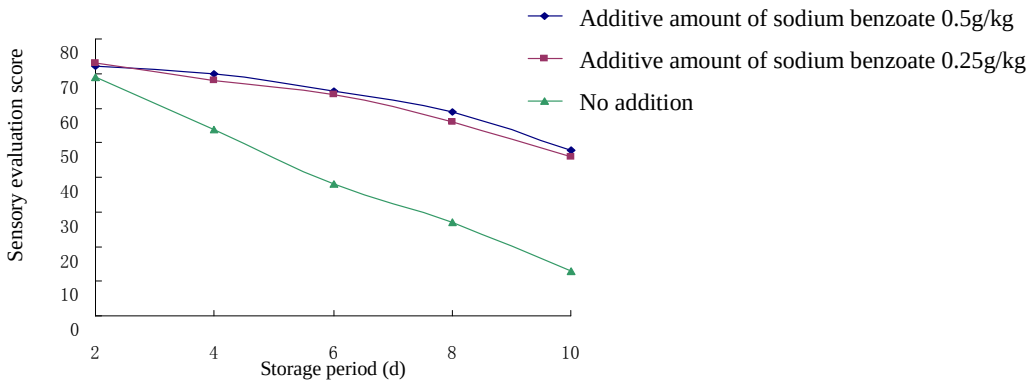

Fig.4 Effect of the amount of Sodium benzoate on natto Shelf life

It can be found out from Fig. 4 that with time going on, natto quality tends to decline. Among them, declining speed of natto quality of the group without adding corrosion remover is the fastest, and when it is sixth day, obvious odorous spoiled odor comes out with severe bitter taste; the two groups adding corrosion remover is just with sligth bitter taste and still with natto perfume which indicates adding corrosion remover can effectively lenghten natto guarantee period. Results of the two groups separately adding 0.25g/kg and 0.5g/kg sodium benzoate have little difference which indicates when additive amount of sodium benzoate is 0.25g/kg, natto guarantee period can be obviously lengthened.

7 Determination of natto kinaseactivity

(1) Tyrosine standard curve

Use spectrophotometer to absorbance value of determine tyrosine at 680nm, take blank tube (only water, sodium carbonate solution and phenol reagent added) as contast, and take absorbance value as Y-axis and microgram number of tyrosine as X-axis to draw standard curve as shown in Fig. 5:

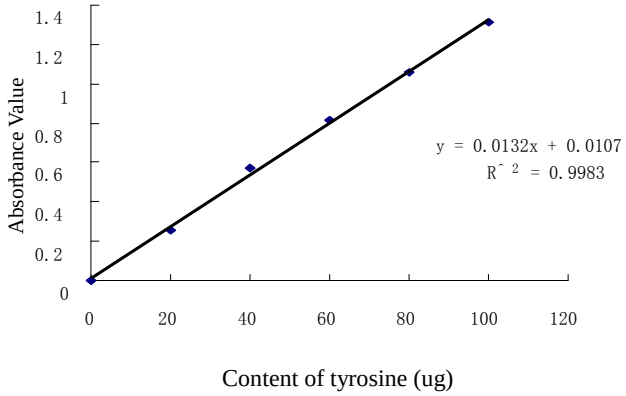

Fig.5 tyrosine standard curve

(2) Calculation of natto kinaseactivity

In order to measure to gain accurate natto kinaseactivity and reasonably arrange time after 24h natto postripeness, instantly add corrosion remover sodium benzoate as per 0, 0.25g/kg and 0.5g/kg dose and determine natto kinaseactivity by taking 2, 4, 6, 8 and 10 days as the period. The result is as shown in Fig. 6:

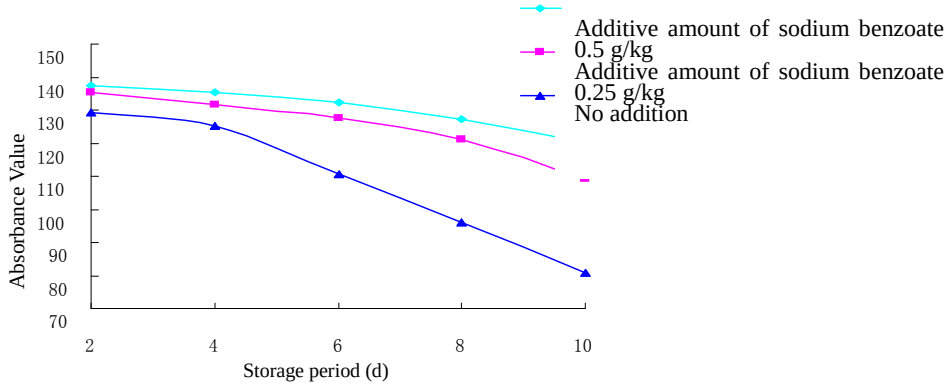

Fig.6 The determination of natto kinase activity

It can be found out from Fig. 6 that with time going on, natto quality tends to weaken. Among them, decreased degree of the group without adding corrosion remover natto kinase activity is obviously quickening as of the fourth day which indicates that it begins to spoil after the fourth day; while the two groups adding corrosion remover tends to slowly decline which means natto spoiling can be effectively postponed by adding corrosion remover. In addtion, Results of the two groups separately adding 0.25g/kg and 0.5g/kg sodium benzoate have little difference which indicates when additive amount of sodium benzoate is 0.25g/kg, natto guarantee period can be obviously lengthened.

8 Natto total number of colonies

Natto continues to conduct fermentation after postripeness, for the purpose of accurately determining natto total number of colonies and facilitating determination, reasonably arrange time before experiment and instantly determine natto total number of colonies after natto postripeness. Separately determine natto total number of colonies of 9 groups of finished natto product of orthogonal experiment and determine mean value, natto total number of colonies contained by natto per gram can be worked out. The result is as shown in Fig. 7:

It can be found out from Fig. 7 that with time going on, natto total number of colonies gradually reduces with less experimental result difference, maybe there are personal errors, but the result can reflect that compared to no addition, natto spoiled speed can be effectively delayed after adding sodium benzoate. When additive amount of sodium benzoate is 0.25g/kg, more obvious effect appears, while increasing additive amount, there is no distinct effect increase, which means when concentration of sodium benzoate is 0.25g/kg, better anticorrosion effect can be obtained.

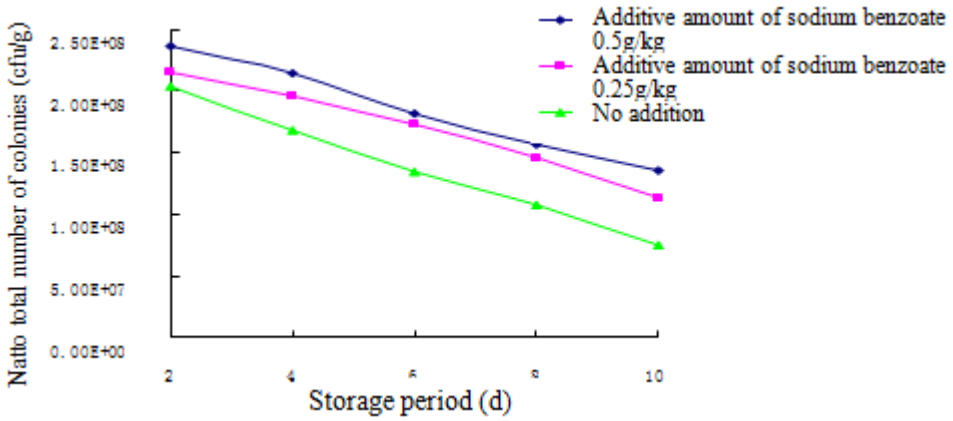

Fig.7 Natto total number of colonies

CONCLUSION

The goal of the experiment is to study optimization and preservation of natto manufacturing technique. As for natto bacteria purified in the lab used in this experiment, factors having a greater effect on its quality can be determined by single factor experiment in the process of natto fermentation and then optimal experiment conditions for natto fermentation is studied by orthogonal experiment to make drawing, taste, flavour, color, etc. of natto impoved. And then conduct preservation experiment under optimal condition and study what influence it will have on natto guarantee period in the way of adding corrosion remover sodium benzoate, specific experimental results are as follows:

Optimal natto manufacturing technique: by conducting sensory evaluation and intuitive analysis for orthogonal experiment, determine optimal manufacturing technique conditions: culture temperature 39□, fermentation time 13h and inoculating scale 11%.

Natto preservation result: conduct sensory evaluation, determine natto kinase activity and natto total number of colonies for three experimental groups by adding 0, 0.25g/kg and 0.5g/kg corrosion remover sodium benzoate to finished natto products and taking 2, 4, 6, 8 and 10 days as the period, and a conclusion can be drawn that when adding corrosion remover sodium benzoate, natto guarantee period can be effectively lengthened, and when additive amount is 0.25g/kg, obvious anticorrosion effect comes out.

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