Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.91522

Correlation analysis between soluble sugars of soybean and mucus drawing length of fermented natto

Delin YAN[1], Yongshan LIN[1], Jing QIU[2], Liangjing LIN[1], Cunyi YANG[3], Xiangyang GAO[1*]

Abstract

Natto is a traditional fermented food from Japan that is made from soybean fermented by *Bacillus subtilis natto*. The mucus drawing length of natto determines the sensory quality of natto, and there are differences in the mucus drawing length and sensory quality of natto fermented with soybeans with different contents of soluble sugars. In this study, soybeans with different contents of soluble sugars were used to ferment natto, and the differences and correlations between the mucilage composition, the mucus drawing length and the sensory quality of natto were investigated. The contents of oligosaccharides and total sugars in soybean were significantly positively correlated with the mucus drawing length ($R = 0.921$, $R = 0.956$), and the correlation between the drawing length of mucilage and total sugars was stronger. Fermenting soybeans with high contents of total sugars could make mucus drawing of natto longer. The contents of oligosaccharides and total sugars in soybean were significantly positively correlated with the sensory index score ($R = 0.886$, $R = 0.925$). The natto fermented from soybean with high contents of soluble total sugars had better overall sensory quality. Soluble sugars had a positive influence on mucus drawing length and sensory quality of natto.

Keywords: soybean; natto; soluble sugars; mucus drawing length; sensory quality; correlation analysis.

Practical Application: The research results reveal another aspect of natto sensory quality control and provide a reference for the breeding of soybean fermented natto varieties.

1 Introduction

Natto is made by fermenting seeds with a bacterium, *Bacillus subtilis (natto)* (Kimijima et al., 2022), which contains various active substances, such as vitamin K2 and nattokinase, and has health care functions, such as preventing cardiovascular disease and lowering blood pressure (Syahbanu et al., 2020; Lee et al., 2015; Zhou et al., 2020; Zhang et al., 2021; Nagata et al., 2017; Nozue et al., 2017). Approximately 60% of carbohydrates are insoluble, and 40% are soluble sugars in soybean. The soluble sugar mainly comprises sucrose, raffinose and stachyose, representing approximately 5%, 1.5% and 3% of soybean dry content, respectively, and contains a small amount of glucose and fructose (< 1%) (Wang et al., 2014). Fermented natto from soybean varieties with low protein, low fat and high sucrose has higher organoleptic qualities and a longer shelf life (Yoshikawa et al., 2014). Sucrose is easily degraded by natto bacilli during fermentation, and fermentation could stop too soon if the sucrose content is high; however, raffinose and stachyose are difficult to degrade by the organisms, and fermentation would proceed adequately if they are present (Pradhananga. 2018). Therefore, the contents of soluble sugars should be given more attention in the breeding of special soybeans for natto fermentation.

The most distinctive feature of natto is that its surface is covered with white viscous material, which can form mucus after stirring. High-quality natto mucus is white, with a light or slightly sour smell, light yellow color, and soft and sticky texture, which has become an important criterion for judging high-quality natto (Yoshikawa et al., 2014; Cao et al., 2021; Wei & Chang, 2004). The stickiness and drawing properties of natto mucus are due to the strong viscous poly-γ-glutamic acid (γ-PGA) and levan, which have a stabilizing effect (Yoshioka et al., 2007). γ-PGA is the main viscous substance produced during natto fermentation (Araki et al., 2020; Ratha & Jhon, 2018; Nguyen et al., 2018). γ-PGA uses *L*-glutamic acid and *D*-glutamic acid as monomers and is a polypeptide polymerized by an amide bond at the γ-position, and its degree of polymerization is between 1000-15000 (Yong et al., 2011). The higher the molecular weight is, the higher the viscosity (Li et al., 2022). Pyruvate is the precursor for γ-PGA in many bacterial species, and its secretion is tightly associated with cell growth. Therefore, suitable culture media could support vigorous cell growth and hence generate sufficient precursor for γ-PGA synthesis. (Gao et al., 2016; Luo et al., 2016). Hence, the carbon source as the main substrate directly or indirectly affects the production of γ-PGA, and the addition of glucose, sucrose, fructose and sugarcane molasses can increase the production of γ-GPA (Luo et al. 2016; Huang et al., 2011; Li et al., 2022). γ-PGA can be used as a carrier, filler, and

Received 09 Aug., 2022
Accepted 02 Oct., 2022
[1] *Guangdong Provincial Key Laboratory of Nutraceuticals and Functional Foods, College of Food Science, South China Agricultural University, Guangzhou, China*
[2] *Guangzhou Dublin International College of Life Sciences and Technology, South China Agricultural University, Guangzhou, China*
[3] *Guangdong Provincial Key Laboratory of Molecular Plant Breeding, College of Agriculture, South China Agricultural University, Guangzhou, China*
*Corresponding author: gaoxiangyang@scau.edu.cn

binder in complex matrices (Li et al., 2022; Lee & Kuo, 2011; Xie et al., 2020; Liang et al., 2006). Levan is an exopolysaccharide comprising fructose, a type of fructan (Wang et al., 2015). Levan is produced by the fructosylation of sucrose by *Bacillus subtilis* using *L*-sucrase (E.C. 2.4.1.10) (Melo et al., 2012), which is one of the few furanoses, and the furan ring structure imparts enhanced flexibility to polysaccharide molecules (Srikanth et al., 2015). Levan mainly comprises β-2,6-linked *D*-fructofuranosyl residues whose side chains are linked to *D*-glucosyl terminal residues (Ragab et al., 2019). The intertwined branched structure of levan is beneficial for enhancing its cohesive strength and contains a large number of hydroxyl groups, making it more viscous (Srikanth et al., 2015). The levan content has an important impact on rheological properties, and edible films made from the combination of levan and other packaging materials have higher tensile strength, solubility and elongation (Hundschell et al., 2020; Mummaleti et al., 2020).

Because food evaluations are based on psychological rather than field or laboratory factors, sensory evaluation is necessary (Yoshikawa et al., 2014). In the sensory experiments of natto, the drawing performance of mucus is mostly used as the measurement standard (Cao et al., 2021; Yoshikawa et al., 2014; Yang et al., 2021). In a sensory test aimed at the elderly in Tokyo, participants rated soft, sticky natto as better than what they generally believed to be natto, and a newly developed soft, sticky natto proved to be useful as food for the elderly (Mitsuboshi et al., 2006). The good quality characteristics of natto depend on soybean varieties, processing conditions and strains. Therefore, the morphophysiological characteristics and seed composition of seeds play an important role in the production of high-quality natto, and the development of soybean varieties with improved natto quality characteristics is crucial for maintaining and expanding the natto market (Escamilla et al., 2019).

The presence of soluble sugars can improve the sensory quality of natto, and the longer the mucus drawing length, the better the sensory evaluation. However, there has been no systematic in-depth study on the relationship between soluble sugars and mucus drawing of natto. Hence, the purpose of this study was to explore the quality differences of soybean fermented natto with different soluble sugars and to determine the effect of the presence of soluble sugars on the drawing length of fermented natto to provide ideas for the breeding of special soybeans for high-quality natto and further deepen the research on the production of natto.

2 Materials and methods

2.1 Production and collection of samples

The soybean samples (BX13, BX10, GX2, BX3, YXH4, YXH2 and YXH3) were provided by the Guangdong Provincial Key Laboratory of Molecular Plant Breeding, College of Agriculture, South China Agricultural University, China. *Bacillus subtilis natto* was obtained from the Guangdong Provincial Key Laboratory of Nutraceuticals and Functional Food, College of Food Science, South China Agricultural University, China. *Bacillus subtilis natto* was inoculated into the seed medium (beef extract 0.5%, peptone 1%, sodium chloride 0.9%) after activation by slant medium (beef extract 0.5%, peptone 1%, sodium chloride 0.9%, agar 1.5%) and placed in a constant temperature shaker at 37 °C and 180 r/min for 24 h; it served as the inoculum for the cooked soybeans. 1) After drying, the soybean samples were washed and soaked in water at room temperature (21 to 23 °C) for approximately 18 h. 2) The soaked natto was steamed (121 °C, 35 min) and cooled. 3) It then underent inoculation (4% V/W of soybean mass after steaming) and fermentation (37 °C, 90% RH, 24 h). 4) Postripening (4 °C, 24 h) was the last step (Hu et al., 2010; Wei et al., 2001). The fermentation process was repeated three times, and a sample was taken as the valid sample during each repetition.

2.2 Determination of the contents of soluble sugars in soybean

The soluble sugars were analyzed using the method of Ahmad et al. (2022) and Lee et al (2022) with some modifications. Briefly, 0.15 g of crushed soybean powder and 1.5 mL of deionized water were added to a 2 mL centrifuge tube, which was placed in a constant temperature shaker at 37 °C for 2 h at 180 r/min and then centrifuged at 12,000 r/min for 10 mins. Then, 750 μL of supernatant was mixed with 750 μL of acetonitrile solution, placed at room temperature for 10 min, and centrifuged at 12,000 r/min for 10 min. Finally, the supernatant was passed through a 0.45 μm filter membrane and detected. The soluble sugar concentrations were determined using an HPLC system (Shimadzu LC-10A high-performance liquid chromatography system) equipped with a refractive index detector (RID). Analyses were performed at 35 °C using an Agilent ZORBAX carbohydrate column (4.6×150 mm, 5 μm) eluted with 75% acetonitrile at a flow rate of 1.0 mL/min, with an injection volume of 20 μL. The sugar concentrations were calculated from calibration curves obtained from soluble sugar standard solutions of known concentrations (2, 4, 6, 8, and 10 mg/mL) and peak values. The soluble sugar content of the soybeans was calculated according to Equation 1 below:

$$\text{Soluble sugars (mg/mL)} = \frac{\text{Conc. detected by HPLC (mg/mL)}}{\text{Conc. soybean powder (g/mL)}} \quad (1)$$

2.3 Determination of mucus composition of natto

The levan content was measured by the phenol−sulfuric acid method (PSA) with minor modifications (Kondo et al., 2021). In brief, 1 mL of natto mucus (diluted to a certain number) was mixed with 1 mL of distilled water, 1 mL of 5% phenol solution and 5 mL of concentrated sulfuric acid. Then, the mixture was reacted in a water bath at 30 °C for 30 mins, and the absorption value was measured at 275 nm. The standard curve was drawn with the glucose standard solution of 0.02–0.1 mg/mL concentration as the abscissa and the absorption value as the ordinate. The levan content in natto mucus was calculated according to Equation 2 below:

$$\text{Levan (\%)} = \frac{\text{Conc. detected by PSA (mg/mL)} \times \text{Sample volume (mL)}}{\text{Mucus quality (g)} \times 10^3} \times 100 \quad (2)$$

The γ-PGA content was measured by the cetyl trimethyl ammonium bromide (CTAB) method. γ-PGA can react with a sodium hydroxide solution of CTAB in turbidity, and the solution turbidity can be reflected by the absorption value. Briefly, referring to the method of Zhang et al. (2012), 2 mL of natto mucus (diluted to a certain number) was mixed well with 2 mL of CTAB solution (during the process, no bubbles appeared in the reaction solution as much as possible). Then, the mixture was placed at room temperature for 3 min, and the absorption value was measured at 224 nm. The standard curve was drawn with the γ-PGA standard solution of 10-50 μg/mL as the abscissa and the absorption value as the ordinate. The content of γ-PGA in natto mucus was calculated according to Equation 3 below:

$$\gamma\text{-PGA }(\%) = \frac{\text{Conc. detected by CTAB (ì g/mL)} \times \text{Sample volume (mL)}}{\text{Mucus quality (g)} \times 10^6} \times 100 \quad (3)$$

2.4 Determination of mucus drawing length of natto

The mucus on the surface of approximately 5 g of natto was gently scraped off, stirred with a glass rod for approximately 2 min, and then pulled vertically at a constant speed to record the height when it broke.

2.5 Sensory properties

The sensory characteristics of natto were evaluated using the procedure described by Yang et al. (2021) and Feng et al. (2015) with some modifications. A sensory team consisting of 10 teachers and graduate students majoring in food-related courses with sensory evaluation experience was recruited. The sensory evaluation was conducted in a ventilated food laboratory with sufficient light and space. The products were labeled with different numbers, randomly disrupted, and finally replicated for three consecutive days under the same conditions. Color, smell, taste, and organization form are key determinants of natto's sensory properties. The product color was evaluated by color intensity, color consistency, lightness, and gloss. The smell of natto was evaluated by aroma, ammonia, beaniness and bitterness, and bitterness was unappealing. Taste was determined by how soft and hard the product was. The organization form of mucus refers to the production of mucus, the size of the mucus and the length of the string. These four sensory attributes were independently assessed using a rating value from 1 to 10 (8~10 = excellent, 6~8 = good, 4~6 = moderate, 2~4 = poor, 0~2 = extremely poor). Finally, each sensory property rating was multiplied by a factor of 2.5 to obtain an index score out of 100 points. A greater index score indicated better natto quality (Yoshikawa, et al. 2014).

2.6 Data analysis

Data are expressed as the mean ± standard deviation and were obtained from three independent experiments. All calculations were implemented using SPSS 20.0 software, the data were statistically analyzed by Pearson's correlation coefficient test and one-way ANOVA plus post hoc Duncan's multiple-comparison test, $p < 0.05$ was defined as statistical significance, and Origin 9.0 was used in drawing (Huang et al., 2022; Yan et al., 2021).

3 Result

3.1 Analysis of the soluble sugar contents and their correlation in soybean

Table 1 shows the standard curve of the soybean soluble sugars. We compared the contents of five soluble sugars in different soybean varieties, as shown in Figure 1. We found that among the 7 different soybean varieties, the sucrose and stachyose contents were the highest, reaching 18.57~40.08 and 18.78~31.34 mg/g, respectively. Following fructose and glucose, the contents were 5.12~8.99 and 3.25~6.93 mg/g, respectively. The raffinose content was the lowest, with a value of only 1.83~3.39 mg/g. The contents of fructose, glucose and raffinose were not significantly different among YXH, BX and GX soybeans; however, the sucrose content of YXH soybeans was significantly higher than that of BX and GX soybeans, and the stachyose content of YXH soybeans was significantly higher than that of BX and GX soybeans. Except for YXH2, the contents of oligosaccharides and total sugars were the highest in YXY3 soybean, reaching 71.05 and 84.00 mg/g;

Table 1. Standard curves of soluble sugars and natto mucus.

Varieties	Linearity (mg/mL)	Equation	R^2
Fructose	2.0-10.0	y=143039x	0.9992
Glucose	2.0-10.0	y=129251x	0.9993
Sucrose	2.0-10.0	y=177314x	0.9990
Raffinose	2.0-10.0	y=129894x	0.9977
Stachyose	2.0-10.0	y=108454x	0.9986
Levan	0.02-0.10	y=8.8935x+0.0664	0.9997
γ-PGA	0.01-0.05	y=0.0169x+0.2070	0.9969

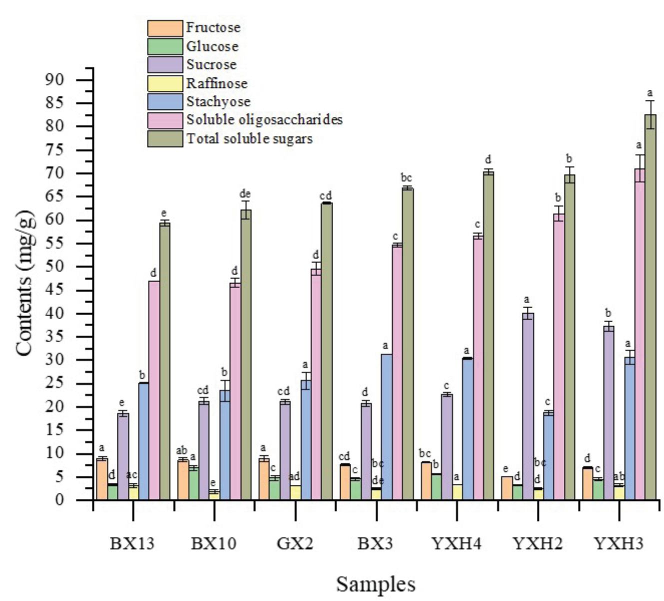

Figure 1. Analysis of soluble sugars in soybean. Values are presented as the mean ± standard deviation. Different letters (a-e) in the table indicate significant differences determined by ANOVA, followed by Duncan's multiple-range test (p value < 0.05).

meanwhile, the contents of oligosaccharides were the lowest for BX10 at 46.55 mg/g, and the contents of total sugars were the lowest for BX13 at 59.25 mg/g. Oligosaccharides were the sum of sucrose, raffinose, and stachyose, and total sugars were the sum of five soluble sugars. Overall, the contents of soluble oligosaccharides and total soluble compounds in YXH soybeans were higher than those in BX and GX soybeans.

Table 2 shows the soluble sugar correlation analysis. The contents of sucrose and fructose in soybean had a very significantly negatively correlated (R = -0.906); the higher the sucrose content was, the lower the fructose content. The soluble oligosaccharide content was significantly positively correlated with the content of soybean sucrose (R = 0.846), and soybeans with high soluble oligosaccharide contents also had higher contents of total soluble sugars (R = 0.972). It can be divided into three levels according to the of total sugar contents: low levels include BX13, BX10, and GX2 (< 65%); medium levels include BX3, YXH4, and YXH2 (65%–70%); and high levels of YXH3 (> 80%).

3.2 Mucus composition and drawing length of Natto

The standard curve of natto mucilage is shown in Table 1. We evaluated the viscous substances in natto mucilage (Figure 2). The γ-PGA content was generally higher than the levan content. Among the seven natto varieties, the γ-PGA contents from high to low were YXH3 > YXH2 > BX13 > YXH4 > GX2 > BX3 > BX10. The γ-PGA content of YXH3 was 15.64%, while that of BX10 was as low as 6.00%. The levan content in natto mucus was higher in YXH4, BX10, and YXH3, at 4.55~4.59%, and the difference was not significant under the condition of p < 0.05, followed by GX2, BX3, and BX13 natto, at 3.57~3.85%. The difference was also not significant, and the YXH2 content was the lowest at less than 3%. The sum of the contents of γ-PGA and levan in natto mucus followed the order YXH3 > BX13 > YXH2 > YXH4 > GX2 > BX3 > BX10.

The mucus drawing length was the most unique quality characteristic of natto mucus, and the mucus drawing length of natto was analyzed (Figure 3). We found that YXH3 had the longest mucus drawing length of natto, with 112.5 cm, followed by YXH4, YXH2, BX3 and BX10, with lengths of 106 cm, 105 cm, 104.5 cm and 101 cm, respectively. GX2 and BX13 had the shortest mucus drawing length at 98 cm and 96 cm, respectively. Most of the natto fermented with YXH soybean had a longer mucus drawing length than natto fermented with BX and GX soybeans.

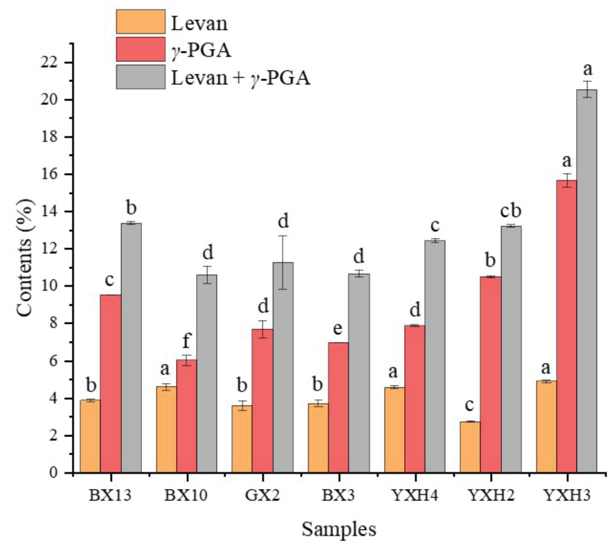

Figure 2. Analysis of mucus components in natto. Values are presented as the mean ± standard deviation. Different letters (a-f) in the table indicate significant differences determined by ANOVA, followed by Duncan's multiple-range test (p value < 0.05).

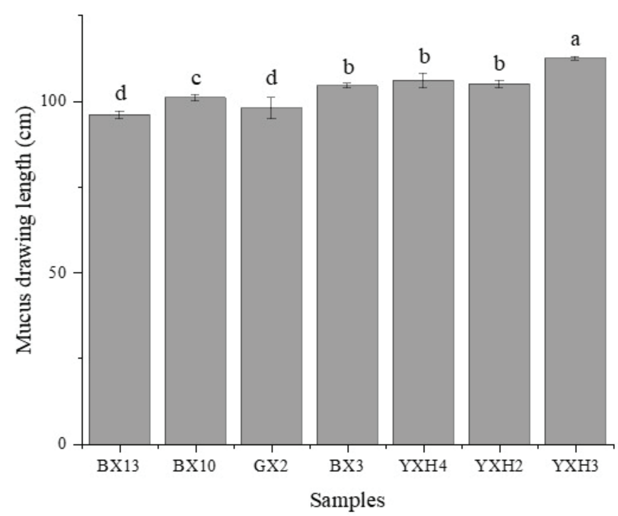

Figure 3. Analysis of mucus drawing length. Values are presented as the mean ± standard deviation. Different letters (a-d) in the table indicate significant differences determined by ANOVA, followed by Duncan's multiple-range test (p value < 0.05).

Table 2. Pearson's correlation analysis of the soluble sugar contents in soybean.

	Glucose	Sucrose	Raffinose	Stachyose	Soluble oligosaccharides	Total soluble sugars
Fructose	0.455	-0.906**	0.121	0.335	-0.722	-0.574
Glucose		-0.386	-0.393	0.239	-0.286	-0.080
Sucrose			0.031	-0.296	0.846*	0.744
Raffinose				0.445	0.328	0.333
Stachyose					0.256	0.395
Soluble oligosaccharides						0.972**

Pearson's correlation coefficient: level of statistical significance: *P < 0.05; **P < 0.01.

3.3 Sensory properties of natto

The sensory profiles of the samples were evaluated in terms of color and smell, taste and organization form, and the average scores of the sensory attributes are reported in Table 3. Based on the results, a spider plot was created to provide a graphic representation, and the differences and similarities were easily identified, as shown in Figure 4. We found that the seven natto sensory scores were as follows: YXH3 > BX3 > YXH4 > YXH2 > BX10, GX2 > BX13. In terms of natto color, BX10 and YXH3 natto were yellow, with a glossy surface and higher scores. BX13, GX2, BX3, YXH4 and YXH2 natto were also yellow; however, their surfaces were not glossy, and their scores were slightly lower. In terms of smell, YXH3 natto had a lighter ammonia smell, a strong unique aroma and a high score. YXH4 and YXH2 natto had slightly lower scores. Although there was a natto aroma, the ammonia smell was slightly heavier. The aroma of BX13, BX10, BX3 and GX2 natto was not as prominent as that of YXH soybeans, and the ammonia smell was also stronger. It was speculated that the fermentation of YXH soybeans was more likely to form the unique aroma of natto. In terms of taste, BX3, YXH2, YXH3, GX2 and YXH4 natto were soft, glutinous, and slippery and had better taste. BX13 and BX10 natto were harder, and their taste was not as good as that of other natto. The organization form was the overall evaluation of natto mucus. BX3, YXH4, YXH3 and BX10 natto mucus were the most viscous and could produce more filaments when stirred, and the drawing was thinner and longer. The viscosity of GX2 and YXH2 natto mucus was very strong, and the amount of filaments and the drawing performance were not as good as those of the 4 aforementioned natto. The BX13 natto had a lower score, and the BX13 natto mucus was very viscous, producing more filaments but a shorter drawing length. Based on the above results, the sensory score of natto fermented with YXH soybean was higher, and it had an advantage in being favored by consumers.

3.4 Correlation analysis of soluble sugars of soybean, mucus drawing length, mucus composition and sensory index score of natto

The correlation analysis between the soluble sugars of soybean, mucus drawing length and mucus composition and sensory index score of natto is shown in Table 4. Levan and γ-PGA were the basis for the formation of mucus, and we found that the γ-PGA content was positively correlated with the sum of the γ-PGA and levan contents ($R = 0.977$). The mucus drawing

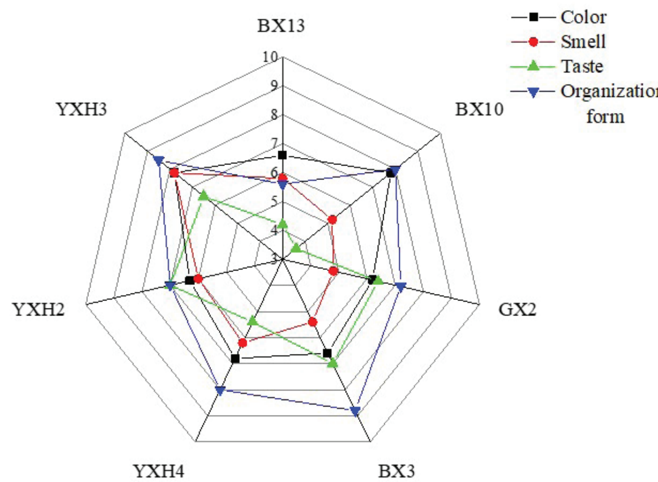

Figure 4. Sensory analysis radar map of different varieties of natto.

Table 3. Sensory evaluation of natto

Varieties	Sensory scores					Index
	Color	Smell	Taste	Organization form	Total scores	
BX13	6.6 ± 0.9[ab]	5.8 ± 0.8[bc]	4.2 ± 0.8[c]	5.6 ± 0.9[c]	22.2 ± 2.3[e]	55.5
BX10	7.8 ± 0.8[a]	5.2 ± 0.8[bc]	3.6 ± 0.5[c]	8.0 ± 0.7[ab]	24.6 ± 1.3[de]	61.5
GX2	6.2 ± 0.4[b]	4.8 ± 0.8[c]	6.4 ± 0.5[ab]	7.2 ± 0.8[b]	24.6 ± 1.5[de]	61.5
BX3	6.6 ± 0.5[ab]	5.4 ± 0.5[bc]	7.0 ± 0.7[a]	8.8 ± 0.8[a]	27.8 ± 1.1[bc]	69.5
YXY4	6.8 ± 0.8[ab]	6.2 ± 0.8[bc]	5.4 ± 0.9[b]	8.0 ± 1.0[ab]	26.4 ± 2.1[cd]	66
YXY2	6.3 ± 0.5[b]	6.0 ± 0.8[bc]	7.0 ± 0.8[a]	7.0 ± 0.8[b]	26.3 ± 1.5[cd]	65.8
YXY3	7.8 ± 0.5[a]	7.8 ± 0.5[a]	6.5 ± 0.6[ab]	8.5 ± 0.6[ab]	30.5 ± 1.3[a]	76.3

Values are represented as the mean ± standard deviation. Different letters (a-e) in the table indicate significant differences determined by ANOVA, followed by Duncan's multiple-range test (p value < 0.05).

Table 4. Pearson's correlation analysis among the soluble sugars of soybean and mucus drawing length, mucus composition and sensory index score of natto

	γ-PGA	Levan + γ-PGA	Mucus drawing length	Index score	Soluble oligosaccharides	Total soluble sugars
Levan	0.181	0.387	0.360	0.298	0.132	0.341
γ-PGA		0.977**	0.673*	0.686*	0.838*	0.797*
Levan + γ-PGA			0.682*	0.703*	0.814*	0.821*
Mucus drawing length				0.952**	0.921**	0.956**
Index score					0.886**	0.925**

Pearson's correlation coefficient: level of statistical significance: *$P < 0.05$; **$P < 0.01$.

length of natto was significantly positively correlated with the γ-PGA content and the total contents of levan and γ-PGA (R = 0.673 and R = 0.682, respectively). The index score of natto was significantly positively correlated with the γ-PGA content and the total contents of levan and γ-PGA (R = 0.686 and R = 0.703, respectively), and the formation of viscous substances in natto mucus was related to the formation of sensory quality. The mucus drawing length of natto was significantly positively correlated with the index score (R = 0.952); that is, the natto with good sensory quality was also long.

The mucus of natto is formed by the transformation of nutrients in soybean by *Bacillus subtilis natto* during the fermentation process. The fermentation strain and fermentation conditions of natto are the same, and the difference in mucus is mainly caused by soybean. Table 4 shows that the γ-PGA content in the natto mucus was significantly positively correlated with the oligosaccharide and total sugar contents (R = 0.838 and R = 0.797, respectively), the total contents of levan and γ-PGA in mucus were significantly positively correlated with the oligosaccharide and total sugar contents (R = 0.814 and R = 0.821, respectively), and high contents of soluble total sugars were conducive to the formation of viscous substances. The mucus drawing length was significantly positively correlated with the contents of oligosaccharides and total sugars (R = 0.921, R = 0.956), and the correlation between the mucus drawing length and total sugars was stronger. Soybean fermentation with high contents of total sugars could make natto mucus draw longer. As soluble sugars are the initial carbon and energy source for natto, their high content promotes microbial growth and makes the finished product taste sweeter (Wei & Chang, 2004). The soybean oligosaccharide and total sugar contents were significantly positively correlated with the index score (R = 0.886, R = 0.925), and the correlation with total sugars was stronger. The fermented natto with high contents of total sugars had a more ideal flavor, and the overall organoleptic quality was better. The results showed that the presence of soluble sugars played an important role in the drawing performance of natto mucus and sensory evaluation of natto.

4 Discussion

The chemical quality traits of soybean seeds are the key to determining suitable natto varieties to produce high-quality natto. Among the carbohydrates contained in soybean seeds, up to 1.6% of the carbohydrates are soluble sugars, of which high sucrose and low stachyose types are the most suitable for cultivation. Specialty soybeans are the most valuable (Hou et al., 2009; Escamilla et al., 2019). In the present study, the sugar content of soybean seeds was quite different in terms of individual sugar and total sugars; sucrose and stachyose accounted for most of the soluble sugars, the differences between different varieties were large, and the raffinose content was the lowest. Sucrose is one of the most important flavor components in soy products because it contributes to the sweetness of soy products. High-yielding breeding lines with high sucrose content are most likely to be accepted by natto producers. Raffinose and stachyose are considered undesirable sugars because they are not easily digested and can cause flatulence or diarrhea (Yoshikawa et al., 2014; Wang et al., 2014; Hou et al., 2009; Escamilla et al., 2019). We found a positive correlation between the contents of soluble oligosaccharides and total sugars and the sucrose content of soybean using Pearson's correlation analysis, and these results were consistent with the study by Hou et al., in which total sugars were positively correlated with glucose, fructose and sucrose (Hou et al., 2009). Therefore, it can be concluded from our study that sucrose has an important position in soluble sugars.

The mucilage and drawing properties of natto are important criteria for judging the quality of natto (Escamilla et al., 2019). A high-quality natto product should have a characteristic flavor, a soft and palatable texture, a pale-yellow color, and a silky sticky mass when stirred with chopsticks (Wei et al., 2001). Levan and γ-PGA are the basis for the formation of mucus strands. Microorganisms play a transformative role in the production of sticky substances, and levan is synthesized by *L*-sucrase (EC 2.4.1.10) in *Bacillus subtilis* in medium containing high amounts of sucrose (Meng & Futterer, 2003). Various metabolic activities in life are closely related. Pyruvate is an intermediate product of sugar metabolism and a precursor of γ-PGA. *Bacillus subtilis natto* converts glucose into pyruvate through sugar metabolism and further undergoes other metabolic processes, such as protein metabolism. The pathway generates γ-PGA (Luo et al., 2016; Li et al., 2022). Therefore, glucose and sucrose can promote the production of γ-PGA, and the contents of soluble sugars play an important role in mucus stickiness. Our study found that the contents of oligosaccharides in soybean were significantly positively correlated with the γ-PGA and total contents of levan and γ-PGA, and total sugars were also positively correlated with γ-PGA and the total contents of levan and γ-PGA. Pearson's correlation analysis showed that high contents of soluble sugars were conducive to the formation of viscous substances, which further confirmed that soluble sugars are converted into sticky substances during the fermentation of natto. The contents of oligosaccharides and total sugars in soybean were significantly positively correlated with the mucus drawing length. Soybean fermentation with high contents of total sugars could make the draw of natto mucus longer. In addition, we found that the γ-PGA content was generally higher than the levan content in the two viscous substances, and the γ-PGA content was positively correlated with the sum of the two viscous substances. The mucus drawing length of natto was significantly positively correlated with the γ-PGA content and the total contents of levan and γ-PGA.

Whether the texture and flavor of natto are suitable for the natto market is usually determined by a sensory panel (Zhang et al., 2008), and fructose, glucose, and sucrose contribute significantly to the flavor and sweetness of soybean products (Wang et al., 2014; Hou et al., 2009). The good sensory quality of natto has been reported to be positively correlated with sucrose, and the contents of total sugars and sucrose enhance the taste of soy products. During the natto fermentation process, high contents of total sugars and sucrose help to produce natto with better flavor (Yoshikawa et al., 2014). In the present study, we used Pearson's correlation analysis to determine that the sensory index scores were significantly positively correlated with the contents of oligosaccharides and total sugars of soybean and had a stronger correlation with total sugars. Therefore, increasing the contents of total sugars and sucrose is very important to achieve an appropriate fermentation rate and improve the taste and flavor of natto and tofu (Hou et al., 2009). In addition to the original characteristics of natto, mucus drawing also has the effect of auxiliary flavoring (Yoshioka et al., 2007). We studied the correlation between the mucus composition, mucus drawing length and index score of natto, and the index score of natto was significantly correlated with the γ-PGA content and total contents of levan and γ-PGA. The index score showed a very significant positive correlation with the mucus drawing length of natto. The formation of viscous substances in natto mucus could enhance the sensory quality of natto.

5 Conclusion

The soluble sugars in soybean, mucus composition, mucus drawing length and sensory quality of natto were studied by Pearson's correlation analysis, and there were significant correlations among them. Soluble sugars such as glucose, fructose and sucrose are the precursors or main components of natto mucus composition (levan and γ-PGA), and soluble sugars can promote the production of natto mucus composition. The sensory quality of natto was not only affected by the taste and flavor of natto but also related to the mucus drawing length of natto. The longer the natto mucus was drawn, the better the sensory quality of natto. Natto fermented from soybeans with high contents of soluble sugars had high contents of mucus composition, longer mucus drawing length and better sensory quality. Soybean germplasms with unique sugar contents are a useful source of diversity for future soybean sugar breeding and genetic studies. Selecting soybeans with a high soluble sugar content for natto fermentation can meet the market demand for high-quality natto and is conducive to the market development of natto.

Acknowledgements

This work was supported by the Guangdong Province Key Area Research and Development Projects (Project No. 2020B020220008).

References

Ahmad, W., Nasir, A., Sattar, F., Ashfaq, I., Chen, M. H., Hayat, A., Rehman, M. U., Zhao, S., Khaliq, S., Ghauri, M. A., & Anwar, M. A. (2022). Production of bimodal molecular weight levan by a lactobacillus reuteri isolate from fish gut. *Folia Microbiologica*, 67(1), 21-31. http://dx.doi.org/10.1007/s12223-021-00913-w. PMid:34453701.

Araki, R., Yamada, T., Maruo, K., Araki, A., Miyakawa, R., Suzuki, H., & Hashimoto, K. (2020). Gamma-polyglutamic acid-rich natto suppresses postprandial blood glucose response in the early phase after meals: a randomized crossover study. *Nutrients*, 12(8), 2374. http://dx.doi.org/10.3390/nu12082374. PMid:32784412.

Cao, L., Kou, F., Zhang, M. C., Jin, X. J., Ren, C. Y., Yu, G. B., Zhang, Y. X., & Wang, M. X. (2021). Effect of exogenous melatonin on the quality of soybean and natto products under drought stress. *Journal of Chemistry*, 8847698, 1-8. http://dx.doi.org/10.1155/2021/8847698.

Escamilla, D. M., Rosso, M. L., Holshouser, D. L., Chen, P., Zhang, B., & Singh, R. (2019). Improvement of soybean cultivars for natto production through the selection of seed morphological and physiological characteristics and seed compositions: a review. *Plant Breeding*, 138(2), 131-139. http://dx.doi.org/10.1111/pbr.12678.

Feng, C., Jin, S., Luo, M., Wang, W., Xia, X. X., Zu, Y. G., Li, L. P., & Fu, Y. J. (2015). Optimization of production parameters for preparation of natto-pigeon pea with immobilized Bacillus natto and sensory evaluations of the product. *Innovative Food Science & Emerging Technologies*, 31, 160-169. http://dx.doi.org/10.1016/j.ifset.2015.08.002.

Gao, W., Zhang, Z., Feng, J., Dang, Y., Quan, Y., Gu, Y., Wang, S., & Song, C. (2016). Effects of MreB paralogs on poly-γ-glutamic acid synthesis and cell morphology in Bacillus amyloliquefaciens. *FEMS Microbiology Letters*, 363(17), fnw187. http://dx.doi.org/10.1093/femsle/fnw187. PMid:27481703.

Hou, A., Chen, P., Alloatti, J., Li, D., Mozzoni, L., Zhang, B., & Shi, A. (2009). Genetic variability of seed sugar content in worldwide soybean germplasm collections. *Crop Science*, 49(3), 903-912. http://dx.doi.org/10.2135/cropsci2008.05.0256.

Hu, Y., Ge, C., Yuan, W., Zhu, R., Zhang, W., Du, L., & Xue, J. (2010). Characterization of fermented black soybean natto inoculated with bacillus natto during fermentation. *Journal of the Science of Food and Agriculture*, 90(7), 1194-1202. http://dx.doi.org/10.1002/jsfa.3947. PMid:20394001.

Huang, J., Du, Y. M., Xu, G. H., Zhang, H. L., Zhu, F., Huang, L., & Xu, Z. N. (2011). High yield and cost-effective production of poly (γ-glutamic acid) with bacillus subtilis. *Engineering in Life Sciences*, 2011(11), 291-297. http://dx.doi.org/10.1002/elsc.201000133.

Huang, T., Qin, K., Yan, Y. M., He, X. R., Dai, G. L., & Zhang, B. (2022). Correlation between the storability and fruit quality of fresh goji berries. *Food Science and Technology (Campinas)*, 42, e46120. http://dx.doi.org/10.1590/fst.46120.

Hundschell, C. S., Braun, A., Wefers, D., Vogel, R. F., & Jakob, F. (2020). Size-dependent variability in flow and viscoelastic behavior of levan produced by gluconobacter albidus tmw 2.1191. *Foods*, 9(2), 192. http://dx.doi.org/10.3390/foods9020192. PMid:32075024.

Kimijima, M., Mandokoro, K., Ichikawa, Y., Tokumaru, M., Narisawa, N., & Takenaga, F. (2022). Isolation and characterization of Bacillus subtilis from commercially available honey and its application in natto fermentation. *Food Science and Technology Research*, 28(3), 267-273. http://dx.doi.org/10.3136/fstr.FSTR-D-21-00312.

Kondo, M., Mulianda, R., Matamura, M., Shibata, T., Mishima, T., Jayanegara, A., & Isono, N. (2021). Validation of a phenol: ulfuric acid method in a microplate format for the quantification of soluble sugars in ruminant feeds. *Animal Science Journal*, 92(1), e13530. http://dx.doi.org/10.1111/asj.13530. PMid:33638207.

Lee, B. H., Lai, Y. S., & Wu, S. C. (2015). Antioxidation, angiotensin converting enzyme inhibition activity, nattokinase, and antihypertension of bacillus subtilis (natto)-fermented pigeon pea. *Journal of Food and Drug Analysis*, 23(4), 750-757. http://dx.doi.org/10.1016/j.jfda.2015.06.008. PMid:28911492.

Lee, C. Y., & Kuo, M. I. (2011). Effect of γ-polyglutamate on the rheological properties and microstructure of tofu. *Food Hydrocolloids*, 25(5), 1034-1040. http://dx.doi.org/10.1016/j.foodhyd.2010.10.001.

Lee, S., Cho, J. H., Park, K. D., Kim, Y. D., & Yim, S. H. (2022). Assessment of validation and antioxidant activities of novel 12 Korean strawberry cultivars. *Food Science and Technology (Campinas)*, 42, e76121. http://dx.doi.org/10.1590/fst.76121.

Li, D., Hou, L., Gao, Y., Tian, Z., Fan, B., Wang, F., & Li, S. (2022). Recent advances in microbial synthesis of poly-γ-glutamic acid: a review. *Foods*, 11(5), 739. http://dx.doi.org/10.3390/foods11050739. PMid:35267372.

Liang, H. F., Chen, C. T., Chen, S. C., Kulkarni, A. R., Chiu, Y. L., Chen, M. C., & Sung, H. W. (2006). Paclitaxel-loaded poly(gamma-glutamic acid)-poly(lactide) nanoparticles as a targeted drug delivery system for the treatment of liver cancer. *Biomaterials*, 27(9), 2051-2059. http://dx.doi.org/10.1016/j.biomaterials.2005.10.027. PMid:16307794.

Luo, Z., Guo, Y., Liu, J., Qiu, H., Zhao, M., Zou, W., & Li, S. (2016). Microbial synthesis of poly-γ-glutamic acid: current progress, challenges, and future perspectives. *Biotechnology for Biofuels*, 9(1), 134. http://dx.doi.org/10.1186/s13068-016-0537-7. PMid:27366207.

Melo, F. C., Zaia, C. T., & Celligoi, M. A. (2012). Levan from bacillus subtilis natto: its effects in normal and in streptozotocin-diabetic rats. *Brazilian Journal of Microbiology*, 43(4), 1613-1619. http://dx.doi.org/10.1590/S1517-83822012000400046. PMid:24031993.

Meng, G., & Futterer, K. (2003). Sctrutural framework of fructosyl transfer in Bacillus subtilis levansucrase. *Nature Structural Biology*, 10(11), 935-941. http://dx.doi.org/10.1038/nsb974. PMid:14517548.

Mitsuboshi, S., Saito, H., Matsukawa, M., Miyaji, Y., Tanaka, T., Murahashi, A., Muramatsu, K., Watanabe, S., & Kiuchi, K. (2006). Development of soft, sticky itohiki-natto manufactured for senior citizens. *Nippon Shokuhin Kagaku Kogaku Kaishi*, 53(9), 466-473. http://dx.doi.org/10.3136/nskkk.53.466.

Mummaleti, G., Sarma, C., Kalakandan, S. K., Sivanandham, V., & Anandharaj, A. (2020). Optimization and extraction of edible microbial polysaccharide from fresh coconut inflorescence sap: an alternative substrate. *Lebensmittel-Wissenschaft + Technologie*, 138(1), 110619. http://dx.doi.org/10.1016/j.lwt.2020.110619.

Nagata, C., Wada, K., Tamura, T., Konishi, K., Goto, Y., Koda, S., Kawachi, T., Tsuji, M., & Nakamura, K. (2017). Dietary soy and natto intake and cardiovascular disease mortality in Japanese adults: the takayama study. *The American Journal of Clinical Nutrition*, 105(2), 426-431. http://dx.doi.org/10.3945/ajcn.116.137281. PMid:27927636.

Nguyen, S., Inaoka, T., & Kimura, K. (2018). Poly-γ-glutamic acid production by bacillus subtilis (natto) under high salt conditions. *Japan Agricultural Research Quarterly*, 52(3), 249-253. http://dx.doi.org/10.6090/jarq.52.249.

Nozue, M., Shimazu, T., Sasazuki, S., Charvat, H., Mori, N., Mutoh, M., Sawada, N., Iwasaki, M., Yamaji, T., Inoue, M., Kokubo, Y., Yamagishi, K., Iso, H., & Tsugane, S. (2017). Fermented soy product intake is inversely associated with the development of high blood pressure: the japan public health center-based prospective study. *The Journal of Nutrition*, 147(9), 1749-1756. http://dx.doi.org/10.3945/jn.117.250282. PMid:28724661.

Pradhananga, M. (2018). Effect of processing and soybean cultivar on natto quality using response surface methodology. *Food Science & Nutrition*, 7(1), 173-182. http://dx.doi.org/10.1002/fsn3.848. PMid:30680171.

Ragab, T. I. M., Malek, R. A., Elsehemy, I. A., Farag, M. M. S., Salama, B. M., Abd El-Baseer, M. A., Gamal-Eldeen, A. M., El Enshasy, H. A., & Esawy, M. A. (2019). Scaling up of levan yield in bacillus subtilis m and cytotoxicity study on levan and its derivatives. *Journal of Bioscience and Bioengineering*, 127(6), 655-662. http://dx.doi.org/10.1016/j.jbiosc.2018.09.008. PMid:30795878.

Ratha, P., & Jhon, D. Y. (2018). Factors increasing poly-γ-glutamic acid content of cheongguk-jang fermented by bacillus subtilis 168. *Food Science and Biotechnology*, 28(1), 103-110. http://dx.doi.org/10.1007/s10068-018-0424-z. PMid:30815300.

Srikanth, R., Reddy, C., Siddartha, G., Ramaiah, M. J., & Uppuluri, K. B. (2015). Review on production, characterization and applications of microbial levan. *Carbohydrate Polymers*, 120, 102-114. http://dx.doi.org/10.1016/j.carbpol.2014.12.003. PMid:25662693.

Syahbanu, F., Kezia, E., Puera, N., Giriwono, P. E., Tjandrawinata, R. R., & Suhartono, M. T. (2020). Fibrinolytic bacteria of Indonesian fermented soybean: preliminary study on enzyme activity and protein profile. *Food Science and Technology (Campinas)*, 40(suppl 2), 458-462. http://dx.doi.org/10.1590/fst.23919.

Wang, X., Yu, S. H., Zhang, T., Jiang, B., & Mu, W. M. (2015). From fructans to difructose dianhydrides. *Applied Microbiology and Biotechnology*, 99(1), 175-188. http://dx.doi.org/10.1007/s00253-014-6238-x. PMid:25431014.

Wang, Y. Q., Chen, P. Y., & Zhang, B. (2014). Quantitative trait loci analysis of soluble sugar contents in soybean. *Plant Breeding*, 133(4), 493-498. http://dx.doi.org/10.1111/pbr.12178.

Wei, Q., & Chang, S. K. C. (2004). Characteristics of fermented natto products as affected by soybean cultivars. *Journal of Food Processing and Preservation*, 28(4), 251-273. http://dx.doi.org/10.1111/j.1745-4549.2004.23047.x.

Wei, Q., Wolf-Hall, C., & Chang, K. C. (2001). Natto characteristics as affected by steaming time, bacillus strain, and fermentation time. *Journal of Food Science*, 86(1), 167-173. http://dx.doi.org/10.1111/j.1365-2621.2001.tb15601.x.

Xie, X. H., Wu, X. Y., Shen, Y., Song, M., Xu, C., Zhang, B., Aziz, U., & Xu, X. J. (2020). Effect of poly-γ-glutamic acid on hydration and structure of wheat gluten. *Journal of Food Science*, 85(10), 3214-3219. http://dx.doi.org/10.1111/1750-3841.15400. PMid:32857865.

Yan, Z. X., Lu, Z. M., Li, W., Hu, R., & Ma, Q. M. (2021). Differential proteomic analysis to identify proteins associated with Tenderness of Yak meat from different parts based on TMT Proteomic. *Food Science and Technology (Campinas)*, 42, e58721. http://dx.doi.org/10.1590/fst.58721.

Yang, Y., Lan, G. Q., Tian, X. Y., He, L. P., Li, C. Q., Zeng, X. F., & Wang, X. (2021). Effect of fermentation parameters on natto and its thrombolytic property. *Foods*, 10(11), 2547. http://dx.doi.org/10.3390/foods10112547. PMid:34828828.

Yong, X., Raza, W., Yu, G., Ran, W., Shen, Q., & Yang, X. (2011). Optimization of the production of poly-γ-glutamic acid by bacillus amyloliquefaciens c1 in solid-state fermentation using dairy manure compost and monosodium glutamate production residues as basic substrates. *Bioresource Technology*, 102(16), 7548-7554. http://dx.doi.org/10.1016/j.biortech.2011.05.057. PMid:21665467.

Yoshikawa, Y., Chen, P., Zhang, B., Scaboo, A., & Orazaly, M. (2014). Evaluation of seed chemical quality traits and sensory properties of natto soybean. *Food Chemistry*, 153, 186-192. http://dx.doi.org/10.1016/j.foodchem.2013.12.027. PMid:24491719.

Yoshioka, K., Sekine, M., & Otobe, K. (2007). Physical properties of viscous materials in natto prepared at various fermentation temperatures. *Nippon Shokuhin Kagaku Kogaku Kaishi*, 54(10), 452-455. http://dx.doi.org/10.3136/nskkk.54.452.

Zhang, B., Chen, P., Chen, C. Y., Wang, D., Shi, A., Hou, A., & Ishibashi, T. (2008). Quantitative trait loci mapping of seed hardness in soybean. *Crop Science*, 48(4), 1341-1349. http://dx.doi.org/10.2135/cropsci2007.10.0544.

Zhang, Q. Q., Jin, X. Q., Chen, J. X., Zhao, A. B., & Li, H. J. (2012). Study on efficient determination method of poly-γ-glutamic acid in fermentation broth. *Science and Technology of Food Industry*, 33(19), 294-296. http://dx.doi.org/10.13386/j.issn1002-0306.2012.19.025.

Zhang, X. S., Sun, X., Li, W. J., Huang, X., Tao, L., Li, T. P., & Li, S. H. (2021). In vitro and in vivo antioxidant activities of soy protein isolate fermented with bacillus subtilis natto. *Journal of Food Science and Technology*, 58(8), 3199-3204. http://dx.doi.org/10.1007/s13197-020-04823-6. PMid:34294982.

Zhou, L. Q., Hao, N., Li, X. T., Chen, J. X., Yang, R. Y., Song, C. Q., Sun, Y., & Zhang, Q. (2020). Nattokinase mitigated dextran sulfate sodium-induced chronic colitis by regulating microbiota and suppressing tryptophan metabolism via inhibiting ido-1. *Journal of Functional Foods*, 75, 104251. http://dx.doi.org/10.1016/j.jff.2020.104251.