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Review

Natto: A medicinal and edible food with health function

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ABSTRACT

Natto is a soybean product fermented by natto bacteria. It is rich in a variety of amino acids, vitamins, proteins and active enzymes. It has a number of biological activities, such as thrombolysis, prevention of osteoporosis, antibacterial, anticancer, antioxidant and so on. It is widely used in medicine, health-care food, biocatalysis and other fields. Natto is rich in many pharmacological active substances and has significant medicinal research value. This paper summarizes the pharmacological activities and applications of natto in and outside China, so as to provide references for further research and development of natto.

Contents

1. Introduction . . . 350
2. Components of natto . . . 350
   2.1. Volatile components . . . 350
   2.2. Amino acids and proteins . . . 350
   2.3. Vitamins . . . 350
   2.4. Other components . . . 351
3. Pharmacological actions of natto . . . 352
   3.1. Thrombolytic effects . . . 352
   3.2. Antibacterial action . . . 352
   3.3. Antitumor effect . . . 353
   3.4. Hypotensive effect . . . 353
   3.5. Anti-osteoporosis effect . . . 353
   3.6. For other purposes . . . 354
4. Nutritional value of natto . . . 354
5. Application of natto . . . 355
   5.1. Fermentation additive . . . 355
   5.2. Natto food . . . 356
   5.3. Natto health products . . . 356
6. Discussion . . . 356
   6.1. Improvement of bad flavor of natto . . . 357
   6.2. Classification of active ingredients in natto . . . 357
   6.3. Correlation between natto components and efficacy . . . 357
   6.4. Safety and quality control of natto and its products . . . 357

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Declaration of Competing Interest . . . 357
Acknowledgements . . . 357
References . . . 357

1. Introduction

Natto is made from soybeans by cooking them and inoculating them with *Bacillus subtilis* for fermentation. According to the food natto standard SB/T 10528-2009 issued by the General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China and the National Standardization Administration of China, raw soybean shall comply with the provisions of GB 1352. According to GB 1352 national standard for soybeans, soybeans with different colors can be divided into yellow soybeans, green soybeans, black soybeans, etc., which are the seeds of soybean *Glycine* max (L.) Merr (Dadou in Chinese) (Wang et al., 2021). After fermentation, the smooth surface of the soybean is covered with yellow and white sticky material that looks like filamentous lines. In Japan, natto is regarded as one of the important health food that have the concomitant function of both medicine and foodstuff. It is very popular in daily life and is considered to be the secret recipe for longevity of Japanese people (Qin, Hara, Raboy, & Saneoka, 2020). In China, natto, based on its high nutrition and defensive capability of disease, is widely used in food, medicine and health products and so on. Nattokinase (NK) is the most important active substances of natto and it has significant advantage in dissolving blood clots, which has set off a wave of in-depth research on natto and made outstanding achievements (Gallelli et al., 2021).

Soybean, the raw material of natto, is a nutritious food with high protein and eight kinds of essential amino acids. After the cooking and fermentation process of natto, the proteins and polysaccharides in the natto will undergo decomposition and enzymatic hydrolysis under high temperature conditions, producing more short chains and easily absorbed amino acids, active peptides, oligosaccharides, etc (Liu, Su, & Song, 2018). It contains more proteins, cellulose, calcium, iron, potassium and vitamin B2 than soybeans, especially more cellulose, calcium, iron and potassium than eggs. Japanese researchers learned that soy proteins are insoluble. After being made into natto, they become soluble and produce amino acids. In addition, various enzymes that do not exist in the raw materials will be produced by natto bacteria and associated bacteria to help gastrointestinal digestion and absorption (Kanghae, Eungwanichayapant, & Chukeatirote, 2017). At the same time, nattokinase with thrombolytic function is also produced in natto. These changes are caused by the fermentation of soybeans with natto bacteria, which is the reason why the edible value of natto is much higher than that of soybeans. This article only reviewed and discussed developing status and research progress of natto.

2. Components of natto

2.1. Volatile components

Compared with soybeans, natto attracts many people because of its unique taste after fermentation, which is related to the fact that natto contains many volatile components. Many volatile components in natto can be obtained by different extraction and isolation methods, including SPME (solid phase microextraction), SAFE (solvent-assisted flavor evaporation), DHS (dynamic headspace sampling), and SDE (simultaneous distillation and extraction) (Liu, Su, & Song, 2018; Huang et al., 2012). The volatile components of natto include ketones, esters, pyrazines, alcohols, aldehydes, alkanes, amines, acids, benzene, ethers, oxazole, carbazole, piperidine, furan, hydrazine, etc (Kanno & Takamatsu, 1987). The volatile components contained in natto were shown in Table 1. In addition, these ingredients have various different smells, such as cheese-like, fruit-like, minty-like, fresh-like, alcohol-like, cream-like, oil-like, etc. For example, the tetramethylpyrazine compounds have a strong sublimation effect and a strong fragrance. Furthermore, pyrazines and sulfur compounds are important contributors to the characteristic odor of natto, which can mask the beany smell.

2.2. Amino acids and proteins

The reason why natto has good health care effect is closely related to the change of the basic components of soybeans in the production process. Under the action of *Bacillus natto* (*B. subtilis natto*), proteins, peptides and other components in soybeans undergo complex biochemical reactions (Suo et al., 2015). While retaining the original active components, new active substances, such as nattokinase (NK), antioxidant peptides and antibacterial peptides, will be produced (Hu et al., 2010). A variety of cationic peptides and nattokinase in natto peptide have good biological activities, including lipopolysaccharide neutralization activity, thrombolytic effect and angiogenic activity (Taniguchi et al., 2019). In addition, natto is rich in superoxide dismutase (SOD), which is the natural enemy of oxygen free radicals in the body, and can inhibit cardiovascular and cerebrovascular diseases, anti-aging, and treat autoimmune diseases (Cui, Jiang, & Li, 2011).

During the fermentation of natto, some soybean proteins will be decomposed into water-soluble nitrogen compounds, including a variety of amino acids. Amino acids play an important role in human metabolism. Natto contains 18 amino acids, of which eight are essential amino acids (Qi, Xi, & Chen, 2004), such as tryptophan, phenylalanine, threonine, leucine, valine, etc. After fermentation of soybeans by *B. natto*, protein hydrolysis was promoted by protease. A total of 50%–60% of soybean proteins is converted into peptides and amino acids. Glutamic acid in natto is polymerized to form the main component of the mucilage unique to natto γ-polyglutamic acid. γ-Polyglutamic acid is also an excellent raw material for processing environment-friendly plastics, which can be used for food packaging, disposable tableware and other industrial purposes. In addition, the special odor component of natto is mainly isovalerenic acid, which increases with the maturity of natto, but decreases slowly during the preservation of the preparation. The content of some amino acids in natto was shown in Table 2 (Gao, 2014; Qi, Xi, & Chen, 2004; Onishi, Abe, Honma, & Aida, 1987).

2.3. Vitamins

Natto is rich in vitamins, including vitamin B2, vitamin B6, vitamin B12, vitamin E and vitamin K. The content of vitamin B and vitamin E is higher than that of soybeans, especially the content of vitamin B2 is more than six times of that in soybeans (Xu, Cai, & Xu, 2017). Vitamin B2 is a component of the coenzyme of flavases in the body (flavases play a role of hydrogen transfer in biological oxidation and reduction), when it is lacking, it will affect the

Table 1
List of volatile components of natto.

No.	Compounds	Formulas	No.	Compounds	Formulas
1	Ethanol	C_2H_6O	57	3-Octanol	$C_8H_{18}O$
2	Phenol	C_6H_6O	58	Amyl ethyl ether	$C_7H_{16}O$
3	*p*-Xylene	C_8H_{10}	59	Hydroxyacetone	$C_3H_6O_2$
4	*m*-Xylene	C_8H_{10}	60	Methyl heptenone	$C_8H_{14}O$
5	*o*-Xylene	C_8H_{10}	61	Trimethyl pyrazine	$C_7H_{10}N_2$
6	Undecane	$C_{11}H_{24}$	62	Phenylacetaldehyde	C_8H_8O
7	Dodecane	$C_{11}H_{26}$	63	Methyl octyl ketone	$C_{10}H_{20}O$
8	Guaiacol	$C_7H_8O_2$	64	2-Piperidin imine	$C_5H_{10}N_2$
9	Pyridine	C_5H_5N	65	Trimethyl-oxazole	C_6H_9NO
10	Hexanal	$C_6H_{12}O$	66	4-Methyl-pyrimidin	$C_5H_6N_2$
11	Nonanal	$C_9H_{18}O$	67	Threo-2,3-octanediol	$C_8H_{18}O_2$
12	Decanal	$C_{10}H_{20}O$	68	2-Phenyl-2-propanol	$C_9H_{12}O$
13	2-Butanol	$C_4H_{10}O$	69	Methyl phenylacetate	$C_9H_{10}O_2$
14	1-Hexanol	$C_6H_{14}O$	70	Tetramethylpyrazine	$C_8H_{12}N_2$
15	Benzaldehyde	C_7H_6O	71	2,5-Dimethylpyrazine	$C_6H_8N_2$
16	Ethyl formate	$C_3H_6O_2$	72	2,6-Dimethylpyrazine	$C_6H_8N_2$
17	2-Ethyltoluene	C_9H_{12}	73	Ethyl methyl disulfide	$C_3H_8S_2$
18	Phenylethylene	C_8H_8	74	2-Ethyl butyric acid	$C_6H_{12}O_2$
19	Ethylbenzene	C_8H_{10}	75	2-Methyl butyric acid	$C_5H_{10}O_2$
20	Methylbenzene	C_7H_8	76	3-Methyl butyric acid	$C_5H_{10}O_2$
21	(+)-Limonene	$C_{10}H_{16}$	77	1-Methyl piperidine	$C_6H_{13}N$
22	Acetophenone	C_8H_8O	78	2,4,5-Trimethyloxazole	C_6H_9NO
23	Naphthalene	$C_{10}H_8$	79	2,3-Dihydrobenzofuran	C_8H_8O
24	Benzopyrrole	C_8H_7N	80	2,3-Dimethylnonane	$C_{11}H_{24}$
25	Benzyl alcohol	C_7H_8O	81	1,2,4-Trimethylbenzene	C_9H_{12}
26	2-Pentylfuran	$C_9H_{14}O$	82	2,3,5-Trimethylpyrazine	$C_7H_{10}N_2$
27	2,3-Butanediol	$C_4H_{10}O_2$	83	2,5-Dimethylpyrazine	$C_6H_8N_2$
28	Furfuryl alcohol	$C_5H_6O_2$	84	2-Ethyl-6-methylpyrazine	$C_7H_{10}N_2$
29	2-Amyl alcohol	$C_5H_{12}O$	85	2,6-Di-*tert*-butyl-*p*-cresol	$C_{15}H_{24}O$
30	3-Amyl alcohol	$C_5H_{12}O$	86	Butanoic acid-ethyl ester	$C_6H_{12}O_2$
31	2-Ethyl hexanol	$C_8H_{18}O$	87	2-Methyl-butanoic acid	$C_5H_{10}O_2$
32	2-Nonanone	$C_9H_{18}O$	88	2-Methyl-3-heptanone	$C_8H_{16}O$
33	Furaldehyde	$C_5H_4O_2$	89	3-Hydroxy-2-Butanone	$C_4H_8O_2$
34	Ethyl propionate	$C_5H_{10}O_2$	90	Hydroxy-2-methyl-4-pyrone	$C_6H_6O_3$
35	Ethenyl ethanoate	$C_4H_6O_2$	91	3,5-Dimethyl-2-ethylpyrazine	$C_8H_{12}N_2$
36	Ethyl isobutyrate	$C_6H_{12}O_2$	92	4-Methyl-1-penten-3-ol	$C_6H_{12}O$
37	Acetic acid	$C_2H_4O_2$	93	3,5-Dimethyl-4-heptanol	$C_9H_{20}O$
38	Isobutyric acid	$C_4H_8O_2$	94	2,5-Dimethyl-3-hexanone	$C_8H_{16}O$
39	Pentanoic acid	$C_5H_{10}O_2$	95	3-Hydroxy-3-methyl-2-butanone	$C_5H_{10}O_2$
40	Diethyl acetic acid	$C_6H_{12}O_2$	96	2,2-Dimethyl-3-heptanone	$C_9H_{18}O$
41	Benzaldehyde	C_7H_6O	97	1,2,4,5-Tetramethylbenzene	$C_{10}H_{14}$
42	2-Nonanone	$C_9H_{18}O$	98	2-Ethyl-3,6-dimethylpyrazin	$C_8H_{12}N_2$
43	2-Butanone	C_4H_8O	99	2-Chloro-1-propyl isopropyl Ether	$C_6H_{13}ClO$
44	2-Pentanone	$C_5H_{10}O$	100	Butanoic acid,2-methyl-hexyl ester	$C_{11}H_{22}O_2$
45	Diallyl trisulfide	$C_6H_{14}S_3$	101	2,3,5-Trimethyl-6-ethylpyrazine	$C_9H_{14}N_2$
46	Diethyl disulfide	$C_4H_{10}S_2$	102	2,5-Dimethyl-3-isopropyl pyrazine	$C_9H_{14}N_2$
47	Isopropyl disulfide	$C_6H_{14}S_2$	103	1,2,4,5-Tetrazine-3,6-diamine	$C_2H_4N_6$
48	2-Heptanone	$C_7H_{14}O$	104	*N*-(dithiocarboxy)-*N*-methyl-glycine	$C_4H_7NO_2S_2$
49	Oct-1-en-3-ol	$C_8H_{16}O$	105	1-(2,4-Dimethyl-furan-3-yl) ethanone	$C_8H_{10}O_2$
50	2-Pentyl-Furan	$C_9H_{14}O$	106	Acetic acid,3-mercapto-3-methylbutyl ester	$C_7H_{14}O_2S$
51	2,3-Butanedione	$C_4H_6O_2$	107	Propanoic acid,2-hydroxy-1-methylethyl ester	$C_6H_{12}O_3$
52	2,3-Heptane dione	$C_7H_{12}O_2$	108	*N*-(2-Methylbutylidene)-2-methylbutylamine	$C_{10}H_{21}N$
53	3-Methyl-2-pentanone	$C_6H_{12}O$	109	3-(2-Oxopropyl) bicyclo[2.2.1]heptan-2-one	$C_{10}H_{14}O_2$
54	4-Methyl-2-pentanone	$C_6H_{12}O$	110	3,6-Bis(*N*,*N*-dimethyl amino)-9-methyl carbazole	$C_{17}H_{21}N_3$
55	5-Methyl-2-hexanone	$C_7H_{14}O$	111	3-Phenylpropionic acid,2,4,6-trichlorophenyl ester	$C_{15}H_{11}Cl_3O_2$
56	6-Methyl-2-hexanone	$C_8H_{16}O$	112	Benzaldehyde,2,4-dinitro-2,2-dimethyl hydrazone	$C_9H_{10}N_4O_4$

biological oxidation of the body and cause metabolic disorders. The storage of vitamin B2 in the body is very limited, so it should be provided by the diet every day, and natto is an important way to intake vitamin B2.

Vitamin E is a fat-soluble vitamin. Its hydrolysate is tocopherol, which is one of the most important antioxidants. Vitamin E can also inhibit the lipid peroxide reaction in the crystalline body of the eye, expand the peripheral blood vessels, improve blood circulation, and prevent the occurrence and development of myopia (Mohd Zaffarin, Ng, Ng, Hassan, & Alias, 2020). Contained in natto α-tocopherol (vitamin E) can eliminate the harm of highly unsaturated fatty acids to human body.

There are two kinds of vitamin K in natto such as vitamins K1 and K2, of which vitamin K2 is rich (Jeong, Gu, Park, Lee, & Kim, 2022). Natto vitamin K2 is a fat-soluble vitamin, which exists in the viscous substance outside natto. Natto bacteria can produce a large amount of vitamin K2, which can generate bone proteins, increase bone density and prevent fractures (Schurgers et al., 2007; Fujita et al., 2012).

2.4. Other components

Natto is rich in carbohydrates, and its oligosaccharides and crude polysaccharides also have unique biological activities on tumor, hepatitis, cardiovascular, anti-aging and so on. A large amount of food fibers such as hemicellulose, cellulose and lignin in natto are important polysaccharide components in natto, which can promote intestinal peristalsis and adjust intestinal digestion

Table 2
List of vitamin content of natto.

Amino acids	Total amino acids (g/100 g)	Free amino acids (g/100 g)	Dissociation rates (%)
Serine	1.2	0.04	4
Valine	1.0	0.10	10
Proline	1.5	0.07	4.5
Leucine	1.6	0.28	18
Glycine	0.6	0.06	10
Cystine	0.2	0.01	5
Alanine	0.8	0.20	25
Tyrosine	0.5	0.03	6.5
Arginine	0.9	0.09	10
Histidine	0.6	0.08	14
Threonine	0.8	0.22	26
Glutamate	3.4	0.36	11
Isoleucine	1.0	0.12	12
Tryptophan	0.2	0.04	22
Methionine	0.2	0.02	10
Aspartic acid	2.0	0.04	2
Phenylalanine	1.0	0.10	10

and absorption function. Some macromolecules in natto polysaccharides can also be degraded into oligosaccharides of small molecules in the fermentation process of natto bacteria to facilitate absorption (Yang, Yang, & Yang, 2019). The naturally occurring oligosaccharides in natto include sucrose, raffinose, stachyose, etc. Because of their unique bifidobacteria proliferation characteristics, they can increase the beneficial bacteria in the intestine and improve the physiological function (Gao, 2014). There are also unsaturated fatty acids in natto such as linolenic acid, linoleic acid, eicosapentaenoic acid, and docosahexaenoic acids that are not available to the human body and can only be taken from food (Kanghae, Eungwanichayapant, & Chukeatirote, 2017). These unsaturated fatty acids can enhance the fibrinolytic activity of nattokinase and improve blood circulation (Takagaki, Suzuki, Suzuki, & Hasumi, 2020).

3. Pharmacological actions of natto

3.1. Thrombolytic effects

In 1987, an alkaline protease was extracted and isolated from natto for the first time, which was named as nattokinase (NK) and its molecular formula is $C_{20}H_{23}BCl_2N_2O_9$ (Sumi, Hamada, Tsushima, Mihara, & Muraki, 1987). It was confirmed that NK has a strong effect of thrombus dissolution through the dog thrombosis model. Current researches showed that the thrombolytic mechanism of NK mainly includes five aspects (Chen, Sha, Ren, Xi, & Wang, 2003; Weng, Yao, Sparks, & Wang, 2017): (1) Dissolving thrombus by directly dissolving fibrin (skeleton structures of thrombus). Studies have shown that NK can directly act on the enzyme cleavage sites of cross-linked fibrin thrombus len-tyr and Ser-hrs to hydrolyze long-chain skeleton fibrin into soluble small molecules, so as to directly dissolve thrombus. (2) Inhibition of platelet aggregation. NK can reduce the elevated levels of thromboxane (B2) and prostaglandin E_2 (PGE_2) in the plasma of the model group, and increase the level of 6-keto-prostaglandin F1α (6-K-PGF1α) to prevent platelet aggregation and inhibit the formation of thrombosis (Yan, Feng, Xu, & Wu, 2021). Furthermore, preincubating NK and platelet for 10 min in buffer solution could inhibit the increase of Ca^{2+} induced by thrombin to repress platelet aggregation (Ji et al., 2014). (3) NK stimulates vascular endothelial cells to produce tissue-type plasminogen activator (t-PA) and inhibit the production of plasminogen activator inhibitor-1 (PAI-1) of endothelial cells (Ji et al., 2014; Yatagai, Maruyama, Kawahara, & Sumi, 2007). As shown in Fig. 1, t-PA activates fibrinogen *in vivo* to form fibrinolytic enzymes, which dissolve fibrin emboli. T-PA is about 6–7 ng/mL in a normal body, while it is detected to be more than 10 ng/mL 4 h after oral NK. Therefore, it can be inferred that NK can play a thrombolytic role by stimulating the body to produce T-PA and increasing fibrinolytic activity. In addition, a study has shown that the dose dependence of PAI-1 content decreased with the increase of NK concentration in a certain range of concentration, which indicated that to a certain degree NK might play antithrombotic role through reducing the PAI-1 secretion level of endothelial cells (Shah & Minocheherhomji, 2022). (4) Catalyzing the conversion of prourokinase to urokinase. It has been suggested that NK can also activate prourokinase to produce urokinase, another fibrinogen activator, to accelerate thrombolysis *in vivo* (Weng, Yao, Sparks, & Wang, 2017). (5) In addition, the multifunctional cationic peptides from natto extracts exhibit angiogenic activities in tube formation assays, which indicated that the bioactive peptides potentially contribute to antithrombotic effects (Taniguchi et al., 2019).

3.2. Antibacterial action

The substances with antibacterial activity in natto mainly include natto fermentative bacteria and their metabolites. Among

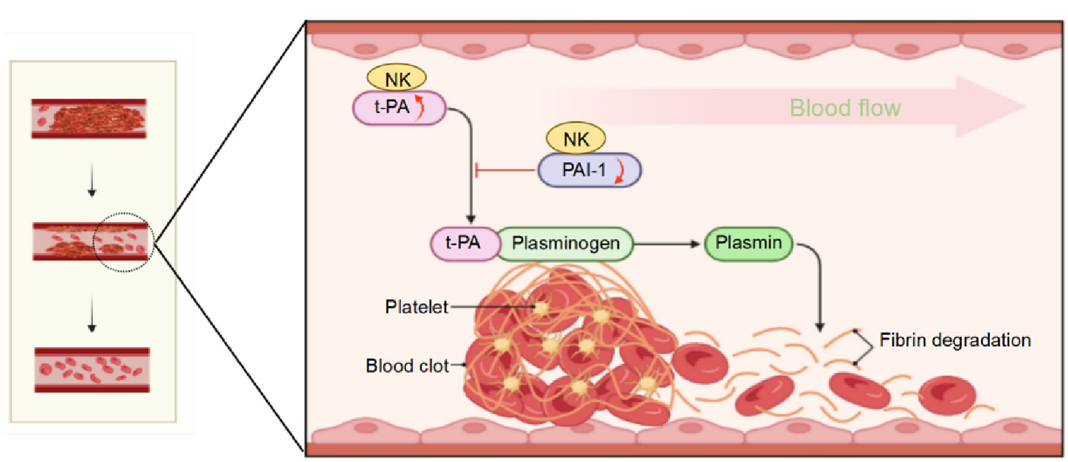

Fig. 1. Thrombolysis mechanisms of nattokinase (NK). NK stimulates endothelial cells to produce t-PA and inhibits PAL-1 T-PA activates fibrinogen to form plasmin, which dissolves thrombus skeleton structure fibrin net, and releases platelets, and blood flow gradually returns to normal.

them, *Bacillus subtilis* natto, as a sort of the most important probiotics, have shown obvious antibacterial activity. Researches have shown the inhibitory effect of *B. subtilis* natto derivatives on *Enterococcus faecalis* that is considered to be the leading cause of hospital-acquired infections (Lin et al., 2021). Some derivatives from the culture fluid of *B. subtilis* natto may be useful for controlling *E. faecalis* biofilms. Furthermore, bacterial poly-γ-glutamic acid (γ-PGA) from *B. subtilis* natto can significantly improve the viability of probiotics and does not contain any of the genes that are usually responsible for toxin production (Bhat et al., 2013). Therefore, it can be inferred that γ-PGA could be used as a food ingredient for the delivery of probiotic bacteria. The antibacterial effects of natto are extremely complex and subtle. The antibacterial activities and immunomodulatory mechanism of Natto were shown in Table 3.

3.3. Antitumor effect

Soybeans, the raw material for natto production, already contain anticancer substances such as flavonoids, isoflavones, phytoestroprotease inhibitors and phytic acids. After fermentation, the contents of anticancer substances of natto are higher than soybeans, such as trypsin inhibitor genistein polyamine (Nan et al., 2017; Oba et al., 2021). Some studies have proved that natto polypeptides extracted from natto powder have significant antibacterial effect and exhibit antitumor activity through cancer cell assay (Cao, Liao, Wang, Yang, & Lu, 2009). In addition, studies demonstrated that natto freeze-drying extract (NFDE) and natto water extract (NWE) could induce cell autophagy at low concentrations (<3 μg/mL) or within a short time while the concentration increases to 5 μg/mL or after a moment, to be replaced by apoptosis (Chou et al., 2021). The process was shown in Fig. 2.

3.4. Hypotensive effect

The viscous material in natto contains a variety of angiotensin converting enzyme (ACE) inhibitors, which can restrain the rise of blood pressure (Okamoto, Hanagata, Kawamura, & Yanagida, 1995). Furthermore, the antihypertensive effect of natto is closely related to its thrombolytic action. The anticoagulant activity of nattokinase enhances blood circulation by dissolving fibrin, reducing blood clotting and soluble fibrin monomer *in vivo*. The water extracts of *B. subtilis*-fermented pigeon pea (a kind of natto) (100 mg/kg bw) markedly attenuated the systolic blood pressure (SBP) and the diastolic blood pressure (DBP) after administration by 21 mmHg and 30 mmHg for 8 h *in vivo* model (Lee, Lai, & Wu, 2015). This phenomenon was closely related to the angiotensin converting enzyme inhibition (ACEI) activity of nattokinase and the antioxidative activities of natto, thereby exerting antihypertensive effect, which indicates that natto has benefits for cardiovascular health. Moreover, a study has confirmed that the culture filtrates from *B. subtilis* natto (CFB) lowers blood pressure via renin-angiotensin system (RAS) in spontaneously hypertensive rats fed with a high-cholesterol diet (Kim, Kim, Kim, & Kwon, 2011). Systolic blood pressure and plasma renin activity were significantly suppressed by the CFB. Renal angiotensin II concentrations were also decreased, whereas whole blood clotting and euglobulin clot lysis time showed insignificant results (Fig. 3). Taken together, natto, after fermentation, could potentially be used as dietary supplement to suppress hypertension.

3.5. Anti-osteoporosis effect

Bones are metabolically active tissues that renew themselves throughout a person's life. Cytokines, together with hormones, nutrients and growth factors, are involved in tightly regulated bone remodeling. Vitamin K, as a multifunctional vitamin, plays a key role in maintaining bone strength and has been confirmed that it has a positive effect on bone metabolism. Vitamin K plays an anabolic role in bone growth through promoting osteoblast differentiation, upregulation of specific gene transcription in osteoblasts, and activating the bone-related vitamin K dependent proteins, which play a pivotal role in extracellular bone matrix mineralization (Akbari & Rasouli-Ghahroudi, 2018; Kubota & Shimizu, 2009). Natto bacteria is the primary bacteria found to produce vita-

Table 3
List of antibacterial action of natto.

Active ingredients	Purpose	Summary of findings	References
B. subtilis natto	Probiotic	*B. ubtilis* natto enhanced the growth and (or) viability of lactobacilli, possibly through production of catalase and subtilisin.	Hosoi, Ametani, Kiuchi, & Kaminogawa, 2000
Natto	Rat feed additive	The treatment of natto significantly improved injury-induced disruption of blood–nerve barrier and loss of matrix component such as laminin and fibronectin. Oral intake of natto has the potential to augment regeneration in peripheral nerve injury, possibly mediated by the clearance of fibrin and decreased production of TNF-α.	Pan et al., 2009
B. subtilis natto	Probiotic	*B. subtilis* natto did not stimulate IgE-mediated allergic reactions, but increased serum IgG and IFN-γ levels in the probiotic-fed calves, which could benefit calf immune function	Sun, Wang, & Zhang, 2010
B. subtilis natto	Probiotic	*B. subtilis* natto improved milk production and milk components yield, decreased SCC and promotes the growth of total ruminal bacteria, proteolytic and amylolytic bacteria, which indicate that *B. subtilis* natto has potential to be applied as a probiotic for dairy cows.	Sun, Wang, & Deng, 2013
B. subtilis natto	Probiotic	*B. subtilis* natto thalli played the important role in promoting rumen fermentation when applied as a probiotic in dairy ration	Sun, Li Bu, Nan, & Du, 2016
Bacillus probiotic spores	Immunomodulator	Ability of *Bacillus* probiotic spores for colonization of the intestinal epithelial cells and their interference with LPS/IL-8 signaling was proposed as main mechanisms of these probiotics for activity on the inflamed human gastrointestinal tract	Azimirad, Alebouyeh, & Naji, 2017
Natto peptide	Antimicrobial	The natto peptides displayed a novel narrow spectrum of bactericidal activity and inhibited cell separation during cell division of *S. pneumoniae*.	Kitagawa et al., 2017
B. subtilis natto	Fungal Antagonism	*B. subtilis* natto can use complex fungal material as a carbon source for growth, and can effectively deconstruct fungal cell walls.	Schönbichler, Díaz-Moreno, Srivastava, & McKee, 2020
B. subtilis NBRC3134	*C. elegans* feed	*B. subtilis* natto regulated host immunity and emphasized its probiotic properties for preventing and alleviating infections caused by gram-positive bacteria.	Katayama et al., 2021
B. subtilis natto BS04	Probiotic	Studies on the use of *B. subtilis* natto as a spore-forming probiotic bacterium in animal nutrition have shown no hazardous effects and have demonstrated the effectiveness of its use as a probiotic, mainly due to its proven antimicrobial, anti-inflammatory, antioxidant, enzymatic, and immunomodulatory activity.	Ruiz Sella, Bueno, De Oliveira, Karp, & Soccol, 2021

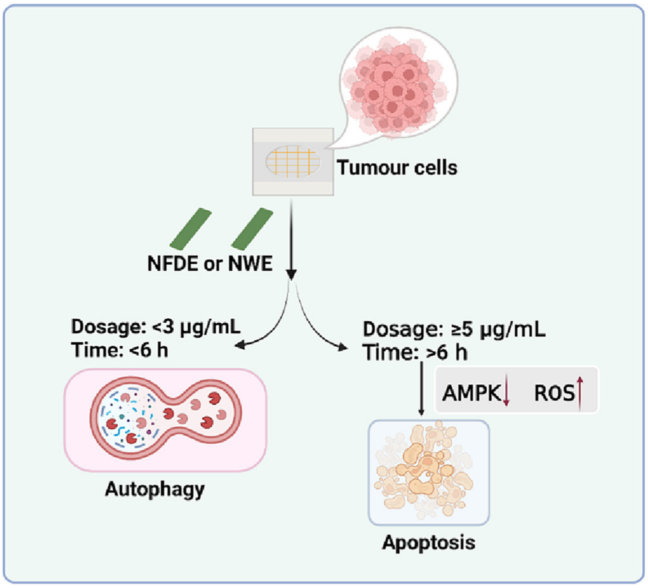

Fig. 2. Antitumor process of natto extracts. NFDE and NWE could induce tumour cell autophagy at low concentrations (<3 μg/mL) or within a short time while the concentration increases to 5 μg/mL or after a moment, to be replaced by apoptosis. NFDE: natto freeze-drying extract; NEW: natto water extract; AMPK: AMP-activated protein kinase; ROS: reactive oxygen species.

min K2, especially menaquinone-7 (MK-7), which can be produced in large quantities in fermented soybean goop to generate osteocalcin, which binds calcium to form bone, thereby enhancing bone density and preventing fractures (Fujita et al., 2012; Kojima et al., 2020). In addition, it has been proved that natto isoflavones (genistein) in natto can prevent and treat osteoporosis caused by lack of estrogen. Genistein can also reduce osteoclastic bone resorption and decrease loss of bone mass in mice by inhibition of protein kinase and protein tyrosine phosphatases, thus genistein can prevent osteoporosis, but there are few experimental materials and further research is still needed (Zhu, Wang, & Su, 2005). Dietary natto intake was significantly indirectly associated with a lower incidence of tooth loss among postmenopausal women, and systemic bone density could be a mediator of this association (Iwasaki et al., 2021).

3.6. For other purposes

In addition to the pharmacological effects described above, natto also have a variety of other effects such as antiviral activity, anti-oxidant action, breast cancer prevention and so on, as illustrated in Table 4.

4. Nutritional value of natto

Natto, as a kind of food, has high nutritional value. During the fermentation process, steamed soybeans will produce a large number of viscous substances, including glutamic acid, fructan, amino acids (such as phenylalanine, tyrosine) and volatile fatty acids (such as butanediol, acetic acid, valeric acid) (El-Safey & Abdul-Raouf, 2004). And natto contains isoflavones, vitamins, dietary fiber, linoleic acid and some minerals which are the same ingredients in natto and soybeans. Among them, isoflavones appear to protect against breast and prostate cancer, menopausal symptoms, osteoporosis and heart disease (Hosoi & Kiuchi, 2003). In addition, it also contains some functional compounds, such as bioactive peptides, enzymes, nattokinase (thrombolytic agent), γ-polyglutamic acid (γ-PGA), etc (Shih & Van, 2001). A research shows that γ-PGA could be used as a food ingredient for the delivery of probiotic bacteria due to its protective effect on probiotic bacteria during

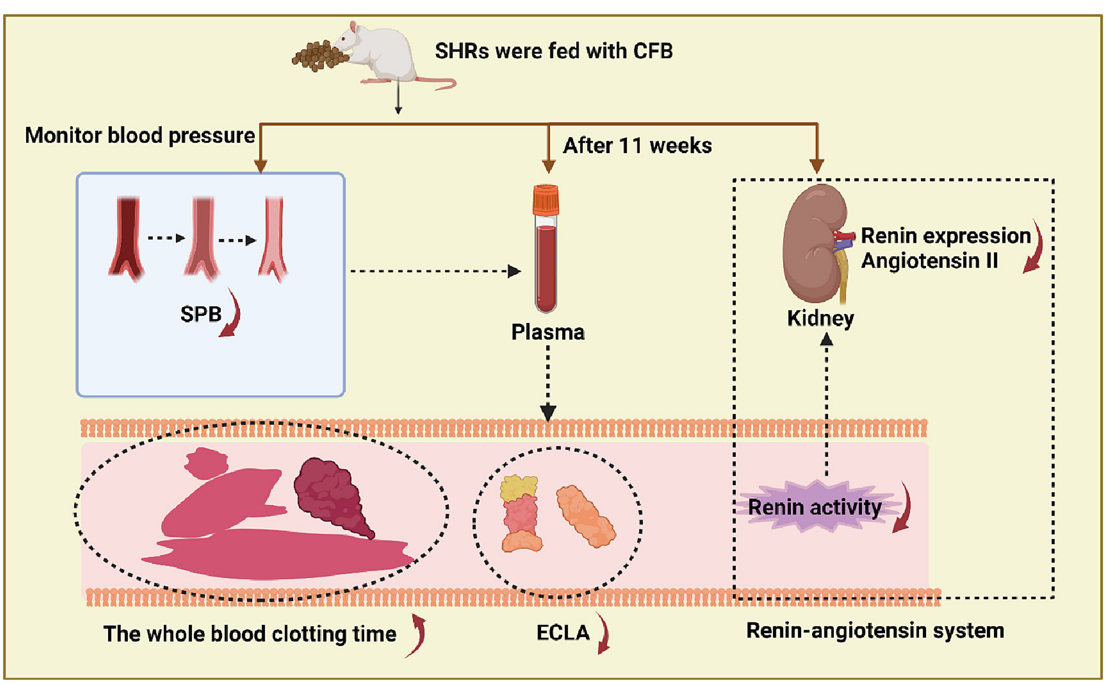

Fig. 3. Antihypertensive mechanism of natto extracts. Feeding CFB to SHRs can reduce blood pressure, including inhibiting plasma renin activity, reducing renal angiotensin II concentration, increasing whole blood coagulation time and reducing euglobulin clot dissolution time. SHRs: the spontaneously hypertensive rats; CFB: the culture filtrates from *B. subtilis* natto; SPB: systolic blood pressure; ECLA: the euglobulin clot lysis time.

Table 4
Summary of other pharmacological functions of natto.

Function	Functional components	Mechanisms of function	References
Anti-oxidation effect	*B. subtilis* natto fermented soy protein isolate (FSPI), Natto extract and natto kinase	FSPI exhibited concentration-dependent free-radical scavenging activity against the $ABTS^+$, hydroxyl radicals (OH), and DPPH radicals (DPPH) *in vitro* and FSPI administration significantly increased the hepatic and serum superoxide dismutase (SOD) activity *in vivo*. Moreover, natto extract and natto kinase can significantly reduce the apoptosis of laser-injured endothelial cells and IL-1β, IL-6, and TNF-α involved in inflammatory reactions. Furthermore, natto extract showed a 60 chelating effect on ferrous ions, which is closely relevant to anti-oxidant activity	Zhang et al., 2021; Chang et al., 2010
Anti-aging effect	Natto extract	The water extract of natto can significantly prolong the adult lifespan of the wild-type worms and render them resistant to oxidative and thermal stress. In addition, treatment with natto extract significantly delay the accumulation of lipofuscin, a characteristic of aging cells	Ibe, Kumada, Yoshida, & Otobe, 2013
Regulation of gut microbiota	Natto, peanut meal extract fermented by *B. subtilis* natto	Natto fermentation products from peanut meal can significantly improve the richness, diversity and uniformity of gut microbiota in antibiotic-induced diarrhoea mice	Jiang et al., 2020; Dimidi, Cox, Rossi, & Whelan, 2019
Prevention of nonalcoholic fatty liver disease (NAFLD)	High γ-polyglutamic acid (high-γ-PGA) natto	Hepatic lipid accumulation typically facilitated the onset of nonalcoholic fatty liver disease (NAFLD). The liver lipid contents, the liver triglyceride contents and the liver cholesterol contents were found to be significantly lower in the PGA group mice fed a 30% high γ-PGA natto diet than those mice served standard diet in the control group	Tamura et al., 2021
Decrease blood sugar, amelioration insulin resitance	Natto and viscous vegetables combined with white rice	The consumption of natto and viscous vegetables combined with WR improved the postprandial blood glucose and insulin profiles in subjects with impaired glucose tolerance (IGT). In addition, the consumption of natto and viscous vegetables in a Japanese style breakfast for two weeks improved insulin sensitivity, lipid profile and oxidative stress in subjects	Taniguchi-Fukatsu et al., 2012
Prevention of breast cancer	Natto lipopeptides	Natto lipopeptides could change the expression levels of estrogen-receptor-α and estrogen-receptor-β and damage DNA of MCF-7 cells, a kind of human breast cancer cell, and therefore inhibit the proliferation of them and induce their apoptosis significantly	Liu, Xiu, Feng, Yong, & Zhang, 2015; An, Liu, Dai, & Zhang, 2014
Antihyperlipidemia effect	Natto enzyme powder	Natto enzyme powder can significantly decrease the levels of total cholesterol, triglyceride and malonaldehyde and low-density lipoprotein cholesterol, and increase the level of total superoxide dismutase and high-density lipoprotein cholesterol. In this way, natto enzyme powder has some antilipidemic effect.	Xuan, Hua, Bin, Sun, & Cui, 2017
Anti-cerebral ischemia effect	6″-*O*-Succinyldaidzin in natto	6″-*O*-Succinyldaidzin had neuro-protective effects through reducing neurological defect and lower infarct volume, and improving cell viability. In addition, 6″-*O*-succinyldaidzin can upregulate the extracellular-signal-regulated kinase (ERK) level and activate nuclear factor E2-related factor 2 (Nrf2) expression in the nucleus	Bao, Chen, Zhang, Huang, & Ding, 2020
Amelioration of obesity	*B. natto*	A study found that *B. natto* reduced the body weight, epididymis fat weight, total cholesterol, triglyceride, low-density lipoprotein while increasing the level of high-density lipoprotein in high-fat diet rats. Therefore, *B. natto* could be used as a potential probiotic supplement to provided new strategy for the prevention and treatment of metabolic diseases such as obesity	Sun et al., 2022; Wang et al., 2020

freeze drying (Bhat et al., 2013). As discussed above, natto contains high-quality proteins and a large amount of bacterial menaquinone-7 (MK-7, a bioactive form of vitamin K2) and intake of MK-7 will contribute to prevent osteoporosis. Furthermore, *B. subtilis* natto, a natural product, is a potential probiotic bacteria that can stabilize intestinal flora and act as human and animal health promoters. Natto bacteria can decompose proteins, carbohydrates, fat and other macromolecular substances, making fermented products rich in amino acids, organic acids, oligosaccharides and other components easily absorbed by the human body. At the same time, natto bacteria can enhance the growth of probiotics, breed fast, consume oxygen in the intestine, and thus inhibit the growth of harmful aerobic bacteria (Ruiz Sella, Bueno, De Oliveiro, Karp, & Soccol, 2021). Natto bacteria can also produce a variety of enzymes to promote the absorption of nutrients by the human body, enhance the immune function of the body, and ensure normal intestinal function (Tsai, Chang, & Kung, 2007). Secondly, the nutritional value of natto is largely reflected in the above-mentioned pharmacological functions. Namely, natto can not only supplement dietary nutrition, but also play a certain role in physiological regulation of cardiovascular and gastrointestinal tract. Therefore, natto is a kind of medicinal food homologous food with potential value.

5. Application of natto

5.1. Fermentation additive

Fermented food is very popular in various countries. It can not only improve the sensory characteristics of products, reduce undesirable ingredients, make nutrients easy to absorb, but also improve the nutritional properties. Due to the fermentation characteristics of *B. natto*, it can be widely used as a fermentation additive in the fermentation industry. For example, compared with unfermented chestnut, fermented chestnut with *B. natto* has significant functional activities, such as fibrinolytic activity, antioxidant level and hypoglycemic effect. In addition, the functional components such as total flavonoids, total phenols and vitamin C were increased significantly, indicating that fermented chestnut has good physical and chemical properties and functional properties, and can be developed as a new healthy functional food in the field of food industry (Dong et al., 2020). Additionally, some scholars (Guo et al., 2019) used *B. natto* strain to produce ginkgo seeds through solid-state fermentation (SSF). The fermented ginkgo seeds showed superior potential to produce nattokinase and higher total flavonoids and lower ginkgolic acids contents, which can improve biological activity and ensure food safety. Furthermore, diets supplemented with *B. subtilis* natto N21 secreting protease, amylase and lipase can improve weight gain and feed efficiency therefore enhanced growth performance of broilers (Chen et al., 2009). Also, it has also been reported that feeding Holstein dairy cows with *B. subtilis* natto can affect hindgut fermentation and microbial bacteria. After *B. subtilis* natto was supplemented, fecal *Alistipes* sp., *Clostridium* sp., *Roseospira* sp., beta-proteobacterium decreased and bifidobacterium increased, which indicating that *B. subtilis* natto has a tendency to change the balance of fecal microbiota (Song, Kang, Wang, Peng, & Bu, 2014).

5.2. Natto food

Natto is a low-cost and nutritious functional food. At present, there are many related food products, and examples are listed below (Fig. 4). (1) Natto compound beverage (Li & Ding, 2012). The product was prepared with natto pulp, millet and sesame soup, honey and citric acid as raw materials and gelatin and sodium alginate as composite stabilizers. (2) Natto chewable tablets (Jiang, Zou, & Xiong, 2007). Maltodextrin, lactose, mannitol and xylitol are used as excipients, and natto lyophilized powder in different proportions is used for tablet pressing. Natto chewable tablets maintain the main nutrients of natto. The products are cool and delicious, easy to carry, and conducive to promotion. (3) Natto pressed candy (Han, Xiang, Tan, Xiao, & Yu, 2015). Natto freeze-dried powder, isomaltulose alcohol, malic acid and mannitol were used as its raw materials. The product fully guarantees the nutritional components and active substances of natto, with sweet and sour taste, unique flavor and easy to carry. (4) Natto sauce. Natto is used as raw material and other ingredients are added to prepare natto sauce, which retains the characteristic nutrients of natto, and neutralizes the bad smell of natto. Natto sauce is conducive to improving the taste, enrich the nutrition, and can be more popular with consumers, such as natto garlic sauce (Yang et al., 2022), natto mushroom sauce (Zuo et al, 2022).

5.3. Natto health products

In addition to the above pharmacological effects, other components of natto also have the functions of maintaining beauty, keeping young and health benefits. Therefore, it can not only be used as food, but also as a health care product. Natto, in addition to retaining the original nutritional components of soybeans, is easier to be digested and absorbed by the human body after fermentation. It also produces many bioactive components, which greatly improves the nutritional and health care efficacy.

Health products include natto soft capsules, natto monascus capsules, etc. Among them, natto soft capsule is made of natto freeze-dried powder, fructooligosaccharide and linseed oil as the main functional components (Pan, Xia, Ye, Qu, & Yu, 2022). Natto *Monascus* capsules are made from natto and *Monascus* spp. as the main raw materials, containing lovastatin, *Monascus* pigment, natto kinase, natto bacteria, isoflavones and other active substances, with dual biological activities of *Monascus* spp. and natto, and significant lipid-lowering and health care effects (Mu et al., 2018).

The raw materials of natto include green beans, yellow beans, black beans, etc., which can be directly consumed after being prepared into natto. Natto products can also be prepared by processing natto. The examples of natto products in Fig. 4 are mainly food and health products. Among them, foods include natto probiotic milk, natto soy sauce, natto monascus tablet candy, etc., and natto health products include nattokinase, natto soft capsules, natto monascus capsules, etc. It can provide certain nutritional value and auxiliary health functions, but cannot replace medication in treating diseases. The products with similar colors in the above image are close to each other.

6. Discussion

Natto, as a traditional daily food with both medicine and food, has a long history of application in medicine and health care, diet and catering. Its extract and isolated components have a wide

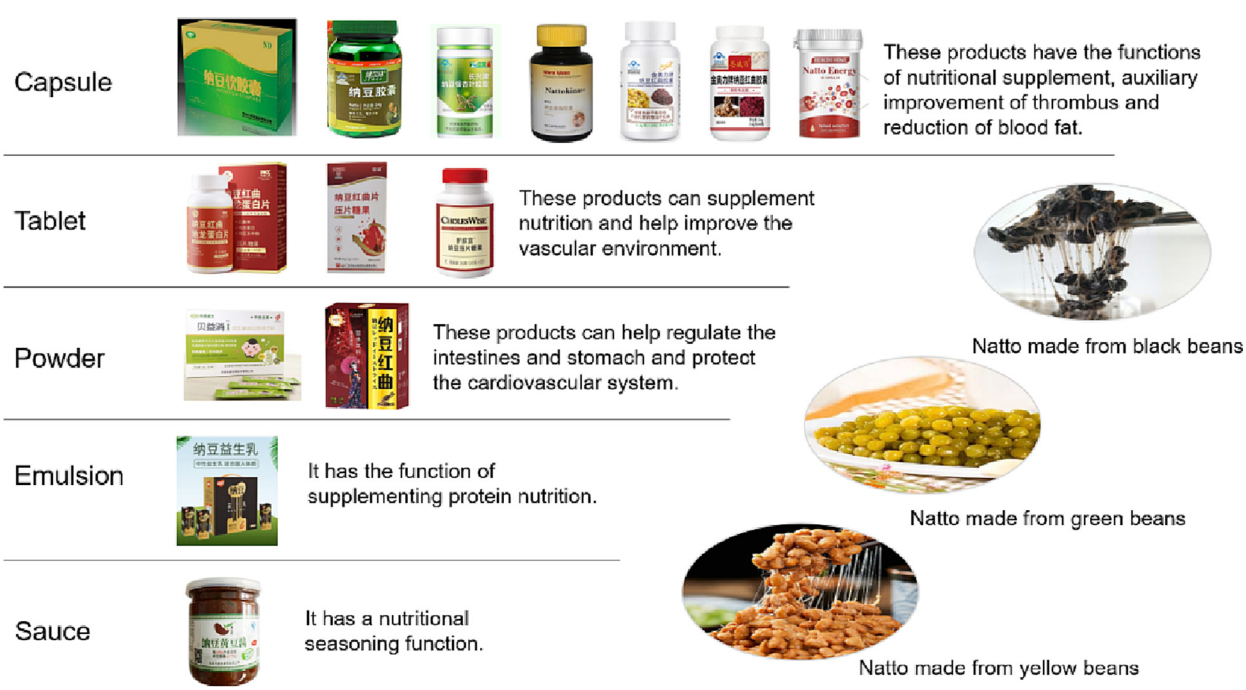

Fig. 4. Natto products in China.

range of biological activities. Scholars in and outside China have conducted extensive research on the isolation and analysis methods and biological activities of natto, and developed related drugs and health food, but there are still some problems in the research of natto as follows.

6.1. Improvement of bad flavor of natto

The unpleasant "ammonia odor" produced by natto itself has deterred some people from natto dietary products, which is also one of the factors limiting the development of natto (Liu et al., 2021). Therefore, especially for natto oral pharmaceutical products or health care products, we should eliminate the "ammonia odor" in the process of preparation as much as possible.

6.2. Classification of active ingredients in natto

Nattokinase in soybean products from different sources has rich biodiversity. The identification of natto protease, the analysis of different kinds of fermentation products and the study of the activity of different components are of great significance to the development and utilization of natto.

6.3. Correlation between natto components and efficacy

The modern pharmacological research of natto mostly focuses on some aspects of thrombolytic function and effective components, while there are less researches on other active substances and mechanisms that can reduce blood pressure, prevent osteoporosis, antibacterial, anticancer, antioxidant and other effects. At present, in the pharmacological research of natto, except thrombolytic components and their mechanisms are relatively clear, other pharmacological effects lack abundant clinical research evidence, and the mechanisms of action are not clear. The toxicology and drug interaction of its main pharmacological active substances need to be further studied.

6.4. Safety and quality control of natto and its products

The original fermentation method of natto does not stipulate standard operation for the fermentation process, but only covers the soybeans with rice withered grass after cooking, until the surface is covered with viscous filaments according to personal judgment. Such a rough traditional operation will inevitably lead to that the quality of the fermentation process cannot be guaranteed. In addition, there are many kinds of strains produced after soybean fermentation, which leads to great controversy. It is necessary to improve the relevant standards of natto preparation and strengthen the systematic, scientific and consistent research of natto standards.

At present, the application of natto is more focused on health food, but its high medicinal value has not been fully served to people's lives in the form of products. Most of the current research is based on animal or cell experiments, while there are few studies in humans, which are needed to confirm the pharmacological action. Future research should focus on the functional mechanism of natto's pharmacodynamic substances and drug interactions, especially on its anticancer and bactericidal effects, prevention of osteoporosis, antioxidation and other effects. We should also improve the fermentation process, screen high-yield strains, improve quality standards, and repeatedly develop the medicinal value of natto to improve the health level of human.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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