Safety of Fermented Foods

Assessing risks in fermented food processing practices and advice on how to mitigate them

- 1 | Introduction to fermented food safety
- 2 | Starter cultures & fermented food standards

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Section 3 | Food safety reviews of fermented foods

A national working group of health inspectors, food safety specialists, and industry fermentation experts reviewed this food safety guidance.

Each fermented food review includes:

- background on the food,
- a description of the food preparation,
- a food flow chart,
- a review of the potential issues with the food preparation, and
- food safety control points.

Foods covered in this guidance are sorted in order of increasing complexity and fermenting agent.

Figure 1	Fermented foods reviewed in this guideline described by	v fermentation agent and complexity
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Complexity	Foods	Fermenting Agent	Section
high	Sausage	Added LAB ¹ , spontaneous moulds & yeasts	3.13
	Kefir, Kombucha	SCOBY ² based: <i>Acetobacter</i> , yeast & mould	3.11-3.12
	Koji, Miso	Aspergillus, spontaneous or added yeast & LAB	3.10
	Tempeh	Rhizopus	3.9
	Natto	Bacillus	3.8
	Yogurt, Plant-based cheese	Added LAB	3.6-3.7
	Dosa, Idli, Fesikh	Spontaneous LAB and Yeast	3.4-3.5
low	Vegetables, Sauerkraut, Kimchi	Spontaneous or added LAB	3.1-3.3

^{1–}LAB-lactic acid bacteria; ^{2–}SCOBY-symbiotic culture of bacteria and yeast

A non-fermented, high alkalinity processed food is also included in this guidance. Pidan century egg (Section 3.14).

Box 1 | How to use the information in this food safety review

The information presented here lays out best practices for a variety of fermented foods, however, it does not replace or supersede federal and provincial guidance or regulatory requirements for fermented foods. Health inspectors, food safety staff, owner and operators of food processing facilities should follow federal and provincial food safety requirements. This work intends to assist food safety staff (health inspectors) to evaluate the safety of fermented foods and fermentation processes encountered during inspections. Owners and operators of food processing facilities may also find this guidance helpful as it reviews critical control points and measures recommended to produce safe fermented foods. The best available evidence guided this work at the time of publication. The application and use of this document is the responsibility of the user.

This guidance does not include information about good manufacturing practices, labelling practices, or management control programs for cleaning and sanitation, pest control, employee training etc. It is expected that operators will follow approved guidance and seek this information elsewhere.

3.8 Natto

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Overview

Description	Natto is a traditional Japanese food made with soaked, boiled and fermented soybeans. It is characterized by the formation of long sticky threads between the soybeans. Natto is ready-to-eat and served without further cooking, usually eaten with rice. Image: trade t
Starter culture	 Bacillus subtilis natto bacteria are the traditional starter culture used to make Japanese style natto. Mould starter cultures are also used depending on variety of natto, including Aspergillus oryzae (also known as koji mould) and Mucor racemosus. Commercially purchased culture is required. Spontaneous fermentation with rice straw is not recommended for safety concerns.
Key features	 Poly-γ-glutamic acids (PGA) are created between soybeans during fermentation which cause formation of characteristic sticky long threads. Alkaline fermentation occurs at temperatures of approximately 40°C to 50°C. pH increases from 7 to 9 with production of ammonia.
Hazards of concern	 Bacillus cereus and Clostridium perfringens can grow in soybeans that are improperly cooked and cooled. This occurs when cooking fails to destroy spores and/or the cooling period is too long allowing growth of these microbes before inoculation of culture. Although rare, delayed onset allergenic reactions to biogenic amines and PGA have been reported.
Important control points	 Soybeans should be soaked in potable water at temperatures of 4°C or less. Do not acidify water for control as this promotes growth of lactic acid bacteria which interfere with <i>B. subtilis</i> starter culture. Cook step is pressurized at 15 to 20 psi for 20 to 30 minutes. Relative humidity is held at 85 to 95%, then reduced to 75% after 15 hours. After initial cook step, control for cooling once temperature falls below 60°C to 2 hours or less before <i>B. subitilis</i> natto culture is added. After fermentation, soybeans are cooled to 10°C or less within 4 hours to prevent secondary fermentation and development of excess ammonia.

Background

Natto is a traditional Japanese food made with boiled and fermented soybeans characterized by the formation of long sticky threads between the soybeans.

The exact origin of fermented soybean products is still unclear, but fermented soybean products have been consumed in Asian countries throughout their written history, going back nearly one thousand years to 1052.1 The majority of natto is made with a bacterial starter culture Bacillus subtilis var. natto (abbreviated as *B. subtilis* natto). Natto has a strong smell, flavour, and viscous texture. Sticky threads occur from formation of poly-y-glutamic acids (PGA) during fermentation process. The two main types of natto in Japan are non-salted natto (itohiki-natto or sticky natto shown in Figure 2) and salty natto (also known as: shiokara-natto/daitokuji-natto/hama-natto based on geographic names where these types of natto originated).¹ Shiokara-natto (salted natto) is made with fermented black soybeans and inoculated with a koji mould (Aspergillus oryzae) starter culture.

Other starter cultures to ferment natto include two types of mould culture: *Rhizopus oligosporus*, the same mould used to make tempeh and *Mucor racemosus* used to make douchi, the name used for natto in China. Sticky natto is more popular in Japan, and it is often served as a topping to rice or by itself accompanied by raw eggs, soy sauce, mustard, or onion.² Natto is consumed under different names in many Asian countries: douchi in China, tua nao in Thailand, chongkukjung in Korea, and as dozens of other names from various countries.³ Non-salted natto made with *B. subtilis* natto is reviewed in this guideline.

Natto is a fast food in many countries and often served packaged with condiments such as soy sauce and mustard as shown in Figure 3.

Figure 2 | Sticky natto



Sticky natto (non-salted natto or itohiki-natto)



From Nagai (2012). Photo A: modern packaged natto with soy sauce and mustard. Photo B: sticky threads of PGA.

Photo C: traditional rice straw, sterilized with commercial starter applied.⁴

In the past, natto was made from steamed soybeans wrapped with rice straw. The rice straw provided the source of starter culture that began spontaneous fermentation (with wild bacterial *B. subtilis*) and also served to absorb the pungent smell of ammonia released during fermentation.⁵ When pure-culture bacteria was isolated for natto (*B. subtilis* natto), the old method of using rice straw was discontinued, and commercial natto manufacturing began.³ The traditional method is still employed in some areas today as a marketing feature using modern techniques, pasteurized straw and commercial culture. Traditional methods using spontaneous fermentation are not recommended because it is difficult to control the quality of natto from possible contamination with unwanted microorganisms in the rice straw.⁵

Outbreaks and Recalls

In a review of soy product recalls, natto has not been recalled in Canada or elsewhere.⁶ However, natto was mentioned in one recall in Taiwan in 2016 that arose over concerns that the prepackaged soy sauce was produced in a prohibited area in Japan with radiation concerns (Table 1) following the Fukushima Daiichi nuclear power plant destruction after the tsunami in 2011. A search of the outbreaks in the *Publically Available International Foodborne Outbreak Database* and in the U.S. foodborne illness database prior to 2022 did not find any foodborne illnesses related to natto products.^{7,8} However, the Canadian Food Safety Information Network (CFSIN) environmental scanning tool found one case of *Bacillus subtilis* bacteraemia related to consumption of natto in an elderly, immunocompromised case.^{9,10} (Table 2). Late-onset anaphylaxis related to allergenic response to natto and natto PGAs have also been reported, rarely, in this product.

Year(s)	Hazard Category	Hazard detail	Number Recalls	Country (s)	Product Description
2016 ¹¹	Other	Radiation	1	Taiwan	Soy sauce present in the pre-packaged natto recalled

 Table 1
 Recalls related to natto products

 Table 2
 Illness case reports related to natto products

Date	Country	Pathogen/agent causing illness	No. of cases	Reason
2023 ⁹	Japan	Bacillus subtilis	1	Immunocompromised case (due to cancer) developed bacteraemia following natto consumption
2020 ¹²	Japan	Allergen	2	PGA-specific IgE antibodies were detected using an enzyme linked immunosorbent antigen (ELISA) test in addition to traditional skin prick test to diagnose natto allergies
201413	Japan	Allergen	1	Late-onset anaphylaxis linked to natto
201014	Japan	Allergen	1	Late-onset anaphylaxis linked to natto
2004 ¹⁵	Japan	Allergen	1	Late-onset anaphylaxis linked to natto

Description of food preparation for natto

The main steps for making natto, are soaking, boiling, cooling, packaging and fermenting. Soybeans need to be selected and cleaned to strain out damaged beans and extraneous materials. Small round types of soybeans are often preferred because small beans have a higher capacity to absorb water, which offer a more desirable texture to natto.¹⁶ The soybeans are then rinsed in potable water to wash away dirt and other contaminants on the surface of soybeans.

After the process of washing, the soybeans are soaked in a tank with tap water at 0 to 4° C to allow thorough absorption. The weight of soaked soybeans should be 2.2 to 2.5 times more than the weight of the dry soybeans.³

After soaking is completed, hydrated soybeans should be steamed or boiled until soft at 15 to 20 psi (lbf/in² and equivalent to 1-1.5 kg/cm²) for 20 to 30 minutes.³ This step is recommended to denature proteins in soybeans that would otherwise impart undesirable flavours to the final fermented product (trypsin inhibitor and hemagglutinin). From a food safety perspective, it is also beneficial to inactivate spore forming microbes. The cooked natto is next cooled to 40°C and inoculated with the pure starter culture, *B. subtilis* natto. In large commercial operations, starter culture is sprayed onto natto as it is tipped out of the cooking vat. Natto bacterial starter culture must be acquired from a commercial manufacturer who can provide a certificate of analysis for the inoculant bacteria.¹⁷ Following inoculation and mixing, in large commercial automated factories the soybeans are portioned into consumer sized containers that have two sides to them, called poly styrol packages (PSP).

Generally, 30 to 50g inoculated soybeans are portioned into one side of the container and covered with a perforated polyethylene film. The other side of the container has space for a small pack of condiments to be placed on the film.¹ The packages used for natto are hygienic improvements over the traditional packaging method of using rice straw, however, use of plastics and disposables are a concern for waste in the environment.

Box 2 | Cooking under pressure

Cooking foods under pressure allows the food to be heated to temperatures greater than the boiling point of water (>100°C). To increase atmospheric pressure foods must be processed in a canner. Canning equipment is sealed and vented to allow build up of pressure inside the equipment. Atmospheric pressure can be described in several ways: using imperial measurements, psi or lbf/in², using metric measurements as kg/cm², or using other terms specific to pressure such as the Pascal, for example, as mPa (mega Pascals). Use conversion software to switch between measures depending on the equipment settings. Pressure canning allows specific conditions of temperature, pressure, and time to reduce risks from spore forming microbes.

The fermentation process requires incubation for approximately 24 hours at 40 to 50°C.¹⁸ Traditional fermentations (prior to commercialization) would take place at room temperatures during winter months. With modern methods, temperature and humidity must be controlled to produce high-quality natto, between 40°C and 50°C with high humidity.^{1,19} The initial relative humidity should be controlled between 85% to 90% during the first stage of fermentation (for six to 16 hours), and then reduced to 75% after 16 hours to avoid hardness of natto.¹⁹ The internal temperature of the natto should reach between 48°C to 52°C by hour 14 for optimal natto quality.¹⁹ Once fermentation is complete, the natto should be cooled within four hours to at least 10°C or lower.¹⁹ Fermentation usually finishes in 20 to 24 hours, with maturation of the natto at cooler temperatures for an additional 8 to 24 hours.^{1,19}

After incubation, a white mucilaginous coating can be found on the fermented soybeans signifying the natto fermentation process is complete and the natto is ready for consumption. At hour 24, the fermented natto should be cooled and matured at temperature below 10°C to lower the production of ammonia.²⁰ Natto pH will rise from pH 7 to a pH of 9. Following incubation natto is transferred from the fermentation chamber to the cooling chamber and stored at or less than 10°C. Natto is packaged, labelled and maintained at or less than 10°C before shipping. Natto is usually transported within one day since it is a perishable product, and its shelf life is about 7 to 12 days if refrigerated below 10°C.¹⁹ Refrigerated temperatures of 4°C and less should be used for shipping and refrigerated display. Natto can be stored for several months if it is kept frozen at temperatures of -18°C or lower.^{4,11} Natto is considered a ready-to-eat product, served without further cooking, usually with rice.²¹

A one page guidance for operators created in Alberta identifies several steps as control points to limit growth and introduction of pathogenic micro-organisms: soaking under 4°C to control for *B. cereus*; cooking/boiling to control for growth of spore-forming bacteria *B. cereus* and *C. perfringens*; hygienic conditions so cross-contamination with other pathogens is avoided (in the cooling step after boiling, during packaging and portioning), and the use of commercial starter culture to ensure spoilage and harmful microbes are not introduced.

Starter culture and natto quality concerns. Issues for quality of natto food products include contamination of starter culture and natto with lactic acid bacteria (LAB) and bacteriophages (phages) during manufacture. Studies that surveyed phage contamination in natto producing factories found contamination was mainly detected in factories with poor sanitation practices in the processing environment of these facilities.⁴ The first report of natto products contaminated with a *B. subtilis* (natto) phage (PN-1 phage) was reported by Fujii *et al*, in 1967, who observed contaminated natto products lose their characteristic viscous substance poly-γ-glutamic acid (PGA), resulting in a product often referred to as abnormal natto.^{4,22} Phage contamination was rarely detected in modern factories⁴ but can be found on factory floors.²³ LAB have been detected in soaking tanks and are of similar concern as their presence may lead to contaminated *B. subtilis* culture.¹⁹ Phages and LAB can be avoided by good hygiene practices and regular facility sanitation of equipment and processing areas, such as cleaning soybean soak tanks.

Natto food flow chart | Process flow and controls



Potential issues with natto food preparation

lssue	Description
Soaking of soybeans	All soybeans must be soaked in water at 4°C or less for about 24 to 30 hours to achieve full absorption of water into the soybeans. The lower soaking temperature inhibits growth of <i>B. cereus</i> . The weight of soybeans should increase by ~2.5-fold, water absorption into soybeans prevents natto hardness.
	Regular cleaning of the soaking tanks is required to avoid LAB contamination. In this type of fermentation LAB are spoilage agents and may impair fermentation by <i>B. subtilis</i> natto ¹⁹ .
Incomplete steaming/cooking	Cooking is a check/control point in this process (not a CCP), check that cooking is sufficient by prolonged boiling, steaming or cooking under pressure. Pressure cooking should reach 15 to 20 psi (lbf/in ²) for 20 to 30 minutes. ^{3,19} This step is recommended to reduce spore-forming pathogens such as <i>B. cereus</i> and <i>C. perfringens</i> , and to denature undesirable soybean proteins (trypsin inhibitor and hemagglutinin). ^{24,25}
Cooling	Soybeans should be cooled to 40°C prior to inoculation of starter culture. If culture is added before cooling has occurred, this may kill the natto bacteria, resulting in a failed fermentation. If cooling takes longer than six hours, spoilage or pathogenic contaminants may grow in the cooked soybeans, before the natto culture is established. Cooling is a CCP, and should be controlled to 2 hours or less once the temperature falls below 60°C, with a critical limit of 6 hours.
Commercial starter cultures	Pure culture, <i>B. subtillis</i> natto is required for natto fermentation. Starter cultures must be acquired from specialist manufacturers who can offer a certificate of analysis (COA) demonstrating purity. Natto culture is available in powdered or liquid form to directly mix into the cooled soybeans.
Bacteriophage contamination	Good hygiene practices and sanitation of facilities is required to prevent contamination. Abnormal natto will be produced if contaminated with PN-1 phage (loss of PGA). ⁴
Portion and Package	Equipment (utensils and containers) must be properly cleaned and sanitized to avoid introducing spoilage or pathogenic microorganisms.
Fermentation time and temperature	Temperature must be controlled to between 40°C to 50°C for 22 to 24 hours. The humidity should be maintained between 85% and 90% initially, and then reduced to 75% from 6 to 16 hours, and to 55% at 16 to 24 hours. ¹⁹
	Avoid secondary fermentation. It will create an ammonia smell, negatively effect natto quality, and promote spoilage by other organisms. ⁵
Cooling	Soybeans must be cooled to 10°C or lower following fermentation for 24 hours.
	Cooling helps inhibit the growth of natto bacteria and lower the ammonia production. ²⁰ Cooling is a CCP and should occur within two to four hours after fermentation is completed.
Storage & Transport	Once all processing steps of natto are completed, natto must be stored at or below 4°C.
Shelf life	The recommended shelf life for commercially fresh natto is between 7 and 12 days at temperature 10°C or lower, ¹⁹ in Canada we recommend 4°C or lower. Cold storage at frozen temperature (-18°C) may extend the shelf life for several months to a year.
Food Handling Hygiene practices	Good hygiene practices must be implemented during food production, processing and storage of natto to reduce possibly introducing spoilage microorganisms or contaminants into natto.
Biogenic amine formation	Biogenic amines may form in natto causing delayed onset anaphylaxis. Temperature control is the main preventative measure, cool natto following fermentation within 4 hours, during transportation, storage and consume within the product shelf life.

Natto food safety control points

- To limit contaminants, soybeans must be inspected through a selection process to strain out damaged beans and extraneous materials (rocks etc.),
- Wash, then soak soybeans in cooled water at temperature below 4°C (this is a CCP) to minimize growth of *Bacillus cereus*. Note this check point: avoid use of acidified water, normally recommended during this step, because it may create conditions for LAB growth and is not recommended for *B. subtilis* alkaline fermentations;
- Fully cook soybeans by prolonged boiling or under pressure 15 to 20 psi (lbf/in²) or 1-1.5 kg/cm² at or above 121°C for 20 to 30 minutes to prevent formation of undesirable substances (1) trypsin inhibitor (e.g., hemagglutinin) and (2) inhibit growth of heat-stabile harmful microorganisms.
- Control cooling once temperature falls below 60°C to 2 hours, and within six hours (critical limit) before adding starter culture to soybeans.
- Select commercial *B. subtilis* natto active starter culture with a COA. Operators are recommended to ensure these cultures do not actively create biogenic amines;²⁶
- Spontaneous and back-slopped natto starter cultures are not recommended;
- Clean and sanitize all utensils, containers and equipment used for natto to avoid introducing contaminants;
- Control fermentation time and temperature between 40°C to 50°C for 22 to 24 hours, and maintain humidity between 85% and 90% for the first 6 to 16 hours, then reducing humidity to 75% (note: these conditions control for optimal natto stickiness and quality);
- Cool finished natto to at least 10°C or colder within 4 hours after the main fermentation step to inhibit growth of natto bacteria culture and lower ammonia production;
- Ensure all equipment, environment and surfaces in the food premises are clean and sanitary to avoid introducing microorganisms and contaminants during all processing steps. Sanitary hygiene and safe food handling are critical throughout natto production to prohibit introduction of contaminants through handling (food hand contact),²⁷ to prevent formation of toxic amines from contaminants, and to prevent poor quality natto from LAB or phage contamination during processing.

Potential health issues with natto

Although natto is a popular food around the world, there are occasional reports of histamine and allergenic reactions associated with natto products. Microbial concerns shared by all soybean based products include formation of toxins from moulds (mycotoxins) and *B. cereus* bacterial spores not eliminated during the cooking process.¹⁸ Anti-nutritional factors, such as trypsin inhibitors, can be controlled by full cooking of soybeans and fully fermenting natto.²⁴

Box 3 | Biogenic amines in fermented foods

Biogenic amines (BAs) can be produced by microbes in fermented foods, such as fermented soybean products, vegetables, cheeses, sausage, and fish. Normal BA intake does not cause illness as intestinal amine oxidases break down and detoxify the BAs.^{28,29} If large amounts of BA are ingested, or if amine oxidase activity is inhibited, then acute toxic symptoms can occur such as nausea, respiratory distress, hot flushing, sweating, heart palpitations, headache, bright red rash, burning sensations in the mouth, alterations in blood pressure, diarrhea and hypertensive crises.^{28,30,31} The toxic effects of BA may vary between individuals depending on individual sensitivity and on the consumption of alcohol or drugs that are monoaminooxidase inhibitory.^{32,33}

The main BAs are histamine, tyramine, β -phenylethylamine, putrescine, cadaverine and spermidine. Health Canada has set action levels for histamines in anchovies, and fermented fish sauces and pastes at 200 mg/kg and for other fish and fish products at 100mg/kg.³⁴ However, there are no guidelines set for other fermented food products and BAs other than histamines in Canada, or elsewhere in the world. At present, the toxic doses in food are suggested only for three biogenic amines: 100-200 mg/kg for histamines, 100-800 mg/kg for tyramine and 30 mg/kg for phenylethylamine.²⁹

Operators manufacturing fermented foods are not required to test for BAs in their products. Operators are recommended to list BAs as a potential chemical hazard in their food safety plan. Operators can address risks of BAs by: (*continued on page 12*)

(1) ensuring preventative measures are in place, the facility is clean and sanitary, handling practices are hygienic to limit bacteriophages and bacteria that interfere with the culture process;

(2) optimizing the fermentation: regulating time, temperature, moisture content, salt concentrations, and storage conditions; using good quality ingredients;

- (3) purchasing commercial starter culture and/or verifying quality of the starter culture;
- (4) monitoring that the expected culture activity occurs within correct timeframe; and
- (5) monitoring for expected pH.

If a fermented food is linked to foodborne illness in consumers, inspectors are recommended to consider testing for BAs if symptoms and onset of illness in cases fit suspected BA illness. Further information about BAs and testing is found in Section 2 of this guidance.

Biogenic amines in natto: Because natto is a fermented soybean product containing nitrogenous amino acid precursors of biogenic amines, biogenic amine activity in natto products is an important food safety risk.^{26,35} Histamine concentrations in individual natto products (as high as 457mg/kg) were found in products in Taiwan at levels much higher than the recommended limit of 100mg/kg.^{27,35} Consumers ingesting levels of histamine above 100 mg/kg in natto may experience symptoms of scombroid poisoning. Scombroid refers to a category of fish (e.g., tuna) that have high levels of histidine in their muscle tissues. Under temperature abuse conditions, bacterial conversion of muscle histidine to histamine in the flesh of the fish may causes allergenic reactions. Other types of biogenic amines detected in natto products include high concentrations of phenylethylamine (51 mg/kg) and tyramine (300 mg/kg), values exceeding suggested dose limits of 30 mg/kg for phenylethylamine and 100 mg/kg for tyramine.^{26,35}

Most commercial natto products have safe levels of biogenic amines. However, microorganisms capable of producing biogenic amines during soybean fermentation could potentially result in food poisoning should biogenic amine levels exceed 1000 mg/kg, considered dangerous for health and the upper tolerance limit of most consumers from all biogenic amine sources.²⁸ Therefore, formation of harmful biogenic amines should be considered as a chemical hazard in the food safety plan for these products. Strict hygiene practices and control of microbial activity and contamination during storage can help inhibit the formation of toxic amines, such as avoiding temperature abuse of the product during processing and storage.^{33,35,36}

Allergenic issues: Several cases of natto-induced anaphylaxis have been reported. Food-induced allergy symptoms usually occur within two hours of consuming foods, however, natto anaphylaxis symptoms are delayed, occurring 12 or more hours after ingestion. The allergen reactions are usually accompanied by urticaria, dyspnea, or dizziness.¹⁴ Tests revealed that the allergenic component of natto is poly-γ-glutamic acid (PGA), which is the main viscous substance in natto. The cause of delayed-type hypersensitivity of natto may be due to the large molecular weight of PGA, which requires longer time for digestion and absorption.^{13,37,38} Although delayed-type hypersensitivity disease is uncommon, people who have anaphylaxis to poly-γ-glutamic acid or have a soy allergy should avoid consuming natto products.³⁹

lssue	Description
Histamine formation	Biogenic amines may be produced during fermentation. Avoid temperature abuse, practice proper hygiene during processing, and control secondary microbial activity with cold storage of 10°C or less to inhibit biogenic amine formation. ^{33,36}
Late-onset anaphylaxis	Although these conditions occur rarely, ensure allergen labelling for the soy-based food is present. Natto-induced late onset anaphylaxis presents with symptoms of urticaria, dyspnea, or dizziness. ¹⁴ Several studies have found poly- γ -glutamic acid is the major allergenic component of natto. ^{13,37,38}
Nutritional factors	Trypsin inhibitors are considered anti-nutritional factors (ANF) because they reduce absorption of dietary proteins, and are (for example, hemagglutinin) linked to excess flatulence during digestion. ^{24,25} Fully cooked (boiled) soybeans and fully fermented natto will prevent formation of ANFs.

Table 3 Summary of health issues with natto

References

- Sarkar PK, Nout MJR, CRC Press. Chapter 2. Diversity of plant-based food products involving alkaline fermentation. In: Handbook of Indigenous Foods Involving Alkaline Fermentation [Internet]. Boca Raton, FL: CRC Press, Taylor & Francis Group; 2015. Available from: <u>https://www.taylorfrancis.com/books/edit/10.1201/b17195/handbook-indigenous-foods-involving-alkaline-fermentation-prabir-sarkar-robert-nout?refId=88d9646a-25ba-4d8f-81f6-97fb6353a316&context=ubx
 </u>
- Jaine T, editor. Natto. In: The Oxford Companion to Food [Internet]. 3rd ed. Oxford, UK: Oxford University Press; 2013. p. 921. Available from: <u>https://www.oxfordreference.com/display/10.1093/acref/9780192806819.001.0001/acref-9780192806819;j-</u> sessionid=D4845F00FC1AF7F3DAC664D48E3EF619
- Shurtleff W, Aoyagi A. History of natto and its relatives (1405-2012): extensively annotated bibliography and sourcebook [Internet]. Lafayette, CA: Soyinfo Center; 2012 [cited 2021 Jun 19]. Available from: <u>http://www.soyinfocenter.com/pdf/151/</u> <u>Natt.pdf</u>
- 4. Nagai T. Chapter 6. Bacteriophages of Bacillus subtilis (natto) and their contamination in natto factories. In: Kurtboke I, editor. Bacteriophages [Internet]. IntechOpen; 2012. p. 270. Available from: <u>https://www.intechopen.com/chapters/32281</u>
- Liu K. Chapter 14. Food use of whole soybeans. In: Johnson LA, White PJ, Galloway R, editors. Soybeans [Internet]. Urbana, Illinois: AOCS Press; 2008 [cited 2021 Jun 19]. Available from: <u>http://link.springer.com/10.1007/978-1-4615-1763-4</u>
- 6. Dale K, McIntyre L. Canadian and international fermented food recalls extracted from food recall databases. Regina, SK: Saskatchewan Ministry of Health; 2022.
- 7. Mascarenhas M. Foodborne outbreaks associated with fermented products from 1985 to Feb 7, 2022, recorded in Publically Available International Foodborne Outbreak Database (PAIFOD). Guelph, ON: Public Health Agency of Canada; 2022 p. 11.
- 8. McIntyre L. Foodborne outbreaks associated with fermented foods from 1998 to 2018 recorded in the National Outbreak Reporting System (NORS) Centers for Disease Control and Prevention Database. Vancouver, BC: Environmental Health Services, BC Centre for Disease Control; 2022 p. 6.
- Hashimoto T, Yahiro T, Khan S, Kimitsuki K, Hiramatsu K, Nishizono A. Bacillus subtilis bacteremia from gastrointestinal perforation after natto ingestion, Japan. Emerg Infect Dis [Internet]. 2023 Oct [cited 2023 Sep 28];29(10). Available from: <u>https://wwwnc.cdc.gov/eid/article/29/10/23-0084_article</u>
- 10. Government of Canada. The Canadian Food Safety Information Network (CFSIN) [Internet]. [cited 2023 Sep 28]. Available from: <u>https://inspection.canada.ca/science-and-research/cfsin/eng/1525378586176/1525378959647</u>
- 11. Natto products recalled on suspicion over origin Taipei Times [Internet]. 2016 [cited 2022 Jul 4]. Available from: https://www.taipeitimes.com/News/taiwan/archives/2016/12/12/2003661067
- 12. Suzuki S, Nakamura Y, Kawano Y, Nishioka K. A case of "late-onset" anaphylaxis caused by fermented soybeans; natto. Arerugi [Internet]. 2006;55(7):832. Available from: <u>https://pubmed.ncbi.nlm.nih.gov/16883111/#:~:text=Natto%20is%20a%20Japa-nese%20traditional,eating%20a%20meal%20containing%20natto.</u>
- 13. Honda T, Michigami M, Miyachi Y, Kabashima K. A case of late-onset anaphylaxis to fermented soybeans (natto). J Dermatol [Internet]. 2014;41(10):940–1. Available from: <u>https://onlinelibrary.wiley.com/doi/10.1111/1346-8138.12616</u>
- 14. Matsubayashi R, Matsubayashi T, Yokota T, Ohro Y, Fujita N, Nakashima Y, et al. Pediatric late-onset anaphylaxis caused by natto (fermented soybeans). Pediatr Int [Internet]. 2010;52(4):657–8. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1442-200X.2010.03083.x
- Inomata N, Osuna H, Yanagimachi M, Ikezawa Z. Late-onset anaphylaxis to fermented soybeans: the first confirmation of food-induced, late-onset anaphylaxis by provocation test. Ann Allergy Asthma Immunol [Internet]. 2005;94(3):402–6. Available from: <u>https://www.annallergy.org/article/S1081-1206(10)60995-7/pdf</u>
- 16. Wei Q, Chang SKC. Characteristics of fermented natto products as affected by soybean cultivars. J Food Process Preserv [Internet]. 2004;28(4):251–73. Available from: <u>https://ifst.onlinelibrary.wiley.com/doi/10.1111/j.1745-4549.2004.23047.x</u>
- 17. Prakash V, Martín-Belloso O, Keener L, Astley SB, Braun S, McMahon H, et al., editors. Regulating Safety of Traditional and Ethnic Foods [Internet]. 1st ed. Waltham, MA, USA and Kidlington, Oxford, UK: Academic Press, Elsevier; 2016. 489 p. Available from: https://www.sciencedirect.com/book/9780128006054/regulating-safety-of-traditional-and-ethnic-foods

- Chen F, Zhang X, Li Z. Chapter 15. Safety of fermented products based on soybean. In: Prakash V, Martin-Belloso O, Keener L, Astley S, McMahon H, Lelieveld H, editors. Regulating Safety of Traditional and Ethnic Foods [Internet]. 1st ed. Waltham, MA, USA and Kidlington, Oxford, UK: Academic Press, Elsevier; 2016. p. 311–8. Available from: <u>https://www.sciencedirect.com/</u> <u>science/article/abs/pii/B9780128006054000153</u>
- Hosoi T, Kiuchi K. Chapter 9. Natto a food made by fermenting cooked soybeans with Bacillus subtilis (natto). In: Farnworth ER, editor. Handbook of Fermented Functional Foods [Internet]. 2nd ed. Boca Raton, FL: CRC Press, Taylor & Francis Group; 2008. p. 267–90. Available from: <u>https://www.routledge.com/Handbook-of-Fermented-Functional-Foods/Farnworth/p/ book/9781420053265</u>
- Kada S (Mizkan GCorp Handa, Aichi (Japan)), Yabusaki M, Kaga T, Ashida H, Yoshida K. Identification of two major ammonia-releasing reactions involved in secondary natto fermentation. Biosci Biotechnol Biochem [Internet]. 2008;72(7):1869–76. Available from: <u>https://academic.oup.com/bbb/article/72/7/1869/5941165</u>
- 21. Tamang JP. Chapter 4. Plant-based fermented foods and beverages of Asia. In: Hui YH, Evranuz EÖ, editors. Handbook of Plantbased Fermented Food and Beverage Technology [Internet]. 2nd ed. Boca Raton, FL: CRC Press, Taylor & Francis Group; 2012 [cited 2023 Jun 2]. p. 51–80. Available from: <u>https://www.taylorfrancis.com/chapters/edit/10.1201/b12055-8/plant-based-fermented-foods-beverages-asia-jyoti-prakash-tamang</u>
- Fujii H, Oki M, Keshino J, Takeya R. On the formation of mucilage by Bacillus natto. Part VII. Isolation and characterization of a bacteriophage active against "natto"-producing bacteria. J Agric Chem Soc Jpn [Internet]. 1967;41(1):39–43. Available from: https://www.jstage.jst.go.jp/article/fstr/15/3/15_3_293/_article
- 23. Nagai T. Overview of studies on Bacillus subtilis (natto) bacteriophages and the prospects. JARQ Jpn Agric Res Q [Internet]. 2012;46(4):305–10. Available from: <u>https://www.jstage.jst.go.jp/article/jarq/46/4/46_305/_article</u>
- 24. Avilés-Gaxiola S, Chuck-Hernández C, Serna Saldivar SO. Inactivation methods of trypsin inhibitor in legumes: a review. J Food Sci [Internet]. 2018;83(1):17–29. Available from: https://ift.onlinelibrary.wiley.com/doi/10.1111/1750-3841.13985
- 25. Soetan K, Oyewole O. The need for adequate processing to reduce the anti-nutritional factors in plants used as human foods and animal feeds: a review. Afr J Food Sci [Internet]. 2009;3(9):223–32. Available from: https://academicjournals.org/article/article1380638739_Soetan%20and%20Oyewole.pdf
- 26. Kim B, Byun BY, Mah JH. Biogenic amine formation and bacterial contribution in natto products. Food Chem [Internet]. 2012;135(3):2005–11. Available from: <u>https://www.sciencedirect.com/science/article/abs/pii/S0308814612010643?via%3Di-hub</u>
- Tsai YH, Chang SC, Kung HF. Histamine contents and histamine-forming bacteria in natto products in Taiwan. Food Control [Internet]. 2007;18(9):1026–30. Available from: <u>https://www.sciencedirect.com/science/article/abs/pii/ S0956713506001770?via%3Dihub</u>
- Doeun D, Davaatseren M, Chung MS. Biogenic amines in foods. Food Sci Biotechnol [Internet]. 2017 Dec 13 [cited 2021 Oct 14];26(6):1463–74. Available from: <u>https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6049710/</u>
- 29. Ruiz-Capillas C, Herranz AH, editors. Biogenic amines on food safety [Internet]. MDPI- Multidisciplinary Digital Publishing Institute; 2019 [cited 2023 Jan 26]. Available from: <u>https://directory.doabooks.org/handle/20.500.12854/42202</u>
- Tofalo R, Perpetuini G, Schirone M, Suzzi G. Biogenic amines: toxicology and health effect. In: Caballero B, Finglas PM, Toldrá F, editors. Encyclopedia of Food and Health [Internet]. Oxford: Academic Press; 2016 [cited 2023 Jan 26]. p. 424–9. Available from: <u>https://www.sciencedirect.com/science/article/pii/B9780123849472000714</u>
- Gardini F, Özogul Y, Suzzi G, Tabanelli G, Özogul F. Technological factors affecting biogenic amine content in foods: a review. Front Microbiol [Internet]. 2016 Aug 12 [cited 2023 Jan 26];7. Available from: <u>https://www.frontiersin.org/articles/10.3389/</u> <u>fmicb.2016.01218/full</u>
- Barbieri F, Montanari C, Gardini F, Tabanelli G. Biogenic amine production by lactic acid bacteria: a review. Foods [Internet].
 2019 Jan [cited 2023 Jan 26];8(1):17. Available from: <u>https://www.mdpi.com/2304-8158/8/1/17</u>
- 33. Santos MS. Biogenic amines: their importance in foods. Int J Food Microbiol. 1996;29(2–3):213–31.
- 34. Health Canada. Health Canada's maximum levels for chemical contaminants in foods [Internet]. 2005 [cited 2023 Jan 26]. Available from: <u>https://www.canada.ca/en/health-canada/services/food-nutrition/food-safety/chemical-contaminants/maxi-mum-levels-chemical-contaminants-foods.html</u>

- 35. Saha Turna N, Chung R, McIntyre L. A review of biogenic amines in fermented foods: occurrence and health effects (in press). 2023;
- 36. ten Brink B, Damink C, Joosten H, In't Veld JH. Occurrence and formation of biologically active amines in foods. Int J Food Microbiol [Internet]. 1990;11(1):73–84. Available from: <u>https://www.sciencedirect.com/science/article/abs/ pii/016816059090040C</u>
- 37. Inomata N, Osuna H, Ikezawa Z. Late-onset anaphylaxis to Bacillus natto–fermented soybeans (natto). J Allergy Clin Immunol [Internet]. 2004;113(5):998–1000. Available from: <u>https://www.jacionline.org/article/S0091-6749(04)01069-3/fulltext</u>
- Suzuki K, Futamura K, Sato N, Nakamura M, Matsunaga K, Yagami A. Two cases of fermented soybean (natto) allergy diagnosed using the skin prick test and enzyme linked immunosorbent assay for poly-γ-glutamic acid. J Dermatol [Internet]. 2020;47(12):e429–30. Available from: <u>https://onlinelibrary.wiley.com/doi/10.1111/1346-8138.15601</u>
- 39. Health Canada. Soy A priority food allergen [Internet]. 2012 [cited 2021 Oct 15]. Available from: <u>https://www.canada.ca/en/</u> <u>health-canada/services/food-nutrition/reports-publications/food-safety/priority-food-allergen.html</u>

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Figure 2: Sticky natto, Getty Images.

Figure 3: Modern natto (with permission): https://www.intechopen.com/chapters/32281