Journal of Advanced Scientific Research
Available online through https://sciensage.info
ISSN
0976-9595
Review Article

NATTOKINASE: ARMOUR AGAINST THROMBOSIS

Shah Nisha R.*[1], Minocheherhomji Farida P.[2]
[1]Dolat-Usha Institute of Applied Sciences and Dhiru-Sarla Institute of Management & Commerce, Valsad, Gujarat, India
[2]B.P Baria Science Institute, Navsari, Gujarat, India
*Corresponding author: nisharshah67@gmail.com

Received: 05-03-2022; Revised: 13-06-2022; Accepted: 20-06-2022; Published: 30-06-2022
© Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License https://doi.org/10.55218/JASR.202213504

ABSTRACT
In this review paper, an emphasis is made to focus on the history, source, structure, mode of action, and application of Nattokinase. The review reveals that Nattokinase can act as a potent thrombolytic agent as compared to other agents which are commercially available. Studies on NK as a thrombolytic agent have also be proved it as an useful for dissolving blood clots by converting plasminogen to plasmin, which further act on fibrin clots. Nattokinase is been isolated from *Bacillus subtilis* varnatto. Various studies been done on the application of NK has shown to overcome the undesirable effects caused by other tissue plasminogen activator, Streptokinase, Staphylokinaseetc and increased its area of application.

Keywords: Nattokinase (NK), Tissue Plasminogen Activator, Thrombolytic agents, Fibrin.

1. INTRODUCTION
Cardiovascular diseases (CVDs) are the leading cause of death globally, taking an estimated 17.9 million lives each year [1]. One person dies every 36 seconds from cardiovascular disease. Heart disease costs the United States about $363 billion each year from 2016 to 2017. This includes the cost of health care services, medicines, and lost productivity due to death [2]. Thrombosis is referred to the undesirable blood clots in blood vessels. Due to the increasing rate of cardiovascular diseases observed by WHO in 2017, the demand of this agents has increased considerably. Many agents such as anti-platelet, anti-coagulant drugs, tissue plasminogen activators, enzymes etc. are used for the treatment of thrombosis, but unwanted side effects like allergy, bleeding complication, hemorrhage etc. have change the focus of research to obtain an effective agents will less or no complication. Moreover, the cost involved in the production of other thrombolytic agents such as Staphylokinase, Streptokinase, Urokinaseetc make them unavailable for the people in countries with low income [3]. Enzymes are powerful biomolecular weapons having wide range of applications. Among these, fibrinolytic enzymes are the enzymes that have proteolytic activity and reduce thrombosis. Fibrinolytic enzymes catalyze the degradation of fibrin clots by converting inactive plasminogen to active plasmin or by fibrin degradation process [4]. Fibrinolytic enzymes have been produced from different organisms such as *Staphylococcus*, *Streptococcus*, *Bacillus*, *Pseudomonas*, *Aspergillus* etc. On the basis of catalytic mechanism microbial fibrinolytic enzymes have been classified into three types: serine protease, metallo protease, both serine and metallo-protease [5].

In recent years, an attempt is made to produce fibrinolytic enzyme from fermented foods such as Japanese food Natto, Tofuyo, Korean Chungkook-Jang soy sauce, Shrimp paste and edible honey. The enzymes obtained from such Asian food have found to have considerable nutritional property and can act in thrombolytic therapy. Even the studies have revealed that this type of food are cost efficient, minimize the health risk and can be used in treatment along with the supplements of nutrition obtained from such foods [6,7].

2. HISTORY
Before 1980s, no one knew about the exact mechanism by which natto consumption can be helpful to treat cardiovascular diseases. Sumit, a Japanese researcher at the Chicago University medical school and his colleague in 1980 discovered nattokinase as a potent fibrinolytic

enzyme present in natto. They extracted enzyme from natto that can degrade fibrin and at the same time act as a plasmin substrate. Hiroyuki Sumi named this novel enzyme as "Nattokinase" (NK). Since then, the research on nattokinase has been started in Japan, Korea, China and other countries. NK is a serine protease purified and extracted from natto, a traditional Japanese food produced from the fermentation of soyabeans with the organism *Bacillus subtilis* (natto) [8].

3. PREPARATION

In 1960s, natto was earlier made by cooked soyabean wrapped in dried rice straw having *Bacillus subtilis* but now it is replaced by a polystyrene foam box due to which the preservation and transport is easy. The surface of natto contains mucilaginous fluid consisting of polyglutamic and polyfructan which forms thread like structure. Due to this sticky mucilage and its odor, NK is not accepted for consumption [9]. The process includes thefollowing steps: soaking soyabeans in water, steaming, dewatering, cooling at 40°C, and wrapping treated soyabean in rice straw for 1-2 days in a warm place, obtain natto (Fig 1). This production process is cost effective and environment friendly [10]. This is the traditional way of producing natto in Japan as the soyabean used act as a good nitrogen source which increases the effective production of Nattokinaseand still the same procedure is followed worldwide.

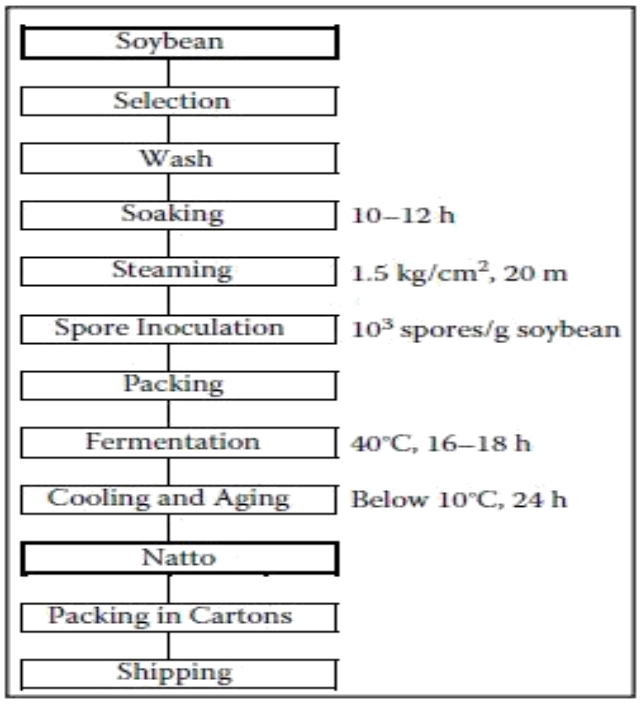

Fig. 1: Preparation flow chart [11]

4. STRUCTURE

NK is a single polypeptide chain composed of 275 amino acids (molecular weight 27,724) which shows strong fibrinolytic activity (Fig. 2). NK is a serine proteases belonging to subtilisin family.

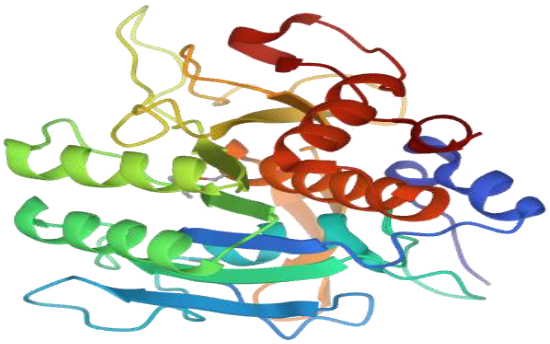

Fig. 2: Structure of Nattokinase [12]

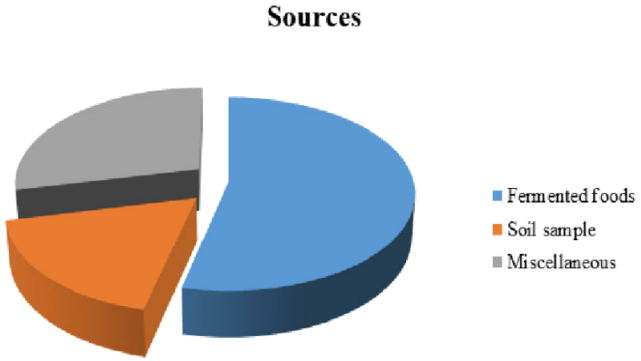

Fig. 3: Sources to obtain Nattokinase producing *Bacillus subtilis* [13]

5. MODE OF ACTION OF NATTOKINASE

Nattokinase is obtained from soil, water, fermented foods (Natto, Douchietc), soya containing products, sea foods etc produced by *Bacillus subtilis*. Oral administration of Nattokinase and its beneficial effects have recently drawn attention on considering NK as a novel thrombolytic agent [14]. NK act by hydrolyzing fibrin and plasmin substrate, converting inactive pro-urokinase to urokinase, degrade plasminogen activator inhibitor-I, and increase the efficacy of tissue plasminogen activator anf thereby increasing the fibrinolytic activity. Research carried out on the application of NK has shown that Nattokinase has considerably little or no side effects as compared to other thrombolytic agents (Staphylokinase, Strpetokinase, Urokinase etc.) which are commercially available.

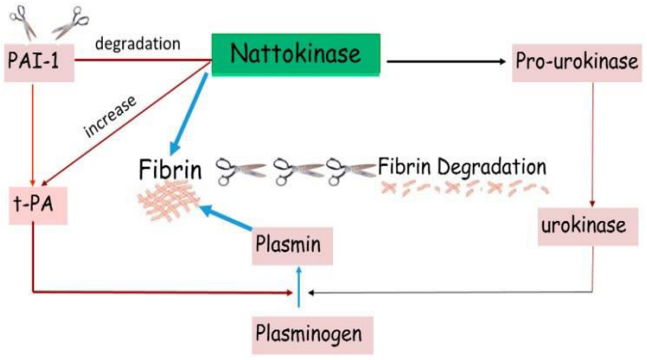

Fig. 4: Mechanism of action of Nattokinase [15]

6. APPLICATIONS

6.1. Reduces risk of heart diseases

NK dissolves the blood clot, which maintain good blood vessel's structure, improve blood flow and lower the risk of heart diseases. It was studied that NK-01 was able to inhibit the activity of coagulation factors through the up-regulation of proteinase C inhibitors and protein S. NK-01 also inhibit the angiotensinogen conversion to Ang-II and promote the degradation of kininogen to reduce the blood pressure and thereby reducing the risk of occuring of heart diseases [16].

6.2. Food supplement

Natto food made from soyabean is rich in protein, vitamin (K2, C), iron, zinc, selenium and copper. Nattō is high in fiber and delivers over 20% of the recommended daily intake of iron, magnesium, calcium, vitamin C, and potassium [17]. NK due to its rich content of superoxide dismutase act as anti-oxidant and counter act on free radical and reduces the oxidative stress. NK prevent free radicals from tearing inside of the blood vessels and prevent from heart stroke [18].

6.3. Improve sinus health

It has been studied that extensive fibrin deposition due to impairment in fibrin degradation result in nasal polyps which can lead to chronic diseases. NK can shrink blockage-causing nasal polyps and thin mucus, improving air flow and reducing discomfort [19]. So, NK can be used to treat symptoms of respiratory conditions like bronchitis and chronic obstructive pulmonary disease.

6.4. Improve gut microbiome and metabolism

NK can maintain a good balance of bacteria presentin our guts. The poor gut health can weaken the immune system and increase the risk of chronic diseases. Due to the probiotic properties of NK, it has the potential to reduce body fat, regulate and prevent our body from metabolic disorders such as diabetes and obesity. To support this study, it was found out the *Bacillus* natto regulates gut microbiota and adipose tissue accumulation in high fat diet mouse model of obesity. B. natto significantly reduces the body, epididymal fat, perirenal fat, liver, and spleen weight of obese mice, and reduces serum triglyceride and total cholesterol [20].

B. natto also reduces alanine transferase, aspartate aminotransferase, total bilirubin, and total bile acid, and decreases fat cell area and fat droplets in the liver, when compared with the untreated high fat diet group. *Bacillus subtilis* var. natto can inhibit fatty acid synthesis and promote fatty acid catabolism in the liver through expression of Pparα and Srebp-1c. The studies revealed that Metagenomic analysis of gut microbiota showed that B. natto increased the ratio of bacteroidetes to firmicutes and increased the abundance of verrucomicrobia to improve obesity [20].

6.5. Oral administration

NK consumption of 10 mg/kg/day for 4 wk was well tolerated in healthy human volunteers suggesting that the oral consumption of NK is of low toxicological concern [21].

6.6. Anti-hypertension

NK can reduce the risk of cardiovascular diseases by reducing the hypertension. It was studied that NK contains strong inhibitor that suppress angiotension-converting enzyme, that is responsible for hyper-tension peptide hormone, angiotension-II, present in renin-angiotension system [22].

6.7. Digestive capability

In recent research, it has been proved that the reduction in digestion result in poor availability of nutrient which can affect pumping of heart and lead to thrombosis. Some foods contain antinutrients that make it more difficult for nutrient to get absorbed and digested. NK contains more probiotics and less antinutrients which help to absorb nutrients and reduce digestive symptoms [23-25].

7. CONCLUSION

The present review explains about the action, sources, production of Nattokinase enzymes and its application as a thrombolytic agent. Cardiovascular diseases affect the human health in great amount due to the cost involved in the treatment. With the

available information in NK, research need to be focused on exploring novel nattokinase producing organisms with great efficiency and more benefits. Moreover, using *Bacillus* sp. producing NK in food supplements can reduce the undesirable effects and this enzyme can be used as an alternative as compared to other agents available.

8. REFERENCES

1. World Health organization, Cardiovascular diseases (CVDs),https://www.cdc.gov/heartdisease/facts.html, accessed on 11 June 2021.
2. Centre for disease control and prevention, Heart disease facts, https://www.cdc.gov/heartdisease/facts.html, accessed on 7 February 2022.
4. Altaf F, WuS, Kasim V. *Frontiers in Molecular Biosciences*, 2021; 8:476.
5. Krishnamurthy A, Belur D, Subramanya, B.*Journal of Analytical Science and Technology*, 2018; **9(1)**:1-11.
6. Rajagopalan S,GoniasSL, Pizzo SVA. *J. Clin. Invest 1985; 75:413 419*.
7. Yoshinori M, Ada HKW, Jiang B. *Food Research International*, 2005; **38(3):**243-250.
8. Dubey RJ, Kumar D, Agrawala T, Char, PuspP. *African Journal of Biotechnology,* 2011; **10(8)**:1409-1420.
9. Weng Y, Yao J, Sparks S, Wang KY. *International journal of molecular Sciences*, 2017; **18(3)**:523.
10. Nishinari K, Fang Y, Nagano T, Guo S, Wang R. In *Proteins in food processing*, 2018; 149-186.
11. Liu K. Food Use of Whole Soybeans. In Soybeans, AOCS Press; 2008, p. 441-481.
12. Atheltime, The natto flow chart, https://nattomatrika.wordpress.com/2013/05/27/the-natto-flow-chart/, accessed on 27 May 2013.
13. Chatake T, Yanagiswa Y. Crystal Structure of Nattokinase from *Bacillus subtilis*natto, https://www.rcsb.org/structure/4DWW, accessed on 14 March 2012.
14. Selvarajan E. Nattokinase: an updated critical review on challenges and perspectives, https://www.researchgate.net/figure/Fig-1-Pie-chart-depicting-the-preferences-of-sample-type-for-isolation-of-nattokinase_fig1_321687198, accessed on 24 January 2018.
15. Yogesh D, Halami PM. *International Food Research Journal*, 2017; **24(1)**:1.
16. Yungi W, Jian Y, Sawyer S, Kevin W, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5372539/, accessed on 28 February 2017.
16. Pan X, Liang P, Teng L, Ren Y, Peng J, Liu W, Yang Y. *Food Science & Nutrition*, 2019; **7(10)**:3185-3193.
17. Cathy W, What is Nattokinase, https://www.verywellhealth.com/what-is-nattokinase-89831, accessed on 28 April 2021.
18. Heidi M, How antioxidant can prevent stroke, https://www.verywellhealth.com/antioxidants-help-prevent-stroke-3145895, accessed on 19 January 2021.
19. Tokunaga T, Ninomiya T, Kato Y, Imoto Y, Sakashita M, Takabayashi T, Fujieda S. *Allergology International*,2017; **66(4)**:610-616.
20. Wang P, Gao X, Li Y, Wang S, Yu J, Wei Y. *Journal of Functional Foods*, 2020; 68.
21. Lampe BJ, English JC. *Food ChemToxicol*, 2016; **88:**87–99.
22. Okamoto A, Hanagata H, Kawamura Y, Yanagida F. *Plant Foods Hum Nutr,* 1995; **47:**39–47.
23. Yokota T, Hattori T, Ohishi H, Hasegawa K, Watanabe K. *LWT-Food Science and Technology*, 1996; ***29*(8)**:751-755.
24. Alina P, Why natto is super healthy and nutritious, https://www.healthline.com/nutrition/natto#:~:text=6%2C%2016).Summary%3A,body%20absorb%20 nutrients%20more%20easily, accessed on 31 March 2017.
25. Dan B, Health benefits of nattokinase, https://www.webmd.com/diet/health-benefits-nattokinase#1, accessed on 6 November 2020.