ISSN: 2644-2981

Global Journal of Nutrition & Food Science

DOI: 10.33552/GJNFS.2025.06.000626

Iris Publishers

Research Article

The Health Effects of Natto, A Fermented Soybean Food That Efficiently Enhances Isoflavone Bioavailability in Humans

Hiroaki Takeuchi[1]*, Yu Yoshikane[2], Satoe Watanabe[1], Takeru Kojima[1], Mayu Okada[1], Masaya Shiraishi[1], Aoi Okamoto[1], Nanaka Morita[1], Asako Kimura[1]

[1]Department of Medical Laboratory Sciences, Health and Sciences, International University of Health and Welfare Graduate School, 4-3 Kouzunomori, Narita-City 286-8686, Chiba, Japan

[2]Department of Human Living Sciences, Notre Dame Seishin University, 2-16-9 Ifuku-cho, Kita-ku, Okayama-City 700-8516, Okayama, Japan

***Corresponding author:** Hiroaki Takeuchi, Department of Medical Laboratory Sciences, Health and Sciences, International University of Health and Welfare Graduate School, 4-3 Kouzunomori, Narita-City 286-8686, Chiba, Japan

Received Date: November 04, 2025

Published Date: November 11, 2025

Abstract

Fermented soybean-based products are globally recognized for their health-promoting effects, particularly through modulation of the intestinal microbiota. Natto, a traditional Japanese fermented soybean food, is widely consumed in Japan and valued for its potential health benefits. However, few clinical studies have investigated the effects of natto on the intestinal environment or the relationship between its health benefits and isoflavone bioavailability. Nigari, a mineral-rich byproduct derived from deep seawater, plays an important role in human health and is known to promote fermentation.

In this study, we examined the effects of natto supplemented with nigari on isoflavone bioavailability and digestive health. Eighty-two Japanese participants consumed either natto containing isoflavones (Natto group) or placebo-natto lacking isoflavones (p-Natto group) for four weeks. Urinary isoflavones (daidzein, genistein, and equol) were measured to assess isoflavone bioavailability. Daidzein and genistein levels significantly increased in the Natto group but not in the p-Natto group following the intervention. This trend was more pronounced in females, although not statistically significant. Furthermore, equol levels and the conversion efficiency of daidzein to equol significantly increased among equol producers in the Natto group but not in the p-Natto group during the intervention period. No significant differences were observed in constipation improvement between the two groups, and no adverse effects were reported.

In conclusion, these findings indicate that nigari-supplemented, isoflavone-containing fermented natto may serve as a promising functional food that effectively enhances isoflavone bioavailability, potentially by supporting a beneficial intestinal environment.

Keywords: Natto; Urinary isoflavones; Equol; Intestinal environment; Bioavailability

Introduction

Natto is a traditional Japanese food made by fermenting soybeans with *Bacillus subtilis* var. *natto*. Although its distinctive flavor, odor, and sticky texture make it unique, recent biomedical research has highlighted its remarkable nutritional and functional properties. The bioactive effects of natto were reported in the fields of cardiovascular health, metabolism, bone integrity, and aging. Such bioactive effects are due to vitamin K2, nattokinase, and antioxidant activity [1-9]. There are also few clinical studies on the beneficial effects of natto on gut microbiota and gastrointestinal function [10, 11]. Kono et al. demonstrated that intake of natto fluctuated the intestinal microbiota. Ingestion of natto increased the relative abundance of *Bifidobacterium* and *Blautia* as well as the relative abundance of *Bifidobacterium* in males and females, respectively. Natto potentially improves the intestinal environment via microbiome modulation and suggests it be associated with lifestyle-related diseases [10]. Kikushima et al. demonstrated that the changes in pain during defecation as well as the stool color and odor was greater in the test group than in the placebo group. Natto powder reduced fatigue and improved sleep quality more effectively than the placebo powder [11].

Natto includes soybean isoflavones that are well known as beneficial heath effect in humans [12,13]. A clinical study with fermented soybean products suggests that the beneficial effects of soy isoflavones and aglycones occur through their fermentation, digestion, and absorption and modulation of the intestinal microbiota [14]. The relationship between isoflavone intake and absorption of functional aglycones mainly depends on the individual intestinal environment, including the presence of specific digestion/metabolization bacteria and β-glucosidase activity. Aglycones absorbed from the intestine then circulate and act throughout the body [15], meaning the pharmacokinetics/dynamics of isoflavones vary greatly depending on the individual. Therefore, to determine the health benefits of isoflavones in natto intake, it is important that not only the intake/consumption of isoflavones and/or aglycones, but also absorption of aglycones, is examined by using clinical samples such as blood and urine. There are two types of soybean products: fermented products (miso, soy sauce, soymilk, etc.) and unfermented products (tofu, etc.). Tofu, a healthy product, is prepared by coagulating soymilk and natural mineral ingredients obtained from surface seawater such as calcium sulfate, magnesium chloride, calcium chloride, and others, known as nigari (bittern) in Japan [16]. The nigari is rich in minerals and plays an important role in maintaining human health. The consumption of unfermented foods (Tofu) supplemented with nigari from deep seawater (DSW) has also been reported to have beneficial biological effects in humans [17]. Furthermore, it has been demonstrated that nigari from DSW can promote fermentation [18]. A case-study intervention with a fermented product natto containing *B. subtilis* var. *natto* documented shifts in gut microbiota composition after ingestion. However, the human evidence is still small. In the present study, we evaluated the effects of natto supplemented with nigari from DSW through a clinical trial with health adults.

Material and methods

Preparation for natto and intervention: The natto used in this study was produced by a licensed company. During the manufacturing process, natural nigari derived from DSW collected offshore of Muroto, Kochi, Japan (patent No. 6486529; Murotokaiyoushinsousui Co., Ltd., Kochi, Japan), was added at a final concentration of 0.8%. A commercially available placebo natto (p-natto) that did not contain soy isoflavones was used as the control. Participants consumed 40 g per day of either natto (Natto group) or p-natto (p-Natto group) for 4 weeks. A serving of 40 g of natto contained 23.4 mg of isoflavones, whereas 40 g of p-natto contained no detectable isoflavones.

Study design: This randomized, double-blind, controlled trial aimed to compare the intestinal environment between healthy adults who consumed natto and those who consumed p-natto. Assessments were performed using a self-administered questionnaire and urinary isoflavone analyses. The study was carried out in Muroto, Kochi, Japan, in 2024. Although the study protocol was restricted by limited time and budget, it was ethically approved and conducted in accordance with the ethical standards of the 1964 Declaration of Helsinki and its subsequent amendments.

Participants: Eighty-one of the 83 healthy adults initially enrolled were included in this study. Participants with any illnesses, prescription medications, or commercial drug use were excluded. In addition, pregnant women and individuals taking isoflavone-related supplements were excluded. After obtaining written informed consent, the 81 eligible participants were randomly assigned to one of two groups: the Natto group (n = 41; mean age, 43 years; range, 24–75 years; 19 males and 22 females) or the p-Natto group (n = 40; mean age, 45 years; range, 19–72 years; 19 males and 21 females). Throughout the study period, all participants maintained their usual daily routines without any special restrictions, apart from the assigned natto consumption.

Measurement for urinary isoflavones: Urinary isoflavones were quantified using high-performance liquid chromatography (HPLC) as previously described [19]. Briefly, 800 μL of urine was mixed with 80 μL of 1 M sodium acetate buffer (pH 4.5) and 8 μL of β-glucuronidase/sulfatase solution (Sigma-Aldrich, Tokyo, Japan). The mixture was incubated at 37 °C for 4 h to hydrolyze conjugated isoflavones. Subsequently, 80 μL of propyl 4-hydroxybenzoate (100 μg/mL) was added as an internal standard. Samples were extracted with 1.5 mL of dichloromethane, evaporated at 50 °C, and the residues were dissolved in 400 μL of methanol. A 20 μL aliquot was injected into the HPLC system (Shimadzu Co., Ltd., Koto, Japan). The detection limits were 100 ng/mL for genistein and daidzein, and 200 ng/mL for equol. Isoflavone concentrations were corrected for creatinine and expressed as mg/g creatinine (Cre). The conversion efficiency of daidzein to equol was calculated as equol/ (equol + daidzein) (g/g E+D).

Dietary Intake and Health Assessment: Dietary intake and health status were assessed using a self-administered questionnaire. The questionnaire evaluated general health conditions, intestinal

environment (with a focus on constipation), consumption of unusual foods or beverages, and the use of supplements or medications during the study period.

Statistical analyses: Baseline characteristics, including urinary isoflavones such as daidzein, genistein, and equol, were compared between groups using the Mann–Whitney U test. Within-group differences in creatinine-corrected urinary isoflavone levels before and after the intervention were analyzed using the Wilcoxon signed-rank test. Changes in questionnaire responses pre- and post-intervention were evaluated using the chi-square test. All statistical analyses were performed using BellCurve for Excel, version 3.20 (Social Survey Research Information Co., Ltd., Tokyo, Japan). A p-value of < 0.05 was considered statistically significant.

Results

Baseline characteristics of the participants are summarized in Table 1. There were no statistically significant differences in any parameters between the Natto and p-Natto groups. Furthermore, no adverse effects related to the consumption of natto or p-natto were observed in any participants throughout the study period.

Self-administered questionnaire

The constipation improvement rate was 16/41 (39%) and 18/40 (45%) in individuals in the Natto and p-Natto groups without statistical significance, respectively. Three participants in p-Natto group experienced slight worsening of constipation conditions. Overall, females in both groups tended to have a higher improvement rate than males, but the difference was not statistically significant. Next, we analyzed the difference in constipation improvement rate between equol-producers and non-equol-producers in the two groups. Equol producers were defined as individuals with ≥ 200 ng/mL urinary equol. No statistically significant differences were observed between equol-producers and non-equol-producers in the two groups.

Urinary isoflavones analysis

Urinary isoflavones such as daidzein and genistein were detected in all 81 participants. Daidzein and genistein significantly increased in the Natto group after the intervention (all $p < 0.01$; Table 2), but there was no change in the p-Natto group. Equol, the isoflavone-derived aglycone with the highest physiological activity, generated from daidzein through intestinal microbiota, was detected in 14 and 13 participants in the Natto and p-Natto groups, respectively. The proportion of equol-producers in this study was 27/81 (33.3 %), which was relatively low compared with the average of approximately 30–50% in Asian countries [20]. In equol-producers, equol values increased in the Natto group ($p < 0.1$) after the intervention. In contrast, in the p-Natto group, equol values slightly increased after the intervention but not significantly ($p > 0.1$). Similarly, equol conversion efficiency from daidzein after the intervention in Natto group increased ($p < 0.05$), while equol conversion efficiency slightly increased in p-Natto group without statistically significant ($p > 0.1$). Taken together, the significantly increase in equol levels in equol-producers from the Natto group appears to have resulted from both higher daidzein levels and higher conversion efficiency.

Table 1: Characteristics of participants in preintervention of two groups (median)

Natto			p-Natto		
	Total (n=41)		Total (n=40)		
Urine Isoflavones					
Daidzein (mg/g-Cre)	0.72	(0.15-3.07)	1.09	(0.25-5.41)	n.s.
Genistein (mg/g-Cre)	0.79	(0.23-2.54)	1.55	(0.30-5.28)	n.s.
	Total (n=14)		Total (n=13)		
Daidzein (mg/g-Cre)	0.93	(0.26-3.03)	3.37	(0.66-5.50)	n.s.
Genistein (mg/g-Cre)	0.77	(0.25-1.23)	1.74	(0.30-4.96)	n.s.
Equol (mg/g-Cre)	0.25	(0.06-1.41)	0.40	(0.32-2.42)	n.s.
Equol (g/g-Da)	0.37	(0.13-1.65)	0.51	(0.10-1.38)	n.s.
Equol (g/g-E+D)	0.27	(0.11-0.59)	0.34	(0.09-0.58)	n.s.

Table 2: The values of urinary isoflabones of two groups with intervention (median)

Natto (n=41)						p-Natto (n=40)				
preintervention			postintervention			preintervention			postintervention	
Urine Isoflavones										
Daidzein (mg/g-Cre)	0.72	(0.15-3.07)	3.43	(0.63-6.21)	↑**	1.09	(0.25-5.41)	1.28	(0.46-4.71)	n.s.
Genistein (mg/g-Cre)	0.79	(0.23-2.54)	2.71	(1.31-6.14)	↑**	1.55	(0.30-5.28)	1.40	(0.67-3.76)	n.s.
Equol-producer										
Natto (n=14)						p-Natto (control) (n=13)				
preintervention			postintervention			preintervention		postintervention		
Urine Isoflavones										
Daidzein (mg/g-Cre)	0.93	(0.26-3.03)	2.75	(0.60-5.80)	↑#	3.37	(0.66-5.50)	1.36	(0.87-2.85)	n.s.
Genistein (mg/g-Cre)	0.77	(0.25-1.23)	2.65	(1.58-4.94)	↑*	1.74	(0.30-4.96)	2.30	(0.87-3.34)	n.s.
Equol (mg/g-Cre)	0.25	(0.06-1.41)	1.78	(1.09-8.45)	↑#	0.40	(0.32-2.42)	1.01	(0.64-2.70)	n.s.
Equol (g/g-Da)	0.37	(0.13-1.65)	1.48	(0.65-4.54)	↑*	0.51	(0.10-1.38)	1.04	(0.35-1.70)	n.s.
Equol (g/g-E+D)	0.27	(0.11-0.59)	0.60	(0.39-0.82)	↑*	0.34	(0.09-0.58)	0.51	(0.26-0.63)	n.s.
**, $P < 0.01$ (Willcoxon signed-rank test)										
*, $P < 0.05$ (Willcoxon signed-rank test)										
*, $P < 0.05$ (Willcoxon signed-rank test)										
n.s., $P > 0.10$ (Willcoxon signed-rank test)										

Discussion

Although numerous studies have investigated the health effects of soybean-based products, including both fermented and unfermented foods, only a few have specifically examined the impact of natto on the intestinal environment and microbiome [10, 11]. To our knowledge, the present study is the first clinical trial to evaluate the health effects of natto supplemented with nigari derived from DSW (marine resources). Importantly, by measuring urinary isoflavone levels and analyzing equol production together with the efficiency of daidzein-to-equol conversion, we were able to assess potential changes in the intestinal environment. This study demonstrated that consumption of nigari-supplemented natto significantly increased equol levels and equol conversion efficiency, indicating that such natto may promote human health by supporting a favorable intestinal environment. However, intake of nigari-supplemented natto did not convert equol non-producers into producers in this study. Therefore, further investigations are required to clarify the physiological functions of nigari-supplemented natto, particularly focusing on its effects on intestinal microbiota composition and activity.

A study using natto powder (*Bacillus subtilis* var. *natto*) demonstrated that ingestion of natto powder improved the intestinal microbiota, as analyzed by 16S rRNA gene sequencing using next-generation sequencing. After 62 days of natto powder intake, increases in *Bifidobacterium* and *Blautia* were observed, contributing to improved health status among participants [10]. Another clinical study with powdered natto over four weeks showed improved bowel movement, including defecation frequency, as assessed by a questionnaire [11]. Natto is rich in soybean-derived protein, vitamin K, dietary fiber, and *Bacillus subtilis* var. *natto* itself, which acts as a probiotic [21]. The effect of natto on the gut microbiota has been shown to include an increase in *Bifidobacterium* abundance following consumption [22]. Furthermore, natto contains isoflavones, well-known functional compounds. These components are thought to act synergistically to regulate and maintain a healthy intestinal environment. Nigari from DSW was reported to enhance fermentation [18], suggesting that the addition of nigari not only shortens the fermentation process but also alters the compositional and metabolic profile of the fermented products. In fact, the isoflavone concentration in nigari-supplemented natto (58.6 mg/100 g) was higher than that in natto without nigari (49 mg/100 g), indicating that nigari enhanced isoflavone accumulation during fermentation. Nigari-supplemented natto may serve as an effective food source for efficient isoflavone intake.

Isoflavones are metabolized by intestinal microbiota, and the physiologically active aglycones produced are subsequently absorbed into the bloodstream. In particular, equol is produced from daidzein by specific intestinal bacteria; thus, the efficiency of isoflavone digestion and absorption is an important factor when evaluating their health effects. In this study, urinary isoflavone levels, equol, and equol conversion efficiency were significantly increased in the nigari-supplemented natto group during the intervention. Consumption of natto is a common dietary habit in Japan to obtain isoflavones for health benefits. Isoflavones show biphasic plasma and urinary appearance after ingestion [23], and the bioavailability of daidzein and genistein is similar in both plasma and urine [24]. However, clinical data on urinary isoflavone levels remain limited; in particular, to our knowledge, no studies have investigated the relationship between natto consumption and urinary isoflavone excretion. According to the Ministry of Health, Labour and Welfare (2002), the estimated daily intake of soy isoflavone aglycones from soy and soy products in Japan is 16–22 mg/day [25]. However, due to the westernization of dietary habits, this intake has been decreasing. Recently, the Ministry of Agriculture, Forestry and Fisheries (2020) recommended a daily intake of 40–50 mg of isoflavone aglycones, with an upper limit of 70–75 mg, although excessive intake from soy foods alone is unlikely [26]. The absorption of isoflavone aglycones is influenced by an individual's intestinal microbiota composition. In the present study, participants consumed nigari-supplemented natto containing 23.4 mg of isoflavones, and urinary isoflavone levels were increased compared with those in the *p*-Natto group (containing no isoflavones). These findings indicate that consumption of nigari-supplemented natto may modulate the intestinal environment and contribute to human health. Nevertheless, further research is needed to clarify the relationship between absorbed isoflavone aglycone levels and specific health outcomes, including clinical parameters in a larger cohort. This study demonstrated that consumption of nigari-supplemented natto containing 23.4 mg of isoflavones increased urinary isoflavone levels, suggesting a positive influence on the intestinal environment.

There were several limitations to this study. First, the clinical trial was conducted over a relatively short period of four weeks, and a longer study duration may reveal more significant or additional effects. Second, equol producers were defined as participants with urinary equol concentrations ≥ 200 ng/mL, which may not fully capture inter-individual variability in equol metabolism. Finally, some variables could not be statistically analyzed due to the limited number of participants. Further large-scale and long-term clinical studies are warranted to confirm these findings and elucidate the mechanisms underlying the health effects of nigari-supplemented natto.

Conclusion

This clinical trial indicates that nigari-supplemented, isoflavone-containing fermented natto may serve as a promising functional food that enhances isoflavone bioavailability, potentially by supporting a beneficial intestinal environment. However, further studies with larger cohorts are needed to confirm these findings and to clarify the relationship between increased absorption of isoflavone aglycones and clinically relevant health outcomes.

Acknowledgements

We would like to express our sincere gratitude to all organizations and individuals who supported this study. We are deeply grateful to all participants who generously consented to take part in this study.

Conflicts of interest

There are no conflicts of interest to declare.

Ethics approval and consent to participate

The study was ethically approved and was conducted in accordance with the ethical standards described in the 1964 Declaration of Helsinki and its later amendments. This study was conducted with written informed consent from all participants.

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