

平成 30 年度 学位論文

Study on utilization of processed soybean food  
to increase dietary fiber intake  
and its effect on health

食物繊維摂取量を増加させるための大豆加工食品の活用と  
それが健康におよぼす影響に関する研究

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## GENERAL ABSTRACT

### Background:

Currently, major health problems world-wide, including in Japan, are lifestyle-related diseases such as diabetes, dyslipidemia and myocardial infarction. Fiber deficiency is one of the major factors for these problems. In the Dietary Reference Intakes for Japanese (2015 edition), the target amount (adult) of dietary fiber is 20g/d or more for men and 18g/d or more for women, but in the National Health and Nutrition Survey (2017), the average intake amount is 14.6g/d and 14.3g/d, respectively. School lunch in Japan is the most respected in the world. The reason for this is that dietitians are placed at each school. Even given this highly effective system, it is difficult to supply adequate dietary fiber in school lunches. According to the dietary standard for fiber intake from school lunches 4g or more at 6-7 years old, 5g or more at 8-9 years old, 5g or more at 10-11 years old, and 6.5g or more at 12-14 years old. However, in the nutritional report of the Japanese Ministry of Education, the average intake of dietary fiber is low at 4.6g (elementary school, 6-11 years old) and 5.9g (junior high school, 12-14 years old). An increase in dietary fiber intake can usually be expected from increased consumption of vegetables and grains but not from main dishes. Many main dishes, such as meat, fish, etc. are low in dietary fiber content and therefore have little potential as a source of fiber and have not been studied well. I conducted two studies on ways to increase dietary fiber through main dishes and one study to observe the effect on blood glucose concentration in patients with type 2 diabetes mellitus (DM).

In the first study, tempeh (about 5.0g/100kcal and 10.2g/100g of dietary fiber), which is a traditional soybean fermented food of Indonesia, was studied whether it is possible to make tasty main dishes that Japanese would find acceptable, and in the second study acceptability of Okara (about 10.4g/100kcal and 11.5g/100g of dietary fiber) mixed with fish that look like a hamburger as school lunch by children.

In the third study, we developed main dishes with Okara that were well accepted by Vietnamese, and observed the effect of Okara fiber on blood glucose level of type 2 DM.

### Purpose:

To develop main dishes with fiber-rich tempeh or Okara that are well accepted by Japanese and to investigate whether the blood glucose level of Vietnamese type 2 DM patients can be reduced by foods with Okara.

## Methods:

Study 1: The subjects were female students, faculty and staff, security guards and cleaners (totaling 60 people) at Jumonji University. Ten Japanese-style main dishes were prepared using the usual principal ingredients, such as eggs and meats, and ten dishes that replaced those ingredients with tempeh. The ten dishes were: rolled thick omelet, teriyaki, Chinese-style fried chicken, keema curry, piccata, hamburger steak, croquette, tofu in meat-and-chili sauce, sweet-and-sour pork, and stuffed pouches of deep-fried tofu. We offered each control dish (normal ingredients) and its parallel tempeh-based dish on the same plate for lunch. Overall taste, aroma, texture and basic taste were evaluated by scores from 1 to 4 (4 very good; 3 good; 2 poor; 1 very poor).

Study 2: As a preliminary study, we conducted various trials to make a tasty mixture of fish and Okara that looked like hamburger; one portion (80g) of the final mixture could supply 2.2g of fiber. We called it a “hamburger style of fish and Okara”. It was served to all the 349 children from the first to 6<sup>th</sup> grade as part of a school lunch at a Japanese school and evaluated by comparison with an ordinary Japanese-style hamburger made with pork and chicken (control). Evaluation was made in three levels (good, ordinary and bad) about overall taste, easy to eat, basic taste, aroma and appearance.

Study 3: We contacted 300 type 2 DM outpatients at a hospital and selected 60 of them in Vietnam. We formed 30 pairs matched by gender, age, BMI and years with DM and divided them randomly into an intervention group and a control group. The intervention group consumed about 6g of fiber from Okara per day for two weeks. As a way of ingesting it, we made ten kinds of Okara menus that match the tastes of Vietnamese. The ten menus were: fried rice with Okara, rice porridge with Okara, Okara hamburger, meatballs with Okara, stir fried piper lolot rolls with pork and Okara, omelette with Okara, cabbage rolls with Okara, stir fried bean sprouts with Okara, miso soup with Okara, Okara patties. At the baseline and final periods, anthropometric measurements, blood withdrawal and a 3-day weighing method nutrition survey were conducted.

## Results:

Study 1: The average evaluations of the 10 control dishes and tempeh dishes were 3.7 and 3.5 points for overall taste, 3.7 and 3.4 points for aroma, 3.4 and 3.1 points for texture and 3.7 and 3.4 points for basic taste, respectively. Evaluations of tempeh dishes were close to those of the control dishes. Especially for keema curry, hamburger steak, and stuffed pouches of deep-fried tofu, the tempeh dishes received high evaluations. In these three dishes, we used finely chopped tempeh, which suggests that the optimal use of tempeh was in a finely chopped form. The amount of dietary fiber increased by about

2g with tempeh dishes.

Study 2: The evaluation about hamburger and “hamburger style of fish and Okara” were respectively; Overall taste is good 94 and 85%, good for easy to eat 90 and 77%, basic taste is good 93% and 83%, aroma is good 82 and 68%, appearance is good 89 and 76%. Although the results of ordinary hamburger were more favorable than those of “hamburger style of fish and Okara”, the evaluation of “hamburger style of fish and Okara” was high (68%). Waste of them was only 2.2 and 4.6% by weight, respectively.

Study 3: Dietary fiber intake increased from 6.9 to 12.6g ( $p<0.01$ ) in the intervention group, but there was no change in the control group. Fasting blood glucose and fructosamine in the intervention group dropped from 113 to 97mg/dL ( $p<0.05$ ) and from 319 to 301 $\mu$ mol/L ( $p<0.05$ ), respectively but they remained unchanged in the control group.

#### Conclusion:

Tempeh and Okara were made palatable by devising appropriate cooking methods. This suggests that dietary fiber intake can be increased by developing main dishes using them. Furthermore, as a health effect due to an increase in dietary fiber intake, it was possible to improve the blood glucose level of type 2 DM patients.

## GENERAL ABSTRACT

### 論文要旨

背景：

現在、日本のみならず世界の健康問題は、糖尿病、脂質異常症、心筋梗塞などの生活習慣病の増加であろう。食事性要因のひとつとして、食物繊維の摂取不足が考えられている。しかしながら食物繊維は摂取しにくい栄養素であり、日本人の食事摂取基準（2015年版）における食物繊維の目標量（成人）は男性20g/日以上、女性18g/日以上だが、国民健康・栄養調査（2017年）の結果では平均摂取量は男性14.6g/日、女性14.3g/日である。日本の学校給食は、世界でも最も評価の高いものでであろう。その理由として、各学校や給食施設に栄養士が配置されているためである。そのような学校給食でも十分な食物繊維を供給することは困難である。学校給食の食物繊維摂取基準では、6～7歳で4g以上、8～9歳で5g以上、10～11歳で5g以上、12～14歳で6.5g以上とされているが、文部科学省の学校給食栄養報告では、小学校（6～11歳）の平均摂取量は4.6g、中学校（12～14歳）の平均摂取量は5.9gと低い。食物繊維摂取量の増加は穀類や野菜に求めることが多いが、摂取基準値を満たすことは難しい。多くの主菜、例えば肉、魚などは、食物繊維含有量が低いために食物繊維の供給源としての期待が薄く、ほとんど研究されていない。そこで、主菜から食物繊維摂取量を増加させる方法に関する2つの研究と、食物繊維摂取量が増加したことによる2型糖尿病（DM）患者の血糖値への影響に関する1つの研究を実施した。

1つめの研究では、インドネシアの伝統的な大豆発酵食品であるテンペ（食物繊維含量 約5.0g/100kcal、約10.2g/100g）によって日本人に受け入れられる主菜を作製することが可能であるか、そして2つめの研究では、オカラ（食物繊維含量 約10.4g/100kcal、約11.5g/100g）と魚肉を使用してハンバーグ風の主菜を作製し、それが学校給食で子供たちに受け入れられるかについて検討した。

3つめの研究では、ベトナム人に受け入れられるオカラを使用した主菜を開発し、2型DMの血糖値に対するオカラ由来の食物繊維の影響を観察した。

## 目的：

日本人に受け入れられる、食物繊維含量が豊富なテンペまたはオカラを使用した主菜を開発すること、オカラを使用して食物繊維摂取量を増加させることでベトナム人の2型DM患者の血糖値を低下させることができるかを調べること。

## 方法：

研究1：被験者は、女子大学生、大学教職員、十文字学園女子大学で働く警備員および清掃員（計60名）とした。おもな食材として卵や肉などが使用されている、日本人が日常的に良く食べる主菜を10種類選択した（対照）。そして、それらの食材をテンペに置き換えた10種類の主菜を作製した（テンペ料理）。10種類の料理は次の通りである：卵焼き、照り焼き、油淋鶏、キーマカレー、ピカタ、ハンバーグ、コロッケ、麻婆豆腐、酢豚、きんちやく。完成したテンペ料理は、その対照と同時に、被験者に昼食の主菜として提供した。被験者は、美味しさ、香り、食感、味について、4段階評価により比較した。評価点は4点：とても良い、3点：良い、2点：良くない、1点：とても良くないとした。

研究2：予備調査として、魚とおからを使用したハンバーグのような主菜を作製することを目指して、生地の検討を重ねた。完成した生地からは、80g/食で2.2gの食物繊維を供給できるものとなった。それを「魚とオカラのハンバーグ風」と名付けた。349名（1～6年生）の小学生に学校給食の主菜として提供し、豚肉と鶏肉で作製されたハンバーグと比較評価した。被験者は、美味しさ、食べやすさ、味、香り、見た目について、3段階評価により比較した。評価は良い、ふつう、悪いとした。

研究3：300人の2型糖尿病外来患者に連絡を取り、そのうち60人を選択した。60人の2型糖尿病患者について、性別、年齢、BMI、糖尿病の罹患歴から、30組のペアを作り、介入群と対照群に無作為に分けた。介入群は2週間にわたって、オカラから約6g/日の食物繊維を摂取した。その摂取方法として、ベトナム人の嗜好に合った10種類のオカラ料理（主食、主菜、副菜）を作製した。10種類の料理は以下の通りである：オカラチャーハン、オカラ入りお粥、オカラハンバーグ、オカラミートボール、オカラと豚肉の胡椒葉巻き、オカラ入りオムレツ、オカラ入りロールキャベツ、オカラともやしの炒め物、オカラ入りみそ汁、オカラパティ。介入前（ベースライン）と介入期間の最終において、身体測定、採血および3日間の秤量法による栄養調査を実施した。

結果：

研究 1：10 種類の対照およびテンペ料理の平均評価点は、それぞれ、美味しさが 3.7 および 3.5 点、香りが 3.7 および 3.4 点、食感が 3.4 および 3.1 点、味が 3.7 および 3.4 点であった。テンペ料理は対照と近い評価を得ることができた。特に、キーマカレー、ハンバーグ、きんちやくは高い評価が得られた。これら 3 種類のテンペ料理では、テンペをみじん切りにして使用した。これはテンペの最適な使い方がみじん切りであることを示唆するものである。また、食物繊維の量はテンペ料理で約 2g 増加した。

研究 2：対照および「魚とオカラのハンバーグ風」の評価は、それぞれ、美味しいが 94% および 85%、食べやすいが 90% および 77%、味が良いが 93% および 83%、香りが良いが 82% および 68%、見た目が良いが 89% および 76% であった。対照のほうがより好まれたが、「魚とオカラのハンバーグ風」は被験者の半数以上から良い（68%）という評価を得ることができた。総重量あたりの廃棄率は、それぞれ、2.2% および 4.6% にすぎなかった。

研究 3：食物繊維の摂取量は、介入群で 6.9g から 12.6g に増加した ( $p < 0.01$ ) が、対照群に変化はなかった。介入群の空腹時血糖値とフルクトサミン値は、それぞれ、113mg/dL から 97mg/dL ( $p < 0.05$ ) と 319  $\mu$  mol/L から 301  $\mu$  mol/L ( $p < 0.05$ ) に低下したが、対照群では変化しなかった。

結論：

テンペやオカラは、調理方法の工夫によって美味しく食べられることがわかり、それらを用いて開発した主菜により、食物繊維摂取量を増加させることができると示唆された。さらに、食物繊維摂取量の増加による健康影響として、2 型 DM 患者の血糖値を改善させることができた。



## GENERAL INTRODUCTION

Currently, major health problems world-wide, including Japan, are lifestyle-related diseases such as diabetes, dyslipidemia and myocardial infarction<sup>1)</sup>. Fiber deficiency is one of the major factors for these conditions<sup>2)</sup>. In the Dietary Reference Intakes for Japanese (2015 edition), the target amount (adult) of dietary fiber is 20g/day or more for men and 18g/day or more for women, but in the National Health and Nutrition Survey (2017), the average intake amount is 14.6g/day and 14.3g/day, respectively<sup>3,4)</sup>. It is assumed that these diseases can be prevented by ingesting a sufficient amount of dietary fiber from childhood<sup>5)</sup>. According to the dietary standard of fiber intake for school lunch, intake should be 4g or more at 6-7 years old, 5g or more at 8-9 years old, 5g or more at 10-11 years old, and 6.5g or more at 12-14 years old<sup>6)</sup>. However, in the recent nutritional report of the Japanese Ministry of Education, the average intake of dietary fiber was low at 4.6g (elementary school, 6-11 years old) and 5.9g (junior high school, 12-14 years old)<sup>7)</sup>.

Various attempts have been made to increase the intake of dietary fiber. Many studies use cereals (staple food) and vegetables (side dishes) etc.<sup>3)</sup>, but still not enough to meet the Japanese Dietary Reference Intake. Rice is often refined to make it more palatable. Since the bran layer rich in dietary fiber is scraped off from the layer, the dietary fiber content of refined rice is only 0.5g per 100g. As the residual proportion of the bran layer increases, the dietary fiber content increases, but a unique texture and smell are added, and the taste tends to deteriorate. Therefore rice is often eaten in milled form and cannot be considered an adequate source of dietary fiber. An average of 350g of vegetables per day is recommended. This amount is based on investigations of the intakes of potassium, dietary fiber and vitamins. However, even if 350g of vegetables is ingested, only about 8g of dietary fiber can come from this source. The average value of current vegetable intake is 288.2g (males 295.4g, females 281.9g), and it has flat in the past 10 years<sup>3,4)</sup>. Therefore, we can say that it is difficult to satisfy dietary fiber intake with vegetables. In school meals, opportunities to provide root vegetables and beans as side dishes increase to satisfy the fiber requirement. However, children often dislike root vegetables and legumes. This leads to an increase in uneaten vegetables<sup>8)</sup>. New sources of dietary fiber are needed apart from staple foods and side dishes.

In Study 1 and Study 2, we examined ways to increase dietary fiber intake by using main dishes as a source of new dietary fiber. Tempeh (about 5.0g/100kcal and 10.2g/100g of dietary fiber) and Okara (about 10.4g/100kcal and 11.5g/100g of dietary

fiber) were selected as food materials which are rich in dietary fiber and can be used in main dishes. If we can develop tasty main dishes using tempeh or Okara, we believe that we can increase dietary fiber intake without difficulty.

Study 3 confirmed the blood glucose level lowering effect by dietary fiber from Okara in Vietnamese type 2 DM patients. As in Japan, Vietnam manufactures a lot of tofu, but does not have a food culture of eating Okara. As a new food culture, if Vietnamese people can realize that Okara can be a tasty ingestible ingredient, it will lead to effectively lowering the blood glucose levels of the increasing numbers of type 2 DM patients.

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## **STUDY 1**

**Indonesian tempeh from soybeans can be included  
in Japanese main dishes  
and can increase fiber intake**

## ABSTRACT

**Background:** Currently, major health problems, not only in Japan, include lifestyle-related diseases such as diabetes, dyslipidemia and myocardial infarction. Insufficient fiber intake is one of the main factors for these conditions. In the Dietary Reference Intakes for Japanese (2015 edition), the target amount (adult) of dietary fiber is 20g/day or more for men and 18g/day or more for women, but in the National Health and Nutrition Survey (2017), the average intake amount is 14.6g/day and 14.3g/day, respectively. An increase in dietary fiber intake is usually expected from increased consumption of vegetables and grains but not from main dishes such as meat, fish, eggs, dairy products, or soybean protein products, because these contain little fiber. In this study, we focused on the use of tempeh (about 5.0g/100kcal and 10.2g/100g of dietary fiber), Indonesian fermented soybeans, as a main dish. **Purpose:** An increase in dietary fiber intake through main dishes using tempeh. **Method:** The subjects were female students, faculty and staff, security guards and cleaners (totaling 60 people) at Jumonji University. Ten Japanese-style main dishes were prepared using the usual principal ingredients, such as eggs and meats, and ten dishes that replaced those ingredients with tempeh. The ten dishes were: rolled thick omelet, teriyaki, Chinese-style fried chicken, keema curry, piccata, hamburger steak, croquette, tofu in meat-and-chili sauce, sweet-and-sour pork, and stuffed pouches of deep-fried tofu. We offered each control dish (normal ingredients) and its parallel tempeh-based dish on the same plate for lunch. Overall taste, aroma, texture and basic taste were evaluated by scores from 1 to 4 (4 very good; 3 good; 2 poor; 1 very poor). **Results:** The average evaluations of the 10 control dishes and tempeh dishes were 3.7 and 3.5 points for overall taste, 3.7 and 3.4 points for aroma, 3.4 and 3.1 points for texture and 3.7 and 3.4 points for basic taste, respectively. Evaluations of tempeh dishes were close to those of the control dishes. Especially for keema curry, hamburger steak, and stuffed pouches of deep-fried tofu, the tempeh dishes received high evaluations. In these three dishes, we used finely chopped tempeh, which suggests that the optimal use of tempeh was in a finely chopped form. The amount of dietary fiber increased by about 2g with tempeh dishes. **Conclusion:** We found that tempeh can be used as a part of Japanese main dishes to increase dietary fiber by about 2g from each meal.

Key words: tempeh, fiber, main dish, Japanese

## INTRODUCTION

Lifestyle-related diseases, such as diabetes, dyslipidemia, and myocardial infarction, have been increasing, not only in Japan but also globally<sup>1)</sup>. One of the important dietary factors for this is insufficient fiber intake<sup>2,3)</sup>. However, it is usually difficult to meet sufficient amounts of fiber. According to the Dietary Reference Intakes for Japanese (2015 edition), the target amount (adult) is 20g/day or more for men and 18g/day or more for women<sup>4)</sup>, while the actual intake of fiber reported by a recent National Health and Nutrition Survey is 14.6g/day for men and 14.3g/day for women in 2017<sup>5)</sup>. This means that actual daily fiber intake is about 5g short. It is difficult to satisfy the target amount of dietary fiber from staple food (grains) and side dishes (vegetables and legumes). We need to find ways to increase new dietary fiber intake. In this study, we examined ways to increase fiber intake from main dishes. For this purpose, we chose tempeh, which is rich in dietary fiber and can be used as a main dish.

Tempeh is a traditional soybean fermented food of Indonesia that has been eaten for about 400 years. In Indonesia, tempeh can be easily obtained, and it is about 400g/50Yen (half a US dollar) in the market. It is mainly used as fried in oil and used as soup and stir-fried materials. In Japan there is a traditional fermented soybean food “natto”, but it uses a different type of microorganism from that used in tempeh. The microorganism for natto is a bacterium, “*Bacillus subtilis* var. *natto*”, whereas for tempeh it is a mold, “*Rhizopus oligosporus*”<sup>6-8)</sup>. It is a filamentous fungus that belongs to the genus *Rhizopus*. It is called Spider Monkey (Japanese name) because its hypha looks like a spider's web when it is cultured. In Indonesia, it is a common mold which naturally occurs on the underside of banana and hibiscus leaves. In Japan, we can get the domestic tempeh now. In this research, we used tempeh from Japan.

Many of the previous studies report on the analysis of the functionality possessed by tempeh, the analysis of nutritional components contained in tempeh, changes in nutritional components due to differences in fermenting microorganisms. As a result, it was found that tempeh has strong antioxidant property, that the amount of each nutrient is increased by fermentation, that tempeh contains more dietary fiber than natto. Fiber concentrations of tempeh are high<sup>6-10)</sup>. The content of dietary fiber of tempeh is about 5.0g/100kcal and 10.2g/100g, natto is about 3.4g/100kcal and 6.7g/100g<sup>11)</sup>. As an influence on health, thrombolytic activity using artificial thrombus and improvement effect of iron deficiency state using rat have been reported<sup>12,13)</sup>. In Indonesia, an improvement effect of lipid abnormality on postmenopausal women by intervention study of tempeh has been reported<sup>14)</sup>. There are also some studies that carried out sensory

evaluation of tempeh. Tempeh hamburger made from two kinds of beans (White bean tempeh and Soybean tempeh) is given to 82 panels, and the taste is evaluated in 9 stages<sup>15)</sup>. Cookies with different tempeh fermentation times (0, 24 and 48hr) and blending ratios (0, 6.5 and 14.5%) were given to 20 panels, and evaluated for taste in 6 stages<sup>16)</sup>. Tempeh dishes with different recipes (raw, unglazed, roasted, fried and steamed) were given to 20 panels, and evaluated for taste in 5 stages. In this study, recipe was considered<sup>17)</sup>. Five kinds of commercially available tempeh were given to 33 panels in raw condition, and evaluated for taste by scoring and ranking. In this study, the degree of recognition and food characteristics of tempeh were considered<sup>18)</sup>.

In these previous studies, there is no report that developed a main dish using tempeh and considered whether it matches the taste of the Japanese. Therefore, in this research, for the purpose of increasing the intake of dietary fiber from main dish of tempeh (tempeh dish), 10 different tempeh dishes were given to 60 panels, and evaluated for taste in 4 stages.

## METHODS

### 1. Subject

The subjects were female students, faculty and staff, security guards and cleaners at Jumonji University where the researchers work. We explained the purpose of this research and those who agreed to participate signed the informed consent form (60 people in total). The recognition of Japanese tempeh is very low. None of the subjects in this study had eaten tempeh. Therefore, subjects tasted raw tempeh before starting this study. There was also a meaning to alleviate the caution against the subject's first food to eat.

### 2. Development of tempeh dishes

Prior to the main study, we conducted a pilot study to find the best cooking methods for 10 types of tempeh dishes. These dishes were familiar to Japanese and did not require special cooking skills. For some dishes, the ingredients were completely replaced with tempeh, while in others they were partially replaced. Details of the cooking methods for the 10 dishes are shown below.

- 1) Rolled thick omelet: Do a trial to find out that a lot of tempeh can be added to the egg solution. The tempeh was not finely chopped, but it was ground and mixed with

raw beaten eggs. The trial tests started with 30% of the total solution but this resulted in a hard and dry texture. Trial production and tasting were repeated, gradually decreasing the proportion of tempeh. As a result, we found that an 80% egg and 20% tempeh mixture was the most delicious (Fig 1-1).

- 2) Teriyaki: Chicken was replaced with tempeh. When tempeh was coated with potato starch and fried, the texture became soft. This would be good for people with chewing difficulties. When tempeh was covered with wheat flour and fried, it was chewy and more delicious. In this study, wheat flour was used. A flavorful sweet sauce went well with the tempeh, so fried the tempeh and simmered it with the sauce; this was the best tasting version and the one used in the study (Fig 1-2).
- 3) Chinese-style fried chicken: Chicken was replaced with tempeh. When tempeh was coated with potato starch and fried, it was smooth and easy to eat. The tempeh was delicious with reduced acid, especially when seasoned with soy sauce before cooking. This taste was best and was the version used in this study (Fig 1-3).
- 4) Keema curry: Mixed ground meat and tempeh in various proportions and found that 30% meat and 70% tempeh was most delicious (Fig 1-4).
- 5) Piccata: Chicken was replaced 100% with tempeh and seasoned with soy sauce. When egg and grated cheese were added, the taste was markedly improved (Fig 1-5).
- 6) Hamburger steak: Started with 70% ground meat and 30% tempeh. This tasted good, but it was difficult to form the hamburger patty. Tried to find the best combination for taste and shaping the burger and decided on 50% chopped meat and 50% tempeh (Fig 1-6).
- 7) Croquette: Used 100% tempeh without meat. The taste was good and cooking was not difficult. With soy sauce and sugar, the taste became much better (Fig 1-7).
- 8) Tofu in meat-and-chili sauce: At first: Used 100% tempeh without meat. The taste was too bland without any fat. Therefore: Increased the meat and found finally that 70% tempeh and 30% meat was best (Fig 1-8).
- 9) Sweet-and-sour pork: Pork was completely replaced with tempeh and the taste was as good as the original pork. However, the tempeh was easily broken up when we cooked it with vegetables; therefore, we cooked the tempeh and vegetables separately (Fig 1-9).
- 10) Stuffed pouches of deep-fried tofu: At first: Used 100% tempeh but this was too soft. Tried to find the best combination and found 50% ground meat and 50% tempeh was most delicious (Fig 1-10).





Fig 1-1. Rolled thick omelet lunch  
right : with tempeh  
left : only eggs (control)



Fig 1-2. Teriyaki lunch  
right : tempeh  
left : chicken (control)



Fig 1-3. Chinese-style fried chicken lunch  
right : tempeh  
left : chicken (control)



Fig 1-4. Keema curry lunch  
right : with tempeh  
left : ground beef and pork (control)



Fig 1-5. Piccata lunch  
right : tempeh  
left : chicken (control)



Fig 1-6. Hamburger steak lunch  
right : with tempeh  
left : ground beef and pork (control)



Fig 1-7. Croquette lunch  
right : tempeh  
left : ground beef and pork (control)



Fig 1-8. Tofu in meat-and-chili sauce lunch  
right : with tempeh  
left : ground pork (control)



Fig 1-9. Sweet-and-sour pork lunch  
right : tempeh  
left : pork (control)



Fig 1-10. Stuffed pouches of deep-fried tofu lunch  
right : with tempeh  
left : ground chicken (control)

Fig 1. Ten lunches given to subjects

### 3. Main test

Evaluated the 10 traditional Japanese dishes made with the usual ingredients such as egg, meat, etc. and the same dishes with the main ingredient partially or fully replaced with tempeh. The control and tempeh dishes were placed on the same plate. We did not inform subjects which was the tempeh dish and which was the control dish.

Overall taste, aroma, texture and basic taste were evaluated by scores from 1 to 4 (4 very good; 3 good; 2 poor; 1 very poor). The questionnaire form is shown in Fig 2. Fig 3 shows the subjects answering the questionnaire.


Questionnaire about stuffed pouches of deep-fried tofu		Age	Male	Female
Please mark the following questions and tell us the reasons.				
(1) I will ask about the left side.				
① How was the aroma?	Very good • Good • Poor • Very poor			
	↳The reason			]
② How was the texture?	Very good • Good • Poor • Very poor			
	↳The reason			]
③ How was the basic taste?	Very good • Good • Poor • Very poor			
	↳The reason			]
④ Was it delicious?	Very good • Good • Poor • Very poor			
	↳The reason			]
(2) I will ask about the right side.				
① How was the aroma?	Very good • Good • Poor • Very poor			
	↳The reason			]
② How was the texture?	Very good • Good • Poor • Very poor			
	↳The reason			]
③ How was the basic taste?	Very good • Good • Poor • Very poor			
	↳The reason			]
④ Was it delicious?	Very good • Good • Poor • Very poor			
	↳The reason			]
(3) Please compare two stuffed pouches of deep-fried tofu.				
① Which aroma do you like? → Left side - Right side				
	↳The reason			]
② Which texture do you like? → Left side - Right side				
	↳The reason			]
With back side				
③ Which basic taste do you like? → Left side - Right side				
↳The reason				
]				
④ Which was tasty? → Left side - Right side				
↳The reason				
]				
⑤ Which do you want to eat again? → Left side - Right side				
↳The reason				
]				
⑥ Which of the following do you think you would like your family to eat? → Left side - Right side				
↳The reason				
]				
(4) Do you want to try different tempeh dishes?				
Yes - No - Under consideration				
↳The reason				
]				
(5) Do you think you want to use tempeh at home (use it)?				
Yes - No - Under consideration				
↳The reason				
]				
(6) About the impression of tempeh.				
① How many times have you participated in the questionnaire survey?				
→ First time - 2th - 3th - 4th - 5th - 6th - 7th - 8th - 9th - 10th				
⑤ First time people, how did you feel when you eat tempeh?				
[ ]				
⑥ Has the impression of tempeh changed in the second and subsequent times? How did it change?				
Especially, is there a difference in impression between when you eat for the first time and this time?				
[ ]				
(7) Please write if you notice something.				
Thank you very much				
				

Fig 2. Questionnaires used for main test



Fig 3. The subjects answering the questionnaire

#### 4. Calculation of nutritional value

We cooked lunch with control and tempeh cuisine as main dish. We compared the amount of dietary fiber of each lunch box.

#### 5. Statistical analysis

Statistical analyses were conducted by paired Student *t*-test and Turkey test using excel.

## 6. Ethical considerations

This research was approved by the ethics committee of Jumonji University (number 2017-007) on 25th July 2017.

## RESULTS

Table 1 shows the number of subjects and average age by gender. Total number of subjects was 60 (13 males and 47 females). The average age was about 39y (58y for males and 33y for females).

Table 1. Number of subject and average age by gender

	Number	Age (year) Mean±SD
Male	13	58 ± 10
Female	47	33 ± 18
Total	60	39 ± 19

Table 2 shows the comparison of scores of overall taste, aroma, texture and basic taste between tempeh and control foods in 10 different menus. Among 10 dishes, 6 dishes were evaluated higher for the control than for the tempeh ( $p < 0.05$ ), however, the difference was not large. On average for the 10 dishes, the scores for tempeh and control were respectively 3.5 and 3.7 for overall taste, 3.4 and 3.7 for aroma, 3.1 and 3.4 for texture and 3.4 and 3.7 for basic taste.

Fig 4 shows the comparison of 3 tempeh cooking methods (chopped, cut in blocks and ground). The order was consistent regardless of the 4 evaluation items, with chopped highest, block second, and ground third.

Table 2. Scores comparison of overall taste, aroma, texture and basic taste between tempeh and control foods in 10 different menus

	n		Overall taste		Aroma		Texture		Basic taste	
Rolled thick omelet	46 (M:10,F:36)	Tempeh	3.2±0.7	*	3.1±0.7	*	2.5±0.7	*	3.2±0.7	*
		Control	3.6±0.6		3.6±0.5		3.5±0.6		3.6±0.5	
Teriyaki	50 (M:10,F:40)	Tempeh	3.3±0.7	*	3.3±0.7	*	2.7±0.8	*	3.4±0.8	*
		Control	3.8±0.5		3.7±0.4		3.4±0.6		3.8±0.5	
Chinese-style fried chicken	51 (M:12,F:39)	Tempeh	3.4±0.7	*	3.6±0.5	ns	3.1±0.8	*	3.3±0.7	*
		Control	3.7±0.5		3.7±0.4		3.5±0.7		3.7±0.6	
Keema curry	50 (M:14,F:36)	Tempeh	3.8±0.4	ns	3.6±0.5	ns	3.3±0.8	ns	3.7±0.5	ns
		Control	3.7±0.5		3.5±0.7		3.3±0.7		3.6±0.6	
Piccata	45 (M:12,F:33)	Tempeh	3.5±0.5	ns	3.4±0.6	ns	3.0±0.8	ns	3.4±0.6	ns
		Control	3.7±0.5		3.5±0.6		3.3±0.7		3.5±0.5	
Hamburger steak	45 (M:14,F:31)	Tempeh	3.5±0.6	ns	3.5±0.6	ns	3.3±0.7	ns	3.5±0.6	ns
		Control	3.7±0.4		3.7±0.5		3.3±0.6		3.7±0.5	
Croquette	50 (M:12,F:38)	Tempeh	3.5±0.7	*	3.5±0.6	ns	3.3±0.8	ns	3.5±0.7	*
		Control	3.9±0.4		3.6±0.6		3.4±0.7		3.8±0.5	
Tofu in meat-and-chili sauce	60 (M:13,F:47)	Tempeh	3.5±0.7	*	3.3±0.8	*	3.1±0.8	*	3.3±0.6	*
		Control	3.7±0.5		3.8±0.5		3.4±0.6		3.8±0.5	
Sweet-and-sour pork	51 (M:12,F:39)	Tempeh	3.2±0.7	*	3.4±0.6	*	3.0±0.9	ns	3.3±0.7	*
		Control	3.6±0.6		3.7±0.5		3.3±0.8		3.7±0.5	
Stuffed pouches of deep-fried tofu	50 (M:12,F:38)	Tempeh	3.8±0.4	ns	3.6±0.5	ns	3.5±0.7	ns	3.8±0.4	ns
		Control	3.8±0.4		3.8±0.5		3.3±0.8		3.8±0.4	
Average	50 (M:12,F:38)	Tempeh	3.5±0.7	*	3.4±0.6	*	3.1±0.8	*	3.4±0.7	*
		Control	3.7±0.5		3.7±0.5		3.4±0.7		3.7±0.5	

Values are mean ± SD

\* Significant difference by paired Student *t*-test between tempeh and control groups in each dish at  $p < 0.05$   
M and F stand for male and female, respectively

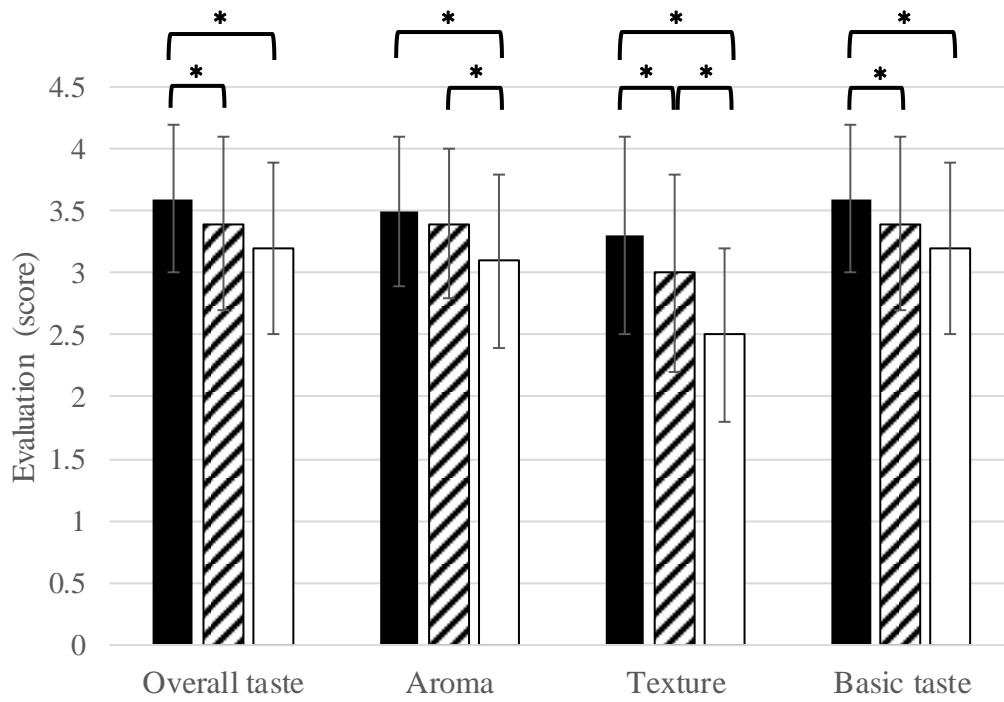


Fig 4. Comparison of 3 cooking methods in tempeh score

\* Significant difference by Turkey test at  $p < 0.05$

■: chopped    ▨: cut in block    □: ground

Table 3 shows comparison of evaluations for the best 4 tempeh dishes by age. Age differences were not observed.

Table 3. Comparison of evaluation for best 4 tempeh dishes by age

		20~30's (n=26)		40~50's (n=9)		60~70's (n=12)	
		Control	Tempeh	Control	Tempeh	Control	Tempeh
Keema curry	Overall taste	3.9±0.3	3.9±0.3	3.6±0.5	3.7±0.5	3.3±0.8	3.6±0.5
	Aroma	3.5±0.7	3.7±0.5	3.4±0.7	3.2±0.4	3.5±0.8	3.5±0.5
	Texture	3.4±0.7	3.3±0.9	3.1±0.8	3.0±0.7	3.1±0.7	3.6±0.5
	Basic taste	3.7±0.5	3.8±0.4	3.3±0.7	3.6±0.5	3.3±0.8	3.5±0.5
Piccata	Overall taste	4.0±0.0	3.8±0.4*	3.4±0.5	3.2±0.7	3.3±0.5	3.3±0.5
	Aroma	3.8±0.5	3.6±0.6	3.4±0.5	3.2±0.7	3.1±0.7	3.3±0.6
	Texture	3.5±0.7	3.2±0.9	3.1±0.8	2.7±0.7	3.1±0.8	2.9±0.8
	Basic taste	3.8±0.4	3.5±0.5	3.2±0.4	3.1±0.8	3.3±0.5	3.4±0.5
Hamburger steak	Overall taste	3.9±0.3	3.7±0.6	3.8±0.4	3.2±0.7	3.4±0.5	3.5±0.5
	Aroma	3.9±0.3	3.7±0.5	3.3±0.7	3.1±0.8	3.5±0.5	3.5±0.5
	Texture	3.5±0.7	3.5±0.7	3.1±0.8	3.1±0.8	3.2±0.4	3.3±0.7
	Basic taste	4.0±0.0	3.7±0.6*	3.4±0.5	3.0±0.5	3.4±0.8	3.4±0.6
Stuffed pouches of deep-fried tofu	Overall taste	3.9±0.3	4.0±0.0	3.8±0.4	3.7±0.5	3.7±0.5	3.6±0.5
	Aroma	3.9±0.4	3.7±0.5	3.7±0.5	3.6±0.5	3.6±0.7	3.7±0.5
	Texture	3.1±0.9	3.5±0.7	3.7±0.5	3.6±0.7	3.4±0.7	3.3±0.8
	Basic taste	3.9±0.3	4.0±0.2	3.7±0.5	3.8±0.5	3.7±0.5	3.6±0.5

Values are mean ± SD

\* Significant difference by paired Student *t*-test between tempeh and control groups in each dish at *p* < 0.05

Fig 5 shows fiber in one serving of lunch. Average dietary fiber included in the control and tempeh dishes was 4.2 g and 6.3 g for lunch. Teriyaki lunch increased 3.4g. By using tempeh dish as the main dish, it is possible to increase the dietary fiber intake by 2.1g.

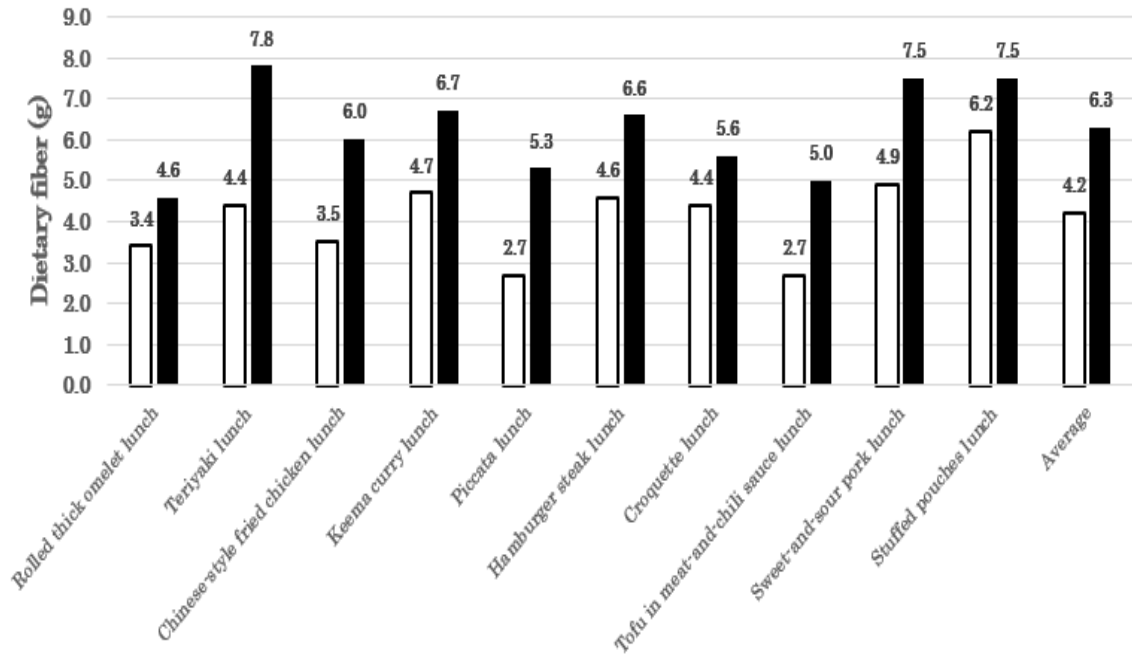


Fig 5. Dietary fiber in one serving lunches  
 □ : Control lunch    ■ : Tempeh lunch  
 Values are dietary fiber content



## DISCUSSION

With this study we found that tempeh can be used as a part of Japanese main dishes and can increase dietary fiber by about 2g from each meal (Fig 5).

The increase of fiber was quite significant. In the Dietary Reference Intakes for Japanese (2015 edition), the target amount (adult) of dietary fiber is 20g/day or more for men and 18g/day or more for women<sup>4)</sup>, but in the National Health and Nutrition Survey (2017), the average intakes amount were 14.6g/day and 14.3g/day, respectively<sup>5)</sup>. An increase in dietary fiber intake is usually expected from increased consumption of vegetables and grains but not from main dishes such as meat, fish, eggs, dairy products, or soybean protein products, because these contain little fiber. If we increase the amount from white rice, we have to eat 400g/d of white rice (uncooked). On the other hand, if we use brown rice, only 67g/d is sufficient; however, many people dislike its hardness and smell, so it is not easy to increase fiber by this method. The Japanese recommendation for vegetables is 350g/d but the current average intake is 288.2g, which has flat in the past 10 years<sup>5)</sup>. Vegetables (350 g) contain about 8g of dietary fiber. It indicates that it is necessary to add about 88g of vegetables to supply 2g of fiber from Japanese common vegetables. This method is not easy. When we have difficulties in supplying fiber, tempeh is really a good main dish.

In this research, we used a Japanese-made tempeh. In Japan, tempeh is manufactured with bacteriologically pure cultured *Rhizopus oligosporus* (called tempeh fungus). Tempeh made in Japan is manufactured in hygienic environment, so we can eat it raw. Observing the appearance of tempeh, the white hyphae are covered. White hypha enters between the grains and fills without gaps (Fig 6). Tempeh has a high dietary fiber content because the fungus cells themselves are fibrous. The main component of the mycelia is thought to be “chitin”<sup>6,7)</sup>. Chitin is an insoluble dietary fiber distributed in fungi, animals and plants. Chitin in fungi exists on the cell wall of yeast and mold. Chitin is a polysaccharide in which 2-asetoamido-2-deoxy-D-glucose is linearly bonded with  $\beta$ -1,4 bond, and natural chitin has a part of it residues bound to proteins. Chitin has a function as dietary fiber such as serum triglyceride, cholesterol and blood pressure lowering action.



Fig 6. The appearance of tempeh  
One grain of soybean is bound in a block shape by mycelium, its surface also covers hyphae, filling the gap between soybean and soybean with no gaps

Tempeh can be a high-dietary fiber-containing food because there are many dietary fibers in the constituents of the mycelia (Table 4). Since tempeh, unlike natto, has little smell or stickiness, it can be adapted to various dishes. It is soft and easy to chew for persons with chewing difficulties (Table 5). From these facts, we believe that if we can use tempeh as the prime ingredient in Japanese main dishes, we can increase dietary fiber intake to the level meet the DG.

Table 4. Tempeh's nutritional value

	Weight	Protein	Lipid	Carbohydrate	Cholesterol	Fiber
	(g)	(g)	(g)	(g)	(mg)	(g)
Tempeh	50	7.8	4.5	7.6	0	5.0
Natto	50	8.3	5.0	6.1	0	3.4
Tofu (momen)	139	9.2	5.8	2.2	0	0.6
Okara (law)	90	5.5	3.2	12.4	0	10.4
Chicken (thigh)	49	8.1	7.0	0.0	44	0.0
Egg	66	8.1	6.8	0.2	278	0.0
Milk	149	4.9	5.7	7.2	18	0.0
Salmon	75	16.8	3.1	0.1	44	0.0

Comparison with major protein source food (amount per 100kcal of energy)  
Standard tables of food composition in Japan (2015)

Table 5. Characteristics of tempeh

Tempeh		Natto
Light bean taste (no habit)	Taste	Dark bean taste
None	Smell	There (strong)
None	Stickiness	There
Soft	Firmness	Soft
Block (can be separated)	form	Grain (to divide)
Can be stored frozen	Save	Can be stored frozen

Although tempeh is a new food for Japanese and can be a significant fiber source, it will be accepted only if people like the taste. Ten Japanese-style main dishes were prepared using the usual principal ingredients, such as eggs and meats, and ten dishes that replaced those ingredients with tempeh. The ten dishes were: rolled thick omelet, teriyaki, chinese-style fried chicken, keema curry, piccata, hamburger steak, croquette, tofu in meat-and-chili sauce, sweet-and-sour pork, and stuffed pouches of deep-fried tofu. We thought that the ordinary food dishes would have higher evaluations than the tempeh dishes, but surprisingly the tempeh dishes had quite high evaluations, with 4 out of 10 dishes evaluated as high as the ordinary dishes. Among the best 4 tempeh dishes, finely chopped tempeh was used in 3 dishes. These were keema curry, hamburger steak, and stuffed pouches of deep-fried tofu (Table 2).

The reason why the taste of finely chopped tempeh dishes is rated highly is that the taste of the soybeans themselves was not noticed when the tempeh was mixed with sufficient amounts of other ingredients. If we eat tempeh itself, not finely chopped, we sense the smell and taste of the soybeans. In the case of croquettes, which are fried, tempeh is less greasy than meat and may be easier to eat.

Tempeh piccata was evaluated one of the best 4 (Table 3), but the tempeh was left as it was and not chopped. The reason for this may be the browning of the grated cheese added to the seasoned egg mixture. Browning grated cheese in the egg mixture in a

frying pan produces a fragrant aroma, which may reduce the awareness of the soybean smell of tempeh.

The limitations of this study, if there are any, are the number of male and female subjects and also the difference in the age groups (Table 1). In this study, we evaluated 10 types of tempeh dishes that were tested by about 50 people in average. This total number is similar to the published papers using panel test<sup>14-20</sup>. Although the number of male and female subjects and also the age structure were different, the results in Table 3 show similar scores for 20-30's, 40-50's and 60-70's except for minor differences, indicating that the results obtained in this study were not affected by such factors.

In conclusion, tempeh dishes will be easily accepted by Japanese with proper cooking. High fiber and also soybean protein can be expected to control blood glucose and lipids significantly. Further studies are expected.

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## **STUDY 2**

### **Acceptability of fish meat with Okara as school lunch**

## ABSTRACT

**Background:** At present in Japan, fish consumption is decreasing. It is desirable that through their school lunch children will learn that fish is tasty. Deficiency of fiber intake is also a problem in Japanese people. Supplying enough fiber is a difficult task in school lunch. Okara is a byproduct created during the process of tofu production. It is mainly fiber and has the potential for beneficial uses. Since in the past there has been little study on supplying fiber from main dishes, we were challenged to address this topic. **Purpose:** To develop tasty main dishes from fish meat with Okara. **Methods and results:** As a preliminary study, we conducted various trials to make a tasty base of “fish and Okara” that looked like hamburger shaped, that has one portion (80g) of the final base could supply 2.2g fiber. We named it “hamburger style of fish and Okara”. It was served to all the 349 children from the first to 6<sup>th</sup> grade in a school lunch at a Japanese school and evaluated by the comparison with ordinary Japanese-style meat hamburger (ordinary hamburger). Their evaluation about ordinary hamburger and “hamburger style of fish and Okara” were respectively; Overall taste is good 94 and 85%, good for easy to eat 90 and 77%, basic taste is good 93% and 83%, aroma is good 82 and 68%, appearance is good 89 and 76%. Although the results of ordinary hamburger were more favorable than those of “hamburger style of fish and Okara”, the evaluation of “hamburger style of fish and Okara” was high. Waste of them was only 2.2 and 4.6% by weight, respectively. **Conclusion:** “Hamburger style of fish and Okara” was highly evaluated by school students and these dishes helped to meet the fiber requirement from school lunch.

Key words: Okara, fish, hamburger, fiber, school lunch



## INTRODUCTION

As a problem of the current Japanese dietary habits, there is a lack of dietary fiber intake. In the Japanese Dietary Reference Intake reported in 2015 by the Ministry of Health, Labor and Welfare, the target amount of dietary fiber was 20g/day or more for men and 18g/day or more for women aged 18 to 69 years<sup>1)</sup>, however, according to the National Health and Nutrition Survey in 2017, the average intake was as low as 14.6g/day for men and 14.3g/day for women, and it did not meet the target amount<sup>2)</sup>. There is a similar problem even in the young generation. The reference value of dietary fiber for school lunch is 4g or more at 6-7 years old, 5g or more at 8-9 years old, 5g or more at 10-11 years old, and 6.5g or more at 12-14 years old<sup>3)</sup>. However, the actual intake was only 4.6g at elementary school (6-11 years old), 5.9g at junior high school (12-14 years old)<sup>4)</sup>. Dietary fiber is one of the nutrients that are difficult to meet the requirement. In school meals, opportunities to provide children with root dishes and legumes to increase fiber intake. The ingredients used for these side dishes are often foods which children dislike. This leads to an increase in waste of vegetables<sup>5)</sup>. "Okara" is generated as a by-product in the production of tofu<sup>6)</sup>. Okara is a food containing a lot of dietary fiber (about 10.4g/100kcal and 11.5g/100g)<sup>7)</sup>, and its value to use as a source of dietary fiber is very high. However, in Japan, less than 1% of Okara is being used for edible<sup>8)</sup>.

Another problem is the decreased intake of fish in Japan. Consumption of fish that has been eaten as a protein source has been decreasing for a long time<sup>9)</sup>, and meat intake exceeds that of fishes and shellfishes in 2010.

## METHODS

### 1. Development of "hamburger style of fish and Okara"

We tried to develop a mixture of Okara and fish meat as a hamburger-style main dish. However, fishy smell and rough mouth feeling of Okara's texture became a problem, so we examined whether it can be eliminated by cooking method. First of all, we examined the fish odor reduction about Surimi (paste) of Lizard fish, Surimi of Scabbard fish, Surimi of Alaska pollack and Otoshimi (fragment) of Alaska pollack which are easily available as materials. Five methods were compared, namely immersed in sake, immersed in mirin, dipped in milk, dipped in ginger root juice, and covered with salt. As the results we found that dipping in milk is the best for fish odor reduction. Next,

for the reduction of roughness of texture, we tried three methods (weight reduction of Okara, increase of tofu, increase of milk) and found that the increase of milk was best. By the above studies, we concluded to use Alaska pollack paste as a main source, dipping the fish paste in milk for odor reduction and increasing milk for making the product smooth mouth feeling. In order to adjust the solidness of the dough, we decided to add rice flour. In order to chew up and improve the coloring, carrots, edamame, burdocks were carved into about 7 mm (Fig 1).

In this way, we made the basic fabric and developed the “hamburger style of fish and Okara” as the main dish.

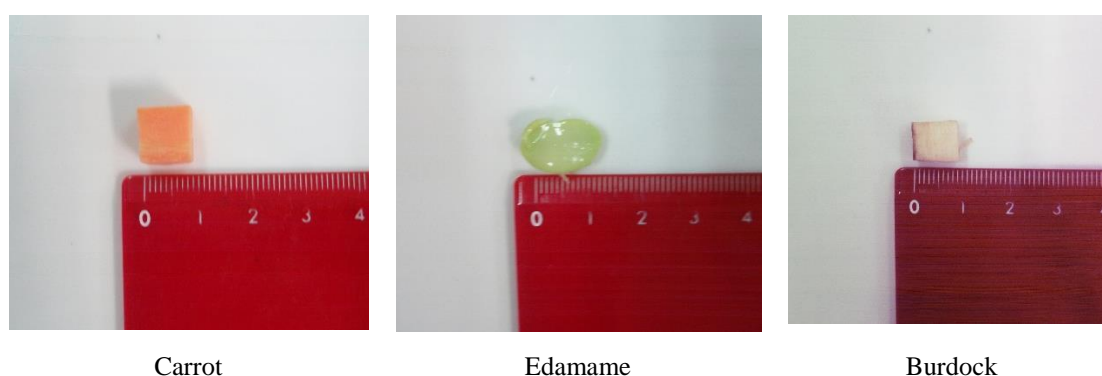


Fig 1. Carrots, edamame, burdock carved in about 7 mm

## 2. Preference test

The preference test was conducted on November 17, 2014. Subjects were the first to sixth grade (total of 349 people) elementary school children at a school near Tokyo. As a main dish school lunch, “hamburger style of fish and Okara”, and the tastes of ordinary hamburgers made from pork and chicken ordinary used were compared (Fig 2) (Fig 3).

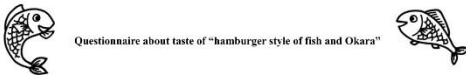





Fig 2. Cooking in a kitchen



Fig 3. “Hamburger style of fish and Okara” provided for school lunch

Questionnaire 1 is about the taste of “hamburger style of fish and Okara” and Questionnaire 2 is about the taste of ordinary hamburger (Fig 4). The recovery rates were 95.7% and 97.3%, respectively. In order to make it easy for elementary school children to understand, questions items and their expression methods were examined. Evaluation was made in three levels about overall taste, easy to eat, basic taste, aroma and appearance. Three levels are: good, ordinary and bad.

<div style="text-align: center;">  <p>Questionnaire about taste of “hamburger style of fish and Okara”</p> </div> <p>Please answer the question about “hamburger style of fish and Okara” or ☑ enter the appropriate one.</p> <ol style="list-style-type: none"> <li>1. What grade are you in ____ .    <input type="checkbox"/>male    <input type="checkbox"/>female</li> <li>2. Was the total taste favorable? <input type="checkbox"/>Good    <input type="checkbox"/>ordinary    <input type="checkbox"/>bad</li> <li>3. Was it easy to eat? <input type="checkbox"/>Good    <input type="checkbox"/>ordinary    <input type="checkbox"/>bad</li> <li>4. Was it tasty? <input type="checkbox"/>Good    <input type="checkbox"/>ordinary    <input type="checkbox"/>bad</li> <li>5. How was the smell? <input type="checkbox"/>Good    <input type="checkbox"/>ordinary    <input type="checkbox"/>bad</li> <li>6. How was the looking? <input type="checkbox"/>Good    <input type="checkbox"/>ordinary    <input type="checkbox"/>bad</li> </ol> <p>Please write in the box below whatever you felt.</p> <div style="border: 2px solid yellow; border-radius: 15px; height: 40px; margin: 10px 0;"></div> <p style="text-align: right;">Thank you very much for your cooperation. </p>	<div style="text-align: center;">  <p>Questionnaire about taste of “hamburger”</p> </div> <p>Please answer the question about “hamburger” or ☑ enter the appropriate one.</p> <ol style="list-style-type: none"> <li>1. What grade are you in ____ .    <input type="checkbox"/>male    <input type="checkbox"/>female</li> <li>2. Was the total taste favorable? <input type="checkbox"/>Good    <input type="checkbox"/>ordinary    <input type="checkbox"/>bad</li> <li>3. Was it easy to eat? <input type="checkbox"/>Good    <input type="checkbox"/>ordinary    <input type="checkbox"/>bad</li> <li>4. Was it tasty? <input type="checkbox"/>Good    <input type="checkbox"/>ordinary    <input type="checkbox"/>bad</li> <li>5. How was the smell? <input type="checkbox"/>Good    <input type="checkbox"/>ordinary    <input type="checkbox"/>bad</li> <li>6. How was the looking? <input type="checkbox"/>Good    <input type="checkbox"/>ordinary    <input type="checkbox"/>bad</li> </ol> <p>Please write in the box below whatever you felt.</p> <div style="border: 2px solid yellow; border-radius: 15px; height: 40px; margin: 10px 0;"></div> <p style="text-align: right;">Thank you very much for your cooperation. </p>
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Questionnaire 1.

Taste of hamburger style of fish and Okara

Questionnaire 2.

Taste of ordinary hamburger

Fig 4. Questionnaire

Table 1 shows the food material weight of “hamburger style of fish and Okara”. For one person is about 80g. Since it was deep-fried and provided, the amount of oil absorption is set to about 5.6g (oil absorption rate 7%). In addition, we offered a sauce of grated Japanese radish. The amount for one person is about 28g. Therefore, the total amount of “hamburger style of fish and Okara” that we offered to children was about 114g.

Table 1. The food material and their weights of “hamburger style of fish and Okara”

	Food material	Weight (g)	Food material	Weight (g)
Dough (80g)	Alaska pollack Surimi	22.7	Onion	12.1
	Okara (law)	13.6	Carrot	2.3
	Tofu (Momen)	9.1	Burdock	2.3
	Bread crumb	3.0	Green soybean	2.3
	Rice powder	2.0	Nutmeg	0.05
	Milk	6.3	Salt	0.5
	Egg	3.8	Pepper	0.05
Deep-fry (5.6g)	Oil absorption	5.6		
Sauce (28g)	Japanese radish	20.0	Soy sauces	4.0
	Mirin	2.0	Sugar	1.8

Sauce : Grated Japanese radish seasoned with soy sauce

Table 2 shows the food material weight of ordinary hamburger. For one person is about 45g. We baked by steam convection oven and offered it.

Table 2. The food material and their weights of ordinary hamburger

	Food material	Weight (g)	Food material	Weight (g)
Dough (45g)	Pork (thigh)	0.8	Bread crumb	3.2
	Chicken (thigh)	18.0	Miso (red)	2.4
	Onion	12.0	Sugar	0.8
	Ginger	0.8	Sesame	0.8
	Egg	6.0		

Table 3 shows energy and nutrient concentrations. Calculated in Standard Tables of Food Composition in Japan (2015)<sup>7)</sup>.

Table 3. Energy and nutrient concentrations of “hamburger style of fish and Okara” and ordinary hamburger

	Energy	Protein	Lipids	Ca	Fe	Fiber	Salt
	kcal	g	g	mg	mg	g	g
Hamburger style of fish and Okara							
Raw dough	81	7.0	1.8	37	0.4	2.2	0.8
Fried dough	133	7.0	7.4	37	0.4	2.2	0.8
Fried dough and sauce	152	7.4	7.4	43	0.5	2.5	1.4
Ordinary hamburger	157	11.7	9.3	23	0.6	0.6	0.4

Standard Tables of Food Composition in Japan (2015)

### 3. Statistical analysis

Statistical analysis was conducted by Chi-square test using Excel Statistics.

### 4. Ethical considerations

We explained to the school the purpose and methods of the research and that would make every effort so that nobody can identify the school and individuals. We used only common foods. The school explained the above information to the teachers and parents and obtained their agreement.

## RESULTS

Their evaluation about ordinary hamburger and “hamburger style of fish and Okara” were respectively; Overall taste is good 94 and 85%, good for easy to eat 90 and 77%, basic taste is good 93 and 83%, aroma is good 82 and 68%, appearance is good 89 and 76%. Fig 5 shows the comparison of taste between ordinary hamburger and “hamburger style of fish and Okara”. Although the results of ordinary hamburger were more favorable than those of “hamburger style of fish and Okara”, the evaluation of “hamburger style of fish and Okara” was also high. The lowest evaluation among Okara hamburger is aroma (68%). However, the evaluation means that 68% of the children answered that they smelled good.

Out of 43.49kg of the total amount of “hamburger style of fish and Okara”, the amount of waste was 2.01kg and the waste rate was 4.6%. On the other hand, out of the total volume of ordinary hamburger 26.7kg, the amount of waste was 0.6kg and the waste rate was 2.2%. From Table 3, it was found that 2.2g of dietary fiber is contained in 80g of “hamburger style of fish and Okara” (law dough). This indicates that it contains dietary fiber about 4 times more than ordinary hamburger.

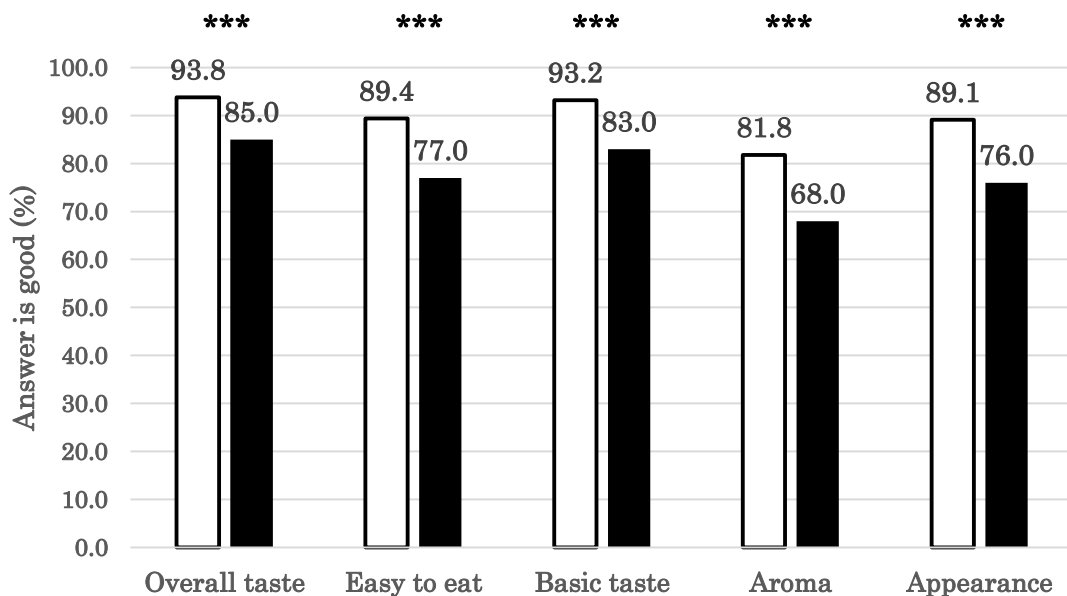


Fig 5. Comparison of taste between ordinary hamburger and “hamburger style of fish and Okara”

\*\*\* Significantly different by Chi-square test at  $p < 0.001$

□: Ordinary hamburger, ■: Hamburger style of fish and Okara



## DISCUSSION

“Hamburger style of fish and Okara” developed by us was able to obtain high evaluation as a result of providing it as school meal to elementary school students. Fish and Okara contain nutrients that are likely to be deficient in modern society, and various advantages can be considered, but their utilization is insufficient. Especially Okara is discarded in making tofu, but it is a food containing a lot of dietary fiber. In order to use fish and Okara as school lunch, it was an issue how to deliciously cook the odor of fish and the texture of Okara.

Dietary fiber is one of the nutrients deficient in school lunch<sup>4,5</sup>. Root crops and legumes have many dietary fiber, but it is difficult to ingest dietary fiber sufficiently from these vegetables because there are many children left behind. It is possible to use staple food such as brown rice, but it is not realistic because it requires a special rice cooker. Pre-germinated brown rice can be cooked with ordinary rice cooker, but the price becomes somewhat higher. For these reasons, it is desired that intake of dietary fiber is taken from the main dish.

In this research, “fish and Okara” was main dishes and we examined how to make delicious dishes with them. Surimi (paste) of Alaska pollack had a considerable fish odor. We examined using sake, mirin, milk, salt and ginger. Alcohol contained in sake has the function of evaporating trimethylamine which is a component of the fish odor. Mirin also has a similar function<sup>10</sup>. Colloidal particles of milk protein and fat tend to adsorb odor components<sup>11</sup>. Salt exerts moisture rich in trimethylamine out of fish body due to osmotic pressure effect. Strong fragrance ginger component cineol becomes a component without smell when mixed with trimethylamine. For these reasons, we tried five methods. As a result, we were able to confirm that milk is a food ingredient that eliminates of the fish odor. It was considered that the evaluation of “aroma” is high because it was able to suppress the fish odor.

We studied the use of Okara to increase dietary fiber. Okara has three types, (1) Okala obtained by traditional production method and contains 81.1g/100g of water<sup>12</sup>, (2) Okala obtained by the new manufacturing method and contains 75.5g/100g of water<sup>7,12</sup>, and (3) Okara obtained by drying and contains 7.1g/100g of water<sup>7</sup>. In this study, (2) Okara was used. This Okara has a characteristic that the fiber is long, and I expected a role as a “tether” such as croquette and hamburger. In order to reduce roughness due to Okara, the blending ratio of Okara, tofu and milk was examined. As a result, milk was most effective. It is thought to be an effect by lipid contained in milk.

In the cooking process, we were aware of adding salt to surimi. The fish meat has the

property that when it is well kneaded by adding salt, the binding property increases<sup>13)</sup>. As a result, I was able to alleviate more roughness.

In order to regulate the firmness of the dough, the ingredients and its blending amount were examined. As a result, 3g/80g of bread crumbs and 2g/80g of rice flour gave the soft texture of the law dough. It seems that the characteristics of the rice flour were also related. The rice flour used this time is made in Japan, sucks water well and thinks it was connected to a soft texture<sup>14)</sup>.

We examined the addition of vegetables to add texture, crunchy, coloring to this fabric. A combination of carrots, burdocks, and green soybeans was evaluated with good texture and color. As a result of examining the composition of the seasoning, it was the best evaluation with salt 0.5g/80g, nutmeg and pepper 0.05g/80g of the law dough.

By repeating trial production like this, we were able to produce more delicious “hamburger style of fish and Okara”. However, we must pay attention to the amount of salt contained in “hamburger style of fish and Okara”. Law dough contains 0.8g/80g of salt. When I offered it to children, I attached a sauce of grated Japanese radish. Therefore, it was decided that fried dough and grated Japanese radish, contained 1.4g/110g of salt. The salinity of the whole serving meal was 3.2g. The reference value of salt equivalents for school lunch is 2g or less at 6-7 years old, 2g or less at 8-9 years old, 2.5g or less at 10-11 years old, and 2.5g or less at 12-14 years old<sup>3)</sup>. Even compared with the average salt intake of elementary school, it is high<sup>4)</sup>. We need to consider ways to lower salt while maintaining taste.

The target school of this research is a medium-sized elementary school with 349 children (1st to 6th grade children), the ratio of men and women is almost the same. Even in comparison with the results such as surveys of family situations and students' tastes, physique of height/weight, lifestyle surveys such as sleeping hours and absence of breakfast<sup>15)</sup>, national physical fitness · exercise ability · exercise habit survey<sup>16)</sup> etc. that school was the average school in the area.

In this research, we aimed to be able to ingest “fish” and “dietary fiber” lacking in the eating habits of present children at the same time, and to be able to offer development of “delicious main dish” especially for lunch. The evaluation about ordinary hamburger and “hamburger style of fish and Okara” were respectively; Overall taste is good 94 and 85%, good for easy to eat 90 and 77%, basic taste is good 93% and 83%, aroma is good 82 and 68%, appearance is good 89 and 76%. Although the results of ordinary hamburger were more favorable than those of “hamburger style of fish and Okara”, the evaluation of “hamburger style of fish and Okara” was high. The lowest evaluation among Okara hamburger is aroma (68%). However, the evaluation means that 68% of

the children answered that they smelled good. It can not be said that “hamburger style of fish and Okara” is not tasty. In addition, “hamburger style of fish and Okara” contains 2.2g of dietary fiber per 80g. This suggests that “hamburger style of fish and Okara” may meet the requirement of dietary fiber. However, I think that whether Surimi of Alaska pollack can obtain the necessary amount at a price that can be used for school lunches throughout the year is a major task and a limit to incorporate into the menu as new meal.

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## **STUDY 3**

**Okara improved blood glucose level in Vietnamese  
with type 2 diabetes mellitus**

## ABSTRACT

**Background:** Diabetes mellitus (DM) has been increasing rapidly in Vietnam. We hypothesized that the main reason may be low fiber intake. Regarding sources, fiber comes mainly from vegetables. However, vegetables popular in Vietnam have low fiber (<2g fiber/100g vegetable), so it is difficult to supply sufficient fiber only from vegetables. Therefore, in this study we tried to increase fiber intake a day by using 60g of Okara foods, containing about 6g of fiber per day, and assess the effects on the blood glucose levels of DM patients. **Method:** We contacted 300 type 2 DM outpatients at a hospital and selected 60 of them. We formed 30 pairs matched by gender, age, BMI and years with DM and divided them randomly into an intervention group and a control group. The intervention group consumed about 6g of fiber from Okara per day for two weeks. At the baseline and final periods, anthropometric measurements, blood withdrawal and a 3-day weighing method nutrition survey were conducted. **Results:** Dietary fiber intake increased from 6.9 to 12.6g ( $p<0.01$ ) in the intervention group, but there was no change in the control group. Fasting blood glucose (FBG) and fructosamine in the intervention group dropped from 113 to 97mg/dL ( $p<0.05$ ) and from 319 to 301 $\mu$ mol/L ( $p<0.05$ ), respectively but they remained unchanged in the control group. **Conclusion:** Vietnamese people consumed about 60g of Okara per day from various menus and increased fiber intake to about 6g/day in 2 weeks, which improved blood glucose in DM patients.

Key words: Diabetes mellitus, Okara fiber, blood glucose, fructosamine, Vietnamese

## INTRODUCTION

Diabetes is a serious, chronic disease that occurs either when the pancreas does not produce enough insulin (a hormone that regulates blood sugar or glucose), or when the body cannot effectively use the insulin. Diabetes and its complications bring about substantial economic loss to patients and their families and to health systems and economies<sup>1)</sup>. The prevalence of DM is increasing worldwide; type 2 DM is the most common form of diabetes (90% of cases) and Vietnam is not an exception<sup>2,3)</sup>. During the last decade, along with rapid economic growth and urbanization, the Vietnamese lifestyle and dietary habits have changed<sup>4)</sup>. There are studies suggesting that the risk factors for DM in Vietnam are high intake of white rice, abdominal fat, and a sedentary lifestyle<sup>5-7)</sup>. Worldwide, the BMIs of type 2 DM patients are usually high ( $>25 \text{ kg/m}^2$ )<sup>8)</sup> but some studies have reported that the BMI of Vietnamese DM is usually not high ( $<25 \text{ kg/m}^2$ )<sup>5-7)</sup>. Taiwan and Vietnam are located in the same region (Asia); however, according to a previous study, there are differences in the BMIs of newly diagnosed type 2 DM patients in the two countries (Taiwanese 26 and Vietnamese 23)<sup>5)</sup>. In addition, Vietnam has recently been facing the problem of “Nutrition Transition”, including changing dietary patterns, a different meal structure such as increases in meat and fat, and decreasing vegetable consumption<sup>4)</sup>. Therefore, we think that dietary factors may be more important than genetic factors.

Although it is a fact that drug therapy is mandatory for most type 2 DM patients, dietary treatment is essential in diabetes management. Meta-analysis studies have shown that increasing dietary fiber intake in patients with type 2 DM decreases HbA1c and FBG levels<sup>9,10)</sup>. Nhung BT showed that increasing fiber intake from 6.7g/day up to 13.1g/day with pre-germinated brown rice was effective in controlling blood glucose and lipid levels as well as body weight in people who consume rice as a staple food<sup>11)</sup>. White rice has a higher glycemic index and less fiber than brown rice. In addition, our previous study also indicated that an increase in fiber intake from vegetables also has an effect on blood glucose of type 2 DM patients<sup>12)</sup>. Therefore, fiber may be effective in controlling blood glucose.

The main source of fiber in Vietnamese cuisine is vegetables. However, the amount of fiber in the vegetables widely in Vietnam used is low (average less than 2g fiber/100g vegetables)<sup>13)</sup> (Table 1).

Table 1. Comparison of dietary fiber content of Okara and vegetables (per 100g edible portion)

Okara and vegetables	Dietary fiber (g)
Okara (traditional product)	9.7
Wax gourd (raw)	1.0
Water convolvulus (raw)	1.0
Kidney beans (immature pods, raw)	1.0
Cabbage (raw)	1.6
Broccoli (raw)	3.2
Bitter gourd (raw)	1.1
Asparagus (shoots, raw)	2.3
Bamboo shoots (raw)	4.1

Vietnamese food composition table, 5<sup>th</sup> Ed.

Okara (traditional product): Standard tables of food composition in Japan, 2010

So it is difficult for people to eat large amounts of vegetables every day to reach sufficient fiber intake. According to a Vietnamese general national survey (2010), the average consumption of vegetables was about 190g/person/day<sup>4</sup>. This was less than the Vietnamese recommended daily allowances for adults (300g/day)<sup>14</sup> and less than the Dietary Treatment for type 2 DM patients (300-500g/day)<sup>15</sup>. In addition, a variety of foods rich in fiber will provide interest in the daily diet. For these reasons, we focused on raising fiber intake from Okara, a fiber-rich food. The content of dietary fiber of Okara (traditional product) is about 10.9g/100kcal and 9.7g/100g, greater than many kinds of vegetables<sup>16</sup>. Okara remains after pureed soybeans are filtered in the production of soy milk and tofu and contains a large proportion of fiber. Okara contains three beneficial components: insoluble dietary fiber,  $\beta$ -conglycinin and isoflavones. Studies on rats have shown that a diet containing higher amounts a higher amount of  $\beta$ -conglycinin and isoflavones has an effect on blood glucose control<sup>17,18</sup>. However, Okara contains only a small amount of these ingredients ( $\beta$ -conglycinin and isoflavones), so we think that fiber would be the main factor to consider when using Okara for humans.

Okara is used as animal feed in Vietnam but Japanese eat it in their daily meals in various dishes. Hence, we wanted to take advantage of abundant Okara sources in Vietnam to make various new menus, which would help to increase fiber intake by DM patients. Two previous studies indicated that increasing fiber intake by about 6g to reach



12-13g fiber intake has a positive effect on blood control<sup>11,12</sup>). So in this study we set a target of about 6g Okara fiber/day with each Okara menu containing about 2g fiber and each main meal including one Okara dish. The purpose of the present study, therefore, was to find out whether 6g Okara fiber/day for 2 weeks decreases blood glucose in type 2 DM.

## METHODS

### 1. Setting and study subjects

The study was designed as a randomized controlled trial. The study population consisted of type 2 DM outpatients being treated at hospital located in Hanoi City (northern Vietnam). After screening data for about 2000 type 2 DM outpatients at the hospital, we selected about 300 type 2 DM who met the inclusion criteria: (1) type 2 DM out-patients aged 45-70 years old and with a diabetic history of more than 2 years; (2) patients were receiving only oral medication and had not changed oral medication for 3 months; (3) they did not suffer from other diseases (except hypertension and dyslipidemia). Through contact by phone and direct meetings at the out-patient clinic, 60 subjects who agreed to participate in this study were enrolled. Then 30 matched pairs were created by sex, age, BMI and years with DM. The subjects were divided into 2 groups: an intervention group (Okara group) and a control group (Fig 1).



Fig 1. Photograph selecting subjects at a hospital

We made 10 Okara menus (staple dishes, main dishes and side dishes). The ten menus were: fried rice with Okara, rice porridge with Okara, Okara hamburger, meatballs with Okara, stir fried piper lolot rolls with pork and Okara, omelette with Okara, cabbage rolls with Okara, stir fried bean sprouts with Okara, miso soup with Okara, Okara patties. For example, for the hamburger we mixed 50g meat and 30g Okara. As another example, to 50g rice porridge, we added 20g Okara. Okara patties were made with 70g Okara, 5g starch and 30g tofu and 12 g mayonnaise (Fig 2).



Fried rice with Okara



Rice porridge with Okara



Okara hamburger



Meatballs with Okara



Stir fried piper lolot rolls with pork and Okara



Omelette with Okara



Cabbage rolls with Okara



Stir fried bean sprouts with Okara



Miso soup with Okara



Okara patties

Fig 2. The 10 Okara menus

Each menu contains about 2g fiber from Okara. Three Okara dishes per day provide sufficient fiber (6g); maintaining this diet for 2 weeks was expected to decrease blood glucose levels in subjects in this study. We carried out a pilot study on 12 people at HMU to assess the taste of the Okara dishes. Okara menus were thus modified to be acceptable to Vietnamese.

One week before intervention day, all the patients were assembled to be introduced to the research, to sign consent forms and to be instructed on how to record dietary items by the weighing method. And the patients recorded all the food they consumed 3 non-consecutive days (Fig 3).



Fig 3. Explanation of how to measure the meal

After conducting a dietary record for 3 days, both groups received nutrition education about dietary guidelines for DM patients, especially the role of fiber, and were given nutrition education materials. For the Okara group only, participants received further instruction on Okara menu cooking methods to increase fiber intake. Subjects tasted Okara dishes and gave positive feedback about implementing the study (Fig 4). Subjects were encouraged to maintain their usual vegetable and fruit intakes. All the patients underwent anthropometric measurements and blood tests and submitted dietary records to the researchers at this time (baseline data).



Fig 4. Subjects in the Okara group taste Okara dishes

During the intervention period (2 weeks), the Okara group included the Okara menus in their diet but the control group continued their diet without Okara. All patients recorded dietary information for the last 3 days of the research period. During the whole study period, investigators contacted the subjects every day by telephone and found that the rough intake of Okara was close to the results of the nutrition survey during the last 3 days. After 2 weeks, all patients were assembled to collect final data on anthropometric measurements, blood tests and dietary records during the research period.

## 2. Anthropometric measurement

Weight, height, and waist and hip circumferences and body fat percentage were measured twice, at the beginning and on the last day, and the average value was calculated for each individual. Body weight and height were measured in light clothing and without shoes. Body mass index (BMI) was computed as the ratio of weight (kg) per height squared ( $m^2$ ). Waist circumference was measured at the minimum circumference between the umbilicus and iliac crest; hip circumference was measured at the widest circumference around the buttocks. Waist-hip ratio was calculated as waist circumference (cm) divided by hip circumference (cm). Body weight, percent body fat, percent muscle and visceral fat were measured by bioelectrical impedance using a body

fat analyzer Tanita BC-526-WH scale (Fig 5).



Fig 5. Anthropometric measurement

### 3. Blood collection

Intravenous fasting blood samples were taken in the morning at baseline and final (after 2 weeks) (Fig 6). The blood samples were separated by centrifuge and kept frozen for analyses of glucose and fructosamine.



Fig 6. Blood collection

#### 4. Nutrition survey

A nutrition survey was conducted for 3 days by the weighing method before and last the intervention period. Each patient was given a scale to weigh the foods they ate and wrote dietary records by themselves. Energy and nutrient intakes were calculated based on the Vietnamese Food Composition Table 2007<sup>13)</sup>. Energy and nutrient in Okara was calculated by using the data published in the Japanese Food Composition Table 2010<sup>16)</sup>.

#### 5. Statistical analysis

Quantitative variables were checked for normal distribution and compared by the Student *t*-test (paired and unpaired). The *p-values* of less than 0.05 were considered statistically different for all the analyses. The above statistical procedures were performed using Stata Version 12.0.

#### 6. Ethical considerations

This research was approved by the ethical committee of Hanoi Medical University (HMU) (number 65/HĐĐDHYHN) on 28th March 2017.

## RESULTS

Eleven of 60 subjects dropped out of the study; thus 49 patients (23 in interventions and 26 in controls) completed the follow-up. The reason for dropping out of the study were: did not have time to prepare the Okara menus, had serious illnesses, had already eaten before coming to have blood glucose checked, lost interest in the study, cited personal reasons.

Table 2 shows the comparison of physical characteristics at baseline and final of control and Okara groups. The average BMI in all the subjects was 23.2-23.7, which was within the normal range. There were no significant differences in values between the control and Okara group in other items such as age, weight, BMI, body fat, visceral fat before and after study period ( $p>0.05$ ).

Table 3 indicates the comparison of biochemical parameters at baseline and final of control and Okara groups. In the Okara group, FBG decrease showed significant difference between at baseline and final. Fructosamine for both genders was

significantly reduced after intervention, compared to the baseline. Such reduction was not found in the control group.

Table 4 shows energy and nutrient intakes of both groups at baseline and final; data within the group were not significant different.

Table 2. Comparison of physical characteristics at baseline and final of control and Okara group

	Control group (n=26)				Okara group (n=23)			
	Baseline		Final		Baseline		Final	
	Male (n=9)	Female (n=17)	Male (n=9)	Female (n=17)	Male (n=6)	Female (n=17)	Male (n=6)	Female (n=17)
Age (year)	61.7±7.2	64.2±4.8	61.7±7.2	64.2±4.8	62.8±3.7	61.3±8.5	62.8±3.7	61.3 ± 8.5
Weight (kg)	66.1±6.4	53.8±7.2	66.1±6.4	53.9±7.3	59.0±14.0	54.8±6.6	59.3±13.8	54.5±6.7
Height (m)	1.64±0.06	1.53±0.04	1.64±0.06	1.53±0.04	1.61±0.07	1.54±0.05	1.61±0.06	1.54±0.05
BMI (kg/m <sup>2</sup> )	24.6±2.9	23.0±3.3	24.6±2.9	23.0±3.3	22.6±4.3	23.1±2.2	22.7±4.2	23.1±2.3
Body fat (%)	24.5±3.1	34.1±4.5	24.4±3.3	34.2±5.7	21.8±7.2	34.5±3.5	21.5±7.3	34.5±3.4
Visceral fat (%)	13.5±2.7	6.3±2.0	13.5±2.7	6.4±2.2	10.8±5.5	6.6±1.5	10.8±5.5	6.5±1.3
Waist (cm)	90.8±5.7	86.1±9.5	90.9±5.8	86.1±9.6	83.9±12.8	85.1±5.6	84.5±12.6	83.9±5.3
Hip (cm)	98.0±4.7	93.5±6.5	98.3±5.0	93.5±6.6	94.5±7.6	92.4±4.9	94.0±8.5	93.4±4.6
Waist/hip ratio	0.93±0.05	0.92±0.05	0.93±0.05	0.92±0.05	0.88±0.07	0.92±0.05	0.90±0.07	0.90±0.05

Values are mean±SD



Table 3. Comparison of biochemical parameters at baseline and final of control and Okara group

	Control group (n=26)				Okara group (n=23)			
	Baseline		Final		Baseline		Final	
	Male (n=9)	Female (n=17)	Male (n=9)	Female (n=17)	Male (n=6)	Female (n=17)	Male (n=6)	Female (n=17)
	All		All		All		All	
FBG (mg/dL)	124±25	126±31	122±41	124±31	97±27	117±29	95±38	103±27*
	126±29		124±34		113±29		97±27*	
Fructosamine (µmol/L)	309±44	311±36	317±44	318±49	331±28	315±23	301±22*	301±32*
	311±38		318 ± 47		319 ± 25		301 ± 29*	

Values are mean±SD

\* Significant difference by Student *t*-test between the baseline and final data (of same gender) within same group ( $p<0.05$ )

Table 4. Energy and nutrient intakes of control and Okara groups at baseline and final

	Control group (n=26)				Okara group (n=23)			
	Baseline		Final		Baseline		Final	
	Male (n=9)	Female (n=17)	Male (n=9)	Female (n=17)	Male (n=6)	Female (n=17)	Male (n=6)	Female (n=17)
Energy (kcal)	1574 ±486	1255±251	1765±738	1330±305	1630±254	1338±214	1524±303	1409±216
Energy (kcal/kgBW)	23±76	23±35	26±114	25±42	28±18	24±32	26±22	26±32
Protein (g/d)	68.5±28.1	66.2±53.1	93.0±46.7	61.5±14.9	81.4±13.2	66.3±11.0	83.9±24.3	68.2±14.3
Protein (g/kgBW)	1.0±4.4	1.2±7.4	1.4±7.2	1.1±2.0	1.4±0.9	1.2±1.7	1.4±1.7	1.3±2.1
Lipid (g/d)	56.3±27.1	36.5±12.4	53.6±38.6	34.7±8.3	39.4±22.4	24.8±9.0	42.4±22.1	28.9±8.3
Lipid (g/kgBW)	0.9±4.2	0.7±1.7	0.8±6.0	0.6±1.1	0.7±1.6	0.5±1.4	0.5±1.8	0.5±1.2
Carbohydrate (g/d)	227±81	176±41	227±97	191±59	237±20	212±52	202 ± 47	219±42
Carbohydrate (g/kgBW)	3.4 ±6.4	3.3±5.7	3.4±15.0	3.5±8.2	4.0±1.5	3.8±7.9	3.4±3.4	4.0±6.3

Values are mean±SD

Control group were compared to Okara group by unpaired Student *t*-test

Fig 7 shows that the base of both groups is similar in dietary fiber intake. However, the Okara group consumed about 6.9g at baseline and 12.6g at final, this difference is significant ( $p<0.01$ ). From our daily contact, these values were maintained throughout the whole period. Additionally, our study indicates 70% of subjects ate 60g Okara or more per day. There was 20% of people eating from 50 to 59g/day and 10% who ate less than 50g/day during the 2 week intervention. After the study finished, we gave an Okara taste questionnaire to the 23 subjects in the Okara group. Twenty one people (91%) answered favorably, and 2 unfavorably (9%).

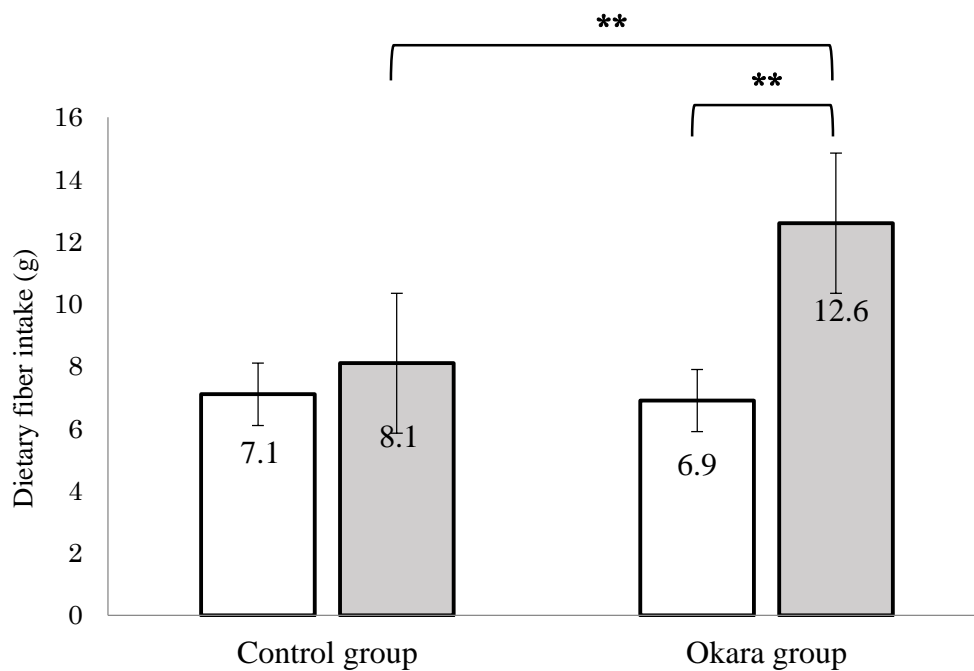


Fig 7. Fiber intake of control and Okara groups

□ : Baseline    ■ : Final

\*\*Significant difference by Student *t*-test between and within the groups at the baseline and final ( $p<0.01$ )

## DISCUSSION

Dietary fiber is recommended for diabetes and reduce risk of DM because of reducing postprandial glucose respond, decrease gastric emptying feeling and beneficially influence certain on blood lipid<sup>19)</sup>. 100g Okara (traditional product) contains about 0.3g soluble fiber and 9.4g insoluble fiber (about 0.3g soluble fiber and 10.6g insoluble fiber at 100kcal)<sup>16)</sup>. Beyond successful experiments on rats<sup>17,18)</sup>, this study was carried out on type 2 DM patients and brought about an initial effect on blood glucose control by using Okara. Okara is a by-product in the production of tofu and soymilk. It is considered a functional food in Asia<sup>20)</sup>. Okara is used in Japanese foods; however, in Vietnam it is used only as animal feed. One reason may be the abundance of vegetables in Vietnam. However, with the recent development of the economy and a new food culture of foreign fast foods such as hamburger, beefsteak, and chicken tenders, vegetable intake has decreased about 190g/person/day<sup>4)</sup>. In such a situation, people have paid increased attention to fiber-rich foods such as Okara. The success of this study lies in its good cooking methods.

In this study we tried to develop 10 Okara menus and did a pilot study to assess acceptability. This helped to prevent monotony in the menu and also provided participants or other preparers some interest in varied cooking methods based on Vietnamese cooking methods. For example, the taste of Okara hamburger was similar to that of a meat-only hamburger. As another example, the taste of the porridge which is added more Okara was not spoiled. Okara patties were also very well accepted, because DM patients are not encouraged to eat sweet cakes but our Okara patties are not only tasty but also the energy intake is very low. Therefore, subjects did not have to worry about maintaining a low energy diet. In addition, the cabbage rolls with Okara received favorable comments because an umami taste was created by the combination of foods as pork, cabbage, tomato, fish sauce and Okara. This menu increased fiber intake from Okara and vegetables significantly. We succeeded in incorporating a new food material (Okala) without greatly departing from routine recipes and tastes of Vietnamese. As a result, 70% of the subjects were able to eat more than 60g Okara/day. We suggested to eat 60g Okara/day, but 30% of the subjects were less than 60g/day. After two weeks of the intervention, we asked the subjects (Okara group) whether they liked the Okara dishes. More than 90% of the subjects (Okara group) answered that they liked them. According to the nutrition survey for 3 days at the baseline and the last, energy intake did not change, indicating that the Okara dishes did not adversely affect subjects' taste for other foods in the menus. We think Okara is readily accepted and may be become a new

food culture in Vietnam in the future.

We suggested that the intervention group consume 60g Okara (about 6g fiber) a day and they took about 59g Okara (5.7g fiber). Total fiber intake was  $12.6 \pm 2.8$ g at final. This was still much smaller than the recommended dietary allowance for Vietnamese (20-22g/day)<sup>21</sup>). Nevertheless, we observed a very favorable effect of Okara on blood glucose. There are studies that have also observed a similar favorable effect in type 2 DM patients by increasing fiber intake to reach 12-13g<sup>11,12</sup>). These results may suggest that the fiber requirement is not so high as the Dietary Recommendation Intake for Vietnamese (20-22g/day) but we need further studies to find the proper requirement.

Our participants were patients who cared about the diet for type 2 DM and had some knowledge about nutrition. Understanding that the amount of fiber in Okara was good for their health, they readily accepted Okara. In this study the period of intervention was only 2 weeks because the turnover rate of fructosamine is 2-3 weeks. Fortunately, we could see a clear decrease in it in the intervention group. Energy and nutrient intake was estimated by the weighing method for 3 non-consecutive days, which placed a heavier burden on the subjects but brought reliable results about patients' actual intake. The average energy intake of both groups was 1548kcal (control group) and 1467kcal (Okara group), respectively. We found that the energy ratio of protein, fat and carbohydrate (P:F:C ratio) at final was about 20%:26%:54% (control group) and about 21%:22%:57% (Okara group). The energy intakes (25kcal/kg/day) and P:F:C ratio (15-20%:25-30%:50-60%) in the two groups almost met the Dietary Treatment for DM (Vietnamese)<sup>15</sup>). There is a report that shows that there were no significant differences in fructosamine levels between males and females in both groups<sup>6</sup>). Although the number of male and female subjects was different in this study, we likewise did not observe differences in blood biochemistry.

Another possible conclusion from the results of this study is that perhaps one of the main factors in the low BMI of Vietnamese type 2 DM is high white rice intake together with low fiber intake. In Vietnam, intake of white rice is the main source of carbohydrate intake. The P:F:C ratio of general Vietnamese people is about 15%:18%:67% (2010). It has improved considerably from about 11%:6%:83% (1985), but still the intake of white rice is high<sup>4</sup>). Our study may be useful for the prevention of type 2 DM in Vietnam in the future.

In conclusion, we found in this study that Vietnamese people were satisfied with new menus with Okara prepared by various cooking methods and could increase their fiber intake about 6g/day during 2 weeks, which improved blood glucose in type 2 DM patients.

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