

# Is the Quality of Sushi Ruined by Freezing Raw Fish and Squid? A Randomized Double-Blind Trial With Sensory Evaluation Using Discrimination Testing

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**Background.** Sushi is a traditional Japanese cuisine enjoyed worldwide. However, using raw fish to make sushi may pose risk of certain parasitic infections, such as anisakidosis, which is most reported in Japan. This risk of infection can be eliminated by freezing fish; however, Japanese people are hesitant to freeze fish because it is believed that freezing ruins sushi's taste.

**Methods.** A randomized double-blind trial with discrimination testing was conducted to examine the ability of Japanese individuals to distinguish between frozen and unfrozen sushi. A pair of mackerel and squid sushi, one once frozen and the other not, was provided to the participants, and they were asked to answer which one tasted better.

**Results.** Among 120 rounds of discrimination testing involving the consumption of 240 pieces of mackerel sushi, unfrozen sushi was believed to taste better in 42.5% (51 dishes) of cases, frozen sushi was thought to taste better in 49.2% (59 dishes), and the participants felt the taste was the same in 8.3% (10 dishes). The odds ratio for selecting unfrozen sushi as "tastes better" over frozen sushi was 0.86 (95% confidence interval [CI], .59–1.26;  $P = .45$ ). For squid, unfrozen sushi was believed to be superior 48.3% of the time (58 dishes), and frozen sushi, 35.0% of the time (42 dishes). They were felt to be the same in 16.7% (20 dishes) (odds ratio, 1.38; 95% CI, .93–2.05;  $P = .11$ ).

**Conclusions.** Freezing raw fish did not ruin sushi's taste. These findings may encourage the practice of freezing fish before using it in sushi, helping to decrease the incidence of anisakidosis.

**Keywords.** sushi; anisakidosis; freezing; sensory evaluation.

Sushi is a traditional Japanese cuisine, which is enjoyed not only in Japan but also worldwide. However, using raw fish to make sushi may pose a risk of certain parasitic infections. *Anisakis* infection, or anisakidosis, is one of the parasitic infections associated with sushi eating, which was first observed in the Netherlands in 1960 [1].

Most human infections are caused by *Anisakis simplex*, *Anisakis pegreffii*, and *Pseudoterranova decipiens* [2]. Gastric anisakidosis is the most common form and is characterized by severe epigastric pain, resulting from larval invasion of the gastric mucosa. Anisakidosis is usually self-limited and not life threatening. However, the pain caused by *Anisakis* infection is excruciating. *Anisakis simplex* larvae are white or milky colored, 19–36 mm in length, and 0.3–0.6 mm in width [2], and can be detected and removed by upper gastrointestinal endoscopy (Figure 1). However, *Anisakis* can sometimes infect the small intestine. In this case, it can be difficult to remove and can be mistaken for acute abdomen, possibly resulting in an unnecessary laparotomy [3]. Rare cases of intestinal perforation or bleeding have been reported [3].

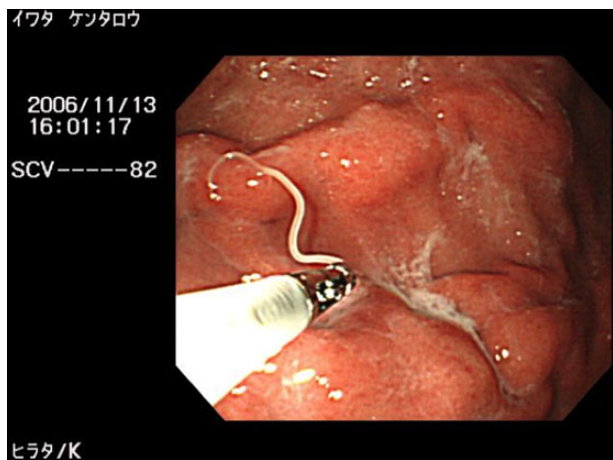
The European Union recently made it obligatory for raw fish to be frozen to a temperature of at least  $-20^{\circ}\text{C}$

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**Figure 1.** An endoscopic finding of *Anisakis* on the stomach wall of author K. I., 2006.

for >24 hours to prevent parasitic infections, including anisakidosis [4]. The US Food and Drug Administration also recommends that fish be frozen before consuming it raw. It recommends freezing and storing at an ambient temperature of  $-20^{\circ}\text{C}$  or below for 7 days (total time), or freezing at an ambient temperature of  $-35^{\circ}\text{C}$  or below until solid and storing at an ambient temperature of  $-35^{\circ}\text{C}$  or below for 15 hours, or freezing at an ambient temperature of  $-35^{\circ}\text{C}$  or below until solid and storing at an ambient temperature of  $-20^{\circ}\text{C}$  or below for 24 hours [5].

Japan does not have such regulations. Sushi masters are concerned that freezing raw fish ruins sushi's delicious taste. These chefs argue that freezing and then defrosting the fish can ruin its quality, especially the texture [4].

More than >90% of anisakidosis cases are reported from Japan, most likely due to widespread use of raw fish in traditional Japanese cuisine such as sushi and sashimi, without freezing their ingredients [2]. Common sources of infection in Japan are mackerel, squid, or sardine, which can carry and maintain L3 larvae of *Anisakis* [2].

Because the potential for taste deterioration is the only reason for Japan's refusal to recommend freezing of fish to prevent anisakidosis, we decided to conduct a randomized double-blind trial to investigate the ability of Japanese people to discriminate between sushi with frozen fish and that made with fresh, never frozen (hereafter "unfrozen") fish.

## METHODS

### Study Design and Participants

We conducted this double-blind trial on 13 July 2014 at Kobe University, Japan. Forty healthy adult medical students and postgraduate residents volunteered to participate in the trial.

Those who could eat 12 sushi pieces were eligible for this trial. Those who had a known allergy to mackerel or squid or those who detest sushi were excluded from the trial. Individuals who eat sushi more than once a week were also excluded from the trial, as they were judged to be gourmets, beyond the average Japanese palate. The participants were asked to avoid eating or drinking too much the day before the experiment, to take plenty of sleep, and to eat a usual breakfast on the day of the experiment.

The participants provided written informed consent. The study was approved by the ethics committee at Kobe University Graduate School of Medicine.

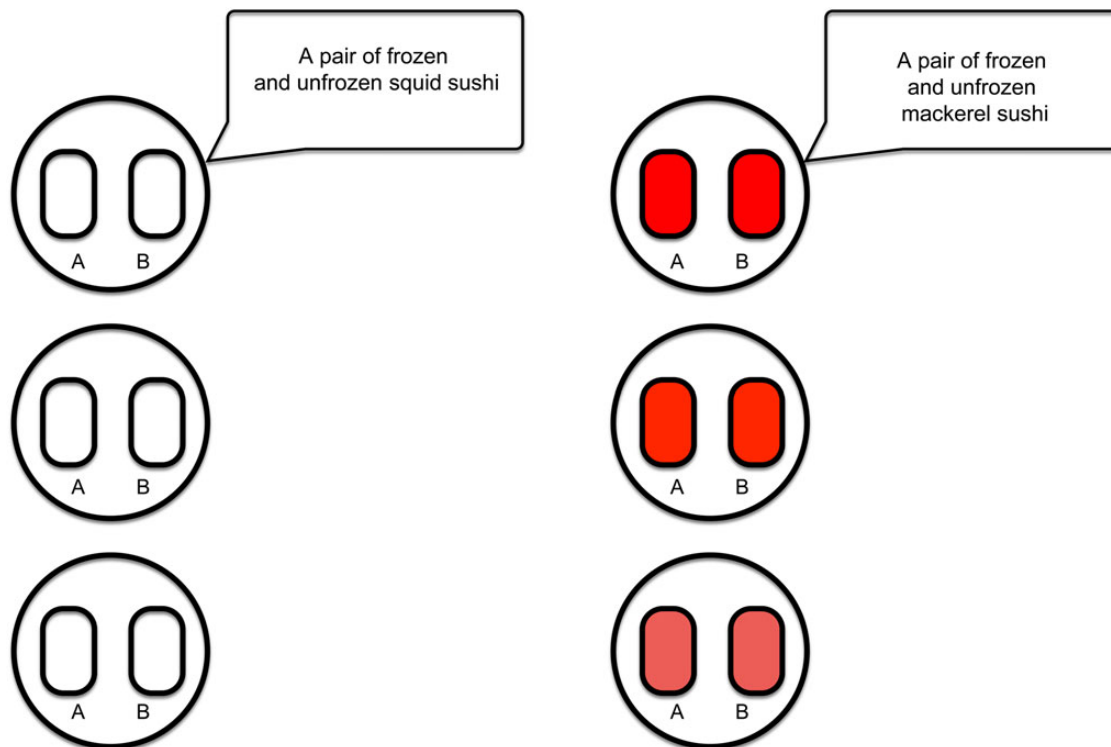
Two professional sushi chefs with >5 years of experience were recruited for the trial. One made mackerel sushi and the other made squid sushi.

### Ingredients Preparation

Mackerel and squid were selected as sushi material, as both are known to possess *Anisakis* and are used frequently for sushi. One week prior to the experiment, both mackerel and squid were purchased at a market in Osaka prefecture, and they were frozen in a freezer at  $-40^{\circ}\text{C}$ . Two days prior to the experiment, the same mackerel and squid were purchased at the same market and were kept in a usual refrigerator. The frozen fish were moved to the refrigerator on the night before the experiment and were defrosted there overnight. For mackerel, *Masaba*, the vernacular name for *Scomber japonicas*, caught at Wakayama prefecture was selected. For squid, *Kensaki-ika*, the vernacular name for *Photololigo edulis*, caught at Tottori prefecture was selected. The species and the site of the fisheries were identical for both the frozen and unfrozen ingredients. On the morning of the experiment, 2 sushi chefs cut mackerel and squid into sushi-sized pieces. They also cooked sushi rice (brand name, Koshi Hikari, made in Ishikawa prefecture), and the same rice was used for every piece of sushi for the experiment.

### Randomization and Masking

Two pieces of sushi, consisting of one once frozen and one unfrozen, were randomly placed on a plate, with a marking "A" on the left side and "B" on the right of the dish by the study assistants. The order of the mackerel and squid allocation was randomly determined using a random number list, with the identification number on the top of each dish. The dishes were then brought to 2 sushi chefs, who were blinded to the selection of ingredients. One chef made mackerel sushi and the other made squid. The participants were also blinded to the allocation of ingredients. Only salt, from Hiroshima prefecture, was used as flavoring, and no soy sauce, wasabi, ginger, or other ingredients were used in this experiment to avoid any confounding of the taste. Sushi was then placed back on the



**Figure 2.** Study diagram. One dish contained 2 pieces of sushi, 1 once-frozen and 1 fresh, placed in random order. Participants were blinded, and ate 3 dishes each of mackerel (in red) and squid (in white). Hence, each participant ate 6 dishes, totaling 12 pieces of sushi. As there were 40 participants, a total of 480 pieces of sushi was consumed.

dishes in the same order of the allocation and was served to the participants.

### Procedures

After being given the dishes, the participants were asked to write down the identification number on the top of each dish on the response sheets, eat the 2 pieces of sushi on the dish, and then mark either A or B based on their preference. They were free to eat either sushi A or B first. If they felt that there was no difference in taste, they were asked to mark so on the response sheets. Three rounds of eating sushi for both mackerel and squid were undertaken—that is, each participant ate 6 pieces of mackerel and 6 pieces of squid, totaling 12 pieces of sushi each (Figure 2). This meant that a total of 120 rounds each for mackerel and squid for comparison between frozen and unfrozen sushi were conducted.

The participants were only allowed to drink bottled water during the experiment to rinse their mouth. It was determined that if someone found *Anisakis* larvae in the ingredients, that dish would be excluded from the trial (in fact, however, no parasites were found during the experiment).

We prepared beforehand to send sickened participants during the experiment to Kobe University Hospital Emergency

Department, and requested the participants to report any adverse reactions during and after the experiment.

### Statistical Analysis

The primary endpoint was participants' sensory evaluation of sushi with once-frozen ingredients compared with those with unfrozen (fresh) ingredients. With an assumed hypothesis that frozen raw fish ruins sushi taste, we expected that the participants would select unfrozen sushi as tasting better in more than two-thirds of cases and that they would report no difference in taste in around 10% of cases. With this assumed number, with power of 90% at an  $\alpha$  error of 5% (2-sided), a total of 120 rounds (240 pieces) eating for each ingredient was needed. Analysis was performed using discrete choice model with binary choice, which is frequently used in consumer preference research as an analogue of the conditional logistic model [6]. No difference in taste was treated as a censored case. Based on a mathematical equivalence between discrete choice model and Cox proportional hazards model, Cox partial likelihood was used for estimating  $P$  values and odds ratios (ORs) [7, 8]. Fisher exact test was used for evaluating learning effect of sequential tasting. All statistical analysis was performed by an independent academic statistician (K. Y.) using SAS software, version 9.3

(SAS Institute, Cary, North Carolina). All *P* values were 2-sided, and *P* values <.05 were considered significant.

## RESULTS

The mean age of the participants was 25.5 years (range, 20–50 years), and 77.5% were male (Table 1). All 40 participants completed the experiment of eating 6 pairs of sushi (12 pieces). No adverse reactions occurred during or after the experiment.

Among 120 rounds of discrimination testing including 240 pieces of mackerel sushi, participants thought that unfrozen sushi tasted better in 42.5% (51 dishes) of cases and frozen sushi in 49.2% (59 dishes) of cases; participants felt the taste was the same in 8.3% (10 dishes). The OR for selecting unfrozen sushi as “tastes better” over frozen sushi was 0.86 (95% confidence interval [CI], .59–1.26; *P* = .45).

For squid, unfrozen sushi was thought to be superior in 48.3% (58 dishes) of cases, and frozen sushi in 35.0% (42 dishes); they were felt to have the same taste 16.7% of the time (20 dishes) (OR, 1.38 [95% CI, .93–2.05]; *P* = .11).

For mackerel, only 13 of the 40 participants demonstrated consistent answers on their evaluation: 5 favored unfrozen sushi 3 times consecutively, 7 favored frozen, and 1 participant answered that they tasted the same all the time. For squid, only 7 participants answered consistently: 6 favored unfrozen sushi and 1 favored frozen. Only 1 participant showed consistency in all 6 dishes, and this person favored unfrozen sushi all the time.

Our sushi chefs also tasted the sushi in a blind fashion, but only 1 round each was given. One chef favored unfrozen sushi on both mackerel and squid, and the other favored unfrozen mackerel and frozen squid.

For potential learning effects during the experiment, the comparison was made between the choice on the first and last rounds for each participant (Tables 2 and 3).

For mackerel, 11 participants favored unfrozen sushi, 24 favored frozen sushi, and 5 thought that both were the same

**Table 2. Change in Participants’ Preference Between the First and the Last Rounds, for Mackerel**

Preference		No. of Participants
First Round	Last Round	
Frozen sushi	Frozen sushi	10
Frozen sushi	Unfrozen sushi	11
Frozen sushi	Both tasted the same	3
Unfrozen sushi	Frozen sushi	3
Unfrozen sushi	Unfrozen sushi	8
Unfrozen sushi	Both tasted the same	0
Both tasted the same	Frozen sushi	0
Both tasted the same	Unfrozen sushi	4
Both tasted the same	Both tasted the same	1

upon eating for the first time. However, on the third round, 23 favored unfrozen, 13 favored frozen, and 4 said they tasted the same. This change was statistically significant (*P* = .02). For squid on the first round, 20 participants favored unfrozen sushi, 1 favored frozen sushi, and 19 thought they tasted the same. However, on the third round, 11 selected unfrozen sushi, 29 favored frozen sushi, and none felt both were equal. Again, the alteration of their judgment was statistically significant (*P* < .0001). Even though there was no statistical difference between frozen and unfrozen mackerel on each test, there was reversal of preference on squid over time, from favoring unfrozen sushi to frozen (Tables 4 and 5).

## DISCUSSION

Japan began to experience an increase in the diagnosis of anisakidosis in the 1970s, likely due to advances in diagnostic techniques such as upper gastrointestinal endoscopy [9, 10].

*Anisakis simplex* belongs to a group of nematodes that accidentally infect humans during their reproductive cycle. The

**Table 1. Baseline Characteristics of Participants**

Variable	Participants, No. (%)
Mean age (SD)	25.5 (6.5)
Male sex	31 (75.5)
Sushi-eating frequency	
Rarely	1 (2.5)
Several times a year	9 (22.5)
Every 3 mo	2 (5)
Every 2 mo	5 (12.5)
Monthly	10 (25)
Several times a month	11 (27.5)
No answer	2 (5)

Abbreviation: SD, standard deviation.

**Table 3. Change in Participants’ Preference Between the First and the Last Rounds, for Squid**

Preference		No. of Participants
First Round	Last Round	
Frozen sushi	Frozen sushi	1
Frozen sushi	Unfrozen sushi	0
Frozen sushi	Both tasted the same	0
Unfrozen sushi	Frozen sushi	14
Unfrozen sushi	Unfrozen sushi	6
Unfrozen sushi	Both tasted the same	0
Both tasted the same	Frozen sushi	14
Both tasted the same	Unfrozen sushi	5
Both tasted the same	Both tasted the same	0

**Table 4. Participants' Preference for Mackerel on Each Test**

Type of Ingredient	First Test	Second Test	Last Test
Frozen	24	22	13
Unfrozen	11	17	23
Same	5	1	4
OR, unfrozen over frozen (95% CI)	0.46 (.23–.94) P = .03	0.77 (.41–1.46) P = .42	1.77 (.90–3.49) P = .10

Abbreviations: CI, confidence interval; OR, odds ratio.

larvae recognize marine mammals as a primary host, but they may be ingested by fish and then accidentally eaten by humans. They can stay in the stomach and intestine by breaking the mucosal layer but they die spontaneously in a few weeks [1].

Beginning in 2012, anisakidosis was added to reportable food-borne diseases according to Japan's food and sanitation act, mandating physicians to report anisakidosis. In 2013, only 89 cases of anisakidosis were reported [11]. However, it appears to be significantly underreported. Based on health insurance claims data, >7000 diagnoses of anisakidosis are made annually in Japan [11], which is a much higher number than reported in the previous literature [2]. In addition, because anisakidosis may be easily mistaken for common acute gastroenteritis, the actual number of anisakidosis cases in Japan may be much higher, and prevention by freezing of raw fish is worthy of consideration.

The Dutch are rare among Europeans as they have the tradition of eating raw fish (herring from the head) [12]. There have been cases of anisakidosis in the Netherlands since the 1960s, but it has been almost eliminated since the law mandated raw fish to be frozen. We were afraid freezing might impair the taste of the fish, but our Dutch acquaintance denied this possibility, stating that it would not change the taste at all (T. Daha, Dutch Working Party on Infection Prevention, personal communication). To make sure this assertion applies to Japanese people who love to eat raw fish, we decided to conduct the current study using Japanese participants.

Our results suggest that ordinary Japanese people are not able to distinguish between sushi with once-frozen ingredients and sushi with fresh fish. We conducted a variation of the classic discrimination testing conducted by Fisher, well known as the

**Table 5. Participants' Preference for Squid on Each Test**

Type of Ingredient	First Test	Second Test	Last Test
Frozen	1	12	29
Unfrozen	20	27	11
Same	19	1	0
OR, unfrozen over frozen (95% CI)	20.00 (2.68–149) P = .004	2.25 (1.14–4.44) P = .02	0.38 (.19–.76) P = .006

Abbreviations: CI, confidence interval; OR, odds ratio.

“lady tasting tea” [13]. In this experiment, the lady in question declared that by tasting a cup of tea she could tell whether the milk or tea was first added to the cup. To prove this assertion, Fisher proposed to give her 8 cups, 4 of each variety, in random order. Four of them were prepared by adding milk first, 4 prepared by adding the tea first. Fisher then counted the number she got correct to make sure she had the ability to distinguish between the 2 types of teas, or if it was just by chance. This is quoted as the pioneering figure of randomized controlled trial in a blind fashion [14]. By applying the same principle, that is, a prospective randomized trial in a blind fashion, we were able to demonstrate that freezing raw fish would not ruin sushi's taste. This practice could help lead to the elimination of anisakidosis, as well as other parasitic infections such as *Kudoa*, another parasite recently found in Japan to cause gastrointestinal symptoms [15].

This study has several limitations. First, our medical students and residents may not represent the average Japanese person. However, it is difficult to assume that hypothesis to be true, as they were not selected to medical school based on tasting ability (or lack of it). Second, we found that there may be some learning effects by repeating the same procedure. The participants may have learned to distinguish between frozen and unfrozen sushi by repeating the tasting. However, the primary purpose of this study is not distinguishability, but the hypothesis of ruining the taste of sushi. At postexperiment surveillance, many of the participants wrote that they enjoyed eating excellent sushi (results not shown). It is hard to believe the sushi they enjoyed in the first round suddenly became detestable on the third round. We surmise that medical students and residents may have learned the difference between the 2 sushi by their appearance, texture, or taste, and guessed the one they thought was “unfrozen,” accurately deducing this for mackerel but not for squid. It is also possible that the mackerel and squid purchased 1 week before the experiment were totally different from the fish purchased 2 days before, therefore tasting different from the outset and confounding the results. However, 2 professional sushi chefs confirmed that both were genuine ingredients. Fourth, the results may not be applicable to more astute gourmets. They may continue to eat unfrozen sushi and risk parasitic infections as a gastronomic tradeoff. Further studies are needed to evaluate consumers' ability to discriminate frozen fish from fresh fish. Fifth, there were more male participants than female, reflecting the low number of female physicians in Japan [16], and this might have affected the results of our study.

In conclusion, our results indicate that freezing raw fish will not ruin sushi's taste. Japan should consider recommending raw fish to be frozen before consumption.

## Notes

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**Author contributions.** All authors contributed to the research design. K. I. led the experiment, and drafted and edited the manuscript. T. F. edited the manuscript. K. Y. conducted statistical analyses.

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**Potential conflicts of interest.** All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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