ORIGINAL

Acceptability Evaluation by Vietnamese about Non-toxic Cultured Pufferfish in Comparison with Grouper and Mackerel

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ABSTRACT *Background and purpose.* World-wide there are few countries in which pufferfish (fugu) is eaten as in Japan. In Vietnam, pufferfish has been banned since it's toxic ocurred due to lack of knowledge about distinguish non-toxic species. Consequently, there is a huge amount of pufferfish inhabiting in Vietnamese water, but whenever accidentally catching it, we have to throw or use as fertilizer. To develop culinary culture of non-toxic pufferfish in Vietnam, it is very important and essential to recognize safe species and culture them in good condition, then apply proper method to process that become safety foods. In order to initial setting up for such purpose, we carried out a sensory study in Vietnamese to test whether they can accept foods made from fugu by method of Japanese. *Methods.* We compared the sensory reaction to Japanese cultured *Takifugu rubripes* and fish from Vietnamese waters: grouper and mackerel. The 107 panelists were Vietnamese volunteers working in the field of nutrition, employees of marine companies, or government officials who could influence the relevant laws. Each panelist tried 10 dishes which were prepared by chefs holding a Japanese fugu license. After eating, they were asked to fill out the sensory questionnaire, used a five-point scoring method, with 5 as the highest score, and reaction rating questionnaire. *Results.* The average score for the various puffer dishes was 4.40 in the good rank flavor was rated best. For questions such as "would you like to eat more Fugu, do you want to introduce it to others, do you want fugu to become a new food culture?", Over 90% of the panels answered "Yes". *Conclusion.* Fugu's acceptable flavor was confirmed for Vietnamese when it was prepared properly by fugu-licensed Japanese chefs.
Key words: pufferfish, fugu-licensed, sensory test, food culture, takifugu rubripes, Vietnam, Japan

INTRODUCTION

Worldwide, only Japan is well known for a special pufferfish (fugu) food culture. Japanese Ministry of Health and Welfare enacted a guideline of edible species, in that only 22 species of fugu are allowed, and only the people who has fugu license can cook (1–3). Marine fugu are believed to accumulate *tetrodotoxin* (TTX) but non-toxic fugu can be produced if they are raised with TTX-free diets in net cages at sea or aquaria on land, where the invasion of TTX-bearing organisms is completely shut off (4). By cultured fugu, some studies showed that it was delicious and rich in nutrients (5,6). Fugu is high class fish and expensive in Japan and now Japanese are trying to import fugu from the other countries such as China or Korea.

There are more than 350 species of fugu (7), and according to a report by the Research Institute for Marine Fisheries, Vietnam has about 60 species, resources are over 37,000 tons (8, 9). There are hundreds of food poisoning related to toxic fugu per years (10) so that the Ministry of Fisheries (now the Ministry of Agriculture and Rural Development) has implemented a ban on fugu since 2003 (11). In order to lift the law which prohibits the harvesting and using Vietnamese fugu, it is necessary to carry out a toxicity test and clarify the type and parts of fugu which can be eaten. Even if a toxicity test, which is difficult, is carried out, the effort would be pointless unless fugu is accepted as a part of food culture by Vietnamese people. Therefore, first of all, in this research, a sensory tests were carried out with various dishes of Japan cultured tiger fugu (*takifugu rubripes*) that was confirmed to be safe and got permission of Vietnam government.

METHODS

Study design: We evaluated Japanese fugu dishes and Vietnamese luxury fish dishes of mackerel and grouper, by using a sensory test two times in June and December of 2017. The first study was conducted at the Vietnam National Institute of Nutrition in Hanoi and the second study at a hotel in Da Nang city. After trying each dish, the panel were asked to score their reaction on the response sheets. We used 5 point scale (extremely good:5, good:4, neither good or bad:3, bad:2, extremely bad:1) to evaluate the flavor of each dish at 3 items: overall taste, aroma and texture. The panel were free to eat all dishes randomly and in unrestricted quantities. After that, panelists were asked to fill in the questionnaire of sensory test (Table 1).

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Ethical committee and customs clearance permission of Japanese fugu: Currently the use of puffer fish is banned in Vietnam so that the safety has not been guaranteed. Therefore, we can not use Vietnamese puffer fish in this study. To conducting research in addition to ethical permission, it was often necessary to get approval from the Vietnamese Ministry of Health. Japanese cultured puffer fish is a high-class fish, which was tested safety in Japan and passed the customs clearance, so it was allowed to bring into Vietnam.

Materials Preparation: Cultured *Takifugu rubripes* for the study were purchased at an aquaculture company in Japan and viscera were removed and then the fish was kept in a freezer under −20°C. The Fugu was delivered from Miyazaki, Japan to Hanoi and Da Nang, Vietnam by air. We used cold gel and dry ice to keep the fish under −20°C during shipping. In Vietnam, the fugu was kept in a freezer under −20°C. One day prior to the experiment, both mackerel and grouper were purchased at a market in Vietnam, viscera were removed and the fish were kept in refrigerators under 4°C. The frozen fugu was moved to a refrigerator and defrosted at about 4°C in more than 10 hours. Other materials were chosen and prepared under the control of Japanese fugu cooking license holders (Picture1). We used the same recipe for fugu, mackerel and grouper in the hotpot dish and Japanese style fried dish to make the comparison among them.

Prior to the main study, in a pilot study (n=10), various Japanese Fugu recipes (2) were evaluated. Based on that pilot, the menu items for the main study were determined: Japanese style fried dish, hot pot, sashimi (dishes with raw fish), hirezake (Japanese sake with grilled Fugu fin in it), nikogori (a kind of jelly made from fugu skin), skin mix (boiled fugu skin was sliced and mixed with chili, miso, and vinegar), tataki (fish grilled on the outside and kept raw inside), and shirako tofu (a kind of mousse made with fugu milt) (Picture 2). Total the amount of fugu for 1 serving set of sensory test with 8 fugu dishes was 400g (50g per dish × 8 dishes = 400g) so at least 42.8 kg needed to be cooked. In case something happen we brought about 90kg of frozen Japanese fugu to Vietnam. By same way, we calculate the amount of grouper and mackerel which were used for taste test. All the dishes were made as close as the time of the test at lunch time that why most of work had to be done since previous night.

Panel selection: To evaluate Vietnamese acceptance of Fugu dishes and to lift the law banning fugu in Vietnam in future, all 107 panelists were Vietnamese who may have influence on the law concerning fugu. In addition, they had experience with the sensory evaluation of food, especially fish, particularly the staff of National Institute of Nutrition, the Research Institute for Marine Fisheries and the government officials. They were adults, in good health, had no allergies to any materials used in this test, were not anomic, were not ageusic, had normal colour vison and able to detect anomalies in the appearance of fish and fish food in a consistent manner, and were able to rely on sensory perceptions and to report them appropriately. The panel were asked to avoid eating or drinking too much the day before the experiment, to get enough sleep, and to eat their usual breakfast on the day of the experiment. Informed consent was obtained from participants. We requested participants to report any adverse reactions during and after eating the food items.

Statistical Analysis: Data were statistically analyzed by Student *t*-test, and Tukey test, $p < 0.05$ was significant different.

Picture 1. Fugu preparation by Japanese fugu cooking license holders

Picture 2-1. Fugu sashimi

Picture 2-2. Fugu tataki

Picture 2-3. Japanese style fried fugu

Picture 2-4. Left: Nikogori; middle: Skin mix; right: Shirako tofu

Picture 2. Fugu dishes for Sensory test

Date Place
Age Sex: 1 Male 2 Female

1. Evaluate the items below to the gradual increase of the following characteristics on a scale from 1 to 5 and please check (✓) in the box

No	Dish		Item	5	4	3	2	1
1	Fugu sashimi		Overall taste					
			Texture					
			Aroma					
2	Hirezake		Overall taste					
			Aroma					
3	Hot pot	Fugu	Overall taste					
			Texture					
			Aroma					
		Mackerel	Overall taste					
			Texture					
			Aroma					
		Grouper	Overall taste					
			Texture					
			Aroma					
4	Japanese style fried	Fugu	Overall taste					
			Texture					
			Aroma					
		Mackerel	Overall taste					
			Texture					
			Aroma					
		Grouper	Overall taste					
			Texture					
			Aroma					
5	Nikogori		Overall taste					
			Texture					
			Aroma					
6	Skin mix		Overall taste					
			Texture					
			Aroma					
7	Tataki		Overall taste					
			Texture					
			Aroma					
8	Shirako tofu		Overall taste					
			Texture					
			Aroma					

2. Questionnaire on acceptance of Fugu dishes
Please circle the number of the answer that matches your opinion

2.1 Would you want to eat the fugu dishes again?
1. Yes 2. No 3. No each other

2.2 If we do other sensory investigations, would you be willing to participate in other taste tests?
1. Yes 2. No 3. No each other

2.3 Would you recommend Fugu dishes to your friends?
1. Yes 2. No 3. No each other

2.4 Would you introduce Fugu dishes to your family?
1. Yes 2. No 3. No each other

2.5 Do you think Fugu dishes can become a new food in Vietnam?
1. Yes 2. No 3. No each other

2.6 Do you like fish dishes?
1. Yes 2. No 3. No each other

2.7 Do you like Grouper dishes?
1. Yes 2. No 3. No each other

2.8 Do you like Mackerel dishes?
1. Yes 2. No 3. No each other

Table 1. Questionnaire of sensory test

RESULTS

The sensory evaluation panelists (age 28 - 38) consisted of 61 males and 46 females, more than 80% of panel liked the fish dishes, mackerel or grouper dishes (Table 2)

Table 2. Characteristics of Panelists

	Panelists	
	n	%
Like fish dishes	98	92
Like mackerel dishes	93	87
Like grouper dishes	103	96

Figure 1 showed the results of Japanese style fried dish, the overall taste of these three fish showed significant difference ($p<0.05$). When compared to grouper, the texture of fugu was the same, aroma was little lower but still in the good rank flavor was rated best; the overall taste of both was very good and even in the hotpot dish the score for fugu was higher in overall taste.

Figure 2 shows the hot pot dish, overall taste of these three fish was significantly different ($p<0.05$) and fugu got the highest score; texture of fugu compared to grouper or mackerel had a significant difference. In comparison with mackerel, fugu was

more delicious, with no fish smell as with mackerel in the hotpot dish, and texture was the same in the fried dish and better in the hotpot dish. In flavor, fugu was not only good but also better than these 2 luxury fish.

Table 3 showed the sensory evaluation of the 8 fugu dishes, in average point, the overall taste was higher than 4.42, the texture was higher than 3.84, the aroma was higher than 4.49. All of the points were at the good rank. The overall taste of sashimi was 4.82 - the highest score.

Figure 3 showed how the panelists accept fugu dishes; more than 92% of the panel wanted to eat them again and would recommend them to other people, 89% panel thought that fugu could become a new food in Vietnam.

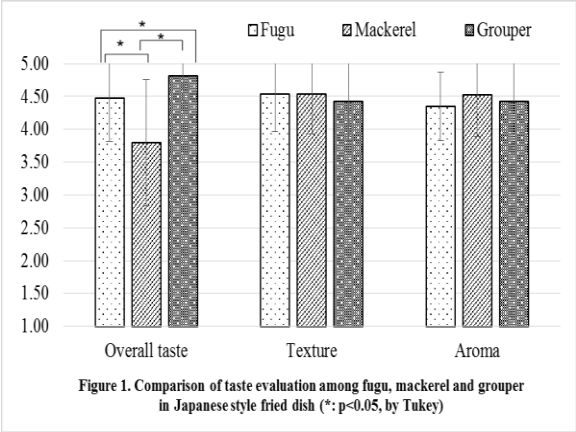

Figure 1. Comparison of taste evaluation among fugu, mackerel and grouper in Japanese style fried dish (*: p<0.05, by Tukey)

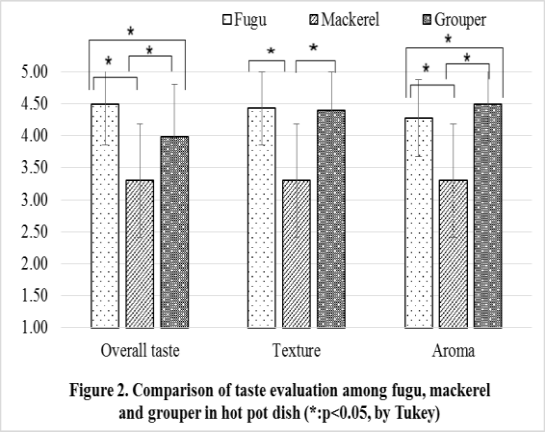

Figure 2. Comparison of taste evaluation among fugu, mackerel and grouper in hot pot dish (*:p<0.05, by Tukey)

Table 3. Average score of sensory evaluation of 8 fugu dishes

	Fugu sashimi	Hirezake	Fugu hot pot	Fugu fried	Skin mix	Nikogori	Shirako tofu	Tataki
Overall taste	4.82±0.39	4.42±0.69	4.50±0.64	4.48±0.66	4.56±0.62	4.56±0.62	4.41±0.71	4.59±0.50
Texture	4.46±0.54		4.50±0.57	3.84±0.96	4.48±0.54	4.48±0.54	4.44±0.57	4.48±0.57
Aroma	4.49±0.54	4.49±0.67	4.28±0.60	4.82±0.39	4.64±0.48	4.64±0.48	4.56±0.57	4.53±0.54

Values are mean ±SD

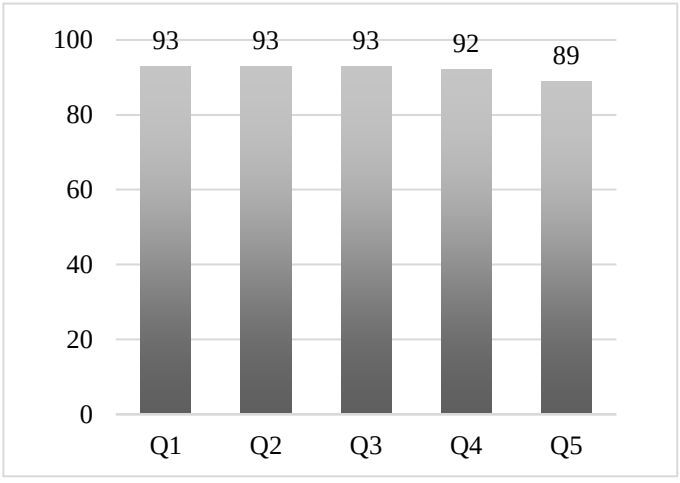

Figure 3. Percent of panelist answered "Yes" about questions on acceptance of Fugu dishes (%)

DISCUSSION

In Vietnam, the Government strictly prohibits processing, trading, and using of pufferfish; therefore, nobody knows the flavor of fugu dishes. However, with this sensory test, more than 90% of the panelists scored fugu dishes as high or best flavor, 80% said they would like to eat fugu again and hoped it would become a new food culture in Vietnam.

Eating and cooking Vietnamese fugu is illegal in Vietnam; therefore, when conducting this research we used Japanese fugu which was confirmed as safe in Japan. Every time when we did study we needed the permission of Vietnam government; all cooking period was under the control of National Research Institutes.

On the other hand, only Japan has a law for fugu culture as well as a fugu chef license system, that why this study followed Japanese cooking methods and all dishes were cooked by professional fugu chefs.

Grouper and mackerel are two of the most esteemed fish in Vietnam, considered to be excellent in overall taste, aroma, and texture. Three fish are white meat fish, the texture of these are considered as similar. Vietnamese usually use the muscle of fish to cook some dishes, such as: hot pot, fried; to compare among 3 kind of fishes we chose these 2 dishes only.

In this study, we invited 107 adults from Hanoi and Da Nang, the number of the panelists were sufficient for this study. According to "Sensory evaluation – guide of food practice", at ranking test the minimum size of panel participating in the test was 12 (12). According to the guideline number JIS Z 9080:2004 of Japan Industrial Standards Committee about sensory evaluation, in ranking test them minimum sample size are 7 if they are professional, 20 if they have experiences, 30 if they have no experience (13). Moreover as similar previous studies about sensory of sushi or food from liver of Japanese cultured fugu (14, 15) in this study we tried to collect more than 50 panel per test.

There are many method to evaluate sensory, for example hedonic scaling - 9 point scale, magnitude estimation, category-ratio scales (16) and 5 point scale. Each method has its own feature. We used the 5 point scale which suitable with the untrained panel and situation of this study as shown in similar studies (5, 6, 17).

In Vietnamese food culture state, most traditional dishes are cooked well. Recently, food safety is a major problem in Vietnam; every year there are 250-500 cases of food poisoning: 7,000-10,000 victims and 100-200 deaths, 33-49% from microorganisms (18–20). Vietnamese do not eat raw food much, especially raw fish. Fish intake in Vietnam in 2010 was only 59g (15.8% of protein intake) (21) lower than in Japan: 73g (20.7% of protein intake) (22). Fish in Vietnam is a luxury item and many times more expensive than meat or eggs. This may be the reason for low fish intake. In this study, the Japanese fugu dishes were very highly evaluated and appreciated by Vietnamese panel. Furthermore fugu has no small bones, usually has no fishy smell, is delicious and is easy for even the elderly or children to consume.

In particular, sashimi got the highest evaluation even though Vietnamese are not familiar with raw fish; in future we hope that fugu will become a part of Vietnamese food culture, and by Japanese experien - ces and cooking methods, Vietnamese can develop the new fish food culture.

The study results also helped the government and National Research Institutes acknowledge that "it was possible to make delicious and safe Vietnamese fugu food, moreover it could become a popular food culture in Vietnam". As a result, the National Research Institutes will send the government proposals for promptly legalizing safe fugu and propagating Japanese fugu culture in Vietnam.

Previous study showed that Fugu consume *tetrodotoxin* (Ttxs) holdings organisms finally become toxic fish. If fugu are prevented from Ttxs holdings organisms, fugu will be nontoxic (≦10MU) (1, 3, 23). Japan have developed technique to cultivate nontoxic Fugu (5, 6) and strictly manage - ment fugu food safety system. These should be established in Vietnam in parallel with fugu new food culture.

In the future, we will analyze toxic in tissue and clarify the types of puffer in Vietnam can be used as food. From those results, safe species of fugu could be chosen to expand the new food culture in Vietnam. We hope these will not only be a new food but will also play a part in "international humanity and cultural understanding", "economic development", "the knowledge society, "technology knowledge" and "practicing globalization".

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