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Problem-Solving Exercise for Undergraduate Students Involving the Japanese Fermented Food Natto †

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Natto is a traditional Japanese food made from soybeans fermented by *Bacillus subtilis natto*. Because natto is high in protein and contains healthful bacteria, dieticians and producers have campaigned for higher consumption of natto in areas such as Kansai, where our university is located. One of the reasons for the low consumption of natto is its distinctive flavor. As a problem-solving exercise, undergraduate students attempted to make natto more marketable. Students set a goal of modifying natto flavor by adding spices to achieve flavors deemed palatable by organoleptic tests. During the exercise, they noticed that lemongrass, *Cymbopogon citratus*, had a flavor-masking ability that reduced the intensity of natto flavor, and they finally determined that it was a useful food additive to make natto marketable. The exercise was an active learning process that effectively induced voluntary student effort of to solve problems originating from microorganisms.

納豆は、納豆菌 *Bacillus subtilis natto* により大豆を発酵させた日本の伝統食品の 1 つである。納豆にはタンパク質と有益な細菌が多く含まれており、栄養士や生産者らによる納豆の消費促進キャンペーンが行われてきた。しかし、筆者らの所属する大学が所在する関西地方では、依然として納豆の消費率は低い。その原因の 1 つは、納豆の有する独特の臭いにある。そこで、「より市場性の高い納豆の開発」を課題とした課題解決型アクティブラーニングを行った。アクティブラーニングにおいて参加学生は、スパイス添加により納豆の臭いを改善することを最終目標として設定した。納豆の臭いの確認は食味試験によって行われた。参加学生は、レモングラス（*Cymbopogon citratus*）は、納豆の臭いをマスキングする効果があり、市場性の高い納豆の開発につなげられる可能性を示した。本アクティブラーニングでは、微生物の関与する食の問題の解決を通じて、学生の自主的な学習意欲を高めることに結びついた。

INTRODUCTION

Natto is a traditional Japanese food made from steamed soybeans fermented by *Bacillus subtilis natto* (1). It is characterized by poly-γ-glutamate stickiness and distinctive flavor produced during the fermentation process (1). The flavor is derived from branched short-chain fatty acids, sulfur-containing substances, and amines (2, 3). In Japan, natto is sold with packets of soy sauce and Japanese mustard. Natto is stirred before eating to increase the stickiness (4). Japanese mustard is generally used to modify the flavor.

Natto contains high-quality absorbable vegetable protein and large amounts of bacterial menaquinone 7 (5). The health effects include slowing osteoporosis and reducing cholesterol (6, 7). While natto consumption is high in the eastern regions of Japan, it is much lower in the western regions (8), possibly due to its distinctive flavor.

As an active-learning exercise, our faculty, with the cooperation of House Foods Corporation, Ltd (Tokyo, Japan), asked undergraduate students to explore ways to

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make commercial foods more marketable using spices. It was a part of an introductory course for all students in the Faculty of Agriculture. One of the student groups in the Department of Plant Science selected fermented foods as a target for the exercise. Through discussion with instructors, they chose natto, which is less popular in Kansai, where our university is located. We describe how they set goals and solved the problems in sequence.

PROCEDURE

Safety issues

To ensure the safety of the students, we used commercial products and food grade materials only. All subjects were not allergic to soybean in organoleptic tests. Packages of natto (Mizkan, Ltd., Handa, Japan) were purchased at a grocery store. Soy beans and *B. subtilis natto* used in these commercial products were not genetically modified. *B. subtilis natto* is treated at BSL1 and is recognized as safe by the U.S. Food and Drug Administration (9, 10). Sample preparation and organoleptic tests were carried out in a seminar room where scientific experiments are never conducted. Institutional review board (IRB) approval was not required because organoleptic tests are not subject to IRB approval in Japan. The rights and welfare of humans participating as subjects were protected at a level equivalent to IRB.

Primary goal setting and the first experiment

The students set their primary goal as identifying spices that improve natto flavor through an organoleptic test. Twenty-four spice powders were provided by the food company (Table 1). The effects of spices on natto flavor were evaluated with soy sauce, because natto is generally served with soy sauce in Japan (4). Each spice powder was added to 45 g of natto with 4.6 g of soy sauce and stirred 20 times to prepare a sample (Table 1). Natto with soy sauce prepared accordingly was used as a negative control. The evaluation was performed by eight subjects, including five students from the group. Samples were tasted following the negative control in sequence (1–24, Table 1) approximately 10 minutes after preparation (nonblinded). The tasting intervals were about 30 seconds, and subjects rinsed their mouths thoroughly with water. Samples were evaluated on a five-point scale relative to the control (5 = much better; 4 = somewhat better; 3 = not much difference; 2 = somewhat worse; 1 = much worse) for the following five aspects: appearance, flavor, taste, texture, and total quality (11).

Several spices, such as paprika and basil, marked high scores, positively modifying natto flavor (Table 1). During the test, one student noticed that lemongrass (*Cymbopogon citratus*) had a de-odorizing effect on natto, while the flavor of the lemongrass itself was not much perceived, even though it marked a low score in the evaluation (Table 1). The other students confirmed the effect, and the group revised the goal to investigate the flavor-masking effect of lemongrass instead of flavor modification by spices.

Quantitative investigation of the effect of lemongrass

The students performed a quantitative investigation of the effect of lemongrass on natto flavor. Seven subjects were recruited from the Kansai region and were asked whether they perceived natto flavor or lemongrass flavor through a single blind experiment. Four samples were prepared (Table 2). The first four subjects tasted samples A to C sequentially. The other three subjects tasted the four samples (A–D) sequentially. The ratio of subjects who perceived the de-odorizing effect was highest at the 0.1% addition (Table 2, treatment B), while the lemongrass flavor was felt to be too strong at the 0.5% addition (Table 2, treatment D). The result indicated that the flavor-masking effect was optimal at 0.1% lemongrass addition, where natto flavor was de-odorized while lemongrass flavor was not much perceived.

During the investigation, students noticed that the natto flavor returned some time after the lemongrass addition and confirmed this by comparing samples immediately after and 30 minutes after adding lemongrass. They next set a goal to extend the longevity of the effect of lemongrass by, for example, coating the powder with gelatin. They also plan to examine the effect of lemongrass on other fermented foods with strong flavors, such as funa-zushi, traditional fermented fish in the Shiga prefecture, a part of the Kansai region.

Students' evaluation of the exercise

The students considered the exercise to be efficient and helpful in consolidating some basics of microbiology and food science. They recognized the importance of discussion with instructors and other students in order to make progress. Overall, they enjoyed the exercise while realizing the challenges of scientific procedures.

Previous studies have shown that active-learning programs result in greater learning than lecture-based programs (12, 13). The students successfully achieved the goal of finding spices that improve natto flavor. The results are potentially applicable to other fermented foods with a strong flavor, such as cheese. This exercise enhanced their knowledge of traditional fermented foods and taste, with a potential for further fruitful ideas from the initial problem.

CONCLUSIONS

During the problem-solving exercise, the students set novel goals at each step and attempted to achieve them, discussing the results thoroughly with the instructors. They discovered and qualitatively investigated the flavor-masking effect of lemongrass. Careful attention to serendipitous findings by students is important in such problem-solving

TABLE 1.
List of 24 spices evaluated in an organoleptic test.

Number	Spice name	Weight added mg	Score		Impression by subjects
			Avg	SD	
1	Allspice	216	3.1	1.3	It is similar to nutmeg
2	Garlic	218	3.6	1.3	
3	Cardamom	192	2.4	0.9	Bitter
4	Cumin	159	3.0	0.6	Strong smell
5	Clove	181	1.6	0.8	
6	Cinnamon	166	1.1	0.4	Strong smell, bitter
7	Coriander	135	3.9	0.9	Fresh
8	Star anise	124	1.8	0.5	Chinese style
9	Celery seed	194	2.7	1.4	Bitter aftertaste
10	Laurel	143	2.5	0.8	Bitter
11	Nutmeg	158	3.1	1.3	It is similar to allspice
12	Thyme	178	1.9	0.9	
13	Oregano	146	2.6	1.0	Not Japanese style bitter
14	Sage	162	2.4	0.9	Strong smell
15	Rosemary	171	2.9	0.9	
16	Basil	228	4.1	1.1	Good smell
17	Lemongrass	111	2.9	1.4	Removes odor
18	Cayenne pepper	169	3.3	1.3	Spicy
19	Black pepper	212	3.5	1.3	Bitter aftertaste
20	White pepper	233	3.1	1.3	
21	Ginger	158	1.9	1.1	
22	Japanese pepper	146	3.6	0.9	Refreshing
23	Turmeric	226	2.8	1.3	Curry flavor
24	Paprika	229	3.7	0.9	Changes to red

Spices were added to 46 g of natto with 4.6 g of soy sauce and the subjects' impressions were recorded. The scores (out of 5) represent averages of five aspects (appearance, flavor, taste, texture, and total quality) provided by eight subjects.

TABLE 2.
Quantitative investigation of the effect of lemongrass on natto flavor.

Treatment	Lemongrass added (%)	The ratio of subjects who felt flavors disappeared (%)	Impression by subjects
A	0.00	0	
B	0.10	86	
C	0.05	57	
D	0.50	75	Lemongrass flavor was too strong

Seven subjects evaluated samples A, B, and C, and four subjects evaluated samples A, B, C, and D to determine whether both natto and lemongrass flavors disappeared through an organoleptic test.

exercises. They considered the exercise helpful for consolidating some basics in microbiology and food science. Our teaching activity could be adapted for use in other active-learning contexts.

SUPPLEMENTAL MATERIALS

Appendix 1. List of 24 spices evaluated in an organoleptic test

Appendix 2. Quantitative investigation of the effect of lemon grass powder on natto flavor

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