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Running Head: ANTIMICROBIAL HYPOTHESIS

Adaptive Ingredients against Food Spoilage in Japanese Cuisine

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Antimicrobial Hypothesis

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Abstract

Billing and Sherman (1998) proposed the antimicrobial hypothesis to explain the world-wide spice use pattern. The present study explored whether two antimicrobial ingredients (i.e., spices and vinegar) are used in ways consistent with the antimicrobial hypothesis. Four specific predictions were tested: (1) meat-based recipes would call for more spices/vinegar than vegetable-based recipes; (2) summer recipes would call for more spices/vinegar than winter recipes; (3) recipes in hotter regions would call for more spices/vinegar; and (4) recipes including unheated ingredients would call for more spices/vinegar. Spices/vinegar use patterns were compiled from two types of traditional Japanese cookbooks. Dataset I included recipes provided by elderly Japanese housewives. Dataset II included recipes provided by experts in traditional Japanese foods. The analyses of Dataset I revealed that the vinegar use pattern conformed to the predictions. In contrast, analyses of Dataset II generally supported the predictions in terms of spices, but not vinegar.

Keywords: Antimicrobial Hypothesis, Food Spoilage, Japanese Cuisine, Spice, Vinegar

Adaptive Ingredients against Food Spoilage in Japanese Cuisine

1. Introduction

Food spoilage is considered as one of the fundamental adaptive problems with which our ancestors were constantly confronted. Recently, proposing the antimicrobial hypothesis, Sherman and colleagues maintained that spice use was one of the solutions to the problem of food spoilage (Billing & Sherman, 1998; Sherman & Hash, 2001). Noticing that most spices contain secondary compounds that kill bacteria and fungi or inhibit their growth, the antimicrobial hypothesis posits that we use spices because they protect us from food borne illnesses. The antimicrobial hypothesis predicts certain patterns of spice use around the world. Some of the predictions confirmed by Sherman and colleagues' studies are follows. First, the antimicrobial hypothesis predicts that spice use will be heaviest in hot climates, where bacteria and fungi grow more rapidly. Second, it predicts that meat-based recipes will require more spices than vegetable-only recipes because meats are more vulnerable to spoilage due to bacteria and fungi than are vegetables. To test these predictions, Sherman and colleagues collected 96 traditional cookbooks from 36 countries to examine spice use patterns in those countries. When meat-based recipes around the world were analyzed, Billing and Sherman (1998) found that recipes from hotter climates called for more spices. The same pattern held when vegetable-only recipes from the 36 countries were analyzed. Consistent with the second prediction, vegetable-only recipes called for significantly fewer spices than meat-based recipes in most of the 36 countries (Sherman & Hash, 2001).

Discussing the antimicrobial hypothesis' implications, Billing and Sherman (1998) noted an interesting contrast between modern Japan and Korea. Regardless of their similar climatic conditions, Korean recipes call for more spices than Japanese recipes. At the same time, Billing and Sherman also noted that the food-poisoning rate was higher in Japan than Korea during the period from 1971 to 1990. They surmised that a small amount of spice used

in Japanese cuisine might have been sufficient when all the seafood came from local waters, and was thus less vulnerable to food spoilage. In these days, however, the amount of spice may not be sufficient because a substantial amount of seafood consumed in modern Japan is imported from abroad. Some archival evidence nevertheless indicates that Japanese people did not always eat fresh seafood. For example, Koizumi (2002) pointed out that Japanese people developed various types of dried fish to protect seafood from spoilage. Therefore, it seems reasonable to assume that traditional Japanese cuisine also had to be somehow protected from food spoilage.

Billing and Sherman (1998) noted that there are other antimicrobial ingredients, such as salt. Accordingly, it is possible that traditional Japanese cuisine incorporated antimicrobial ingredients other than spices. The primary purpose of the present study is to explore this possibility. Employing the basic methodology of Sherman and Billing's cookbook study, the present study analyzed the vinegar use pattern in addition to the spice use pattern in traditional Japanese cuisine. I decided to include vinegar in the analyses for the following reasons. First, vinegar is known to have an antibiotic effect on various types of bacteria causing food poisoning (e.g., Tsuburaya, 1998). Second, vinegar has a long history in Japanese culinary culture. In Japan, vinegar began to be produced around A.D. 600, and was considered to be one of the four important seasonings along with *hishio* (i.e., the original form of soy sauce), *sake* (i.e., an alcohol beverage produced from rice) and salt (Koizumi, 1999). Third, salt and soy source were excluded from the present analyses, not because I do not think they were important, but because most Japanese recipes call for these ingredients, and thus these variables yield little variance as far as the cookbook study method is used (see Billing & Sherman, 1998, for a similar argument).

The present study tested the following specific predictions, each of which was derived from the antimicrobial hypothesis, with Japanese spices/vinegar use data.

- Prediction 1. Meat-based recipes will call for more spices/vinegar than vegetable-based recipes.
- Prediction 2. Summer recipes will call for more spices/vinegar than winter recipes.
- Prediction 3. Recipes from hotter regions will call for more spices/vinegar than those from cooler regions within Japan.
- Prediction 4. Recipes with unheated main ingredients will call for more spices/vinegar than recipes with only well-heated ingredients.

Predictions 1 through 3 were Sherman and colleagues' original predictions, although Prediction 2 was not tested by their cookbook studies. One might suspect that the variance in the mean annual temperature within Japan would be too small to test Prediction 3. However, because Japan covers several degrees of latitude, the mean annual temperatures vary from north to south at least to the extent that it seems worth attempting to test Prediction 3. In the present study's data set, the mean annual temperatures associated with various recipes were distributed from 5.5 to 23.3 in Celsius (Mean±SD = 14.30±2.41, median = 14.60; cf. the range of mean annual temperatures was 2.8 to 27.6 in Billing and Sherman's data). Prediction 4 was added given the Japanese common habit of eating raw fish (e.g., *sushi*). Because heating is one of the human repertoires of adaptations against food spoilage, it was predicted that unheated recipes would call for more spices/vinegar.

2. Methods

2.1. Data Coding Scheme

The methods were similar to those used in Sherman and colleagues' studies.

Traditional Japanese cookbooks included in the present study were divided into two categories: one containing recipes collected from ordinary Japanese housewives (henceforth referred to as Dataset I) and the other containing recipes provided by experts in traditional Japanese cuisine (Dataset II). In the following, I shall first describe the data coding scheme

common to both datasets. Then, I shall delineate characteristics of each dataset.

For each recipe, (i) whether any spice or vinegar was called for and (ii) what types of spices, if any, were called for was recorded. A recipe was considered as meat-based when its main ingredient was meat or fish. In fact, because meat-eating was prohibited for a long time, until the middle of the 19th century, by Japanese rulers (Nagayama, 2003), most of the traditional Japanese meat-based recipes were fish-based recipes. However, we call them meat-based recipes following Sherman and colleagues' terminology. A recipe was considered as vegetable-based when its main ingredient was a vegetable(s), seaweed(s) or products of soybeans (e.g., *tofu*). Apart from one of Sherman and Hash's criteria, I did not exclude recipes with a meat-based stock from vegetable-based recipes as far as they did not call for other meat/fish as their main ingredient. This decision was made for the following reason: Two of the most common Japanese meat-based stocks are boiled and dried small fish (*iriko* or *niboshi*) and fermented skipjack tuna (*katsuobushi*), which is dried due to mold activities.

Both are made for preservation, contain little fat, and are less vulnerable to food spoilage than meat-based stocks in other countries (Koizumi, 1999).

2.2. Dataset I

Dataset I was compiled from a set of four volumes containing recipes collected by members of the Rural Culture Association around the 1980s (Rural Culture Association, 2003a, 2003b, 2003c, 2003d). They interviewed elderly Japanese women, who took charge of housework in their family (extended-family in most cases) around the 1920s, in about 350 regions of Japan. In particular, the interviewers asked those women to remember what dishes they had prepared in the 1920s. The recipes were originally published in different forms, but were re-published in 2003 being organized in terms of season (i.e., each of the four volumes contains either spring, summer, autumn, or winter recipes). Importantly, in the 1920s, electronic refrigerators were not available (Murase, 2005): it was 1965 when more than half

of Japanese households came to possess an electronic refrigerator (Cabinet Office, Government of Japan, 2004). Therefore, it is reasonable to assume that traditional ways of protecting foods from bacteria and fungi were still effective in the 1920s.

Dataset I contained 825 recipes (396 meat-based recipes, 292 vegetable-based recipes). There were 137 recipes coded as neither meat-based nor vegetable-based because they called for substantial portions of both meat/fish and vegetables. Complementing this dataset, the mean annual temperatures were taken from the Japan Meteorological Agency's internet site. For each recipe, the mean annual temperature was taken from the record of the observation point closest to the region where the interview was conducted. The mean temperatures were computed based on the records from 1971 to 2000 (Japan Meteorological Agency, n.d.).

2.3. Dataset II

The second dataset, Dataset II, was compiled from two cookbooks containing recipes introduced by two experts in traditional Japanese cuisine, Masaru Doi (1995) and Ichiko Sawamura (1998). Doi served as a presenter on a televised short (approximately 4 minutes) cooking program for 21 years beginning in 1973. In the preface of *Japanese dishes: Selected 500*, Masaru Doi's son, who is also an expert in Japanese cuisine, says that his father's recipes in the book were inherited from older generations. Sawamura is considered to be an expert on traditional Kyoto cuisine. She writes that her recipes are based on what she ate during her childhood (around the 1930s) in her uncle's house. Her uncle was a successful merchant in Kyoto (i.e., the former Japanese Capital) and while there, she ate various meals delivered from traditional Kyoto restaurants. Because the Kyoto restaurants at that time primarily served upper class families, the recipes in Sawamura's book may be considered as the authorized recipes of traditional Japanese cuisine. It may be reasonable to assume that traditional Japanese cookbooks available in the United States contain some experts' recipes

rather than ordinary housewives' recipes. Accordingly, one may consider this dataset, Dataset II, closer to the Japanese recipes included in Sherman and colleagues' analyses. Dataset II contained 439 recipes (123 meat-based, 89 vegetable-based). Because the recipes included in these books were organized in terms of season but not of region, Prediction 3 was unable to be tested with this dataset.

3. Results

3.1. Spice Grouping

The types of spices that are called for in Japanese cuisine were examined first. The two datasets included spices uniquely used in Japan or in a few Asian countries. What would count as a spice was determined by consulting a list presented by a Japanese expert on spices, Takemasa (1990, 2001). Some of the spices, which many western readers might find unfamiliar, were as follows: Japanese pepper (Xanthoxylum piperitum DC; Sansho in Japanese), perilla (Perilla frutescens Britton var.acuta Kudo; Shiso in Japanese), water pepper (Polygonum hydyopiper Opiz; Tade in Japanese), mioga (Zingiber mioga Rosc.), and wasabi (Wasabia japonica). These Japanese spices also contain antimicrobial compounds. Japanese pepper, which contains alpha-sanshool, is shown to have an inhibitive effect on methicillin-resistant staphylococcus aureus (Ogawa et al., 2001). A compound of perilla, perillaldehyde, has been found to have a growth inhibitory effect on bacteria and fungi (Oda, Tanaka, & Beppu, 1982). Water pepper contains polygodial, which shows some antifungal effect (Kubo, Fujita, & Lee, 2001). Mioga is shown to have several antimicrobial compounds, such as miogadioal (Abe et al., 2004). For wasabi, which contains allyl isothiocyanate, it was found that its antibacterial activity was greater than that of coriander leaf and mustard seed (Ono, Tesaki, Tanabe, & Watanabe, 1998).

To categorize the spices included in the Japanese cookbooks, I basically followed Billing and Sherman's (1989) grouping scheme. For example, leeks and some chives were

categorized as onions. Also, several types of citrus fruits including Japanese ones (e.g., *Yuzu*; *Citrus junos* Siebold ex Tanaka) were categorized as lemons. However, I departed from their grouping scheme for green pepper. Although green pepper was assigned one category in the Billing and Sherman study, it was included in the chili pepper category because only one recipe in Dataset II called for a green pepper. I also consulted Takemasa's (2001) categorization when categorizing some uniquely Japanese spices, for which Billing and Sherman's categorization schemes did not readily apply. Figure 1 shows the relative frequency of recipes calling for each type of spice as a function of dataset. The category of "others" in Figure 1 includes spices infrequently called for (e.g., pepper, water pepper) and a mixed-spice (i.e., *Shichimi Togarashi*) commonly used in Japan. The correlation between the relative frequencies in the two datasets (excluding the "others" category) was significant, r(15) = .63, p = .013. The correlation was also significant when spices with different names in Japanese were treated as different spices: r(35) = .69, p < .001.

3.2. Spice Use Pattern in Dataset I

It was noted that spices were infrequently used by Japanese housewives around the 1920s. Of 825 recipes, 392 recipes (47.5%) called for no spices at all. In addition, 327 recipes (39.0%) called for only one spice. Because 87.2% of recipes in Dataset I included only one or no spice, the spice use variable was treated as a binary variable, indicating whether a given recipe called for at least one spice. Also, many spiced-recipes called for Japanese radish (*daikon*), which is, in many cases, eaten as a vegetable. Faced with a similar problem with onions and green peppers, Sherman and colleagues treated them as spices regardless of the quantity called for in the recipes. However, it is know that the antimicrobial potency of Japanese radish's compound (4-methilthio-3-butenyl-isothiocyanate; Uda, Matsuoka, Kumagami, Shima, & Maeda, 1993) depends on how it is cooked (e.g., strongest when being grated). Also, as we shall see in the following, exclusion of Japanese radish from spices

caused substantial changes in the analytical results. Accordingly, I shall report the results of two sets of analyses: one including Japanese radish as a spice and one excluding it. In addition, notice that Predictions 1 and 2 require different subsets of the entire recipes: recipes involving both meat/fish and vegetables are not relevant to test Prediction 1; spring and autumn recipes are not relevant to test Prediction 2. Hence, Predictions 1 and 2 were tested with separate analyses and Predictions 3 and 4 were tested in a single analysis including the entire recipe sample.

Whether meat-based recipes would more frequently call for at least one spice (with or without Japanese radish) than vegetable-based recipes was tested by two separate χ^2 -tests. When Japanese radish was included as a spice, 44.5% of meat-based recipes (130 of 292) called for at least one spice while 51.5% of vegetable-based recipes (204 of 396) did so. This difference, opposite to Prediction 1, was marginally significant, $\chi^2(df=1)=3.29$, p=.07. This opposite tendency could be due to the fact that a substantial portion of vegetable-based recipes included Japanese radish as their main ingredient. When Japanese radish was not included as a spice, the corresponding percentages were reduced to 35.6% (104 of 292) and 36.6% (145 of 396) for meat-based and vegetable-based recipes, respectively, $\chi^2(df=1)=.07$, ns. In either case, the spice version of Prediction 1 was not supported.

To test Prediction 2, two separate χ^2 -tests were conducted to test whether summer recipes would more frequently call for at least one spice than winter recipes. With Japanese radish as a spice, winter recipes rather than summer recipes more frequently called for at least one spice, 34.1% (75 of 220) and 74.2% (158 of 213) for summer and winter recipes, respectively, $\chi^2(df=1)=69.97$, p<.001 (see Figure 2a for spice use patterns in other seasons). When Japanese radish was not included, there was no significant difference between summer recipes (31.8%; 70 of 220) and winter recipes (36.6%; 78 of 213), $\chi^2(df=1)=1.11$, ns. Accordingly, it can be said that in Japan around the 1920s, Japanese radish was widely

consumed during winter, and apparently increased the spice use in winter. In fact, even today, Japanese radish is considered to be a winter vegetable. However, as can be seen in the analyses of Dataset II, its winter consumption is not now as dominant as it used to be (see Figures 2a and 2b).

To test Predictions 3 and 4, two separate logistic regression analyses were conducted with spice use (with or without Japanese radish) as the dependent variable and mean annual temperature and heating as the independent variables. The variable of heating was a dummy coded variable, 0 indicating unheated and 1 indicating heated recipes. When Japanese radish was included as a spice, neither independent variable was significant, $B\pm SE = -.019\pm.029$, Wald's $\chi^2(df=1)=.45$, ns for the mean annual temperature and $B\pm SE=-.28\pm.19$, Wald's $\chi^2(df=1)=2.37$, ns for heating. On the other hand, when Japanese radish was excluded from spices, the effects of both variables were marginally significant, but the effect of mean annual temperature was in the opposite direction, $B\pm SE=-.059\pm.030$, Wald's $\chi^2(df=1)=3.74$, p=0.053 for the mean annual temperature and $B\pm SE=-.35\pm.19$, Wald's $\chi^2(df=1)=3.45$, $\chi^2(df=1)=3.45$,

In sum, the analyses of Dataset I (i.e., recipes of Japanese housewives around the 1920s) provided little support for the spice versions of the predictions. Although Prediction 4 was weakly supported, the opposite tendency was marginally significant for Prediction 3.

3.3. Vinegar Use Pattern in Dataset I

It was impossible to quantify the amount of vinegar used in each recipe. Accordingly, it was analyzed as a binary variable (i.e., whether vinegar is called for in a given recipe). The statistical analyses were comparable with the ones in the previous section.

Consistent with Prediction 1, vinegar was more frequently called for in meat-based

recipes (27.7%; 81 of 292) than in vegetable-based recipes (23.2%, 92 of 396). However, the difference was not statistically significant, $\chi^2(df=1)=1.81$, ns. Consistent with Prediction 2, vinegar was more frequently called for in summer recipes (28.6%; 63 of 220) than in winter recipes (18.8%; 40 of 213), $\chi^2(df=1)=5.80$, p=.016 (see also Figure 2d for other seasons). Predictions 3 and 4 were tested by a logistic regression analysis. The result supported both predictions, $B\pm SE=.079\pm.036$, Wald's $\chi^2(df=1)=4.87$, p=.027 for the mean annual temperature and $B\pm SE=-1.92\pm.20$, Wald's $\chi^2(df=1)=95.04$, p<.001 for heating. The effect of the mean annual temperature was corroborated by a χ^2 -test with the mean annual temperature treated as a categorical variable (i.e., low vs. middle vs. high). The proportions of recipes calling for vinegar were 20.5% (57 of 278), 26.3% (66 of 251), and 30.1% (89 of 296) for low, middle, and high temperature areas, respectively, $\chi^2(df=2)=6.94$, p=.031. The effect of heating reflects the more frequent use of vinegar in unheated recipes (60.3%; 88 of 146) than in heated recipes (18.3%; 124 of 679).

In sum, when the vinegar use pattern in Dataset I was subjected to the analyses, all the predictions except for Prediction 1 were confirmed. However, one might notice that a relatively small portion of recipes called for vinegar (i.e., 25.7%; 212 of 825). I shall return to this issue in the discussion section.

3.4. Spice and Vinegar Use Patterns in Dataset II

It is noteworthy that Sherman and colleagues' analyses included Japanese recipes and their Japanese data supported Prediction 1: More spices were called for in Japanese meat-based recipes than in Japanese vegetable-based recipes (Sherman & Hash, 2001). This pattern was not found in Dataset I. As we already noted, Dataset II might include recipes more similar to Sherman and Hash's Japanese recipes. I tested both spice and vinegar versions of Predictions 1, 2, and 4 with Dataset II. In this set of analyses, the spice variable was treated as a continuous variable (i.e., the number of spices called for in each recipe), while the vinegar

variable was the binary variable as in the analyses of Dataset I. This change in the spice variable was made because most of the recipes in Dataset II (77.2%; 339 of 439) included at least one spice and thus the binary variable did not work well (cf. Figures 2b and 2c).

The spice versions of the three predictions (1, 2, and 4) were tested first. As in the analyses of Dataset I, these predictions were tested with separate analyses because these predictions required different subsets or the entire set of the recipe sample. The mean numbers of spices were 1.24 (SD = 1.01, N = 123) for meat-based recipes and 1.03 (SD = .73, N = 89) for vegetable-based recipes, t(210) = 1.76, p = .04 (one-tailed, equal variance was not assumed). A comparable t-test with the number of spices without Japanese radish revealed a marginally significant difference: 1.11 (SD = .91) for meat-based recipes vs. .94 (SD = .66) for vegetable-based recipes, t(209.99) = 1.50, p = .068 (one-tailed, equal variance was not assumed). Two planned contrasts were conducted to compare summer recipes and winter recipes. With Japanese radish as a spice, the mean numbers of spices were 1.37 (SD = 1.03, N= 117) for summer recipes and 1.24 (SD = .94, N = 141) for winter recipes, and this difference was not statistically significant, t(435) = 1.11, ns (see Figure 2c). As we already noted, however, winter recipes may include more Japanese radish, which is used as a winter vegetable rather than a spice. When Japanese radish was not included, the mean numbers of spices were 1.25 (SD = .95) for summer recipes and 1.00 (SD = .81) for winter recipes, and this difference was statistically significant, t(435) = 2.43, p = .016. The mean numbers of spices were 1.31 (SD = 1.05, N = 88) for unheated recipes and 1.15 (SD = .89, N = 351) for heated recipes, and this difference was marginally significant, t(119.70) = 1.32, p = .094(one-tailed, equal variance was not assumed). The mean numbers of spices without Japanese radish were 1.15 (SD = .95) for unheated recipes and 1.00 (SD = .80) for heated recipes, and this difference was also marginally significant, t(119.70) = 1.34, p = .092 (one-tailed, equal variance was not assumed).

The vinegar versions of the three predictions were tested by a series of χ^2 -tests. Meat-based recipes called for vinegar more frequently (26.0%; 32 of 123) than vegetable-based recipes (19.1%; 17 of 89). However, this difference was not statistically significant, $\chi^2(df=1)=1.39$, ns. Contrary to Prediction 2, winter recipes more frequently called for vinegar (34.0%; 48 of 141) than summer recipes (23.9%; 28 of 117). This difference was marginally significant, $\chi^2(df=1)=3.15$, p=.076 (see Figure 2d). Unheated recipes called for vinegar more frequently (51.1%; 45 of 88) than heated recipes (17.9%; 63 of 351). This difference was statistically significant, $\chi^2(df=1)=41.78$, p<.001.

In sum, when Dataset II (i.e., recipes provided by experts in traditional Japanese cuisine) was subjected to analyses, spice versions rather than vinegar versions of the predictions received stronger support. When spice versions of the three predictions were tested, the analyses supported Predictions 1 and 2 at the statistically significant level and Prediction 4 at the marginally significant level. When the vinegar versions were tested, on the other hand, only Prediction 4 was supported.

4. Discussion

The present study demonstrated apparently contradictory patterns of antimicrobial ingredient usage in traditional Japanese cuisine. When recipes practiced by ordinary housewives around the 1920s (i.e., Dataset I) were analyzed, the vinegar use pattern accorded more closely with the antimicrobial predictions: Meat-based recipes more frequently called for vinegar than vegetable-based recipes (although this was not statistically significant), summer recipes more frequently called for vinegar than winter recipes, recipes in hotter climates more frequently called for vinegar than those in cooler climates, and unheated recipes more frequently called for vinegar than heated recipes. On the other hand, when recipes provided by experts in traditional Japanese cuisine (i.e., Dataset II) were analyzed, the spice use pattern was closer to the predictions: Meat-based recipes called for more spices than

vegetable-based recipes, summer recipes called for more spices than winter recipes (if Japanese radish was excluded), and unheated recipes called for more spices than heated recipes.

One possible explanation for the discrepancy between the two types of datasets is availability. Recipes in Dataset I, in particular, appeared to call only for locally available ingredients. Although Billing and Sherman (1998) pointed out that local availability was not a sufficient explanation for the world-wide spice use pattern, ordinary citizens' spice use pattern might have been more strongly constrained by local and seasonal availability. Moreover, spices might have often been too expensive for ordinary citizens to consume on a daily basis. Seasonal variation in the spice use pattern in Dataset I is also supportive of the constraint imposed by availability. As shown by darker bars in Figure 2a, the largest proportion of recipes includes at least one spice in spring, if Japanese radish, mostly consumed as a winter vegetable, is excluded. This may be because some of the Japanese spices become available during spring. For example, a species of spring onion, wakegi (Allium wakegi Araki), is consumed mostly in spring (i.e., 17 of 18 recipes calling for it were spring recipes). Such a pattern suggests that spice use by Japanese housewives around the 1920s was more or less constrained by availability. On the other hand, expert chefs might have been able to afford to use rather expensive spices, and thus recipes inherited by authorities might have been less constrained by availability.

We have noted that vinegar was called for relatively infrequently in recipes in Dataset I, although its usage pattern was consistent with the antimicrobial predictions. Also, only a small portion of the recipes in Dataset I called for spices. Note, however, that we need not exclusively depend on one type of antimicrobial ingredients. When the spice and vinegar variables were combined, 62.4% (515 of 825) of recipes in Dataset I included at least one antimicrobial ingredient. This proportion could increase if we take other antimicrobial

ingredients, such as salt, into account. In fact, Japanese fish preservation methods often call for salt as well as vinegar (Nagayama, 2003). Another potential antimicrobial ingredient in Japanese cuisine is *sake*, a Japanese alcohol beverage. Collecting recipes from fishers in various regions of Japan, Nomura (2005) noted that one of the simplest fishers' recipes prescribes merely boiling fish in *sake*. More detailed analyses including various types of antimicrobial ingredients and their complementary usage patterns are yet to be conducted.

There are several limitations in the present study. First, in testing the predictions, all types of spices were treated as a single variable. As Billing and Sherman (1998) noted, some spices are more potent against certain types of pathogens. Accordingly, it would be more informative if the analyses included such an interaction effect between spice-type and pathogen-type. Second, it is likely that spices and vinegar have different antimicrobial effects contingent on quantity and timing of use. Third, in addition to the aforementioned complementary uses of different antimicrobial ingredients, their potential synergic effects would be interesting to explore. There seem to be some synergic effects between spices and vinegar: an experiment conducted by the Aichi Industrial Technology Institute shows that certain combinations of them increased the antimicrobial effect (cited in Takemasa, 1990). According to Nagayama (2003), Japanese historical archives indicate that the combined uses of spices and vinegar (e.g., wasabi and vinegar, mustard and vinegar) began during the *Muromachi* era (1338—1573 according to one definition). Analyses targeting more specific effects of spices/vinegar or interaction effects between them are needed to obtain a full picture of how traditional Japanese cuisine combated food spoilage.

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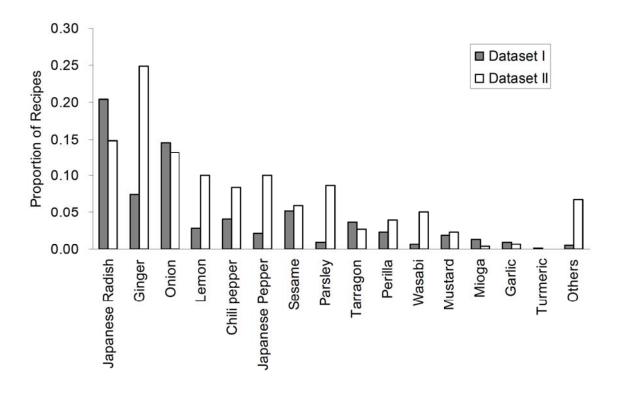
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Figure Captions

Figure 1. Proportions of recipes that call for each of 16 spices as a function of dataset type.

Figure 2. Seasonal difference in spice/vinegar use pattern of Japanese recipes: (a) Proportions of recipes in Dataset I that called for at least one spice as a function of season. Numbers placed above the bars show the numbers of recipes calling for at least one spice (i.e., numerators of the proportions shown in the figure). Numbers in parentheses placed bottom of the bars show the number of the given season's recipes (i.e., denominators of the proportions shown in the figure). (b) Proportions of recipes in Dataset II that called for at least one spice as a function of season. (c) The mean numbers of spices that were called for by recipes in Dataset II as a function of season. Error bars represent standard errors of means. (d) Proportions of recipes that called for vinegar as a function of dataset and season.



<Figure 1>

